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Evaluation of the potential astringency of the skins and seeds of different grape varieties based on polyphenol/protein binding

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Abstract

The potential astringency of eight grape cultivars was evaluated based on polyphenol/protein binding. The parameters such as weights, sizes, total phenols, flavanols and tannins of different grape tissues were determined, and the polyphenol/protein binding was analyzed by SDS-PAGE and spectroscopy. The Cabernet Sauvignon had the highest skin proportion, while the Blue French had the highest seed proportion. The Blue French seeds had the highest total phenolic content, while the Cabernet Sauvignon skins had the highest total phenolic content. SDS-PAGE showed that the seed extracts had a better affinity than skin extracts for saliva proteins, but fluorescence spectra demonstrated that the interactions of skin polyphenols with protein were more complex. These results may provide some useful information on grape potential astringency's evaluation and wine-making in China.

Keywords: grape cultivars; polyphenols; interaction; electrophoresis; spectroscopy.

Practical Application: This study may facilitate the evaluation of potential astringency of grapes and the improvement of quality wine.

1 Introduction

Astringency has been reported to be an oral tactile sensation contains a complex set of sensations related to drying, roughing and puckering of the mouth epithelium and caused primarily by the interaction of polyphenols with salivary proteins (Gawel, 1998; Vidal et al., 2018; Ferrer-Gallego et al., 2014). The presence of phenolic compounds and their ability to interact with salivary proteins are concerned with wine astringency. The compound of grapes berries is well recognised to be a potential astringency of wine.

The skins and seeds of grapes accumulate the most phenolic compounds (Kyraleou et al., 2016), which are important quality components contributing to the color, aroma and mouth-feel in both grapes and wines (Cáceres et al., 2012; Harrison, 2017; Kyraleou et al., 2016; Zhang et al., 2015). However, phenolic compounds in the two parts are qualitatively and quantitatively different among the grape cultivars (Katalinić et al., 2010; Santos et al., 2011). Previous studies showed that grape skin polyphenol content could amount to 28% ~ 35% and seed polyphenol up to 60% ~ 70% (Pantelić et al., 2016). These compositions from grape skins and seeds transfer to the wine during the wine-making process (Nogales-Bueno et al., 2017). The phenols are released more easily and quickly from grape skins than from seeds, but their phenolic concentration is lower than that of seeds (Lomolino et al., 2010). Anthocyanidins and flavonoids are the most abundant phenolic compounds in the skins (Yu & Ahmedna, 2013). Anthocyanidin is a red pigment that is primarily present in red grape cultivars and often imparts red or blue color. Flavonoid is a yellow pigment, which is present in both red and white grape cultivars. Moreover, grape seeds are rich in flavan-3-ols, catechins and procyanidins; catechins contain three monomers including catechin, epicatechin and epicatechin gallate (Mattivi et al., 2009; Obreque-Slier et al., 2010).

The quality of grape is paramount as it is the material for wine-making. Nevertheless, the quality of grapes and wines is influenced by many factors (region, climate, cultivation, and grape variety) (De Pascali et al., 2014; Pantelić et al., 2016), and irrespective of the type vinification, the grape variety ultimately determines the quality of the wine (Meng et al., 2017). This is primarily due to the phenolic compounds in the grape skins and seeds. And the phenolic composition of skins and seeds varied with ripening and defcit irrigation regimes (Perestrelo et al., 2018; García-Esparza et al., 2018). During the wine fermentation process, skin phenolics are released into the wine immediately. The anthocyanin content increased rapidly and reached the highest amount after 2 to 3 days. Seed phenolics started to leach out five days the commencement of fermentation. Therefore, the maceration time would directly influence the quality of the wine (Zhang et al., 2015).

Many studies have focused on the total phenolic content, antioxidant capacity, anthocyanins, fatty acid composition, and specific phenolic (caffeic acid, gallic acid, resveratrol, and catechin) contents in the skins and seeds of different grape cultivars (Balik & Kumsta, 2008; Lutz et al., 2011). Moreover, the extracts from skins and seeds of different grape varieties have been analyzed by one or more of the following methods: HPLC-MS,

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electrophoresis, FT-NIR and so on. And little attention has been paid to the interactions between proteins and polyphenols from grapes skins and seeds (Obreque-Slier et al., 2012).

The main aim of the study was to investigate the phenolic compounds from skins and seeds of eight grape cultivars (*Vitis vinifera* L.) growned in the Taigu area of Shanxi Province, China, and widely cultivated in China. Electrophoresis and spectroscopy were used to analyze the interactions between salivary proteins and polyphenols, which would help to understand the properties of grape skin and seed phenolic compounds and provide some useful information to evaluate the wine-making potential of the different grape cultivars.

2 Materials and methods

2.1 Chemicals

Folin-Ciocalteu's phenol reagent (99%), p-dimethylaminocinnamaldehyde (DMACA) (98%), and bovine serum albumin (BSA) (96%) were obtained from the Sigma Chemical Co. (St. Louis, MI, USA). The SDS-PAGE kit was purchased from Shanghai Solarbio Bioscience & Technology Co., Ltd (Shanghai, China).

