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Taste and Mouthfeel Properties of Red Wines Proanthocyanidins and Their Relation to the Chemical Composition

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ABSTRACT: The aim of this work is to assess the relationship between the in-mouth sensory properties of proanthocyanidins (PAs) and its chemical composition. To achieve such a goal, the proanthocyanidin fraction from six different young commercial red wines was obtained by gel permeation chromatography. A sensory panel, selected on the basis of their PROP status and trained in taste and mouthfeel sensations, described both the wines and fractions. MALDI-TOF−MS and UPLC−MS were used to identify thoroughly the polyphenolic composition of each proanthocyanidin fraction. The results showed that the PAs fractions were exclusively described as astringent and persistent. The astringent subqualities studied (velvety and puckering/drying) were mainly related to the quantity of proanthocyanidins and the proportion of the extension flavanol units linked to proanthocyanidins. A significant negative correlation was found between both of the astringencies (velvety and puckering/drying). Furthermore, both subqualities appeared to contribute to the persistence. A significant correlation was observed between the astringency and the persistence data of the wines and fractions. Significant multiple linear regressions were found between the sensory astringency data and the chemical compounds analyzed. The concentration of proanthocyanidins present in young red wines is the major determinant of the differences perceived in the astringency. Additionally, the extension flavanol units linked to the proanthocyanidins seem to have a different impact on the astringent subqualities.

KEYWORDS: *wine, proanthocyanidins, sensory analysis, astringency, persistence*

INTRODUCTION

The relationship between the sensory evaluation and the nonvolatile composition of wines, especially of red wines, is a critical subject in current enological research.[1−6] Regarding the nonvolatile composition of red wines, the polyphenolic compounds play an important role because of their contribution to interesting orosensory properties such as bitterness and astringency. This mouthfeel attribute has been reported as a relevant sensory attribute in the overall quality of red wines.[7,8] Astringency is a complex oral sensation described with different terms such as drying, roughing, puckering, velvety, and so forth by experienced wine tasters and winemakers. Gawel et al.[9] developed a hierarchically structured vocabulary of mouthfeel sensations to subqualify the astringency, which was published as a "mouthfeel wheel". A comprehensive understanding of the chemical composition related to the different subqualities of the astringency in the wines would allow us to extend the knowledge of the chemical basis of astringency and to develop tools for the vinification of different red wines. The strategy pursued to determine the taste-active compounds in wines and in other foods and beverages has usually been the fractionation of the different studied matrix together with the sensory evaluation and chemical characterization of the obtained fractions.[5,10−13] Proanthocyanidins (PAs) or condensed tannins are oligomeric and polymeric flavonoids composed of elementary flavan-3-ols units linked by C−C bonds. Tannins (higher than the tetramer) have been mainly reported to elicit astringency.[11,14] Several papers suggest a positive correlation between the astringency and the PAs concentration.[1,15−18] Besides the quantity of PAs, the size and composition also seem to affect the astringency perceived as well as its subqualities. In this context, Gawel et al.[15] noticed that astringency subqualities might be related to the content of polyphenols. Jones et al.[19] found that the polymeric phenolic composition was correlated with sensory terms such as roughness and drying. Hufnagel and Hofmann[4] described as puckering astringent a polymeric fraction exhibiting molecular masses above 5 kDa. Other papers[1,10] focused on the same goal have established that the degree of polymerization is positively correlated with the astringency. Besides, on one hand, an increase in the percentage of galloylation might be responsible for the increase in coarseness. On the other hand, these authors have also observed that the trihydroxilation of the B-ring could decrease the coarseness perception. Quijada-Morin et al.,[20] studying wine proanthocyanidins, have concluded that the astringency perceived is more affected by the subunit composition than by the total concentration or the average degree of polymerization.

Even though neither the flavan-3-ol monomers nor dimers and trimers seem to contribute to astringency, there are other nonvolatile compounds of low molecular weight such as certain phenolic acids, flavonols, and even aconitic acid that can

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contribute together with PAs to the astringency perceived by the panelists tasting both the wine fractions and wines.[4,5,21,22]

In this context, an unresolved issue is to establish the quantitative and qualitative (subqualities) contribution of the tannic fraction in the astringency perceived in different wines. Thus, the aims of the present work are (1) to screen the sensory properties (taste, astringency, and persistence) of the PAs fraction obtained from six different red wines, (2) to determine whether the sensory properties of these six fractions of the PAs are related to the sensory properties of the original wines, and (3) to assess to what extent the chemical composition of these fractions is related to their sensory properties.

MATERIALS AND METHODS

Chemicals and Reagents. Tannic acid, vanillin, 6-propyl-2-thiouracil, benzyl mercaptan (toluene-α-thiol), (−)-epicatechin-3-*O*-gallate (ECG), cesium carbonate, 2,5-dihydroxybenzoic acid (DHB), and ovalbumin (V grade) were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-Catechin, (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and quercetin-3-*O*-galactoside were supplied by Extrasynthese (Genay, France). Quinine sulfate dehydrate was from Alfa Aesar (Karlsruhe, Germany), and tartaric acid, potassium, aluminum sulfate, and sodium chloride were purchased from Panreac (Barcelona, Spain). TSK Toyopearl gel HW-50F was from TosoHaas (Montgomeryville, PA, U.S.A.).

HPLC-grade methanol, ethanol, acetone, dichloromethane, and sulfuric acid were obtained from Scharlab (Barcelona, Spain). Formic acid and hydrochloric acid were from Sigma-Aldrich (Steinheim, Germany), and deionized water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use.

Wines. To select young red wines with significant differences in their phenolic composition and astringency perceived, 35 commercial young red wines from different Spanish Denominations of Origin and wineries were analyzed. Six wines were selected for this study out of the 35 wines on the basis of their significant differences in the total polyphenol index (TPI), the protein-precipitable proanthocyanidins, and the astringency perceived.

Sample Preparation. *Elimination of Wine Volatiles Compounds.* Wines were dealcoholized and dearomatized according to Sáenz-Navajas et al.[22] to obtain an odorless tastant fraction from each wine. The nonvolatile extract obtained from 50 mL of wine was then redissolved in 2 mL of ethanol/water (13:87, v/v) before being chromatographed.

Isolation of Proanthocyanidins. TSK Toyopearl gel HW-50F was suspended in milli-Q water, and, after swelling, it was packed in a Millipore (Bedford, MA, U.S.A.) Vantage L column (120 mm × 12 mm i.d.) at atmospheric pressure. The system used was an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany) equipped with a peristaltic pump (Agilent 61311A), Rheodyne injector (2 mL loop), diode array detector (Agilent, G1315D), and Agilent Chemstation software. Two milliliters of the nonvolatile extract were directly applied to the column at a flow rate of 2 mL/min. The method of fractionation used was adapted from Guadalupe et al.[23] The compounds of low molecular weight (sugars, anthocyanins, phenolic acids, organic acids, flavonols, and flavanols) were washed with 240 mL of ethanol/water/formic acid (55:45:1, v/v/v). The proanthocyanidin fraction was eluted with 40 mL of acetone/water (60:40, v/v) and manually collected in round-bottomed flasks. The acetone was evaporated under vacuum, the proanthocyanidin fraction (PAsF) was freeze-dried to obtain a powder of tannins, and the powder was redissolved in 32 mL of bottled water. The fractionation was carried out six times for each wine. PAsF were stored at 4 °C until chemical and sensory analyses were performed.

Chemical Analysis. *Analysis of Conventional Enological Parameters in Wines.* The total polyphenol index (TPI) was estimated as the absorbance at 280 nm multiplied by 100.[24] The analysis of reducing sugars, ethanol content, pH, and titratable and volatile acidities were determined by Infrared Spectrometry with Fourier Transformation using a WineScan FT 120 (FOSS, Barcelona, Spain), which was previously calibrated using official OIV methods.

