JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Reactivity of Polymeric Proanthocyanidins toward Salivary Proteins and Their Contribution to Young Red Wine Astringency

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ABSTRACT: Recent studies have indicated the presence of significant amount of highly polymerized and soluble proanthocyanidins in red wine and such compounds interacted readily with proteins, suggesting that they might be particularly astringent. Thus, the objective of this work was to verify the astringency of polymeric proanthocyanidins and their contribution to red wine astringency. The precipitation reactions of the purified oligomeric procyanidins (degree of polymerization ranging from 2 to 12−15) and polymeric procyanidins (degree of polymerization ranging from 12−15 to 32−34) with human salivary proteins were studied; salivary proteins composition changes before and after the reaction was verified by SDS-PAGE and procyanidins composition changes by spectrometric, direct HPLC and thiolysis-HPLC methods. The astringency intensity of these two procyanidin fractions was evaluated by a sensory analysis panel. For verifying the correlation between polymeric proanthocyanidins and young red wine astringency, the levels of total oligomeric and total polymeric proanthocyanidins and other phenolic composition in various young red wines were quantified and the astringency intensities of these wines were evaluated by a sensory panel. The results showed that polymeric proanthocyanidins had much higher reactivity toward human salivary proteins and higher astringency intensity than the oligomeric ones. Furthermore, young red wine astringency intensities were highly correlated to levels of polymeric proanthocyanidins, particularly at low concentration range (correlation coefficient r = 0.9840) but not significant correlated to total polyphenols (r = 0.2343) or other individual phenolic compounds (generally r < 0.3). These results indicate the important contribution of polymeric proanthocyanidins to red wine astringency and the levels of polymeric polyphenols in red wines may be used as an indicator for its astringency.

KEYWORDS: *proanthocyanidins, salivary proteins, red wine, astringency, reactivity*

INTRODUCTION

Astringency is defined as "the complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as tannins".[1] It is generally accepted that polyphenols, particularly proanthocyanidins (or condensed tannins), are responsible for astringency of plant-derived foods owing to their complexation with salivary proteins. Astringency is an essential characteristic of red wine. Chemically, this sensation is related to the ability of wine proanthocyanidins to precipitate human salivary proteins.[2,3] The interactions between salivary proteins and tannins have been intensively studied.[4−10] Protein−tannin reactions depend on the pH and the composition of the proteins and the tannins.[6,11] The proline-rich proteins, as found in saliva, were confirmed to be the essential agent for these reactions.[6] Alternatively, it was reported that the relative astringency of proanthocyanidins was related to their degree of polymerization (DP).[2,12] According to these authors, the intensity of astringency of proanthocyanidins increases with molecular size at least up to DP = 6−7 then decreases because higher proanthocyanidins became, or no longer soluble[12] or too bulky to bind to the protein.[13] However, several studies have shown that proanthocyanidins presented in grape and red wine were essentially in higher polymerized forms.[14−18] Grape seed tannins are composed of water-soluble procyanidins ranging from 2 to 33;[19] grape stems consist of mainly procyanidins, with small amount of prodelphinidins, with the degree of polymerization ranging from 2 to 28 in aqueous solution,[15,20] while grape skins contain tannins consist of soluble procyanidins and prodelphinidins, with degree of polymerization from 2 up to 83.[14] In red wine, the mean degree of polymerization of polymeric proanthocyanidins fraction was determined to be 22.[21] Furthermore, such water-soluble and highly polymerized proanthocyanidins could be selectively precipitated by fining proteins,[8,22] indicating that they can readily interact with proteins and thus suggesting that they could be particularly astringent.[23]

The usual method for estimating astringency of red wine is sensory analysis by a tasting panel, which is always subject to certain subjectivity.[24] Analytical methods for this estimation are generally based on protein-polyphenol interactions. The most suitable proteins for estimating astringency would be human salivary proline-rich proteins,[25] but there is no such product commercially available, probably due to their highly complicated purification procedure.[26] Thus, gelatin[26] and more recently ovalbumin,[25] the two types of the most used proteins for fining red wine, have been selected as precipitation agents to estimate red wine astringency. However, the published data showed that both these analytical methods were not well correlated with sensorial astringency estimation of red wines,[27] although the latter method, by using ovalbumin to estimate red wine astringency, presented better reproducibility and better correlation with sensory analysis than that using gelatins.[25]

Received: August 28, 2012
Revised: January 7, 2013
Accepted: January 7, 2013
Published: January 7, 2013

