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Relationship between Nonvolatile Composition and Sensory Properties of Premium Spanish Red Wines and Their Correlation to Quality Perception

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The correlation of nonvolatile composition in wines with quality perception is a critical subject in current enological research, and it is far from being clear. Thus, the present work aims at (1) defining the chemical composition and in-mouth sensory properties of a set of wooded premium Spanish red wines and (2) assessing the implication of their chemical composition in the sensory perception of quality. Therefore, 24 wines were analyzed by sensory descriptive analysis and chemical analysis for nonvolatile composition, and their correlations have been discussed. In parallel, a panel of wine experts performed a quality evaluation based on overall perception. Multivariate statistical analysis has revealed that quality was primarily related to wines without defective aroma and secondarily to the presence of nonvolatile components such as reducing sugars and alcohol content as well as some phenolic compounds: proanthocyanidins linked to polysaccharide, *trans*-caffeic, *trans*-coutaric, and *trans*-caftaric acids, quercetin-3-*O*-glucuronide, and malvidin−catechin dimer. The results show that wines evaluated as high-quality wines by experts present higher concentrations of these compounds except for *trans*-caffeic acid, which accumulates higher concentration levels in low-quality wines.

KEYWORDS: Quality; nonvolatile composition; PLS; wine

INTRODUCTION

Wine is a product for hedonic consumption and, thus, its sensory quality is positively related to its sensory appeal, quality perception being complex and multidimensional (*1*). The determination of the minor (quantitatively) chemical components is a promising approach to assess the stability of wine (*2*), its origin (*3*) and authenticity (*4*), and thus its commercial quality. It is widely reported that not only chemical composition but also molecular interactions among wine components play a determinant role in the chemical stability of wine and affect the sensory properties (*5*−*7*). The overall wine sensory quality perception is elicited by the simultaneous stimulation of several senses that provide precise properties for color, odor, taste, and mouthfeel. The color of a wine is the first characteristic perceived. Color seems to be a very important parameter in red wines as it enables tasters to anticipate the gustatory and/or olfactory properties on the basis of their previous experience (*8*). Boselli et al. (*8*) reported the characterization of phenolics in Lacrima di Morro d'Alba wine showing that copigmentation was positively related to the sour taste of wine, but was negatively related to astringency. CIELAB color space is considered to be the most homogeneous and, therefore, possibly the best model to evaluate wine color (*9*).

The volatile composition influences the organoleptic characteristics of wines, particularly the aromatic sensory perception. However, the overall aroma of a wine presents an extremely complex chemical pattern in both qualitative and quantitative terms (*10*). One research aiming at studying the influence of odorants on the quality of a set of premium Spanish red wines showed that wine quality was related to its aroma composition, primarily to the absence of defective or negative odorants and secondarily to the presence of a relatively large number of fruit-sweet odorants (*11*).

Aroma-active volatiles as well as nonvolatile chromophores of red wine have been thoroughly investigated in recent decades, but only a small number of studies were targeted toward the study of taste-active and/or astringent-active nonvolatile molecules able to affect the overall perceived flavor (*12*, *13*).

Astringency, which is a complex mouthfeel sensation, has been found to positively influence quality evaluation of flavor (attack, evolution, and persistence) and overall quality. Although in many wines astringency is regarded as a defect, in the particular case of wines from the variety Tannat (*14*), astringency was described as one of the sensations associated with high quality. Besides, it has been reported (*15*) that among the sensory characteristics of red wines that are involved in quality perception, both acidity and astringency constitute important parameters of red wines. Indeed, studies on phenolic compounds have shown that these

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compounds constitute one of the most important quality parameters of red wines because they contribute to their organoleptic characteristics, particularly, color, astringency, and bitterness (*12*, *13*, *16*). According to these studies, a close link exists between high-quality wines and astringent and acid wines and hence phenolic composition (*15*).

In this context, the aims of this work are (1) to evaluate the relationship between the nonvolatile composition and sensory properties (taste and astringency) of a set of 24 premium wines and (2) to assess to what extent the chemical composition of this set of wines is related to its general sensory quality. For this, the nonvolatile composition of a relatively large number of standard market samples of premium Spanish red wines have been screened, and such nonvolatile profiles have been related to the measured sensory quality evaluated by wine experts through multivariate statistics tools.

MATERIALS AND METHODS

Wine Samples. Twenty-four Spanish red aged wines from 13 different Spanish Denominations of Origin have been evaluated. All wines were premium products with a price ranging between 15 and 20 euros/bottle and were selected on the basis of sales records to obtain a random sample representative of the Spanish high-quality red wine market. The detailed list of samples, including sample information, is shown in the Supporting Information.

Reagents. All chemicals used were of analytical reagent grade. All chromatographic solvents were of HPLC grade. Ultrapure water was obtained from a Milli-Q purification system (Millipore, Molsheim, France). TSK Toyopearl gel HW-50F was from Tosohaas (Montgomeryville, PA). Methanol, formic acid, ethanol, acetonitrile, and sulfuric acid were purchased from Scharlau (Scharlab, Spain). Quinine sulfate dihydrate (98%) was obtained from Alfa Aesar (Karlsruhe, Germany). Potassium and aluminum sulfate and tannic acid were purchased from Panreac (Barcelona, Spain). Ovalbumin (V-grade), tartaric acid, catechin, epicatechin, *trans*-aconitic acid (98%), *cis*-aconitic acid, syringic acid, myricetin, kaempferol (90%), vanillin, protocatechuic acid ethyl ester (97%), gallic acid (97%), *trans*-caffeic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO). Malvidin-3-*O*-glucoside, syringetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucuronide, isorhamnetin-3-*O*-glucoside, epicatechin gallate, epigallocatechin gallate, epigallocatechin, syringetin-3-*O*-galactoside, isorhamnetin-3-*O*-glucoside, ferulic acid, and *p*-coumaric acid were provided from Extrasythèse (Genay, France). Vanillic acid and trifluoroacetic acid (TFA) were supplied by Fluka (Buchs, Switzerland).

Fractionation of Wine Phenolics by Size Exclusion Chromatography. Wine samples were directly fractionated by means of a TSK Toyopearl gel packed in a HW-50F Millipore (Bedford, MA) Vantage L column (120 mm × 12 mm i.d.) at atmospheric pressure as described elsewhere (*17*). Therefore, 2 mL of wine was directly injected in the column, and the flow rate was regulated at 1 mL min^{-1} using a peristaltic pump. The first fraction (F1) was eluted with 60 mL of ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v). A second fraction (F2) was recovered by elution with 50 mL of acetone/water (60:40, v/v). The two fractions collected were brought to dryness under vacuum. Fraction 1 and 2 were redissolved in 2 mL of formic acid/water (5:95, v/v) and 7 mL of methanol, respectively. Fraction F1 was further analyzed by HPLC-DAD and HPLC-MS, and F2 was employed for the analysis of proanthocyanidin content using the vanillin assay. All wines were fractionated three times and passed through a 0.45 μL filter before analysis.