Analysis of Protein-Precipitable Proanthocyanidins (PPAs). PPAs were measured in both the wines and PAsF using ovalbumin as the precipitation agent and tannic acid solutions as standards in accordance with a previously described method.[25] The analyses were carried out at room temperature (20 ± 2 °C) in triplicate.

MALDI-TOF−MS of PAs Fraction. The MALDI-TOF−MS spectra were obtained using a MicroFlex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument was equipped with a pulsed nitrogen laser (337 nm, 3 ns pulse width) and a time-delayed extracted ion source. Spectra were acquired in the positive-ion mode using the reflectron in a mass range of 800−4060 Da, suppressed up to 800 Da, with a 20 kV accelerating voltage. The PAs fraction was dissolved in a suitable quantity of methanol, and the spectra were run with 2,5-dihydroxybenzoic acid and MicroScout Plates Anchorchip (Bruker Daltonics). The matrix solution was prepared at a concentration of 10 mg mL^{-1} in acetone. The sample/matrix ratio was 2:10, v/v. After brief mixing, 0.5 μL of the mixture was added on the MALDI target and allowed to air-dry. Mass calibration was performed with poly(ethylene glycol) (PEG 2000) as the internal standard. Each recorded spectrum was the sum of 900 laser shots.

Thiolysis-UPLC-UV/vis−MS of the PAs Fraction. Acid-catalyzed degradation in the presence of toluene-α-thiol was performed according to the method described by Labarbe et al.[26] but with some modifications. PAsF (100 μL) was mixed with an equal volume of the thiolysis reagent (5%, v/v) benzyl mercaptan in methanol containing HCl (0.2 N). After sealing, the mixture was shaken and heated at 60 °C in a water bath for 10 min. Before UPLC analysis, 150 μL of milli-Q water was added to the reaction mixture to avoid asymmetrical peaks.

UPLC Acquity (Waters) with an MS detector (Bruker Daltonics, Bremen, Germany) was used. UPLC conditions were adapted from the HPLC method described by Monagas et al.[27] The separation was performed on a reversed-phase Waters Nova-Pak C18 column (100 mm × 2.1 mm, 1.7 μm) at 30 °C. A binary gradient consisting of solvent A (water/formic acid, 98:2, v/v) and solvent B (acetonitrile/water/formic acid, 80:18:2, v/v/v) was applied at a flow rate of 0.45 mL/min as follows: 5−30% B linear from 0 to 5 min, 30−32% B linear from 5 to 5.5 min, 32−60% B from 5.5 to 10 min, 60−99% B from 10 to 12 min, and 99−100% B from 12 to 12.5 min followed by washing (solvent B) and reequilibration of the column from 12.5 to 16.5 min.

Quantification was done in the negative mode from the extracted ion chromatogram (EIC). The area under the peaks from both the flavan-3-ols monomers (terminal units) and toluene-α-thiol adducts (extension units) released from the depolymerization reaction were integrated. Calibration curves were established with (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, and (−)-epigallocatechin. In the absence of the standards of the thiol derivatives and considering the fact that the thiolytic derivatives were shown to have similar response factors as the correspondent monomeric units,[28] these were calculated from the respective monomer calibration curves. The mean degree of polymerization (mDP) as well as the percentage of procyanidins (PC), prodelphinidins (PD), and galloylation (G) were calculated as the molar ratio of the total units to terminal units, the molar ratio of catechin, epicatechin, and their extension units to total units, the molar ratio of epigallocatechin units to total units, and the molar ratio of galloylated units to total units, respectively. All samples were analyzed in triplicate.

Analysis of Total Proanthocyanidins (TPAs). This determination was performed using vanillin according to the method described by Guadalupe et al.[23] The spectrophotometric measurements were performed on a UNICAM UV2 spectrophotometer (Burladingen, Germany). For the quantification, a standard curve ranging from 100 to 350 mg L^{-1} of catechin ($y = 0.0029x - 0.1541$, $R^2 = 0.9921$) was obtained. PAsF were analyzed in triplicate.

Sensory Analysis. *Selection of Sensory Panel.* Eighteen volunteers out of 34 (12 males and 6 females, ranging in age from 21 to 45 years), all students or staff from the University of La Rioja

Table 1. Definitions and Composition of Reference Standards for Panel Training on Astringency Subquality Attributes

attribute	definition	reference standard[a]	concentration
velvety astringency	A silky and finely textured kind of astringent sensation (notably in the tip of the tongue and in front of superior teeth).	quercetin-3-*O*-galactoside[56]	0−2.5−5−7.5−10 mg/L
puckering astringency	A reflex action of the cheek surfaces being brought together and released in an attempt to lubricate the mouth surfaces (all over the tongue, including laterals and palate).	tannic acid[56]	0−0.01−0.1−0.5−0.8 g/L
drying astringency	Feeling of desiccation or lack of lubrication. Perceived in all parts of the mouth.	potassium and aluminum sulfate[8]	0−1−2−3−4 g/L

[a]Dissolved in mineral water "Solán de Cabras".

Table 2. Denomination of Origin, Vintage, Varietal Composition, Conventional Analysis, Total Polyphenol Index (TPI), Protein Precipitable Proanthocyanidin (PPAs) and Astringency Mouthfeel Sensory Score of the Six Wines Selected for This Study

wine codes	denomination of origin or region	vintage	grape variety	ethanol (v/v)	pH	volatile acidity[a]	total acidity[a]	reducing sugars[b]	TPI[c]	PPAs[d]	astringency[e]
W1	Rioja	2008	Tempranillo	12	3.62	0.32	4.81	1.18	30.0	105	3.16
W2	VT Cangas	2008	Mencía. Albarín negro	12	3.47	0.31	5.05	1.22	36.8	220	3.37
W3	Valencia	2008	Bobal. Shyrah	12.5	3.54	0.30	5.25	3.98	40.8	334	4.22
W4	VT Castilla y Leon	2007	Tempranillo	13	3.57	0.31	5.14	1.66	55.3	503	4.59
W5	Valdepeñas	2008	Tempranillo	13.5	3.73	0.26	5.33	1.59	59.0	679	5.65
W6	Toro	2008	Tempranillo	14.5	3.89	0.36	4.33	1.69	62.5	966	6.43

[a]Expressed as grams of tartaric acid per liter. [b]Expressed as grams per liter. [c]Total polyphenol index expressed as the absorbance at 280 nm × 100. [d]Protein-precipitable proanthocyanidins were measured as the ovalbumin index and expressed as milligrams per liter of tannic acid. [e]Scale for rating the astringency (0−9).

(Spain), were selected by their ability to taste bitter on the basis of their PROP status (3 supertasters and 15 medium tasters). PROP status was determined using 6-propyl-2-thiouracil according to the Tepper's test.[29] The intensity of the taste was recorded on a labeled magnitude scale. Instructions for using the scale were given according to Green et al.[30] Subjects were sorted into nontasters, medium tasters, and supertasters. Subjects who rated NaCl higher in intensity than PROP were considered nontasters. Those who gave similar ratings to NaCl and PROP were medium tasters, and those who rated PROP as more intense than NaCl were classified as supertasters.[29] Panelists were not informed about the nature of the samples evaluated.

Panel Training. The training period included two phases: a general and a product-specific training phase. During the general training, different reference standards solutions, representative of taste and astringency terms, were presented to be recognized and discriminated as described by Saenz-Navajas et al.[8] During wine-specific training, different Spanish young red wines were presented to the judges. A single reference standard for the astringency attribute (potassium and aluminum sulfate) was used to evaluate the wines. Another specific training was carried out with the proanthocyanidin fractions. For it, the different PAs fractions obtained as described were presented, and the different astringency subqualities were studied. The panelists were asked to describe the different oral sensations perceived in four different PAs fractions with their own words. A group discussion with the panel leader was carried out to agree on the definitions and the reference standards of the selected terms (Table 1). Velvety, puckering, and drying astringency were rated on a 10-point scale (0 = absence, 1 = very low, and 9 = very high).