Moreover, Cliff et al.[28] proposed a predictive model for astringency from anthocyanin, phenolic, and color analyses of red wines. However, proanthocyanidin levels were not quantified and no satisfactory correlation between chemical and sensory analysis was established. Since polymeric proanthocyanidins were suggested to be extremely astringent,[23] it is a reasonable assumption that the levels of these compounds in red wine might be mostly correlated to its astringency intensity. In our previous work,[21] we have established one method for quantitative separation of grape and wine proanthocyanidins into monomeric, oligomeric, and polymeric fractions. The modified vanillin assay as described[29] permits quantification of proanthocyanidins in each fraction. Furthermore, the same separation procedure was extended in preparative scale, allowing us to obtain two highly pure proanthocyanidin fractions—oligomers (DP ranging from 2 to 12−15; mean DP = 7.2) and polymers (DP ranging from 12−15 to 32−34; mean DP = 25.2) from grape seed.[19] Thus, the objective of the present work was to give some experimental evidence about astringency of polymeric proanthocyanidins and to study the relationship between polymeric proanthocyanidins and astringency in red wines. For this reason, we have first verified and compared the reactivity of purified oligomeric and polymeric proanthocyanidins fractions toward human salivary proteins; the astringency intensity of these two fractions was evaluated by a sensory panel. Then we determined the levels of total oligomeric and total polymeric proanthocyanidins and other phenolic compounds in various selected red wines. The astringency intensities of these wines were analyzed by a tasting panel.

MATERIALS AND METHODS

Reagents. All solvents used were of analytical or HPLC grade. (+)-Catechin and (−)-epicatechin were purchased from Fluka A. G. (Buchs, Switzerland). Procyanidins B1, B2, B3, B4, B1−3-O-gallate, B2−3-O-gallate, B2−3′-O-gallate, trimer C1, and trimer T2 were isolated from grape seeds, in our laboratory, by column chromatography on Toyopearl TSK HW-40 (F) and semipreparative HPLC, as described earlier.[30] Oligomeric and polymeric procyanidin fractions were isolated and purified using column chromatography on LiChroprep RP 18 as described.[19] Reversed-phase HPLC, thiolysis-HPLC, ESI−MS analyses and elemental analysis showed that the two procyanidin fractions were highly pure (93.0 ± 1.3% and 92.2 ± 1.8%, respectively), with mean degrees of polymerization of 7.2 and 25.2, and percentages of galloylation of 28.8 and 35.1, respectively.[19]

Red Wine Samples. Eleven one-year-old young red wines (2010 harvest) of different wine regions of Portugal were purchased from the local market, of which four from Alentejo region (Parameiras, Galitos, Nevegante, and Monte Grande), one from Dão region (Dão), one from Setubal Peninsula region (Regões), two from the Lisbon region (Cerejeira and Vinho Regional de Lisboa) and one from the Tejo region (Monte Casaleiro).

Preparation of Salivary Proteins. Human saliva was collected from 5 healthy volunteers in an ice-cooled tube, centrifuged at 10 000 *g* for 10 min at 2−4 °C. The supernatant, using as salivary protein sample,[1] was carefully taken and used immediately.

Preparation of Oligomeric and Polymeric Procyanidins Solutions. Oligomeric and polymeric procyanidins solutions were prepared by dissolving the purified oligomeric or polymeric procyanidins in a model wine solution with final concentration ranging from 1.5 to 6.0 g/L. The model wine solution was ethanol−water (10/90; v/v) solution with 2 g/L tartaric acid and at pH 3.5 adjusted by KOH, as reported by Yokotsuka and Singleton.[31]

Procyanidins−Salivary Proteins Precipitation Reaction. In test tubes, 0.5 mL of purified oligomeric procyanidin or polymeric procyanidin solution in varying concentrations (1.5−6.0 mg/mL) was mixed with 0.5 mL of freshly prepared salivary proteins. The tubes were kept in a bath at 30 °C during 30 min with agitation, followed by centrifugation at 10 000 *g* for ten minutes to separate supernatant and residue. For verifying the reactivity, procyanidins contents in the initial reaction solution and in the supernatant after the reaction were determined by Folin−Ciocalteau assay and by HPLC; structural compositional changes in procyanidins before and after the reaction between 0.5 mL of procyanidins (6 mg/mL) and 0.5 mL freshly prepared salivary proteins were also verified by thiolysis-HPLC and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) methods, as described below.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Separation and analysis of salivary proteins before and after their reaction with procyanidins were carried out by SDS-PAGE using the Mighty Small II SE 250 vertical electrophoretic system from Hoefer/Pharmacia. Twenty-five microlitres of the sample to be electrophoresed were mixed with equal volume of loading buffer (Tris-HCl 0.125 M, SDS 4%, glycerol 20%, DTT 0,2 M, bromophenol blue 0.02%, pH 6.8) and heated in a water bath at 100 °C for 5 min. Electrophoresis of 10 μL of each treated sample was run on a 8 × 7 cm and 0.75-mm thick, 12.5% w/v denaturating polyacrylamide gel, overlaid with 2,5% w/v polyacrylamide stacking gel, according to Hames.[32] Two molecular weight markers (SigmaMarkers), high range (molecular weight 36 000−205 000 Da) and low range (molecular weight 6500−66 000 Da) were also loaded in the gel. Electrophoresis was carried out at 4 °C with a constant voltage of 100 V, until the tracking dye (bromophenol blue) reached the bottom of the gel (approximately 2 h). After electrophoresis separated proteins were revealed by using a silver stain protocol based on the procedure proposed by Merril et al.[33] The gel was photographed and the relative mobility (Rm) of the bands was calculated as the quotient between the distance migrated by the protein over the distance migrated by the tracking dye, bromophenol blue.