HPLC-DAD Analysis. Anthocyanins, acids, flavanols, and flavonols were analyzed by direct injection of fraction F1, obtained from the SEC, into the HPLC system. HPLC-DAD analyses were performed using an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany) equipped with a G1313A injector, a G1311A HPLC quaternary pump, an online G1379A degasser, a G1316A oven, a G1315B photodiode array detector, and Agilent Chemstation software. The column was a reversed-phase Kromasil 100-C18 (5 μm packing, 250 mm × 46 mm i.d.), protected with a guard column of the same material (Teknokroma, Barcelona, Spain). Compounds were eluted under the following conditions: 1 mL min^{-1} flow rate; oven at 40 °C; solvent A, formic acid/water (5:95, v/v); solvent B, acetonitrile (100%); gradients, isocratic 0% B in 2 min, from

0 to 8% B in 3 min, from 8 to 20% B in 55 min, from 20 to 30% B in 10 min, from 30 to 50% B in 1 min, from 50 to 100% B in 2 min, isocratic at 100% B for 7 min, from 100 to 0% B in 1 min, and then isocratic at 0% B for 9 min, followed by washing and reconditioning of the column. Thirty microliters of F1 (in formic acid/water, 5:95 v/v) was directly injected in the HPLC system and chromatographed. UV−vis spectra were recorded from 210 to 700 nm, with a bandwidth of 2.0 nm.

Quantification was carried out by peak area measurements at 520 nm for anthocyanins, at 365 nm for flavonols, at 310 nm for hydroxycinnamic acids, at 280 nm for flavanols and phenolic acids, and at 220 nm for aconitic acids. Identification of compounds was performed by comparing their retention times and UV−vis spectra to those of authentic standards and also confirmed by HPLC-MS analysis. Their quantification was performed in triplicate using an external standard calibration curve for each compound. Quantification of noncommercial compounds was made using the calibration curves and response factor of a structurally closer compound such as *trans*-caffeic acid for *trans*-caftaric acid or *trans*-coumaric acid for *trans*-coutaric acid. Calibration curves were obtained by injecting different concentrations of standards. The range of the linear calibration curves (r^2 > 0.994 in all cases) was from 0.01 (limit of detection) to 1 mg L^{-1} for the lower concentration compounds and from 1.0 to 100 mg L^{-1} for the higher concentration compounds. Concentrations were determined from the linear regression equations. Quantitative data of the identified compounds were obtained by interpolation of the relative areas in calibration curves built for pure reference compounds.

HPLC-ESI-MS Analysis. MS analysis was performed by coupling the Agilent 1200 liquid chromatograph described above to a Hewlett-Packard 5989A quadrupole mass spectrometer equipped with an electrospray interface (HP 59987A) and controlled by the MS Agilent 1200 software. Chromatographic separation was performed under the same conditions described above. To ensure a flow of 19 μL min^{-1} into the ESI interface during LC-MS, the LC effluent was split by means of a zero dead volume T-piece. This flow was found to be optimum under these conditions. Nitrogen was used as nebulizing gas at an inlet pressure of 80 psi and a temperature of 225 °C. All mass spectrometry data were acquired in positive ionization profile mode from *m/z* 150 to 700.

Determination of the Total Proanthocyanidin (PA) Content by the Vanillin Assay. The vanillin assay was performed according to the method described elsewhere (*18*) in F2, which contains practically all of the polymeric PAs. The absorbance of the colored adducts formed between vanillin (4-hydroxy-3-methoxybenzaldehyde) and proanthocyanidins (flavanols with a single bond at the 2,3-position and free meta-oriented hydroxy groups on the B ring) was measured at 500 nm.

Analysis of Conventional Enological Parameters in Wines. Conventional enological parameters of wines were determined in accordance with official OIV practices. Malic and lactic acids were determined by enzymatic methods in accordance with official AOAC analysis methods (*19*). Total polyphenol index (TPI) was estimated as absorbance at 280 nm (*20*). The analysis of reducing sugars, ethanol content, pH, and titratable and volatile acidities was made by infrared spectrometry with Fourier transformation (IRFT) with a WineScan FT 120 (FOSS, Barcelona, Spain), which was previously calibrated according to official OIV methods.

Analysis of Protein−Precipitable PAs. The protein-precipitable PAs were estimated using ovalbumin as the precipitation agent and tannic acid solutions as standards in accordance with a previously described method (*21*). All experiments were carried out at room temperature (20 ± 2 °C) and in triplicate.

Analysis of Ethanol Index (ETI). The ETI reflects the tannin/polysaccharide condensation; an increase in value indicates stronger combination between tannins and polysaccharides, and it was calculated as described elsewhere (*22*). One milliliter of wine was placed in one tube and 9 mL of ethanol was added; after 24 h, the solution was centrifuged to eliminate the precipitates. The supernatant was diluted 1/10 with distilled water. Absorbance was measured immediately at 280 nm (A_2) (Hewlett-Packard 8453) in a quartz bucket with an optical path of 10 mm. The original wine was also diluted 1/100 with distilled water, and the absorbance was measured at 280 nm (A_1). Measurements were carried out in triplicate. The ETI was calculated as follows:

$$\text{ethanol index (ETI)} = 100 \times (A_1 - A_2)/A_1$$

Colorimetric Measurements. The whole visible spectrum (380–770 nm) of each sample was recorded ($\lambda = 1$ nm). The CIE 2004 standard observer (10° visible field) and the CIE standard illuminant D65 were used as references according to the OIV (*23*, *24*).

Descriptive Sensory Analysis. A total of 35 students or staff members from the University of La Rioja (Spain) were recruited on the basis of their interest and availability during 13 weeks (one 60 min session per week). They were not paid for their participation. A 1 h selection session was devoted to choose the best panelists suited to performing descriptive tasks. Those candidates showing sensory deficiencies in identifying basic tastes or a manifest inability in verbal description were not selected to participate in the study. Among the 35 panelists recruited, 32 were selected to carry out the measuring sessions (12 males and 20 females from 21 to 62 years old) and with them a three-way ANOVA involving sample (S), judge (J), and replicate (R) as fixed factors; all first-order interactions was calculated to confirm the panel performance. None of the replicate effect was significant, indicating a consistent assessment of attributes and reflecting the reproducibility of the panel. The wine-by-replicate interaction (W × J) was significant for the term bitterness. This indicates that there are differences in the interpretation of the term bitterness and that assessors may need more training with respect to this attribute. Hence, this term was not considered in subsequent analysis.

Panelists attended eight descriptive sensory training sessions over a period of two months, during which they worked in subgroups. During training, different reference standards representative of taste and astringency terms were presented. Solutions containing different concentrations of table sugar (0–12 g L^{-1}) for sweetness, tartaric acid (0–1.5 g L^{-1}) for acidity, quinine sulfate (0–10 mg L^{-1}) for bitterness, and potassium and aluminum sulfate (0–5 g L^{-1}) for astringency stimuli (*5*, *25*) were presented to the panel to aid with recognition and discrimination between the different oral sensations. During the training sessions definitions for aromatic intensity (defined as the intensity generated orthonasally), global intensity (defined as the intensity generated by aroma, taste, and mouthfeel attributes evaluated in-mouth), and persistence (defined as the time the perceptions lingered in the mouth after the wine was expectorated) were provided.