Sample Evaluation. The six wines (10 mL) and the six PAs fractions (4 mL) were described in duplicate and in a single session, respectively. Both the wines and fractions were served in dark ISO-approved wine glasses[31] that were labeled with three-digit random codes and covered by plastic Petri dishes according to a random arrangement to balance the presentation order and carry-over effect.

During evaluation, a sip and spit protocol was used.[32] Therefore, 10 s after sample was sipped it was expectorated and recorded. Ten seconds later, an apple pectin solution (1 g L^{-1}) was sipped, left in the mouth for 10 s, and spat out. Between samples-rinse combinations, the subjects rinsed twice with deionized water for 20 s. All fractions were served at room temperature and were evaluated in individual booths using paper ballots to rate the attributes. Samples were stored at 4 °C.

Statistical Analysis. Simple linear regressions were calculated between the sensory and chemical variables. Statistically significant differences in the results were tested by Tuckey's test at $P < 0.05$. A three-way ANOVA involving the fraction (F), judge (J), and replicate (R) as fixed factors and all first-order interactions were calculated to assess the panel performance. Significant differences between the samples for each of the sensory attribute were determined by a one-way analysis of variance and principal component analyses (PCA), and a multiple linear regression (MLR) was performed on the mean of all of the chemical and significant sensory data of the PAs fractions using SPSS software (IBM Statistics, version 19).

RESULTS AND DISCUSSION

Wines. The detailed list of the studied wines, including the region of origin, varietal, vintage, and basic compositional data obtained following standard chemical measures, is shown in Table 2.

The six selected wines were Spanish young wines from vintages 2007 and 2008. All of the wines had average values of ethanol (12.0−14.5%) with respect to other Spanish red wines. The reducing sugars content was below 2 g/L except for W3, which presented at 4 g/L; nonetheless, W3 is classified as a dry wine. The pH values ranged from 3.5 to 3.9, whereas the lowest value for the titratable acidity was observed for W6 (4.33 g/L). The W5 and W6 wines had the highest total polyphenol index (TPI) and protein-precipitable PAs (PPAs).

Sensory Characterization of Wines. The ANOVA performed on the sensory data of the studied wines determined that the trained panelists were reproducible and consistent (data not shown). The results of the sensory evaluation carried out on the six wines considered in this study are shown in Figure 1. According to this, the six wines were evaluated with high scores in acidity, bitterness, astringency, and persistence, whereas the mean scores given for the sweetness attribute in all the wines were 2.5 out of 9 points. It should be noted that neither the reducing sugars content nor the ethanol content was correlated with the sweetness perceived in this set of

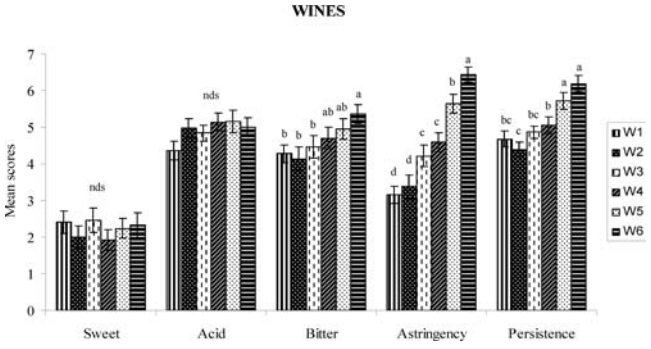

Figure 1. Mean sensory ratings for the six wines. Error bars are calculated as $s/(n)^{1/2}$, where (s) is the standard deviation and (n) is the number of panelists. The different letters indicate the existence of a significant difference between wines ($\alpha \leq 0.05$) (Tukey's test); nds, no significant difference.

samples. These results were also observed in the sensory characterization of the dry aged red wines.[33] The W5 and W6 wines were the most astringent and persistent. The results obtained showed that the wines astringency was correlated with the TPI (R^2 = 0.8932; P = 0.0044), PPAs (R^2 = 0.9886; P = 0.00005), ethanol (R^2 = 0.9712; P = 0.0003), and pH (R^2 = 0.7076; P = 0.0358). These findings are consistent with the results found by others[19,34,35] where significant correlations were observed between the astringency and the TPI, PPAs, and ethanol content, whereas a less-significant correlation was found with the pH. Similarly, Demiglio et al.[36] concluded that the ethanol content played a more dominant role in modifying most oral sensations than pH. The influence of ethanol on the mouthfeel sensations has also been studied by several authors who highlight the influence of alcohol in the intensity and in the in-mouth oral sensations perceived.[36−39] Nevertheless, the higher ethanol content in wines W5 and W6 could be responsible for the higher TPI and PPAs in these wines, possibly because of a higher extraction of phenolic compounds from the grape skins and seeds, which at the same time increases the astringency perception of these samples. The astringency of the wines was not correlated with either the titratable acidity or the reducing sugars.

Concerning the persistence, this attribute turned out to be correlated with the astringency (R^2 = 0.9497; P = 0.001) and with the bitterness (R^2 = 0.9752; P = 0.0002), although significant correlations were not found either with the acidity or the sweetness. Similar results have been observed in previous works carried out in our research group between the persistence/astringency and persistence/acidity.[8]

Sensory Characterization of the Wine Proanthocyanidin Fraction (PAsF). The ANOVA results for the in-mouth-assessed sensory properties of the proanthocyanidin fraction obtained from the six studied wines is shown in Table 3. The judge effect was significant ($P < 0.05$) for all attributes because judges have unique physiological perceptions.[40] This effect is commonly found in sensory analysis and can be explained by interindividual differences. The replicate effect was not significant, indicating a consistent assessment of replicates by the judges. However, the fraction-by-judge interaction (F*J) was significant for the puckering astringency term. The PCA run on the puckering astringency data revealed that the first component accounted for 34% of the explained variance. For this term, the judges' projections were spread over the loading plot (data not shown). This indicated that during the assessment of the six proanthocyanidins fractions there were differences in the interpretation of this term and that assessors may need more training with respect to this attribute. Looking closer at the data given by the panelists for the velvety, puckering, and drying astringency attributes, and with the purpose of retaining the values that the panelists gave for the puckering astringency term, combined terms were constructed from the three individual terms of astringency, evaluating the consistency of the judges for each new term. Only the combined term resulting from considering the puckering and drying astringency showed consistency in the ANOVA analysis (Table 3). The proanthocyanidins fractions were described with low scores (<1) for the sweet, acid, and bitter terms, with no significant differences between them (Figure 2a). These low scores obtained for the sweet and acid attributes in these fractions were expected and can be explained by the remove of alcohol, aroma, and low-molecular-weight compounds, such as the sugars and the organic acids, among others. The low scores also observed in the bitter taste in these fractions can be due to the lack of monomer phenols and other compounds that are considered more bitter than proanthocyanidins.[41,42] These results are consistent with other work carried out in proanthocyanidins fractions from apple as well as grape seed and skin.[10] It is interesting that the panelists that were selected because of their ability to detect bitter taste on the basis of their PROP status did not detect bitterness. Therefore, the results clearly showed that the proanthocyanidin fractions were exclusively described as astringent and persistent, showing significant sensory differences for these terms. The scores of the

