# Effects of the fertilizer and water management on amino acids and volatile components in Cabernet Sauvignon grapes and wines

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**Abstract:** The amino acids and the volatile substances in grapes and wines play important roles in their quality, and the concentrations of these substances can be changed by how a vineyard is managed, e.g., irrigation and fertigation regimes. This study aimed to evaluate the effect of fertilizer and water management on the distribution of amino acids, the volatile component profiles, and the sensory characteristics of Cabernet Sauvignon grapes and wines. The results showed that the amino acid concentration in grape berries was the highest under the 100% local fertilizer rate (HF) and 100% water irrigation quota (HW) treatment, and the volatile component concentration in wine was the highest under HF and 80% water irrigation quota (MW) treatment. The effect of irrigation on the amino acid content in grapes was greater than that of fertigation. The synergistic effect of fertilizer and water on arginine, serine, and glutamine in grape berries was significant. The interactive effect of fertigation and irrigation on the volatile substance in grapes was greater than that of fertigation and irrigation alone. The influence of irrigation on volatile substances in wines was greater than that of fertigation. In addition, there was also a correlation between the concentrations of multiple amino acids in grapes and volatile components in wines. Principal component analysis showed that the wine from the HFMW treatment had the best quality among all treatments.

**Keywords:** fertilizer and water management, grape, wine, amino acids, volatile components
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## 1 Introduction

Amino acids in grapes can serve as nitrogen sources for yeast and play an important role in yeast growth and development during alcoholic fermentation[1]. Some amino acids are also the precursors of volatile compounds that are converted into volatile aroma compounds during fermentation, such as higher alcohols, volatile fatty acids, and ethyl esters[2,3], which contribute to the expression of wine aroma. For example, isoamyl alcohol, isobutyl alcohol, and phenethyl alcohol are derived from leucine, valine, and phenylalanine, respectively[4,5].

Amino acid concentrations in grapes and wines are affected by various factors, such as grape variety, soil properties, climatic conditions, and vineyard management[6]. Volatile compounds can accumulate in grapes during grape ripening depending on temperature and water availability[7,8]. Currently, the main wine-producing regions in China have a continental monsoon climate, and there are many challenges in the grape and wine industry of China. The accumulation of sugars, organic acids, amino acids, and volatile compounds has been unsatisfactory[9,10], owing to the insufficiency of rainfall during the ripening processes. Nowadays, extensive irrigation management often leads to water stress or overgrowth of grapevines and consequent decreases in the yield and quality of grapes[11-13].

More and more studies on increasing the concentration of amino acids and volatile compounds in grapes through vineyard management (e.g., precise irrigation and fertigation scheduling) have begun attracting the attention of researchers[14-16]. Novel irrigation and fertigation strategies to control grapevine vigor have been shown to influence berry metabolism, essential sugar, organic acids, amino acids, volatile compounds, and so on, which consequently impact the sensory characteristics of wines[17]. It was also reported that irrigation had a significant effect on some amino acids and volatile compounds in grapes and wine, but it did not greatly improve the quality of wine[18]. The application of fertilization in soil can not only increase nitrogen content in grape berries, but also enhance aroma expression[19], but too little or too much fertilizer in the soil can reduce the quality of grapes and wines, damage vines, and increase the cost of plant protection[20]. Vilanova et al.[21] acknowledged that fertilization had a specific effect on the volatile components of grapes and wines, especially terpenes, which improved the aroma of wines. Fertigation and irrigation in vineyards can affect grapevine physiology and grape fruit quality. Therefore, fertigation and irrigation management practices in vineyards should not only be conducted carefully to avoid negative impacts but also with best management schedules for grapevines to improve the grape yield and wine quality[22]. Thus, fertilizer and water management offer a common and effective strategy to supply water and nutrients required by grapes[23].

Cabernet Sauvignon is already one of the most popular red grape varieties in China. It is mainly used for dry red wine, with a

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taste that is soft, delicate, and fruity[24]. The amino acids and volatile components of wines produced from Cabernet Sauvignon have been described[25,26], but the effects of fertilizer and water management on amino acids and volatile compounds of grapes and wines during grape fruit development have rarely been studied.

To address the shortage of research in this field and consider that grape quality depends on key factors, such as water and fertilizer, the current study aimed to evaluate the effect of irrigation and fertigation on the amino acids, volatile compounds, and sensory characteristic profiles of Cabernet Sauvignon grapes and wine and to investigate the intrinsic relations between amino acids and volatile compounds. Ultimately, these results provide optimal water and nutrient management schedules in order to increase fertilizer and water use efficiency.

## 2 Materials and methods

### 2.1 Chemical reagents

Ultrapure water was produced using Millipore equipment (Millipore, Bedford, MA, USA). Acetic acid, sodium hydroxide, sodium acetate, potassium metabisulfite, sodium chloride, and pectinase were obtained from Merck (Shanghai, China). The acetonitrile (HPLC grade), tryptophan, tyrosine, citrulline, arginine, phenylalanine, histidine, methionine, glutamic acid, glutamine, lysine, aspartic acid, asparagine, leucine, isoleucine, cysteine, threonine, valine, proline, serine, gamma-aminobutyric acid (GABA), alanine, and glycine were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The amino acid solution was prepared with ultrapure water. Both $C_8$-$C_{30}$ *n*-alkane standards and 2-octanol standards were purchased from Merck and prepared from ethanol solutions.

### 2.2 Experimental conditions

The experiment was carried out in Huayang Vineyard, Xuanhua District, Hebei Province, China. The vineyard is located at 40°64′N and 114°90′E, with an average altitude of 629 m, and the area has a typical East Asian continental monsoon climate. The annual average temperature is about 7.6°C, the annual sunshine duration is about 2881 h, and the annual rainfall is about 381 mm, most of which occurs in July and August. Cabernet Sauvignon vines were planted in 2010, and the rows were established in a north-south orientation with plant spacing of 0.6 m and 2.8 m respectively. The texture of the soil was sandy loam, the average bulk density of the top 0-60 cm soil layer was about 1.56 g/cm$^3$, the field water holding capacity was about 27.5%, and the soil nitrate-nitrogen content was between 24.70 and 48.40 mg/kg; see Table 1 for more specific data. Agrometeorological data were obtained from small weather stations at the experimental site.

**Table 1 Base physicochemical properties of the 0-60 cm soil**

| Soil Depth/cm | Texture | Particle mass fraction/% | | | Field Capacity/% | Bulk Density/g·cm$^{-3}$ | Total N/g·kg$^{-1}$ | Total P/g·kg$^{-1}$ | Total K/g·kg$^{-1}$ | Alkaline hydrolyzed N/(mg·kg$^{-1}$) | Available P/mg·kg$^{-1}$ | Available K/mg·kg$^{-1}$ | Organic Matter/g·kg$^{-1}$ | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Clay | Silt | Sand | | | | | | | | | | |
| 0-20 | Sandy loam | 2.26 | 27.08 | 70.67 | 28.28 | 1.54 | 0.43 | 1.26 | 15.40 | 48.40 | 84.80 | 230.33 | 5.06 | 7.90 |
| 20-40 | Sandy loam | 2.76 | 34.58 | 62.66 | 23.11 | 1.62 | 0.43 | 0.68 | 14.20 | 24.70 | 21.10 | 177.48 | 4.54 | 7.96 |
| 40-60 | Loamy sand | 1.36 | 18.16 | 80.48 | 31.21 | 1.52 | 0.33 | 0.63 | 15.20 | 27.60 | 20.20 | 166.13 | 3.45 | 8.02 |

### 2.3 Experimental design and treatments

Two treatment factors, fertilization and irrigation, were set in the experiment. During the grape growing period, three fertilization levels were set at 60% (LF), 80% (MF), and 100% (HF) of the local fertilization rate, respectively, and three irrigation levels were established at 60% (LW), 80% (MW), and 100% (HW) of the irrigation quota, respectively. There were nine treatments, namely LFLW, LFMW, LFHW, MFLW, MFMW, MFHW, HFLW, HFMW, and HFHW, and each treatment was replicated three times. The specific implementation scheme is listed in Table 2. The irrigation cycle was once every two weeks, and the fertilization cycle involved fertilizer application during the shoot growth period, flowering period, fruit expansion period, and coloring period. The experimental layout adopted a completely random block design, with three rows per plot and 50 grapevines in each row (20 in the middle of the middle row were used for measurement and sampling, while the remaining plants served as buffers). Field drip irrigation was applied to the vineyard plots, and the irrigation pipes were arranged 40 cm below the surface of the vineyard soil; the flow rate of the dripper was controlled at 2.8 L/h per plant. Separate irrigation systems and water meters were installed in each plot to control specific irrigation and fertilization.

### 2.4 Grape samples and fermentation

Following the method of Vilanova et al.[21], during the harvest season in 2021, grape samples (15 kg) were manually harvested at their optimum maturity from nine treatment blocks on the same day, respectively. Grape juice samples (200 mL) were collected from each treatment and stored at −30°C for subsequent analysis.

The fermentation was carried out by traditional methods[27]. The grapes were destemmed and crushed to obtain must with the addition of 20 mg/L of pectinase and 50 mg/L of sulfur dioxide ($SO_2$). After standing for 24 h, the must was inoculated with 200 mg/L of commercial *Saccharomyces cerevisiae* (ZYMAFLORE F15, Bordeaux, France). The fermentation was performed at 26°C-28°C. After alcohol fermentation, the free $SO_2$ was adjusted to 60 mg/L. After being naturally clarified at 4°C for 1 month, the wines were filtered, bottled, and stored for further analysis. All experiments were carried out in triplicate.

