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EFFECT OF POLYPHENOL CONCENTRATIONS ON ASTRINGENCY PERCEPTION AND ITS CORRELATION WITH GELATIN INDEX OF RED WINE

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ABSTRACT

The aims of this work were to investigate the effect of polyphenol concentrations on astringency perception and gelatin index measurements in noncommercial red wines, as well as evaluate astringency evolution over time. Spearman coefficients showed a positive correlation between polyphenols at low concentration with gelatin index ($P < 0.001$*), and astringency (*$P < 0.05$*). Gelatin index values and polyphenol concentrations were related by a power function at low polyphenol levels, but no correlation was shown when total polyphenol levels overcame 5.20 g/L. Similar relationships were found between perceived astringency/gelatin index, and astringency/polyphenol concentrations. It was evident that gelatin index was a better estimator of astringency when polyphenol levels were low, and astringency intensity did not increase when polyphenol concentrations were higher than 5.20 g/L. Time-intensity measurements of astringency showed that maximum intensity governed the evolution of sensation.*

PRACTICAL APPLICATIONS

The findings from this research will aid winemakers to understand the availability of an *in vitro* assay to estimate the astringent sensation. This method's comparison and crossbreeding with sensory data will allow a better interpretation of what happens when wine is drunk.

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INTRODUCTION

Polyphenols are recognized as substances that provide astringency sensation. However, other substances such as organic acids, sugars and ethanol can also influence this sensation. Red wine contains all of these compounds to provide astringency, the most important quality of wine (Lesschaeve and Noble 2005).

Astringency is a complex tactile sensation produced by binding polyphenols with saliva proteins, which later precipitate. It is a highly dynamic process that continuously changes during wine ingestion. Wine tannins – a class of polyphenols – derive from solid parts of grape and are partially extracted during red wine maceration (Kennedy *et al*. 2006). Consequently, astringency plays a crucial part in this kind of wines.

Descriptive analysis makes it possible to evaluate how changes in wine composition affect sensory attributes perception; yet, this unique method alone is not enough to describe all relevant aspects of such a time-dependent process. Time-intensity technique was used to evaluate astringency in several studies of wines by comparing effects of phenolic compounds (Robichaud and Noble 1990) or sugars (Ishikawa and Noble 1995). However, limited information was found about astringency evolution through time in both wine and water media, when tested with the same levels of pH, reducing sugars, ethanol levels and polyphenol concentrations. For this reason, a dynamic method in both media was performed in the current study, so as to obtain more complete information about influence of wine components on astringency sensation.

Many studies have been carried out, in which scientists have had to correlate sensory with analytical data. Kennedy *et al*. (2006) analyzed the astringency of Merlot and Syrah wines with a trained panel, and by using five different estimation methods: absorption at 280 nm, phloroglucinolysis, gel chromatography and protein precipitation, noting that with protein precipitation got better correlation between the two. Monteleone *et al*. (2007) proposed a predictive model by measuring the polyphenol-mucin reactivity; they found a linear relation between perceived astringency and the astringency mucin index in experimental and commercial red wines. Llaudy *et al*. (2004) proposed another method using ovalbumin as the precipitation agent and tannic acid solutions as standards.

Gelatin index (Ribéreau-Gayon and Glories 1986) approximates astringency by measuring the extent of tannin precipitation with gelatin. This is an *in vitro* method, which was selected because gelatin is used to reduce harshness (astringency) and improve clarity (Braga *et al*. 2007). In addition, proline is an aminoacid that is common to gelatine and saliva protein (Bajec and Pickering 2008). However, Llaudy *et al*. (2004) affirmed that it is not very reproducible because there are many gelatins on the market with heteroge-

neous composition. Maury *et al*. (2001) compared three types of gelatins with different molecular weight and found that the lower the molecular weight gelatin was more effective in removing large tannins than the higher molecular weight tannins.

Siebert (2006) stated that the formation of protein-polyphenol complex depends on haze-active proteins, previous stage of sedimentation, and the number and location of hydroxy groups of phenols. There are four factors to influence protein-polyphenol interaction in model systems: protein concentration, polyphenol concentration, pH and alcohol content, but at pH corresponding to wine, ethanol effect was more modest.

