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Inter- and intra-varietal clonal differences influence the aroma compound profiles of wines analyzed by GC–MS and GC-IMS

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ABSTRACT

To investigate the impact of genetic factors on wine aroma, wines made from 22 clones of five grape varieties (*Vitis vinifera* L.) were used to analyze the volatile compounds by headspace solid phase microextraction gas chromatography mass spectrometer (HS-SPME-GC–MS) and headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS). Results showed that 52 and 49 aroma compounds were identified from 22 clones of wines by two technologies, respectively. Esters were the most abundant compounds, followed by alcohols and aldehydes. The aroma profiles demonstrated significant varietal and clonal diversity, the clones with the highest aroma compound content were CH VCR6, PN VCR20, CS VCR11, ML VCR101, and CF 678. Partial least squares discriminant analysis (PLS-DA) identified decanoic acid, 1-heptanol, diethyl succinate, ethyl octanoate, and octanal as key biomarkers for distinguishing 22 clones of wines. Our results revealed that white wine CH VCR6 and red wine CS VCR11 possessed the most complex aromas. These findings address the research gap concerning the genetic determinants of wine aroma, highlighting the significance of grape variety and clone selection in developing wines with desirable sensory attributes.

1. Introduction

The quality of wine is primarily determined by its balance, intensity, complexity, aftertaste, and aroma (He et al., 2020). Moreover, aroma is a crucial parameter for evaluating wine quality. At the plant level, volatile aromas act as antioxidants, protecting plants from the damaging effects of reactive oxygen species (ROS) generated under abiotic stresses like intense light and high temperatures (Liu et al., 2024). Under high-temperature exposure, the emission of isoprene, monoterpenes, and C6 compounds stabilizes the photosynthetic membranes of grapes, thereby protecting the photosynthetic apparatus from lipid peroxidation (Wang et al., 2023). Aromas originate from various volatile compounds, including higher alcohols, esters, terpenes, pyrazines, phenols, aldehydes, and thiols. The sources of wine aroma are primarily classified into varietal aroma (primary aroma), fermentation aroma (secondary aroma), and aging aroma (tertiary aroma) (Yin et al., 2023).

The aroma of wine is affected by factors such as cultivar, light exposure, temperature, cultivation practices, and yeast varieties (Arrizabalaga-Arriazu et al., 2020; Liu et al., 2024; Xie et al., 2023). These factors influence the types and concentrations of aromatic compounds in wine by influencing grape growth and the fermentation process (He et al., 2020). Studies reported that under warm climate conditions, the total content of norisoprenoids and terpene compounds in wines decreased significantly due to elevated berry temperatures (Wang et al., 2020). However, the genotype (grape variety and clone) plays a critical role in shaping the aroma characteristics (Ascrizzi et al., 2022; Ju et al., 2021). Gao et al. (2024) analyzed six grape varieties from the Weibei Plateau and found that ‘Beibinghong’ and ‘Gongniang’ grapes and wines contained higher levels of aldehydes and esters. ‘Sauvignon Blanc’ cultivated in the Loess Plateau region was found to have significantly higher concentrations of C6/C9 compounds (Chen et al., 2024). Additionally, the aroma fingerprints of varietal wines have been proposed as analytical tools for origin traceability (Sikuten et al., 2022; Zhang et al., 2023).

Grape varieties exhibit genetic complexity and can be further subdivided into clones (Li et al., 2024; Qian et al., 2022). Clones typically

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originate from spontaneous somatic mutations during vegetative propagation, leading to phenotypic variations within a variety (Longo et al., 2021; Zombardo et al., 2022). As a result, different clones of the same grape variety can produce wines that differ not only in morphological traits and phenolic composition but also in the specific volatile aroma compounds that define their sensory profiles (Ren et al., 2023). These differences may manifest as variations in the concentrations of key aroma-active compounds such as norisoprenoids, terpenes, and esters, ultimately influencing the intensity, complexity, and characteristic aromatic attributes of the resulting wines (Ju et al., 2021). For instance, different clones of 'Sauvignon Blanc' have been reported to exhibit varying concentrations of thiols in wine (Zhu et al., 2021). Qian et al. (2022) analyzed the volatile components of two 'Cabernet Sauvignon' varieties from Xinjiang. The concentrations of several norisoprenoids and terpenes were significantly higher in CS169 wine than in CS191 wine. Additionally, researchers have identified 2-dihydronaphthalene and 1,1,6-trimethyl-1 as important aroma compounds distinguishing wines from eight 'Riesling' clones (Ziegler et al., 2020). Therefore, the selection of varieties and clones is crucial for obtaining wines with specific sensory attributes. Previous studies have analyzed the volatile aroma compounds and sensory characteristics of different grape varieties and clonal red wines (Sikuten et al., 2022; Zang et al., 2022). In particular, Zang et al. (2022) identified (Z)-3-hexen-1-ol and ethyl caproate as key contributors that positively influence the floral aromas of 'Merlot' and 'Malbec' wines. However, the study by Zang et al. (2022) limited to red wines and did not employ advanced analytical techniques, such as headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS), for more detailed volatile analysis.

In recent years, HS-GC-IMS has been widely applied to determine volatile components in food (Zhu et al., 2021). HS-GC-IMS combines the separation capabilities of gas chromatography (GC) with the rapid response and high sensitivity of ion mobility spectrometry (IMS), allowing the detection of numerous compounds with various chemical groups (Zhu et al., 2023). HS-GC-IMS enhances the accuracy of qualitative analyses and generates three-dimensional spectra comprising retention time, drift time, and signal intensity. It has been successfully used to analyze the flavor and quality of foods by detecting various volatile organic compounds and their metabolites (Cao et al., 2022; Yin et al., 2023). However, the use of HS-GC-IMS for detecting target compounds and identifying volatile components in wine samples has been seldom reported.

Genetic diversity among grape (*Vitis vinifera* L.) varieties and clones significantly influences wine quality, particularly through variations in volatile aroma compounds. Understanding these variations is crucial for the production of high-quality wines with distinctive aromas. However, the specific volatile markers associated with both inter-varietal and intra-varietal differences are not fully characterized. In this study, wines produced from 22 clones across five grape varieties were analyzed using headspace solid phase microextraction HS-SPME-GC–MS and HS-GC-IMS. These advanced analytical techniques allowed for the identification and quantification of a broader range of aroma compounds. Furthermore, partial least squares discriminant analysis (PLS-DA) was employed to identify potential volatile markers linked to genetic diversity. This study aims to enhance the characterization of wines' volatile components and to support the future selection of suitable grape varieties and clones for producing high-quality wines with characteristic aromas.

