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Characterization of wine astringency contributed by acetaldehyde-mediated condensation between flavan-3-ols and grape skin/seeds polyphenol

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Abstract

Since the grape skin and seed contain different phenolic substances, the proportion of the skin and seed will affect the wine mouthfeel during the wine making process. During the wine ageing, the interaction between flavanols with anthocyanins is an important reaction and considered linked to astringency. Hence, a major question is whether changing the skin-seed ratio will affect the polymerization of phenolic substances in wine, thus affecting the astringency of wine. To answer this, astringency of wine was studied by changing the content of grape skins and seeds by SDS-PAGE, fluorescence quenching and sensory taste. Besides, acetaldehyde-mediated condensation reaction between different flavan-3-ols and malvidin-3-glucoside were studied in model solution.

The result showed that grape skins and seeds contribute differently to astringency sensation, mainly because of the difference in the type and content of flavanols and anthocyanins contained in them. In addition, acetaldehyde-induced flavan-3-ols: anthocyanin mixture presents a strong effect toward the interaction with BSA when compared to other phenolic combinations. Furthermore, it was observed that the protein-binding ability of the polyphenols in red wine has a strong correlation with the wine astringency intensity.

Keywords: skin; seed; astringency; flavan-3-ols; malvidin-3-glucoside; acetaldehyde.

Practical Application: Astringency is one of the most important and complex mouthfeel and quality attributes of red wine. This study has important value for improving the mouthfeel and quality of red wine.

1 Introduction

Astringency is one of the most important and complex mouthfeel and quality attributes of red wine. Condensed tannins (proanthocyanidins; flavan-3-ol oligomers and polymers), extracted from grape seeds and skins during wine making, were widely accepted as the major contributor to astringency (Harbertson et al., 2014; Sun et al., 2013; García-Estévez et al., 2017b). However, knowledge about grape skins and seeds is not sufficient to explain the mechanism of astringency in wine production. In particular, the degree of ripeness of polyphenols in some producing areas is not enough, which makes the wine taste defective (Ferrer-Gallego et al., 2010; Montealegre et al., 2006). The seeds mainly contain (+)-catechin, (-)-epicatechin, epicatechin gallate and other flavan-3-ols, while grape skins mainly provide anthocyanins and many other important proanthocyanidins like procyanidins and prodelphinidins for wine production (McRae & Kennedy, 2011; Chira et al., 2011). In addition, the skin also contains EGC that is not contained in seed, while the ECG content in skin is significantly less than that in seed. Grape skin tannins consist of long polymeric chains ranging from 3 to 83 flavanol subunits (degree of polymerization, DP), while the size of seed tannins has been reported as between DP 2 to 17 (González-Manzano et al., 2004). It is generally accepted that seed proanthocyanidins are more astringent than skin proanthocyanidins because they have a greater degree of galloylation (Vidal et al., 2004). Due to the difference in polyphenols contained in grape skins and seeds, wine astringency is governed by the extraction of skin and seed phenolic substances in optimal proportions.

García-Estévez et al. (2017a) suggested that, along with tannins, other polyphenols present in red wine, namely pyranoanthocyanins, could actively contribute to red wine global astringency. In fact, anthocyanins extracted from the skin are not only directed at the color of the wine, but also the potential effects on the wine astringency. Ma et al. (2014) indicated anthocyanin is usually incorporated as a terminal unit of the pigmented polymer in condensation reactions of wine to make the shorter tannin chains and hold back further polymerization, benefiting a better astringency experience in the aged wines. Several studies suggested that condensation reactions

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between anthocyanins and flavan-3-ols to form more stable pigments were believed to be main reason for the transform in color during red wine ageing (Cheynier et al., 2006; Liu et al., 2019). There are two confirmed mechanisms of the formation of new anthocyanin-flavan-3-ols aggregates (Dueñas et al., 2006; He et al., 2012; Santos-Buelga et al., 1999). One is the direct condensation reaction between anthocyanins and flavan-3-ols through the formation of a vinyl-flavan-3-ols intermediate or an interflavan bond, which produces the direct condensation products, shch as F-A^+ (Salas et al., 2003; Sánchez-Ilárduya et al., 2014). The other is the indirect condensation reaction through nucleophilic addition of the flavan-3-ols to a protonated acetaldehyde, resulting in a new carboca-tion intermediate, which undergoes nucleophilic addition of anthocyanins in the form of hemiketals and eventually produces ethyl-linked F-Et-A adducts (Es-Safi et al., 1999a; Sánchez-Ilárduya et al., 2014). Besides, the indirect condensation reaction is fast and important in red wine (Lee et al., 2004). Acetaldehyde, produced by yeast metabolism or ethanol oxidation, can improve the condensation reaction rate of anthocyanins and flavan-3-ols (Es-Safi et al., 1999b). The condensation reaction between anthocyanins (i.e., malvidin-3-glucoside, cyanidin-3-glucoside, and peonidin-3-glucoside) and (–)-epicatechin, mediated by acetaldehyde, was conducted in model wine solutions to obtain six ethyl-linked anthocyanin-flavanol pigments, and their chemical structures were identified (Li et al., 2018). As mentioned earlier, the reaction mechanism of various phenols in wine has been thoroughly studied, and previous studies about those reactions mainly focused on the wine color, with few on astringency. Liu et al. (2019) demonstrated the reaction of the five primary wine anthocyanins towards (-)-epicatechin to form stable anthocyanin-ethyl-(-)-epicatechin and their color characteristics. However, the study of Soares et al. (2019) indicated the mixture of epicatechin: mv-3-glc presents a greater interaction with both salivary PRPs when compared to individual polyphenols, which has implications for the study of the effects of the reaction between flavanols and anthocyanins on astringency. Furthermore, during the wine making, the content of various phenolic substances in the wine can be affected by adjusting the proportion of each part of the grapes, while how this adjustment affects the astringency is still unknown.

