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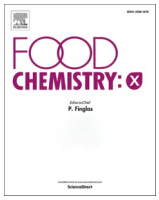

Impact of combined grape maturity and selected *Saccharomyces cerevisiae* on flavor profiles of young 'cabernet sauvignon' wines

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ABSTRACT

Grape maturity and yeast strains are crucial to determining young wine quality. This study evaluates the impact of three grape maturity levels with sugar contents of 22, 25, and 28°Brix combined with two *S. cerevisiae* strains selected from distinct terroirs on the Cabernet Sauvignon wine profile in the Ningxia Qingtongxia region in China. Physicochemical parameters and volatile aroma compounds were analyzed and quantitative descriptive analysis was performed on wine samples. The results indicated that berry ripeness primarily influenced physicochemical profiles, while aroma characteristics were affected by both grape maturity and yeast strain. Some esters and higher alcohols increased with grape maturity. Late-harvest wines scored significantly higher in aroma and taste quality than early-harvest wines. The CECA strain yielded wines with elevated medium-chain ester levels, reduced higher alcohols, improved balance and purity, and enhanced the typical aroma of blackberry, spice, and dark chocolate.

1. Introduction

The sensory profile of wines depends on the concentration and interaction of various compounds in wine, which is closely related to the composition of grape juice or must at harvest (van Leeuwen et al., 2022). Therefore, harvesting grapes at the appropriate level of maturity has always been a crucial factor in ensuring wine quality. In addition to assessing technical maturity based on parameters such as sugar content, total acidity, and pH, wine-makers now also consider phenolic and aromatic maturity in grapes at the time of harvest (van Leeuwen et al., 2022). This broader perspective allows winemakers to better understand the overall composition and potential flavor profile of the grapes, leading to the production of wines with enhanced sensory characteristics. Nonetheless, there are relatively few studies regarding the influence of aromatic maturity on wine quality and typicity. With regards to red wines, many winemakers tend to delay harvest dates to minimize green aromas and obtain riper, and fuller-bodied fruit-driven aromas in the wine (Williamson et al., 2012). Whilst the resultant benefits from late harvest grapes on wine flavor are quite limited, it, however, would lead to greater ethanol levels and insufficient acidity in wines (Schelezki et al., 2018), at the same time, there may be issues with late seal fruit dehydration, characterized by berry mesocarp cell death and water loss that leads to an increase in total soluble solids (TSS) concentration (Deloire et al., 2021). Notedly, such risks have been exacerbated by the compression of the growing season due to rising global temperatures in recent years (van Leeuwen et al., 2024), and have led to the appearance of dried fruit and even oxidized and cooked aromas in the young red wines. At the same time, high temperatures may decouple technical, phenolic, and aromatic maturity (van Leeuwen et al., 2022). Thus, it is necessary to analyze the composition of volatiles and evaluate the organoleptic properties of wines made from grapes of different harvesting periods in a given region.

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As a representative red grape variety, the dichotomy between the 'green/ vegetative' and 'fruity' attributes of Cabernet Sauvignon is particularly obvious (Robinson et al., 2011). Studies have shown that green characteristics in Cabernet Sauvignon are mainly due to two types of compounds. The first is grape-derived methoxypyrazine, such as isobutyl methoxypyrazine (IBMP). The other is C6 alcohols and their derivatives such as hexanal and (Z)-3-hexen-1-ol (Escudero et al., 2007), some of which are present in the berries, and most of the derivatives originate in the wine production process (Kalua & Boss, 2009). However, studies of the contribution of compounds to green characteristics are poorly understood and complex (Forde et al., 2011). This can be due to the interaction between IBMP and C6 volatiles (Escudero et al., 2007), and/or the suppression by other substances that impart fruity aromas (King et al., 2011).

Esters synthesized by yeast during wine fermentation are the main contributors to fruity aromas of wine (Gammacurta et al., 2014). Bindon et al. (2013) reported that the levels of fatty acid ethyl esters and acetates in Cabernet Sauvignon wines increased in line with enhanced ripeness of the grape. One possible explanation for this phenomenon is the higher availability of metabolic substrate for ester production in the grape must, which is attributed to late harvest. However, some studies have also shown that only 30 % of esters are affected by harvest date and sugar concentration (Antalick et al., 2015). Besides grape maturity, fermentation temperature, and predominant yeast strains also influence the production and composition of esters (Sumby et al., 2010). In terms of yeasts, it is believed that indigenous wine yeasts have a better ability to adapt to the unique characteristics of local grapes habitats. They contribute to the expression of wine typicity and become an integral component of the wine terroir (Foroni et al., 2017). For the fermentation of Cabernet Sauvignon wine, the winery in Qingtongxia region typically employs the commercial yeast selected from the Bordeaux region of France, which has notably different terroir conditions compared to the experimental vineyard. Conversely, commercial active dry yeast strains, CECA, selected from a habitat most similar to that of the experimental vineyards, exhibits great potential for enhancing wine quality in Qingtongxia region. However, it has received limited research attention.

Accordingly, for a specific climate region, selecting the optimal harvest dates and yeast strains can effectively enhance the expression of the wine typicity of the region. The Eastern Foothills of the Helan Mountains in Ningxia, China, holds significant prominence as a vital wine-producing region. The Qingtongxia region, located in its core area, has developed well in recent years. Cabernet Sauvignon is the main variety planted in this region. Research indicates that climate conditions in this region have led to a decoupling between phenolic maturity and technological maturity (Zhou et al., 2019), complicating the determination of the optimal harvest time. Many winemakers choose to delay harvest until the sugar content reaches around 25°Brix after achieving technological maturity (22–23°Brix) to enhance phenolic maturity. However, at this stage, acidity levels fall below the desired threshold. Moreover, the escalating effects of global climate warming have significantly increased the rate of sugar accumulation. Consequently, when phenolic maturity aligns with winemakers' perceived level, sugar content might soar to 28°Brix, while acidity drops to 4-5 g/L, resulting in an imbalance with heightened alcohol levels and reduced acidity. Additionally, prevailing studies have predominantly focused on phenolic maturity, neglecting the impact of these harvesting strategies on aroma maturity and the stress induced by elevated sugar concentrations on yeast, as well as yeast's pivotal role in aroma maturity within this domain. Therefore, the objective of this study was to combine grape harvest date and yeast metabolism to ascertain the optimum aromatic maturity under the current climate dynamics, and to provide insights into expressing the finest terroir typicality inherent to Cabernet Sauvignon in the region.

2. Materials and methods

2.1. Site location and sampling

Samples were obtained at four representative plots located within the expansive vineyard of Imperial Horse International winery, covering an approximate area of 200 ha in Ganchengzi county (latitude:27°43′-39°05′ N, longitude: 105°45–106°27′ E, altitude: 1100 m), within Qingtongxia region during the harvest season. The vineyard is located in an area of Helan Mountain's East Foothill with sandy gravel loam soil and a typical continental climate. The annual sunshine is more than 3000 h, and the annual rainfall ranges between 150 and 300 mm (data from Ningxia statistical year book). The *Vitis vinifera* L. cv. 'Cabernet Sauvignon' used in this experiment was planted in 1998.