2. Materials and methods

2.1. Grape materials

The grape used in this study was cultivated in 2020 in a commercial vineyard located in Wuwei City, Gansu Province, China (38°3′45″–39°27′37″). The region is characterized by a temperate continental desert climate. Own-rooted grapevines planted in 2015 were used; the vineyard is oriented north-south with vine spacing of 2.5 m × 0.8 m. The canopy was trained using a vertical shoot positioning (VSP) trellis system, and standard field management practices, including consistent irrigation and yield management, were followed. Wines from 22 clones of five grape varieties were studied: 'Chardonnay' (CH), 'Pinot Noir' (PN), 'Cabernet Sauvignon' (CS), 'Merlot' (ML), and 'Cabernet Franc' (CF). All clones were sourced from certified nurseries and cultivated within the same vineyard to ensure their authenticity and genetic distinctiveness, as detailed in our previous study (Ren et al., 2023).

2.2. Winemaking protocol

At harvest, grapes were manually harvested for microvinification at optimal technological maturity, determined by the sugar-to-acid ratio (Table. S1). Harvesting was performed in triplicate, with each replicate consisting of 10 kg of grapes. Winemaking was performed using the method described by Gao et al. (2024). Grape berries of each variety were crushed, then transferred into three 10 L glass fermentation tanks. The musts were treated with 60 mg/L sulfur dioxide and maintained at 20 °C during a 24 h maceration period. Agitation was performed twice daily using gentle stirring to ensure uniform extraction of phenolic compounds. After maceration, active commercial yeast (200 mg/L, *Saccharomyces cerevisiae* CECA, Angel Yeast Co., Ltd., China) was added to initiate alcoholic fermentation. Alcoholic fermentation was carried out in a temperature-controlled room at 23–25 °C. The caps were punched down manually, and the specific gravity was measured twice daily. When the specific gravity dropped below 0.997, the skins and seeds were removed, and fermentation was allowed to continue. When the reducing sugar level fell below 4 g/L, the wines were bottled in 750 mL bottles containing 60 mg/L SO_2 and stored at −4 °C prior to chemical analysis, which was conducted after three months.

2.3. Physicochemical parameters of wine samples

The physicochemical parameters of grapes and wines were determined according to the methods described by Li et al. (2021). Reducing sugar and titratable acidity were measured using the Fehling reagent reduction method and acid-base titration, respectively, with titratable acidity expressed as grams per liter of tartaric acid. The alcohol content, volatile acidity, and dry extract of the wines were determined according to Tian et al. (2022). Each sample was analyzed in triplicate.

2.4. HS-SPME-GC–MS analysis of volatile profile in wine samples

Volatile aroma components and their concentrations in the wines were determined according to the method of Yin et al. (2023) using headspace solid-phase microextraction (HS -SPME) combined with gas chromatography–mass spectrometry (GC –MS). For sample preparation, wine samples (8 mL) were mixed with 8 μL of isooctanol (internal standard at 40 μg/L) and 2 g of sodium chloride, then placed in a 20 mL headspace vial. The SPME fibers (PDMS/DVB, 50/30 μm, Supelco) were exposed to the headspace at 40 °C for 30 min, then desorbed at the GC inlet (230 °C) for 5 min. The carrier gas (helium) flow rate was set to 1.5 mL/min. The column temperature was programmed as follows: initially held at 40 °C for 5 min, then increased to 130 °C at a rate of 2 °C per minute and held for 45 min, and finally raised to 220 °C at 5 °C per minute and maintained for 18 min. The total run time was 68 min. The sample was injected using split injection. The ion source temperature was 200 °C, and the transfer line temperature was 220 °C. The electron impact ionization voltage was 70 eV, with a scan range of m/z 30–350. An Agilent 7890 GC system coupled with a 5977B Mass Selective Detector (MSD) equipped with a DB -WAX capillary column (60 m × 0.25 mm × 0.25 μm; Agilent J&W, USA) was used for the separation and identification of aroma components. Aroma compounds were identified by comparing their retention times and mass spectra with those of authentic standards. Compounds without authentic standards were preliminarily identified by comparison with the AMDIS 2.73 (2017) and NIST 2.3 (2017) databases. Compound identification was validated by calculating Kovats retention indices (RIs) and comparing them with literature values. Compounds exhibiting matching or closely aligned RIs were confirmed (Li et al., 2021). The Kovats RIs, CAS numbers, and retention times were presented in Table. S2. Each analysis was performed independently in triplicate.

2.5. HS-GC-IMS analysis of volatile profile in wine samples

With slight modifications to the method of Wang et al. (2023). A FlavourSpec® gas analysis system (G.A.S.) equipped with an MXT −5 capillary column was used for the analysis. Prior to injection, 3 mL of wine sample was placed in a 20 mL headspace vial and incubated at 40 °C for 10 min. Specific parameter settings were provided in Tables S3 and 4. Volatile compounds were identified using Laboratory Analytical Viewer (LAV) software and GC –MS library searches by comparing their retention indices (RI) and drift times (DT) with those of standard n-ketones (C4 —C9) in the IMS library and built-in databases. Volatile compounds were quantified based on the peak intensities obtained from HS –GC –IMS, assuming that concentrations were proportional to peak intensities. The identified volatile compounds were annotated, with detailed information provided in Table. S5.

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 22.0, followed by Tukey's test ($P < 0.05$). Histograms were generated using Origin 2022. Heatmaps and principal component analysis (PCA) plots were generated using ChiPlot v2.1 (https://www.chiplot.online). Hierarchical clustering dendrograms were generated using R Studio software (version 4.3.3).

3. Results and discussion

3.1. Physicochemical parameters

The chemical parameters of wines are influenced by grape berry characteristics and winemaking processes (Ren et al., 2023). The reducing sugar content of wines from different clones ranged from 2.27 g/L to 3.61 g/L (Table. S6). The volatile acidity of wine reflects its stability and any changes in quality. According to Tian et al. (2022), the volatile acidity of wine should be less than 1.20 g/L (expressed as acetic acid). As shown in Table. S6, the volatile acidity of the wines ranged from 0.34 g/L (CS VCR19) to 0.75 g/L (PN792). Among all varieties, the CF clonal wines had the highest alcohol content, averaging 14.08 % (*v*/v), with CF 678 significantly higher than other clones and varieties at 14.80 % (v/v). Acidity is a key element in wine taste, imparting refreshing and lively characteristics (Cheng et al., 2015; Li et al., 2022). The titratable acidity of PN VCR18 wine was significantly higher than that of other clones, demonstrating significant differences in titratable acidity among varieties and clones. Specifically, the titratable acidity of PN VCR18 wine was 1.36 times higher than that of PN VCR20. ML VCR13 exhibited the lowest titratable acidity, at only 5.20 g/L.

