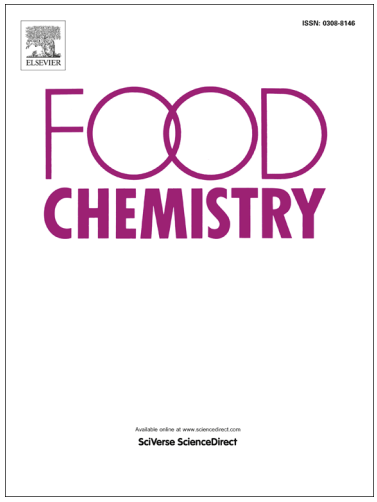

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Aroma modulation of Cabernet Gernischt dry red wine by optimal enzyme treatment strategy in winemaking

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Abstract Cabernet Gernischt (CG) is a famous Chinese wine grape cultivar, the red wine of which is known for its green trait, especially when produced from grapes cultivated in regions with monsoon climate. To modify CG wine aroma, three enzyme preparations (*H. uvarum* extracellular enzyme, AR2000, and pectinase) were introduced in different winemaking stages with *Saccharomyces cerevisiae*. Free and bound aroma compounds in young wines were detected using headspace solid-phase micro-extraction and gas chromatography-mass spectrometry, and aroma characteristics were quantified by trained panelists. Results showed that simultaneous inoculation of enzymes and yeasts improved wine aroma. Partial least-squares regression revealed that the green trait was due mainly to varietal compounds, especially C_6 compounds, and could be partly weakened by fermentative compounds. Moreover, *H. uvarum* enzyme treatments enriched the acid fruit note of CG wine by enhancing the synergistic effect of varietal volatiles and certain fermentative compounds, such as esters and phenylethyls.

Keywords: Cabernet Gernischt, enzyme treatment, *Hanseniaspora uvarum*, wine aroma

enhancement, partial least-squares regression

Chemical compounds studied in this article

1-Hexanol (PubChem CID: 8103); α-Terpinene (PubChem CID: 7462); β-Damascenone (PubChem CID: 5366074); Ethyl acetate (PubChem CID: 8857); Phenylethyl acetate (PubChem CID: 7654).

1. Introduction

Cabernet Gernischt (*Vitis vinifera* L. cv.), also called "Shelongzhu" in Chinese, is a mutation of the Cabernet grape, and is one of the most important red wine cultivars in China (Fan, Xu, Jiang, & Li, 2010). The dry red wines made from CG are popular with customers because of their attractive color and taste, whereas CG wines also exhibit a green note, which is seldom appreciated by consumers who generally prefer wines with fruity aroma. Especially for viticulture in wet regions, grapes are often harvested early because of rainy weather during the maturation time, which enhances the green trait of the resulting wines. To meet the increasing demand for CG wines, methods of improving the aromatic profile of CG wine is now a major research interest in the Chinese wine industry.

Mixed fermentation and flavor enzyme treatment are the prevailing approaches of bio-based processes for modulating the fruity aroma of wine. Many studies have investigated the effect of specific non-*Saccharomyces* yeasts on wine aroma by using them as a co-inoculum or sequential inoculum with *Saccharomyces* yeasts. Mixed fermentation produces higher amounts of volatile components, leading to stronger aroma intensity and complexity than pure fermentation (Renault, Coulon, Revel, Barbe, & Bely, 2015; Ciani & Comitini, 2015). However, the inoculum amount of the non-*Saccharomyces* yeast in mixed fermentation has to be controlled as it may interfere with wine flavor when inoculated at

higher ratios (Wang, Li, Marta, Niamat, Sun, & Tao, 2017).

Apart from mixed fermentation, application of exogenous enzymes in winemaking has recently attracted attention. Commercial enzyme preparations from *Aspergillus niger* and *Trichoderma harzianum* have been widely used for aroma release, color extraction, must clarification, and filtration in enological practice (Fia, Canuti, & Rosi, 2014). Previous studies showed that these enzymes can positively affect wine aroma by increasing aromatic compound concentration (Vázquez, Pérez, & Cabezudo, 2002; Cabaroglu, Selli, & Canbas, 2003; Palomo, Hidalgo, González-Viñas, & Pérez-Coello, 2005). Glycosidases especially enhance the aroma typicality and style by hydrolyzing odorless aroma precursors into volatile varietal compounds, such as terpenes and C_{13}-norisoprenoids (Robinson, Boss, Solomon, Trengove, Heymann, & Ebeler, 2014). However, reports showed that certain glycosidase preparations of fungal origin are a mixture of non-specific enzymes, which may trigger collateral hydrolysis reactions or form undesirable flavors (Villena, Iranzo, & Perez, 2007; Hernandez-Orte, Cersosimo, Loscos, Cacho, Garcia-Moruno, & Ferreira, 2009). In addition to the use of fungal glycosidase, recent studies have focused on screening, enzyme properties, and aroma release of glycosidases from non-*Saccharomyces* yeasts (González-Pombo, Fariña, Carrau, Batista-Viera, & Brena, 2011; Wang, Zhang, Li, & Xu, 2013; López, Mateo, & Maicas, 2015). Nevertheless, the influence of enzyme treatment strategy on wine aroma has rarely been reported.

In our previous study, we identified a novel glycosidase from an indigenous *H. uvarum* isolate, which had considerable potential for wine aroma enhancement (Hu, Qin, Tao, Zhu, Peng, & Ullah, 2016a). It is well-documented that high sugar and ethanol content inhibit glycosidase activity (Madrigal et al., 2013), and it is well-known that the sugar concentration decreases, whereas ethanol level increases during fermentation. This study attempted to enhance fruity aroma of CG wine by addition of *H. uvarum* glycosidase at three stages of the

enological process and compared it to wines produced using two commercial enzyme preparations and fermentation without enzyme addition. To illustrate the modulation effect, free and bound aroma compounds in CG wines were analyzed, and aroma characteristics were quantified by trained panelists. Further, the mathematical relationship between representative aroma traits and volatile compounds was built using partial least squares regression to highlight the main aroma contributors of wines.

2. Material and methods

2.1. Commercial yeast and enzyme preparations

Saccharomyces cerevisiae (Actiflore F33) was purchased from Laffort Co. (Bordeaux, France). Three commercial enzyme preparations, AR2000 (DMS Food Specialties B.V., Delft, The Netherlands) containing 0.25 U/mg *β*-D-glucosidase activity, macerating pectinase (Optizym®, Laffort Co. Bordeaux, France) containing 0.276 U/mg *β*-D-glucosidase activity, and *β*-D-glucosidase (G4511, Sigma-Aldrich, Beijing, China; 40 U/mg) were used.

2.2. Chemical standards

p-Nitrophenyl-*β*-D-glucopyranoside (98%), *p*-nitrophenyl-*α*-L-arabinofuranoside (98%), *p*-nitrophenyl-*α*-L-rhamnopyranoside (98%), *p*-nitrophenyl-*β*-D-galactopyranoside (98%), and *p*-nitrophenyl-*β*-D-xylopyranoside (98%) were obtained from Yuan Ye Bio-Technology Co. Ltd. (Shanghai, China). *p*-Nitrophenyl hexanoate (C_6, 98%) was purchased from Sigma-Aldrich (Shanghai, China).

Forty-two chromatographically pure standards (98%, Sigma-Aldrich, Shanghai, China), such as 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, nerolidol, *β*-damascenone, 1-butanol, phenethyl alcohol, ethyl acetate, 2-phenylethyl acetate, hexanoic acid, and octanoic acid, were

used for identification and quantitation. Water was purified using a Milli-Q system (Millipore, Billerica, MA).

