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 combining a complex set of sensations related to drying, roughening and puckering of the mouth epithelium and caused primarily by the interaction of polyphenols with salivary proteins (Gavel, 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 deficit 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,
The total phenolic content (TPC) in the grape skins and seeds was measured by the Folin-Ciocalteu method, as described in previous reports (Oora et al., 2015; Xu et al., 2010). A volume of 0.1 mL skin or seed extract was mixed with 3 mL 0.1% Folin-Ciocalteu solution (0.1% in 1 mol/L HCl in methanol), the mixture was allowed to stand for 10 min. The absorption at 760 nm was measured in a 751-GD model UV/Vis spectrophotometer (Shanghai, China), every extract was replicated twice.

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 510 nm was measured at a 751-GD model UV/Vis spectrophotometer (Shanghai, China), each sample was measured two times.

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 FeCl₃ 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
Table 1. Some parameters of different grape cultivars.

| Cultivars | Grain weight (g) | Grain size (mm) | Fruit shape index | Skin | Weight (g) | percentage (%) | Seed | Weight (g) | percentage (%) | Pulp | Weight (g) | percentage (%) |
|-----------|------------------|-----------------|-------------------|------|-------------|---------------|------|-------------|---------------|------|-------------|---------------|
| G1        | 1.08 ± 0.10      | 12.57 ± 0.13    | 12.94 ± 0.70      | 0.13 | 0.27 ± 0.02  | 25.13         | 0.07 ± 0.005 | 6.17          | 0.74 ± 0.014 | 68.70 |           |                |
| G2        | 2.60 ± 0.03      | 17.05 ± 0.30    | 16.54 ± 0.41      | 0.99 | 0.32 ± 0.024 | 12.47         | 0.19 ± 0.003 | 7.26          | 2.15 ± 0.053 | 80.27 |           |                |
| G3        | 1.41 ± 0.09      | 14.24 ± 0.48    | 13.86 ± 0.56      | 0.97 | 0.15 ± 0.012 | 10.67         | 0.09 ± 0.004 | 6.07          | 1.17 ± 0.095 | 83.26 |           |                |
| G4        | 1.66 ± 0.07      | 14.10 ± 0.55    | 14.21 ± 0.35      | 1.01 | 0.18 ± 0.017 | 10.66         | 0.11 ± 0.002 | 6.39          | 1.37 ± 0.046 | 82.95 |           |                |
| G5        | 2.40 ± 0.10      | 15.60 ± 0.42    | 16.53 ± 0.48      | 1.06 | 0.21 ± 0.022 | 8.68          | 0.06 ± 0.004 | 2.55          | 2.13 ± 0.049 | 88.77 |           |                |
| G6        | 1.99 ± 0.07      | 15.02 ± 0.58    | 15.99 ± 0.53      | 1.06 | 0.17 ± 0.009 | 8.64          | 0.12 ± 0.005 | 5.82          | 1.71 ± 0.022 | 85.54 |           |                |
| G7        | 2.40 ± 0.05      | 16.12 ± 0.34    | 17.22 ± 0.42      | 1.07 | 0.20 ± 0.016 | 8.34          | 0.08 ± 0.004 | 3.49          | 2.12 ± 0.054 | 88.17 |           |                |
| G8        | 2.05 ± 0.11      | 15.32 ± 0.45    | 15.58 ± 0.51      | 1.02 | 0.16 ± 0.025 | 7.67          | 0.06 ± 0.004 | 2.59          | 1.83 ± 0.057 | 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 | 1.65 |           | 83.38       |

Note: Different letters in each column represent significant differences at p ≤ 0.05.
Table 2. Total phenolic, flavanol and tannin contents of different grape skins and seeds.