Due to differences in wine styles, the dry extract content of white and red wines varies. A lower dry extract content renders dry white wines more refreshing. A higher dry extract content imparts dry red wines with a fuller body and richer structure (Ascrizzi et al., 2022). In our study, CH clonal wines were found to have the lowest average dry extract content and the highest average titrable acidity among the wine varieties examined. These characteristics contribute to a fresher and more vibrant image of the wines, enhancing their overall balance and appeal (Tian et al., 2024). Conversely, CS VCR169 and ML VCR101 were found to have significantly higher dry extract contents compared to other varieties. Dry extract consists of non-volatile substances such as phenolics and tannins. Phenolics and tannins function as antioxidants, slowing oxidation reactions (Piombino et al., 2024). Additionally, tannins contribute to the structural complexity and mouthfeel of the wines, enabling the development of more nuanced flavors and maintaining stability over extended storage periods (Tian et al., 2024; Ziegler et al., 2020). Lemos et al. (2020) found that the chemical parameters of different wines mainly depend on varietal differences, whereas in this study, variations between and within varieties jointly affected the basic quality of the wines.

3.2. HS-SPME-GC–MS analysis of volatile profile in wines

The volatile components in the grape samples were qualitatively and quantitatively analyzed using HS-SPME-GC–MS. Table. S7 shown that 54 volatile compounds were detected and classified into six categories: higher alcohols (14), esters (18), fatty acids (5), terpenes and norisoprenoids (9), aldehydes and ketones (4), and other aroma compounds (4).

Higher alcohols originate during the fermentation process of wine. Their synthesis begins with glucose undergoing glycolysis to form pyruvate. Pyruvate enters the amino acid biosynthesis pathway under the action of acetohydroxy acid synthase, forming α-keto acid intermediates. These intermediates are further reduced to form the corresponding higher alcohols, catalyzed by specific enzymes (Gonzalez-Barreiro et al., 2015). The average content of higher alcohols in CS clones was relatively high (261,546.16 μg/L to 473,266.35 μg/L), with clonal differences ranging from 1.31- to 1.81 -fold. A significant intra-varietal difference of up to 2.45-fold was observed between clones CF 678 and CF VCR10. Specifically, CS VCR11 had a significantly higher content of higher alcohols than other varieties, while CF VCR10 had the lowest content at only 123,492.17 μg/L. Sagratini et al. (2012) analyzed higher alcohols in monovarietal Montepulciano wines from Abruzzo and Marche (four different areas from two regions) and found that 3-methyl-1-butanol had the highest content, which is similar to our results. Among the 14 higher alcohols studied, 3-methyl-1-butanol was the most abundant, with an average content of 221,219.06 μg/L. Many higher alcohols have pleasant aromas. For example, phenylethyl alcohol is described as having honey and rose aromas. Longo et al. (2021) demonstrated that viticultural interventions in 'Pinot Noir', such as leaf removal or cluster thinning, can increase the concentration of phenylethyl alcohol. These compounds are associated with pleasant fruity and floral aromas, which can benefit winemakers. Zhang et al. (2023) found that in three wines from Ningxia, the concentration of phenylethyl alcohol exceeded its odor threshold and was significantly higher in 'Cabernet Sauvignon' and 'Merlot' wines compared to 'Chardonnay' wines. 3-Methylthiopropanol was not detected in certain clones such as CH VCR8, CH VCR11, PN VCR20, and CF 396, reflecting specificity and variability between and within varieties.

Esters mainly originate during fermentation, with a small portion formed during aging (Gao et al., 2024). Esters in wine are usually ethyl esters (condensation of ethanol and fatty acids) or acetate esters (condensation of ethanol and acetic acid). These compounds directly affect the aromatic characteristics and sensory perception of wine (Wang et al., 2020). Previous studies found that esters were the most abundant volatile aroma compounds in Cabernet Sauvignon and Merlot wines from Ningxia (Zhang et al., 2023). Similarly, in the present study, 18 esters were detected. Among the total esters, ML VCR3 and ML VCR101 had significantly higher contents than other varieties, while CH VCR8 had the lowest content at only 23,320.52 μg/L. Additionally, the intra-varietal difference between CS VCR11 and CS 170 reached 2.03-fold. Among the 18 esters studied, ethyl acetate had the highest content, with an average of 49,862.31 μg/L. Researchers have found that 'Hasan' grapes have higher ethyl acetate content than 'Beibinghong', 'Cabernet Sauvignon', and 'Syrah' grapes (Cao et al., 2022). In our study, the Merlot variety contained higher levels of ethyl acetate, with ML VCR101 and ML VCR3 significantly higher than other varieties, exhibiting rich tropical fruit flavors and floral aromas. The concentration of esters in wine depends on several factors: yeast strains, fermentation temperature, aeration, and sugar content (Qian et al., 2022; Zhao et al., 2022). Studies have found that in 'Riesling' wines, natural fermentation significantly increases the levels of ethyl hexanoate, ethyl octanoate, and propyl acetate compared to inoculated fermentations (Wang et al., 2023). In our study, the average contents of ethyl 2-methylpropanoate, isobutyl acetate, and other esters ranged from 0.80 to 7.30 μg/L. Although present at low concentrations, these compounds significantly contribute to the fruity aromas of pineapple, apple, and other fruits in wine due to their low olfactory thresholds (Chen et al., 2024; Yue et al., 2021).

Fatty acids are synthesized by the repetitive condensation of acetyl-CoA catalyzed by the fatty acid synthase complex (Ju et al., 2021). The average fatty acid content in ML clones was relatively high, ranging from 1532.57 to 6181.87 μg/L; ML VCR1 had significantly higher fatty acid content than other varieties, while PN VCR9 had the lowest content. No significant differences in fatty acid content were observed among the CF clones. Among the five fatty acids, hexanoic acid had the highest average content across all varieties and clones at 1156.25 μg/L, while decanoic acid had the lowest average content at only 180.87 μg/L. Reports indicate that the concentration of medium-chain fatty acid ethyl esters depends on the concentration of their fatty acid precursors (Zang et al., 2022). Both hexanoic acid and ethyl hexanoate contents in ML VCR1 were higher than in other varieties and clones in our study. Acetic acid is abundant; it is produced during alcoholic and malolactic fermentations. Volatile fatty acids can enhance the complexity of wine aroma even at levels below the sensory threshold, but excessive acetic acid can impart unpleasant sourness and bitterness to wine, reducing its quality (Gonzalez-Barreiro et al., 2015).