The experiment was conducted in 2018, and climatic conditions during this vintage (March to October) are listed in Table S1. The row direction of the vineyard is north-south, the spacing is 1.0 m × 3.0 m, drip irrigation, short pruning, and modified vertical shoot position, and the thickness of the leaf curtain is controlled at 40 cm.

The samples were collected on three different harvest dates. The first harvest date was determined when the TSS of the berries reached approximately 22°Brix, indicating that they met the requirements for technological maturity and were ready to be picked. The second harvest date was determined when the TSS of the berries reached around 25°Brix. The final harvest occurred when the TSS of the berries exceeded 28°Brix. These dates were recorded as D1, D2, and D3, the interval between D1 and D2 was 11 days, and the interval between D2 and D3 was 5 days. And the samples were labeled as M1, M2, and M3, respectively. At each harvest date, approximately 60 kg of grape bunches were randomly sampled and mixed from 5 plots (approximately 1300 square meters each) within the vineyard to ensure representativeness. Post each harvest, 200 berries were randomly selected for the assessment of their basic physiochemical parameters, as detailed in Table S2. The remaining grape samples were used for small-scale vinification purposes.

2.2. Materials

Analytical grade chemicals, including sodium chloride, sodium hydroxide, sodium carbonate, ammonium sulfate, potassium chloride, sodium acetate, and hydrogen chloride (37 %) were purchased from Xilong Chemical Co. Ltd. (Sichuan, China). Methylcellulose, methanol, Folin-Phenol, and 4-(Dimethylamino) cinnamaldehyde were purchased from Solebo Technology Co., Ltd. (Beijing, China). Pure standards (purity ≥ 95 %) of Gallic acid, quercetin, caffeic acid, and catechins were purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Standards (purity > 95 %) used for the identification and quantification of organic acids and volatile compounds were obtained from Sigma–Aldrich.

The commercial active dry yeast strains used in the vinification process were uvaferm® BDX (Lallemand, France), which is a yeast strain selected from the Bordeaux region of France, and CECA, a yeast strain selected from the eastern foothills of the Helan Mountain region in Ningxia, China, and produced by Angel Yeast.

2.3. Wine making process

At each harvest date, for each replicate, approximately 15 kg grapes with uniform quality were de-stemmed and partially crushed into a 20 L wide-mouth glass jar (50 % crushing rate). To ensure proper preservation and prevent oxidation, 50 mg/L of sulfur dioxide was added to the grape must immediately after crushing. This was achieved by supplementing potassium metabisulphite. Next, pectolytic enzyme (EX, Lallemand, France) was added to the must, which was then allowed to soak for 24 h. Following the soaking period, the must was inoculated with 200 mg/L of activated yeast, either CECA or BDX, depending on the specific treatment. Before inoculating the selected yeast strains, the

amount of spontaneous yeasts present in the grape must was assessed using a plate-based method with the WLN medium (Table S3). The active dry yeast was rehydrated at 37 °C for approximately 30 min before inoculation. The inoculum was added in an amount equivalent to 4×10^6 CFU/mL according to the yeast's instructions. This ensured the establishment of numerical and metabolic dominance by the inoculated yeast strains right from the beginning of fermentation. Given the remarkable similarity in fermentation rates observed across the triplicate fermentations (Fig. S1) and the small standard deviation in the chemical composition of the triplicate ferments, it can safely be reasonably assumed that all fermentations were conducted by the inoculated yeast strains (Serafino et al., 2023). During fermentation, the temperature was controlled at 22–25 °C, and the cap was plunged down every 8 h. Temperature and density measurements were taken every 12 h. Once the reducing sugar fell below 4 g/L, the wines were manually pressed and transferred into a clean 10 L glass jar. Immediately after transfer, wines were added potassium metabisulphite to adjust sulfur dioxide level to 50 mg/L without malolactic fermentation. After clarification, the wines were bottled into 750 mL bottles and sealed with cork closures. They were then stored at 15 °C for subsequent analysis. The samples from grapes fermented by CECA were labeled as C1, C2, and C3, while the samples from grapes fermented by BDX were labeled as B1, B2, and B3. All component analysis and sensory analysis of the wine samples were completed within six months.

2.4. Analysis of physicochemical and color indices

A subsample of 100 berries was selected to determine berry weights. The berries were manually crushed to obtain must, and then the supernatant was used for analysis. The total sugar, titratable acid, and pH of grapes, as well as the residual sugar, titratable acid, volatile acid pH, and alcohol of wine, are determined according to OIV (2022) guidelines. Spectrophotometric methods are used to determine phenolic profiles of wine using Cary 60 UV–Vis spectrophotometer (Agilent, USA). This included quantifying the total phenols content (TPC) through the Folin-Ciocalteu method, derived from standard curves with gallic acid, at 760 nm, as endorsed by the International Vine and Wine Organization (OIV-MA-BS-19). Total tannin content (TTC) using the methylcellulose precipitation method (catechins) at 280 nm, tartaric ester (caffeic acid), flavonol (quercetin), and, total anthocyanin content (malvidin-3-glucoside), by measuring the absorbance of wines at 320, 360, and 520 nm. While flavanols were assessed using the *p*-dimethylaminocinnamaldehyde-HCl method (catechin) at 640 nm (Hosu et al., 2014). The wine color was characterized using CIELAB parameters analyzed by Spectrophotometer CM-5 (D65, 10°, Konica Minolta, Tokyo, Japan), following the OIV-MA-AS2–11 guidelines.

2.5. Organic acids analysis

Concentrations of organic acids were measured using HPLC. Wine samples were filtered by PTFE 0.22 μM syringe filters and diluted to ensure that the concentration of the specific organic acid being tested in the sample falls within the detection range. An Agilent 1260 system equipped with the quaternary pump, autosampler, column thermostat, HPX-87H column (300 mm × 7.8 mm, 9 μm), and UV–Vis detector were used. The column temperature was set at 55 °C and the detection wavelength was 210 nm. Injected sample volume was 5 μL. The mobile phase was 0.005 M H_2SO_4 with isocratic elution at a flow rate of 0.6 mL/min. For the analysis of organic acids, individual standards of each acid were weighed 100 mg and diluted to 100 mL with deionized water to prepare the stock solutions. Serial dilutions of the stock solutions were then prepared and analyzed. Calibration curves were generated by plotting the peak areas against concentrations of the standards.

2.6. Volatile aroma compounds determination

Volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) and quantitated by GC–MS as described in the previous study (Qian et al., 2024) with some modifications. Gas chromatographic analyses were performed with an Agilent 7890B GC equipped with an Agilent 5975B MS. An HP-INNOWAX capillary column (J&W Scientific, Folsom, CA, USA) (60 m × 0.25 mm i.d., 0.25 μm film thickness) was used. A CTC CombiPAL autosampler with a 2 cm DVB/CAR/PDMS 50/30 μm SPME fiber (Supelco, Bellefonte, PA) was used for the automatic HS-SPME. Full scan MS was used to quantitate all the aforementioned aroma compounds. A 5.0 mL wine sample was mixed with 10.0 μL 4-methyl-2-pentanol (internal standard, 1.0018 g/L) and 1.00 g NaCl. The mixture was placed into a 20 mL vial capped with a PTFE-silicon septum. Following equilibration at 40 °C for 30 min, the sample was extracted by an SPME fiber at 40 °C for 30 min with stirring at 500 rpm. The coated fiber was then thermally desorbed by insertion into the injection port for 8 min. The flow rate of carrier gas, helium, was 1 mL/min. The injector temperature was kept at 250 °C. The split mode (5,1) was used for injection. The GC temperature program was as follows: initial temperature 50 °C, held for 1 min and increased by 3 °C/min to 220 °C, held for 5 min. The MSD transfer line heater was set at 250 °C. The temperature of the ion source and quadrupole were 250 °C and 150 °C, respectively. The mass detector was operated in the full scan mode (*m/z* 30–350) with electron ionization (EI) mode at 70 eV.