The goal of this research were therefore to study the effect of adjusting the proportion of skin and seed on wine astringency through SDS-PAGE, fluorescence quenching and sensory taste, and the final purpose is to provide a reference for the regulation of wine flavor. Moreover, possible mechanisms instigated by the reaction between the standard malvidin-3-glucoside and six phenol induced by acetaldehyde in the model solution.

2 Materials and methods

2.1 Regents and chemicals

Unless otherwise stated all solvents were of HPLC grade and all chemicals of analytical grade. Procyanidin B2, (+)-catechin (C), (-)-epicatechin (EC), epicatechin gallate (ECG), gallic (G), malvidin-3-glucoside and BSA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, formic acid and ethanol were purchased from Fuyu Fine Chemical (Tianjin, China). Acetonitrile was purchased from Aladdin (Shanghai, China). SDS-PAGE gel preparation kit and protein loading buffer were purchased from Suo Laibao (Beijing, China). Deionized water was obtained from a Milli-Q Gradient water purification system (Millipore, Billerica, MA, USA).

2.2 Wine samples preparation

Cabernet Gernischt (*Vitis vinifera L.cv.*) grapes were collected from Ningxia Eastern Helan Mountain region in China on September 30, 2016. Fifty kilograms Cabernet Gernischt grape berries were destemmed and pressed by a cold crusher to obtain grape juice. The grape skins and seeds residue were separated by a porous sieve. The skins, seeds and juices yield were weighed and calculated as 8.94%, 5.28% and 84.63%, respectively, which reflected the original proportion of each part in grape.

Thereafter, the skin and seed were added to juice under different ratios and then fermented according to the methods of (Li, 2002) to obtain six wine samples of different skin-seed ratio. In the wine samples P, Z1, Z2, Z3, Z4 and Z5, the content of skin remained unchanged, while the content of seed was 0, 4%, 8%, 16%, 32% and 40%, respectively. The wine samples P*, Z2* and Z4* were obtained by reacting 50 mL of each of the wine samples P, Z2 and Z4 with 1 mL of acetaldehyde (60%) for 2 weeks. The complete process is presented in supplementary Figure S1 (Supplementary Material).

2.3 Determination of phenolic substances in wine

The total phenol (TP) content of the wine samples was measured by the previously described Folin-Ciocalteu method (Singleton & Rossi, 1965). The absorbance was measured at 765 nm, and the resulting values were expressed in gallic acid equivalents (GAE) using units of mg/L. The total flavanol (TFA) content of the samples was determined by spectral analysis, as previously described (Li et al., 1996). The absorbance was measured at 640 nm, and the total flavanol content was calculated through a calibration curve using catechin as the standard, with the results expressed as catechin equivalents (CTE). The total anthocyanins (TA) contents of the wine sample were determined using the pH differential method (Lee et al., 2005). In short, 2 samples diluted 20 times were prepared; 1 with HCl-KCl buffer (pH 1) and the other with HAC-NaAC buffer (pH 4.5). Samples were then equilibrated at room temperature for 15 min. Absorbance was measured at 510 and 710 nm in buffers at pH 1 and 4.5, using $A = (A510 - A710)\text{pH1} - (A510 - A710)\text{pH4.5}$. Cyanidin-3-glucoside (c3g) with a molar extinction coefficient of 26900 was used as standard with results expressed as milligrams of c3g equivalents per kilogram dry weight.

2.4 HPLC-DAD

HPLC-DAD analysis was using a Thermo Scientific UltiMate 3000 Closed Sampler XRS0 system (Thermo Fisher Scientific, Waltham, USA), which consists of a model LPG-3400SD HPLC pump, a TCC-3000 SD column compartment, a WPS-3000SL analytical splitloop well plate auto-sampler, and a DAD-3000 diode array detector, run by Chromeleon CDS Software (version 6.8). The column used was a Waters XBridge Shield RP18 (4.6 mm × 250 mm, Agilent, Netherlands).

30 mL of each wine sample was extracted three times with two volumes of ethyl acetate to obtain an organic phase. These graded phases were combined, concentrated to dryness under reduced pressure, and the dry matter was dissolved in methanol with a chromatographic solution and placed in a 10 mL volumetric flask and and stored in a refrigerator at -20 °C. Samples were filtered using a 0.22 μm micropore filter prior to injection. The HPLC working parameters were as follows: column temperature 30 °C, sample injection volume 10 μL, and flow rate 0.6 mL/min with solvent A (acetonitrile containing 2% glacial acetic acid) and solvent B (water containing 2% glacial acetic acid). Gradient elution was performed with the following proportions of solvent A: 5% to 15% from 0 to 60 min, 15% from 60 to 65 min, 15% to 20% from 65to 66 min, 20% from 66 to 73 min, 20% to 30% at from 73 to 74 min, 30% from 70 to 80 min, 30% to 40% from 80 to 81 min, 40% from 81 to 93 min, 40% to 5% from 93 to 100 min.