2.2 Planting materials and Sample preparation

Eight grape cultivars including Cabernet Sauvignon (G1), Blue French (G2), Merlot (G3), Cabernet Franc (G4), Cinsaut Cehco (G5), Ruby Cabernet (G6), Carignane (G7) and Cabernet Gernischet (G8) were picked from Taigu, Shanxi Province, China in 2015. The fruits were separated into skins (P1, P2, P3, P4, P5, P6, P7, and P8), seeds (Z1, Z2, Z3, Z4, Z5, Z6, Z7, and Z8) and pulp manually and were immediately frozen and stored at -40 °C until analysis.

First, 7.0 g grape skins, 2.5 g seeds, and 10 g pulp samples were separately immersed in 20 mL methanol and digested for 24 h at room temperature. Secondly, the residue was extracted in methanol/ water (80/20, V/V) for 4 h, and the extraction was collected. Thirdly, the extracted residue was again, submerged in methanol/ water (50/50, V/V) for 4 h, and the last extraction was collected. Finally, all extractions were merged and prepared for subsequent analysis.

2.3 Determination of the weights and sizes of grapes and different grape tissues

One hundred berries were selected randomly from each cultivar as the samples, and the grain weight and size were measured. Then the weight of the skins, pulp and seeds fractions was determined separately, and the average value was recorded.

2.4 Determination of the total phenolics, flavanols and tannins

The total phenolic content (TPC) in the grape skins and seeds was measured by the Folin-Ciocalteu method, as described in previous reports (Iora et al., 2015; Xu et al., 2010), with some modifications. The extracts (0.1 mL) were mixed with 7 mL distilled water and 0.5 mL Folin–Ciocalteu reagent, homogenized and incubated for 1 min at room temperature. Then, 1.50 mL 20% Na_2CO_3 solution and 0.9 mL distilled water were added and oscillated sufficiently. The mixture was incubated for 60 min in the dark at room temperature. The absorbance was determined at 765 nm in a 751-GD model UV/Vis spectrophotometer (Shanghai, China) using gallic acid (GAE) as a standard and each sample was measured two times.

The total flavanol content (TFA) was determined by DMACA, and results were expressed as catechin equivalents (CTEs) (Xu et al., 2010). A volume of 0.1 mL skin or seed extract was mixed with 3 mL 0.1% DMACA solution (0.1% in 1 mol/L HCl in methanol), the mixture was allowed to stand for 10 min. The absorption at 640 nm was measured in a 751-GD model UV/Vis spectrophotometer (Shanghai, China), every extract was replicated twice.

The total tannin content (TA) was measured according to the method of Adams-Harbertson (Brooks et al., 2008; Mercurio & Smith, 2008). Tannins from grape skins or seeds extraction were precipitated by adding 1 mg/mL bovine serum albumin (BSA) solution, and centrifuged at 13,500 g for 5 min. The supernatant was carefully removed, and the precipitate was collected; the buffer solution (200 mM acetic acid and 170 mM sodium chloride, pH adjusted to 4.9 with sodium hydroxide) was slowly added to the precipitate and centrifuged for 1 min, and the process was repeated. The buffer solution (5% (v/v) triethanolamine and 5% (w/v) sodium dodecyl sulfate, pH adjusted to 3.3 with 10.18 M hydrochloric acid) was added to the precipitate and incubated for 10 min. The centrifugal tube was agitated until the precipitate was dissolved and incubated for 10 min. The background absorbance was measured at 510 nm, and the tannin absorbance was measured after adding the $FeCl_3$ solution. The absorbance was standardized to catechin equivalents (CTEs), and each sample was determined in triplicate.

2.5 SDS-PAGE

The human saliva was collected as the report (Rinaldi et al., 2010). Four non-smoking volunteers (two males and two females) with no oral disease were selected and their saliva was obtained between 10 to 11 a.m. Volunteers were not permitted to eat any food or drink within 1 h before saliva collection. Eventually, the saliva was centrifuged for 10 min at 10,000 g to remove any insoluble material, and the supernatant was the human saliva sample.