Folin−Ciocalteau Assay. Folin−Ciocalteau assay was used for total soluble procyanidins before and after procyanidins-salivary proteins precipitation reactions, according to Singleton and Rossi.[34]

HPLC Analysis. HPLC analysis of individual catechins and procyanidins (dimers and trimers) were performed by HPLC as described by Sun et al. [21]

Thiolysis-HPLC. Acid-catalyzed degradation of procyanidins in the presence of toluene-α-thiol, followed by HPLC analysis, in order to verify the structural compositional changes in procyanidins before and after procyanidins-salivary proteins interaction, was performed as described earlier.[35] The residue was dissolved in 0.2% SDS methanol before thiolysis-HPLC, as described by Sarni-Manchado et al.[8]

Vanillin Assay. Vanillin assay for oligomeric and polymeric proanthocyanidins in red wines was carried out according to Sun et al..[21,29]

Total Polyphenols Analysis. Total polyphenols content of red wines was determined by spectrophotometric method using catechin as reference standard as described by Ribereau-Gayon.[36]

Sensory Evaluation. The sensory panel was composed of 12 judges who participated, at least once a week, in the wine sensory sessions. The judges were requested not to smoke or eat for one hour before each sensory session. Before sensory analysis of oligomeric and polymeric proanthocyanidin fractions and red wines, two training sessions were sequentially conducted using commercial grape seed procyanidins (>95% HPLC purity) for sensory panel to familiarize with astringency induced by these phenolic materials: one training session with lower concentration range (0, 75, 150, 300, 450 mg/L in water) and another one with higher concentration range (900, 1200, 1400, 1600, 2000 mg/L in water). Beyond the concentration at 1400 mg/L, the sensory panel could not detect the differences in astringency among the solutions, indicating that at this concentration level, the saturation is attained.

For verifying the astringency intensity of oligomeric procyanidins and polymeric procyanidins, the isolated oligomeric procyanidins and polymeric procynidins were dissolved respectively in distilled water in three different concentrations (250, 500, and 1000 mg/L) and evaluated by the previously trained sensory panel. Because alcohol,

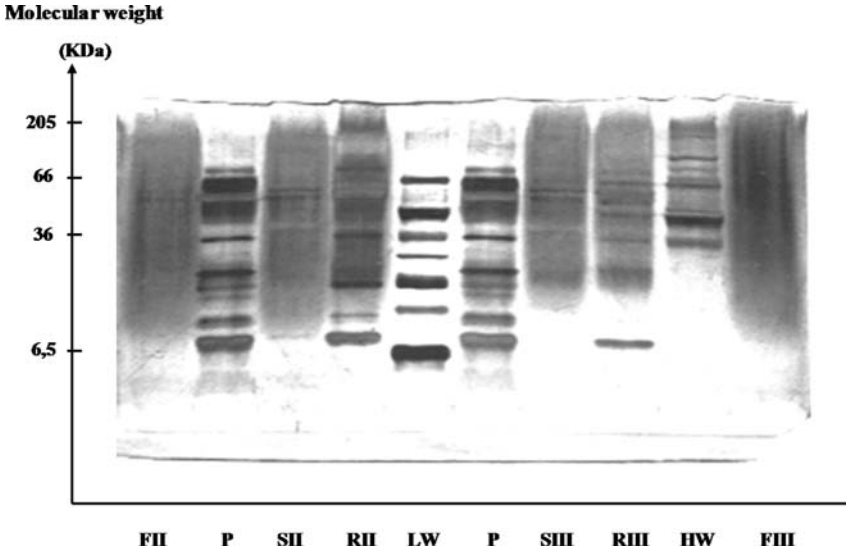

Figure 1. Separation of human salivary proteins before and after their reaction with procyanidins by gel polyacrylamide electrophoresis. FII, Oligomeric procyanidins; P, Human salivary proteins; SII, Supernatant after reaction between oligomeric procyanidins and salivary proteins; RII, residue after reaction between oligomeric procyanidins and salivary proteins; LW, low-molecular-weight marker (MW = 6.5−66 KDa); SIII, Supernatant after reaction between polymeric procyanidins and salivary proteins; RIII, residue after reaction between polymeric procyanidins and salivary proteins; HW, high-molecular-weight marker (MW = 36−205 KDa); and FIII, polymeric procyanidins.