Terpenes and norisoprenoids are major components with floral and fruity aromas and are among the main contributors to the aroma of non-aromatic grape varieties such as 'Cabernet Sauvignon', 'Merlot', 'Syrah', and 'Chardonnay' (He et al., 2020; Yue et al., 2021). Regarding the total amount of terpenes and norisoprenoids, the content in CH clones was relatively higher than in other varieties and clones, ranging from 204.33 to 429.02 μg/L. The varietal differences ranged from approximately 1.64 to 2.10 -fold. CF VCR10 had a significantly lower content than other varieties, at only 32.15 μg/L. For farnesol, the average content was highest in CH, followed by PN. Specifically, α-terpineol in CH VCR6 and β-ionone in CH VCR11 were significantly higher than in other varieties. Linalool, described as having a "floral" aroma, was significantly higher in CH VCR6 and CH VCR11 than in other varieties. However, linalool was not detected in some clones of CS, ML, and CF. Previous studies on grape varieties from different fruit positions found that linalool was only detected in wines made from grapes grown in the middle canopy position, including 'Merlot', 'Chardonnay', and 'Riesling' (Cheng et al., 2015). This indicates that linalool exists in trace amounts in grapes and wines, related not only to the variety and clone but also to the fruit position. Yin et al. (2023) found that linalool exhibits strong sensitivity to ultraviolet radiation. Studies also show that ultraviolet light may increase linalool levels and promote its conversion to oxidative derivatives. Although present only at trace levels, most norisoprenoids have very low sensory thresholds. Therefore, these compounds make important contributions to the aroma potential of many wine varieties. Previous studies found that geraniol content in 'Malbec' was significantly higher than in other varieties, with significant differences between two clones of this variety; the geraniol content in Malbec 598 was 1.50 times higher than that of Malbec VCR6 (Zang et al., 2022). In our study, CS VCR11 had significantly higher geraniol content than other varieties at 18.07 μg/L. CS 170 and CS VCR19 did not have detectable levels of geraniol. This variation suggests that different genotypes markedly influence geraniol biosynthesis. The absence of geraniol in CS 170 and CS VCR19 may be attributed to genetic differences that affect the expression or functionality of key enzymes involved in the geraniol synthesis pathway (He et al., 2020). Such varietal and clonal distinctions highlight the importance of genetic regulation in determining the volatile compound profiles of these varieties.

In terms of total aroma, CS VCR11 was significantly higher than other varieties, while CF VCR10 had the lowest total aroma content. The clones with the highest aroma content for each variety were CH VCR6, PN VCR20, CS VCR11, ML VCR101, and CF 678.

3.3. OAV-based characteristic aroma analysis

Although numerous volatile substances were detected in each wine sample, not all components significantly impact the wines' overall aroma characteristics (Cao et al., 2022). To evaluate the contributions of various volatile compounds to the wine's olfactory impression, odor activity values (OAVs) were calculated using average analytical concentrations and published odor thresholds (Gao et al., 2024; Li et al., 2021; Zhang et al., 2023). Typically, only compounds with OAVs exceeding 1 are considered to contribute to the wine's aroma (Tables S8 and 9). Above it was mentioned that 20 aromas had OAV higher than 1, suggesting they may contribute to the wines' aromas.

However, among the six compounds analyzed, Odor Activity Values (OAVs) exceeding 1 were observed only in certain grape variety clones. For example, 1-hexanol, a C6 compound classified as a green leaf volatile (GLV) that imparts typical herbal aromas, exhibited an OAV exceeding 1 exclusively in the CF VCR10. Previous studies have reported that the OAV of 1-hexanol in 'Cabernet Sauvignon' from the Loess Plateau was significantly higher than in 'Merlot', which contrasts with our findings where 'Merlot' showed higher levels (Chen et al., 2024). This discrepancy may be attributed to the profound influence of climatic conditions on wine aroma characteristics; specifically, the Loess Plateau's lower temperatures and unique precipitation patterns may favor the biosynthesis of 1-hexanol. Additionally, factors such as soil composition and viticultural practices could contribute to these differences. Furthermore, 1-octen-3-ol, a straight-chain fatty alcohol and the primary contributor to mushroom aroma, was found in higher concentrations in CS and CF compared to other varieties. The OAV range for CS was 0.85–2.21, while for CF, it was 1.46–4.69. Notably, OAVs exceeding 1 for 1-heptanol were observed only in PN clones, reflecting the distinct aroma profiles associated with different grape varieties.

The remaining 14 compounds with OAVs exceeding 1 include five alcohols, four esters, three fatty acids, and two aldehydes and ketones. Among the higher alcohols, 2,3-butanediol exhibited relatively high OAVs ranging from 6.47 to 39.11. The highest OAV for 2,3-butanediol was observed in CS 15. This compound imparts slight sweetness and a creamy sensation to wine, potentially affecting taste and complexity (Liu et al., 2024; Zhao et al., 2022). Esters are synthesized through the condensation of alcohols and carboxylic acids (Ascrizzi et al., 2022; He et al., 2023). Short- to medium-chain esters typically exhibit fruity aromas, while longer-chain esters can also display these characteristics. Ethyl hexanoate showed the highest OAV among esters, with ML VCR1, ML VCR3, and PN 375 exhibiting significantly higher values than other varieties. Ethyl hexanoate is known for its sweet fruity aromas, featuring unique scents of pineapple and green apple (Ilc et al., 2016). As demonstrated by Tomasino et al. (2015) using addition/omission tests, ethyl octanoate enhances the red fruit aroma of wine and, when combined with ethyl decanoate, contributes to a black cherry aroma. In our study, CF clones exhibited higher OAVs of ethyl octanoate compared to other varieties. This suggests that CF clones may enhance these particular aroma profiles in wine, potentially offering a distinctive sensory experience.

Conversely, terpenes and norisoprenoids, compounds associated with floral and fruity aromas, exhibited lower OAVs across all varieties studied. This reduction may be related to the arid climate conditions in Gansu, which can influence the biosynthesis of these compounds (Yue et al., 2021). Previous studies similarly found that regions like Xinjiang and Ningxia, which also experience arid climates, present lower terpene and norisoprenoid levels compared to more temperate regions like Hebei (Li et al., 2021; Wang et al., 2023).

These findings highlight the significant impact of grape variety clones and regional climate on the aromatic profiles of wines. Understanding these influences can aid vintners in clone selection and vineyard management to optimize desirable aroma characteristics. Future research could focus on exploring the underlying biochemical pathways affected by climate and how viticultural practices might mitigate these effects.