The quantification of individual compounds was performed by using the linear equation obtained with the standard. Standard curves (R2 ≥ 0.990 in all cases) were obtained by HPLC-PDA injection of C, EC, EGC, ECG, and G standard solutions in methanol/water (1:1) with six different concentrations ranging from 3 to 60 μg/mL. The peak area was used as the analytical signal for compound quantification.

2.5 SDS-PAGE

Saliva was collected from two males and two females volunteers who are healthy and do not smoke. The collection time was from 10 am to 11 am. Volunteers were required to limit eating and drinking anything 2 hours before saliva collection. The insoluble matter from mixed saliva was removed by centrifugation at 10,000 × g for 10 min, where the obtained supernatant was the salivary protein sample (HS) required for the test. The salivary protein sample (HS) was added in the model wine in a ratio of 4:1(v/v) 5 min of reaction and centrifuged at 10,000 × g for 10 min. 20 μL of the obtained supernatant was mixed with 80 μL of each wine sample, then boiled in water for 5 min and stored in a refrigerator at -20 °C (Rinaldi et al., 2012).

SDS-PAGE was performed in tricine buffer (pH 8.2) using a 12% separation gel and 5% stacking gel according to the instructions of the SDS-PAGE gel preparation kit instructions. Bands were visualized by Coomassie brilliant blue R-250 staining. The initial voltage was set to 100 V. After approximately 20 minutes, the voltage was adjusted to 120 V.

2.6 Fluorescence measurement

Components of model wine solutions were selected on basis of those in real red wines (Wang et al., 2018).

The acetaldehyde stock solution (4.55×10^{-2} M) was prepared by spiking acetaldehyde in distilled water. For flavanol standards of EC, C, EGC, ECG, an aqueous stock solution of each (1×10^{-4} M) was prepared respectively in 10 mL ethanol in a brown volumetric flask. All the solutions above were stored at -30 °C before use. Three milliliter of BSA working solution (1.0×10^{-6} M) were added to a cuvette (1 cm), followed by a certain amount of each polyphenol standard stock solution (1×10^{-4} M) by micro-injector. The reaction solution was subjected to a dark reaction in a fluorescence sample chamber, and was measured after 2 minutes of rapid action. Each sample was blanked during the reaction, and the blank was subtracted from the calculation`n to exclude the error caused by the fluorescence of the phenolic sample emitted itself.

The standard reaction working solutions containing each flavanol were performed in F-7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with a thermostatic bath and a 1.0 cm quartz cell. The excitation and emission slit width was set to 5 nm. The excitation wavelength was set at 280 nm, and the emission spectra were recorded from 285 to 450 nm at a scan rate of 1200 $nm.min^{-1}$. Appropriate blanks corresponding to the model wine solution (without polyphenol) were employed to subtract the fluorescence background. The data analysis was performed with the method reported by Li & Hao (2015).

Fluorescence quenching method was also conducted to explore the astringency of red wine samples with different grape seeds-skins ratios. Each 200 μL of the wine sample was mixed with 3 mL of BSA working solution for 5 min and then subjected to fluorescence spectrum scanning in the same procedures as above (Zhang & Wang, 2017).

2.7 Sensory evaluation

The triangle tasting method was used for this sensory evaluation (Green, 1955). There were eight panelists (4M and 4F, age range 25-32) participating in the sensory evaluation session. Each wine sample (10 mL) was subjected to sensory descriptive analysis by the panelists at room temperature after it was poured into a standard red wine glass. In this research, the 12 wine samples were divided into 6 groups, in which the wine before and after the induction of acetaldehyde was a group like Z1 and Z1*. When each group was tasted, the wine samples of this group were labeled as 1, 2, and 3. Samples 1 and 3 were alike. The wine samples astringency were evaluated using quantitative descriptive analysis involving a 0-9 ten-point interval scale. In a group, the scores of the same wine sample were averaged. To counterbalance the study, the choice of repeated wine samples and the order of each group were presented randomly. Before tasting each sample, the panelists were required to rinse their mouth thoroughly with purified water (Yu et al., 2015).

2.8 Statistics analysis

The data were presented as the means ± standard deviations of triplicate measurements and evaluated by one-way analysis of variance (ANOVA) using SPSS 18.0 Statistics (SPSS Inc., Chicago, USA). Differences were considered significant at $p < 0.05$. Drawings and tables were created using Origin 8.5 mapping software and the results were analyzed by DPS 7.05.

3 Results and discussion

3.1 Total phenols, total flavanols and total anthocyanins of different wine samples

It can be seen from Figure 1a that the total phenol content in the whole skin wine is 1125.71 ± 8.89 GAE mg/L, and the total

phenolic content of the wine sample increased with the increase of the proportion of grape seed. As shown in Figure 1b, the total flavanol content of the wines sample increased significantly from wine sample P to Z5, which means that the grape seed contained a lot of flavanol. However, the total anthocyanin content was relatively stable in Figure 1c from wine P to wine Z5*, which indicates that the anthocyanins in those wine samples are mainly from the grape skin.