tartaric acid, and metal ions—components composing model wine solution, can strength or alter significantly astringency and bitterness intensity, we used distilled water instead of model wine solution for preparing polyphenols solutions to avoid any possible interferences. For evaluation of the astringency of total 11 red wines, two separate sensory sessions with 6 and 5 red wines were carried out, respectively, by the same sensory panel. The judges were asked to score the aqueous procyanidins solutions or red wines according to their astringency intensity on a scale from 0 to 10 (0 = none and 10 = extreme).

Statistical Analysis. Chemical and sensory analyses were performed in triplicate. One-way analysis of variance and comparison of means (LSD, 99% level) were carried out using Statistica v´98 edition (StatSoft Inc. Tulsa, U.S.).

RESULTS

In our previous works, oligomeric and polymeric procyanidin fractions were isolated from grape seeds by column chromatography on LiChroprep RP-18 in a semipreparative scale.[30] The range of polymerization degree (DP) of oligomeric procyanidin fraction was determined to be from 2 to 12−15 (mean DP = 7.2) and that of polymeric proanthocyanidins, from 12−15 to 33−35 (mean DP = 25.2), both with high purity (>92%).[19] In this work, we have first verified the reactivity of these two procyanidin fractions toward human salivary proteins. Figure 1 showed the results obtained by separation of salivary proteins before and after their reaction with procyanidins using SDS-PAGE electrophoresis.

By comparison with molecular markers, human salivary proteins are essentially composed of low molecular proteins (6.5 − 66 kDa). For both reactions with oligomeric procyanidins (FII) and polymeric procyanidins (FIII), the migrations of supernatant and residue showed that procyanidins react nearly all salivary proteins, particularly those of lower molecule weights (proximately 6.5−36 kDa), suggesting that lower-molecular-weight proteins have higher reactivity than higher-molecular-weight proteins. Sarni-Manchado et al.[8] reported that by SDS-PAGE of human salivary proteins, the bands ranging from 16 to 67 kDa corresponded to proline-rich proteins (PRP), which were considered to be the major target for the reaction with procyanidins.[6,9] Furthermore, the results obtained by SDS-PAGE electrophoresis do not permit distinguishing clearly the reactivity of FII and FIII toward salivary proteins.

Figure 2 presents the results obtained by quantification of procyanidins before and after reaction using Folin−Ciocalteau method.

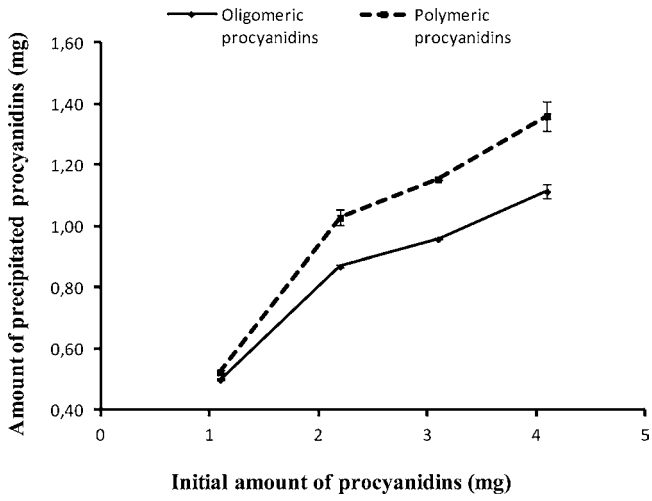

Figure 2. Reactivity of oligomeric and polymeric procyanidins toward salivary proteins evaluated by Folin−Ciocalteau assay.