Table 3. Fixed ANOVA Model of the Attribute Ratings (18 Judges) of the PAs Fraction Evaluated in Mouth[a]

	judge (J) (df = 17)		fraction (F) (df = 5)		replicates (R) (df = 1)		F*J (df = 83)		J*R (df = 17)		F*R (df = 5)	
attribute	F	P	F	P	F	P	F	P	F	P	F	P
sweet	15.13	**<0.001**	1.559	0.181	1.749	0.191	0.719	0.932	1.083	0.384	1.19	0.321
acid	7.564	**<0.001**	2.347	0.060	1.581	0.212	1.459	0.071	0.676	0.817	0.23	0.949
bitter	9.895	**<0.001**	0.547	0.740	0.066	0.798	1.289	0.125	0.259	0.999	0.351	0.880
velvety astringency	5.764	**<0.001**	5.383	**<0.001**	0.023	0.88	1.103	0.328	0.747	0.746	1.547	0.184
puckering astringency	4.995	**<0.001**	4.086	**0.002**	0.079	0.779	1.613	**0.015**	1.051	0.414	0.102	0.991
drying astringency	7.863	**<0.001**	22.236	**<0.001**	1.326	0.254	1.332	0.129	1.791	0.064	1.213	0.313
puckering/drying astringency (combined term)	5.078	**<0.001**	23.781	**<0.001**	0.189	0.665	1.149	0.266	0.869	0.611	0.828	0.534
persistence	14.983	**<0.001**	11.855	**<0.001**	0.020	0.888	0.930	0.630	1.185	0.295	0.224	0.951

[a]df, degrees of freedom; F, F ratios; and P, P values. Significant P values (5% level) are highlighted in bold.

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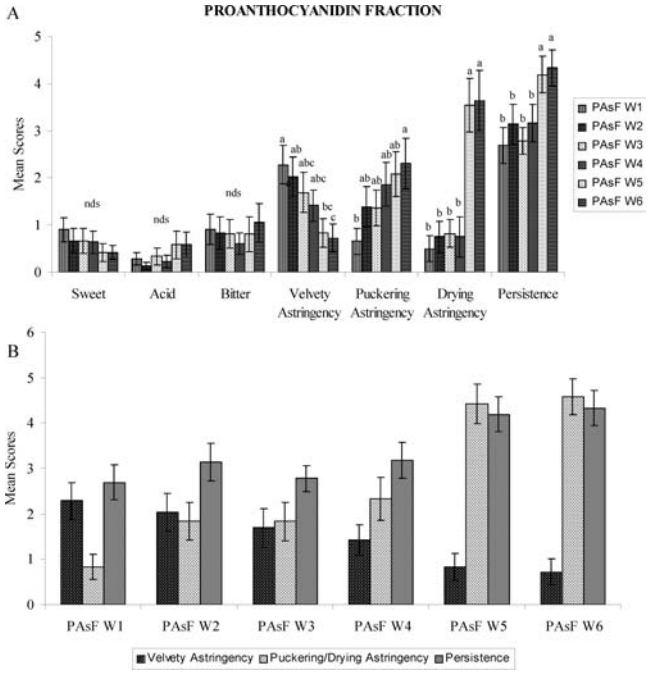

Figure 2. (a) Mean sensory ratings for the six proanthocyanidin fractions. Error bars are calculated as $s/(n)^{1/2}$, where (s) is the standard deviation and (n) is the number of panelists. The different letters indicate the existence of a significant difference between fractions ($\alpha \leq 0.05$) (Tukey's test); nds, no significant differences. (b) Mean sensory ratings of astringency subqualities and persistence evaluated in the proanthocyanidin fraction. Error bars are calculated as in panel a.

velvety and the puckering/drying antringency subqualities as well as the persistence evaluated are shown in Figure 2b. Fractions from W4, W5, and W6 wines were evaluated with higher scores in puckering/drying astringency than in velvety astringency. From this data, we can see that the velvety astringency of PAsF W5 and PAsF W6 was rated with values next to 0 compared to the puckering/drying astringency values; however, in PAsF W4 both attributes were scored with close values. Wine fractions W2 and W3 were evaluated with similar values in both astringent subqualities, whereas wine fraction W1 was mainly described with the velvety astringency term. Indeed, both of the tactile sensations (velvety and puckering/drying) were negatively correlated (R^2 = 0.9432; P = 0.0012). A negative relation was also observed by Vidal et al.[10] in a study of mouthfeel properties of proanthocyanidins fractions. These authors claimed that the term "fine grain" related to the feel of silk cloth was opposed to the term "dry". This result indicates that the quantity and/or type of compounds present in this fraction may induce different perceptions of the astringency attribute. Furthermore, it is worth noting that both astringent subqualities clearly play a significant role in persistence. As can be seen, the six fractions were scored as persistent (Figure 2b). The less persistent fractions were those corresponding to wines W1, W2, W3, and W4, and the most persistent fractions were those of wines W5 and W6. The latter two were mainly evaluated as puckering/drying, so its persistence might be attributed to this astringency. On the contrary, the persistence of the other four fractions does not seem to be exclusively resulting from the puckering/drying astringency because these fractions presented lower values for this cited attribute than for the persistence. It is possible that the velvety astringency is contributing to the persistence of these fractions, as is clearly observed in the case of PAsF W1 (Figure 2b). Thus, both astringency subqualities appear to contribute to in-mouth persistence.

Relationship between the Sensory Characteristics of Wines and their PAsF. Although wines and fractions are different matrices, hydroalcoholic and water, respectively, significant correlations were found among them. Regarding the astringency attribute, a positive correlation was found between the astringency of the wines and the puckering/drying astringency of the fractions (R^2 = 0.9172; P = 0.0026) as well as a negative correlation between the astringency of the wines and the velvety astringency evaluated in the fractions (R^2 = 0.9731; P = 0.0003). Although the first correlation was expected because the panel was trained with the same reference standard (potassium and aluminum sulfate) in both types of samples, it is worth noting that the scores of the PAs fractions retained the astringency differences found in the wines. Both astringent subqualities, puckering/drying and velvety, were described with lower scores than those given to rate the astringency attribute in the wines. This might be due to several reasons. On one hand, the nonvolatile compounds not collected in this fraction could increase the astringency shown by the proanthocyanidins. The absence of ethanol, acidity, and different pH between the wine and the PA fractions in water might have a significant impact on the astringency ratings. On the other hand, it is possible that a dumping effect has taken place because of the use of one scale for rating the astringency in the wines and three scales for rating the astringency subqualities in the fractions. This last statement agrees with what Valentin et al.[43] have also observed. Similarly, a positive correlation has been found between the persistence scores of both kinds of samples (R^2 = 0.8050; P = 0.0153), with the lower persistence scores found in the fractions than in the corresponding wines. Contrary to that discussed above for the astringency, the dumping effect for the persistence seems less likely to happen because both of them were evaluated with the same scale. Therefore, the nonvolatile compounds not present in the fraction samples might have an influence on the increase of the wines persistence. Thus, these results confirm that in red wines the persistence attribute is strongly related to the astringency attribute and also that the compounds present in this fraction play an active sensory role in both attributes. Further research in this sensory field regarding the addition of different taste-active compounds to the proanthocyanidin fraction would be needed to explore other compounds that could enhance the astringency and persistence perceived in these fractions.