**Table 2 Experimental scheme of fertigation and irrigation**

| Fertigation level | Irrigation level | Fertilizer rate (N-$P_2O_5$-$K_2O$)/kg·hm$^{-2}$ | Irrigation amount/m$^3$·hm$^{-2}$ |
|---|---|---|---|
| LF | LW | 51.03-39.69-100.17 | 1500 |
| | MW | 51.03-39.69-100.17 | 2000 |
| | HW | 51.03-39.69-100.17 | 2500 |
| MF | LW | 68.04-52.92-133.56 | 1500 |
| | MW | 68.04-52.92-133.56 | 2000 |
| | HW | 68.04-52.92-133.56 | 2500 |
| HF | LW | 85.05-66.15-166.95 | 1500 |
| | MW | 85.05-66.15-166.95 | 2000 |
| | HW | 85.05-66.15-166.95 | 2500 |

Note: LF, MF, and HF, 60%, 80%, and 100% of the fertilizer amounts locally recommended, respectively. LW, MW, and HW, 60%, 80%, and 100% of the designed irrigation quota, respectively.

### 2.5 Analysis methods

#### 2.5.1 Determination of physical and chemical parameters

Conventional physical and chemical indexes were determined according to the Chinese National standard GB/T 15038-2006 “General Analytical Methods for Wine and Fruit Wine”[28]. The single-fruit weight of grapes was measured with an electronic balance. Determination of the single fruit volume of grapes was performed using the drainage method. Total soluble solid (TSS) content was detected with a hand-held refractometer. Measurement of the pH value was carried out with a pH meter. The titratable acid and volatile acid content in wine were determined by titrimetric methods. The alcohol content of wine was analyzed by the alcohol meter method. The sum of the absorbance values of wine was measured at 420 nm, 520 nm, and 620 nm by a UV-5800 spectrophotometer, respectively. The indicators were each measured three times. The analytical determination of the wines was carried out 3 months after bottling[18].

2.5.2 Determination of amino acids

Amino acid analysis was performed by a pre-column derivatization-high performance liquid chromatography on an Agilent 1290 series HPLC system (Agilent Technologies, USA) according to the methods of Wang et al.[29] The separation was carried out on an Agilent Zorbax SB-$C_{18}$ Rapid Resolution HT column (3 mm×50 mm, 1.8 $\mu$m). Mobile phase A was 0.025 M sodium acetate buffer (pH 5.8), 0.02% sodium hydroxide; mobile phase B was 100% acetonitrile. The eluent gradient was as same as that used by Wang et al.[29] The other conditions were as follows: column temperature, 16°C; injection volume, 2 $\mu$L; flow rate, 0.6 mL/min; photodiode array detector, 280 nm.

Under these conditions, 22 amino acids were isolated, identified, and quantified. The compounds were identified according to the retention time of the standard, and the quantitative analysis was carried out by calculating the peak area on the linear regression curve of the standard and integrating the automatic identification of chromatographic peaks.

2.5.3 Determination of volatile compounds

Determination of volatile compounds in grape juices and wines was carried out by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS)[30]. The Agilent GC 7890B instrument equipped with an Agilent 5977A MS was used to analyze the samples. The HP-INNOWAX capillary column (30 m×0.25 mm×0.25 $\mu$m) was used with helium as the carrier gas at a 1 mL/min flow rate. The oven temperature was initially programmed at 50°C for 5 min and then increased at 5°C/min to 220°C, at which it was maintained for 5 min. The mass spectrometer inlet temperature was 250°C, and the temperature of the quadrupole and ionization source was 150°C and 230°C, respectively. The ionization mode was electron impact ion source (EI, 70 eV), with an acquisition range from 35 to 350 m/z.

For each of the treatments, 5 mL samples of juice or wine were placed in 20 mL vials, together with the addition of 5 $\mu$L of 2-octanol internal standard (100 mg/L) and 1.5 g of NaCl. The extraction procedure was performed with a 50/30 $\mu$m DVB/CAR/PDMS fiber (Supelco, Inc., Bellefonte, PA, USA). The fiber was inserted into the headspace for 30 min at 50°C under agitation and desorbed in the injector at 250°C for 5 min.

Identification was carried out using the GC/MS Chem-Station Software (Agilent), by comparing mass spectra with those of the mass library (NIST) and retention indices with those of the standard compounds. Quantitative data were obtained according to the internal standard method. The odor activity value (OAV) of each of these compounds was calculated by dividing the compound concentration by its corresponding odor threshold odor threshold to assess its contribution to wine sensory[31,32].

### 2.6 Statistical analysis

All data are presented as the mean±standard deviation (SD) values of three replicate experiments and processed with Microsoft Excel 2019 (Microsoft Corp., USA). Analysis of variance (ANOVA) was used to evaluate the difference and interaction effects between fertigation and irrigation treatments. Duncan's multiple range test ($p$<0.05) was applied to determine significant differences between treatment means. The volatile compounds and amino acids in treatments were analyzed using principal component analysis (PCA) to distinguish the effects of fertigation and irrigation. All statistical analyses were performed using SPSS 26.0 (IBM Corp., USA). The graphs presented were drawn using Origin 2022 (OriginLab, Northampton, MA, USA).

## 3 Results and discussion

### 3.1 Weather conditions

The temperature (°C), precipitation (P), and reference evapotranspiration (ETo) from May to October 2021 are shown in Figure 1. From May 1 to the harvest period, the average temperature was 20.32°C, the total rainfall was 221 mm, and the average evapotranspiration was 3.28 mm/d. From May to June, the precipitation was reduced, the average temperature was lower, and the evapotranspiration was higher. From July to August, the precipitation increased, and the average temperature was higher, but the evapotranspiration decreased. From September to October, precipitation was lower, the average temperature was lower, and evapotranspiration was at its lowest. The precipitation, temperature, and evapotranspiration across these seasons influenced the aromatic composition and amino acids in grapes. When rainfall or soil moisture was insufficient, irrigation water was required to meet the evapotranspiration needs of grapevines[16,25]. Thus, irrigation water was necessarily adjusted throughout the growth and development stage of grape berries based on annual precipitation, average temperature, and evapotranspiration in the experiment.

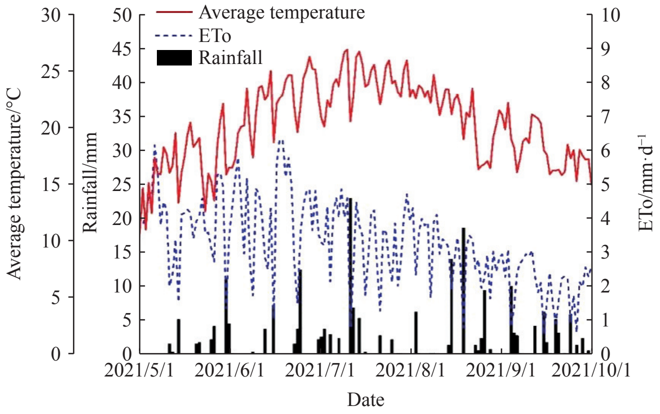


Figure 1 Weather data map of the test site during grape fruit development in 2021

### 3.2 General parameters of grapes and wines

Table 3 lists the characteristic parameters of grapes presented according to the different fertilizer and water treatments. Irrigation had a significant effect ($p$<0.05) on berry volume of grapes and an extremely significant effect ($p$<0.01) on titratable acid content; fertilizer had a significant effect ($p$<0.05) on grape berry weight, volume, and TSS, but other grape parameters were not significantly affected. In addition, fertigation and irrigation had no significant interactive effect of the basic parameters of grape berries. Overall, the volume and weight of single grape berries increased with the irrigation amount and fertilizer amount, and the degree of increase weakened with subsequent increases. The TSS in grape berries decreased with increases in the irrigation water at the same fertilizer level, but on the contrary, it increased with fertilizer application at the same irrigation level. It has been reportedthat under water deficit conditions TSS increased in red varieties (Cabernet Sauvignon) but not white varieties (Chardonnay), which indicates that the metabolic response to water deficit differs among varieties[33]. The change in titratable acid content in this study was consistent with previous studies, and it has been acknowledged that the titratable acid value increases with irrigation water[34].

**Table 3 Research on the general parameters in Cabernet Sauvignon grapes**

| Samples | Treatments | Average weight per berry/g | Average volume per berry/mL | Total soluble solids/(°Bx) | Titratable acidity/($g\cdot L^{-1}$) | pH |
|---|---|---|---|---|---|---|
| Grape fruits | HFHW | 1.49±0.05 | 1.52±0.04 | 22.0±2.2 | 6.86±0.47 | 3.97±0.14 |
| | HFMW | 1.49±0.07 | 1.50±0.05 | 23.1±2.6 | 5.95±0.23 | 3.89±0.04 |
| | HFLW | 1.44±0.09 | 1.43±0.03 | 23.4±2.4 | 5.67±0.77 | 3.92±0.16 |
| | MFHW | 1.44±0.16 | 1.47±0.14 | 21.8±2.9 | 6.68±0.69 | 3.90±0.13 |
| | MFMW | 1.47±0.09 | 1.47±0.09 | 22.3±2.0 | 5.90±0.42 | 3.99±0.17 |
| | MFLW | 1.40±0.02 | 1.43±0.02 | 22.6±0.9 | 5.41±0.23 | 3.97±0.06 |
| | LFHW | 1.36±0.01 | 1.40±0.02 | 20.5±0.6 | 6.48±0.65 | 3.97±0.07 |
| | LFMW | 1.43±0.05 | 1.46±0.03 | 21.8±1.3 | 5.90±0.16 | 3.96±0.08 |
| | LFLW | 1.28±0.02 | 1.30±0.03 | 22.0±0.5 | 5.91±0.88 | 3.90±0.10 |
| Factorial analysis | Irrigation | ns | * | ns | ** | ns |
| | Fertigation | * | * | * | ns | ns |
| | Irrigation× Fertigation | ns | ns | ns | ns | ns |

Note: Value: mean±standard deviation. Significance: irrigation, fertigation and their interaction are expressed as ns=not significant; * and ** indicates at $p$<0.05 and $p$<0.01, respectively.