The volatiles were identified by comparing their retention index and mass spectra with those of pure standards using the National Institute of Standards and Technology Library (NIST 17). Quantification depended on the standard curves (Table S5), Volatiles without standard curves were estimated with equations for those of the same functional group and/or with a similar number of carbon atoms. Volatile compounds from the wines were identified in μg/L. Odor activity values (OAVs) were calculated as the ratio between the concentration of an individual compound and the perception threshold.

2.7. Sensory analysis

Informed consent was obtained from each subject before they participated in the study, and ethical permission was not required to conduct this sensory evaluation. The participants were selected from the Tasting Panel of the College of Enology, Northwest A&F University, all of whom have more than one and a half years of wine-tasting experience. We conducted three rounds of triangular tasting, and those with accuracy rates of over 80 % in all three rounds were selected to form the tasting panel for this experiment. Ultimately, 7 females and 4 males passed the tests, formed into a sensory evaluation panel for Quantitative Descriptive Analysis (QDA). Afterward, these 11 participants underwent weekly training, focusing on their ability to differentiate various aroma characteristics with a 54-aroma kit (Le Nez du Vin®, France). The final requirement for the panelists was to achieve a scent recognition success rate of over 95 %. After two months of training, all participants met the standard.

Once groups were built and trained, participants were asked to describe and rate the intensity of descriptors that applied to the sample on a ten-point scale according to QDA methodology, which provides a complete sensory description of wine (Gomis-Bellmunt et al., 2024). To ensure accuracy, three samples were randomly selected for four rounds, with each sample appraised twice. When appraising the sample wine, the panelists needed to smell the fragrance of the still wine sample and then shake the wine glass. In principle, the evaluation time for each sample should not exceed 3 min. When evaluating between samples, panelists needed to rinse their mouths with water to ensure accuracy. The group members evaluated and discussed the wine samples freely under the leadership of experienced researchers. The descriptors with a passing rate of more than 50 % were retained and defined for the attributes. In the screening process, the panelists considered several

factors: first, there must be a basic aroma that can best summarize a wine; second, it must be reduced to a smaller number of representative words; and finally, all sensory descriptors that were not applicable to this sample should be reduced according to the specific situation. For example, the group agreed that there was no difference in complexity in a wine fermented by grapes of uniform quality as a new vintage, so this descriptor was no longer used. After the unanimous discussion of the panelists, descriptors, and definitions of the sensory characteristics of the experimental wine samples were established by the QDA method (Table S4).

The modified frequency (MF %) of each aroma characteristic was calculated according eq. (1).

$$MF\% = \sqrt{F(\%)I(\%)} \quad (1)$$

in which F% is the average perception frequency of the described reference terms in an aroma group by the panel, and I% is the average intensity of the described reference terms in the group expressed as the percentage of maximum intensity.

2.8. Statistical analysis

Significant differences in some parameters among wine treatments were evaluated by one-way analysis of variance (ANOVA) and Two-factor multivariate analysis of variance (Two-way Manova) using Tukey's test ($p < 0.05$) by the SPSS 19.0 software (SPSS Inc., Chicago IL, USA). The contributions of volatile categories to final wine aromas were expressed as regression coefficients in the partial least square regression (PLSR) model. The PLSR model used cross validation and the variables were standardized using Unscrambler X 10.1 (Camo, Trondheim, Norway). All other statistical analyses were conducted in R version 4.1.3. The permutational multivariate analysis of variance (PERMANOVA) was performed by 'adonis2' function using the R package 'vegan' version 2.6.4. The principal co-ordinates analysis (PCoA) was performed by 'pcoa' function in the 'ape' package version 5.7.1. The distribution of aroma components in different treatments was subjected to principal component analysis (PCA) by the 'PCA' function in the'FactoMineR' package version 2.8. Correlation analysis was performed using 'corrplot' package version 0.92. All figures were prepared based on the R package 'ggplot2' version 3.4.3.

3. Results and discussion

3.1. Physicochemical parameters of berries and wines

The basic physicochemical parameters of berries were determined and shown in Table S2. As the harvest was delayed, sugar content gradually increased while acid content decreased, both in accordance with the ripening process of grapes. However, from D2 to D3, the 100-berry weight decreased from 219.17 g to 210.37 g, signaling the occurrence of late-season fruit dehydration (Deloire et al., 2021), which can also lead to an elevation in sugar levels. Previous studies have identified a plateau phase in berry sugar accumulation, where subsequent increases in sugar concentration are primarily due to dehydration (Antalick et al., 2021). In this experiment, the berries at D3 lost 4 % of their weight compared to D2, yet exhibited a 12 % increase in sugar content, and acid levels continued to decline. This suggests that the rise in sugar content was mainly due to berry ripening. Antalick et al. (2021) proposed that sequential harvesting should rely on berry sugar accumulation (mg/berry) rather than sugar concentration, with the post-plateau period serving as a more accurate indicator of harvest timing, aligning better with the aromatic and sensory development of Cabernet Sauvignon and Syrah wines during maturation. However, in this experiment, the berries in this region did not show a distinct sugar accumulation plateau. Physicochemical parameters, color indices, and organic acids of wines are in Table 1. These parameters were found to be influenced by the ripeness of the berries rather than the two yeast strains used in the current study. Specifically, as the berries ripened, there was an observed increase in the pH and alcohol levels of the wines, while the titratable acidity decreased. These findings align with previous studies (Bindon et al., 2013; Gao et al., 2019). Since 50 mg/L of sulfur dioxide was added to the grape must immediately after crushing, the inoculated plate culture tests showed that microorganisms were nearly absent in the grape must. The high volatile acid content of C1 and B1 may be because the berries harvested at D1 have already carried acetic acid produced by decay bacteria such as acetic acid bacteria, thus increasing the volatile acid content of wine, while C3 may be due to the high sugar condition leading to the increase of acetic acid production (Ferreira et al., 2006).

The analysis also revealed the presence of various organic acids in the wines. Tartaric acids and malic acids, derived from the grape berries, were the predominant organic acids and their concentrations decreased as the grapes ripened. Phenols, which are greatly influenced by grape

Table 1
Physicochemical parameters, color indices, and organic acids of the wines.