On the basis of the investigation carried out by several authors previously mentioned, it can be stated that gelatin index method has been modified, principally by changing the protein to achieve more reproducible results. However, given that wine is highly complex, no unanimous opinion has been reached regarding the optimum conditions to carry out this method. For this reason, in the present work, the more traditional method of gelatin index was used.

All these works were made in model solutions or commercial wines, but no experiments had been made on noncommercial wines taken directly from the fermentation tank. This permitted the evaluation of astringency and polyphenols naturally present in wines before adding gelatine for fining process. These wines had a wide range of polyphenols that could not be obtained in commercial samples. Therefore, it was vital to conduct a study on noncommercial wines, which were not modified by any enological practices.

The aims of this work were to investigate the effect of polyphenol concentrations at two levels, low (1.40–4.70 gallic acid equivalent [GAE] g/L) and high (5.20–7.20 GAE g/L), on the astringency perception and its correlation with gelatin index measurements in noncommercial red wines. Likewise, astringency evolution through time in model systems of wine and water media was evaluated by using the same chemical conditions.

MATERIALS AND METHODS

Experiment I

Wine Samples. Twenty-nine noncommercial 100% Malbec wines (2004 vintage) were obtained from fermentation tanks, after maceration, before clarification and filtration; they were produced under conditions without wood treatment, malolactic fermentation, carbonic gas or additives.

Grapes were manually harvested at 23–25°B during March 2004, from vineyards that were 10–12 years old; fermentation and maceration times were

7–10 days and 13–21 days, respectively (data supplied by wineries).

Wines were selected according to their total polyphenol levels: low (range 1.40–4.70 GAE g/L) and high (range 5.20–7.20 GAE g/L). These two levels were separated taking into account maximum concentrations found in commercial wines (approximately 5.0 g/L). Sensory evaluations were first performed, and then samples were conserved at −18C to avoid esters hydrolysis.

Physicochemical Analysis

Physicochemical characteristics of wines – pH, dry extract (g/L), titratable acidity (g/L), alcohol, reducing sugars and density – were determined by official methods of AOAC (1990).

Total Polyphenols. Total polyphenols were determined by the Folin-Ciocalteau method (Folin-Ciocalteau reagent, Merck KgaA Darmstadt, Germany) and concentrations were expressed as gallic acid equivalent (GAE) in g/L. Absorbance at 760 nm (spectrophotometer Shimadzu PharmaSpec UV-1700) of wine samples (5 mL diluted 1:10) were measured against water in duplicate. Polyphenol concentratios of samples were derived from a standard curve of gallic acid ranging from 0.05 to 5.00 g/L.

Gelatin Index. The gelatin index of wines was measured by using the methodology described by Glories (1984). 2.5 mL of gelatin solution (35 g/L) was added to every 25 mL of wine. After 3 days, samples were centrifuged at 1,500 rpm during 15 min and tannin concentration was determined on supernatants. Results were expressed as a percentage, which was calculated by referring the difference between total tannins (550 nm) and protein-reactive tannins to total tannins.

Sensory Analysis

Panel Training. Ten paid not-sighted assessors (four females and six males, 21–55 years old) from the panel of Staffing and Training Group (S & TG), Buenos Aires consulting company, were trained in the descriptive analysis of Malbec wines (Goldner and Zamora 2007). During training period (five sessions, 2 h each), judges performed the following task: (1) taste identification using standard solutions; (2) order tastes in ascending scale using sucrose (1.5 and 3.0%; food grade), tartaric acid (0.2, 0.4 and 0.6%, Alcor analytical reagent, Buenos Aires, Argentina), caffeine (0.004 and 0.008%, Merck analytical reagent, Darmstadt, Germany) and gallic acid for astringency (0.50, 1.40, 3.40 and 7.20 g/L Anedra analytical reagent, Buenos Aires, Argentina) and (3) use of structured scales working with standard solutions.

Descriptive Analysis. Quantitative descriptive analysis of mouthfeel attributes – sourness, sweetness, bitterness, persistency, pungency and astringency (Stone and Sidel 1993) – was developed. A 9-point intensity scale for each attribute was used. Samples (50 mL) were presented at 18 ± 2C in tulip-shaped transparent glasses, covered with glass Petri dishes and identified by random three-digit codes. The samples were spat, and mineral water was provided for oral rinsing, along with unsalted crackers. A randomized incomplete block design was used to evaluate all the wines (Goldner and Zamora 2007).