2.3. Extracellular enzyme extract from the selected strain

Extracellular enzyme was extracted from *H. uvarum*, according to Hu, Zhu, Mu, Ma, Ullah, and Tao. (2016b). The strain was incubated in fermentation medium (10 mL/L Tween 80, 10 g/L yeast extract powder, 20 g/L peptone, 20 g/L D-glucose, 3 g/L ammonium nitrate, 0.5 g/L $MgSO_4 \cdot 7H_2O$, and 4 g/L KH_2PO_4) for 72 h (28 °C, 150 rpm), after which the supernatant was obtained by centrifugation (9,338 *g*, 4 °C, 15 min). The extract was purified using finely ground $(NH_4)_2SO_4$ to 80% saturation and kept overnight; it was centrifuged at 16,200 *g*, 4 °C for 10 min and dialyzed (dialysis membrane cut off <10 kDa) in 0.1 mol/L sodium acetate buffer (pH 5.0) at 4 °C. Finally, the enzyme was concentrated 20 times (0.6 U/mL *β*-D-glucosidase activity) by PEG 20,000.

Enzyme activities of different glycosidases were measured following the method proposed by Fia et al. (2014) with some modifications. Enzyme extract (200 μL), 750 μL citric-phosphate buffer (pH 5.0), and 250 μL of 1 mmol/L corresponding glycosides were mixed together and reacted for 30 min at 40°C. Na_2CO_3 (1 mol/L, 1 mL) was immediately added to the mixture to terminate the reaction. Esterase activity was determined using the method of Pérez-Martín, Seseña, Izquierdo, and Palop (2013) with some modifications. For the assay, 1,720 μL 0.1 mol/L citrate/phosphate buffer (pH 5.0), 80 μL stock substrate solution (25 mmol/L *p*-nitrophenyl hexanoate in ethanol), and 200 μL of extracellular enzyme were mixed and incubated at 37 °C for 1 h, and terminated with 200 μL of 0.5 mol/L NaOH. The absorbance value of the released *p*-nitrophenyl (*p*-NP) was measured using an ultraviolet (UV) spectrophotometer (Agilent Technologies, Santa Clara, CA) at 400 nm. One unit (U) of enzyme activity was defined as the amount of enzyme that released 1 μmol of *p*-NP per min

under the experimental conditions.

2.4. Winemaking process and enzyme treatment

Two hundred kilograms of CG grapes, containing 193.6 g/L sugar and 7.8 g/L acid (as tartaric acid), were normally harvested from the Yuma vineyard (37°36′N, 105°21′E), Qingtongxia, Ningxia, in September 2014. As shown in the schema (Supplementary Fig. 1), the grapes were destemmed and crushed, 50 mg/L SO_2 was added, followed by maceration at 15 °C for approximately 24 h. The homogenized must was equally divided into twenty parts for different enzyme treatments (each treatment was 20 kg in duplicate), and inoculated with 200 mg/L activated yeasts to initiate fermentation. Sucrose (22 g/L) was added to obtain 12% *v/v* alcohol. During the fermentation, cap management was conducted thrice a day by pushing the skins into the juice. The fermentation was performed at 25–27 °C for 8 days until the reducing sugar level dropped below 2 g/L. After fermentation, wines were separated with skins and lees, followed by addition of 50 mg/L SO_2, and stored in clean tanks at 4 °C until analysis after 6 months.

Enzyme treatments were conducted using 0.6 mU/mL β-D-glucosidase of each enzyme preparation. More specifically, *H. uvarum* extracellular enzyme (H), AR2000 (AR) and pectinase (P) were added at three winemaking stages, namely, maceration (1), simultaneous inoculation with yeast (2), and end of fermentation (3). Pure inoculation of *S. cerevisiae* served as control (CK). Each treatment was conducted in duplicate.

2.5. Isolation and enzymatic hydrolysis of glycosidically-bound aroma compounds

Aroma glycosides were extracted using a Lichrolut EN column (Lichrolut EN; Merck, Darmstadt, Germany) according to Hu et al. (2016b), with some modifications. Clarified wine samples (100 mL) were percolated on the activated column at a flow rate of 1 mL/min, followed successively by 50 mL pure water, 8 mL pentane/dichloromethane (2/1, *v/v*), and 8

mL ethyl acetate/methanol (9/1, *v/v*) to elute the aroma compounds. The last eluate was dissolved in methanol, evaporated to dryness by N_2 gas, and reconstituted in 8 mL citrate-phosphate buffer (0.2 M, pH 5.0), followed by treatment with commercial *β*-D-glucosidase (5 U/mL) at 40 °C for 18 h to release volatile aromatic compounds.

2.6. Volatile aroma analysis

The free and released volatiles were extracted using DVB/CAR/PDMS fiber (50/30 μm film thickness, 2 cm Stableflex) assembled with a 57330-U holder (Supelco, Bellefonte PA,), followed by gas chromatography-mass spectrometry (GC-MS) according to Wang et al. (2017).

Wine samples (8 mL), 0.4 mg/L internal standard (2-octanol), and 2 g NaCl were held in the 15 mL-headspace bottle and stirred by a magnetic bar in a 40 °C water bath for 15 min. After that, the fiber was exposed for 30 min and desorbed in the gas chromatography (GC) injector at 250 °C for 8 min. The TRACE 1310 GC was coupled with an ISQ LT single quadrupole MS (Thermo Scientific, Waltham, MA) equipped with a DB-WAX capillary column (60 m × 0.25 mm × 0.25 μm; Agilent J&W, Santa Clara, CA). The GC-MS operating conditions were: splitless/split injection with 0.8 min delay; carrier gas was ultrapure helium (99.999%) at 1 mL/min flow rate; temperature program: initial temperature 40 °C increased to 130 °C at 3 °C/min, then ramped to 250 °C at 4 °C/min, and maintained at this temperature for 5 min. The temperatures of the injector, transfer line, and ion source were at 250 °C. Mass detector conditions: electron impact (EI) in positive ionization mode at 70 eV and scanning range was *m/z* 25–350 with a 0.2-s interval.

Qualitative analysis for aroma compounds was based on the comparison of retention times and mass spectra with those of authentic standards and/or those in the Wiley 275.L database (Agilent Technologies Inc.). Quantitative data for the identified compounds were obtained by

interpolation of the relative areas *versus* the internal standard area in the calibration graphs for the pure reference compounds. The calibration graphs were drawn using a 5-point method for synthetic wines containing exact volumes of the standard chemicals (12% *v/v* ethanol, 6 g/L tartaric acid, pH 3.2,). The concentration of any volatile compound for which a pure reference compound was not available was obtained using the calibration graphs for compounds with similar chemical structure.

2.7. Sensory analysis

Wine sensory analysis was performed in duplicate according to Peng et al. (2013). Aroma characteristics were analyzed by the panelists (18 females and 12 males) who were well-trained with a 54-aroma kit (Le Nez du Vin®, France) until their accuracy of identification for each trait exceeded 95%. Panelists were asked to describe wine aroma using 4-6 terms from the aroma kit, which had been classified into different groups, and grade the intensity using a five-point scale (1, very weak; 2, weak; 3, medium; 4, intense 5, very intense). On the basis of the material source, the terms were classified into several aroma groups, such as citrus (orange, lemon, and grapefruit), acid fruit (green apple, apple, and pear), sweet fruit (peach, plum, and apricot), berries (raspberry, blackberry, redcurrant, and blackcurrant), floral (rose, violet, and acacia), green (grass, green beans, green pepper), spicy (clove, licorice, pepper, cinnamon, and vanilla), smoky (smoky, tobacco, and coffee), and earthy (earth and mold).The mixture of intensity and frequency of detection was expressed as modified frequency (MF%), which was calculated using the formula:

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

where *F%* is the detection frequency of an aroma group, and *I%* is average intensity expressed as the percentage of maximum intensity.

2.8. Statistical analysis

Volatile and sensory data were compared using one-way analysis of variance (ANOVA) with Duncan test at the 95% level of confidence. Volatile data were also evaluated with two-way ANOVA. The summed data of volatiles from respective chemical categories were used as variables in principal component analysis (PCA) using a correlation matrix (SPSS 19.0 software; SPSS Inc., Chicago IL). Partial least squares regression (PLSR) was conducted using PLSR 1 with Unscrambler 9.7 (Camo, Trondheim, Norway). The PLS model used cross validation and variables were standardized. The *X*-variables were the summed concentrations of aroma volatiles with OAVs ≥ 0.1 from respective chemical categories, and the *Y*-variables were MF values of aroma traits.