In ML clones, all OAVs of ethyl decanoate were less than 1. Ethyl decanoate imparts a black cherry aroma, indicating that this aroma is lacking in ML. Among the fatty acids, CH VCR8, CH VCR6, and CH VCR11 had higher OAVs of acetic acid, reflecting a greater contribution to the overall aroma. For benzaldehyde, except in CH VCR8, the OAVs in CS, ML, and CF clones were higher than in CH and PN. These results underscore the varietal influence on the concentration of key aroma compounds, enhancing our understanding of how clone selection can impact wine aroma. Based on OAV thresholds, significantly higher concentrations of ethyl hexanoate were detected in MV VCR1 and ML VCR3, contributing to their pronounced fruity aromas. Additionally, CF VCR10 exhibited markedly elevated levels of 1-octen-3-ol, imparting a distinctive green character to the wine. These findings summarize the primary aroma differences among the wine varieties, highlighting the key volatile compounds responsible for their unique sensory attributes.

3.4. Clustering analyses reveal varietal and clonal differences in wine aroma profiles

Heatmap clustering analysis was utilized to identify similarities and differences in the aroma profiles of various clones. This method groups similar aroma compounds into distinct clusters, facilitating the visualization of complex data patterns (Fig. 1A). In CH clones, the levels of α-terpineol, linalool, β-ionone, ethyl-2-acetoxybenzoate, and acetic acid were higher overall compared to other varieties. These compounds are known to enhance floral and fruity aromas, contributing to the overall sensory appeal of Chardonnay wines. Elevated levels of acetone, 1-heptanol, octanal, diethyl succinate, ethyl octanoate, and cis-2-hexen-1-ol were observed in PN clones. Higher levels of benzaldehyde, phenylethyl alcohol, geraniol, and 3-methylthiopropanol were found in CS clones compared to other varieties. Phenylethyl alcohol and geraniol contribute positively to the floral and fruity aromas of wine, while 3-methylthiopropanol is typically associated with reductive winemaking or sulfur-related compounds (Zhao et al., 2022). At low concentrations, this compound can add desirable complexity, but at high concentrations, it may result in off-flavors (Lu et al., 2022; Wang et al., 2020). In ML clones, relatively higher levels of 4-methyl-1-pentanol, 4-ethyl-2-methoxyphenol, and geranyl acetate were observed, with geranyl acetate enhancing rose-like floral notes and fruit complexity. Geranyl acetate is known to enhance rose-like floral notes and fruit complexity, potentially improving the sensory quality of ML wines. Higher levels of decanoic acid, ethyl octanoate, and ethyl decanoate were detected in CF clones, which contribute fruity, sweet, and tropical aromas such as pineapple, pear, and apple. These compounds enhance the fruity complexity of CF wines, especially in younger vintages (Gao et al., 2024).

As expected, hierarchical cluster analysis (HCA) further distinguished the grape varieties and clones. Based on the distance relationships among 24 individual components, all wine genotypes were grouped into two subgroups (Fig. 1B). Notably, CF VCR10 was independent from other genotypes. Our study had found that CF VCR10 had significantly lower total aroma compounds compared to other varieties, suggesting limited potential for aromatic intensity in wines produced from this clone. This may be due to lower biosynthetic activity of compounds such as higher alcohols and esters in CF VCR10 or restricted activity of key enzymes in the biosynthetic pathway (Xie et al., 2023). CH clones clustered together, whereas PN375, PN VCR18, and PN VCR9 formed a separate cluster, indicating intra-varietal variations. In CS clones, two distinct clusters were observed: CS15, CS VCR11, and CS169 grouped together, characterized by elevated levels of higher alcohols; in contrast, CS VCR19 and CS170 formed a different cluster with a higher proportion of esters compared to other CS clones. These results indicate that both inter-varietal and intra-varietal variations significantly affect the aroma profiles of wines, thereby further amplifying the genetic differences among grape varieties. Understanding these variations can inform clone selection and breeding programs aimed at enhancing desirable aromatic characteristics.

3.5. Principal component analysis and partial least squares discriminant analysis

Principal component analysis (PCA) was employed to visualize differences in volatile compounds among grape varieties and their clones. The analysis was performed on normalized volatile compound data. The PCA results indicated that the first (PC1) and second (PC2) principal components accounted for 68.18 % and 18.24 % of the total variance, respectively (Fig. 2A). The grape varieties were well separated, indicating that variety plays a more significant role than genotype in determining the wine's aroma profile. CH clones were primarily separated from other varieties along PC1, suggesting significant differences in the composition and concentration of their volatile compounds. Similarly, PN varieties were mainly separated along PC2, reflecting unique characteristics in their aroma compounds. The loading plot revealed positive correlations between PC1 and compounds such as β-ionone, 4-terpineol, linalool, and α-terpineol, suggesting that PC1 is primarily influenced by terpenes and norisoprenoids (Fig. 2B). These compounds are abundant in CH wines and contribute violet, clove-like floral, and citrus aromas (Cantu et al., 2021). PC2 showed positive correlations with 1-heptanol, octanal, and acetone, and negative correlations with 4-methyl-1-pentanol, 3-methylbutanoic acid, and benzaldehyde. Positively correlated compounds like 1-heptanol and octanal are known to contribute to fruity and floral aromas, while negatively correlated compounds such as benzaldehyde impart almond-like notes, affecting the overall sensory profile.

To further clarify the flavor profiles, partial least squares discriminant analysis (PLS—DA) was conducted based on the PCA results. Variable importance in projection (VIP) scores were used to identify the most influential compounds (Fig. 2C), with compounds scoring greater than 1 considered key contributors. A total of 17 compounds were identified as key contributors, with the top five being decanoic acid, 1-heptanol, diethyl succinate, ethyl octanoate, and octanal. These compounds are critical for distinguishing volatile compound differences among varieties and clones. The identification of these key metabolites presents significant practical implications for winemakers and grape breeders. Winemaking processes and vineyard management practices can be tailored using this information to enhance or mitigate specific aroma compounds, thereby optimizing the desired flavor characteristics of the wines. Future studies could focus on manipulating vineyard conditions or fermentation processes to enhance desirable aroma compounds identified in this analysis. Furthermore, compounds with VIP scores exceeding 1 may serve as reliable markers for wine quality. These markers can facilitate the authentication of wine varieties, thereby verifying the origin and quality claims of premium wines.