| Cultivars | TPC (mg·100 g~1 FW) | TFA (mg·100 g~1 FW) | TA (mg·100 g~1 FW) | Titratable acid (g·L~1) | Soluble solid (g·L~1) |
|-----------|---------------------|---------------------|---------------------|-------------------------|-----------------------|
|           | Skin                | Seed                | Skin                | Seed                    |                       |
| G1        | 125.11 ± 1.16~a     | 153.70 ± 3.81~b    | 14.99 ± 0.92~b     | 172.71 ± 4.84~c        | 81.08 ± 7.81~d        |
| G2        | 84.37 ± 0.78~c     | 305.62 ± 6.84~d    | 8.78 ± 0.32~c      | 410.14 ± 7.43~e        | 25.98 ± 6.51~f        |
| G3        | 61.44 ± 0.87~g     | 186.10 ± 0.43~h    | 7.38 ± 0.47~i      | 229.73 ± 8.67~j        | 26.10 ± 14.14~k       |
| G4        | 42.32 ± 0.38~l     | 114.12 ± 0.58~m    | 6.06 ± 0.22~n      | 123.07 ± 13.79~o       | 33.94 ± 10.13~p       |
| G5        | 47.07 ± 0.91~q     | 32.27 ± 0.77~r     | 6.15 ± 0.30~s      | 60.22 ± 3.87~t         | 17.52 ± 1.97~u        |
| G6        | 73.34 ± 4.44~v     | 125.83 ± 0.50~w    | 27.46 ± 4.03~x      | 129.43 ± 8.78~y        | 17.52 ± 1.97~u        |
| G7        | 47.99 ± 0.45~z     | 90.09 ± 2.62~aa    | 5.56 ± 0.11~ab      | 98.76 ± 5.59~ac        | 26.73 ± 6.44~ad       |
| G8        | 59.88 ± 0.30~bb    | 55.79 ± 0.33~cc    | 4.80 ± 0.12~dd      | 49.08 ± 0.48~ee        | 28.44 ± 2.18~ff       |
| Range     | 42.32 - 125.11     | 32.27 - 305.62     | 4.80 - 27.46       | 49.08 - 410.14          | 17.52 - 81.08         |
| Average   | 67.69               | 132.94             | 10.15              | 159.14                  | 33.81                 |

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

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 addition, 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 grapes were not affected by the contents of titratable acid and soluble solid.

As shown in Table 2, the vast majority of grape were localized in these results; these were in agreement with previous studies (Curko 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.

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.

Spirulina 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.

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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 anthocyaninds, 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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Reference

Balik, J., & Kumsta, M. (2008). Evaluation of Colour Content in Grapes Originating from South Moravia. Czech Journal of Food Sciences, 26(Special Issue), S18–S24. http://dx.doi.org/10.17221/240/2008-CJFS.

Brooks, L., McCloskey, L., McKesson, D., & Sylvan, M. (2008). Adams-Harbertson Protein Precipitation-Based Wine Tannin Method Found Invalid. Journal of AOAC International, 91(5), 1090-1094. PMid:18980123.

Cáceres, A., Pena-Neira, A., Galvez, A., Obreque-Slier, E., Lopez-Solis, R., & Canals, J. M. (2012). Phenolic compositions of grapes and wines from cultivar cabernet Sauvignon produced in Chile and their relationship to commercial value. Journal of Agricultural and Food Chemistry, 60(35), 8694-8702. http://dx.doi.org/10.1021/jf301374t. PMid:22860632.

Chen, W. K., He, F., Wang, Y. X., Liu, X., Duan, C. Q., & Wang, J. (2018). Influences of Berry Size on Fruit Composition and Wine Quality of Vitis vinifera L. cv. ‘Cabernet Sauvignon’ Grapes. South African Journal of Enology and Viticulture, 39(1), 67-76. http://dx.doi.org/10.21548/39-1-2439.

Cinthia, C. B., Correa, L. C., Silva, J. K., Batista, A. G., Furlan, C. P. B., Basoto, A. C. T., Pereira, G. E., Rybka, A. C. P., Maróstica Júnior, M. R. (2013). Tropical Isabella Grape Juices: Bioactive compounds and antioxidant power depends on harvest season. Journal of Food Science and Engineering, 3(2), 64-70.

Curko, N., Kovačević Ganić, K., Gracín, L., Dapić, M., Jourdes, M., & Teissedre, P. L. (2014). Characterization of seed and skin polyphenol extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. Food Chemistry, 145, 15-22. http://dx.doi.org/10.1016/j.foodchem.2013.07.131. PMid:24128443.