Data Analysis

Spearman correlation analysis was performed among two levels of polyphenols, gelatin index, physicochemical and mouthfeel attributes. Regression Analysis was used to infer relationships among: (1) gelatin index and total polyphenols, (2) astringency and gelatin index and (3) astringency and total polyphenols. ANOVA of wine samples was applied between both ranges of polyphenols. Variability among assessors was studied using a mixed model ANOVA in which assessors were treated as a random factor.

Partial least-squares regression (PLS2, Infostat versus 2007, Universidad Nacional de Córdoba, Argentina) was used to explore relationships between physicochemical data (X-variables, regressors: predicting) and sensory data (Y-variables, regressands: predicted) at two polyphenol levels.

Experiment II

Samples. Model systems were made using a base wine with the following characteristics: 3.60 g/L reducing sugars, 5.10 g/L titratable acidity, 1.40 g/L gallic acid and 13.5% ethanol. This base wine was modified adding: (1) fructose (Lab. Ciccarelli analytical reagent, Buenos Aires, Argentina) to obtain 5.80 g/L of reducing sugars, (2) tartaric acid to increase the level of titratable acidity up to 6.50 g/L and (3) gallic acid between 4.30 and 7.20 g/L.

Model solutions – with similar amounts of reducing sugars, titratable acidity and polyphenol levels – were also prepared in water media besides another concentration of gallic acid (0.5 g/L). These maximum concentrations were chosen from a previous work by Goldner and Zamora (2007). Model solutions are shown in Table 1.

Sensory Analysis

Panel Training. Ten voluntary assessors (six female, four men; 24–55 years old) were selected according to their relation with the wine and sensory

TABLE 1.
COMPOSITION OF MODEL SOLUTIONS STUDIED BY TI METHOD

n°	Polyphenols (g/L)	Red. sugars (g/L)	Titratable acidity. (g/L)
Wine/water media 13.5% ethanol			
1†	1.40	3.60	5.10
2	1.40	3.60	6.50
3	1.40	5.80	5.10
4	1.40	5.80	6.50
5	4.30	3.60	5.10
6	4.30	3.60	6.50
7	4.30	5.80	5.10
8	4.30	5.80	6.50
9	7.20	3.60	5.10
10	7.20	3.60	6.50
11	7.20	5.80	5.10
12	7.20	5.80	6.50
Water media 13.5% ethanol			
13	0.50	3.60	5.10

† Base wine.

analysis experience: sommeliers and members of the Facultad de Ciencias Agrarias, Universidad Católica Argentina. Training (six sessions, 2 h each) was performed such as was explained in Experiment I and using model solutions.

Paired Comparison. Paired comparison test (ASTM 1977) was performed to investigate differences in astringency among 12 wine model solutions (Table 1). Ten assessors evaluated the samples in duplicate (66 pairs), tasting six pairs per session (30 min each) and they rinsed their mouth with carboxymethylcellulose 0.55% to avoid residual effect of astringency (Brannan *et al*. 2001). Model solutions in water media (13 samples, 78 pairs) were tasted in similar conditions.

Time-Intensity Analysis (TI). Astringency evolution over time was studied according to (ISO TC 34/SC 12 N 385 1999) using a computer software specially designed for this purpose. Assessors used a mouse to move a cursor along a 500-pixel line that represented a 20 cm unstructured line scale on the monitor. Data were automatically recorded every 0.35 s. The software provided the TI curve as well as six parameters that described it: maximum intensity reached (Imax), time elapsed to maximum intensity (Tmax), total duration of sensation (Tdur), time for astringency intensity decline to half its

maximum value (T50max), area under curve (AUC), and plateau time (Tpla). Assessors were prompted by the computer to expectorate the sample at 10 s while continually recording perceived intensity until sensation reached extinction.

Assessors were trained in order to minimize individual differences and standardize TI curves along intensity and time axes. For this purpose, solutions n°1 and 5 – which were perceived as different from paired comparison tests – were used as standards to estimate a reference value for Imax and Tdur. Once assessors replicated the curves for the standard solutions, they could begin testing unknown solutions. The number of training sessions (three to six) depended on each judge's ability to handle the mouse and to replicate the measurements. Trained assessors evaluated samples in triplicate in individual booths under daylight (6,500 K).