Chemical Characterization of PAsF. *Qualitative analysis by MALDI-TOF–MS.* The results concerning the qualitative determination of PAs by MALDI-TOF-MS are shown in Table 4. The mass data for proanthocyanidins has usually been reported as $[M + Na]^+$ and $[M + K]^+$ adducts;[44] in this case, the samples only presented sodium adducts. The assignment of MALDI-TOF mass signals to a particular proanthocyanidin structure can be achieved by the determination of the theoretical or calculated mass. On the basis of the report performed by Monagas et al.,[44] the following equation was formulated to calculate the theoretical monoisotopic mass (as sodium adducts, $[M + Na]^+$): $[M + Na]^+ = (290.08 \times CAT) + (306.07 \times GCAT) + (152.01 \times GALLOYL) - (2.02 \times B) - (4.04 \times A) + 22.99$, where CAT and GCAT are the numbers of (epi)catechin and (epi)gallocatechin units contained in the proanthocyanidin molecule, respectively, GALLOYL is the

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Table 4. Qualitative and Quantitative Data of Wine Proanthocyanidin Fraction (PAsF Wx)[a]

	MALDI-TOF		thiolysis-UPLC/MS											
			terminal units (%)			extension units (%)								
fraction	PC	PD	C	EC	ECG	EGC	C	EC	ECG	mDP	% PC	% PD	% G	PPAs	TPAs
PAsF W1	PC_3–PC_9	PD_3–PD_9	66.70	33.30	tr	11.07	1.99	86.73	0.21	4.62 ± 0.95 b	90.90 ± 1.88 a	8.87 ± 1.86 d	0.23 ± 0.02 c	45 ± 20 b	499 ± 19 c
PAsF W2	PC_3–PC_9	PD_3–PD_9	80.34	18.77	0.89	13.82	1.69	84.15	0.34	5.32 ± 0.87 ab	88.00 ± 1.80 b	11.55 ± 1.76 c	0.45 ± 0.04 b	190 ± 30 b	521 ± 8 c
PAsF W3	PC_4–PC_8	PD_4–PD_8	73.14	26.86	tr	19.12	0.91	79.78	0.19	6.18 ± 0.45 a	82.92 ± 2.18 cd	16.87 ± 2.15 ab	0.21 ± 0.04 c	180 ± 15 b	619 ± 4 c
PAsF W4	PC_3–PC_9	PD_3–PD_9	65.23	34.30	0.46	22.20	3.11	74.44	0.24	6.32 ± 1.01 a	80.72 ± 2.16 d	19.00 ± 2.14 a	0.28 ± 0.02 c	315 ± 35 b	784 ± 71 b
PAsF W5	PC_3–PC_{10}	PD_3–PD_{10}	72.17	27.47	0.36	18.76	4.63	76.34	0.27	6.38 ± 0.16 a	83.77 ± 0.54 c	15.96 ± 0.57 b	0.27 ± 0.07 c	590 ± 20 a	1260 ± 82 a
PAsF W6	PC_3–PC_{10}	PD_3–PD_{10}	74.48	25.21	0.31	20.70	6.66	72.06	0.58	6.32 ± 0.46 a	82.13 ± 0.42 cd	17.34 ± 0.42 ab	0.54 ± 0.05 a	890 ± 70 a	1241 ± 22 a

[a] Abbreviations: tr, traces; C, catechin; EC, epicatechin; ECG, epicatechingallate; EGC, epigallocatechin; mDP, mean degree of polymerization; PC, procyanidins; PD, prodelphinidins; G, galloylation; PPAs, protein precipitable proanthocyanidins expressed as milligrams per liter of tannic acid; and TPAs, total proanthocyanidin content expressed as milligrams per liter of catechin.

numbers of galloyl ester units attached to the flavan-3-ol units, and B and A are the numbers of B-type and A-type linkages between units, respectively. The spectrum of the direct analysis of one PAs fraction is presented (Figure 3). An enlarged spectrum of masses representing prodelphinidins and labels of peaks, referred as A-type linkages, is shown in the inset.

In all samples, the main groups of peaks in the spectra were separated by Δ290 amu (e.g., m/z 889, 1177, 1465, 1753, etc.), corresponding to the presence of procyanidins units (catechin/epicatechin). Procyanidins with a degree of polymerization from 3 to 10 (m/z 889−2907) were detected in PAsF W5 and PAsF W6. In the other samples, the highest polymer detected was the nonamer, except for sample PAsF W3 in which up to only the octamer was observed. Another separation of 152 amu was also observed (e.g., m/z 1041, 1329, 1617, 1905, etc.), corresponding to the addition of one galloyl group at the heterocyclic C-ring, as in (−)-epicatechin-3-*O*-gallate. In all samples, we have noted the presence of these compounds. Another strongly repeated pattern was the signals separated by a Δ16 amu difference. These masses can be produced by prodelphinidins, where the third hydroxyl group introduces differences of 15.99 amu.[45] To verify that the presence of this difference of 16 amu was due to the prodelphinidins and not to the presence of Na^+ and K^+ ions in the sample, they were ion exchanged with Cs^+, demonstrating that the Δ16 amu was from a pattern of hydroxylation. In the enlarged spectrum, it can be observed that this difference of 16 amu is repeated up to three times, which means that three prodelphinidins can be attached to the procyanidins. The mixture of procyanidins and prodelphinidins in the same compound (e.g., m/z 1193) has an inherent problem because this signal could represent either a B-linked tetramer of three (−)-epicatechin and one (−)-epigallocatechin or a B-linked trimer of two (−)-epicatechin-3-*O*-gallate and one (−)-epicatechin units. It is typical of MALDI-TOF−MS that different compositions of the oligomers may yield the same signal, so structure assignment from MALDI-TOF−MS are always tentative.[46] In the enlarged spectrum, we also observed m/z 1175, 1191, and so forth, which corresponds to proanthocyanidins with A-linkages. At least one A-type interflavan bond exists in each oligomer.

In the spectrum obtained, the lower masses have a greater peak response than higher masses even when the more polymerized proanthocyanidins could be present in the sample at similar concentrations. This fact is due to the detector response and its finite capacity, which means that the lowest masses reach the detector first. This pattern was the same for the six samples studied. The detection of lighter ions than m/z 889.4 was not attempted on our MALDI-TOF−MS instrument because of noise and matrix interference problems. In addition, we have observed that the peak response of galloylated tannins are always lower than nongalloylated ones. In accordance with this, we affirm that all PAsF analyzed were constituted by galloylated and nongalloylated procyanidins and prodelphinidins linked by B-type interflavonoid linkages and by at least one A-type linkage. The most important qualitative difference found among this set of fractions was that the PAsF of wines W5 and W6 contained proanthocyanidins with a higher degree of polymerization.

Thiolysis-UPLC-UV/vis−MS. Table 4 shows the results of the structural composition (terminal and extension units), the mDP, and the percentage of procyanidins (% PC), prodelphinidins (% PD), and galloylated tannins (% G) found in the six fractions studied. (−)-Epigallocatechin