As can be seen from Table 1, among the three groups of wines not treated with acetaldehyde, the whole-skin wine P has a lower content of the flavan-3-ols and phenol acid than wine sample Z2 and Z4. When the proportion of grape seed increased, the content of flavan-3-ols and phenolic acids increased significantly. By comparing the three groups of treatments, the content of flavan-3-ols in wine was significantly reduced after the addition of acetaldehyde, especially in wine sample Z2/Z2* and Z4/Z4*, which may because that acetaldehyde promotes various polymerization reactions of phenols in red wine.

3.2 Analysis of combination of wine samples polyphenols and proteins

The SDS-PAGE of human saliva protein after binding wine sample was shown in Figure 2. The land HS is salivary proteins. There are four main protein bands of salivary proteins, and the protein bands with a molecular weight of about 66 kDa ~ 69 kDa are mainly carbonylated ruthenium-rich protein. The protein band with a molecular weight of about 62 kDa to 59 kDa is an amylase, and the low molecular weight protein with a molecular weight of about 25 kDa to 22 kD is mainly a basic prion protein. The most relevant proteins for astringency are bands around 54 kDa ~59 kDa and 15 kDa, so the SPI (saliva precipitation index) can be calculated by the two protein bands.

As can be seen from Figure 2a, 2c, from wine sample Z1 to Z5, the color of the protein bands associated with astringency became lighter, indicating an increase in protein precipitation ability. From the above conclusions, it can be deduced that the content of flavan-3-ols in the wine with more grape seed wine is higher, so their protein binding ability is stronger. In addition, the acetaldehyde-treated wines had an enhanced ability to precipitate proteins (Rinaldi et al., 2012). However, Sheridan & Elias (2015) thought the exogenous acetaldehyde treatment to fermenting musts contributed the condensation between tannins and anthocyanins but lowered tannin-protein precipitation. The possible explains for this difference was that the concentration of acetaldehyde is too high, resulting in an increase in the degree of polymerization of tannin to precipitate, thereby reducing the binding of tannin-protein (Picariello et al., 2017).

As can be seen from Figure 3a, the values of fluorescence intensities were decreasing from the model wines Z1 to Z5, which indicated that with the increase of the grape seeds, the binding strength of polyphenols to protein in wine increased gradually. It can be seen from Figure 3b-3d that the protein binding ability of the wine sample was enhanced after the addition of acetaldehyde. For whole skin wine P, the change in fluorescence quenching ability of BSA before and after acetaldehyde treatment was not very obvious, probably because the content of flavan-3-ols in whole skin wine was low. After the addition of acetaldehyde, the protein binding ability of the wine sample was enhanced, especially in the Z4/Z4* group, which may be related to the higher content of flavan-3-ols (Symoneaux et al., 2014). It can be seen from the above different experiments that acetaldehyde can promote the polymerization of polyphenol and those combination can promote their binding ability to salivary proteins (Soares et al., 2019).

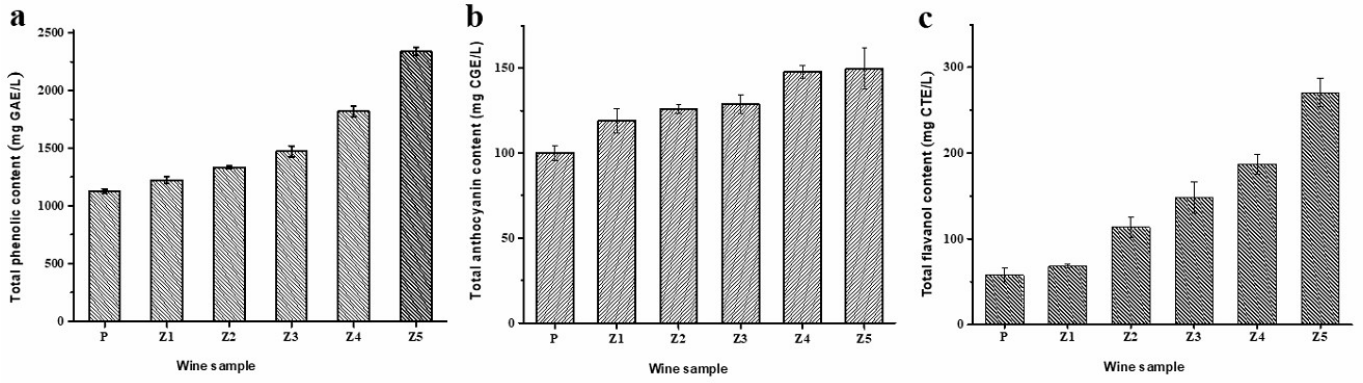

Figure 1. The total phenolic content (a), total anthocyanin content (b) and total flavanol content (c) of six grape skin-seed blend wine sample.

Table 1. Content of monomeric phenol in different wine samples determined by HPLC.