Data Analysis

The significance level of the paired comparison test was calculated by binomial test based on the number of correct answers.

The TI data for triplicates were analyzed separately using noncentered principal component analysis (PCA) (Piggott *et al*. 2000). Characteristic parameters were calculated for each of these new curves obtained by noncentered PCA (in triplicate for each studied model solution) and were analyzed by an ANOVA and Tukey test using replicates and solutions as factors.

All data were processed with Infostat versus 2007, Universidad Nacional de Córdoba, Argentina, except for not centered PCA that was analyzed by Unscrambler versus Demo (CAMO ASA, N-0115 Oslo, Norway).

RESULTS AND DISCUSSION

Experiment I

ANOVA mixed model of sensory and physicochemical data between two polyphenol levels showed that astringency ($P < 0.05$), persistence ($P < 0.05$), bitterness ($P < 0.01$), sweetness ($P < 0.05$) and gelatin index ($P < 0.05$) were different between both polyphenol ranges (Table 2). These variables increased when polyphenol levels raised, except for sweetness, which decrease its intensity.

Assessors showed good reproducibility (replication factor was not significant) and good consensus (but for sourness, polyphenol level × assessor interactions were not significant).

Spearman coefficients (Table 3) showed a positive correlation among total polyphenols at low concentration (1.40–4.70 GAE g/L) with gelatin

TABLE 2.
MEAN MOUTHFEEL ATTRIBUTES AND PHYSICACHEMICAL DATA FROM TWO TOTAL PLOYPHENOLS RANGE OF 29 WINES

Attribute	Mean mouthfeel attributes and physicochemical data from polyphenols range (GAE g/L) ± SEM	
	1.40–4.70	5.20–7.20
Astringency	5.0 ± 0.9	6.3 ± 1.0**
Persistence	4.9 ± 0.5	5.4 ± 0.7*
Sweetness	3.2 ± 0.7	2.4 ± 0.8*
Sourness	4.9 ± 0.7	5.2 ± 0.8
Bitterness	4.6 ± 0.8	5.4 ± 0.7*
Pungency	2.9 ± 0.5	3.4 ± 0.8
pH	3.8 ± 0.2	3.7 ± 0.2
Dry extract	26.4 ± 2.9	27.8 ± 3.9
Tritatable acidity	4.8 ± 0.5	4.8 ± 0.7
Reducing sugars	2.7 ± 0.7	2.5 ± 1.1
Density	0.993 ± 0.001	0.993 ± 0.001
Ethanol	13.1 ± 1.9	13.6 ± 1.9
Gelatin index	38.1 ± 4.1	54.5 ± 5.9*

* $P < 0.05$, ** $P < 0.01$.
GAE, gallic acid equivalent; SEM, standard error of the mean.

index ($P < 0.001$), astringency ($P < 0.05$) and bitterness ($P < 0.05$); and a negative correlation with sweetness ($P < 0.01$). However, the correlations were not significant when polyphenol concentration was high.

In the present work, it was not observed any effect of ethanol levels on astringency perception. Several authors studied this effect, such as Demiglio *et al*. (2002), who found that the combined treatments of ethanol and pH (pH 3.4 and 3.6; 12 and 15% ethanol) did not have an effect of the perception of the astringent subqualities of the wine.

Gelatin index values and polyphenol concentrations were related by a power function at low polyphenol level, but no correlation was shown when total polyphenol level overcame 5.20 g/L (Fig. 1; being $r^2 = 0.728$ and 0.237 for low and high polyphenol level, respectively). Similar relationships were found between perceived astringency and gelatin index (Fig. 2; being $r^2 = 0.563$ and 0.093 for low and high polyphenol levels, respectively). It was evident that gelatin index was better estimator of astringency when polyphenol levels was from 1.40 to 4.70 g/L (low). Moreover, astringency and polyphenol concentrations showed similar behavior (Fig. 3; $r^2 = 0.684$ and 0.004, respectively). Now, polyphenol concentrations higher than 5.20 g/L did not increase the intensity of astringency perception.