Electrophoresis was performed on a DYCZ-24DN electrophoresis apparatus (Beijing, China) using a DYY-6C power supply (Beijing, China) according to previous reports (Ferrer-Gallego et al., 2012; Rinaldi et al., 2015). Human saliva was combined with the grape skin or seed extracts from eight different grape varieties at a ratio of 4:1 ratio, keep the reaction for 5 min at 25 °C, then centrifuged the reaction mixtures (300 μL of HS and 150 μL of sample) for 10 min at 3000 r/min. The supernatant was mixed with an equal volume of 5×electrophoresis sample buffer (1 mol/L Tris–HCl, 4% SDS, 20% glycerol, 0.2 mol/L DTT, 0.1% bromophenol blue, pH 6.8), then boiled 5 min in boiling water and analyzed by SDS–PAGE with 5% acrylamide stacking gel and 12% acrylamide resolving gel. The electrophoresis conditions were as follows: the stacking

gel area was firstly run at 100 mA, and then, the resolving gel was run at 72 mA. The gel was stained with Coomassie Brilliant Blue R250 (0.1% in 45% methanol and 10% acetic acid) for 3~12 h. The staining fluid was washed off, and a mixture of acetic acid, methanol and deionized water (10:10:80) was used to destain the gel. The gels were imaged on the gel imager and pictures were taken. The gels were digitalized in a ChemiDoc-it 510 Chemiluminescence imaging (American UVP company-1b) and the optical density was analyzed.

2.6 Research the interaction of phenolics and BSA by spectrum

Fluorescence spectra were recorded using a 970 CRT fluorescence spectrophotometer (Shanghai, China) (Ferrer-Gallego et al., 2012; Pinto et al., 2010). The extracts of grape skins or seeds were appropriately diluted and added to the BSA solution (BSA was dissolved in 1 mol/L Tris-HCl buffer (pH 7.4), then mixed with 0.5 mol/L NaCl and distilled water to make up 1.0×10^{-6} mol/L BSA solution). The tube were shaken to sufficiently mix the mixture, which was then reacted for 5 min at room temperature (25 °C). The same concentration of BSA solution was set as blank and the samples were scanned in the range 285 to 450 nm. The excitation wavelength was set at 280 nm, and 5.0 nm excitation and 10.0 nm emission slit widths were used.

2.7 Data processing

The results were analyzed by UVP VisionWorks™ LS (Optical density analysis software), Excel 2010 and DPS 7.05 (DPS data processing system).

3 Results and discussion

3.1 Parameters of different grape tissues

Table 1 shows some parameters of the tested grape varieties, and the results showed significant differences ($p \leq 0.05$). The berry weight ranged from 1.08 to 2.60 g, with Cabernet Sauvignon and Blue French being the smallest and largest respectively. Fruit shape index indicated the ratio of the vertical berry size and cross size. These indices ranged from 0.97 to 1.06 and were similar and higher than 0.95. This meant that all grapes were conical or round. Varietal differences were observed in the proportions of grape skins, seeds and pulp (Zhang et al., 2015). According to Chen et al. (2018), wine quality varied with the size of berry, while the wines made from small berries presented the highest content of alcohol and residual sugar, and were more desirable about the colour.

The grape skin percentage in this study varied between 7.67% and 25.13% ($p \leq 0.05$). The highest proportion was found in Cabernet Sauvignon skin, while the lowest was in Cabernet Gernischet skin. Conversely, the seed proportions were found to be ranged between 2.55% (Cinsaut Cehco) and 7.26% (Blue French) ($p \leq 0.05$). Moreover, the pulp proportions of tested grapes were higher than 80% except for Cabernet Sauvignon (68.7%) ($p \leq 0.05$). Specifically, the proportions of grape skin was greater than that of grape seed.

The pulp proportions were found to be notably higher than the skins and seeds ($p \leq 0.05$). However, high proportions of skins and seeds indicate that the grape has a high content of polyphenol, such as Cabernet Sauvignon. Similarly, if the pulp ratio of the grape is higher, the content of phenolic substances in the wine, contributed by the grape, will be less. Therefore, knowledge of the proportion and the phenolic substances content of each type of grape skins and seeds can help determine the varieties of grapes' skins, seeds and pulp, which can be added to make high quality wine.

3.2. Determination of total phenolics, total flavanols and total tannins

The results for total phenolics, flavonols and tannins in grape skins and seeds are presented in Table 2 with significant difference ($p < 0.05$).

Varying contents ($p < 0.05$) of total phenolics, flavonols and tannins were observed in the berry skins. The total phenolic content of the skins ranged from 47 to 125 mg/100 g FW, values that were in the range reported by Pantelić et al. (2016) and Yilmaz et al. (2015) for grapes. Cabernet Sauvignon had the highest TPC (125.11 mg•100 g^{-1}FW), while Cabernet Franc had the lowest TPC (42.32 mg·100 g^{-1}FW). The TFA varied between 4.80 mg•100 g^{-1}FW in Cabernet Gernischet and

Table 1. Some parameters of different grape cultivars.