3. Results and discussion

3.1. Glycosidase activities in extracellular enzymes

Stored glycosides form a significant proportion of aromatic precursors in grapes, and their release requires various enzymes that act sequentially in two steps; first, disaccharide glycosidic enzymes such as *α*-L-arabinosidase, *α*-L-rhamnosidase, and *β*-xylosidase cleave the terminal sugar unit of the glycoside; second, *β*-D-glucosidase liberates volatile odorants (Maicas, & Mateo, 2003). In the multiple glycosidase extract of *H. uvarum*, activities of both disaccharide glycosidic enzymes and *β*-D-glucosidase were detected, with *β*-D-galactosidase (30 mU/mL) and *β*-D-glucosidase (28 mU/mL) showing higher activities than others (Supplementary Fig. 2). Activities of *α*-L-rhamnosidase, *α*-L-arabinosidase, and *β*-D-xylosidase were relatively lower, ranging from 17–25 mU/mL. This glycosidase profile was similar to that reported in a previous study on commercial enzyme preparations used for wine aroma enhancement (Fia et al., 2014), indicating that the extracellular enzymes possessed good potential for application. However, glycosidase activities of different commercial preparations varied largely in enological conditions. As Fia, Olivier, Cavaglioni,

Canuti, and Zanoni (2016) reported, only 2 of 15 commercial enzyme preparations had *β*-D-glucosidase activity in commercial grape juice (1.3 and 0.5 U/g or U/mL) and both preparations were specific for the release of varietal aroma. In addition to the glycosidase activities, commercial enzyme preparations from *Aspergillus spp.* and *Trichoderma spp.* also harbored esterase activity (Fia et al., 2014). The latter was also detected in the *H. uvarum* enzyme extract (23.08 mU/mL, expressed as C_6 chain substrate specificity) in this study, indicating that its addition may affect ester profiles.

In addition to the enzyme activities in these preparations, data in Supplementary Table 1 suggested that with the exception of SO_2, enzyme treatment affected the conventional physico-chemical parameters of CG wine negligibly. Therefore, it is necessary to confirm the modulation effect of enzyme treatment on aroma profiles.

3.2. Varietal aroma compounds

Twelve free varietal compounds were quantified (Table 1) and their total content in enzyme-treated wines ranged between 5.98 mg/L and 6.72 mg/L, which was higher than that in CK (5.91 mg/L). The increase in varietal compound levels suggested that glycosidase activities contributed to their enhancement, especially for AR2, that had the highest concentration (6.72 mg/L), followed by P1 (6.38 mg/L) and H2 (6.34 mg/L). Among these compounds, C_6-compounds accounted for the majority (> 94%), followed by terpenes and C_{13}-norisoprenoids.

C_6 compounds are derived from long-chain fatty acids in grapes during berry ripening and crushing (Palomo et al., 2005; Baumes, 2009). Although the total C_6 compound content varied slightly between wines, an apparent difference in *(E)*-3-hexen-1-ol and *(Z)*-3-hexen-1-ol levels was observed. This suggested that the activity of the enzyme preparation used significantly affected certain C_6 compounds. Terpenes and C_{13}-norisoprenoids are derived

from the activities of glycosidase on grape precursors, and are responsible for the floral and fruity notes in wines (Pons, Allamy, Lavigne, Dubourdieu, & Darriet, 2017). Compounds such as α-terpinene, α-terpineol, (*E*)-geranylacetone, and β-damascenone are detected in higher concentration in most enzyme-treated wines. This observation was supported by previous results showing that exogenous enzymes increased the terpene and C_{13}-norisoprenoid levels (Cabaroglu et al., 2003; Hu et al., 2016b). Two-way ANOVA was applied to evaluate the effect of enzymes, time of addition, as well as their interactions on free varietal aroma compounds. As shown in Supplementary Table 2, enzymes, time of addition, and their interactions all differentially affected the formation of terpenes, but not C_6 compounds. In contrast, only enzymes showed significant differential modulation of C_{13}-norisoprenoid content.

Table 2 shows the volatile components derived from the aroma precursors, including C_6 compounds, terpenes, C_{13}-norisoprenoids, higher alcohols, and phenylethyls. To determine the catalytic specificity of different enzyme treatment strategies, PCA was used to analyze the varietal chemical categories and their precursors (Fig. 1). Wines from H2, AR2, and H1 were located close to free aroma compounds at the negative part of PC1. On the contrary, CK, H3, P3, P2, and P1 were more related to bound compounds at the positive part of PC1, which implied that a large amount of varietal compounds remained unreleased in these wines. Thus, we confirmed that the addition of *H. uvarum* extracellular enzyme and AR2000 at optimal time (simultaneous inoculation with yeast) could accelerate the hydrolysis of glycosidic aroma precursors and increase the concentration of varietal aroma compounds. Nevertheless, the use of pectinase negligibly enhanced varietal aromas.

3.3. Fermentative aroma compounds

Thirty-six fermentative aroma compounds were identified, including 11 higher alcohols,

15 esters, 5 fatty acids, and 5 phenylethyls (Supplementary Table 3). In contrast to the dramatic release of varietal volatiles, the total content of fermentation compounds in most enzyme-treated wines seemed unchanged compared to the control wine (275–304 mg/L), except some slight decrease in wines from H3, AR3, and P2 (255–260 mg/L).

Enhancement of higher alcohol levels may compete with fresh fruity notes and positively relate to vegetal/pepper notes in young red wines, leading to a decrease in wine quality (Aznar, López, Cacho, & Ferreira, 2003; San-Juan, Ferreira, Cacho, & Escudero, 2011; Cameleyre, Lytra, Tempere, & Barbe, 2015). Fatty acids are essential for the perception of fresh fruity notes, and their branched forms can suppress the animal note of ethylphenols (Romano, Perello, Lonvaud, Sicard, & Revel, 2009; San-Juan et al., 2011). However, excessive levels of fatty acids may generate negative aromas in wines (Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012). Esters are known as the major contributors to the fruity trait of wine (Ugliano, 2009) and are mainly categorized into acetate esters and fatty acid ethyl esters. In this study, enzyme treatments negligibly influenced total ester levels. A previous study showed that AR2000 treatment generally reduced the concentration of fermentative aroma compounds in wines (Palomo et al., 2005). In this study, almost all the enzyme-treated wines had lower concentration of higher alcohols and fatty acids than the control wine. However, the *H. uvarum* enzyme extract-induced modulation of C_3–C_5 fatty acid ethyl esters, other esters, and phenylethyls showed an upward trend in CG wines, which was similar to that observed with white wine treated with an enzyme from *Pichia fermentans* (Ma, Yan, Wang, Zhang, & Tao, 2017). The results of two-way ANOVA in supplementary Table 2 suggested that the major profiles of fermentative volatiles were significantly affected by enzymes as well as their interactions with time of addition. In contrast, higher alcohols and acetates were only influenced by time of addition.

PCA was applied for analyzing fermentative aroma compounds to determine the influence

of enzyme treatment on fermentative volatile profile. As shown in Fig. 2, all fermentative compounds were located on the positive part of PC1. Specifically, acetates, higher alcohols, and C_3–C_5 fatty acid ethyl esters were located on the positive part of PC2, whereas other compounds were on the negative part of PC2. The distribution of wine samples implied that *H. uvarum* enzyme treatments had a positive effect on fermentative compounds, such as acetates, C_3–C_5 fatty acid ethyl esters, and phenylethyls, which indicated the possibility of enhancing fruity aroma.

3.4. Correlation between aroma characteristics and volatiles

Fig. 3 shows the sensory profiles of wines obtained from different enzyme treatments, including acid fruit, sweet fruit, green, tropical fruit, floral, berry, and smoky notes (original data is shown in Supplementary Table 4). Among all the treatments, H2 wine received the highest levels of acid fruit, sweet fruit, and floral notes, followed by AR2 wine. However, the latter also exacerbated the green flavor of CG wines. This result was in agreement with that of Hu et al. (2016a), where *H. uvarum* glycosidase significantly improved floral and fruity traits while AR2000 treatment significantly enhanced the green note of wine with the improvement of fruity and floral notes. In contrast, berry note was the prominent aroma of the control wine and the three pectinase-treated wines, whereas the other traits were relatively weak, which imparted a bland wine aroma. This result indicated that the most promising treatment for enhancing fruity aroma in CG wines was simultaneous addition of *H. uvarum* extracellular enzyme and yeast.