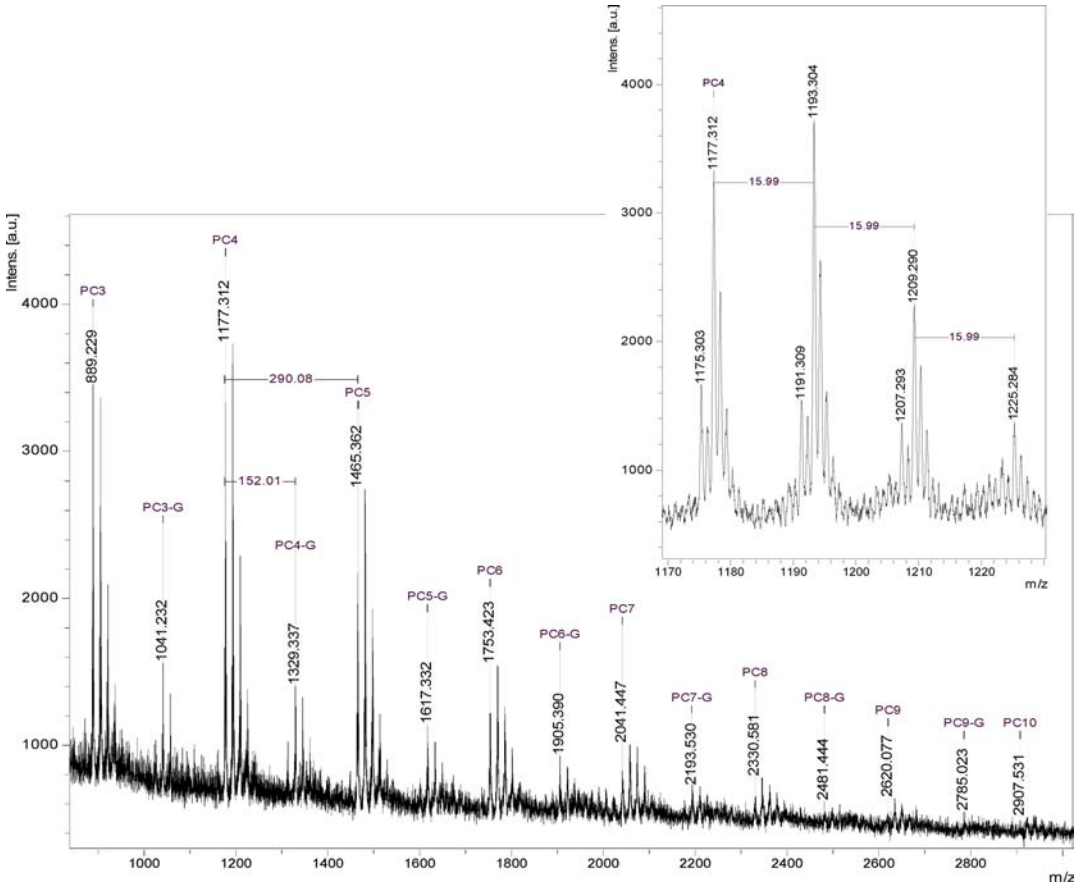

Figure 3. MALDI-TOF mass spectrum in positive reflectron mode showing a procyanidin series [M + Na^+] from trimer to decamer. The inset is an enlarged spectrum of the tretramer as well as the prodelphinidins and peaks derived from A-type linkages.

(EGC), (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin-3-*O*-gallate (ECG) were identified as extension proanthocyanidins units, whereas (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate were identified as terminal units. Several studies performed with different wine varieties showed the presence of C_t and EC_t as terminal units.[27,47] Sun et al.,[48] in the study of different fractions obtained from Castelão wines, identified ECG_t terminal units. In our wine's fractions we have also detected the presence of ECG_t terminal units in all samples, but its quantification has been possible only in four of them because the other two fractions only presented traces.

EC_e was the mayor extension unit. Extension units also contained a high proportion of EGC_e. For the terminal units, C_t and EC_t were the most abundant compounds, with C_t being in higher proportion. These results are consistent with previous works carried out by Monagas et al.,[27] Fernández et al.,[47] and Hanlin et al.[49] As we had already noted with MALDI, thiolysis analyses confirmed that wine proanthocyanidins contained galloylated and nongalloylated procyanidins as well as prodelphinidins. Concerning the results of the % PC, % PD, and % G, we can see that the content of prodelphinidins is higher than the content of galloylated units, with procyanidins presenting the highest content. The PAsF of wines W1 and W2 presented the smallest content in % PD (8.87 and 11.55), with the highest percentage being the PAsF W4, which presented (19.00% PD). The mDP ranged from 4.62 to 6.38, with only PAsF W1 being significantly different from the other calculated mDPs. The mDP values obtained in the proanthocyanidin fraction of these wines were similar to other data reported in the literature found in wines.[47,49−51] The results showed a positive significant correlation between the mDP and the percentage of PD (F = 44.94, P = 0.0026, R^2 = 0.92). These results agree with those obtained by Monagas et al.[27] and Sun et al.[52] In contrast, we have not observed a correlation between the mDP and the percentage of galloylated tannins, as other authors have also observed.[52,53]

Total Proanthocyanidins Content (TPAs). The results of the total proanthocyanidins assay indicated that the fraction characterized by the lowest polymeric proanthocyanidin content was PAsF W1 with a catechin equivalent value of 499 mg/L, whereas the fraction with the highest content was PAsF W5 with a value of 1260 mg/L (Table 4). These values are consistent with the data obtained by other authors[27] who found, using the vanillin index, mean values of 700−800 mg/L for the Tempranillo, Graciano, and Cabernet Sauvignon varieties. Sun et al.[51] used this index for studying the different concentration in oligomeric and polymeric PAs in cv. Tinta Miuda. These authors found values of 300 mg/L in wines elaborated with no stem contact, around 800 mg/L in wines with stem contact, and values around 1400 mg/L in wines elaborated with the carbonic maceration technique. In our study, this index showed a positive correlation with the TPI of the wines (F = 21.84, P = 0.009, R^2 = 0.81).

Proanthocyanidins Precipitable with Ovalbumin (PPAs). The results of PPAs are presented in Table 4. The PPAs values were ranged from 45 mg L^{-1} for PAsF W1 to 890 mg L^{-1} for PAsF W6. These values were, in general, slightly

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lower from those obtained in the wines (Table 2), although a significant correlation between the PPAs of the wines and fractions has been found (R^2= 0.9647, P = 0.0005). Furthermore, as was to be expected considering the previous results in other sets of wines,[33,54] there was a positive correlation between PPAs and TPAs for the six fractions studied (R^2= 0.8807, P = 0.0056).

Relationship between the Sensory and Chemical Data of the Proanthocyanidin Fraction. A principal component analysis was carried out taking into account the chemical data and the sensory attributes (illustrative variables) of the six PAsF. Figure 4 shows the projection of the samples and

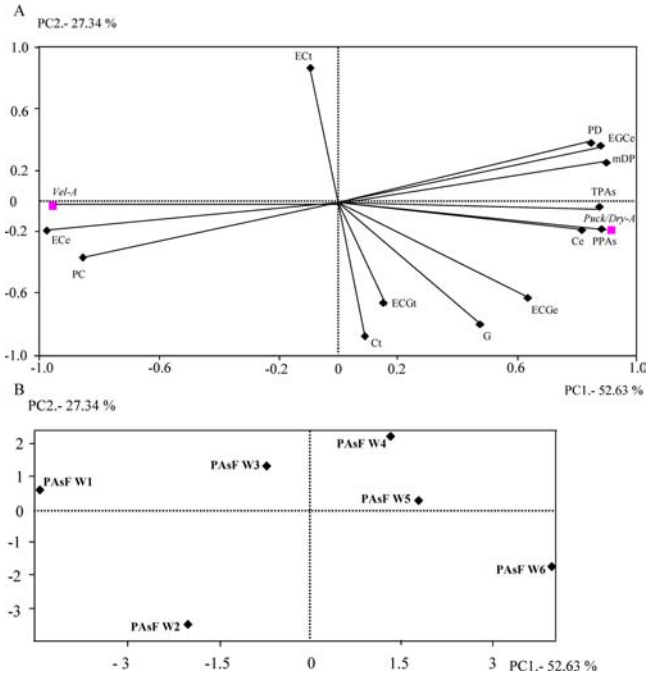

Figure 4. (a) Principal component analysis biplot. Projection of the variables. Abbreviations: t, terminal; e, extension; Vel-A, velvety astringency; Puck/Dry-A, puckering/drying astringency. (b) Projection of the six fractions on the first two PCs of the PCA.

variables on the first two PCA components, which accounts for more than 80% of the original variance. The first principal component (PC1) was positively correlated with the percentage of extension flavanols (C_e and EGC_e) as well as the prodelphinidins, TPAs, PPAs, and mDP and was negatively correlated with the percentage of procyanidins and EC_e. The projection of the illustrative variables, velvety and puckering/drying astringency, on the first two principal axes showed that the velvety astringency had negative scores on PC1 and the puckering/drying astringency had positive scores on this component. It should be emphasized that the velvety astringency was positively correlated with EC_e, negatively correlated with C_e, EGC_e, % PD, mDP, TPAs, and PPAs, and inversely correlated to puckering/drying astringency. The second component was correlated with the type of flavanol attached to the terminal position of the proanthocyanidins and with the PAs galloylated. These variables did not modify the tactile sensations described for these fractions. The projection of the six proanthocyanidins fractions on the first two axes is shown in Figure 4b. This distribution agreed with the subqualities of the astringency evaluated in the fractions. Therefore, PAsF W6 (higher puckering/drying astringency) was projected on the right of the plane and PAsF W1 (higher velvety astringency), on the left. The other fractions were located between them, in accordance with the values awarded by the panelists for these attributes.