Z	Monomeric phenol	Wavelength (nm)	P	P*	Z2	Z2*	Z4	Z4*
Flavanol (mg/L)	C	280	----	0.95 ± 0.03	46.23 ± 1.75	28.44 ± 0.12	89.08 ± 1.76	32.76 ± 0.86
	EC	280	8.81 ± 0.33	4.61 ± 0.12	57.28 ± 1.86	24.39 ± 1.65	76.73 ± 1.35	27.92 ± 0.35
	EGC	280	----	----	8.47 ± 0.98	4.37 ± 0.08	14.86 ± 0.36	7.08 ± 0.05
	ECG	280	2.00 ± 0.15	1.80 ± 0.05	4.72 ± 0.52	4.74 ± 0.12	14.62 ± 0.23	13.68 ± 0.24
Phenolic acid (mg/L)	G	280	9.50 ± 0.44	50.60 ± 1.35	32.38 ± 2.8	41.47 ± 0.98	53.28 ± 1.07	58.92 ± 0.20

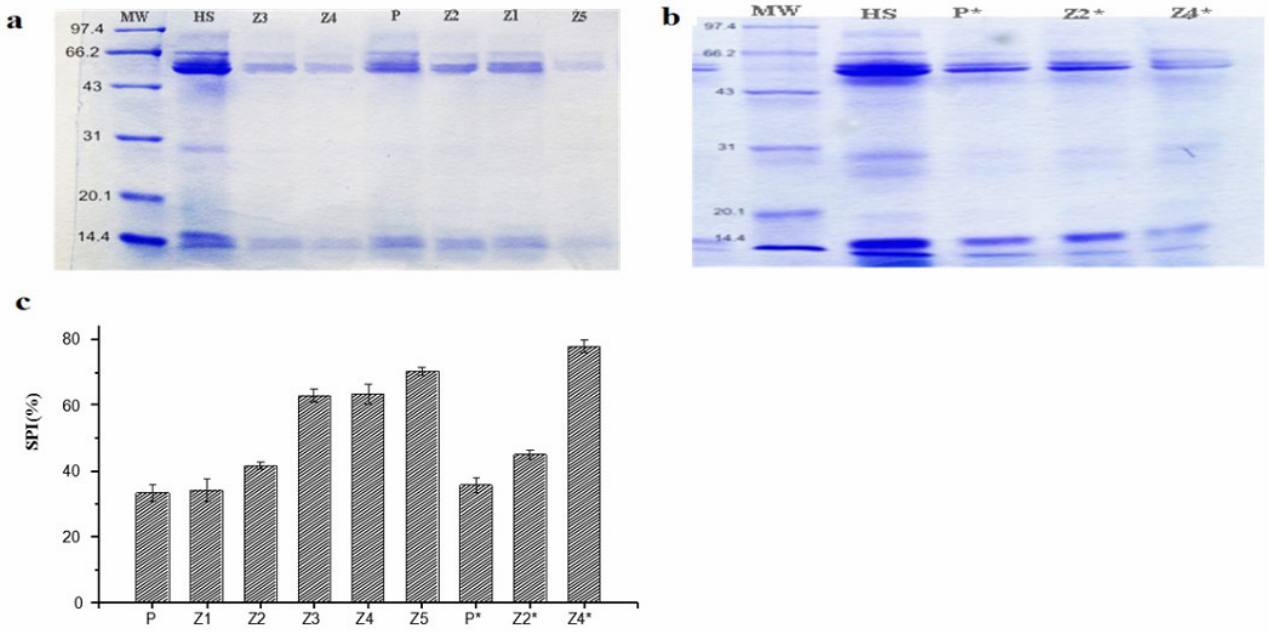

Figure 2. (a) The SDS-PAGE of human saliva after binding to polyphenol in different wine sample. (b) The SDS-PAGE of human saliva after binding to polyphenol in wine sample which was treated with acetaldehyde. (c) SPI value of wines fermented under different ratio of skin. Lane MW: marker; Lane HS: salivary protein. The wine samples P*, Z2* and Z4* were obtained by reacting 50 mL of each of the wine samples P, Z2 and Z4 with 1 mL of acetaldehyde (60%) for 2 weeks.