The training period included two phases: a general and a product-specific training phase. During the general training phase (four sessions), panelists became familiar with intensity rating of sweetness, acidity, bitterness, astringency, and aromatic and global intensity as well as persistence. During a typical session panelists had to evaluate three to five different wines by rating sweetness, acidity, bitterness, and astringency on a 10-point scale (0 = "absence", 1 = "very low", and 9 = "very high") and global and aromatic intensity (1 = "very low" and 9 = "very high") as well as persistence (1 = "very short" and 9 = "very long") on a 9-point scale. The wines selected for this training phase presented intense and easily recognizable properties and included red, white, and rosé wines of diverse grape varieties and origins. The session ended with a discussion during which the panel leader compared the attributes intensity scores given by panelists to describe each wine, and a discussion was organized until a consensus was reached. The specific training phase consisted of four sessions during which panelists became familiar with the type of samples of the study. During this phase, panelists rated the intensity of sweetness, acidity, bitterness, astringency, and global and aromatic intensity, as well as persistence of premium Spanish red wines commercially available different from those of the experiment.

Trained panelists described wines in duplicate during a total of five sessions (9–10 wines per session). Ten-milliliter wine samples were presented in dark ISO (*26*) approved wine glasses labeled with three-digit random codes and covered by plastic Petri dishes according to a random arrangement. Panelists were asked to rate the sweetness, acidity, bitterness, astringency, global and aromatic intensity, and global persistence of the samples using the above-mentioned structured scales for each wine. Trained panelists rated samples using a sip and spit protocol. Ten seconds after wine was sipped, it was expectorated. Ten seconds later, apple pectin solution (1 g L^{-1}) was sipped, which was spat out after another 10 s. Between wine–rinse combinations, subjects rinsed twice with deionized water for 20 s as described by Colonna et al. (*27*).

All wines were served at room temperature and were evaluated in individual booths. The sessions took place in a ventilated and air-conditioned tasting room (at around 20 °C). Panelists were not informed about the nature of the samples to evaluate.

Quality Assessment by Wine Experts. The sensory panel was composed of 8 females and 10 males, 30–60 years of age, all with a long experience as wine tasters but with different backgrounds: 5 were aroma researchers (AR), 4 were winemakers (WM), 5 were sommeliers (S), and 4 were wine retailers (R). Twenty-milliliter wine samples were presented in clear ISO (*26*) approved wine glasses labeled with three-digit random codes and covered by plastic Petri dishes according to a random arrangement. Each panelist participated individually in one session. First, the panelists were required to smell and taste each of the 24 wines, once in the proposed order, to minimize any bias introduced by the order of presentation. Afterward, they could smell and taste the samples as many times as they wanted and in any order. The panelists were asked to sort the wines into groups on the basis of quality (color, odor, and taste). They were asked to form five groups and to put as many wines as they wished in each group. The groups were exceptional (scored as 5 during data recording), good or very good (scored as 4), right or approved (scored as 3), poor or disappointing (scored as 2), and defective or rejectable (scored as 1). The panelists were informed about the general price range of the samples before the tasting session, but no more data were disclosed. The quality index of each wine was obtained by averaging all of the individual scores obtained by each wine after recoding.

Statistical Analysis. Simple lineal regressions were calculated between sensory and chemical variables. Significance was considered at an error threshold of 5% ($P < 0.05$). Statistical analyses were performed using SPSS (version 15.0, SPSS Inc., Chicago, IL).

Partial least-squares regression (PLSR) by means of the PLS1 algorithm was used to evaluate the relationship between sensory astringency or quality and the nonvolatile composition measured in the set of wines studied in the present work. Sweetness and acidity were not predicted because they presented significant correlations with none or only one of the chemical measurements acquired. Therefore, the matrix of the chemical composition data has been scaled to obtain normalized data, and homoscedasticity and linearity have been confirmed to avoid overestimating the goodness of fit in case the distribution of data would have been heteroscedastic. Finally, collinearity between the *X*-variables has been ruled out, because no significant correlations between variables were found. The strategy followed for the prediction of astringency and quality from chemical composition was as follows: A first initial model was built for astringency/quality using all *X* variables (chemical measurements of nonvolatile composition). After this, the existence of outliers was checked, and samples with a clear deviation from the model were eliminated and kept out of the calibration process. The model was then recalculated. An iterative process was then begun, to reduce the number of *X* variables in the model, searching for the simplest model with the best prediction ability. A full cross-validation was carried out to estimate the prediction ability of the models for a new set of samples. The data used for the prediction of astringency and quality with PLS were the average value given by all panelists to each sample. PLS regression was performed with Unscrambler 9.7 (CAMO).

RESULTS AND DISCUSSION

Chemical and Sensory Characterization of Wines. A summary of the sensory attributes, global compositional data, and color parameters determined in the 24 wines are shown in **Table 1**. According to one-way ANOVA with repeated measurements (judges considered as repetition), the effect of wine was highly significant ($P < 0.001$) for all of the sensory attributes evaluated by the trained panel except for sweetness ($F = 1.475$; $P = 0.071$). This indicates that this attribute is not useful in characterizing differences among this set of wines. The sensory thresholds of reducing sugars have been reported to range between 10 and 50 g L^{-1} (*13*) for sweetness; however, the samples studied in the present work are all dry wines with contents of <5 g L^{-1}. This could explain the fact that panelists could not find significant differences for sweetness in this set of wines. Moreover, it should be noted that the sweetness perceived is not correlated either with the content in reducing sugars ($F = 0.960$; $P = 0.760$) or with the ethanol content ($F = 0.01$; $P = 0.975$). With regard to the acidity perceived, among all of the compounds studied, this attribute was

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Table 1. Mean, Maximum, and Minimum Values of the Sensory Attributes, Conventional Enological Parameters, and Global Phenolic Composition of the 24 Wine Samples[a]

parameter	mean	max	min
sensory attributes			
quality	3.00	4.00	1.50
sweetness	2.45	2.80	2.11
acidity	3.98	4.58	3.41
astringency	5.23	6.33	3.92
global intensity	5.55	6.31	4.62
persistence	5.29	5.77	4.42
aromatic intensity	5.90	6.25	5.48
a^* (red color)	40.9	51.7	27.0
b^* (yellow color)	20.1	35.9	11.0
L^* (luminosity)	49.8	66.6	27.7
conventional enological parameters			
pH	3.59	3.95	3.37
total acidity[b]	3.70	4.55	3.07
volatile acidity[b]	0.55	0.95	0.39
malic acid (g L^{-1})	0.19	0.62	0.10
lactic acid (g L^{-1})	1.64	2.43	0.90
reducing sugar (g L^{-1})	2.70	3.96	1.36
total alcohol content (v/v)	14.8	16.0	13.1
polyphenolic composition			
proanthocyanidins (polymeric)[c]	114.5	419.0	0.1
total polyphenol content[d]	65.2	87.7	48.2
proanthocyanidin/polysaccharide complexes[d]	29.1	40.4	5.60
proanthocyanidins precipitable with ovalbumin[e]	1.1	1.9	0.5