De Pascali, S. A., Coletta, A., Del Coco, L., Basile, T., Gambacorta, G., & Fanizzi, F. P. (2014). Viticultural practice and winemaking effects on metabolic profile of Negroamaro. Food Chemistry, 161, 112-119. http://dx.doi.org/10.1016/j.foodchem.2014.03.128. PMid:24837928.

Ferrer-Gallego, R., Goncalves, R., Rivas-Gonzalo, J. C., Escribano-Balión, M. T., & de Freitas, V. (2012). Interaction of phenolic compounds with bovine serum albumin (BSA) and alpha-amylase and their relationship to astringency perception. Food Chemistry, 135(2), 651-658. http://dx.doi.org/10.1016/j.foodchem.2012.04.123. PMid:22868141.

Ferrer-Gallego, R., Hernández-Hierro, M. J., Rivas-Gonzalo, J. C., G. & Escribano-Balión, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. Food Research International, 62, 1100-1107. http://dx.doi.org/10.1016/j.foodres.2014.05.049.

Garcia-Esparza, M. J., Abrisqueta, I., Escrìche, I., Intrigliolo, D. S., Álvarez, I., & Lizama, V. (2018). Volatile compounds and phenolic composition of skins and seeds of ‘Cabernet Sauvignon’ grapes under different deficit irrigation regimes. Vitis, 57, 83-91.

Gawel, R. (1998). Red wine astringency: a review. Australian Journal of Grape and Wine Research, 4(2), 74-95. http://dx.doi.org/10.1111/j.1755-0238.1998.tb00137.x.

Harrison, R. (2017). Practical interventions that influence the sensory attributes of red wines related to the phenolic composition of grapes: a review. International Journal of Food Science & Technology, 53(1), 3-18. http://dx.doi.org/10.1111/ijfs.13480.

Iora, S. R. F., Maciel, G. M., Zielinski, A. A. F., Silva, M. V., Pontes, P. V. A., Haminuki, C. W., & Granato, D. (2015). Evaluation of the bioactive compounds and the antioxidant capacity of grape pomace. International Journal of Food Science & Technology, 50(1), 62-69. http://dx.doi.org/10.1111/ijfs.12583.

Jin, Z.-M., Bi, H.-Q., Liang, N.-N., & Duan, C.-Q. (2010). An Extraction Method for Obtaining the Maximum Non-Anthocyanin Phenolics from Grape Berry Skins. Analytical Letters, 43(5), 776-785. http://dx.doi.org/10.1080/00032710903846351.

Karnopp, A. R., Figueroa, A. M., Los, P. R., Teles, J. C., Simões, D. R., Barbano, A. C., Kubiani, F. T., Oliveira, J. G. B., & Granato, D. (2015). Effects of whole-wheat flour and Bordeaux grape pomace (Vitis labrusca L.) on the sensory, physicochemical and functional properties of cookies. Food Science and Technology (Campinas), 35(4), 750-756. http://dx.doi.org/10.1590/1678-457X.0010.

Katalinić, V., Možina, S. S., Škroza, D., Generalič, I., Abramovič, H., Miloš, M., Ljubekov, I., Piskernik, S., Pezo, I., & Terpinc, P. (2010). Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in...
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Processing and Preservation, 39(6), 1682-1691. http://dx.doi.org/10.1111/jfpp.12399.

Yu, J., & Ahmedna, M. (2013). Functional components of grape pomace: their composition, biological properties and potential applications. International Journal of Food Science & Technology, 48(2), 221-237. http://dx.doi.org/10.1111/j.1365-2621.2012.03197.x.

Zhang, M. X., Liu, C. H., Nan, H. J., & Li, Z. (2015). Phenolic compound profiles in skins of white wine and table grape cultivars grown in the national grape germplasm resource nursery of China. South African Journal of Enology and Viticulture, 36(1), 154-164. http://dx.doi.org/10.21548/36-1-948.

Zhang, N., Liu, X., Jin, X., Li, C., Wu, X., Yang, S., Ning, J., & Yanne, P. (2017). Determination of total iron-reactive phenolics, anthocyanins and tannins in wine grapes of skins and seeds based on near-infrared hyperspectral imaging. Food Chemistry, 237, 811-817. http://dx.doi.org/10.1016/j.foodchem.2017.06.007. PMid:28764071.