Moreover, a multiple linear regression analysis was performed where the projections of the fractions on the PCA analysis were considered as independent variables and the astringency as a dependent variable. Therefore, three PCs were considered (explaining 91.65% of the total variance). The multiple linear regressions provided two significant models, which were explained by the first component. The regressions were

$$\text{velvety astringency} = 1.495 - 0.211 \times \text{PC1}\ (F = 44.15;\ P = 0.003)$$

$$\text{puckering/drying astringency} = 2.655 + 0.470 \times \text{PC1}\ (F = 16.82;\ P = 0.015)$$

The results of the PCA and multiple linear regressions showed that the different content in the total and protein-precipitable PAs and the extension units of flavanols attached to PAs provides sensory differences in the astringency perceived by the panelists. Thus, puckering/drying astringency is positively correlated with the content in total PAs, as reported Gawel et al.,[15] and with the protein-precipitable PAs. This seems to be reasonable because oral sensations such as drying or puckering are commonly attributed to interactions of proanthocyanidins with salivary glycoproteins.

The increase in the degree of polymerization appears to enhance the puckering/drying astringency perceived and decrease the velvety astringency noted. Vidal et al.[10] and Chira et al.[55] pointed out similar findings, relating the increase in the chain length with the higher scores given to the "dry" attribute.

The relationship between the structural characteristics of PAs and its sensory properties can be evaluated according to the different relevant compositional factors, such as the percentage of galloylation (G), the percentage of hydroxylation of the B-ring (PD), and the percentage of monomer flavanols attached (PC). According to our results, the percentage of galloylation does not seem to play an important role in both of the subqualities studied, as was also claimed by Vidal et al.[10] However, the percentage of PD and PC appears to have an effect on the astringency subqualities. Therefore, these results show that an increase in the percentage of PD (i.e., an increase in the hydroxylation of the B-ring) seems to decrease the velvety astringency term and increase the perception of puckering/drying astringency. Differences in the subqualities of astringency resulting from the trihydroxylation of the B-ring have been observed.[10] In addition, as far as we are aware, this is the first evidence for the opposite role played by the extension units of PAs in the subqualities of the astringency perceived. Hence, a higher percentage of C_e and EGC_e lead to an increase in the puckering/drying astringency, whereas an increase in the percentage of EC_e seems to decrease this subquality, enhancing the perception of velvety astringency.

Proanthocyanidins elicit astringency and persistence exclusively. The quantity in which proanthocyanidins are present in the samples and the proportion/type of the extension flavanols attached to the PAs seem to play an important role in the perception of both of the astringent subqualities, with the impact of the concentration of PAs being more important in astringency perception than the structural composition.

Furthermore, both subqualities appear to contribute to persistence. A significant correlation has been observed between the sensory astringency and persistence data from both the wines and fractions. Significant multiple linear regressions have been found between the evaluated sensory astringencies and the analyzed compounds in the subject fraction of the study.

It is prudent to emphasize that because of the small number of wines profiled here, further investigations are required to unequivocally establish causative effects between red wine composition and its mouthfeel. Moreover, there are other compounds, such as polysaccharides and polymeric pigments, that could also contribute to modulating the perception of astringency in this fraction, so the influence of these compounds are worthy of further study.

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Notes

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REFERENCES

(1) Preys, S.; Mazerolles, G.; Courcoux, P.; Samson, A.; Fischer, U.; Hanafi, M.; Bertrand, D.; Cheynier, V. Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Anal. Chim. Acta* **2006**, *563*, 126−136.

(2) Vidal, S.; Francis, L.; Noble, A.; Kwiatkowski, M.; Cheynier, V.; Waters, E. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta* **2004**, *513*, 57−65.

(3) Boselli, E.; Giomo, A.; Minardi, M.; Frega, N. G. Characterization of phenolics in Lacrima di Morro d'Alba wine and role on its sensory attributes. *Eur. Food Res. Technol.* **2008**, *227*, 709−720.

(4) Hufnagel, J. C.; Hofmann, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008**, *56*, 9190−9199.

(5) Hufnagel, J. C.; Hofmann, T. Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *J. Agric. Food Chem.* **2008**, *56*, 1376−1386.

(6) Sáenz-Navajas, M. P.; Fernández-Zurbano, P.; Ferreira, V. Contribution of Nonvolatile Composition to Wine Flavor. *Food Rev. Int.* **2012**, *28*, 389−411.

(7) Gawel, R. Red wine astringency: A review. *Aust. J. Grape Wine Res.* **1998**, *4*, 73−95.

(8) Sáenz-Navajas, M. P.; Martín-López, C.; Ferreira, V.; Fernández-Zurbano, P. Sensory properties of premium Spanish red wines and their implication in wine quality perception. *Aust. J. Grape Wine Res.* **2011**, *17*, 9−19.

(9) Gawel, R.; Oberholster, A.; Francis, I. L. A 'mouth-feel wheel': Terminology for communicating the mouth-feel characteristics of red wine. *Aust. J. Grape Wine Res.* **2000**, *6*, 203−207.

(10) Vidal, S.; Francis, L.; Guyot, S.; Marnet, N.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. J. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.* **2003**, *83*, 564−573.

(11) Sáenz-Navajas, M. P.; Ferreira, V.; Dizy, M.; Fernández-Zurbano, P. Characterization of taste-active fractions in red wine combining HPLC fractionation, sensory analysis and ultra performance liquid chromatography coupled with mass spectrometry detection. *Anal. Chim. Acta* **2010**, *673*, 151−159.

(12) Weber, F.; Greve, K.; Durner, D.; Fischer, U.; Winterhalter, P. Sensory and chemical characterization of phenolic polymers from red wine obtained by gel permeation chromatography. *Am. J. Enol. Vitic.* **2012**, in press.

(13) McRae, J. M.; Schulkin, A.; Kassara, S.; Holt, H. E.; Smith, P. A. Sensory properties of wine tannin fractions: Implications for in-mouth sensory properties. *J. Agric. Food Chem.* **2013**, *61*, 719−727.

(14) Lea, flavor, color, and stability in fruit products: The effect of polyphenols. In *Plant Polyphenols: Synthesis, Properties, Significance*; Hemingway, R. W., Laks, P. E., Eds.; Plenum Press: New York, 1992; pp 827−847.

(15) Gawel, R.; Francis, L.; Waters, E. J. Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. *J. Agric. Food Chem.* **2007**, *55*, 2683−2687.

(16) Kennedy, J. A.; Ferrier, J.; Harbertson, J. F.; Peyrot Des Gachons, C. Analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. *Am. J. Enol. Vitic.* **2006**, *57*, 481−485.

(17) Landon, J. L.; Weller, K.; Harbertson, J. F.; Ross, C. F. Chemical and sensory evaluation of astringency in Washington State red wines. *Am. J. Enol. Vitic.* **2008**, *59*, 153−158.

(18) Boselli, E.; Boulton, R. B.; Thorngate, J. H.; Frega, N. G. Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **2004**, *52*, 3843−3854.

(19) Jones, P. R.; Gawel, R.; Francis, I. L.; Waters, E. J. The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Qual. Prefer.* **2008**, *19*, 596−607.