[a] Quality was evaluated by the panel of wine experts, whereas sweetness, acidity, astringency, global intensity, persistence, and aromatic intensity were evaluated by a specifically trained panel. [b] Expressed as g L^{-1} of sulfuric acid. [c] Expressed as g L^{-1} of catechin. [d] Expressed in %. [e] Expressed as g L^{-1} of tannic acid.

found to be correlated only with the titratable acidity ($F = 5.254$; $P = 0.032$), in accordance with the literature (5, 28). It is also noteworthy that both persistence and global intensity are highly positively correlated with astringency ($P < 0.001$; $F = 107.25$ and 84.66, respectively). These results are in accordance with the literature (5, 29), where it has been demonstrated that in the case of red wine persistence and global intensity are mainly related to its nonvolatile composition, particularly to its phenolic composition, which is mainly responsible for astringency, whereas in white wines both attributes are mainly linked to its volatile composition.

Color properties of these set of wines were evaluated by measuring the CIELab coordinates a^* (representing red color), b^* (representing yellow color), and L^* (representing luminosity), and the three variables were found to significantly vary among this set of wines.

Simple lineal regressions were calculated to determine the correlations between sensory properties (taste, astringency, global intensity, persistence, and color) and chemical composition. For the color properties, it is important to highlight that the L^* coordinate, which is related to the perceived luminosity of wines, was inversely correlated with the PA content ($F = 8.41$; $P = 0.008$). This suggests that a higher content in PAs results in darker wines.

With regard to the sensory scores given by the trained panel, for acidity a significant correlation with titratable acidity was found ($F = 5.234$; $P = 0.032$), which is in accordance with the literature (30); however, it does not present any significant correlation either with malic or lactic acids or with pH. The sensory

perception of sweetness was not correlated with reducing sugars, which could be attributable to the low level of fructose and glucose in this set of dry wines and thus the absence of their sensory implication. Ethanol, which is sometimes considered to elicit sweetness (31), was not significantly correlated with the sensory attribute, which was also observed by Blackman et al. (30). Notwithstanding, the content of ethanol in this set of wines was found to be strongly correlated with the sensory perception of astringency ($F = 24.27$; $P < 0.001$). Indeed, ethanol has been described as an oral desiccant, which could explain its contribution to the perceived drying sensation and roughness of wines (6, 30). Moreover, PAs are widely reported to be responsible for astringency perception ascribed to their interaction with salivary proteins (21, 32). This property is known to vary with both PA structure and degree of polymerization, the larger PA molecules being the most important in the astringency sensation (33). In the present work, significant correlations between sensory astringency and protein-precipitable PAs ($F = 9.130$; $P = 0.006$) as well as with polymeric PAs measured by the vanillin assay ($F = 7.285$; $P = 0.013$) have been observed. Besides, Hufnagel and Hofmann (12) and Sáenz-Navajas et al. (28) have demonstrated that PA monomers, dimers, and trimers seem not to be the key elicitors of the astringent taste of the red wine, instead a variety of different low molecular weight polyphenols as well as a polymeric fraction, which acquire the highest mouthfeel impacts. Hence, the low molecular weight polyphenols reported by Hufnagel and Hofmann (12, 13) to exert high sensory impacts in wine astringency were targeted by HPLC-DAD to study their implication in sensory astringency in this set of wines.

Analysis of Phenolic Compounds with Sensory Properties by HPLC-DAD. Individual monomeric phenols with known sensory properties were identified by HPLC-DAD and HPLC-MS and quantified with standards by HPLC-DAD; among the compounds quantified, five families were distinguished: acids, flavanols, hydroxycinnamic acids, flavonols, and anthocyanins and their derivatives (**Tables 2** and **3**) as reported Hufnagel and Hoffmann (12, 13). In addition, the ranking of compounds in their sensory impact was based on the DoT (dose-over-taste) factor, which was calculated for each compound from the ratio of the concentration and the threshold concentration (34).

With regard to the quantified acids, gallic acid is by far the most abundant, followed by protocatechuic acid, in this set of wines, which is in accordance with data reported for red wines from Madeira (35) or Spanish red wines elaborated with Cabernet Sauvignon (36). Both gallic and protocatechuic acids have been shown to provide a puckering astringency at concentrations of > 50 and > 32 mg L^{-1} (12), respectively. This reveals that gallic acid could have a sensory impact in most of the wines analyzed (average content of 42 mg L^{-1} and maximum concentration of 67.2 mg L^{-1}), whereas protocatechuic acid did not reach in any case the minimum sensory threshold ($DoT_{max} < 1$). Similarly, the ester of protocatechuic acid ($DoT_{max} = 0.60$) does not reach in any of the studied wines its sensory threshold, which suggests that it would not have any sensory impact in this set of samples. For both isomers of aconitic acid it is important to highlight that both have been described as astringent and acid with a quite low sensory threshold for astringency (0.1 mg L^{-1}) and a > 16 times higher threshold for acid sensation (13). This suggests that these acids could have an important implication in the astringency of red wines and thus in the wines examined in this work, where *trans*-aconitic acid reaches a DoT_{max} of 76.6.

Monomeric flavanols have been reported to elicit astringency; however, none of them was found at concentrations higher than their corresponding sensory threshold ($DoT_{max} < 1$ in all cases) in the studied wines. On the contrary, the sensory thresholds of

Table 2. Astringent Compounds Analyzed by HPLC-DAD-MS Found in the 24 Wines[a]