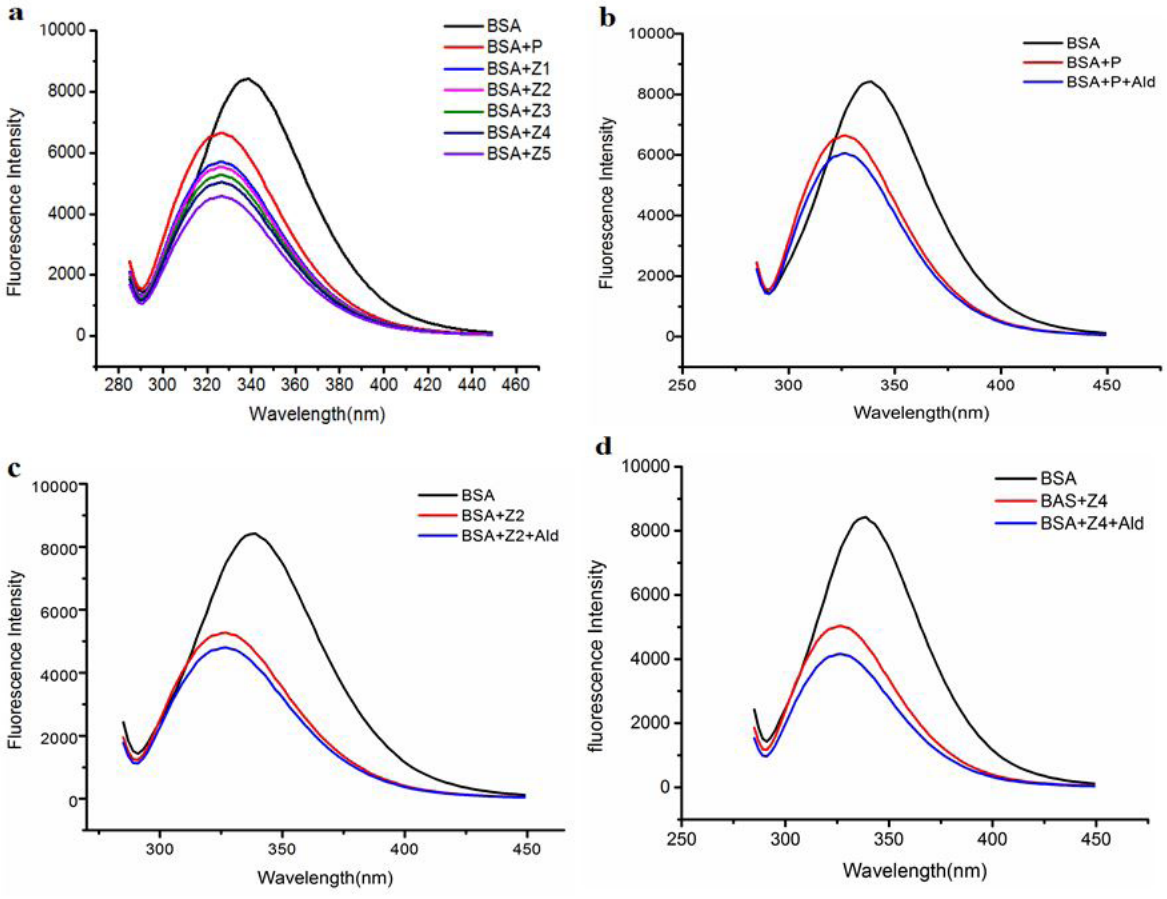

Figure 3. Fluorescence spectra of BSA after binding with polyphenols in five wine samples (a) and fluorescence spectra of BSA after binding with polyphenols in wine sample P (b), Z2(c), and Z4(d) in presence of acetaldehyde. BSA:Bovine Serum Albumin.

3.3 Analysis of fluorescence quenching between the malvidin-3-glucoside and flavan-3-ols and BSA

Zhang et al. (2017) indicated that the ECG can interact with BSA and quench its fluorescence. As can be observed in Figure 4a, the fluorescence intensity of BSA decreased with the addition of six polyphenol, which suggested that the five polyphenol other than ECG also have the ability to interact with BSA and quench its fluorescence. From Figure 4b-4f, the addition of malvidin-3-glucoside and acetaldehyde affected the ability of flavan-3-ols to bind to BSA. For example, in Figure 4c, EC: malvidin-3-glucoside mixture have a weaker BSA quenching ability than EC: acetaldehyde mixture, and the mixture of acetaldehyde: EC: malvidin-3-glucoside has the most powerful BSA quenching ability. In the EC: malvidin-3-glucoside mixed sample, those substances that can bind to BSA are EC, EC self-polymerized product and EC-malvidin-3-glucoside polymer (EC-mv-3-glc). In the EC: acetaldehyde mixed sample, those substances that can bind to the protein are EC, EC self-polymerized product and EC-acetyl-EC. In the EC: acetaldehyde: malvidin-3-glucoside mixed sample, the substance that can bind to the protein are EC, EC self-polymerized product, EC-acetyl-EC, EC-mv-3-glc and EC-acetyl-mv-3-glc. However, previous studies suggested that the presence of acetaldehyde in red wine presents strong competition to direct covalent reactions between tannin and anthocyanin in the formation of polymeric pigments, with reaction rates of ethyl linkages being 14 to 120 times more rapid than direct covalent linkages, as also suggested for polymeric tannin-anthocyanin reactions (Dallas et al., 1996b; Thorngate & Singleton, 1994; Singleton & Trousdale, 1992). Therefore, in this system, acetaldehyde-mediated polymerization can be considered to be dominant. As shown in supplementary Figure S3, the production of EC-acetyl-mv-3-glc and EGC-acetyl-mv-3-glc were detected by HPLC-MS under acetaldehyde-induced conditions.