Cultivars	Grain weight (g)	Grain size (mm)		Fruit shape index	Skin		Seed		Pulp	
		Cross	Vertical		Weight (g)	percentage (%)	Weight (g)	percentage (%)	Weight (g)	percentage (%)
G1	1.08 ± 0.10^{f}	12.57 ± 0.13^{e}	12.94 ± 0.70^{d}	1.03	0.27 ± 0.021^{b}	25.13	0.07 ± 0.005^{de}	6.17	0.74 ± 0.014^{e}	68.70
G2	2.60 ± 0.03^{a}	17.05 ± 0.30^{a}	16.54 ± 0.41^{b}	0.97	0.32 ± 0.024^{a}	12.47	0.19 ± 0.003^{a}	7.26	2.15 ± 0.053^{a}	80.27
G3	1.41 ± 0.09^{e}	14.24 ± 0.48^{cd}	13.86 ± 0.56^{cd}	0.97	0.15 ± 0.012^{d}	10.67	0.09 ± 0.004^{c}	6.07	1.17 ± 0.095^{d}	83.26
G4	1.66 ± 0.07^{d}	14.10 ±0.55^{d}	14.21 ± 0.35^{c}	1.01	0.18 ± 0.017^{cd}	10.66	0.11 ± 0.002^{b}	6.39	1.37 ± 0.046^{c}	82.95
G5	2.40 ± 0.10^{b}	15.60 ± 0.42^{bc}	16.53 ± 0.48^{ab}	1.06	0.21 ± 0.022^{c}	8.68	0.06 ± 0.004^{e}	2.55	2.13 ± 0.049^{a}	88.77
G6	1.99 ± 0.07^{c}	15.02 ± 0.58^{cd}	15.99 ± 0.53^{b}	1.06	0.17 ± 0.009^{cd}	8.64	0.12 ± 0.005^{b}	5.82	1.71 ± 0.022^{b}	85.54
G7	2.40 ± 0.05^{b}	16.12 ± 0.34^{ab}	17.22 ± 0.42^{a}	1.07	0.20 ± 0.016^{c}	8.34	0.08 ± 0.004^{cd}	3.49	2.12 ± 0.054^{a}	88.17
G8	2.05 ± 0.11^{c}	15.32 ± 0.45^{bcd}	15.58 ± 0.51^{b}	1.02	0.16 ± 0.025^{cd}	7.67	0.06 ± 0.004^{e}	2.59	1.83 ± 0.057^{b}	89.38
Range	1.08 - 2.60	12.57 - 17.05	12.94-17.22	0.97-1.07	0.15 - 0.32	7.67 - 25.13	0.06 - 0.19	2.55 - 7.26	0.74-2.15	68.70 - 89.38
Average	1.95	15.00	15.36	1.02	0.21	11.53	0.10	5.04	1.65	83.38

Note: Different letters in each column represent significant differences at $p \leq 0.05$.

Table 2. Total phenolic, flavanol and tannin contents of different grape skins and seeds.

Cultivars	TPC(mg·100g^{-1}FW)		TFA(mg·100g-^{1}FW)		TA(mg·100g-^{1}FW)		Titratable acid (g·L^{-1})	Soluble solid (g·L^{-1})
	Skin	Seed	Skin	Seed	Skin	Seed		
G1	125.11 ± 1.16^{a}	153.70 ± 3.81^{g}	14.99 ± 0.92^{b}	172.71 ± 4.84^{f}	81.08 ± 7.81^{a}	256.21 ± 5.59^{c}	5.09 ± 0.21^{cde}	19.30 ± 0.00^{g}
G2	84.37 ± 0.78^{c}	305.62 ± 6.84^{b}	8.78 ± 0.32^{c}	410.14 ± 7.43^{b}	25.98 ± 6.51^{bc}	526.99 ± 22.88^{a}	5.56 ± 0.21^{cd}	17.60 ± 0.00^{l}
G3	61.44 ± 0.87^{e}	186.10 ± 0.43^{f}	7.38 ± 0.47^{cde}	229.73 ± 8.67^{d}	26.10 ± 14.14^{bc}	302.96 ± 7.00^{b}	3.91 ± 0.71^{fg}	19.60 ± 0.00^{f}
G4	42.32 ± 0.38^{hi}	114.12 ± 0.58^{j}	6.06 ± 0.22^{efg}	123.07 ± 13.79^{g}	33.94 ± 10.15^{b}	207.58 ± 8.18^{d}	5.21 ± 0.41^{cd}	19.03 ± 0.06^{h}
G5	47.07 ± 0.91^{g}	32.27 ± 0.77^{n}	6.15 ± 0.30^{efg}	60.22 ± 3.87^{j}	17.52 ± 1.97^{c}	45.00 ± 5.04^{f}	8.17 ± 0.62^{a}	16.50 ± 0.00^{n}
G6	73.34 ± 4.44^{d}	125.83 ± 0.50^{hi}	27.46 ± 4.03^{a}	129.43 ± 8.78^{g}	30.70 ± 3.18^{bc}	107.80 ± 4.57^{e}	9.00 ± 0.82^{a}	18.63 ± 0.06^{j}
G7	47.99 ± 0.45^{g}	90.09 ± 2.62^{k}	5.56 ± 0.11^{fgh}	98.76 ± 5.59^{h}	26.73 ± 6.44^{bc}	115.19 ± 5.85^{e}	8.64 ± 0.54^{a}	18.73 ± 0.06^{i}
G8	59.88 ± 0.30^{e}	55.79 ± 0.33^{m}	4.80 ± 0.12^{ghi}	49.08 ± 0.48^{k}	28.44 ± 2.18^{bc}	103.81 ± 5.38^{e}	4.02 ± 0.21^{fg}	16.20 ± 0.00^{o}
Range	42.32 - 125.11	32.27 - 305.62	4.80 - 27.46	49.08 - 410.14	17.52 - 81.08	45.00 - 526.99	3.91 - 9.00	16.20 - 19.60
Average	67.69	132.94	10.15	159.14	33.81	208.19	6.20	18.20

Note: The same letters in each row are not significantly different at the 0.05 level; FW: Fruit fresh weigh.