compd no.	compd name	RT	M[+] [m/z]	mean	max	min	max/min[b]	DoT$_{max}$[c]	threshold
	acids and derivatives								
1	*cis*-aconitic acid + unidentified	3.2	175	3.52	4.70	2.34	2.01	**47.0**	0.1 (12)
2	*trans*-aconitic acid	3.7	175	4.90	6.67	3.41	2.00	**76.6**	0.1 (12)
3	gallic acid	4.0	171	42.06	74.27	19.81	1.50	**1.50**	50 (12)
4	protocatechuic acid	6.7	155	6.02	10.21	3.30	*	0.32	32 (12)
5	protocatechuic acid ethyl ester	23.7	183	2.01	5.25	0.08	*	0.60	9 (12)
	flavanols								
6	catechin	9.3	291	78.77	126.20	49.86	1.06	**1.06**	119 (40)
7	epigallocatechin	10.1	307	5.58	7.13	4.40	*	0.04	159 (40)
8	epicatechin	12.4	291	10.16	14.39	7.06	*	0.05	270 (40)
9	epigallocatechin gallate	14.0	459	8.57	13.54	5.04	*	0.12	110 (40)
	hydroxycinnamic acids and derivatives								
10	*trans*-caftaric acid[d]	8.2	311	15.75	51.24	2.56	10.24	**10.26**	5 (12)
11	*trans*-coutaric acid[d]	9.8	295	10.84	20.40	1.99	2.04	**2.04**	10 (45)
12	*trans*-caffeic acid	10.8	181	7.35	21.66	2.89	1.67	**1.67**	13.0 (12)
13	*trans*-coumaric acid	14.6	165	7.58	23.00	3.32	1.01	**1.01**	23.0 (12)
14	ferulic acid	18.8	195	1.89	2.90	1.48	*	0.22	13.0 (12)
	flavonols								
15	quercetin-3-*O*-galactoside	24.6	465	2.29	4.75	1.00	4.75	**23.79**	0.2 (12)
16	quercetin-3-*O*-glucuronide	26.9	479	1.80	15.2	0.50	15.20	**15.20**	1.0 (12)
17	quercetin-3-*O*-glucoside	29.6	465	2.69	3.35	0.61	*	0.38	0.1 (40)
18	myricetin	32.2	319	8.83	13.7	7.03	1.37	**1.37**	10 (39)
19	isorhamnetin-3-*O*-glucuronide[e]	38.2	493	0.42	1.42	1.00	1.42		
20	kaempferol-3-*O*-glucoside	40.6	509	1.74	4.98	0.56	8.82	**16.61**	0.3 (40)
21	isorhamnetin-3-*O*-glucoside	42.5	479	0.23	1.42	0.88	1.29	**1.29**	1.1 (12)
22	quercetin	46.8	303	3.42	7.32	2.17	*	0.7	10.0 (39)
23	kaempferol	61.6	289	5.30	6.45	0.77	*	0.3	20.0 (39)

[a] Retention time (expressed in minutes), molecular ion (M[+]), mean, maximum, and minimum concentrations (expressed in mg L^{-1}), maximum taste-over-dose parameter (DoT$_{max}$), and sensory thresholds (expressed in mg L^{-1}) for astringency (numbers in parentheses are bibliography references for sensory thresholds). [b] If max < threshold: * [c] DoT$_{max}$ > 1 marked in bold. [d] Expressed as mg L^{-1} of *trans*-caffeic and *trans*-coutaric acid, respectively. [e] Expressed as mg L^{-1} of querecetin-3-*O*-glucuronide.

Table 3. Retention Time (Expressed in Minutes), Molecular Ion, and Mean, Maximum, and Minimum Concentrations (Expressed in Milligrams per Liter) of the Anthocyanins Analyzed by HPLC-DAD-MS in the 24 Wine Samples

anthocyanidins and derivatives	RT	M[+] [m/z]	mean	max	min	max/min[a]
peonidin-3-*O*-glucoside-(epi)catechin	10.8	751	0.90	1.06	0.70	1.51
malvidin-3-*O*-glucoside-(epi)catechin	11.6	781	1.07	1.26	0.87	1.45
delphinidin-3-*O*-glucoside	12.8	465	1.42	1.88	0.91	**2.07**
cyanidin-3-*O*-glucoside	14.2	449	0.96	1.05	0.88	1.19
petunidin-3-*O*-glucoside	18.4	479	1.38	2.55	0.70	**3.64**
petunidin-3-*O*-glucoside pyruvate	21.6	547	0.90	0.97	0.70	1.39
peonidin-3-*O*-glucoside	22.3	463	1.15	2.73	0.70	**3.90**
malvidin-3-*O*-glucoside	25.4	493	3.77	6.89	1.25	**5.51**
malvidin-3-*O*-glucoside pyruvate (vitisin A)	30.5	561	1.50	2.34	1.06	**2.21**
malvidin-3-*O*-glucoside-vinylcatechol	37.4	625	0.88	1.05	0.70	1.50
malvidin-3-*O*-glucoside-catechin	40.5	781	0.89	1.50	0.70	**2.14**
malvidin-3-*O*-(acetyl)glucoside	46.6	517	0.98	1.05	0.89	1.18
peonidin-3-*O*-(p-coumaryl)-glucoside	60.1	609	0.89	1.08	0.70	1.54
malvidin-3-*O*-(p-coumaryl)-glucoside	62.0	639	0.88	1.06	0.70	1.51

[a] Max/min > 2 marked in bold.

oligomeric flavanols (PAs) have been reported to be lower than their corresponding monomers, ranging from 3.5 mg L^{-1} (37) to 22 mg L^{-1} for PAs of > 5 kDa (12). Consequently, both groups of PAs studied in the present work (total and protein-precipitable PAs) are well above their sensory thresholds in the major part of the studied wines, which suggests an important sensory implication of these compounds in this set of samples.

Among hydroxycinnamic acids, the tartaric ester of *trans*-caffeic acid, *trans*-caftaric acid, is by far the most predominant hydroxycinnamic acid followed by *trans*-coutaric acid, although the free acids are present in smaller amounts, which is in accordance with the literature that reports this fact in grapes and wine (38). The sensory implication of this family of compounds

has been already reported, where *trans*-coumaric, ferulic, *trans*-caffeic, and *trans*-caftaric acids are described as puckering astringent compounds (12, 13). Four of the five hydroxycinnamic acids studied were found to be at concentrations higher than their corresponding sensory threshold (DoT$_{max}$ > 1). Only ferulic acid was not found to reach a DoT$_{max}$ > 1. Among this family of compounds, *trans*-caffeic and *trans*-coutaric acids were found to present strong significant correlations with the perceived astringency described by the trained panel ($F = 8.939$ and 6.638; $P = 0.007$ and 0.017, respectively). This suggests that these compounds could play an important sensory role in the major part of this particular set of red wines.

The sensory impact of flavonols has been already proved (12, 13), and they have been reported to present velvety astringency in all cases with very low sensory thresholds ranging between 20 mg L^{-1} for kaempferol (39) and 0.1 mg L^{-1} for quercetin-3-*O*-glucoside (40). As can be seen in **Table 2**, most of the flavonols (except for quercetin and kaempferol) analyzed reach their sensory threshold in at least one of the wines studied (DoT$_{max}$ > 1). Besides, simple correlations calculated between astringency and this family of compounds showed significant correlations ($P < 0.01$) with myricetin, quercetin-3-*O*-galactoside, and quercetin-3-*O*-glucuronide, which suggests the probable implication and relevant sensory role played by these three flavonols in this set of Spanish red wines.