In order to analyze the relationships among polyphenol levels, physicochemical and sensory variables, a PLS2 was performed (Fig. 4). The wines

TABLE 3.
SPEARMAN CORRELATIONS BETWEEN MOUTHFEEL AND PHYSICOCHEMICAL ATTRIBUTES OF 29 WINES

	pH	Dry ext.	Titratable Ac	Red. Sugars	Density	Ethanol	Low polyphenols	High polyphenols	Gelatin index	Astringency	Persistence	Sweetness	Sourness	Bitterness
Dry extract	0.057													
Trit. Acidity	−0.182	0.083												
Red. Sugars	−0.379*	0.483**	−0.193											
Density	0.425*	0.377*	0.071	0.101										
Ethanol	0.050	0.291	0.323	−0.108	0.207									
Low polyphenols	−0.023	0.284	−0.011	0.209	0.111	−0.139								
High polyphenols	0.401	−0.018	−0.094	−0.115	0.121	−0.069								
Gelatin index	0.086	0.366	0.162	0.018	0.442*	0.173	0.911***	0.353						
Astringency	0.019	0.097	−0.171	−0.221	0.009	0.075	0.558*	0.140	0.561**					
Persistence	0.384*	0.451*	−0.202	−0.081	0.395*	0.208	0.131	−0.118	0.251	0.469*				
Sweetness	−0.483**	0.081	0.112	0.600**	0.151	−0.023	−0.707**	−0.343	0.043	−0.636***	−0.469*			
Sourness	−0.266	0.045	0.301	−0.023	0.025	0.323	0.232	−0.373	0.043	0.080	0.006	−0.047		
Bitterness	−0.132	0.056	−0.121	−0.249	−0.189	0.001	0.467*	−0.252	0.320	0.432*	0.177	−0.454*	0.288	
Pungency	−0.008	−0.016	−0.024	−0.204	0.100	0.420*	0.013	−0.251	−0.126	0.107	0.319	−0.211	0.405*	0.265

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0$.

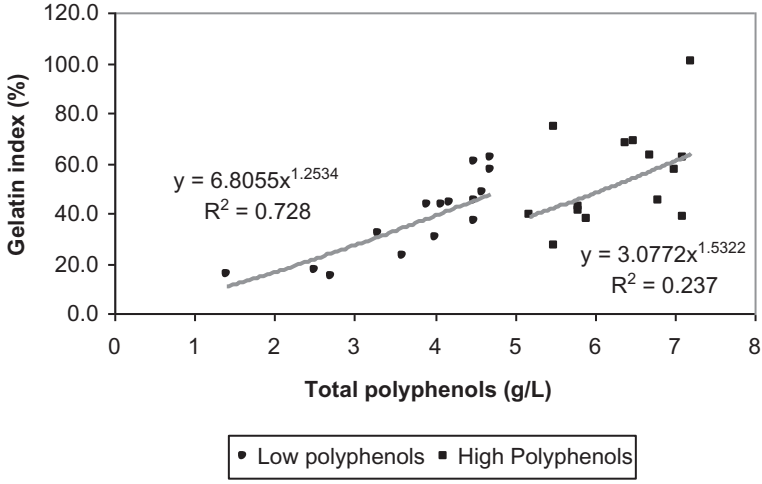

FIG. 1. GELATIN INDEX VALUES AS A FUNCTION OF LOW (1.40–4.70 GAE G/L) AND HIGH (5.20–7.20 GAE G/L) TOTAL POLYPHENOL LEVELS