27.46 mg•100 g-^{1}FW in Ruby Cabernet in the skin. The values of TA in the skins showed no significant differences, except for Cabernet Sauvignon (81.08 mg•100 g-^{1}FW) and Cinsaut Cehco (17.52 mg•100 g-^{1}FW).

In the berry seeds, the contents of total phenolics, flavonols and tannins showed significant difference ($p < 0.05$). The highest TPC was found in Blue French (305.62 mg•100 g-^{1}FW), while the lowest was in Cinsaut Cehco (32.27 mg•100 g-^{1}FW). This result was in line with previous studies (Yilmaz et al., 2015). Moreover, Blue French had the highest TFA (410.14 mg·100 g-^{1}FW) and TA (526.99 mg·100 g-^{1}FW) in the seeds, while Cabernet Gernischet and Cinsaut Cehco had the lowest TFA(49.08 mg•100 g-^{1}FW) and TA (45.00 mg•100 g-^{1}FW), respectively.

In addation, there was no significant changes in soluble solid among the tested grapes, and the titratable acid increased from 3.91 g·L^{-1} to 9.00 g·L^{-1}($p > 0.05$), suggesting that the polyphenols of graps were not affected by the contents of titritable acid and soluble solid.

As shown in Table 2, the vast majority of grape were localized in the seeds; these results were in agreement with previous studies (Ćurko et al., 2014; Santos et al., 2011). The tested grape cultivars were planted in the same area and grown in identical natural conditions. The results showed that the differences in the mass amounts of total phenols, flavanols and tannins in different grapes. This was similar to those reported in previous studies (Pantelić et al., 2016; Trošt et al., 2016). The phenolics of grapes depend on many factors, including climate, ripeness, grape variety and viticulture practices (Cinthia et al., 2013; Mendes Lopes et al., 2016). The profile of the grapes cultivars represents a determinant factor in the phenolics' components.

Grape skins and seeds contain high levels of functional compounds, which are known to play a beneficial role in human health (Karnopp et al., 2015; Niu et al., 2016; Pantelić et al., 2017; Trošt et al., 2016). These compounds can be transferred to wine during fermentation (Nogales-Bueno et al., 2017), further influencing the quality of the wine (Zhang et al., 2017). Grape skins contain abundant anthocyanidins and flavonoids, which mainly influence the color and aroma of the wine (Jin et al., 2010; Obreque-Slier et al., 2010). The most abundant phenolics in grape seeds were flavan-3-ols (Pantelić et al., 2016), which regulation the bitterness, astringency and structure of wines (Rodríguez Montealegre et al., 2006). Thus, it is necessary to pay more attention to the grape skins and seeds during wine-making and in grape products.

3.3 SDS-PAGE experiments

The electrophoresis results are presented in Figures 1-3. Figure 1 shows that the extraction of grape skins or seeds interacted with saliva protein had a different intensity, namely that the phenolic contents in grape skins and seeds have differences.

Figure 1-B shows the optical density values of grape skins' extracts combined with the saliva protein obtained from the gel bindings (Figure 1-A). The values of optical density varied between 16.37 in Blue French skin and 28.67 in Cabernet Franc skin. Similarly, Figure 1-D showed the optical density values of protein bands (Figure 1-C) obtained from the saliva protein interacted with grape seed extract. The Ruby Cabernet seed extraction showed the highest ability to precipitate saliva compared with the others.

SPI value was obtained by calculating the percentage of reduction in the optical density of the stripes (at 55 kDa) after interaction with the sample extracts. Significant differences were found in the SPIs between skin and seed extracts for the eight cultivars from the Figure 2. The SPI values obtained from the grapes' seeds were all higher than 80%, while the SPI values from the grape skins were less than 60%. It is obvious that the seed extracts of all grape cultivars had a better affinity for saliva proteins than the skins.

The skin and seeds from a tested grape variety were randomly selected for the electrophoresis experiment. The results are shown in Figures 3-A and B. The optical density values were 25.20 (skin) and 6.30 (seed), and the SPI values, respectively, were 36.04% and 84.01%. This also showed that the phenolic substances in grape seeds precipitate protein better than in grape skins.