(20) Quijada-Morín, N.; Regueiro, J.; Simal-Gándara, J.; Tomás, E.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T. Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *J. Agric. Food Chem.* **2012**, *60*, 12355−12361.

(21) Sáenz-Navajas, M. P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010**, *58*, 5574−5585.

(22) Sáenz-Navajas, M. P.; Campo, E.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chem.* **2010**, *121*, 1139−1149.

(23) Guadalupe, Z.; Soldevilla, A.; Sáenz-Navajas, M. P.; Ayestarán, B. Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation. *J. Chromatogr., A* **2006**, *1112*, 112−120.

(24) Ribereau-Gayon, P. Le dosage des composés phénoliques totaux dans les vins rouges. *Chem. Anal.* **1970**, *52*, 627−631.

(25) Llaudy, M. C.; Canals, R.; Canals, J.-M.; Rozés, N.; Arola, L.; Zamora, F. New method for evaluating astringency in red wine. *J. Agric. Food Chem.* **2004**, *52*, 742−746.

(26) Labarbe, B.; Cheynier, V.; Brossaud, F.; Souquet, J.-M.; Moutounet, M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1999**, *47*, 2719−2723.

(27) Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo Da Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475−6481.

(28) Vivas, N.; Nonier, M.-F.; Vivas De Gaulejac, N.; Absalon, C.; Bertrand, A.; Mirabel, M. Differentiation of proanthocyanidin tannins

from seeds, skins and stems of grapes (Vitis vinifera) and heartwood of Quebracho (Schinopsis balansae) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and thioacidolysis/liquid chromatography/electrospray ionization mass spectrometry. *Anal. Chim. Acta* **2004**, *513*, 247−256.
(29) Tepper, B. J.; Christensen, C. M.; Cao, J. Development of brief methods to classify individuals by PROP taster status. *Physiol. Behav.* **2001**, *73*, 571−577.
(30) Green, B. G.; Shaffer, G. S.; Gilmore, M. M. Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. *Chem. Senses* **1993**, *18*, 683−702.
(31) ISO 3591; International Organization for Standardization: Geneva, Switzerland, 1977.
(32) Colonna, A. E.; Adams, D. O.; Noble, A. C. Comparison of procedures for reducing astringency carry-over effects in evaluation of red wines. *Aust. J. Grape Wine Res.* **2004**, *10*, 26−31.
(33) Sáenz-Navajas, M. P.; Tao, Y. S.; Dizy, M.; Ferreira, V.; Fernández-Zurbano, P. Relationship between nonvolatile composition and sensory properties of premium Spanish red wines and their correlation to quality perception. *J. Agric. Food Chem.* **2010**, *58*, 12407−12416.
(34) Obreque-Slíer, E.; Peña-Neira, Á.; López-Solís, R. Enhancement of both salivary protein-enological tannin interactions and astringency perception by ethanol. *J. Agric. Food Chem.* **2010**, *58*, 3729−3735.
(35) Sáenz-Navajas, M. P.; Avizcuri, J.-M.; Ferreira, V.; Fernández-Zurbano, P. Insights on the chemical basis of the astringency of Spanish red wines. *Food Chem.* **2012**, *134*, 1484−1493.
(36) Demiglio, P.; Pickering, G. J. The influence of ethanol and pH on the taste and mouthfeel sensations elicited by red wine. *J. Food, Agric. Environ.* **2008**, *6*, 143−150.
(37) Fontoin, H.; Saucier, C.; Teissedre, P.-L.; Glories, Y. Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Qual. Prefer.* **2008**, *19*, 286−291.
(38) Geddes, D. J.; Bell, S. J.; Holzapfel, B. P.; Rogiers, S. Y.; Pickering, G. J. In *Astringency sub-qualities elicited by red wine-the effects of viticultural practises*, Proceedings of the 4th Pangborn Sensory Science Symposium, Dijon, France, July 22−26, 2001; INRA-UMRA: Dijon Cedex, France, 2001; p 86.
(39) Vidal, S.; Courcoux, P.; Francis, L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual. Prefer.* **2004**, *15*, 209−217.
(40) Bartoshuk, L. M. *Psychol. Today* **1980**, *14*, 48.
(41) Lea, A. Bitterness and astringency: The procyanidins of fermented apple ciders. In *Biterness in Foods and Beverages*; Rouseff, R., Ed.; Elsevier: Amsterdam, 1990; Vol. *25*, pp 123−143.
(42) Peleg, H.; Gacon, K.; Schlich, P.; Noble, A. C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **1999**, *79*, 1123−1128.
(43) Valentin, D.; Chrea, C.; Nguyen, D. H. Taste-odour interactions in sweet taste perception. In *Optimising Sweet Taste in Foods*; Spillane, W. J., Ed.; Woodhead Publishing Ltd.: Cambridge, United Kingdom, 2006; pp 66−84.
(44) Monagas, M.; Quintanilla-López, J. E.; Gómez-Cordovés, C.; Bartolomé, B.; Lebrón-Aguilar, R. MALDI-TOF MS analysis of plant proanthocyanidins. *J. Pharm. Biomed. Anal.* **2010**, *51*, 358−372.
(45) Krueger, C. G.; Vestling, M. M.; Reed, J. D. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum [Sorghum bicolor (L.) Moench]. *J. Agric. Food Chem.* **2003**, *51*, 538−543.
(46) Li, C.; Leverence, R.; Trombley, J. D.; Xu, S.; Yang, J.; Tian, Y.; Reed, J. D.; Hagerman, A. E. High molecular weight persimmon (Diospyros kaki L.) proanthocyanidin: A highly galloylated, a-linked tannin with an unusual flavonol terminal unit, myricetin. *J. Agric. Food Chem.* **2010**, *58*, 9033−9042.
(47) Fernández, K.; Kennedy, J. A.; Agosin, E. Characterization of Vitis vinifera L. Cv. Carménère grape and wine proanthocyanidins. *J. Agric. Food Chem.* **2007**, *55*, 3675−3680.
(48) Sun, B.; Leandro, M. C.; de Freitas, V.; Spranger, M. I. Fractionation of red wine polyphenols by solid-phase extraction and liquid chromatography. *J. Chromatogr., A* **2006**, *1128*, 27−38.
(49) Hanlin, R. L.; Kelm, M. A.; Wilkinson, K. L.; Downey, M. O. Detailed characterization of proanthocyanidins in skin, seeds, and wine of Shiraz and Cabernet Sauvignon wine grapes (Vitis vinifera). *J. Agric. Food Chem.* **2011**, *59*, 13265−13276.
(50) Fulcrand, H.; Remy, S.; Souquet, J.-M.; Cheynier, V.; Moutounet, M. Study of wine tannin oligomers by on-line liquid chromatography electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 1023−1028.
(51) Sun, B.; Spranger, I.; Roque-do-Vale, F.; Leandro, C.; Belchior, P. Effect of different winemaking technologies on phenolic composition in Tinta Miúda red wines. *J. Agric. Food Chem.* **2001**, *49*, 5809−5816.
(52) Sun, B.; Leandro, C.; Ricardo Da Silva, J. M.; Spranger, I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1998**, *46*, 1390−1396.
(53) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.
(54) Sáenz-Navajas, M.-P.; Campo, E.; Avizcuri, J. M.; Valentin, D.; Fernández-Zurbano, P.; Ferreira, V. Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: Wine reconstitution strategies and sensory sorting task. *Anal. Chim. Acta* **2012**, *732*, 64−72.
(55) Chira, K.; Schmauch, G.; Saucier, C.; Fabre, S.; Teissedre, P.-L. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *J. Agric. Food Chem.* **2009**, *57*, 545−553.
(56) Scharbert, S.; Holzmann, N.; Hofmann, T. Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *J. Agric. Food Chem.* **2004**, *52*, 3498−3508.