For wines derived after *H. uvarum* enzyme and AR2000 treatments, acid fruit and green traits were the most typical flavor, respectively. Hence, PLSR was conducted to clarify the underlying correlation between the typical aroma notes and aromatic compounds with OAVs ≥ 0.1. Volatiles with coefficient > 0 or < 0 suggested that they affected aroma trait positively

or negatively. As shown in Fig. 4, varietal compounds (C_6 compounds, terpenes and C_{13}-norisoprenoids) had considerable positive contribution to both acid fruit and green notes, while fermentative compounds, such as higher alcohols, esters, and phenylethyls exerted a different effect. In terms of acid fruit note, phenylethyls (coefficient 0.486) and esters (coefficient 0.283) were synergistic with varietal volatiles, whereas higher alcohols (coefficient -0.409) had the opposite effect. In contrast, phenylethyls (coefficient -0.327) and esters (coefficient –0.159) adversely interacted with varietal volatiles and affected the green trait.

Another remarkable observation is that C_6 compounds played a critical role in both notes. Since C_6 compounds are often supposed to have a negative effect on wine quality due to their grassy and herbaceous odors (Ugliano, 2009), their positive contribution to green note (coefficient 0.420) was predictable; however, their significant positive effect on acid fruit note (coefficient 0.323) was interesting, the reason for which might be the synergistic effect mentioned above. Similarly, C_6 compounds were reported to be markers of early maturity stage Shiraz grapes, providing fresh aromas to wine derived from these grapes (Antalick, Šuklje, Blackman, Meeks, Deloire, & Schmidtke, 2015).

4. Conclusions

Enzyme treatment dramatically modulated the volatile concentration in CG wines, thereby affecting their aroma characteristics. The combined result of GC-MS and sensory analysis revealed that simultaneous inoculation with yeast was the optimal application time of the three enzyme preparations because of their better performance in aroma enhancement. PLSR models demonstrated that the green trait, the typical aroma of CG, was mainly generated by varietal compounds, while fermentative compounds, such as phenylethyls and esters, had a negative impact on the trait. The application of *H. uvarum* extracellular enzyme enhanced

fruity and floral aroma, especially the acid fruit note, by partially modulating the fermentative compound profile and facilitating the positive interaction between varietal compounds and fermentative compounds. This study provided an applicable approach for modulating wine aroma, especially for the improvement of aroma quality of CG wine. Further study for elucidating the effect of *H. uvarum* glycosidase on the taste, color, and antioxidant properties of CG wine are warranted.

Author contributions

Sun W. X. and Hu K. contributed equally to this work.

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Conflict of Interest

The authors declare no competing financial interest.

References

Antalick, G., Šuklje, K., Blackman, J. W., Meeks, C., Deloire, A., & Schmidtke, L. M. (2015). Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry, 63*, 4664–4672.

Aznar, M., López, R., Cacho, J., & Ferreira, V. (2003). Prediction of aged red wine aroma properties from aroma chemical composition. Partial least squares regression models. *Journal of Agricultural and Food Chemistry, 51*, 2700–2707.

Baumes, R. (2009). Wine Aroma Precursors. In M. V. Moreno-Arribas & M. C. Polo (Eds.) *Wine Chemistry*

and Biochemistry (251–275). New York: Springer.

Cabaroglu, T., Selli, S., & Canbas, A. (2003). Wine flavor enhancement through the use of exogenous fungal glycosidases. *Enzyme and Microbial Technology, 33*, 581–587.

Cameleyre, M., Lytra, G., Tempere, S., & Barbe, J. C. (2015). Olfactory impact of higher alcohols on red wine fruity ester aroma expression in model solution. *Journal of Agricultural and Food Chemistry, 63*, 9777–9788.

Ciani, M., & Comitini, F. (2015). Yeast interactions in multi-starter wine fermentation. *Current Opinion in Food Science, 1*, 1–6.

Culleré, L., Escudero, A., Juan Cacho, A., & Ferreira, V. (2004). Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry, 52*, 1653–1660.

Fan, W., Xu, Y., Jiang, W., & Li, J. (2010). Identification and quantification of impact aroma compounds in 4 nonfloral *Vitis vinifera* varieties grapes. *Journal of Food Science, 75*, S81–S88.

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*, 1659–1667.

Fia, G., Canuti, V., & Rosi, I. (2014). Evaluation of potential side activities of commercial enzyme preparations used in winemaking. *International Journal of Food Science and Technology, 49*, 1902–1911.

Fia, G., Olivier, V., Cavaglioni, A., Canuti, V., & Zanoni, B. (2016). Side activities of commercial enzyme preparations and their influence on the hydroxycinnamic acids, volatile compounds and nitrogenous components of white wine. *Australian Journal of Grape and Wine Research, 22*, 366–375.

González-Pombo, P., Fariña, L., Carrau, F., Batista-Viera, F., & Brena, B. M. (2011). A novel extracellular β-glucosidase from *Issatchenkia terricola*: Isolation, immobilization and application for aroma enhancement of white Muscat wine. *Process Biochemistry, 46*, 385–389.

Guth, H. (1997). Identification of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*, 3022–3026.

Hernandez-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., Garcia-Moruno, E., & Ferreira, V. (2009). Aroma development from non-floral grape precursors by wine *Lactic acid* bacteria. *Food Research International, 42*, 773–781.

Hu, K., Qin, Y., Tao, Y. S., Zhu, X. L., Peng, C. T., & Ullah, N. (2016a). Potential of glycosidase from non-*Saccharomyces* isolates for enhancement of wine aroma. *Journal of Food Science, 81*, M935–M943.

Hu, K., Zhu, X. L., Mu, H., Ma, Y., Ullah, N., & Tao, Y. S. (2016b). A novel extracellular glycosidase activity from *Rhodotorula mucilaginosa*: its application potential in wine aroma enhancement. *Letters in Applied Microbiology, 62*, 169–176.

Kheir, J., Salameh, D., Strehaiano, P., Brandam, C., & Lteif, R. (2013). Impact of volatile phenols and their precursors on wine quality and control measure of *Brettanomyces/Dekkera* yeasts. *European Food Research and Technology, 237*, 655–671.

Laurence, G., Dianehelene, S., Adeline, D., Melanie, C., & Gabriel, T. (2009). Determination of activity profiles of the main commercial enzyme preparations used in winemaking. *American Journal of Enology and Viticulture, 60*, 322–331.

López, M. C., Mateo, J. J., Maicas, S. (2015). Screening of β-glucosidase and β-xylosidase activities in four non-*Saccharomyces* yeast isolates. *Journal of Food Science, 80*, C1696–C1704.

Ma, D. C., Yan, X., Wang, Q. Q., Zhang, Y. N., & Tao, Y. S. (2017). Performance of selected *P. fermentans* and its excellular enzyme in co-inoculation with *S. cerevisiae* for wine aroma enhancement. *LWT - Food Science and Technology, 86*, 361–370.

Madrigal, T., Maicas, S., Tolosa, J. J. M. (2013). Glucose and ethanol tolerant enzymes produced by *Pichia* (*Wickerhamoces*) isolates from enological ecosystems. *American Journal of Enology and Viticulture, 64*, 126–33.

Maicas, S., & Mateo, J. J. (2003). Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. *Applied Microbiology and Biotechnology, 67*, 322–335.

Noguerol-Pato, R., González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012). Aroma profile of Garnacha Tintorera-based sweet wines by chromatographic and sensorial analyses. *Food Chemistry, 134*, 2313–2325.

Oliveira, J. M., Araújo, I. M., Pereira, Ó. M., Maia, J. S., Amaral, A. J., & Maia, M. O. (2004). Characterization and differentiation of five "Vinhos Verdes" grape varieties on the basis of monoterpenic compounds. *Analytica Chimica Acta, 513*, 269–275.