These polymerizations also occur in other phenolic samples. In combination with Figure 4b, 4c, 4e and 4f, it can be seen that under the conditions of acetaldehyde participation, the sample with the anthocyanin addition has stronger BSA quenching ability than the sample without anthocyanin, indicating that anthocyanins can promote the combination of flavan-3-ols substances and BSA. At the same time, after the addition of acetaldehyde, the BSA quenching ability of the malvidin-3-glucoside: flavan-3-ol mixture was enhanced, and it was speculated that the protein binding ability of ethyl-linked F-Et-A adducts were stronger than that of F-A and self-polymerization product of flavan-3-ol. However, as shown in Figure 3d, the condensation reaction of ECG with malvidin-3-glucoside in the absence of acetaldehyde showed the strongest quenching effect on BSA. The probably reason for the result of Figure 4d may be the non-strongly induced polymerization of acetaldehyde to the esterified flavan-3-ols, resulting in a relatively large amount of monomeric ECG remaining in the reaction system, which has strong binding ability to proteins as the study of Skrt et al. (2012). Previous studies have found that the esterification of proanthocyanidins play a significant role in the protein-

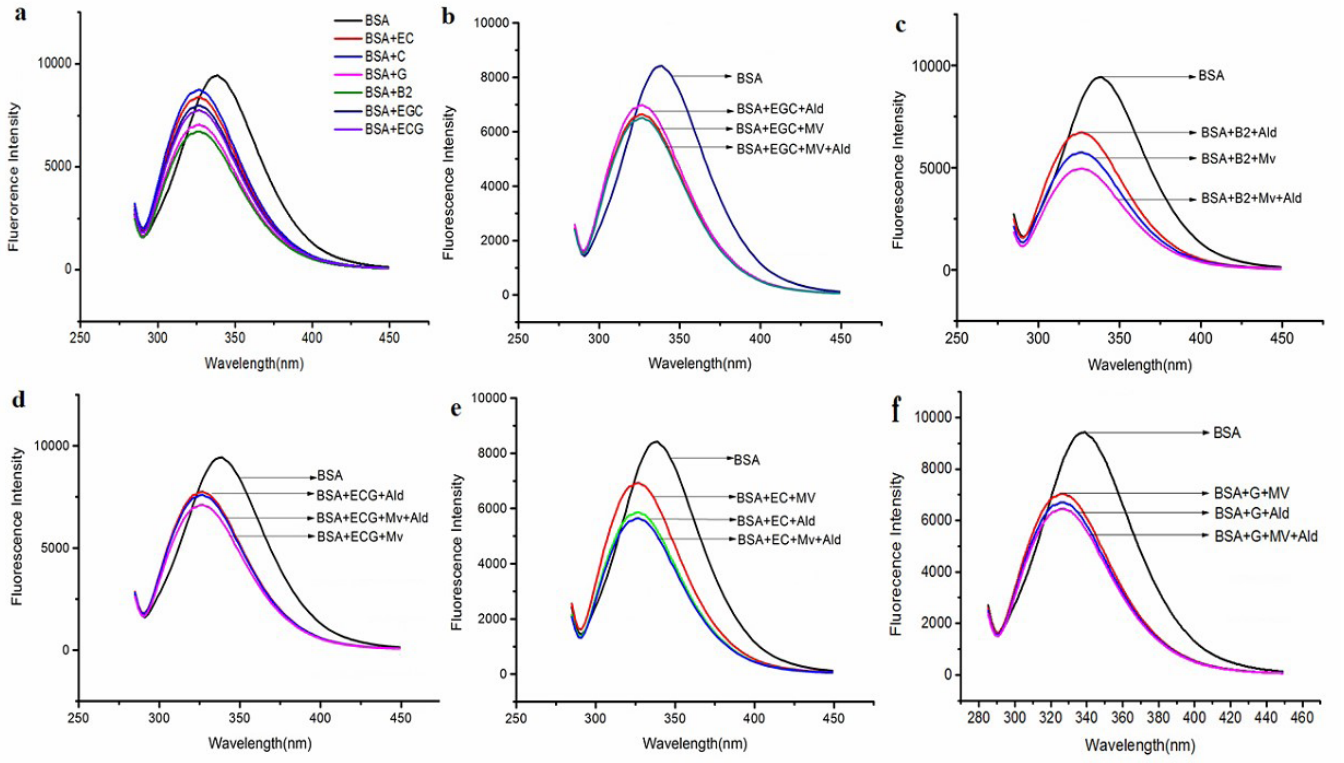

Figure 4. Fluorescence spectra of six polyphenols and BSA (a), flavan-3-ols and BSA in presence or absence of acetaldehyde or malvidin-3-glucoside; (b) epicatechin; (c) epigallocatechin; (d) epicatechin gallate; (e) procyanidins B2; (f) gallate. The wine samples P*, Z2* and Z4* were obtained by reacting 50 mL of each of the wine samples P, Z2 and Z4 with 1 mL of acetaldehyde (60%) for 2 weeks.