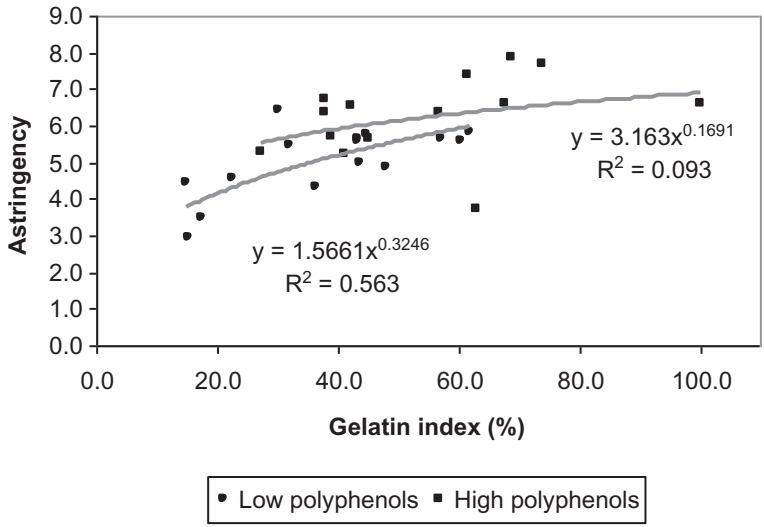

FIG. 2. ASTRINGENCY INTENSITY AS A FUNCTION OF GELATIN INDEX AT TWO TOTAL POLYPHENOL LEVELS

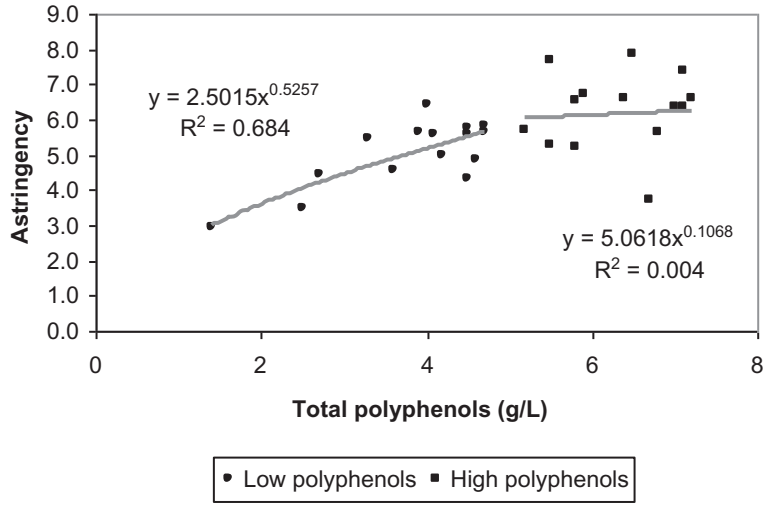

FIG. 3. ASTRINGENCY INTENSITY AS A FUNCTION OF LOW (1.40–4.70 GAE G/L) AND HIGH (5.20–7.20 GAE G/L) TOTAL POLYPHENOL LEVELS

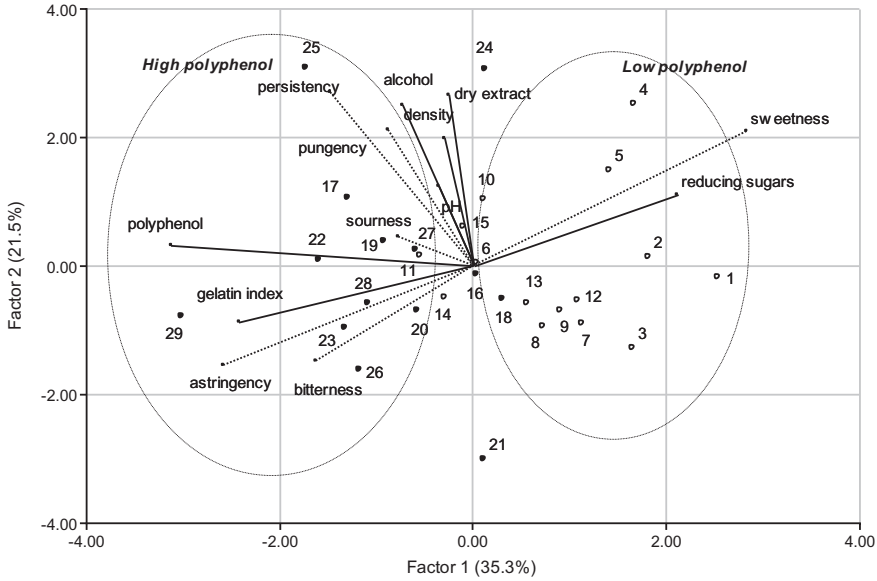

FIG. 4. PARTIAL LEAST SQUARES REGRETION (PLS2) FACTORS FOR SENSORY ATTRIBUTES AND PHYSICOCHEMICAL DATA

with low polyphenol levels (identified as n°1 to 15), were clustered with reducing sugar concentration and sweetness intensity, except for samples n°11 and 14. These samples were perceived very astringent because they had low sugar content. Wines, which had high polyphenol levels (n°16 to 29), were clustered with astringency, bitterness, sourness, pungency, persistency, polyphenol content and gelatin index. Wines n°16, 21 and 24 were out of this group. Samples n°16 and 21 had intermediate concentrations of reducing sugars and sourness intensity; and sample n°24 had high sugar concentration (Fig. 4). As can be notice, sugar content had a great influence on astringency perception. Therefore, it was very complex to explain the relationship between astringency and polyphenols or gelatin index. Consequently, gelatin index method was limited to wine samples with a maximum of total polyphenols about 5.0 g/L.