From Figure 2, it can be seen that increasing the initial amount of procyanidins in the reaction solution significantly increases the amount of precipitated procyanidins after the reaction, which is in agreement with the previous work.[8] Furthermore, the increase in amount of precipitated procyanidins is more accentuated for polymeric procyanidins/salivary proteins reaction than for oligomeric procyanidins/

dx.doi.org/10.1021/jf303704u | J. Agric. Food Chem. 2013, 61, 939−946

Table 1. Structural Composition of Procyanidins before and after Their Reaction with Human Salivary Proteins[a]

fraction		relative percentage of structural units (%)						
		terminal unit			extension unit			
		cat	epi	epiG	*cis*-cat	*trans*-cat	epicat	epiG
oligomeric procyanidins	before reaction	5.5b ± 0.0	4.1b ± 0.3	4.3b ± 0.1	3.1a ± 0.1	11.4b ± 0.3	47.1a ± 0.5	24.5b ± 0.4
	supernatant	6.9c ± 0.3	5.8c ± 0.1	3.5a ± 0.2	3.8b ± 0.1	13.1c ± 0.5	50.4b ± 1.3	16.5a ± 0.0
	residue	3.5a ± 0.3	1.9a ± 0.0	4.4b ± 0.1	3.1a ± 0.2	9.9a ± 0.3	47.4a ± 0.5	29.5c ± 0.6
polymeric procyanidins	before reaction	1.6a ± 0.1	0.8a ± 0.0	1.5b ± 0.0	2.3a ± 0.3	8.1a ± 0.4	52.2a ± 1.6	33.5b ± 0.9
	supernatant	2.7c ± 0.3	1.4b ± 0.1	1.6b ± 0.1	2.3a ± 0.5	8.5a ± 0.6	57.5b ± 2.3	26.0a ± 1.9
	residue	1.7b ± 0.0	0.8a ± 0.1	1.2a ± 0.2	2.6b ± 0.1	9.0b ± 0.9	58.1b ± 2.6	26.7a ± 2.3

[a]Abbreviation: cat = catechin; epicat = epicatechin; epiG = epicatechin 3-O-gallate; for the same procyanidins fraction, means ($n \pm 3$) followed by the same letter in a column are not significantly different (LSD, 5%).

Table 2. Characteristics of Procyanidins before and after Their Reaction with Human Salivary Proteins[a]

fraction		mDP	%G	% procyanidins
oligomeric procyanidins	before reaction	7.2b ± 0.2	28.8b ± 0.4	99.9 ± 0.1
	supernatant	6.2a ± 0.2	20.1a ± 0.2	60.6 ± 0.8
	residue	10.2c ± 0.3	33.9c ± 0.6	39.4 ± 0.7
polymeric procyanidins	before reaction	25.2b ± 0.5	35.1b ± 0.6	100.1 ± 0.6
	supernatant	17.4a ± 1.1	27.7a ± 1.0	17.1 ± 0.8
	residue	27.7c ± 0.3	37.9c ± 0.6	82.9 ± 0.1

[a]Abbreviation: mDP = mean degree of polymerization; %G = percentage of galloylation; %procyanidins = percentage of procyanidins; for the same procyanidins fraction, means ($n \pm 3$) followed by the same letter in a column are not significantly different (LSD, 5%).

salivary proteins reaction, suggesting that polymeric procyanidins present higher reactivity than the oligomeric ones.

Alternatively, the precipitation abilities of oligomeric and polymeric procyanidins fractions with human salivary proteins were also verified by thiolysis-HPLC analysis. Thiolysis-HPLC analysis permitted a determination of structural composition and degree of polymerization of procyanidins. Table 1 presents the data of structural composition of the results of procyanidins before and after their reaction with human salivary proteins.

Table 1 shows clearly the significant variation in structural composition of procyanidins before and after their reaction with human salivary proteins. In order to better explain this variation, mean degrees of polymerization (mDP), percentages of galloylation (%G), and relative amount of procyanidins before and after their reaction were calculated from the data given in Table 1, and the results are presented in Table 2.

From Table 2, it can be seen that for either reaction with oligomeric procyanidins or with polymeric procyanidins, the mDP of procyanidins in the residue after reaction is higher than that in the initial solution before reaction, while the mDP of procyanidins in the supernatant after reaction is lower than that in the initial solution before reaction. These results indicate that human salivary proteins precipitate preferably with higher-molecular-weight of procyanidins than with lower-molecular-weight of procyanidins. These results are in agreement with those of Sarni-Manchado et al.[8] In fact, it can also be observed from Table 2 that on the basis of equivalent initial amount (6 mg/mL), 82.9% of polymeric procyanidins could be precipitated by salivary proteins, while only 39.4% of oligomeric procyanidins could be precipitated. In other words, the precipitation capacity of polymeric procyanidins with human salivary proteins is higher than the oligomeric procyanidins, indicating that polymeric procyanidins have higher reactivity toward human salivary proteins. Moreover, it can be also noted that for both fractions, the residue presented higher percentage of galloylation degree than the supernatant, indicating that the reactivity of procyanidin molecules toward human salivary proteins may be positively related to their galloylation degrees.