As can be seen from Table 4, irrigation only had a significant effect ($p$<0.05) on the volatile acid content in Cabernet Sauvignon wines, and fertigation only had an extremely significant effect ($p$<0.01) on the alcohol content. The interactive effect of irrigation and fertilizer was significant for the basic physical and chemical indicators in wines. The titratable acid, pH, and color of wine were not affected by fertilizer and irrigation. In this study, volatile acid content decreased with the increase of irrigation water, which may be owing to the synthesis of higher concentrations of volatile acids to adapt to drought conditions during grape growth under low water conditions[35]. The TSS of grape berries increased with the increase in fertilizer application amount, which may be caused by the increase of fertilizer application rate promoting the transportation of sugar to berries, thus causing grape berry TSS to show an increasing trend[36]. As listed in Table 4, a similar increase can be found in alcohol content with the amount of fertilizer applied. It was generally believed that the fermentable sugar increased along with the increase in TSS. In addition, the increase in nitrogen compounds promoted an increase in the assimilable nitrogen (YAN) of yeast, thus increasing fermentation activity[37]. The correlation analysis for grape berry TSS and corresponding wine alcohol intensity shows that the two variables are positively correlated. Indeed, water stress during the growth process of grapes may affect their photosynthesis and sugar metabolism transport, thus affecting the physicochemical parameters of resulting wines[38,39]. As previously found, subtle changes in wine attributes observed among treatments can be readily detectable at the sensory level[15]. The results show that irrigation and fertilization can be used as tools to adjust the general parameters of grape berries, which can be utilized to adjust specific properties when producing grape juice and wine[25].

**Table 4 Research on the general parameters in Cabernet Sauvignon wines**

| Samples | Treatments | Alcohol/(% vol) | Titratable acidity/($g\cdot L^{-1}$) | Volatile acid/($g\cdot L^{-1}$) | pH | Chroma |
|---|---|---|---|---|---|---|
| Wines | HFHW | 13.1±0.3 | 6.84±0.19 | 0.18±0.01 | 3.77±0.04 | 10.8±0.7 |
| | HFMW | 12.9±0.4 | 7.23±0.52 | 0.17±0.01 | 3.72±0.05 | 9.3±0.5 |
| | HFLW | 13.3±0.0 | 6.86±0.26 | 0.20±0.01 | 3.73±0.10 | 10.9±1.3 |
| | MFHW | 13.0±0.4 | 6.94±0.41 | 0.16±0.01 | 3.75±0.11 | 11.0±1.3 |
| | MFMW | 12.7±0.1 | 6.81±0.30 | 0.19±0.05 | 3.84±0.04 | 11.3±2.2 |
| | MFLW | 12.6±0.4 | 6.94±0.19 | 0.21±0.01 | 3.72±0.06 | 12.8±1.0 |
| | LFHW | 12.4±0.2 | 6.75±0.41 | 0.16±0.01 | 3.71±0.09 | 10.7±1.2 |
| | LFMW | 12.4±0.3 | 6.97±0.05 | 0.17±0.02 | 3.71±0.04 | 10±0.4 |
| | LFLW | 12.5±0.4 | 7.03±0.77 | 0.18±0.03 | 3.68±0.09 | 10.9±1.4 |
| Factorial analysis | Irrigation | ns | ns | * | ns | ns |
| | Fertigation | ** | ns | ns | ns | ns |
| | Irrigation× Fertigation | ns | ns | ns | ns | ns |

Note: Value: mean±standard deviation. Significance: irrigation, fertigation and their interaction are expressed as ns=not significant; * and ** indicates at $p$<0.05 and $p$<0.01, respectively.

### 3.3 Amino acids in grape berries and wines

#### 3.3.1 Effect of fertilizer and water on amino acids in grape berries

Nitrogen, phosphorus, and potassium are particularly abundant soil-derived macronutrients in grapevines, influencing vine growth and playing an important role in enhancing the quality of grapes for making wine[40]. They increase the content of major compounds in grape berries, such as total N and total amino acids[1]. The content of amino acids in grape juice according to fertilizer and water treatments is listed in Table 5. It can be seen that arginine was the most abundant amino acid, accounting for about 37% of the total amino acids, followed by proline (32%), citrulline (5%), glutamine (6%), and serine (4%) in fruits. Cabernet Sauvignon grapevine could be considered an arginine-accumulating variety, similar to Syrah, Merlot, and Pinot Noir[6]. Indeed, the metabolisms of proline and arginine were linked; specifically, the final concentration of one amino acid was influenced by the level of the other, and it was previously confirmed that at least a portion of proline is the precursor of the arginine that accumulates in grapes[11].

Irrigation had a significant effect ($p$<0.05) on the levels of histidine, glutamine, cysteine, and threonine and had an extremely significant effect ($p$<0.01) on the levels of citrulline, arginine, methionine, lysine, and serine in fruits. Moreover, their concentration increased with the increase of irrigation amount. As far as fertilizer was concerned, it had significant effects ($p$<0.05) on the levels of histidine, methionine, lysine, leucine, isoleucine, serine, and glycine, which first decreased and then increased with increases in fertilizer application. The interaction of fertilizer and irrigation had significant effects ($p<0.05$) on arginine, glutamine, and serine levels. As previously reported, both fertilizer and irrigation had an impact on total amino acid content in grapes[41]. In general, the HWHF treatment had the highest amino acid level, while the LWHF treatment had the lowest amino acid level among all treatments.

**Table 5 Effects of fertilizer and water treatments on amino acid concentration (mg/L) in Cabernet Sauvignon grape juice**

| Compounds | HFHW | HFMW | HFLW | MFHW | MFMW | MFLW | LFHW | LFMW | LFLW | Irrigation | Fertigation | Irrigation× Fertigation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tryptophan | 1.7±0.1 | 1.2±0.5 | 0.9±0.3 | 0.6±0.4 | 0.6±0.3 | 1.0±0.5 | 1.5±1.0 | 0.8±0.4 | 1.1±0.5 | ns | ns | ns |
| Tyrosine | 1.0±0.7 | 0.9±0.5 | 0.8±0.6 | 1.1±0.3 | 1.4±0.7 | 1.8±1.4 | 0.4±0.1 | 0.2±0.1 | 1.7±1.0 | ns | ns | ns |
| Citrulline | 92.5±16.9 | 66.9±11.5 | 46.4±12.4 | 58.1±23.4 | 64.0±22.9 | 41.6±18.2 | 99.0±13.4 | 51.0±24.2 | 50.1±16.9 | ** | ns | ns |
| Arginine | 675.4±29.7 | 601.2±152.4 | 322.4±46.1 | 458.3±14.7 | 553.7±37.9 | 454.5±86.8 | 620.5±32.2 | 433.5±30 | 447.4±71.8 | ** | ns | ** |
| Phenylalanine | 19.7±3.9 | 22.4±4.1 | 11.2±3.8 | 14.3±5.6 | 14.3±5.6 | 12.7±2.0 | 16.8±0.2 | 14.1±5.6 | 14.2±2.6 | ns | ns | ns |
| Histidine | 28.9±1.1 | 30.1±2.4 | 23.2±5.5 | 22.7±2.4 | 24.5±1.6 | 24.7±3.1 | 32.4±2.8 | 27.7±4.1 | 24.5±2.1 | * | * | ns |
| Methionine | 6.0±0.9 | 4.3±0.1 | 2.3±0.6 | 2.6±1.0 | 3.2±1.7 | 2.1±1.0 | 5.4±0.3 | 3.8±2.6 | 2.9±0.4 | ** | * | ns |
| Glutamate | 14.8±1.4 | 14.7±1.7 | 7.7±3.5 | 9.5±5.6 | 11.0±5.8 | 15.0±11.3 | 14.8±3.3 | 12.0±7.1 | 13.9±4.5 | ns | ns | ns |
| Glutamine | 90.6±2.9 | 107.4±27.2 | 44±6.6 | 59.1±16.3 | 60.9±12.1 | 70.9±2.8 | 100.2±21.8 | 65.1±29 | 78.1±6.9 | ns | ns | ** |
| Lysine | 6.6±0.1 | 7.2±1.0 | 3.2±1.0 | 4.1±1.3 | 4.6±1.2 | 3.7±1.6 | 7.2±1.6 | 5.1±1.8 | 5.1±1.4 | ** | * | ns |
| Aspartic acid | 4.7±1.3 | 4.4±1.9 | 3.0±1.6 | 2.3±1.8 | 2.9±1.6 | 3.5±0.6 | 4.3±1.1 | 3.7±0.4 | 3.5±0.3 | ns | ns | ns |
| Asparagine | 5.8±0.3 | 5.4±0.7 | 4.5±0.4 | 5.2±1.3 | 4.6±0.7 | 4.6±0.2 | 5.9±0.9 | 4.8±0.6 | 5.2±0.1 | * | ns | ns |
| Leucine | 57.4±9.5 | 50.8±6.5 | 35.7±9.0 | 35.3±8.9 | 33.3±2.2 | 40.8±8.4 | 51.2±1.8 | 39.5±12.7 | 41.4±3.2 | ns | * | ns |
| Isoleucine | 17.2±5.0 | 14.2±2.3 | 10.8±2.4 | 9.5±4.5 | 8.6±1.8 | 11.7±2.2 | 13.2±1.2 | 10.3±3.0 | 11.4±2.1 | ns | * | ns |
| Cysteine | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.1±0.1 | 0.2±0.0 | 0.2±0.0 | 0.2±0.1 | 0.2±0.0 | * | ns | ns |
| Threonine | 32.3±3.3 | 28.7±1.1 | 19.0±2.5 | 21.7±5.2 | 23.3±4.9 | 20.0±4.9 | 29.4±4.8 | 22.9±6.2 | 23.5±4.0 | * | ns | ns |
| Valine | 6.5±0.2 | 6.7±0.3 | 5.6±0.9 | 6.3±0.9 | 6.2±0.3 | 5.5±1.1 | 6.9±1.2 | 6.2±1.0 | 5.9±0.6 | ns | ns | ns |
| Proline | 456.4±48.7 | 454.7±17.7 | 406.8±69.7 | 431.3±55.7 | 438.8±31.1 | 411.1±77.1 | 477.3±75.7 | 430.9±63.9 | 421.3±10.2 | ns | ns | ns |
| Serine | 67.5±1.3 | 59.5±2.9 | 40.1±3.6 | 45.1±17.5 | 47.3±10.7 | 39.7±4.8 | 66.6±5.0 | 43.0±11.0 | 45.8±0.9 | ** | * | * |
| GABA | 30.8±10.7 | 23.3±3.0 | 20.7±12.0 | 22.2±4.6 | 20.2±0.5 | 20.7±1.2 | 24.5±2.2 | 26.2±3.0 | 23.8±2.3 | ns | ns | ns |
| Alanine | 57.5±11.2 | 55.4±12.2 | 33.0±6.9 | 44.0±9.0 | 43.8±7.3 | 36.5±6.8 | 48.4±10.9 | 39.7±12.3 | 45.8±8.7 | ns | ns | ns |
| Glycine | 1.9±0.2 | 1.7±0.5 | 1.0±0.2 | 1.2±0.8 | 1.0±0.6 | 0.9±0.4 | 1.2±0.2 | 1.0±0.5 | 1.0±0.2 | ns | * | ns |