As reported by other studies (Ćurko et al., 2014; Pantelić et al., 2016; Yilmaz et al., 2015), the seeds contained higher total content of phenolics compared with skins. Astringency is described as a mouth-feel of dryness, roughness and puckering, which results from the interaction of phenolics with salivary proteins. Therefore, grape seed polyphenols have a more significant impact on the wine mouth-feel. Thus, it is necessary to avoid the destruction

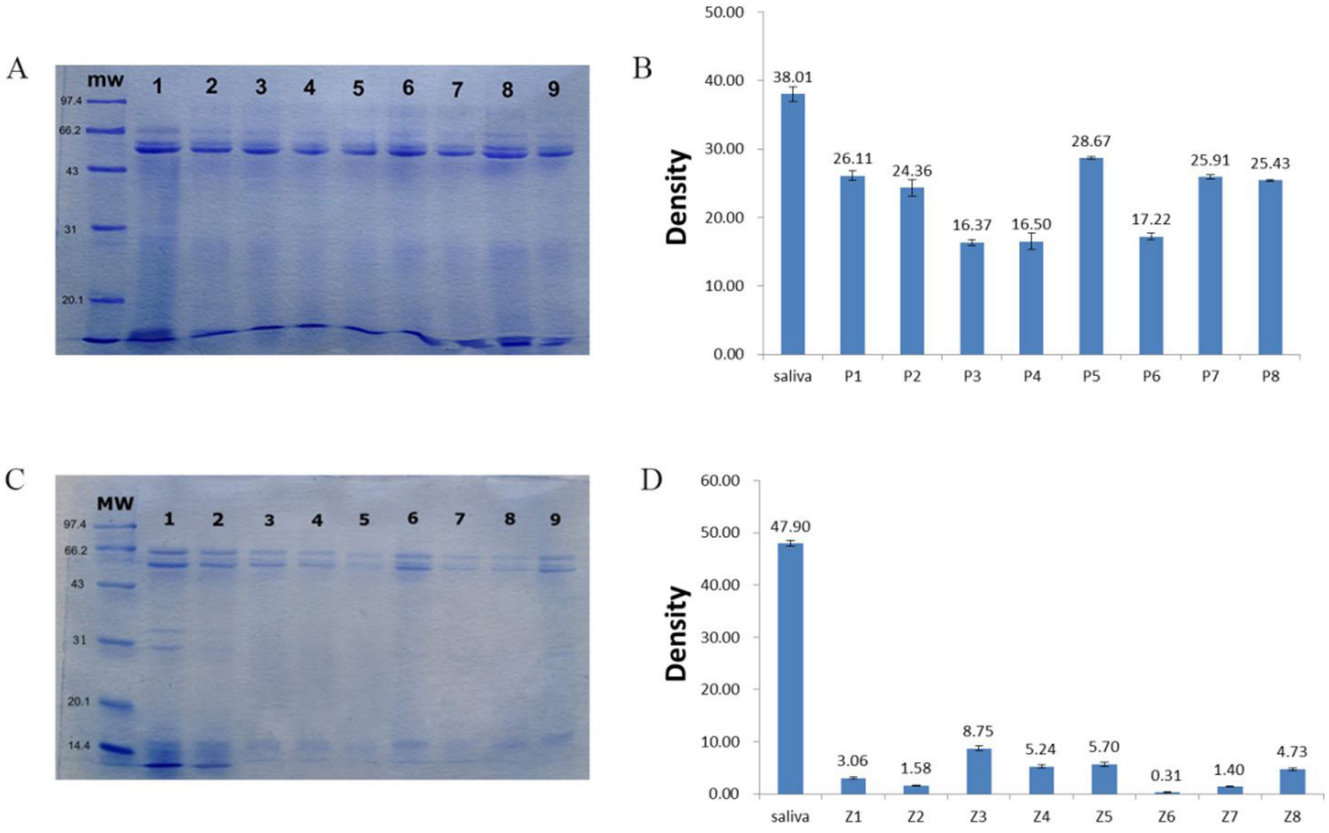

Figure 1. (A) The SDS-PAGE of the saliva supernatant after the binding reaction between the saliva and different skins. MW: markers [molecular mass (kDa) as marked on the left side]; (B) The density (provided by densitometry) of salivary protein bands after the reaction of HS (human saliva) with different skins. (C) SDS-PAGE of saliva supernatant after binding reaction between saliva and different seeds. (D) The density (provided by densitometry) of salivary protein bands after reaction of HS (human saliva) with different seeds.