Experiment II

Table 4 shows the sample pairs that presented significant differences in astringency. It is observed that changes in wine astringency ($P < 0.05$) were found when gallic acid concentration increased from 1.40 g/L to 4.30 g/L without modifications in reducing sugars and tritatable acidity (solution 1 versus 5; Table 4). Another variation was obtained when gallic acid rose from 4.30 g/L to 7.20 g/L, but tritatable acidity was 6.50 g/L (solution 5 versus 10, Table 4). These observations confirm the results obtained in Experiment I, which found sensory differences in the range of low total polyphenol levels (1.40–4.70 GAE g/L). Solution 10 was not evaluated by TI methodology because its polyphenol concentration was higher than that of commercial wines.

Differences were found in water media when gallic and tartaric acid were added – reaching 7.20 g/L and 6.50 g/L, respectively, and reducing sugars were not modified (solution 5 versus 10, Table 4). When gallic acid increased from 0.50 g/L to 1.40 g/L and 4.30 g/L (solution 13 versus 1 and 13 versus 5, Table 1), no differences were found.

TABLE 4.
DISCRIMINATION BETWEEN SAMPLES: PAIR TEST

Samples compared†	Correct answers	Total answers	Significance level %
†Wine media 13.5% ethanol			
1/5	18	20	0.1
5/10	17	20	1
†Water media 13.5% ethanol			
5/10	20	20	0.1

† Significant different pairs among 66 and 78 possible pairs, respectively.

TABLE 5.
MEAN ± SEM OF TEMPORAL VARIABLES EXTRACTED FROM TI CURVES

Samples	Imax	Tmax	Tdur	T50max	Tpla	AUC
Water-0.5 EAG/L	195 ± 3.26b	22.2 ± 0.47a	95.8 ± 5.86a	50.5 ± 1.11a	5.95 ± 1.11a	8,969 ± 232b
Wine-1.4 EAG/L	109 ± 7.37a	21.9 ± 1.02a	85.6 ± 6.33a	43.6 ± 43.6a	7.23 ± 0.31a	4,673 ± 637a
Wine-4.3 EAG/L	191 ± 6.55b	23.0 ± 0.61a	92.6 ± 2.71a	49.4 ± 2.33a	7.23 ± 0.4a	8,572 ± 184b

Means within columns followed by different letters denote differences at $P < 0.05$ according to Tukey's Test.
AUC, area under curve; Imax, maximum intensity reached; SEM, standard error of the mean; Tdur, total duration of sensation; Tmax, time elapsed to maximum intensity; Tpla, plateau time; T50max, time for astringency intensity decline to half its maximum value.

Model solution n°1 and 5 in wine, and n°13 in water with ethanol 13.5% were used to study the media effect.

Time-Intensity Curves

Results of ANOVA for TI parameters showed that Imax and AUC (Table 5) were significantly different at $P < 0.05$. As can be seen, Imax of astringency grew when the gallic acid concentration was increased. In this case, raising the gallic acid concentration from 1.40 to 4.30 GAE g/L approximately doubled the Imax. Similarly, in Experiment I at low polyphenol range, astringency intensity doubled when the polyphenols grew from 1.4 to 4.7 g/L. In addition, this concentration range was necessary to notice differences in astringency perception from paired comparison test.

Taking the last results into account, a great increase in polyphenol concentration was necessary to perceive a difference in astringency sensation. The exponent of power function is a measure of the rate of growth of perceived intensity as a function of stimulus intensity. When the exponent is smaller than 1, the sensation grows more slowly than the stimulus; this was observed in the present work because the calculated exponent in Experiment I was 0.53.