Note: Value: mean±standard deviation. Significance: irrigation, fertilization and their interaction effects were expressed as ns=not significant; * and ** indicates at $p<0.05$ and $p<0.01$, respectively.

Although there were significant differences ($p<0.05$) in amino acid content between fertilizer and irrigation treatments, PCA still provided an important means of examining the results. Specifically, it was used to conduct the covariance analysis for amino acid content, the results of which are shown in Figure 2. The first two principal components (PC) explained 81.5% of the total variance of the data set, among which PC1 accounted for 71.8% and PC2 accounted for 9.7%. PC1 was positively correlated with the contents of many amino acids except tyrosine, while PC2 was positively correlated with the contents of cysteine, tyrosine, glutamate, isoleucine, and tryptophan. It can be seen from Figure 2 that the samples from the fertilizer and water treatments were effectively separated in terms of their amino acid distribution, but when the effect of fertilization was only considered, the sample separation was not obvious. The HFHW-treated grapes had positive PC1 and PC2 values owing to their high contents of threonine, serine, and isoleucine. The amino acid content in the HFLW treatment was generally lower than that of other treatments, so it had a negative PC1 value. Irrigation and fertilization had a great influence on the contents of amino acids in grapes, which significantly changed the distribution of amino acids. This was basically consistent with the findings of Holzapfel et al.[40]

### 3.3.2 Effects of fertilizer and water regulation on amino acids in wines

After alcohol fermentation, the amino acid contents in wines under different irrigation and fertigation treatments differed. Fertilizer had a significant effect ($p<0.05$) on tryptophan content in this study. Irrigation had significant effects ($p<0.05$) on the contents of arginine, asparagine, cysteine, and proline and had an extremely significant effect ($p<0.01$) on tryptophan content. Among all treatments, the wine from the HWHF treatment had the highest amino acid level, and that of the LWMF treatment had the lowest amino acid level. This was similar to the results for amino acids in grape juices. The amino acid contents of grape berries are very important for successful fermentation[42]. Irrigation and fertilizer influence vine nitrogen availability and therefore the fruit amino acid content and subsequent wine quality[43].

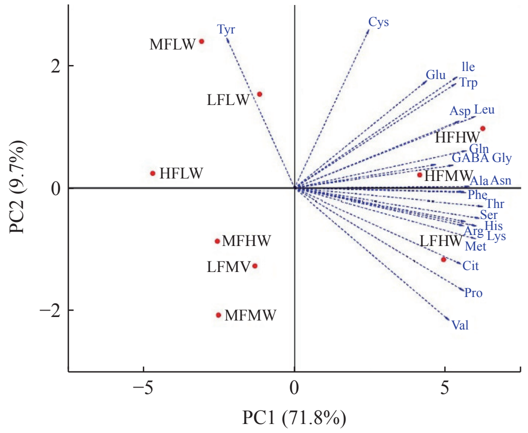


Figure 2 PCA in Cabernet Sauvignon grape fruits: bigraph analysis of the first two components (PC) for the treatments of fertilizer and water and the amino acid distribution

The consumption of amino acids during alcoholic fermentation was not significantly different among fertilizer and irrigation treatments. The most highly consumed amino acids during alcohol fermentation were arginine, citrulline, and glutamine, which represent the assimilable nitrogen sources, and therefore, they were preferentially used by yeast[1]. In contrast, some amino acids, such as arginine, proline, and asparagine, had higher concentrations in wines than the corresponding essential amino acids in this experiment. This condition may be caused by autolysis of yeast[44]. It was found that under normal fermentation conditions, after alcohol fermentation, the concentration of most amino acids in grapes, especially of assimilable amino acids, decreases significantly[41].

The PCA of amino acid content in wines (Figure 3) showed that the first two principal components explained 66.4% of the total variance of the samples, with PCI and PC2 accounting for 48.9% and 17.5% of the variance, respectively. Construction of PC1 was based on the concentrations of phenylalanine, methionine, leucine, glutamine, asparagine, and arginine, while construction of PC2 was dependent on the contents of isoleucine and citrulline. The wine from the HFMW treatment had high phenylalanine, methionine, isoleucine and citrulline concentrations, so it had positive PC1 and PC2 values. Other processed wine samples were also distinguished. The wine samples from the low irrigation treatment all had negative PC1 values, reflecting the distribution of grapes in the same low irrigation treatment. The decrease in amino acid content in wine caused by low irrigation might be because low irrigation tends to reduce the weight of berries and thus affect sugar metabolism, thereby reducing amino acid content in grapes[34]. The distribution of PC1 and PC2 in wine samples treated with fertilization was relatively dispersed, similar to that of the grape samples. Therefore, the effect of fertilization on amino acid content in wine was not obvious. The interaction between high fertilizer and medium water irrigation had the most positive effect on amino acid content in wine. Taking the Mencía variety as an example, Vilanova et al.[45] was also able to use PCA to distinguish wines well.

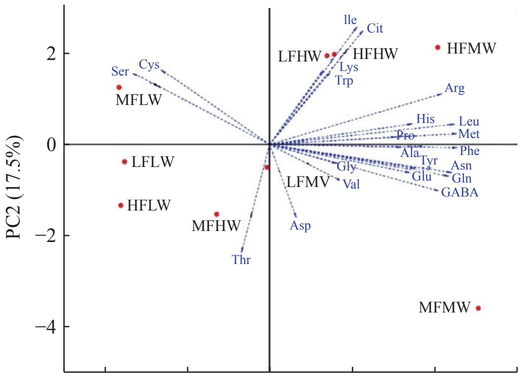


Figure 3 PCA in Cabernet Sauvignon wines: biplot analysis of the first two components for the treatments of fertilizer and water and the amino acid distribution

## 3.4 Volatile components in grape berries and wines

### 3.4.1 Effects of fertilizer and water regulation on volatile components in grape berries

It can be seen from Table 6 that 38 volatile compounds were detected in grape juices by gas chromatography-mass spectrometry, mainly including higher alcohols, fatty acids, esters, terpenes, aldehydes, and ketones. The results of variance analysis showed that neither fertigation nor irrigation had significant effects on the levels of volatile compounds in grape juices, and the interaction of fertigation and irrigation had a significant effect ($p<0.05$) on the content of phenylethyl alcohol. In general, the individual irrigation and fertilization treatments did not have a significant effect on the content of volatile substances in grape juice, while the interaction of irrigation and fertilization had a specific effect on the content of a few volatile substances (Table 6).