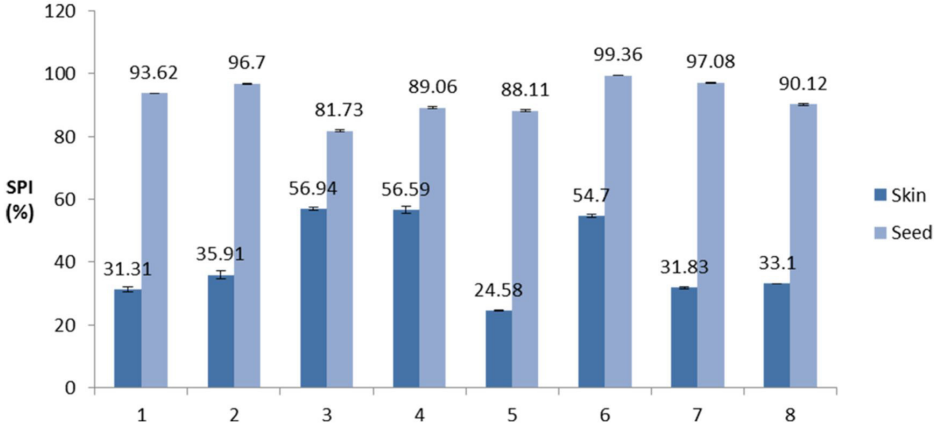

Figure 2. The SPI of skins and seeds of tested grapes.

of seeds by external forces to prevent the release of excessive bitterness into the wine (Nogales-Bueno et al., 2017).

3.4 Fluorescence spectra research

The skin and seed extracts of the grapes were analyzed by fluorescence spectrum. Three kinds of grape varieties including Cabernet Sauvignon, Merlot, and Cabernet Gernischet were demonstrated in the Figure 4. Figure 4 presents the fluorescence emission spectrum obtained from BSA upon addition of the skin and seed extracts; the fluorescence quenching of BSA in the presence of extracts from grape skins and seeds was evaluated.

This study used fluorescence spectrometry based on the simulated conditions of the human physiological conditions (pH = 7.4) to study the phenolics. A decrease in the fluorescence intensity, caused by quenching of skin and seed extracts, was observed. The fluorescence spectrum curves of skin were

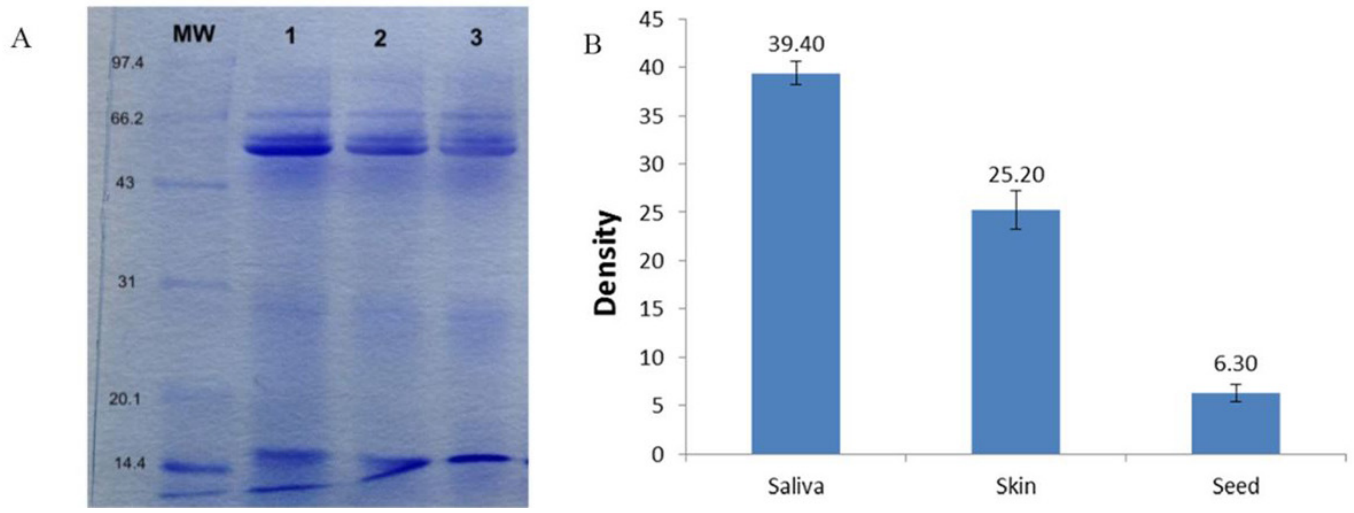

Figure 3. (A) SDS-PAGE of the saliva supernatant after binding reaction between saliva and skins and seeds. MW: markers [molecular mass (kDa) as marked on the left side]; (B) The density (provided by densitometry) of salivary protein bands after reaction of HS (human saliva) with skins and seeds.