3.6. HS-GC-IMS analysis of volatile profile in wines

HS-SPME-GC–MS was utilized due to its high sensitivity and capability to identify a broad spectrum of volatile and semi-volatile compounds, thereby providing detailed molecular information (Liu et al., 2024). Conversely, HS-GC-IMS offers rapid analysis with high resolution, particularly effective in distinguishing isomeric compounds and facilitating real-time monitoring (Wang et al., 2023). The integration of these two advanced techniques facilitated a more exhaustive and precise characterization of the volatile profiles across different grape clones, thereby enhancing the reliability of the findings (Yin et al., 2023). In

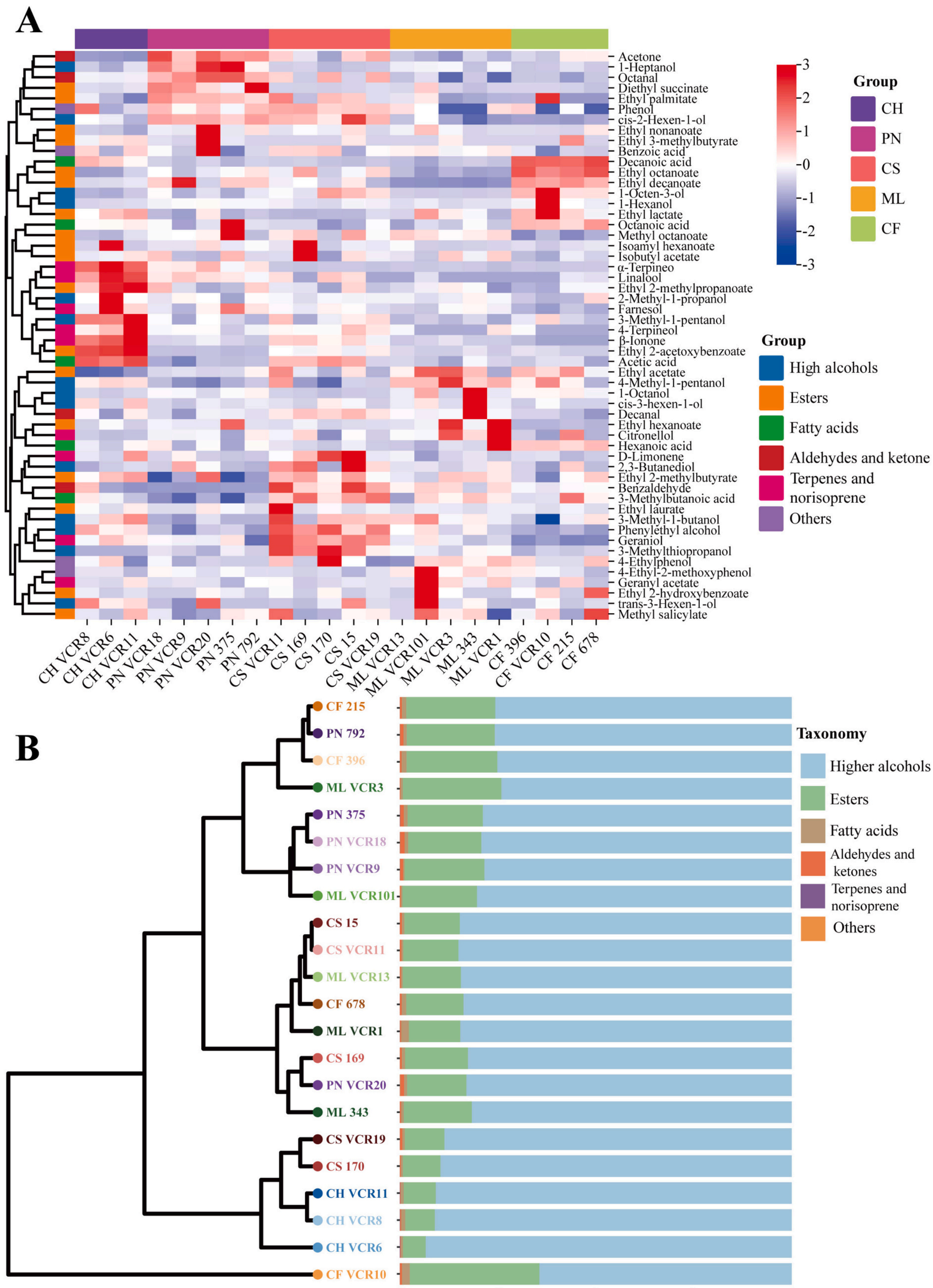

Fig. 1. Heatmap illustrating the quantified volatile compound profiles among different wine varieties and their clones (A). Hierarchical clustering analysis (HCA) of the volatile profiles, grouping wines based on their similarity in volatile compound composition (B). 'chardonnay' (CH), 'pinot noir' (PN), 'cabernet sauvignon' (CS), 'merlot' (ML), and 'cabernet franc' (CF). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend).