Grape-derived aliphatic alcohols/aldehydes have been identified as precursors of acetates in wines, and in particular, the $C_6$ compounds (E)-2-hexenal, hexanal, (E)-2-hex-1-ol, and hexanol were found to be precursors of hexyl acetate[2]. Although most higher alcohols were fermentation by-products of yeast, some are present in grapes and persist during fermentation, such as isoamyl alcohol, octanol, heptanol, hexanol, and phenethyl alcohol[46]. For terpenes, it was found that fertilizer and water regulation had no significant effect in wines, but too much water irrigation reduced the terpene content in this experiment. In general, although fertilizer and water regulation had no significant effect on volatile compounds, it still affected the concentrations of some higher alcohols, fatty acids, esters, and terpenes.

### 3.4.2 Effects of fertilizer and water regulation on the volatile components in wines

A total of 52 volatile compounds were detected in wines (Table 7), including alcohols, acids, esters, and terpenes. There were significant differences ($p<0.05$) in the concentrations of isoamyl acetate, phenethyl acetate, methyl heptanoate, citronellol, and 2-octanone among fertilization treatments. Fertigation and irrigation had significant interactive effects ($p<0.05$) on lauryl alcohol, ethyl tridecanoate, and methyl heptanoate concentrations. In general, the interaction between fertilization and irrigation in this experiment had a small effect on the content of volatile substances in wine, while irrigation had a larger effect on the content of volatile substances.

The contents of 11 compounds detected in wines were higher than their corresponding sensory thresholds, which may contribute to the aroma of wines[30]. Among them, the OAV of phenethyl alcohol was significantly different ($p<0.05$) between irrigation treatments, and the OAVs of isoamyl acetate and citronellol were significantly different ($p<0.05$) between fertigation treatments.

In this study, the higher alcohol levels in wines were all less than 300 mg/L, which would not have a negative impact on the aroma of wines[47]. High isoamyl alcohol concentration in wine did not differ among treatments, likely owing to mild water stress on Cabernet Sauvignon vines, similar to the findings in other grape varieties[48]. In contrast, other subtle higher alcohols, such as phenethyl alcohol (OAV>1), a compound that can give wine a rose fragrance, are obviously affected by irrigation[49]. This experiment shows that MFLW treatment had the highest total content of advanced alcohols, while HFHW treatment had the lowest total content of advanced alcohols, among which, low water irrigation can significantly improve the concentration of phenyl ethanol.

Volatile fatty acids are also one of the important indicators for evaluating wine quality. When volatile fatty acid level remained above their sensory thresholds, wines exhibited rancid and cheesy smells[8]. It can be seen from Table 7 that the total volatile fatty acid content in the HFMW-treated wine was the highest, and its concentration did not change with the fertigation or irrigation treatment. A similar result was found for Tempranillo wines, and it has been confirmed that supplemental irrigation had no significant effect on fatty acid concentrations[50].

In this experiment, acetates of five higher alcohols were determined in wines (Table 7), and their overall concentration was low compared with other research results[24], which may be associated with differences in grape origin and yeast[1]. In the present study, irrigation had no significant effect on acetate concentration, but increased with increasing irrigation. Fertigation

**Table 6 Effects of fertilizer and water treatments on the concentration of volatile compounds in grape juices ($\mu$g/L)**

| | Free compounds | LFLW | LFMW | LFHW | MFLW | MFMW | MFHW | HFLW | HFMW | HFHW | Irrigation | Fertigation | Irrigation× Fertigation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alcohols | 1-Penten-3-ol | 0.8±0.4 | 0.9±0.2 | 0.8±0.2 | 0.8±0.2 | 0.7±0.1 | 0.9±0.0 | 0.9±0.2 | 0.9±0.2 | 0.8±0.2 | ns | ns | ns |
| | Isopentyl alcohol | 11.8±1.2 | 21.2±11.7 | 15.9±2.8 | 20.6±14.9 | 17.5±7.5 | 19.1±8.6 | 25.9±8.4 | 12.0±0.8 | 17.8±6.6 | ns | ns | ns |
| | 2-pentenol | 1.8±0.8 | 1.9±1 | 1.8±0.2 | 1.6±0.5 | 1.4±0.6 | 2.0±1.0 | 2.0±0.5 | 1.5±0.2 | 1.7±0.3 | ns | ns | ns |
| | 1-Octen-3-ol | 2.1±0.4 | 2.4±0.4 | 2.6±0.6 | 2.4±0.2 | 1.7±0.0 | 2.3±0.6 | 2.4±0.3 | 2.3±0.3 | 2.8±0.2 | ns | ns | ns |
| | Heptyl alcohol | 3.4±0.7 | 3.3±0.5 | 3.2±0.5 | 3.4±0.3 | 2.6±0.4 | 2.5±0.6 | 2.3±0.9 | 3.0±0.7 | 2.9±0.6 | ns | ns | ns |
| | 3-Ethyl-4-methylpentanol | 5.2±1.9 | 6.1±1.6 | 5.0±1.9 | 5.1±2.7 | 3.3±0.6 | 4.9±3.2 | 3.9±1.7 | 5.5±3.4 | 5.1±0.7 | ns | ns | ns |
| | Octyl alcohol | 2.8±0.3 | 3.5±0.9 | 2.6±0.2 | 3.1±0.6 | 2.5±0.6 | 3.6±0.9 | 3.9±1.3 | 3.7±0.2 | 8.2±4.1 | ns | ns | ns |
| | Nonyl alcohol | 2.0±0.4 | 3.1±1.2 | 2.2±0.7 | 2.6±0.3 | 2.2±0.4 | 4.5±3.3 | 3.6±1.5 | 3.9±0.4 | 4.6±3.4 | ns | ns | ns |
| | (Z)-6-Nonen-1-ol | 3.4±1.3 | 4.5±3.1 | 3.7±1.5 | 3.7±2.6 | 2.2±0.7 | 4.2±4.8 | 6.5±4.2 | 4.7±1.2 | 4.1±2.0 | ns | ns | ns |
| | Dodecyl alcohol | 1.4±0.9 | 1.1±0.7 | 0.8±0 | 0.5±0.4 | 0.7±0.0 | 1.1±0.3 | 1.2±0.6 | 1.4±0.7 | 2.2±1.8 | ns | ns | ns |
| | Hexyl alcohol | 635.7±232.1 | 634.3±271.3 | 577.3±71.2 | 786.3±178.0 | 563.9±362.8 | 554.6±297.8 | 455.6±143.6 | 589.2±226.1 | 646.0±269.6 | ns | ns | ns |
| | (E)-3-Hexen-1-ol | 11.1±4.3 | 6.7±0.8 | 9.2±2.3 | 9.4±1.6 | 6.2±1.5 | 8.5±2.5 | 7.1±2.6 | 9.4±0.8 | 13.9±6.0 | ns | ns | ns |
| | (Z)-2-Hexenol | 390.8±300.9 | 226.4±168.3 | 196.9±28.0 | 225.0±202.1 | 141.0±53.0 | 259.8±146.6 | 176.8±86.3 | 264.8±79.8 | 282.1±76.8 | ns | ns | ns |
| | Benzyl alcohol | 9.9±2.9 | 9.2±0.7 | 8.5±1.1 | 11.4±1.8 | 7.4±1.6 | 8.6±2.6 | 7.6±1.7 | 10.3±1.1 | 8.5±1.1 | ns | ns | ns |
| | Phenethyl alcohol | 10.9±2.1 | 9.4±3.0 | 11.3±1.6 | 12.2±1.3 | 7.5±1.8 | 9.4±1.5 | 8.1±3.7 | 11.8±2.0 | 15.3±4.6 | ns | ns | * |
| Volatile acid | Hexanoic acid | 5.4±3.0 | 3.4±2.4 | 1.7±0.6 | 11.3±10.9 | 3.2±2.9 | 9.9±1.4 | 5.6±4.8 | 6.1±5.6 | 7.9±6.8 | ns | ns | ns |
| | 2-Hexenoic acid | 1.8±0.8 | 1.4±0.4 | -- | 3.0±2.9 | -- | 1.7±1.5 | -- | 1.9±0.6 | 2.3±0.2 | ns | ns | ns |
| Esters | Isoamyl acetate | 5.6±6.2 | 1.9±0.8 | 2.4±1.8 | 3.8±1.8 | 2.2±1.2 | 3.4±2.6 | 2.9±0.8 | 5.9±4.2 | 3.5±1.5 | ns | ns | ns |
| | (Z)-2-Hexenyl acetate | 5.5±7.5 | 1.7±0.7 | 1.7±0.3 | 3.2±0.5 | 4.2±1.6 | 4.1±3.1 | 1.8±0.8 | 2.0±0.9 | 7.3±8.8 | ns | ns | ns |
| | Ethyl hexanoate | 6.4±3.5 | 4.6±2.3 | 1.8±0.5 | 5.5±3.5 | 3.0±1.4 | 4.1±1.9 | 3.5±1.5 | 5.0±2.2 | 4.1±1.6 | ns | ns | ns |
| | Ethyl octanoate | 42.5±16.6 | 33.4±21.9 | 4.9±2.8 | 28.5±21.3 | 16.6±18.3 | 22.6±8.9 | 32.7±1.4 | 35.3±31.5 | 43.2±13.4 | ns | ns | ns |
| | Ethyl decanoate | 16.3±7.3 | 26.7±18.5 | 3.3±1.2 | 10.7±5.5 | 4.4±3.4 | 47.4±20.1 | 11.3±2.6 | 12.0±2.5 | 19.2±7.8 | ns | ns | ns |
| | Isoamyl butanoate | 5.9±3.6 | 7.5±3.1 | -- | 3.3±1.7 | 5.2±4.2 | 4.3±2.3 | 5.4±1.5 | 10.7±6.3 | 1.7±0.0 | ns | ns | ns |
| | Isopentyl hexanoate | -- | 9.8±7.5 | -- | -- | -- | 4.4±2.8 | 4.5±3.7 | -- | 2.2±0.2 | ns | ns | ns |
| Terpenes-$C_{13}$ | Limonene | 2.2±1.0 | 2.2±0.5 | 2.2±0.4 | 2.3±0.2 | 2.7±0.4 | 2.1±0.1 | 2.6±0.4 | 2.3±0.8 | 1.8±0.4 | ns | ns | ns |
| | Linalool | 5.7±2.4 | 8.4±7.4 | 1.6±0.1 | 3.0±0.6 | 4.8±4.2 | 13.3±10.9 | 8.1±6.2 | 2.9±1.8 | 5.2±4.9 | ns | ns | ns |
| | α-Terpinol | 1.3±1.1 | 3.1±2.1 | -- | 0.8±0.1 | -- | 5.0±3.6 | 1.9±0.4 | 0.8±0.1 | 1.7±1.6 | ns | ns | ns |
| | Citronellol | 3.9±1.4 | 2.6±0.9 | 2.8±2.4 | 2.1±1.4 | 2.7±0.9 | 3.2±1 | 3.8±2.4 | 3.8±1.7 | 1.7±0.9 | ns | ns | ns |
| | β-Damascenone | 46.2±34.0 | 37.7±25.3 | 24.6±11.5 | 34.8±19.4 | 28.7±21.0 | 35.9±10.3 | 30.4±22.7 | 41.1±27.9 | 28.7±20.6 | ns | ns | ns |
| | Neryl alcohol | 1.4±1.1 | 0.7±0.4 | 0.6±0.1 | -- | 0.4±0.0 | -- | 1.2±0.7 | -- | -- | ns | ns | ns |
| Alkanes, Olefins | Hexanal | 2.3±0.4 | 2.1±0.9 | 2.6±1.2 | 3.4±2.0 | 3.6±3.0 | 4.7±3.1 | 3.4±2.1 | 2.3±0.8 | 2.3±2.0 | ns | ns | ns |
| | 2-Heptanone | 1.7±0.9 | 1.6±0.7 | 1.7±0.6 | 4.1±3.7 | 1.7±0.8 | 1.6±0.5 | 2.9±2.8 | 2.0±1.4 | 2.4±1.9 | ns | ns | ns |
| | Leaf aldehyde | 55.4±42.4 | 49.8±19.1 | 44.2±7.9 | 61.1±24.7 | 40.3±17.0 | 47.5±29.9 | 25.8±8.1 | 43.2±15.7 | 50.4±20.0 | ns | ns | ns |
| | 2-Octanone | 2.1±0.6 | 2.7±0.7 | 2.3±0.8 | 3.4±2.8 | 2.2±0.9 | 3.1±1.4 | 3.4±3.0 | 3.5±2.3 | 3.7±3.4 | ns | ns | ns |
| | Prenylacetone | 1.2±0.1 | 1.1±0.5 | 1.1±0.1 | 1.2±0.1 | 1.1±0.6 | 1.2±0.3 | 1.1±0.2 | 1.0±0.1 | 1.7±1.4 | ns | ns | ns |
| | 2-Nonanone | 2.1±0.3 | 1.5±0.1 | 1.8±0.3 | 2.8±2 | 1.2±0.2 | 1.6±0.2 | 1.6±1.2 | 1.9±0.7 | 2.5±1.5 | ns | ns | ns |
| | Benzaldehyde | 16.1±6.0 | 22.9±12.8 | 15.9±0.9 | 12.9±1.8 | 17.8±9.0 | 18.7±7.6 | 17.7±10.1 | 12.5±5.1 | 11.8±7.7 | ns | ns | ns |
| | Benzene acetaldehyde | 1.6±0.1 | 1.5±0.6 | 1.4±0.6 | 1.2±0.0 | 1.2±0 | 1.5±0.5 | -- | 1.8±1.3 | 2.0±0.6 | ns | ns | ns |