It is noticeable that samples with 0.50 GAE g/L in water, and 4.30 GAE g/L in wine, with the same amounts of sugar, acid and ethanol, were isointense (Table 5). It became clear that Imax was higher in water with 0.50 GAE g/L than in wine with 1.40 GAE g/L. So, astringency perception was modulated by components of wine.

Tmax, Tpla and T50max were similar in three samples; therefore newly, astringency Imax – in the range evaluated – was affected by wine components (probably different from fructose, tartaric acid and ethanol, which were used to prepare the model solutions) that modulated perception. Sáens-Navajas *et al.* (2010) measured the effect of different volatile extract compositions on the perception of taste, astringency, global intensity and persistence of wine. The

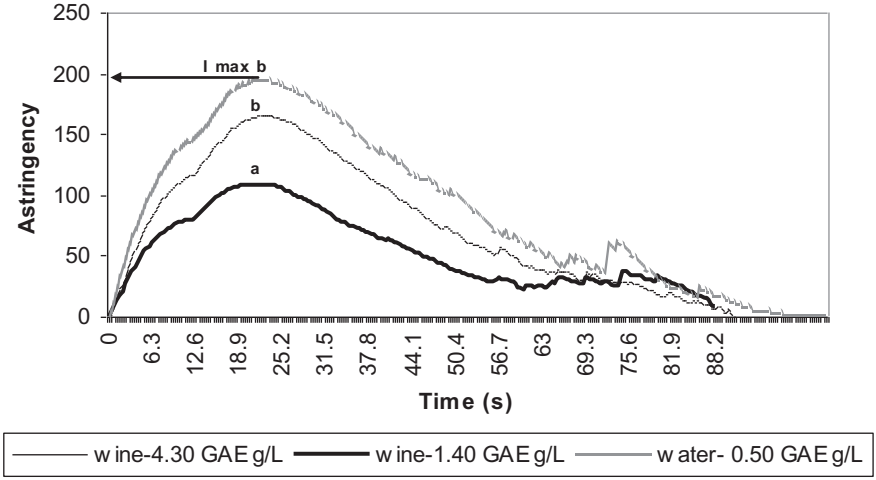

FIG. 5. AVERAGE TI CURVES OF THREE MODELS SYSTEMS AT 13.5% ETHANOL AND 10 ASSESSORS

effects of replacing the volatile fraction of a red wine by volatile extracts from other red wines were small and inconsistent, which confirms that taste and astringency are primarily driven by nonvolatile molecules in these wines. Therefore, minor compounds like glycerol, high molecular weight sugars and alcohols could increase wine viscosity, which may contribute to modulating astringency perception.

In the current study, approximately nine times more gallic acid concentration was needed to match the astringency's intensity in wine versus water (Table 5). This effect was not reflected in temporal variables such as Tmax, Tpla, T50max and Tdur, where neither the increase of gallic acid concentration nor media change modified it. As regards the global sensation through time represented by AUC, it can be seen that it followed the same behavior as Imax's.

Figure 5 shows average curves of not-centered PCA, in which the differences of intensities in astringency perception among samples are noticed. Particularly, there is an inflection at the second 10 in all curves, time at which a song signaled spitting the sample; prior to that instant, the rate of increase – curve slope – was slower, but later, it increased. This is in accordance with Guinard *et al*. (1986), who stated that astringency was not perceived immediately, and that it evolved in the mouth following spitting.

In agreement with Naish *et al*. (1998), who said that one contribution to astringency lay in the fading of a response, the sensation (Fig. 5) disappeared much faster in mixtures that were perceived to be more astringent than others

(slopes were more pronounced). This explained why, though Tdur remained unchanged, kinetic curves were different, so curves reached higher altitude and fell faster.

CONCLUSIONS

Gelatin index was a limited method as estimator of astringency. In non-commercial wines, astringency was expressed through persistence, bitterness and gelatin index when polyphenol concentrations were low (1.40–4.70 g/L). Minor components, which played an important role in its perception, exerted a modulating effect on the maximum intensity, and duplicated when gallic acid concentration was increased from 1.40 to 4.30 GAE g/L at 13.5% ethanol. During astringency perception through time, fading of sensation was more important than duration. Maximum intensity of astringency was the temporal variable that governed the evolution of sensation.

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