Parameters	C1	C2	C3	B1	B2	B3
Alcohol (%.v/v)	11.87 ± 0.06e	14.23 ± 0.06b	16.13 ± 0.06a	12.37 ± 0.06d	13.77 ± 0.06c	16.03 ± 0.06a
Residual sugar (g/L)	3.88 ± 0.03c	3.52 ± 0.03e	5.73 ± 0.06b	2.72 ± 0.03f	3.63 ± 0.03d	5.87 ± 0.06a
Titratable acidity (g/L)	7.57 ± 0.06ab	7.38 ± 0.03c	6.97 ± 0.06d	7.58 ± 0.03a	7.43 ± 0.06bc	7.07 ± 0.06d
Volatile acidity (g/L)	0.52 ± 0.03ab	0.36 ± 0.01bc	0.60 ± 0.01a	0.53 ± 0.03ab	0.30 ± 0.16c	0.30 ± 0.01c
pH	3.36 ± 0.00f	3.52 ± 0.01c	3.63 ± 0.01a	3.39 ± 0.01e	3.46 ± 0.01d	3.55 ± 0.01b
Citric acid (g/L)	0.23 ± 0.01c	0.24 ± 0.01bc	0.26 ± 0.01abc	0.25 ± 0.02abc	0.28 ± 0.02a	0.27 ± 0.01ab
Tartaric acid (g/L)	2.98 ± 0.16b	2.5 ± 0.15de	2.29 ± 0.14e	3.39 ± 0.02a	2.78 ± 0.06bc	2.68 ± 0.01 cd
Malic acid (g/L)	2.06 ± 0.16ab	1.85 ± 0.06bc	1.69 ± 0.05c	2.32 ± 0.02a	1.31 ± 0.03d	1.59 ± 0.13c
Pyruvic acid (g/L)	0.2 ± 0.01a	0.19 ± 0ab	0.14 ± 0.02d	0.17 ± 0.01bc	0.16 ± 0.01 cd	0.16 ± 0.01 cd
Succinic acid (g/L)	1.76 ± 0.1a	1.62 ± 0.09ab	1.45 ± 0.07bc	1.73 ± 0.09a	1.51 ± 0.11bc	1.34 ± 0.03c
Lactic acid (g/L)	0.39 ± 0.04a	0.34 ± 0.02ab	0.26 ± 0.01bc	0.33 ± 0.02ab	0.20 ± 0.12c	0.27 ± 0.02abc
Acetic acid (g/L)	0.41 ± 0.12abc	0.36 ± 0.02bc	0.54 ± 0.04a	0.48 ± 0.08ab	0.33 ± 0.02c	0.36 ± 0.01bc
Total tannin (mg/L)	673.5 ± 4.5d	810.7 ± 11.8c	969.4 ± 1.4b	665.9 ± 4.9d	952.1 ± 22.7b	1110.7 ± 11.8a
Total phenols (mg/L)	1169.5 ± 22.7e	1361.3 ± 4.1c	1627.9 ± 1.9a	1085.8 ± 3.7f	1248.9 ± 0.9d	1597.7 ± 4.2b
Anthocyanin (mg/L)	158.9 ± 11.6d	224.8 ± 4.1c	283.2 ± 1.3a	142.3 ± 2.8e	215.7 ± 1.3c	260.1 ± 4.2b
Flavonol (mg/L)	56.9 ± 2.9f	82.8 ± 4.1d	105.0 ± 1.7b	75.5 ± 0.6e	97.5 ± 2.2c	166.1 ± 1.6a
Tartaric ester (mg/L)	161.2 ± 4d	214.6 ± 4.6bc	243.5 ± 3a	205.6 ± 4.8c	225.6 ± 4.9b	241.1 ± 3.9a
Flavanol (mg/L)	374.0 ± 5.7e	507.8 ± 3.6c	524.0 ± 3.8b	226.0 ± 2.8f	473.5 ± 3.1d	552.5 ± 2.5a
L*	2.72 ± 0.01f	7.56 ± 0.02d	6.60 ± 0.06e	11.47 ± 0.02a	10.55 ± 0.01b	9.91 ± 0.01c
a*	17.85 ± 0.01f	37.28 ± 0d	45.22 ± 0.01a	32.71 ± 0.02e	41.78 ± 0.01b	39.99 ± 0.02c
b*	0.43 ± 0.02b	−0.03 ± 0.01d	−0.12 ± 0.01e	0.56 ± 0.01a	0.01 ± 0.01c	−0.11 ± 0.01e
Color Visualization	●	●	●	●	●	●

ripeness, play a crucial role in determining red wine styles. It was observed that wines made from more mature grapes showed higher levels of phenols (Table 1). The distribution patterns of anthocyanins, the primary pigments in red wine, and flavonols, which contribute to wine color through co-pigmentation with anthocyanins, across samples of varying ripeness levels suggest that wines made from riper grapes exhibit a redder hue and enhanced color stability. (Alves Filho et al., 2022). The accumulation of phenolic compounds may increase as the fruit matures. While, research indicates that although total phenolic content stabilizes after the berry sugar accumulation plateau, the extractability of phenolic compounds increa)ses due to the higher alcohol content in late-harvested grapes, which enhances cell wall permeability (Antalick et al., 2021). Studies have also shown that the impact of alcohol on phenolic compounds in wine differs from that of grape ripeness. Under identical ripeness conditions, treatments aimed at increasing potential alcohol primarily elevated the concentrations of tannins and iron-reactive phenols in Cabernet Sauvignon wines. In contrast, grape ripeness influenced the levels of various phenolic compounds. Notably, the concentration of non-precipitable polymeric pigments was solely affected by ripeness (Feifel et al., 2024). Additionally, in this experiment, the late-harvested grapes had higher sugar levels, leading to longer fermentation times and, consequently, extended maceration times (Fig. S1). Alves Filho et al. (2022) revealed that proanthocyanidins and flavonoids increased in wines made from mature grapes subjected to prolonged maceration. These factors are likely responsible for the observed differences in phenolic content across samples from various harvest periods. Flavonol and tartaric esters levels were higher in BDX yeast-fermented wines (Table 1). The a* values, which represent the red color, were indeed higher in BDX wines compared to CECA wines. BDX wine samples had the same or even shorter maceration time compared to CECA wines. This suggests that the BDX yeast may be responsible for the reddish color of the wine samples by increasing the amount of flavonols. Furthermore, the indices of total phenols, tannins, anthocyanins, and flavanols in CECA wines were higher than those in BDX wines. Except for the 24-h longer maceration time of C3 compared to B3, the data from the other harvest periods suggest that, compared to BDX yeast, CECA yeast may impart a more pronounced convergence and fuller body to the wine.

3.2. Volatile compound profiles of wines

Volatile aroma components in wine samples were determined by HS-SPME-GC–MS, and a total of 64 volatiles were detected and categorized into nine groups: esters, higher alcohols, fatty acids, C6-compounds, aldoketones, terpenes, phenylethyls, phenolic acid esters, C13-norisoprenoids, and volatile phenols (Table S5). The total concentration range of these compounds was found to be 793.03–1204.87 mg/L. For most of the varietal compounds, no significant trend was observed from D1 to D2, but levels either declined or stabilized from D2 to D3. Wine samples fermented by BDX yeast exhibited higher levels compared to those fermented by CECA. Regarding fermentation aroma compounds, the levels of ethyl esters of branched acids (EEBA), higher alcohol acetates (HAA), and fatty acids remained stable or declined from D1 to D2, then increased from D2 to D3. Most ethyl esters of straight-chain fatty acids (EEFA) and higher alcohols showed either minimal variation or continuous growth with delayed harvest. HAAs and major higher alcohols were more abundant in the BDX wine samples, while most medium-chain fatty acid ethyl esters were more abundant in the CECA-fermented samples (Table S5).