Palomo, E. S., Hidalgo, D. M., González-Viñas, M. Á., & Pérez-Coello, M. S. (2005). Aroma enhancement

in wines from different grape varieties using exogenous glycosidases. *Food Chemistry, 92*, 627–635.

Peng, C. T., Wen, Y., Tao, Y. S., & Lan, Y. Y. (2013). Modulating the formation of Meili wine aroma by prefermentative freezing process. *Journal of Agricultural and Food Chemistry, 61*, 1542–1553.

Pérez-martín, F., Seseña, S., Izquierdo, P. M., & Palop, M. L. (2013). Esterase activity of lactic acid bacteria isolated from malolactic fermentation of red wines. *International Journal of Food Microbiology, 163*, 153–158.

Pons, A., Allamy, L., Lavigne, V., Dubourdieu, D., & Darriet, P. (2017). Study of the contribution of massoia lactone to the aroma of Merlot and Cabernet Sauvignon musts and wines. *Food Chemistry, 232*, 229–236.

Renault, P., Coulon, J., Revel, G. D., Barbe, J. C., & Bely, M. (2015). Increase of fruity aroma during mixed *T. delbrueckii* / *S. cerevisiae*, wine fermentation is linked to specific esters enhancement. International Journal of Food Microbiology, 207, 40-48.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., & Ebeler, S. E. (2014). Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture, 65*, 1–24.

Romano, A., Perello, M. C., Lonvaud-Funel, A., Sicard, G., & de Revel, G. (2009). Sensory and analytical re-evaluation of ‘‘Brett character’’. *Food Chemistry, 114*, 15–19.

San-Juan, F., Ferreira, V., Cacho, J., & Escudero, A. (2011). Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of Spanish red wines can be predicted from their aroma-active chemical composition. *Journal of Agricultural and Food Chemistry, 59*, 7916–7924.

Ugliano, M. (2009). Enzymes in winemaking. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine chemistry and biochemistry* (pp. 103–126). New York: Springer.

Vázquez, L. C., Pérez-Coello, M. S., & Cabezudo, M. D. (2002). Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes. *Analytica Chimica Acta, 458*, 39–44.

Villena, M., Iranzo, J., & Perez, A. (2007). β-Glucosidase activity in wine yeasts: Application in enology. *Enzyme and Microbial Technology, 40*, 420–425.

Wang, X. C., Li, A. H., Dizy, M., Ullah, N., Sun, W. X., & Tao, Y. S. (2017). Evaluation of aroma enhancement for “Ecolly” dry white wines by mixed inoculation of selected *Rhodotorula*

*mucilaginosa*and *Saccharomyces cerevisiae*. *Food Chemistry, 228*, 550–559.

Wang, Y., Zhang, C., Li, J., & Xu, Y. (2013). Different influences of *β*-glucosidases on volatile compounds and anthocyanins of Cabernet Gernischt and possible reason. *Food Chemistry, 140*, 245–254.

FIGURE CAPTIONS

Fig. 1. Loadings of free varietal components, aroma precursors, and distribution of different enzyme-treated wines in the first two PCs. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

Fig. 2. Loadings of fermentative components and distribution of different enzyme-treated wine samples on the first two PCs. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

Fig. 3. MF% values of aroma characteristics of different enzyme-treated wine samples. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

Fig. 4. PLS regression of acid fruity (a) and green note (b) with aroma volatiles. Wines treated by *H. uvarum* extracellular enzyme (H) and AR2000 (AR) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

TABLES

Table 1 GC-MS analysis of aromatic compounds in CG wine samples obtained from different enzyme treatment strategies.

Table 2 GC-MS analysis of bound aroma compounds in CG wine samples obtained from different enzyme treatment strategies.

SUPPLEMENTARY DATA

Supplementary Fig. 1. The schema of winemaking process.

Supplementary Fig. 2. Enzyme profiles of *H. uvarum* extracellular enzyme.

Supplementary Table 1 Conventional physicochemical parameters of wine samples obtained from different enzyme treatment strategies.

Supplementary Table 2 Two-way ANOVA of the effects of enzyme types, time of addition, and their interaction on aroma compounds.

Supplementary Table 3 GC-MS analysis of fermentative compounds in wine samples obtained from different enzyme treatment strategies.

Supplementary Table 4 Modified frequency percentage of aroma characteristics of different enzyme-treated wine samples.

Table 1 GC-MS analysis of aromatic compounds in CG wine samples obtained from different enzyme treatment strategies.

RI[§]	Compounds	Concentration[†] (μg/L)										Odor Threshold (μg/L)	Odors
		CK	H1	H2	H3	AR1	AR2	AR3	P1	P2	P3		
C_6 compounds													
1392	1-Hexanol	5460 ± 224^{a}	5526 ± 282^{ab}	5835 ± 252^{ab}	5562 ± 274^{ab}	5784 ± 294^{ab}	6145 ± 292^{b}	5509 ± 278^{ab}	5872 ± 264^{ab}	5601 ± 310^{ab}	5537 ± 263^{ab}	8000[A]	Green, grassy[A]
1401	(*E*)-3-Hexen-1-ol	78 ± 4^{cd}	57 ± 4^{a}	63 ± 6^{ab}	68 ± 4^{abc}	72 ± 6^{bc}	98 ± 6^{e}	77 ± 7^{cd}	85 ± 6^{d}	73 ± 4^{bcd}	66 ± 4^{abc}	400[B]	Green, herb[C]
1415	(*Z*)-3-Hexen-1-ol	155 ± 9^{ab}	192 ± 12^{cd}	179 ± 15^{bc}	142 ± 11^{a}	168 ± 10^{abc}	211 ± 17^{d}	159 ± 9^{ab}	177 ± 9^{bc}	170 ± 16^{abc}	152 ± 12^{ab}	400[B]	Green, cypress[F]
	Subtotal	5692 ± 237^{a}	5776 ± 298^{ab}	6077 ± 272^{ab}	5771 ± 289^{ab}	6024 ± 309^{ab}	6454 ± 316^{b}	5745 ± 293^{ab}	6134 ± 278^{ab}	5844 ± 330^{ab}	5755 ± 279^{ab}		
Terpenes													
1498	Carvyl acetate	7 ± 0^{b}	11 ± 1^{d}	10 ± 1^{c}	9 ± 0^{c}	7 ± 1^{b}	7 ± 0^{b}	9 ± 1^{c}	6 ± 1^{b}	6 ± 1^{b}	4 ± 0^{a}	NF	Mint, fruity, herb[C]
1540	α-Terpinene	9 ± 1^{b}	13 ± 2^{d}	10 ± 1^{bcd}	7 ± 1^{a}	12 ± 1^{cd}	11 ± 1^{bcd}	9 ± 1^{bc}	13 ± 1^{d}	6 ± 0^{a}	9 ± 1^{b}	80[C]	Citrus, lemon[F]
1633	4-Terpinol	9 ± 1^{a}	16 ± 1^{cd}	22 ± 2^{e}	13 ± 1^{b}	16 ± 2^{bcd}	36 ± 2^{f}	15 ± 1^{bc}	18 ± 2^{d}	15 ± 1^{b}	18 ± 1^{cd}	110-400[D]	NF
1706	α-Terpineol	40 ± 2^{cd}	34 ± 2^{ab}	37 ± 3^{bc}	29 ± 1^{a}	31 ± 1^{ab}	48 ± 5^{e}	33 ± 2^{ab}	46 ± 4de	35 ± 3^{abc}	29 ± 3^{a}	250[E]	Pleasant, floral[F]
1869	(*E*)-Geranylacetone	15 ± 1^{bc}	17 ± 1^{c}	22 ± 1^{e}	20 ± 1de	10 ± 1^{a}	17 ± 2^{cd}	14 ± 1^{b}	17 ± 1^{cd}	16 ± 2^{bc}	18 ± 1^{cd}	60[A]	Floral[A]
1558	Nerolidol	5 ± 0^{a}	9 ± 0^{c}	13 ± 1^{d}	7 ± 1^{b}	6 ± 1^{a}	9 ± 1^{bc}	6 ± 0^{a}	12 ± 1^{d}	8 ± 0^{bc}	15 ± 1^{e}	700[A]	Rose, apple, citrus[A]
2037	Cedrol	18 ± 1^{a}	26 ± 2^{c}	25 ± 2^{bc}	23 ± 1^{b}	19 ± 2^{a}	25 ± 0^{bc}	24 ± 1^{bc}	26 ± 2^{bc}	17 ± 1^{a}	24 ± 1^{bc}	NF	NF
	Subtotal	104 ± 5^{a}	127 ± 8^{bc}	139 ± 9^{cd}	108 ± 6^{ab}	101 ± 8^{a}	153 ± 10^{d}	110 ± 8^{ab}	138 ± 11^{cd}	103 ± 9^{a}	117 ± 9^{ab}		
C_{13}-Norisoprenoids													
1591	Vitispirane	72 ± 3^{a}	89 ± 4^{b}	77 ± 4^{a}	73 ± 7^{a}	68 ± 3^{a}	73 ± 6^{a}	66 ± 3^{a}	73 ± 4^{a}	69 ± 5^{a}	71 ± 4^{a}	800[A]	Eucalyptus[A]
1832	*β*-Damascenone	37 ± 3^{ab}	42 ± 2^{bc}	44 ± 3^{c}	37 ± 2^{ab}	47 ± 2^{c}	43 ± 3^{bc}	47 ± 4^{c}	32 ± 3^{a}	37 ± 2^{ab}	35 ± 2^{a}	0.05[A]	Floral, honey, sweet, apple[A]
	Subtotal	109 ± 6^{a}	131 ± 6^{b}	121 ± 8^{ab}	110 ± 9^{a}	115 ± 5^{ab}	116 ± 9^{ab}	113 ± 7^{a}	105 ± 7^{a}	106 ± 7^{a}	106 ± 6^{a}		
Total		5906 ± 248^{a}	6033 ± 313^{ab}	6337 ± 290^{ab}	5989 ± 303^{ab}	6239 ± 322^{ab}	6723 ± 335^{b}	5967 ± 307^{ab}	6378 ± 283^{ab}	6053 ± 346^{ab}	5978 ± 294^{ab}		