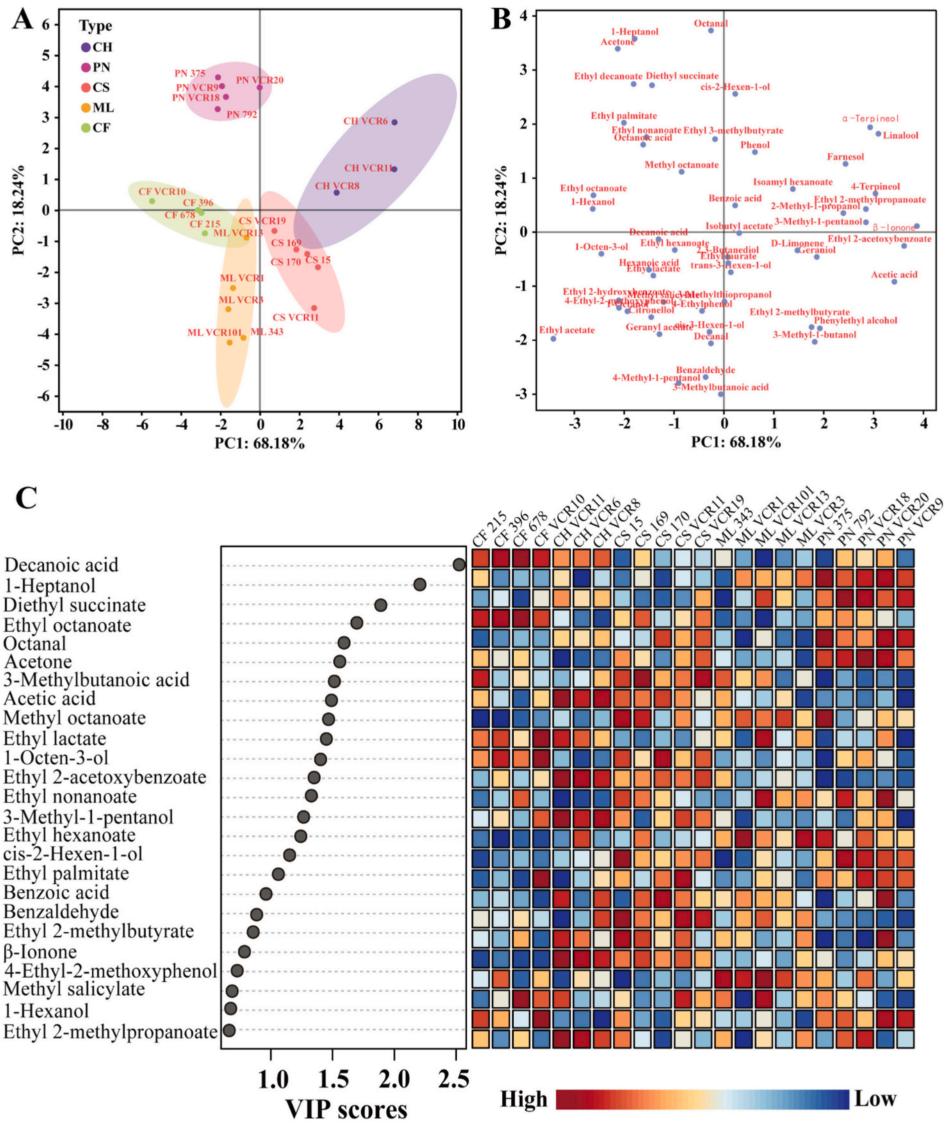

Fig. 2. PCA and PLS-DA analysis of volatile profile among different wine varieties and their clones. PCA scores (A); PCA loadings (B); VIP values from PLS-DA (C). 'chardonnay' (CH), 'pinot noir' (PN), 'cabernet sauvignon' (CS), 'merlot' (ML), and 'cabernet franc' (CF). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend).

addition, HS-GC-IMS enhances the accuracy of qualitative analysis and produces three-dimensional spectra representing retention time, drift time, and signal intensity (Li et al., 2020). This technique has been successfully applied to analyze flavor and quality in food products (Zhu et al., 2021; Zhu et al., 2023). In this study, clones with the highest aroma concentrations from five grape varieties were selected for HS-GC-IMS analysis (CH VCR6, PN VCR20, CS VCR11, ML VCR101, and CF 678). HS-GC-IMS effectively separated the volatile compounds in the five wines, clearly visualizing their differences (Table. S10). As shown in Fig. 3, each detected volatile compound is represented by a dot located to the right of the RIP peak. Using CH VCR6 as a reference, the remaining spectra were subtracted from its signal peaks, resulting in a background of white, blue, and red (Fig. 4A, B). The white background indicates compounds with concentrations similar to the reference; blue indicates lower concentrations than CH VCR6, and red indicates higher concentrations. A fingerprint profile of volatile compounds for the five grape varieties was constructed based on all peaks in the HS-GC-IMS two-dimensional spectra. A total of 49 volatile compounds were detected, of which 46 were identified (Fig. 4C). Esters (18) were the most abundant group, followed by aldehydes and ketones (11), and alcohols (9). Esters accounted for the largest proportion of total volatile compounds, ranging from 41.35 % to 49.57 %, followed by aldehydes and ketones (26.06 %–29.57 %) and alcohols (19.09 %–22.65 %). Although the same types of volatile aroma compounds were present in all five grape varieties, their concentrations varied significantly. CH VCR6 exhibited significantly higher concentrations of volatile compounds than the other varieties, followed by CS VCR11. This finding underscores the pivotal role of grape variety and clone selection in shaping the aromatic profiles of wines. By selecting specific grape varieties and clones, winemakers can strategically influence the composition of volatile compounds, thereby enhancing desirable aromas and overall wine quality. For instance, the elevated levels of certain esters and alcohols in CH VCR6 may contribute to more pronounced fruity and floral notes, which are highly valued in premium wines.

PCA was performed on multiple variables obtained from HS-GC-IMS to highlight differences in volatile compound profiles. The variance contributions of PC1 and PC2 were 47 % and 38 %, respectively, with a cumulative variance of 85 %, significantly higher than the acceptable threshold (Fig. 4D). The PCA results revealed significant differences in aroma compounds among the five sample groups, effectively distinguishing them from each other. For instance, CH VCR6 wines exhibited significantly higher concentrations of 2,5-dimethylpyrazine,

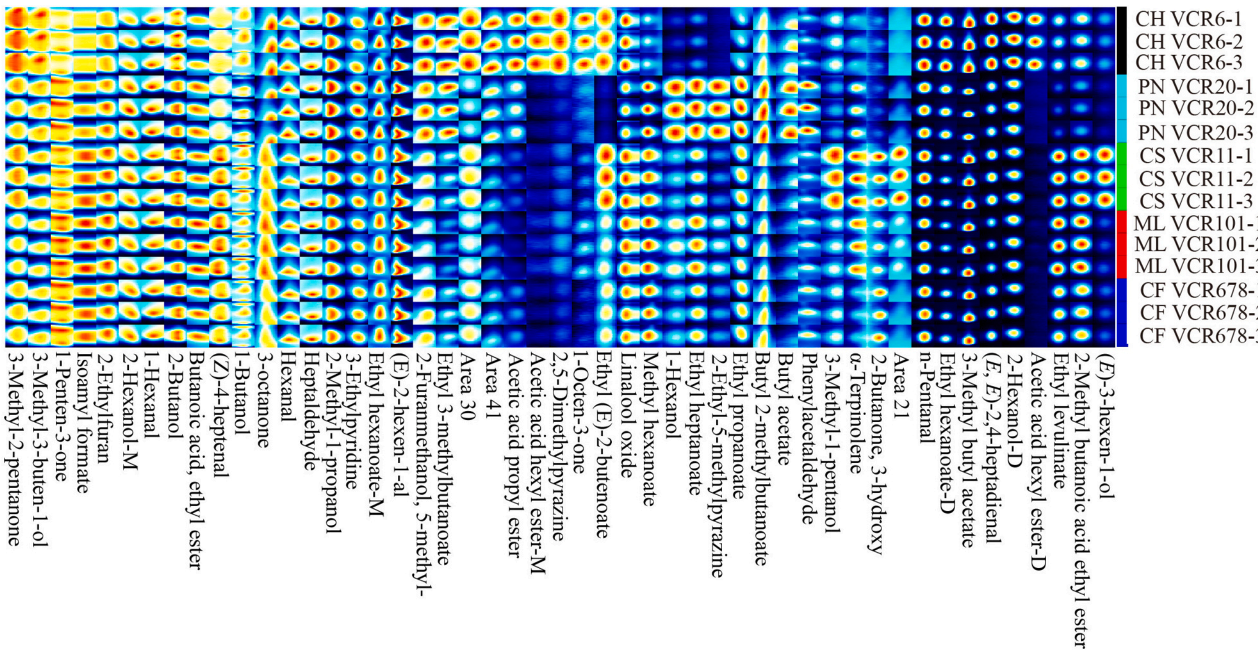

Fig. 3. Fingerprints of volatile compounds by HS-GC-IMS analysis in five wine samples. 'chardonnay' (CH), 'pinot noir' (PN), 'cabernet sauvignon' (CS), 'merlot' (ML), and 'cabernet franc' (CF). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend).