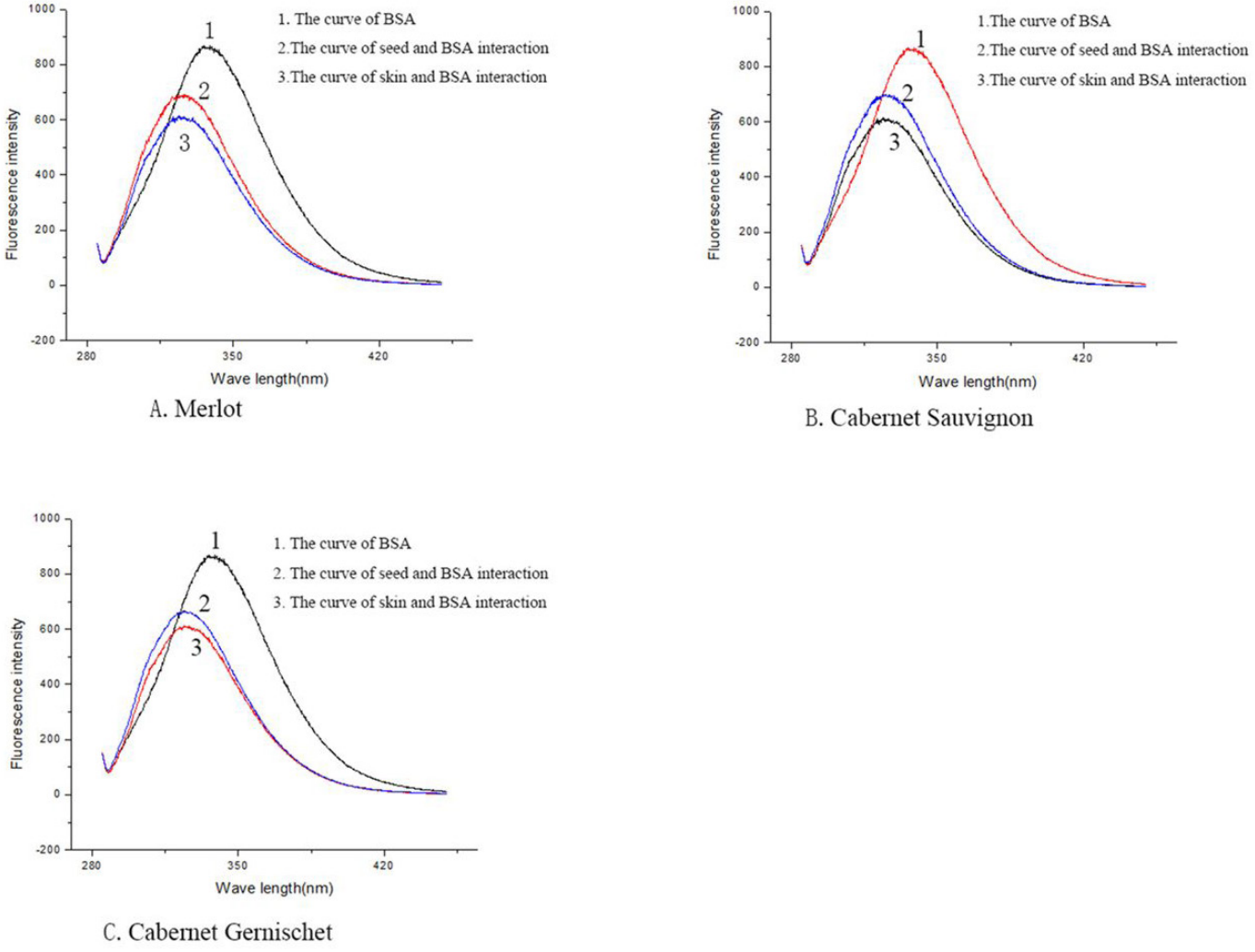

Figure 4. Fluorescence quenching of different skins or seeds and BSA interaction at 25 °C.

demonstrated to be lower than those of seeds. This indicates that grape skin extracts had a better affinity for proteins.

As shown previously, this study showed a higher amount of phenolics are present in grape seeds than skins. Figure 2 and 3 showed that the saliva proteins were more precipitated by the seed extracts than skin extracts. However, the result of Figure 4 presented another phenomenon. The result of the fluorescence spectrometry precisely illustrated that the skin polyphenols combined with BSA were a more complex reaction than the seed polyphenols.

In the grape berry, the content and structure of phenolics vary according to the location of the tissues. Grape skins contain abundant anthocyanidins, which are primarily responsible for the color of the wine. Skin polyphenols are polymerized to a greater extent than the seeds, and the polymeric fraction is also more than in the seeds (Monagas et al., 2003; Pantelić et al., 2016). This could be the reason for the skin phenolics exerting a greater influence on the BSA.

4 Conclusion

This study aimed to investigate the interactions of proteins and phenolics from different tissues of grapes and provide useful information for the potential astringency of grapes and blending them for wine-making. The tested grape varieties showed significant differences in phenolic content, while the grape seeds had more phenolic compounds than the skins. The SDS-PAGE experiment demonstrated that the polyphenols in grape seeds could precipitate more proteins than in grape skins. However, the interaction of skin phenolics and BSA were more complex than the combination of seed phenolics with proteins. These results may help evaluate the potential astringency of grapes and improve the wine-making process. The characteristics of some red wines and properties of grape cultivars make it feasible to use skins and seeds from different varieties individually and directly into the liquor-making raw material to obtain higher quality wine.

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