†: Data are the mean ± SD (n = 2). Values followed by different capital letters in a row are significantly different ($p < 0.05$) by Duncan test.

CK, without enzyme treatment; H, *H. uvarum extracellular* enzyme; AR, AR2000; P, pectinase; "1", addition at maceration; "2", simultaneous inoculation with yeast; "3", addition at end of fermentation.

§: Retention indices (RI) were on a DB-WAX column.

NF: Not found. The letters following the values are references:

A: (Peng et al., 2013), thresholds were in 12% ethanol/water mixture containing 5 g/L tartaric acid at pH 3.2.

B: (Ferreira, López, & Cacho, 2000), thresholds were in a synthetic wine (11% ethanol, v/v, 7 g/L glycerine, 5 g/L tartaric acid, pH 3.4).

C: Odor thresholds and characteristics were measured in the laboratory in a 9.72 g/100 g ethanol/water mixture containing 5 g/L tartaric acid at pH 3.2.

D: (Oliveira et al., 2004.), thresholds were in an 8.10 g/100 g water/ethanol solution containing 5 g/L tartaric acid.

E: (Guth, H, 1997), thresholds were in a water/ethanol (90/10) solution.

F: (Wang et al., 2017), thresholds were measured in the laboratory in 11% ethanol/water mixture at pH 3.2.

Table 2 GC-MS analysis of bound aroma compounds in CG wine samples obtained from different enzyme treatment strategies.

RI[§]	Compounds	Concentration[†] (μg/L)									
		CK	H1	H2	H3	AR1	AR2	AR3	P1	P2	P3
C_6 compounds											
1392	1-Hexanol	1104 ± 57^{a}	1284 ± 71^{bc}	1034 ± 43^{a}	1320 ± 88^{cd}	1490 ± 98de	1307 ± 59^{bcd}	1129 ± 63^{ab}	1530 ± 83^{e}	1708 ± 102^{f}	1336 ± 79^{cd}
1401	(*E*)-3-Hexen-1-ol	42 ± 2^{b}	25 ± 1^{a}	32 ± 2^{a}	100 ± 7^{f}	73 ± 5^{d}	52 ± 3^{c}	41 ± 2^{b}	86 ± 5^{e}	83 ± 4^{e}	67 ± 3^{d}
1415	(*Z*)-3-Hexen-1-ol	118 ± 5^{b}	131 ± 6^{bc}	94 ± 7^{a}	153 ± 9^{cd}	156 ± 11^{d}	75 ± 6^{a}	146 ± 8^{cd}	190 ± 16^{e}	156 ± 8^{d}	133 ± 9^{bc}
	Subtotal	1264 ± 65^{ab}	1441 ± 78^{bc}	1160 ± 52^{a}	1573 ± 104^{cd}	1719 ± 114de	1434 ± 67^{bc}	1316 ± 72^{ab}	1805 ± 104^{ef}	1947 ± 115^{f}	1536 ± 92^{cd}
Terpenes											
1706	*α*-Terpineol	18 ± 0^{e}	15 ± 1^{d}	13 ± 1^{c}	18 ± 1^{e}	7 ± 1^{a}	6 ± 0^{a}	15 ± 0^{d}	9 ± 1^{b}	9 ± 0^{b}	9 ± 0^{b}
1786	*β*-Citronellol	0 ± 0^{a}	2 ± 0^{ab}	7 ± 1^{c}	3 ± 0^{b}	9 ± 0^{d}	10 ± 1^{d}	17 ± 0^{f}	14 ± 1^{e}	9 ± 0^{d}	13 ± 1^{e}