To analyze the importance of aroma compounds and their changing patterns under different harvest periods with two yeasts, the OAV value of each aroma compound was calculated (Table S5). Generally, aroma compounds with content below their olfactory thresholds have minimal impact on the overall sensory profile. However, some studies have shown that certain compounds with subthreshold ($0.1 < OAV < 1$) can still contribute significantly to sensory characteristics due to synergistic effects (Xiao et al., 2019). In this experiment, an unsupervised PCAs method was used to perform dimensionality re-duction analysis on compounds with a content higher than the subthreshold value ($OAV > 0.1$) (Fig. 2). The samples were well separated based on the first two principal components. As the harvest was delayed, the samples position in Fig. 2b is approximately close to the negative direction of PC1 and the positive direction of PC2. The wine samples fermented by different yeasts can be separated along the diagonal of the second and fourth quadrants. With delayed harvest, the main odor activity compounds that increased were mainly esters and alcohols. At the same harvest date, CECA wines had higher contents of ethyl 2-methyl-butyrate, ethyl hexanoate, isobutyrate, ethyl acetate, 3-hexen-1-ol, and benzyl alcohol compared to those of the BDX wines. Notedly, the total content of higher alcohols of CECA wines was significantly lower than that of BDX wines, in particular isobutanol, isoamyl alcohol, and 3-methylthiopropanol.

Different varietal aroma compounds varied with the harvesting delay. Terpenes and C13-norisoprenoids decreased with the delay in harvesting. However, there was an interesting observation that hexanol increased with the delay in harvesting, contrary to other research results (Williamson et al., 2012). Moreover, at the same level of maturity, the content of 1-hexanol was higher in BDX wines than in CECA wines. In general, small amounts of C6 alcohols in wine are derived during the maturation process from linolenic acid esters and linoleic acid esters in grapes via the pathways of lipoxygenase and alcohol dehydrogenase or oxidized during grape crushing operation. A large amount of C6 alcohol is obtained by reducing C6 aldehyde by alcohol dehydrogenase under the action of yeasts (Dennis et al., 2012). In this experiment, the grape-crushing operation was consistent throughout. The difference in hexanol content in fermented wine samples of different strains may be caused by the higher alcohol dehydrogenase activity of BDX compared to CECA. The high content of 1-hexanol in late-harvested wines may be due to that C18 fatty acids or C6 aldehydes, the precursors of C6 alcohols, are still in the increasing stage. This suggests that the aromatic and technological maturity of grapes in this region are decoupled (Escudero et al., 2007), indicating that the rate of accumulation of sugar is faster than the change of aroma compounds.

Esters have a great influence on the aroma of wine (Gammacurta et al., 2014). Ethyl acetate is the most common ester in wine, primarily because of the abundant availability of its substrates(acetic acid and ethanol)and the high reactivity of ethanol. In this study, ethyl acetate concentration varied significantly among the samples. Ethyl acetate's flavor can vary, and it can impart a solvent-like aroma to the wine in excessive amounts. In wines from the D3, particularly C3, ethyl acetate levels exceeded 100 mg/L, which may negatively impact the wine's aroma. The trend of ethyl acetate levels mirrored that of ethanol, which explains its increase with delayed harvests. Differences in acetic acid levels among samples account for the variations in ethyl acetate across wines fermented with different yeast strains, with the high levels in C3 likely due to elevated levels of both acetic acid and ethanol. The concentrations of C4-C10 EEFAs increased during grape ripening, which aligns with findings by Bindon et al. (2013), while long-chain EEFAs remained stable, as observed by Antalick et al. (2015), and there was no significant correlation between EEFA levels and fatty acid substrates, indicating a complex relationship between grape composition and yeast metabolism (Antalick et al., 2021). In this experiment, EEBA levels decreased from D1 to D2, then stabilized or slightly increased from D2 to D3, but did not exceed D1 levels. This pattern is similar to trends observed in studies of Syrah grape ripening, potentially linked to yeast redox metabolism (Antalick et al., 2021). HAA levels decreased from D1 to D2, but increased sharply from D2 to D3, surpassing D1 levels. Existing literature indicates that HAA content can either increase or decrease with ripening. However, in this study, sugar levels varied significantly, with a maximum of 285 g/L. Such high sugar content at harvest is rarely documented, and the substantial alterations in juice parameters may have had a considerable impact on acetyltransferase activity (Šuklje et al., 2016). Ripeness alone does not fully explain the

variations in ester content. Other factors known to influence ester production and concentration include the initial presence of esters or their precursors in grapes, fermentation temperature, yeast strains, and the availability of nutrients, particularly nitrogen compounds and must solids. (Gobert et al., 2019). In this study, variations in ester profiles were observed due to fluctuations in soluble solid content of grapes during different harvesting periods. Furthermore, dissimilarities in ester composition were attributed to the utilization of distinct yeast strains. Studies have shown that the average yield of esters and the relative proportion of each ester are highly dependent on the specific yeast strain (Wang et al., 2024) In this study, the wine samples fermented by CECA exhibited higher content of ethyl acetate, ethyl 2-methylbutyrate, ethyl caproate, and ethyl decanoate.

Higher alcohols are the most abundant aroma compounds in wine. It is widely acknowledged that they can enhance the complexity of wine at low concentrations (approximately 300 mg/L or less), but elevated concentrations can negatively impact wine quality, resulting in a pungent odor. The wine samples in this experiment generally exhibit a high content of higher alcohols, which may explain why most wines produced in this region are somewhat coarse (Lai et al., 2023). Higher alcohols are mainly derived from sugar carbon in the biosynthesis of branched-chain amino acids. In the case of red wine, it should be noted that the concentration of higher alcohol was greatly influenced by the grape solids (Gobert et al., 2019). Therefore, in this study, the increase in grape solids caused by the delay of harvest, resulting in elevated grape solids content, is expected to enhance the higher alcohol content (Gao et al., 2019). Surprisingly, the overall content of higher alcohols, especially isobutanol, isoamyl alcohol, and 3-methylthiopropanol, in wines fermented with CECA was significantly lower compared to those fermented with BDX strain. Elevated levels of isobutanol and isoamyl alcohol contribute to solvent-like aromas in wine, while a high content of 3-methylthiopropanol imparts scents of boiled potato and rubber. The reduction of these compounds contributed to enhancing the purity of the wine samples (Lai et al., 2023). Previous studies have also demonstrated that the yield of higher alcohols varies among different strains of *S. cerevisiae* and should be a crucial factor in yeast selection for industry purposes (Kłosowski et al., 2015). Based on the results of this study, CECA is more suitable for industrial production in this region than BDX.