Note: Value: mean±standard deviation. "--": Not detected. Significance: irrigation, fertigation and their interaction effects were expressed as ns=not significant; * and ** indicates at $p<0.05$ and $p<0.01$, respectively.

had a significant effect ($p<0.05$) on the concentrations of isoamyl acetate and phenethyl acetate. As fertigation increased, the concentrations of both isoamyl acetate and phenethyl acetate tended to rise. For each treatment, both isoamyl acetate and hexyl acetate, with OAV>1, provided fruity flavors such as banana and apple to the wines, while the acetate esters content of other higher alcohols was consistently below their aroma thresholds[51].

Most of the esters identified in this study were fatty acid ethyl esters (Table 7); they were not affected by fertilizer and irrigation, as these compounds are secondary products of sugar metabolism produced by yeast during fermentation[52], usually playing an active role in quality and mainly providing fruity aromas to wines[47]. For example, ethyl butyrate provided fruity aromas, ethyl caproate provided banana and green apple aromas, ethyl octanoate provided banana and pear and other fruit aromas, and ethyl capric acid provided fruity and fatty flavors. These compounds are typical of young red wines, and their concentrations each exceeded the detection threshold, becoming a relevant part of wine aroma.

Terpenes are an important contributor to the aroma of dry red wine, and a correlation between floral sensory characteristics and high concentrations of linalool has been demonstrated previously[7]. In this study, irrigation had a significant effect ($p<0.05$) on $\beta$-damascone concentration, and fertigation had a significant effect ($p<0.05$) on citronellol concentration (Table 7). Of these compounds, linalool and citronellol levels were only found above their sensory thresholds; the former provided floral and fruity aromas, while the latter gave rose and grassy aromas[31]. The terpene level in LFMW-treated wine was the highest, but it was still low compared with the levels of other volatile substances.

All in all, when grouped by family, the contents of volatile compounds differed among treatments. The higher alcohol decreased with the increase of irrigation water. The esters increased