RI[§]	Compounds	Concentration[†] (μg/L)									
		CK	H1	H2	H3	AR1	AR2	AR3	P1	P2	P3
1814	Nerol	14 ± 0^{d}	7 ± 0^{a}	9 ± 0^{b}	12 ± 1^{c}	14 ± 0^{d}	7 ± 0^{a}	11 ± 1^{c}	8 ± 0^{ab}	8 ± 0^{b}	9 ± 0^{b}
1856	Geraniol	38 ± 2^{def}	32 ± 1^{bcd}	30 ± 1^{bc}	44 ± 4^{f}	28 ± 3^{b}	21 ± 2^{a}	35 ± 3^{cde}	32 ± 2^{bc}	39 ± 2^{ef}	33 ± 3^{bcde}
1869	(*E*)-Geranylacetone	10 ± 0^{e}	5 ± 0^{a}	6 ± 0^{b}	8 ± 0^{d}	11 ± 1^{f}	7 ± 0^{c}	8 ± 0^{d}	7 ± 0^{c}	8 ± 0^{c}	6 ± 0^{b}
	Subtotal	80 ± 3de	61 ± 3^{b}	65 ± 4^{bc}	84 ± 7^{e}	69 ± 6^{bc}	52 ± 2^{a}	87 ± 4^{e}	70 ± 4^{bc}	74 ± 2^{cd}	70 ± 4^{bc}
C_{13}-Norisoprenoids											
1832	*β*-Damascenone	75 ± 3^{d}	49 ± 3^{c}	57 ± 4^{c}	56 ± 5^{c}	55 ± 3^{c}	24 ± 1^{a}	34 ± 3^{b}	53 ± 3^{c}	57 ± 3^{c}	51 ± 3^{c}
	Subtotal	75 ± 3^{d}	49 ± 3^{c}	57 ± 4^{c}	56 ± 5^{c}	55 ± 3^{c}	24 ± 1^{a}	34 ± 3^{b}	53 ± 3^{c}	57 ± 3^{c}	51 ± 3^{c}
Higher alcohols											
1165	1-Butanol	92 ± 8^{abc}	81 ± 5^{a}	88 ± 5^{ab}	91 ± 5^{abc}	85 ± 4^{ab}	91 ± 4^{abc}	102 ± 4^{cde}	97 ± 4^{bcd}	113 ± 5^{e}	105 ± 6de
1273	1-Pentanol	49 ± 2^{e}	16 ± 1^{a}	25 ± 3^{b}	54 ± 4^{e}	34 ± 3^{c}	35 ± 3^{cd}	23 ± 3^{b}	41 ± 3^{d}	32 ± 3^{c}	53 ± 3^{e}
1449	1-Octen-3-ol	6 ± 0^{h}	5 ± 0^{e}	4 ± 0^{b}	6 ± 0^{g}	7 ± 0^{i}	3 ± 0^{a}	5 ± 0^{d}	5 ± 0^{c}	5 ± 0^{f}	4 ± 0^{b}
1450	1-Heptanol	281 ± 13^{d}	271 ± 21^{cd}	227 ± 14^{ab}	275 ± 15^{d}	226 ± 17^{ab}	198 ± 12^{a}	248 ± 13^{bcd}	251 ± 15^{bcd}	273 ± 15^{cd}	237 ± 13^{bc}
1676	1-Nonanol	27 ± 1^{e}	22 ± 1^{c}	23 ± 1^{cd}	26 ± 1de	23 ± 2^{cd}	17 ± 0^{b}	13 ± 1^{a}	24 ± 2^{cd}	23 ± 1^{cd}	21 ± 1^{c}
	Subtotal	456 ± 24^{e}	395 ± 29^{abcd}	367 ± 23^{ab}	451 ± 25de	375 ± 26^{ab}	345 ± 19^{a}	390 ± 21^{abc}	418 ± 23^{bcde}	446 ± 21^{cde}	419 ± 23^{bcde}
Phenylethyls											
1555	Benzaldehyde	1050 ± 77^{bcd}	793 ± 43^{a}	1066 ± 85^{bcd}	1137 ± 73^{cd}	963 ± 50^{abc}	920 ± 52^{ab}	1457 ± 79^{e}	1669 ± 85^{f}	2042 ± 145^{g}	1182 ± 73^{d}
1896	Benzyl alcohol	267 ± 12^{b}	167 ± 9^{a}	252 ± 14^{b}	285 ± 21^{bc}	234 ± 21^{b}	270 ± 18^{b}	321 ± 30^{cd}	441 ± 32^{e}	365 ± 28^{d}	286 ± 16^{bc}
1931	Phenethyl alcohol	4103 ± 164^{a}	3413 ± 143^{b}	3259 ± 157^{b}	3205 ± 162^{b}	3314 ± 199^{b}	2090 ± 118^{a}	2259 ± 153^{a}	4769 ± 239^{d}	3978 ± 290^{c}	3013 ± 159^{b}
	Subtotal	5420 ± 253^{c}	4372 ± 194^{b}	4576 ± 257^{b}	4627 ± 255^{b}	4511 ± 270^{b}	3279 ± 187^{a}	4037 ± 202^{b}	6879 ± 356^{d}	6384 ± 462^{d}	4481 ± 248^{b}
Total		7295 ± 348^{c}	6318 ± 307^{b}	6224 ± 340^{b}	6792 ± 396^{bc}	6728 ± 419^{bc}	5133 ± 277^{a}	5864 ± 303^{ab}	9224 ± 491^{d}	8908 ± 603^{d}	6557 ± 370^{bc}

†: Data are the mean ± SD (n = 2). Values followed by different capital letters in a row are significantly different ($p < 0.05$) by Duncan test.

CK, without enzyme treatment; H, *H. uvarum extracellular* enzyme; AR, AR2000; P, pectinase; “1”, addition at maceration; “2”, simultaneous inoculation with yeast; “3”, addition at end of fermentation.

§: Retention indices (RI) were on a DB-WAX column.

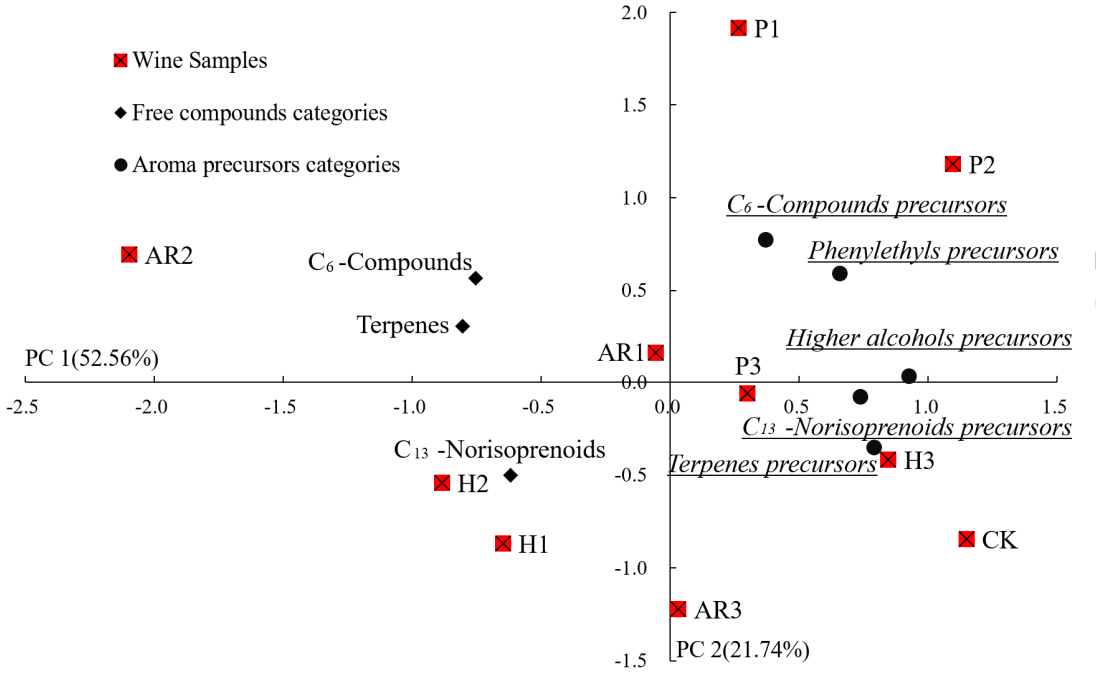


Fig. 1. Loadings of free varietal components, aroma precursors, and distribution of different enzyme-treated wines in the first two PCs. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

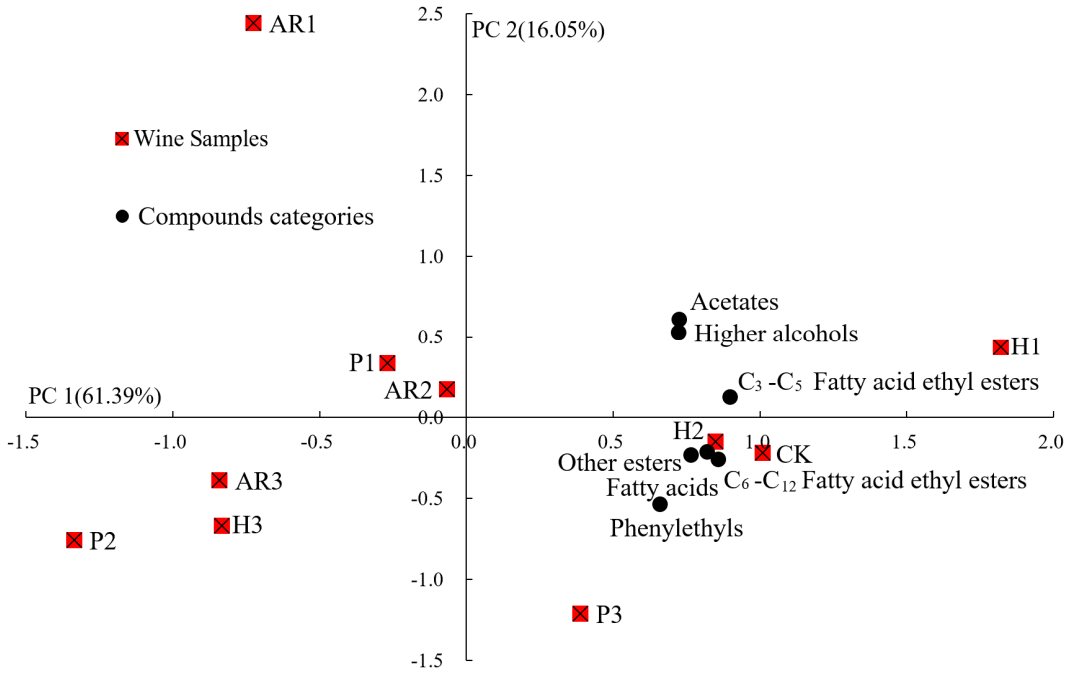
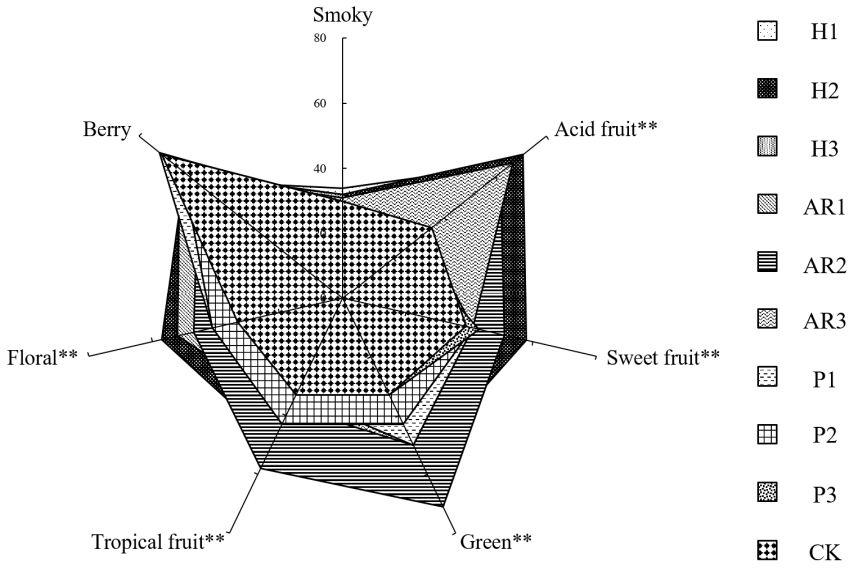