Furthermore, anthocyanins have been reported to increase tannin solubilization in wine (41) and may, in turn, affect tannin perception and hence perceived astringency. Thus, in the present work anthocyanins and some of their derivatives have been quantified by HPLC-DAD as can be seen in **Table 3**. It can be observed that the free anthocyanin content is already very low, as the wines have been aged for a long time (13−24 months). The anthocyanin content was found to present big differences in the
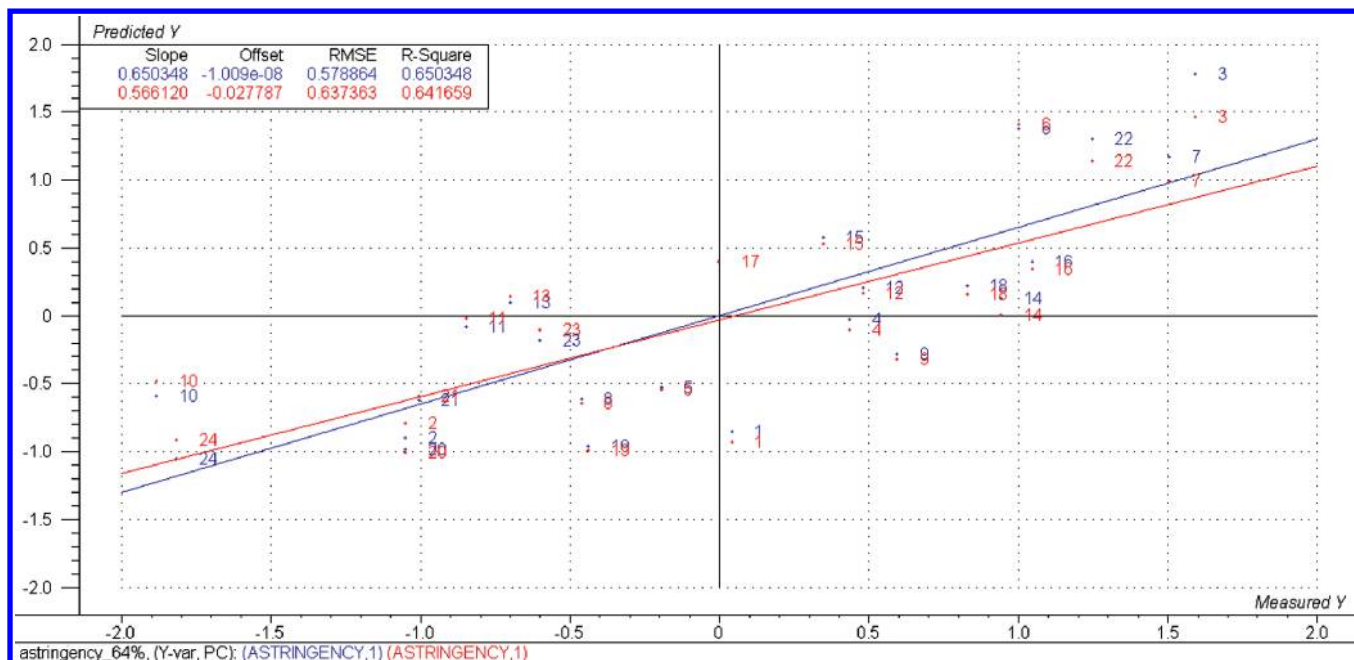
Figure 1. Prediction of sensory astringency from chemical composition: PLS plot of predicted versus measured scores obtained with the model.

wines studied, especially for the glucosides of delphinidin, petunidin, and malvidin as well as vitisin A and the dimer of malvidin-3-*O*-glucoside and catechin, because their maximum/minimum rates were higher than 2 units (*10*) and the ANOVA results showed significant differences ($P < 0.05$) among samples. In an attempt to correlate sensory astringency with the anthocyanin content, a significant correlation (3.606; $P = 0.049$) between the sensory attribute and the malvidin-catechin dimer was found.

In general, these data reveal the sensory implication of different families of both monomeric and polymeric phenolic compounds in the sensory astringency of wines; thus, in an attempt to construct a model for this sensory attribute, PLSR has been used.

Prediction of Astringency from Nonvolatile Composition by PLSR. PLSR has been successfully used to study the relationships between sensory and chemical analyses in wine. Thus, to build a predictive model for sensory astingency (*Y* variable), using nonvolatile composition (*X* variable) as explicative one, PLSR with cross-validation was performed. At first approximation, to reduce the number of compounds that could be responsible for sensory modifications, only the compounds found at concentrations above their thresholds in at least one wine were considered (DoT$_{max}$ > 1, see **Table 2**). Furthermore, it should be expected that the concentrations of the taste-active compounds responsible for effective sensory differences in the studied samples show remarkable differences. Thus, the maximum/minimum concentration rate was taken as a differentiability criterion (*10*). Compounds reaching values above 2 for this parameter were considered to have the greatest capacity to induce sensory modifications and were confirmed by calculation of significant differences by means of ANOVA. As can be seen in **Table 2**, the most discriminant taste-active compounds, on the basis of the quotient maximum/minimum, were found to be protein-precipitable PAs (max/min = 3.50), polymeric PAs measured by the vanillin assay (max/min = 13.10), and *cis*-aconitic acid + unidentified peak, *trans*-aconitic, *trans*-caftaric, and *trans*-coutaric acids, quercetin-3-*O*-galactoside, and kaempferol-3-*O*-glucoside. The variables with the highest capacity to induce modifications are PAs, *trans*-caftaric acid, and kaempferol-3-*O*-glucoside, which present a maximum/minimum > 8 in all cases. A second group is made up of the components with a maximum/minimum ratio ranging

between 5 and 2 composed of quercetin-3-*O*-galactoside, protein-precipitable PAs, *trans*-coutaric acid, *cis*-aconitic acid + unidentified peak, and *trans*-aconitic acid.

Apart from these phenolic compounds the variables alcohol content and reducing sugars as well as the dimer of malvidin-3-*O*-glucoside were used for the prediction of astringency because they were found to be significantly correlated ($F = 24.27$, 9.40, and 3.58; $P < 0.001$, 0.006, and 0.049, respectively) to the sensory perception of astringency.

The best model obtained following the strategy described under Materials and Methods is highly significant ($P = 0.002$), the total explained variance by the first principal component is 65% (64% by cross-validation), and the root-mean-square prediction error (RMSEP) is 0.579, as can be seen in **Figure 1**. According to Martens' uncertainty test (*42*) the model included six significant variables: alcohol content, reducing sugars (RS), PAs measured by the vanillin assay (PAs), *trans*-aconitic acid (t-Acon), *trans*-coutaric acid (Cou), and quercetin-3-*O*-galactoside (Q-Gal). All variables were positively correlated to sensory astringency, and the importance of these variables can be seen in the predicted model:

$$\text{astringency} = 0.269 \times \text{alcohol} + 0.203 \times \text{RS} + 0.185 \times \text{PAs}$$
$$+ 0.167 \times \text{t-Acon} + 0.179 \times \text{Cou} + 0.184 \times \text{Q-Gal}$$