### Table 7 Effects of fertilizer and water treatments on the concentration of volatile compounds in wines ($\mu$g/L)

| Free Compounds | LFLW | LFMW | LFHW | MFLW | MFMW | MFHW | HFLW | HFMW | HFHW | Irrigation | Fertigation | Irrigation× Fertigation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alcohols | | | | | | | | | | | | |
| Isobutyl alcohol | 160.6±16.8 | 160.7±27.9 | 163.3±15.6 | 151.3±18.2 | 154.0±10.0 | 138.4±13.5 | 142.7±9.4 | 145.7±28.8 | 151.2±13.2 | ns | ns | ns |
| Isopentyl alcohol | 10 396.2±952.2 | 10 379.3±373.9 | 10 010.7±387.0 | 10 260.4±181.7 | 10 276.1±273.5 | 10 234.7±603.1 | 10 064.0±233.9 | 10 515.3±979.6 | 9572.5±393.4 | ns | ns | ns |
| Isohexyl alcohol | 9.6±1.7 | 10.1±1.2 | 9.1±1.0 | 9.8±0.6 | 8.7±1.3 | 10.6±1.1 | 10.0±0.9 | 10.1±1.8 | 8.6±1.6 | ns | ns | ns |
| 3-Methylpentan-1-ol | 34.5±8.0 | 35.9±6.6 | 30.6±1.7 | 33.8±1.4 | 30.3±7.0 | 36.5±7.5 | 37.6±7.6 | 35.2±7.1 | 27.5±5.1 | ns | ns | ns |
| Heptyl alcohol | 37.5±9.3 | 58.3±7.2 | 45.1±27.8 | 37.7±8.0 | 66.3±49.2 | 45.2±11.6 | 49.2±8.1 | 49.3±21.0 | 71.3±20.1 | ns | ns | ns |
| 3-Ethyl-4-methylpentanol | 8.2±1.3 | 8.3±1.8 | 7.6±1.2 | 7.4±0.6 | 8.3±0.5 | 8.1±1.8 | 8.0±0.5 | 7.7±1.0 | 8.2±0.1 | ns | ns | ns |
| Octyl alcohol | 39.1±4.7 | 48.1±4.9 | 37.5±4.7 | 34.3±9.1 | 40.9±10.4 | 43.4±0.4 | 43.4±7.2 | 47.7±7.9 | 40.9±3.0 | ns | ns | ns |
| Nonyl alcohol | 20.9±4.6 | 25.2±2.6 | 18.5±3.8 | 20.4±1.1 | 26.0±8.8 | 18.9±1.2 | 20.8±4.8 | 24.8±1.0 | 19.5±2.3 | * | ns | ns |
| 3-Methylmercapto-1-propanol | 9.5±4.0 | 8.3±1.9 | 7.3±3.0 | 8.2±4.2 | 6.3±1.1 | 8.7±4.6 | 9.9±6.5 | 11.8±6.2 | 4.5±1.0 | ns | ns | ns |
| Decyl alcohol | 15.7±3.8 | 21.2±3.0 | 16.7±2.1 | 12.8±4.1 | 17.4±8.8 | 17.8±5.0 | 15.8±3.5 | 24.7±1.3 | 16.2±2.1 | * | ns | ns |
| Dodecyl alcohol | 14.2±7.7 | 28.3±14.5 | 11.1±5.3 | 35.6±4.1 | 33.9±8.7 | 10.4±4.0 | 12.6±4.5 | 23.8±7.2 | 26.7±14.0 | * | ns | * |
| Hexyl alcohol | 501.7±76.1 | 482.2±25.5 | 424.1±92.4 | 487.9±88.0 | 606.1±338.4 | 435.7±22.6 | 474.3±51.1 | 527.2±33.4 | 491.0±50.2 | ns | ns | ns |
| (E)-3-Hexen-1-ol | 9.3±1.7 | 8.9±0.8 | 9.4±2.6 | 9.6±0.5 | 9.8±4.5 | 8.5±0.4 | 8.0±0.3 | 10.2±1.3 | 10.5±2.3 | ns | ns | ns |
| (Z)-6-Nonen-1-ol | 17.6±5.0 | 21.4±4.6 | 15.9±3.8 | 17.7±4.1 | 20.7±5.9 | 15.6±2.2 | 17.0±4.9 | 14.3±12.6 | 17.2±1.4 | ns | ns | ns |
| Benzyl alcohol | 28.7±5.0 | 34.0±0.9 | 31.2±7.0 | 28.6±4.3 | 39.6±4.0 | 30.1±1.5 | 25.4±8.8 | 33.7±3.0 | 33.6±7.0 | * | ns | ns |
| Phenethyl alcohol | 7160.9±1470.7 | 6775.2±874.7 | 5558.3±430.2 | 7916±613.7 | 6883.7±322.1 | 6283.4±1258.0 | 6669.4±1398.2 | 6320.5±1499.6 | 5634.8±1387.9 | * | ns | ns |
| Volatile acid | | | | | | | | | | | | |
| Hexanoic acid | 446.7±73.1 | 510.5±19.8 | 507.5±151.4 | 413.9±48.6 | 454.9±61.8 | 460.2±59.3 | 432.4±122.7 | 591.2±74.5 | 529.8±112.1 | ns | ns | ns |
| Octanoic acid | 1975.6±43.6 | 2235.9±260.8 | 1982.3±635.1 | 1821.9±165.9 | 1550.8±384.9 | 1749.9±487.7 | 1742.6±677.8 | 2305.7±364.3 | 2053.4±347.3 | ns | ns | ns |
| Decanoic acid | 491.0±239.5 | 424.4±96.2 | 322.3±107.5 | 462.8±114.9 | 283.5±67.8 | 313.4±96.3 | 366.9±95.8 | 365.9±53.8 | 317.9±73.1 | ns | ns | ns |
| Dodecanoic acid | 15.2±4.2 | 7.7±2.1 | 7.4±2.6 | 9.0±7.1 | 5.9±1.5 | 6.2±0.8 | 8.5±0.4 | 8.1±1.3 | 7.7±4.1 | ns | ns | ns |
| Esters | | | | | | | | | | | | |
| Ethyl acetate | 549.7±18.4 | 492.2±35.2 | 544.7±138.7 | 552.8±43.7 | 665.7±207.6 | 576.1±50.1 | 509.9±26.4 | 551.2±63.5 | 538.2±63.8 | ns | ns | ns |
| Isobutyl acetate | 10.7±3.7 | 9.0±2.4 | 10.9±4.5 | -- | 11.1±4.5 | 11.6±3.1 | 8.5±0.5 | 15.2±2.1 | 11.7±1.3 | ns | ns | ns |
| Isoamyl acetate | 680.2±179.6 | 729.2±70.3 | 1010.8±478.9 | 625.0±326.3 | 910.1±712 | 1320.1±449.3 | 1127.0±481.6 | 1762.2±403.3 | 1204.4±107.9 | ns | * | ns |
| Hexyl acetate | 47.7±10.4 | 58.0±8.1 | 66.2±35.3 | 50.5±10.7 | 67.8±30.2 | 74.9±22.6 | 73.3±23.8 | 106.4±45.7 | 59.3±21.8 | ns | ns | ns |
| Phenethyl acetate | 65.1±26.4 | 94.3±30.0 | 54.5±25.7 | 72.3±39.3 | 66.4±49.1 | 78.9±68.5 | 121.5±57.0 | 179.1±81.2 | 135.6±69.9 | ns | * | ns |
| Ethyl propanoate | -- | 24.9±5.0 | -- | -- | -- | -- | -- | 18.0±3.3 | -- | ns | ns | ns |
| Ethyl butanoate | 94.5±24.9 | 109.3±17.7 | 127.2±24.6 | 87.8±29.5 | 119.1±65.8 | 133.2±30.7 | 118.5±23.9 | 149.5±16.3 | 131.9±18.5 | ns | ns | ns |
| Ethyl hexanoate | 901.1±249.1 | 1086.7±230.3 | 1151.2±485.2 | 829.9±315.0 | 1247.5±803.3 | 1382.7±563.8 | 1410.4±239.9 | 1624.7±453.6 | 1247.8±403.5 | ns | ns | ns |
| Ethyl heptanoate | 4.6±1.0 | 6.1±1.5 | 6.2±1.6 | 4.7±0.5 | 20.2±17.5 | 6.6±0.3 | 5.9±2.4 | 8.0±2.0 | 7.3±4.4 | ns | ns | ns |
| Ethyl 2-hexenoate | 8.9±4.2 | 7.2±1.6 | 7.1±2.0 | 8.5±2.3 | 15.2±10.3 | 8.1±2.6 | 10.9±3.8 | 8.9±4.6 | 10.5±4.3 | ns | ns | ns |
| Ethyl octanoate | 2471.5±1451.4 | 3903.6±1876.3 | 3966.1±2449.4 | 1544.7±571.5 | 4138.0±3675.2 | 4160.1±2626.9 | 3066.4±1261.3 | 5089.1±3230.8 | 3353.5±3018.0 | ns | ns | ns |
| Ethyl β-hydroxybutyrate | 3.9±0.1 | 4.4±0.5 | 4.5±0.3 | 3.7±0.4 | 4.1±0.9 | 4.6±0.7 | 4.4±0.2 | 4.4±0.2 | 4.4±0.7 | * | ns | ns |
| Ethyl nonanoate | 5.7±2.7 | 12.4±5.3 | 7.3±3.9 | 3.8±0.3 | 11.4±7.4 | 6.6±1.7 | 5.8±0.9 | 9.1±4.5 | 7.0±3.6 | * | ns | ns |
| Ethyl decanoater | 1634.7±842.3 | 2346.1±457.6 | 1924.8±948.2 | 1302.4±146.8 | 2427.4±2006.7 | 1882.8±672.9 | 1526.0±814.7 | 2124.8±1481.2 | 1426.8±1106.8 | ns | ns | ns |
| Diethyl butanedioate | 26.4±2.3 | 34.5±5.6 | 30.6±6.3 | 25.7±2.1 | 45.9±32.2 | 26.9±3.1 | 25.3±2.7 | 30.4±3.7 | 27.2±3.6 | ns | ns | ns |
| Ethyl 9-decenoate | 105.2±68.5 | 280.3±172.9 | 269.7±120.2 | 75.7±36.7 | 425.4±328.4 | 225.6±165.7 | 184.0±125.2 | 361.1±157.3 | 209.3±118.5 | ns | ns | ns |
| Ethyl dodecanoate | 326.1±133.5 | 450.6±211.2 | 347.9±256.5 | 361.2±78.9 | 472.2±235.7 | 323.7±123.9 | 375.7±99.9 | 283.0±153.3 | 255.2±66.7 | ns | ns | ns |
| Ethyl benzenepropanoate | -- | 8.6±3.9 | -- | -- | 12.0±1.7 | -- | -- | 9.5±4.3 | 14.2±1.4 | ** | ns | ns |
| Ethyl tridecanoate | 20.0±1.7 | 26.8±8.3 | 12.0±2.5 | 33.4±11.1 | 22.0±8.0 | 11.6±5.8 | 18.1±5.1 | 17.0±11.7 | 32.3±7.5 | ns | ns | ** |
| Ethyl myristate | 41.9±8.4 | 25.1±6.9 | 34.7±15.3 | 39.3±2.1 | 30.1±7.6 | 23.2±12.3 | 36.4±12.7 | 20.7±2.8 | 36.1±22.8 | ns | ns | ns |
| Ethyl hexadecanoate | 48.2±10.2 | 36.0±7.5 | 43.5±19.2 | 62.0±20.5 | 40.5±8.6 | 50.7±10.4 | 47.0±18.4 | 27.1±4.7 | 38.0±12.0 | * | ns | ns |
| Methyl heptanoate | -- | 9.0±0.6 | -- | -- | -- | -- | -- | 9.3±3.6 | 8.4±1.5 | ** | ** | ** |
| Isoamyl butanoate | 27.1±8.2 | 32.5±3.4 | 39.3±12.5 | 35.3±9.4 | 23.0±19.7 | 29.6±12.1 | 36.1±22.0 | 16.3±9.6 | 33.5±5.4 | ns | ns | ns |
| Methyl octanoate | 10.7±3.5 | 14.3±5.0 | 15.3±8.0 | 8.3±2.1 | 15.4±9.0 | 14.3±6.3 | 13.3±1.9 | 20.2±8.8 | 14.4±6.3 | ns | ns | ns |
| Isopentyl hexanoate | 66.1±29.3 | 64.6±13.0 | 52.0±19.9 | 67.6±10.6 | 69.7±31.9 | 54.1±10.5 | 80.3±36.9 | 69.7±17.6 | 46.0±29.0 | ns | ns | ns |
| Isoamyl caprylate | 30.0±9.3 | 45.4±19.5 | 38.0±13.2 | 22.1±3.7 | 51.3±39.1 | 35.0±10.3 | 26.4±10.3 | 44.8±25.9 | 29.7±15.0 | ns | ns | ns |
| Isoamyl decanoate | 41.8±6.7 | 56.9±19.1 | 47.8±20.0 | 59.0±13.2 | 78.4±16.7 | 47.5±6.8 | 50.6±1.2 | 41.1±35.6 | 41.9±7.2 | ns | ns | ns |
| Terpenes-C13 | | | | | | | | | | | | |
| Limonene | 7.8±3.1 | 6.6±2.5 | 8.1±2.9 | 7.9±2.4 | 9.5±3.3 | 7.0±2.4 | 10.1±2.0 | 6.7±1.9 | 6.8±0.6 | ns | ns | ns |
| Linalool | 58.1±14.6 | 153.4±81.6 | 76.9±21.1 | 42.0±10.9 | 24.9±17.5 | 51.1±45.9 | 28.9±7.4 | 22.7±14.0 | 44.0±19.3 | ns | ns | ns |
| α-Terpinol | 12.5±5.3 | 75.3±13.1 | 13.9±2.5 | 6.5±1.3 | 6.2±4.0 | 12.0±9.1 | 5.3±0.2 | 5.4±0.0 | 7.4±3.2 | ns | ns | ns |
| Citronellol | 40.5±9.0 | 49.6±8.2 | 42.2±15.1 | 36.7±12.8 | 40.0±19.9 | 25.8±9.1 | 24.0±4.5 | 27.4±5.7 | 35.4±16.1 | ns | * | ns |
| β-Damascenone | 42.2±13.8 | 33.0±2.4 | 25.2±2.1 | 34.3±7.1 | 25.6±9.7 | 26.5±5.9 | 25.7±6.6 | 30.0±5.0 | 16.1±14.0 | * | ns | ns |