Moreover, direct HPLC analysis permitted determination of dimer and trimer procyanidins in the oligomeric fraction before and after its reaction with salivary proteins. The results showed that the concentrations of di- and trimer procyanidins maintained identical in the initial solution and in the supernatant after the reaction, suggesting no interaction between these low-molecular procyanidins and salivary proteins. These results are in agreement with earlier published work.[8]

Thus, these results obtained by both spectrophotometric analysis and thiolysis-HPLC may lead one to conclude that polymeric procyanidins have more precipitation capacity with human salivary proteins than oligomeric ones. In other words, the results provided experimental evidence that polymeric procyanidins would have more astringency intensity than oligomeric ones. In order to verify the astringency of oligomeric and polymeric procyanidins fractions, sensory analysis on these two fractions was performed by a sensory panel. The results are presented in Figure 3.

Figure 3 showed that on the equivalent concentrations ranging from 250 to 1000 mg/L, polymeric procyanidins present always more astringency intensity than the oligomeric ones. Thus, the results obtained by sensory analysis are in agreement with those obtained by chemical analysis described above.

Furthermore, from quantitative point of view, polymeric proanthocyanidins are major group of polyphenols in red wine. On the basis the results above, it was reasonable for us to think about the possibility of existing a positive correlation between the contents of polymeric proanthocyanidins with astringency intensity of red wines. For this reason, the concentrations of polymeric proanthocyanidins together with total polphenols and other individual catechins and procyanidins, and the astringency intensity of various one-year-old young red wines

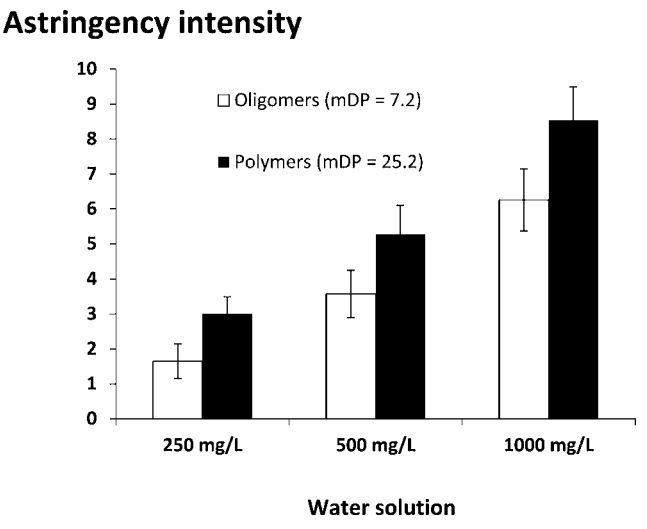

Figure 3. Astringency intensity of oligomeric and polymeric procyanidins fractions.

were evaluated by chemical and sensory analysis respectively. The results are presented in Table 3.

Significant differences in phenolic composition and in astringency intensity were observed for all tested red wines. Total polyphenols are not highly correlated to total polymeric proanthocyanidins. For example, Monte Grande red wine contains 4965.1 ± 12.1 mg/L of total polyphenols with polymeric proanthocyanidins as high as 2006.0 mg/L, while Fialhoza red wine contains 5737.8 ± 35.2 mg/L of total polyphenols with polymeric proanthocyanidins only 1437.1 mg/L. The reason for this should be mainly due to the contribution of free and complex anthocyanins to total polyphenols.

Furthermore, from the data presented in Table 3, the coefficients of correlation between phenolic compounds and astringency intensity of red wines can be determined. The astringency intensities of red wines were highly correlated to their polymeric proanthocyanidins contents (r = 0.9840). Procyanidin B4 has weak correlation with astringency intensity of red wines (r = 0.3810). However, there was no such correlation for total polyphenols (r = 0.2343), and for any other analyzed individual polyphenol (generally r < 0.3). These results would indicate that the major indicator representing red wine astringency is polymeric proanthocyanidins (at least for young red wines), but not total polyphenols, as commonly considered.

In order to better elucidate the correlation between polymeric proanthocyanidins and astringency intensity of red wines, Figure 4 presented the linear regression lines obtained at different ranges of polymeric proanthocyanidins concentrations.