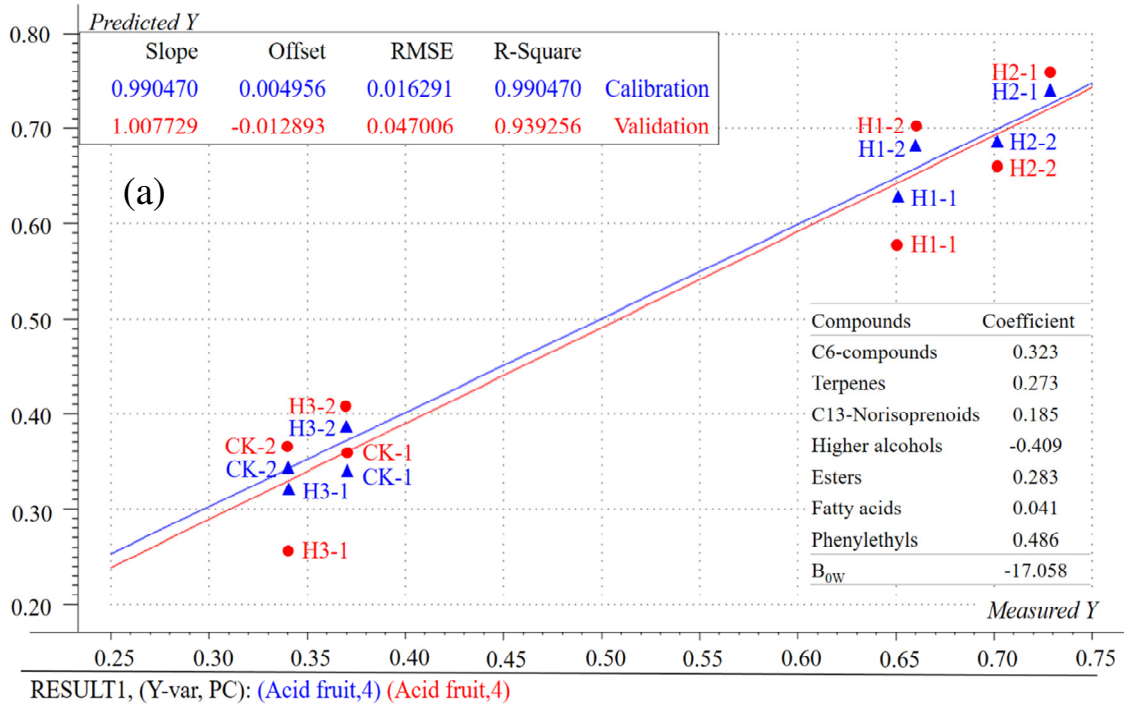
Fig. 2. Loadings of fermentative components and distribution of different enzyme-treated wine samples on the first two PCs. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P)

at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.



** Significantly different at 99% confidence level between wine samples.

Fig. 3. Modified frequency percentage of aroma characteristics of different enzyme-treated wine samples. Wines treated by *H. uvarum* extracellular enzyme (H), AR2000 (AR), and pectinase (P) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.



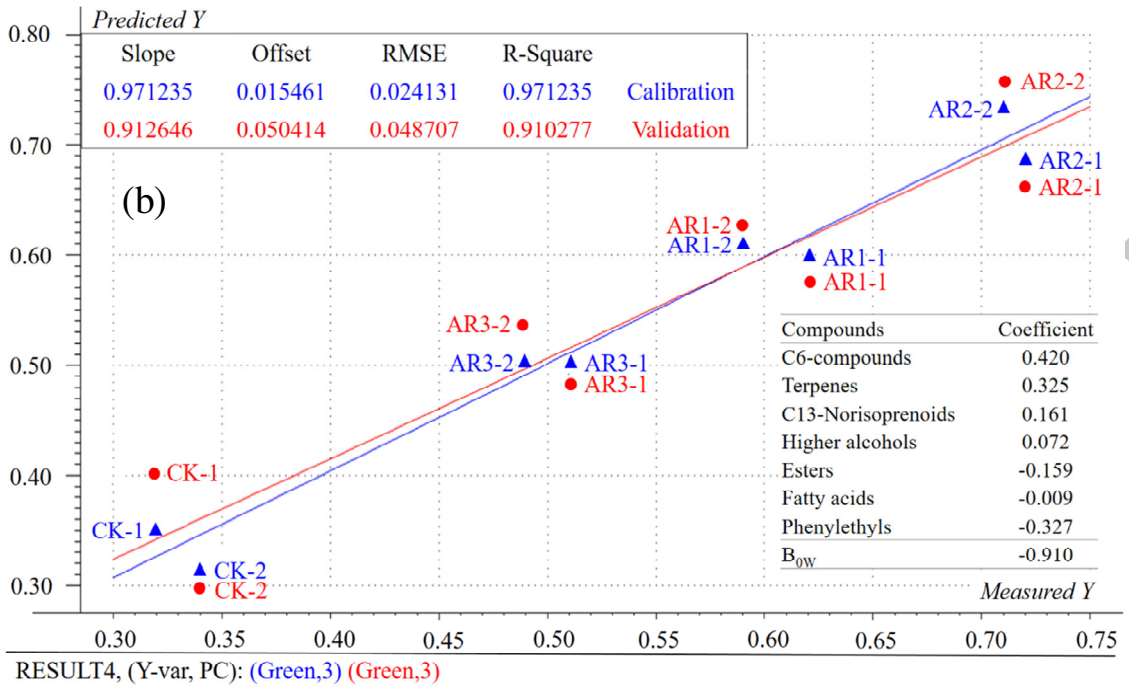


Fig. 4. PLS regression of acid fruit note (a) and green note (b) with aroma volatiles. Wines treated by *H. uvarum* extracellular enzyme (H) and AR2000 (AR) at maceration (1), yeast inoculation (2), and end of fermentation (3). CK was the wine without enzyme treatment.

Highlights

- A novel *H. uvarum* extracellular enzyme was used to modulate aroma of CG wine.
- Green trait of CG dry red wine was mainly given by varietal volatiles.
- Acid fruit flavor was improved by the synergistic effect of varietal and fermentative volatiles.