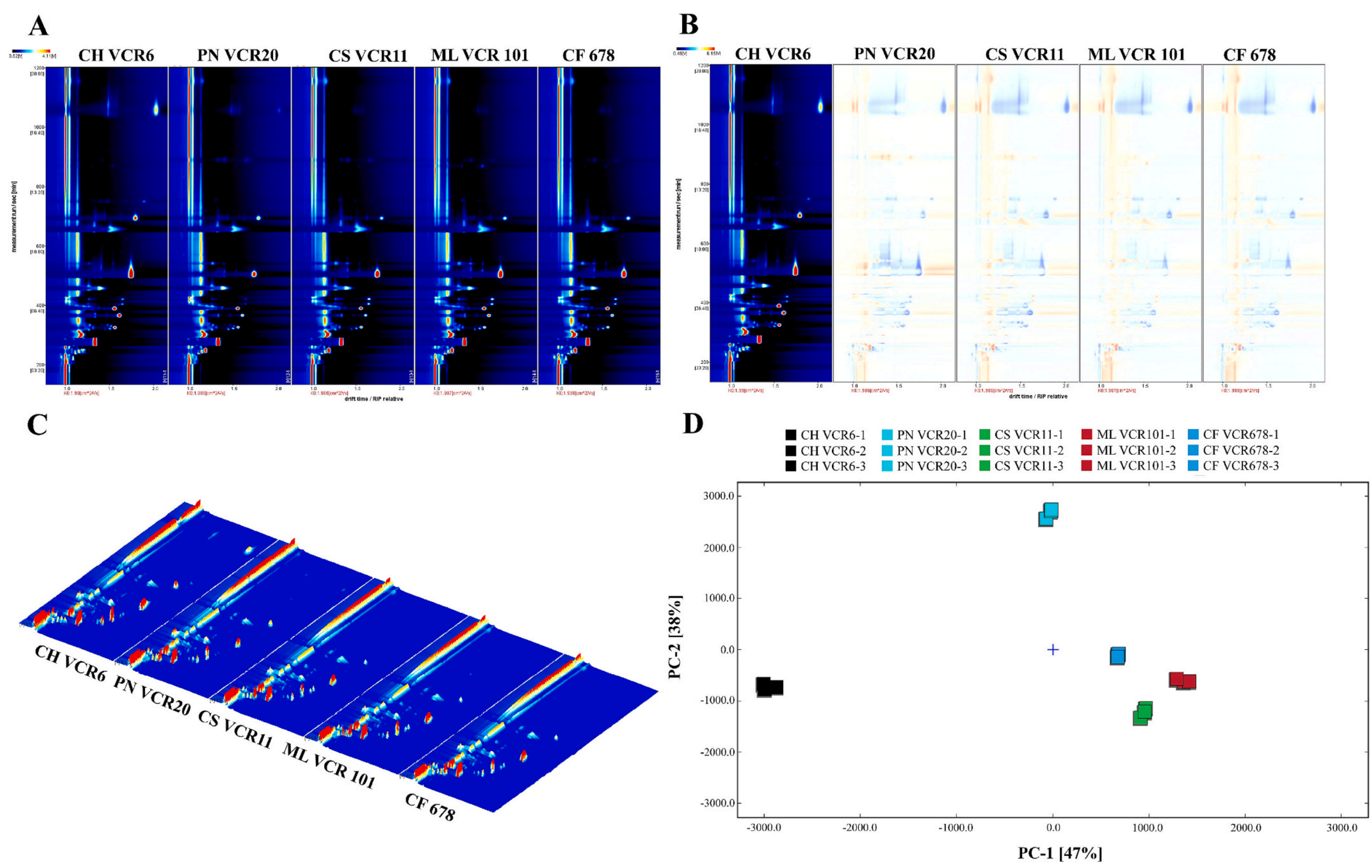

Fig. 4. HS-GC-IMS volatile compound analysis in five wine samples. Two-dimensional topographic plots of volatile compounds in five wine samples (A). PN VCR20, CS VCR11, ML VCR101 and CF 678 were reduced by using CH VCR6 as a reference and the comparative analysis topographic plots (B). Three-dimensional (3D)-topographic of volatile compounds in five wine samples (C). Principle components analysis (PCA) of five wine samples (D). (Consult the online version of this article for an elucidation of the color codes mentioned in the figure legend).

acetic acid hexyl ester-M, acetic acid hexyl ester-D, and 1-octen-3-one compared to others. In ML VCR101, concentrations of (*E*)-3-hexen-1-ol and 3-methyl-1-pentanol were notably higher than in other varieties. These compounds are known to contribute to distinct aroma characteristics, such as nutty, fruity, and green notes, which can influence the sensory perception of the wines.

Thus, the analysis of volatile compound fingerprints using HS-GC-IMS demonstrates that grape variety and clone significantly impact the concentration and composition of volatile compounds in wine. The ability of HS-GC-IMS to effectively separate and visualize these compounds in the five wine samples underscores its utility in enological research and quality control.

4. Conclusion

Genetic variation plays a crucial role in shaping the volatile aroma profiles of wine, affecting its quality and consumer appeal. This study analyzed the volatile compounds of 22 clones from five grape varieties using GC–MS and GC-IMS, detecting 52 and 49 compounds, respectively. Esters, which are key contributors to the fruity and floral aromas in wines, accounted for the largest proportion of the total volatile compounds, followed by alcohols and aldehydes. Notably, clones CH VCR6, PN VCR20, CS VCR11, ML VCR101, and CF 678 exhibited the highest concentrations of aroma compounds, indicating their potential for producing wines with enhanced aromatic qualities. Conversely, CF VCR10 had the lowest levels, indicating limited aromatic intensity. The top five compounds with VIP scores exceeding 1 were decanoic acid, 1-heptanol, diethyl succinate, ethyl octanoate, and octanal.

The integration of GC–MS and GC-IMS data enabled a comprehensive analysis, revealing that white wine CH VCR6 and red wine CS VCR11 possessed the most complex and intense aromatic profiles. These findings highlight the importance of genetic breeding and clonal selection in optimizing wine aroma profiles, offering valuable insights for viticulture and enology.

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CRediT authorship contribution statement

Huawei Chen: Writing – original draft, Methodology, Investigation. **Shijian Bai:** Software, Methodology, Investigation. **Bowei Yang:** Software, Methodology. **Ruihua Ren:** Methodology. **Zizhu Tang:** Investigation. **Zhenwen Zhang:** Writing – review & editing, Funding acquisition. **Qingqing Zeng:** Writing – review & editing, Funding acquisition, Formal analysis.

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.2024.102136.

Data availability

Data is contained within the article.

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