Note: Value: mean±standard deviation. "--": Not detected. Significance: irrigation, fertigation and their interaction effects were expressed as ns=not significant; * and ** indicates at $p<0.05$ and $p<0.01$, respectively.

as fertigation increased, while terpenes decreased with increases in fertigation. These changes could explain why the fertilizer and water treatments each had a distinct effect on the volatile aroma compounds in wines, among which the HFMW-treated wine had the highest concentration of volatile compounds out of all treatments.

#### 3.4.3 Effects of fertilizer and water regulation on sensory characteristics in wines

The PCA of volatile substances with OAV greater than 1 in wines showed that the aroma activity value of volatile substances was closely related to fertilizer and water regulation and exhibited obvious separation (Figure 4). The first two principal components accounted for 71.94% of the total variance of the dataset, with PC1 and PC2 explaining 50% and 21.94% of the variance, respectively. The wine from the HFMW treatment had positive PC1 values, because it had higher concentrations of hexyl acetate, ethyl hexanoate, and isoamyl acetate, which are correlated with higher fruity aroma intensity in wine. In contrast, wine from the MFLW treatment had negative PC1 and PC2 values, which was related to its higher concentrations of phenylethyl alcohol, which imparts aromas of rose and honey[49]. However, wine from the LFMW treatment had a positive PC2 value, which was related to the high concentrations of caprylic acid and ethyl caprate, which impart sour and cheesy tastes to wine, thus affecting its quality[8].

### 3.5 Relationship between amino acids and volatile compounds

In this experiment, the correlation between the contents of amino acids in grape berries and the main volatile components in wines were studied and are displayed in the form of a heatmap in Figure 5. There was a linear correlation between the amino acid content in grape berries and the volatile compounds in the fermented wines. The concentrations of most amino acids in grape berries were positively correlated with concentrations of (E)-3-hexen-1-ol and caproic acid, but negatively correlated with the concentrations of phenethyl alcohol and ethyl laurate.

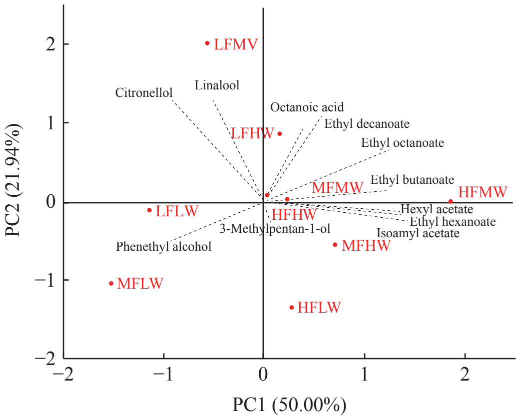
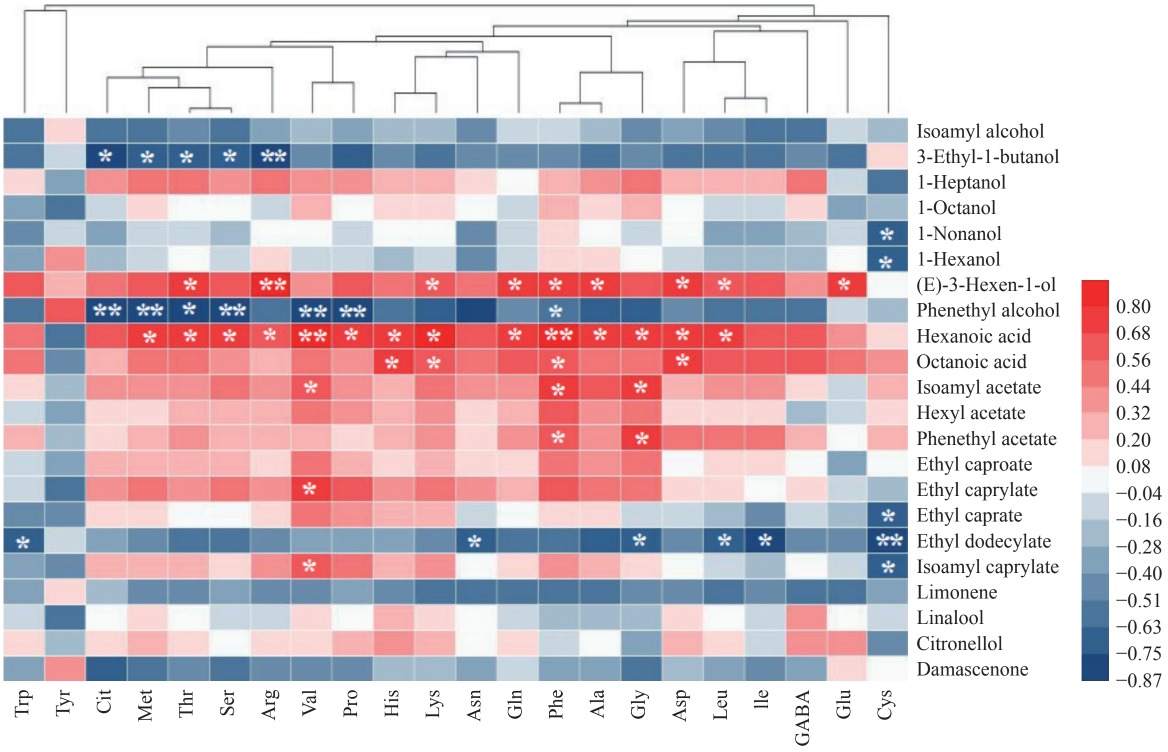


Figure 4 PCA in Cabernet Sauvignon: biplot analysis of the first two components for the treatments of fertilizer and water and the volatile compounds with an odor activity value greater than 1



Note: * denotes a significant difference ($p<0.05$); ** denotes an extremely significant difference ($p<0.01$)

Figure 5 Heat map of the correlation between amino acids in grape juices and aroma in wines

There was a positive correlation between the concentration of esters in wines and the concentrations of amino acids, including phenylalanine, glycine, and valine, in grape berries[4,22]. These compounds contribute fruity aromas and flavors to wines[53], so the concentration of amino acids may affect the organoleptic properties of wines. In the current study, there were distinct differences in the concentrations of acetate and higher acid ethyl ester in the wines under different fertigation and irrigation treatments. Therefore, the fruit aroma and organoleptic properties in wines can be regulated by appropriate fertilizer and water management.

## 4 Conclusions

Fertilizer and water management technology is a common method to meet the water and nutrient requirements of plants. The present work provides data that elucidated the effects of fertilizer and water on the amino acid profiles, volatile components, and sensory characteristics in grape berries and the resulting wines. Fertilizer had significant effects on the weight, volume, and TSS in fruits as well as the alcohol content in wines. Increased irrigation significantly promoted the volume and titratable acid in grape berries and reduced volatile acid content in wines. The effects of irrigation on amino acids in wines and grapes were different. Water significantly impacted histidine, methionine, lysine, and serine concentrations in grape berries, but arginine, asparagine, cysteine, and proline concentrations in wines. Fertilizer and water affected the concentrations of higher alcohols, ethyl esters, and terpenoids in wines, and it was found that the multi-amino acids in grape berries were positively correlated with concentrations of (E)-3-hexen-1-ol and caproic acid, but negatively correlated with concentrations of phenethyl alcohol and ethyl laurate. In addition, the wine from the HFMW treatment was of good overall quality, with abundant aromatic substances and prominent fruit fragrance. This research provides a foundation for improving water and fertilizer use efficiency and quality of grapes and wines.

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