3.3. Effects of harvest date and strain on the overall flavor of wine samples

To assess the overall effects of different factors (harvest date and strains) on the various components, a PERMANOVA was performed. Moreover, PCoA was performed to visualize the differences in the various components of wine samples (Fig. 1). As can be seen from Fig. 1, based on various components, the wine samples had good differentiation in the first two dimensions. The harvest date, yeast strains, and their interaction explained the difference in samples ($R^2 > 0.95$), and there was interaction between the two factors. When considering physicochemical indexes, organic acids, phenolics, and colors, it was evident that the harvest date exerted the greatest impact, accounting for 95.70 %, 76.70 %, and 90.90 % of the variations in the respective samples. The explanation in strain was less than 10 %, while the interaction effect surpassed that of the strains. Nevertheless, the impact of both factors was highly significant. In terms of aroma components, the harvest date and strain contributed similarly, accounting for 45.8 % and 42.7 % of the variations, respectively. The interaction effect accounted for 6.6 %, while the influence of a single factor was more significant. A two-way ANOVA was performed for each specific component. The Tests of Between-Subjects Effects (Table S6) highlighted the individual and interactive influences of the factors on various components. Harvest date significantly affected most components, except for 17.19 % of the aroma compounds. The strain significantly influenced 64.06 % of the aroma compounds (mainly esters and higher alcohols), 80 % of the physicochemical indexes, 71.43 % of the organic acids, as well as all phenolic and color attributes. Interaction effects were not significant for titratable acidity, succinic acid, and anthocyanins but significantly influenced 51.56 % of the aroma compounds.

The significant impact of harvest date on the wine profile in this experiment aligns with previous research findings (Bindon et al., 2013; Ferrero-del-Teso et al., 2020; Gao et al., 2019). However, few studies have considered the influence of yeast, a key contributor to the aroma of young wines during the critical process of alcoholic fermentation, on wine quality (Añón et al., 2014). While Bindon et al. (2013) mentioned the importance of yeast metabolism in experiments on grape maturity, further research specifically targeting the yeast factor has not been conducted. The results of this experiment indicate that yeast's impact on physicochemical parameters, phenolics, and organic acids is less than that of the harvest date. However, a surprising finding was the significant and equivalent impact on the aroma profile, highlighting the importance of yeast in enhancing aroma quality. The levels of heptyl acetate, isopentyl hexanoate, (6Z)-nonen-1-ol, 3-methylthiopropanol, and phenylethyl acetate were influenced solely by the yeast strain. The CECA strain reduced the concentration of 3-methylthiopropanol, a compound with negative effects on the wine, by 50 %. Interaction effects were significant for higher alcohol esters, higher alcohols, medium-chain fatty acid esters, and C6 alcohols. These results provide some insights for research endeavors directed towards enhancing the maturity of wine aroma.

3.4. Wine sensory properties

Sensory evaluation focused on wine's specific characteristic aromas

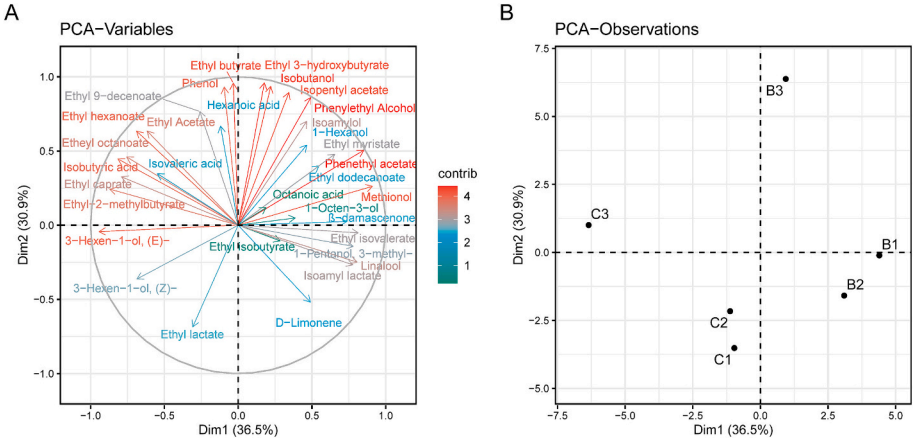

Fig. 1. Principal component analysis of main aroma compounds (OAV > 0.1) (A) Variables plot of Dim 1 and 2; (B) Observations plot of Dim 1 and 2.

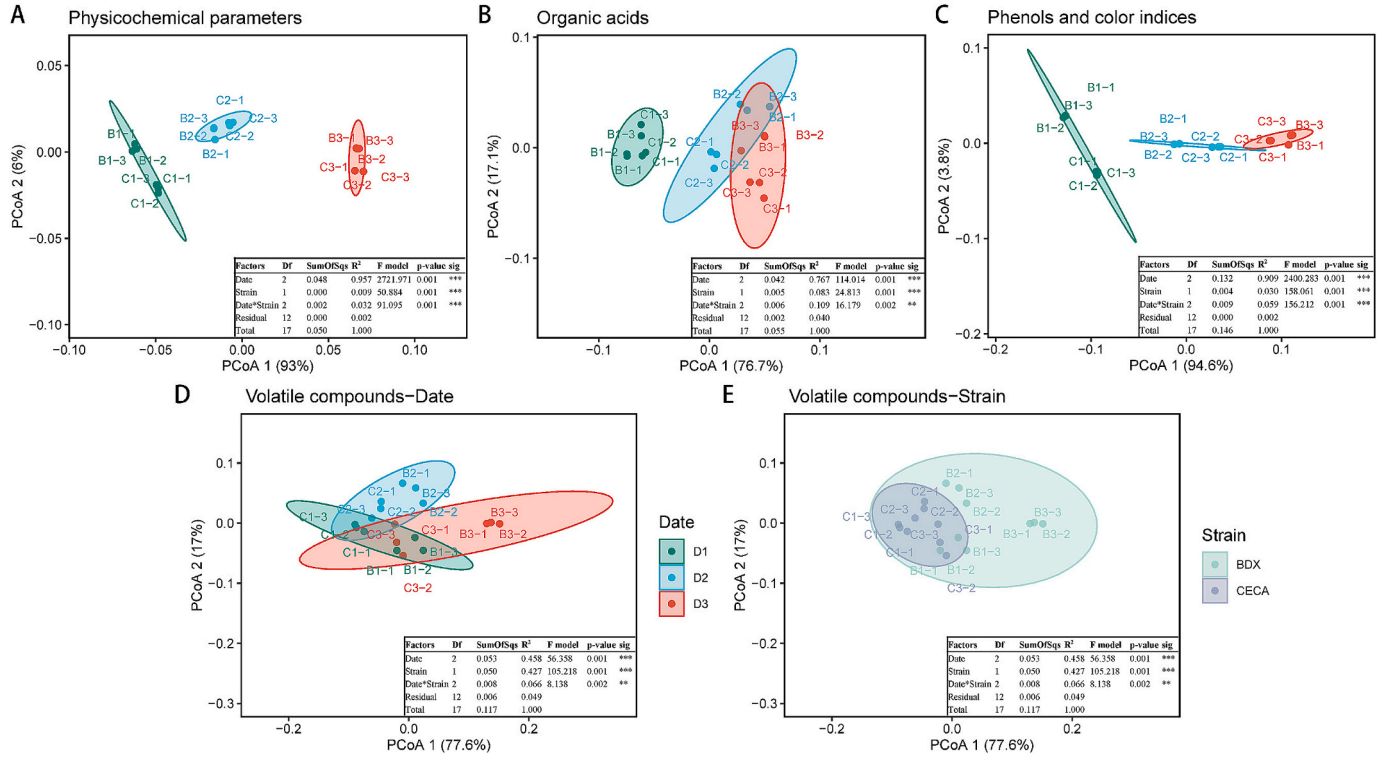

Fig. 2. The PERMANOVA of the effect of grape maturity and strain on wine compositions. (A) PERMANOVA of the effect of grape maturity and strain on physicochemical parameters; (B) PERMANOVA of the effect of grape maturity and strain on organic acids; (C) PERMANOVA of the effect of grape maturity and strain on phenols and color indexes; (D) PERMANOVA of the effect of grape maturity and strain on aroma compounds (display in groups by date); (E) PER-MANOVA of the effect of grape maturity and strain on aroma compounds (display in groups by strain).