When all eleven red wines were considered, the linearity of polymeric proanthocyanidins (concentration ranging from 857 to 1570 mg/L) with astringency was quite acceptable (r = 0.8264) and the t-test conformed the correlation coefficient was significant (at 95% level). However, if all red wines except for the wine with highest polymeric proanthocyanidins concentration (2006.0 mg/L) were considered, then the linearity was much improved (r = 0.9358). Moreover, if only the first six wines with lower range of polymeric proanthocyanidins concentrations from 857 to 1221 mg/L were considered, very good linearity was obtained (r = 0.9840). These results indicate that at low and normal polymeric proanthocyanidins

Table 3. Concentrations (mg/L) of Individual Procyanidins, Total Polymeric Proanthocyanidins and Total Polyphenols, And Astringency Intensity of Various Red Wines[a]

	catechin		epicatechin		B1		B2		B3		B4		T2		C1		total polyphenols		polymeric PA		astringency intensity[b]	
wine samples	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Patameiras	55.2	4.8	91.2	5.3	54.9	5.5	46.9	2.3	17.3	3.8	10.1	1.1	15.8	0.2	20.9	1.8	3902.6	2.1	856.9	60.4	4.5	2.1
Galitos	41.7	8.2	55.8	9.6	48.2	10.9	32.0	7.7	6.5	4.7	10.9	1.9	2.5	0.4	37.9	4.9	3270.6	11.8	779.4	55.7	3.1	1.6
Navegante	56.9	3.3	105.5	12.6	68.3	3.4	38.9	1.0	9.2	0.1	17.5	3.4	4.6	1.5	62.2	0.8	931.7	44.3	1221.2	276.5	6.9	1.9
Vinha do Bispado	45.4	2.6	94.1	5.7	59.1	5.7	46.4	3.8	8.9	1.0	11.6	1.5	3.1	0.5	19.0	2.3	4259.7	7.7	912.6	14.9	4.4	2.0
Fialhoza	25.3	2.9	73.1	8.1	35.1	4.9	24.0	2.9	5.0	3.7	8.7	0.5	2.4	0.4	11.2	2.6	5737.8	35.2	1437.1	80.4	8.2	1.6
Dão	68.6	2.5	81.0	3.2	72.9	6.3	43.0	0.5	7.9	1.3	16.9	0.6	6.1	1.9	19.7	3.0	5151.1	91.6	1378.6	113.1	7.6	1.1
Pegões	66.9	3.8	66.3	0.9	88.4	5.3	43.3	0.5	9.6	1.8	20.3	1.6	6.1	0.9	101.7	1.6	4805.2	11.3	1570.2	59.7	7.2	1.7
Cerejeiras	33.1	1.7	47.2	1.4	60.7	1.6	42.0	2.4	14.3	5.0	12.4	0.6	3.8	0.2	21.5	0.4	1105.0	30.8	1211.5	298.3	6.8	1.6
Casaleiro	69.3	12.9	83.6	15.6	64.3	11.8	40.0	8.0	4.9	1.2	12.6	3.4	3.4	1.1	45.9	9.4	2143.0	62.9	1069.8	44.2	6.0	1.5
Monte Grande	71.5	2.5	69.0	3.6	80.2	8.0	43.0	1.8	9.9	2.4	19.2	1.7	5.4	0.8	18.0	2.5	4965.1	12.1	2006.0	69.1	7.6	1.9
Vinho Reg. Lisboa	12.3	1.1	65.2	0.4	48.6	0.2	28.4	0.3	53.2	0.2	7.5	0.9	1.3	0.7	28.6	0.2	4562.1	8.7	1397.0	87.7	6.7	1.9

[a]B1, B2, B3, B4, T2, and C1 are procyanidins B1, B2, B3, B4, T2, and C1. [b]Astringency intensity of red wine samples was evaluated by a sensory panel composed of 12 judges on a scale from 0 to 10 (0 = none and 10 = extreme).