These results confirm the importance of PAs in astringency perception, but the implication of different monomeric phenolics such as quercetin-3-*O*-galactoside and *trans*-coutaric acid as well as nonphenolic compounds such as *trans*-aconitic is remarkable. It is also noteworthy that the model suggests that alcohol content plays a relevant role in sensory astringency, a fact that has already been revealed by other investigations (*6*, *43*). The positive correlation of reducing sugars to astringency could be the result of an indirect consequence. It is well-known that during alcoholic fermentation, when nitrogen sources are consumed and ethanol concentrations are high, most yeast strains have difficulties in fermenting the remaining reducing sugars (especially fructose) (*44*), which means that wines with a higher content in alcohol could lead to wines with a higher content in reducing sugars and thus the high correlation between both variables ($P < 0.001$).
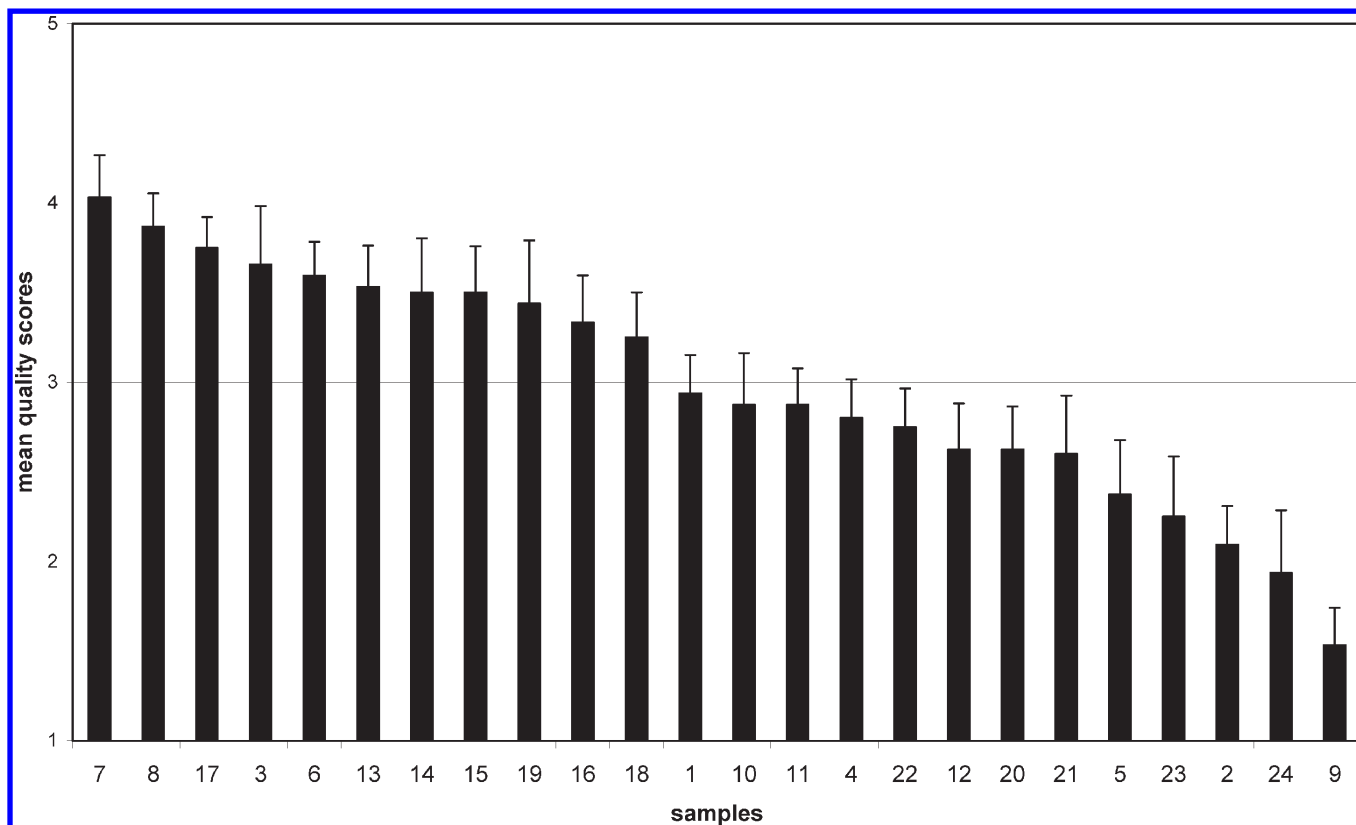
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Figure 2. Mean quality scores obtained for the 24 wines in the study.

Quality Perception and Its Correlation with Chemical and Sensory Properties. The overall sensory quality of wines was assessed by wine experts combining visual and odor−gustatory assessment. In this work, the quality of wine samples was evaluated by a group of 18 panelists with different professional backgrounds as explained under Materials and Methods (AR, WM, S, and R). To assess the degree of agreement among the four different groups of experts, a two-way ANOVA with the kind of expert and wines as fixed factors was performed. No significant differences ($P = 0.160$; $F = 1.693$) were found between the scores provided by the four different groups, which suggests that the quality concept was similar for them. Results of the sensory evaluation carried out with the 24 wines are shown in **Figure 2**. The quality scores range from 1.5 to 4.0 (the proposed scale ranged between 1.0 and 5.0), 3.0 being the average quality score. In an attempt to study to what extent the nonvolatile composition is related to quality perception by wine experts, PLSR was carried out to provide a preliminary predictive quality model. The predictive variables taken into account to accomplish such a goal were conventional enological parameters (**Table 1**) and phenolic composition (**Tables 2** and **3**). First, a reduction of variables was carried out, and only parameters considered to be able to induce significant differences in sensory perception were taken into account ($P < 0.05$). Then, the same strategy as described under Materials and Methods was followed. Sample 8 was considered an outlier due to its abnormal influence on the model. This wine was evaluated very high in quality by experts, probably due to its outstanding color (its L^* parameter was the lowest) and aroma properties (*11*) (fruity vector and defect aroma were highest and lowest, respectively), and thus the sensory properties generated by its nonvolatile composition are not able to explain its high perceived quality. The best model obtained with 23 wines and 8 variables explained 74% of the original variance (70% by cross-validation) ,and the RMSEP was 0.46 (0.56 by cross-validation). The 8 variables with

significant weights according to Marten's uncertainty test (*42*) in the PLS model were alcohol content, reducing sugars, *trans*-coutaric, *trans*-caffeic, and *trans*-caftaric acids, quercetin-3-*O*-glucuronide, PAs linked to polysaccharides, and the malvidin−catechin dimer.

In a recently published work (*11*) dealing with the aroma properties of this same set of wines and their linkage to quality perception, it was found that the presence of compounds such as 3,5-dimethyl-2-methoxypyrazine, 2,4,6-trichloroanisole, 4-ethyl-phenol, 4-ethylguaiacol, and *o*-cresol were the major causes of the low quality scores of these wines. For the purpose of modeling, the olfactometric scores of these five odorants with known negative sensory effect were summed to form a single olfactometric vector. Thus, a closer look at quality prediction from nonvolatile composition revealed that the model was significantly improved by including in the model the vector of defective aroma compounds. The new PLS model calculated with the 23 wines and 9 variables was highly significant ($P < 0.001$), the total explained variance by the first two principal components rose to 85% (84% by cross-validation), and the RMSEP decreased to 0.25 (0.29 by cross-validation) as can be seen in **Figure 3a**. The importance of the significant variables according to Marten's uncertainty test (*42*) can be seen in the correlation loading plot (**Figure 3b**). Quality perception of this set of wines was positively correlated to reducing sugars, ethanol content, PAs linked to polysaccharides (measured by the ethanol index), *trans*-coutaric and *trans*-caftaric acids, quercetin-3-*O*-glucuronide, and the malvidin−catechin dimer, whereas *trans*-caffeic acid and the defective aroma vector contributed negatively to the model.