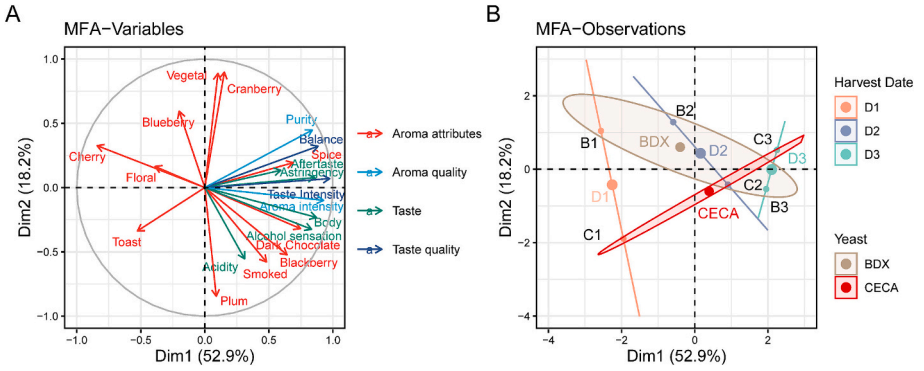

Fig. 3. Multiple factor analysis of sensory indicators. (A) Variables plot of Dim 1 and 2; (B) Observations plot of Dim 1 and 2.

and tastes. The quantitative sensory results were shown in Table S4. The tasting panel summarized the aroma quality of Cabernet Sauvignon from Qingtongxia region as intense and relatively pure with scents of cherries, blueberries, blackberries, black plums, herbs, spices, and dark chocolate. The taste characteristics were summed up as high acidity, medium strong tannins, moderate body, medium intensity of the mouth, slightly obvious alcohol sense, medium length of the aftertaste, and good balance. The specific ratings for each sensory attribute are provided in Table S7.

The quantified tasting results were analyzed through MFA (Fig. 3). In terms of the first two principal components, the samples could be well separated. With the delay of harvesting, the samples were located in the positive direction of PC1 (Fig. 3B). Overall, increased grape maturity resulted in a purer and more intense aroma, which was consistent with previous studies (Bindon et al., 2014; Ferrero-del-Teso et al., 2020). Notably, the herbal odor was prominent in the maturity of 25°Brix, which differed from the results of Bindon et al. (2014), who observed a decrease in herbal odor with increasing alcohol content. This suggested that the aroma maturity of the region did not correspond to the rate of sugar accumulation. Regarding taste, tannins, acidity, body, balance, and sense of alcohol all increased with delayed harvesting. The BDX wines had greater intensity in red berry, blueberry, and herbal aromas (B1 and B2), while the CECA wines had more blackberry, smoky, spice, and dark chocolate aromas. When harvested early, C1 had a more mature aroma compared to B1, which indicated CECA may enhance the ripe berry character of the wine and reduce the green characteristic. BDX fermented wine samples had stronger taste intensity, in which B3 had a stronger acid sensation, while CECA fermented wine samples presented more balance. Overall, compared with BDX, CECA improved the maturity of the wine aroma, making the aroma more intense and purer.

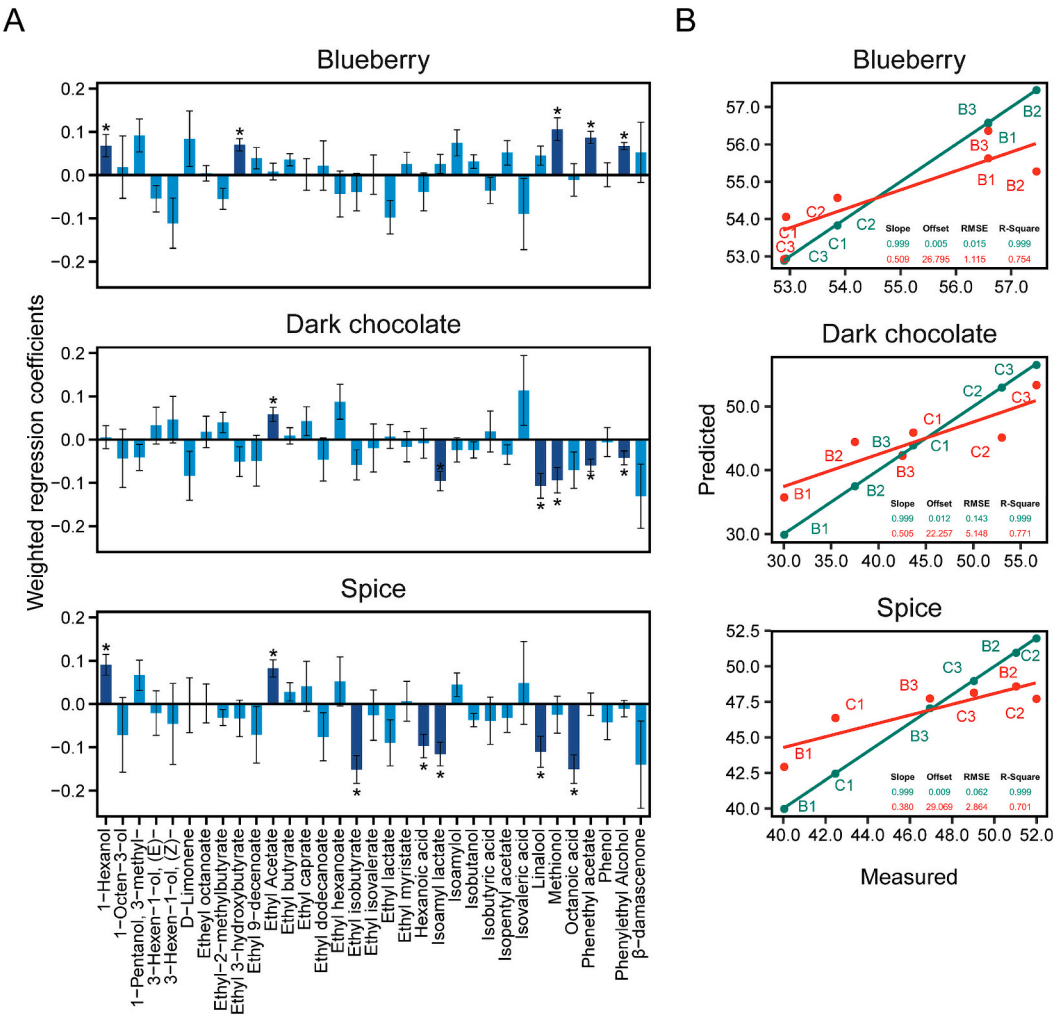

Fig. 4. Partial least squares regression analysis of main aroma characteristics. (A) PLSR weighted regression coefficients of aroma attribute and volatile compounds; (B) PLSR of "blue-berry"," dark chocolate", and "spice" aromas from volatiles (OAV > 0.1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