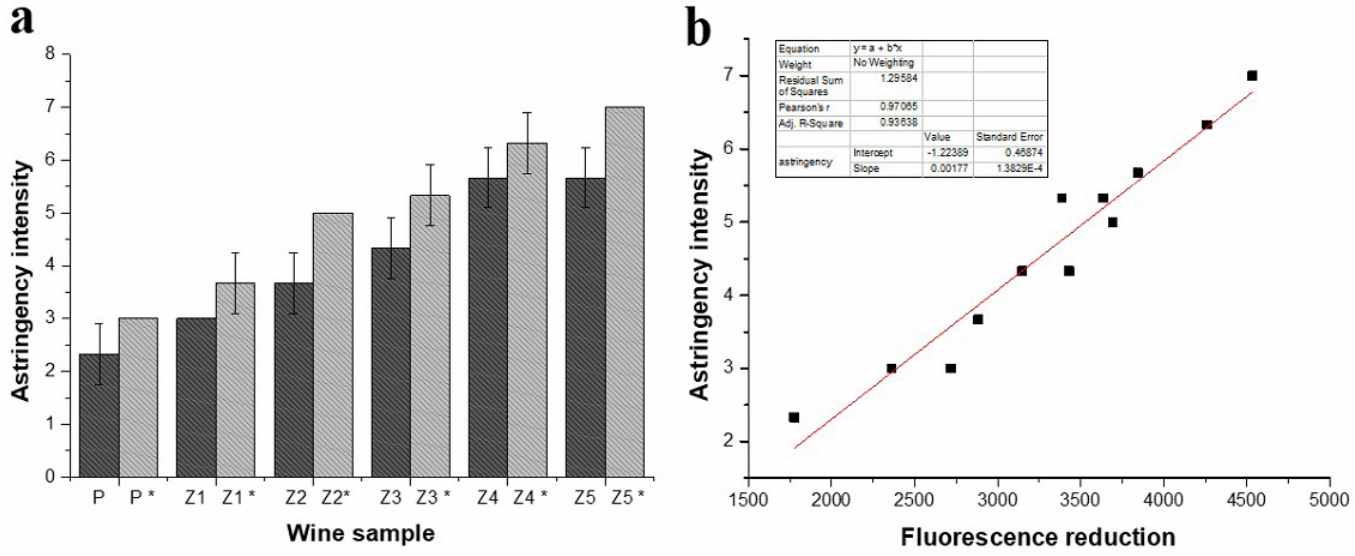

Figure 5. The astringency score of 12 different wine samples (a), and the correlation between the fluorescence difference and the astringency (b). BSA: Bovine Serum Albumin. C: Catechin. EC: Epicatechin. EGC: Epigallocatechin. ECG: Epicatechin gallate.

bind ability, which may be due to the enhanced hydrophobic interaction and the increase of hydrogen bonding caused by the aromatic ring and the hydroxyl group (Soares et al., 2007; Ferrer-Gallego et al., 2010). Acetaldehyde could promotes the polymerization of various phenolic substances in wine, and the polymer product has strong protein binding ability, especially the indirect mediated product of acetaldehyde, so wines with acetaldehyde addition will have stronger protein binding and precipitation capacity (Figure 2 and Figure 3). Similarly, in wine samples with high flavanol content, the polymerization of flavanols and anthocyanins is more intense, resulting in a polymeric pigment with a stronger ability to bind proteins, so from the wine sample P to Z5, the polyphenols in the wine sample have an enhanced protein binding and precipitation ability (Figure 2 and Figure 3).

3.4 Sensory evaluation

As can be seen from Figure 5a, in the wine sample which was not treated with acetaldehyde, astringency increased from the wine sample P to Z5. In acetaldehyde-treated wine samples, the astringency of the wine also increased with the increase of the whole seed wine content. Among the two wine samples of the same skin-seed ratio in each group, the wine astringency with acetaldehyde addition was significantly enhanced compared with the wine without acetaldehyde addition. It can be inferred that the astringency of the seed tannin is stronger than the skin tannin, and the addition of acetaldehyde to red wine can significantly enhance the astringency. Figure 5b displays the correlation between the fluorescence difference of different skin-to-seed ratio wines and their astringency, which indicates that the polyphenol-protein binding ability has a strong correlation with the wine astringency intensity. From the results (Figure 3a and Figure 5a) it is possible to observe that in wine samples that were not treated with acetaldehyde, the stronger the protein precipitation ability, the stronger the corresponding astringency, and the same conclusion was observed in Figure 3b.

4 Conclusion

In this experiment, flavanols were mainly derived from grape seeds, while anthocyanins were mainly supplied from grape skins. In the skin-seed re-design wine, with the grape skin content remaining stable, as the grape seed content increased, the ability of the polyphenolic substance to bind to protein was enhanced, and the astringency of wine was also enhanced. Besides, after incubation with acetaldehyde, the protein binding capacity of phenolic in wine was significantly enhanced, and after tasting, the wine's astringency was also enhanced. Through correlation analysis, it is found that the astringency of wine has a strong correlation with the protein binding ability of phenolic substances in red wine. Therefore, to a certain extent, the study of the interaction of these phenolic substances with proteins could give valuable information to understand astringency sensation. Combined with the reaction of the standard flavan-3-ol with anthocyanin in the induction of acetaldehyde, it was concluded that the astringency of wine is closely related to the interaction between flavan-3-ols and anthocyanins from grapes. Grape skin and seed contribute differently to astringency sensation, and one of the most important reasons is the difference in the type and content of flavanols and anthocyanins contained in skin and seeds. Furthermore, the reason why acetaldehyde can improve the astringency of wine is because acetaldehyde promotes the interaction between phenolic substances in wine, resulting in more adducts with stronger protein binding ability.

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Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Experimental design.

Figure S2. Fluorescence spectra of (+)-catechin and BSA in presence or absence of acetaldehyde or malvidin-3-glucoside.

Figure S3. HPLC chromatogram and mass spectrum of acetaldehyde induced reaction of flavan-3-ols with malvidin-3-glucoside. (a) epicatechin; (b) epigallocatechin. The numbers 1, 2, and 3 represent acetaldehyde, flavan-3-ol, and anthocyanin, respectively.

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