In general, the PLS regression indicates that quality was primarily related to wines without defective aroma and secondarily to the presence of nonvolatile components and more precisely to phenolic composition that is able to modulate quality perception. This is well in line with recent data provided by other
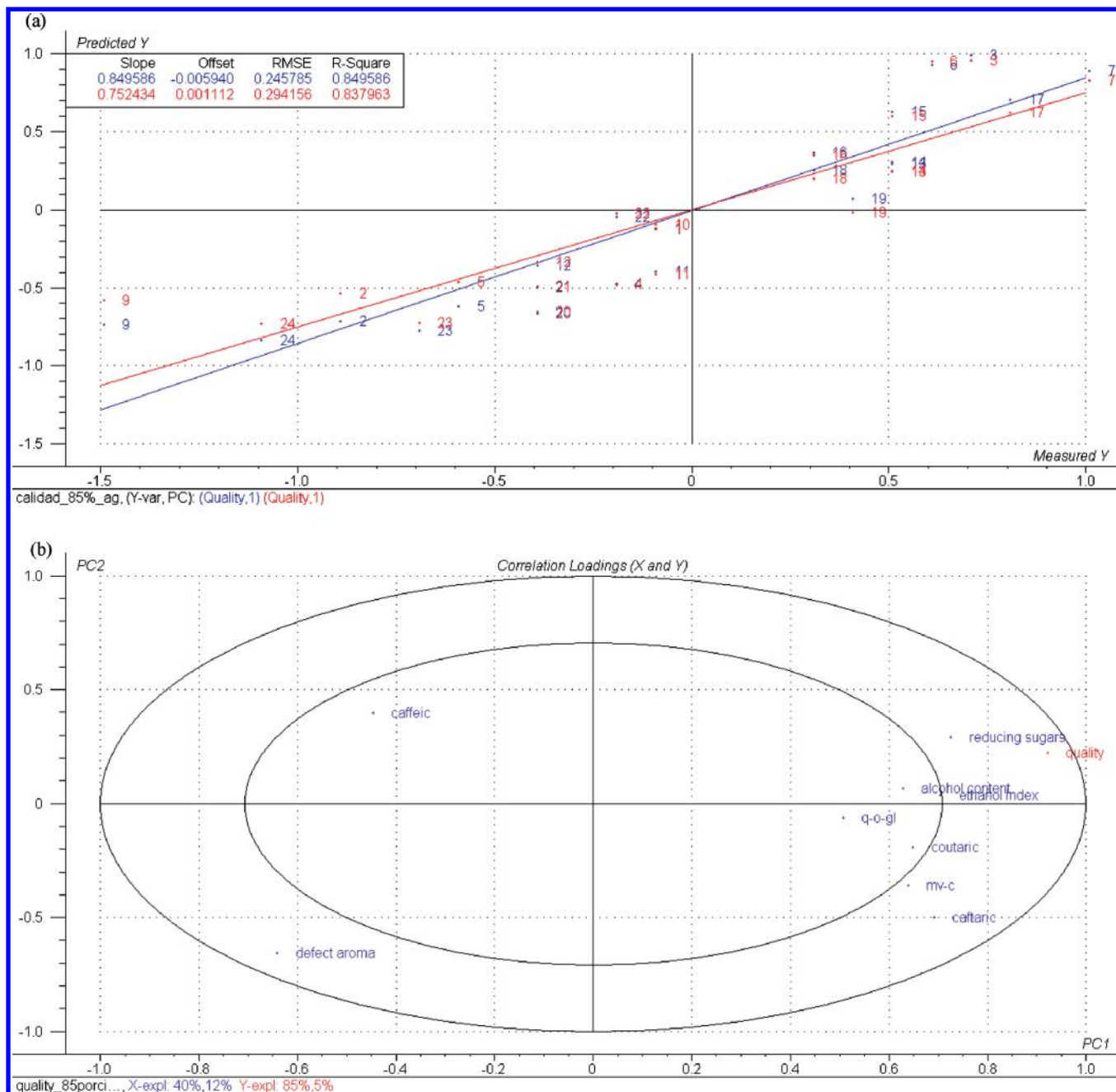
Figure 3. PLS regression: (**a**) plot of predicted versus measured quality scores; (**b**) *X* and *Y* correlation loading plot obtained with the model.

Table 4. Mean Scores (± Standard Deviation) of Nonvolatile Variables Significantly Explaining the Quality Model by PLS in Two Different Sample Subsets (Wines with Low and High Quality Scores), *F* Ratios, and *P* Values for the One-Way ANOVA Calculated on the Two Quality Subsets for Each Attribute

variable	low quality (*Q* < 3)	high quality (*Q* ≥ 3)	*F*	*P*
trans-caftaric acid (mg L^{-1})	12.3 ± 1.8	24.3 ± 4.0	8.365	0.008
trans-coutaric acid (mg L^{-1})	8.7 ± 1.3	13.4 ± 1.2	6.734	0.017
trans-caffeic acid (mg L^{-1})	10.0 ± 1.4	6.3 ± 2.4	3.548	0.073
quercetin-3-*O*-glucuronide (mg L^{-1})	0.2 ± 0.1	2.9 ± 1.5	3.740	0.066
PAs linked to polysaccharides (%)	18.3 ± 1.2	40.0 ± 3.6	33.866	<0.001
alcohol content (%, v/v)	14.5 ± 0.1	15.1 ± 0.2	3.320	0.082
reducing sugars (g L^{-1})	2.3 ± 0.2	3.1 ± 0.2	9.237	0.006
malvidin−catechin dimer (mg L^{-1})	5.2 ± 1.5	14.0 ± 2.9	7.766	0.011

authors (*12, 13*), which demonstrate the importance of phenolic compounds in wine sensory properties and thus in its quality perception. In fact, quercetin-3-*O*-glucuronide has already been described to provide a positive mouthfeel attribute described as velvety astringency (silky and finely textured kind of astringent sensation), whereas *trans*-caffeic acid, negatively correlated to quality in the model, has been related to a negative sensory attribute such as puckering astringency (reflexive action of cheek surfaces being brought together and released in an attempt to lubricate mouth surfaces (*33*)). Moreover, **Table 4** shows the eight

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nonvolatile variables included in the PLS model and their concentration levels according to quality perception, where two wine subsets could be well differentiated (high quality, with $Q > 3$, and low quality, with $Q < 3$).

In conclusion, the study does provide some insight into the implication of nonvolatile composition on the final quality perception. Particularly, the linkage between quality and hydroxycinnamic acids, quercetin-3-*O*-glucuronide, ethanol, and reducing sugar content as well as the PAs linked to polysaccharides has been evidenced, and hence their sensory implication is worthy of further study. Quantitative studies as well as taste reconstruction and omission experiments in wine-like matrices are currently ongoing to get a lead on the importance of the individual contribution of each compound or group of compounds in quality.

Supporting Information Available: Wines analyzed in the experiment including origin, vintage year, varietal composition, and oak aging time. This material is available free of charge via the Internet at http://pubs.acs.org.

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