dx.doi.org/10.1021/jf303704u | J. Agric. Food Chem. 2013, 61, 939−946

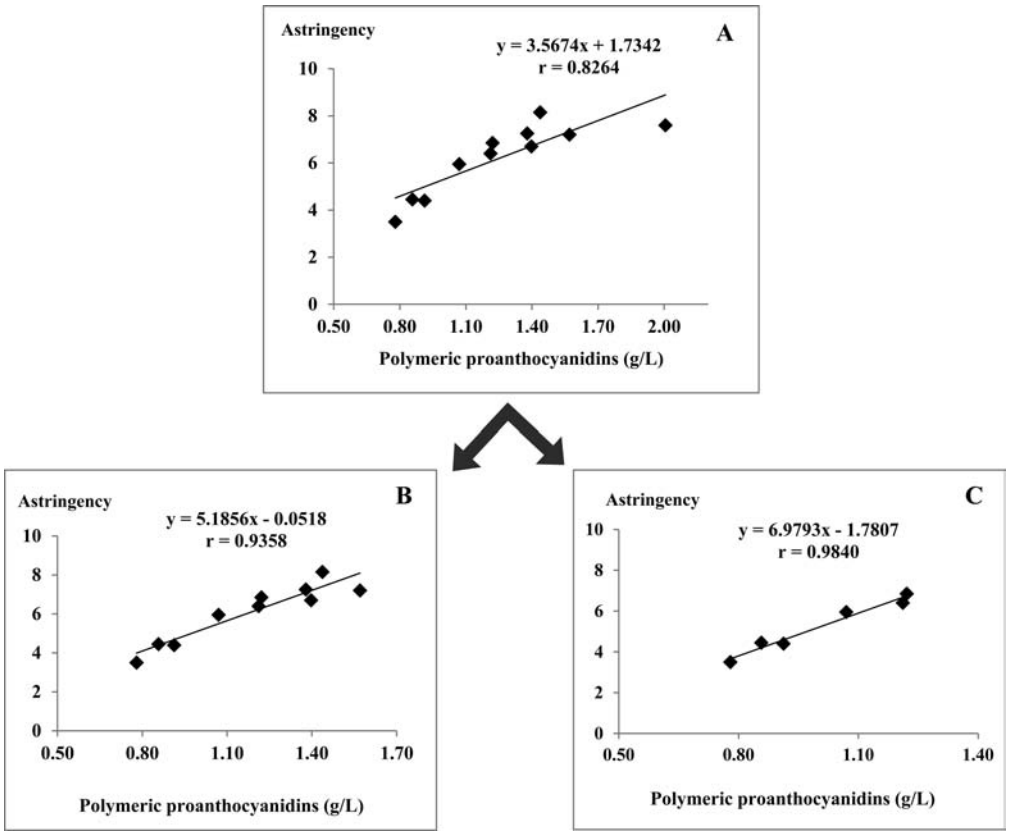

Figure 4. Linear regression lines of polymeric proanthocyanidins in different concentration ranges; A, considering all red wines samples; B, considering all red wines samples except that with highest concentration (2.006 ± 0.069 g/L); and C, considering only the first six red wines with the lowest concentrations (0.857 ± 0.06 to 1.221 ± 0.276 g/L).

concentration range, the astringency of young red wines is perfectly correlated to its polymeric proanthocyanidin concentration. However, this correlation coefficient is reduced as increases the polymeric proanthocyanidin concentration in red wines, probably because at high concentration levels, the astringency intensity was closed to or reached its saturation.

DISCUSSION

Although astringency sensation of red wine has been thought, for a long time, to be produced by the interaction of tannins with salivary proteins and that there has been considerable research work on the relationship between tannin composition and astringency of red wine, no highly satisfactory correlation between phenolic compounds and the astringency intensity in red wine has been established.[37−40] Moreover, the previous assumption about proanthocyanidins with a degree of polymerization above 10 not being water-soluble or too bulky to bind to the protein[12,13] should be excluded because the much higher molecular weight of soluble proanthocyanidins are presented in grape extracts or in red wines.[14,17,21,41−44]

This work studied, for the first time, the astringency of polymeric condensed tannins (proanthocyanidins) in grape and their contribution to red wine astringent sensation. Our previous work related to isolation and quantification of polymeric proanthocyanidins in grape and red wine[17,21] gave the possibility of this study. The first part of the work related to the reactivity of oligomeric and polymeric procyanidins toward salivary proteins provided chemical evaluation of astringency of these two fractions; polymeric procyanidins showed much higher reactivity toward salivary proteins than oligomeric ones; the sensory panel analysis of these two procyanidins fractions further confirmed the higher astringency intensity of polymeric procyandins (mDP = 25) than the oligomeric ones (mDP = 7.2). The more important results obtained by this work was that the astringency intensity of one-year-old red wines is highly correlated to their polymeric proanthocyanidins content. Interestingly, no such correlation was found with total polyphenols or any individual phenolic compounds. These results indicate the important contribution of polymeric proanthocyanidins to red wine astringency and the levels of polymeric polyphenols in red wines may be used as an indicator for its astringency. Although this study was done on the astringency of grape and wine polymeric proanthocyanidins, the results may also be suitable for other food or beverages.

It should be mentioned that in this work, only one-year-old young red wines were selected and analyzed. For old red wines, the correlation between polymeric proanthocyanidins and astringency intensity may not be so good because as aging time increases, astringency sensation would be modified due especially to the condensation reaction between tannins and anthocyanins.[45] As a consequence, astringency intensity of old red wine would depend on not only polymeric proanthocyanidins but also proanthocyanidins−anthocyanins complexes.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the graduate students Francisco Roque do Vale and Susana Cláudia Conceição for technical assistance.

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