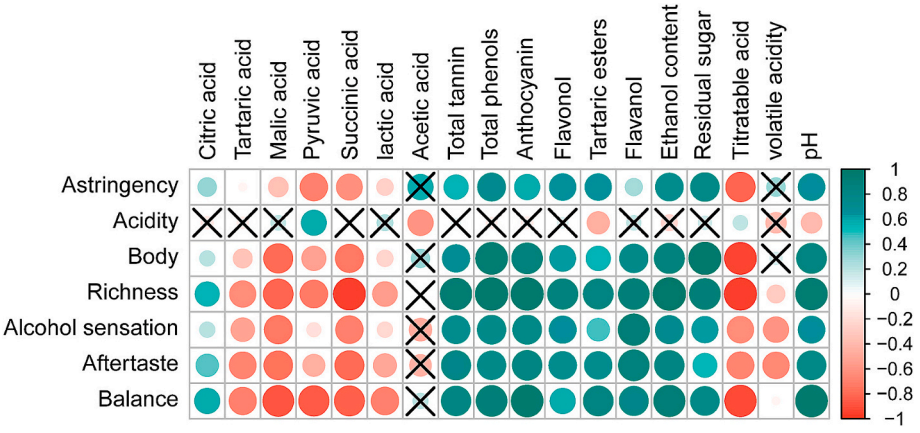

Fig. 5. Correlation analysis of sensory evaluation.

3.5. Relationship between sensory and chemical variables

To better reveal the relationship between volatile compounds and aroma sensory properties, a PLS regression model with substance (OAV > 0.1) as the independent variable and sensory as the dependent variable was established, and a jackknife significance test was conducted. The aroma properties of blueberry, spice, and dark chocolate were well predicted by the model (Fig. 4B), and the weighting coefficients of each substance and aroma properties were shown in Fig. 4A. Among them, the main compounds positively correlated with the aroma characteristics of blueberry were hexanol, ethyl 3-hydroxybutyrate, methyl mercaptan, phenylethyl acetate, and phenylethanol, and the correlation

was significant. The fitting results were consistent with those previously reported (Farneti et al., 2017). Compounds positively correlated with dark chocolate flavors were ethyl acetate, isovaleric acid, and ethyl palmitate. Isovaleric acid has been reported as one of the main aroma compounds of dark chocolate (Chetschik et al., 2019). Ethyl palmitate is likely to have a synergistic effect on the presentation of dark chocolate aroma due to its toast aroma (Siebert et al., 2018). 1-Hexanol, 3-methyl-1-pentanol, and ethyl acetate were positively correlated with the aroma characteristics of spices. As reported in previous studies, 3-methyl-1-pentanol has a pungent and cocoa odor, and ethyl acetate may contribute to the aroma of wine-like spices (Karabegović et al., 2021). Many other compounds showed negative correlations with specific aroma characteristics, which may have an inhibitory effect on the appearance of odor. For example, isoamyl acetate, ethyl isobutyrate, caproic acid, and caprylic acid had a significantly negative effect on the aroma of spices and chocolate. The above regression model explains that the CECA strain may made the chocolate and spice aroma profiles more prominent than BDX-fermented wine samples by elevating ethyl acetate and decreasing isoamyl acetate and caproic acid content of the wine samples. These correlations are solely based on the concentrations of compounds detected using the methods employed in this study. Considering the complex interactions between volatile substances and non-volatile elements such as polysaccharides, polyphenols, and proteins (Zhang et al., 2022). Validating the relationship between aroma compounds and aromatic perception may require additional sensory trials.

At the same time, pearson correlation analysis was conducted for variables and sensory evaluation that may participate in the formation of red wine taste characteristics (Fig. 5). The results showed that titratable acid content was positively correlated with acidity and negatively correlated with pH, but the positive correlation between organic acids and acid sensitivity was not significant, which is consistent with previous studies (Sáenz-Navajas et al., 2010). Citric acids, alcohols, residual sugars, and all phenolic compounds were significantly positively correlated with astringency, body, richness, alcohol sensation, aftertaste, and balance, while other organic acids were significantly negatively correlated with these sensory properties. These findings are in agreement with some of the results reported by Hufnagel and Hofmann (2008). However, other studies have shown that wine astringency decreases with increasing alcohol content, possibly due to the presence of alcohol reducing the intensity of tannin-protein interactions (McRae et al., 2015). Interestingly, in this experiment, with the increase of alcohol and phenol concentration, the sense of convergence first decreased and then increased, which was similar to previous research results (Ferrero-del-Teso et al., 2020). This suggested that ethanol may induce astringence-related sensations through mechanisms other than polyphenol-protein interactions.

4. Conclusions

This study explored the impacts of different harvest dates and *S. cerevisiae* strains on the chemical and sensory profiles of young Cabernet Sauvignon wines in the Qingtongxia region, located at the eastern foot of Helan Mountain, Ningxia, China. Results indicated that the sugar content of Cabernet Sauvignon grapes in this region increased rapidly after reaching 25°Brix driven by the climate and the onset of berry shrivel. This leads to high sugar and low titratable acidity, with the technical maturity decoupled from phenolic and aroma maturity. Both harvest date and yeast strains, as well as their interaction effect significantly influenced the wine profiles. The basic physicochemical indexes, organic acid, and phenolic indexes of the wines are primarily influenced by the ripeness level of grapes. As the harvest date extended, the increased tannins and other phenolic compounds enhanced the astringency of the wine samples, while anthocyanins and flavanols contributed to the color and potential stability of the wines. Volatile aroma components were affected by both the harvest date and the yeast strains used. The levels of key aroma-active compounds, such as C4–C10 fatty acid esters and higher alcohols, increased with grape ripening. The content of HAA and other substances is intricately linked to grape composition across maturity stages, making it difficult to discern a consistent pattern. Notably, the green-aroma compound C6 alcohol exhibited a significant increasing trend, while wines fermented with the CECA strain effectively reduced its concentration. Some medium-chain esters contributing to fruity aromas were found to be higher in wines fermented by CECA, the levels of higher alcohols affecting the purity of the samples were significantly lower than those in wines fermented by BDX. Sensory analysis revealed that CECA enhanced the typical aromas of blackberry, spices, and dark chocolate in the samples, with a purer aroma. In conclusion, the wines made from the ripest grapes and utilizing the CECA strain exhibited superior aroma maturity and overall sensory quality. These findings provide valuable insights for enhancing aroma maturity and overall wine quality of wines in the region with similar terroir conditions.

CRediT authorship contribution statement

Xiaomin Zang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Qing Du:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Jiao Jiang:** Writing – review & editing, Supervision, Formal analysis. **Yan-ying Liang:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Dongqing Ye:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Yanlin Liu:** Writing – review & editing, Supervision, Resources, Project administration, 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.2024.102066.

Data availability

Data will be made available on request.

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