Effect of pH on Astringency in Model Solutions and Wines

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Red wines and model solutions containing either grape seed phenols or (+)-catechin were assessed for perceived astringency by a panel of trained assessors using time−intensity (T−I) methodology. The effect of acid on the perception of astringency was evaluated by using three different sensory protocols. Maximum intensity (I_{max}), total duration (T_{tot}), and time to reach maximum intensity (T_{max}) of astringency were significantly increased when the acid solution was tasted after the astringent model solutions as well as when a mixture of acid and astringent compounds was assessed. However, when the panelists rinsed their mouths with the acid solution before tasting the model solutions, astringency parameters remained unaffected. For wine samples, I_{max} of astringency decreased significantly when the acid was tasted after the control wine, whereas it remained unaffected when it was tasted before. The other T−I astringency parameters of wine were not affected significantly by the tasting of the acid.

Keywords: *Astringency; time−intensity; grape seed phenols; (+)-catechin*

INTRODUCTION

Sensory astringency is defined as a drying or puckering sensation in the mouth. It is not confined to a particular region of mouth or tongue but is perceived as a diffuse stimulus that requires appreciable time for development (Bate-Smith, 1973). It is believed to result from the interaction of tannins with salivary proteins in the mouth (Joslyn and Goldstein, 1964). Lyman and Green (1990) have proposed that this interaction involves cross-linking between tannins and proteins which causes physical changes in the saliva and epithelium proteins which are sensed by the mechanoreceptors in the mouth. Hydrogen bonding and hydrophobic interaction are the most likely mechanisms under physiological conditions (Clifford, 1986).

Although astringency is considered to be a tactile sensation rather than a taste (Breslin et al., 1993), interaction with taste stimuli has been shown to affect its perception. Guinard et al. (1986) showed that acidity significantly increased the intensity of astringency of model solutions. A similar pH reduction did not have a significant effect on the astringency of red wines either because the red wine was so astringent that the small changes induced by acidity adjustments were not detectable or because the limiting factor for tannin−protein complex formation was the quantity of protein in the mouth. They suggested that the addition of acids reduces the pH of a solution and increases the percentage of tannins in the phenolate form and therefore increases the likelihood of hydrogen bonding. Recently, the effect of lactic and malic acids on the perception of astringency was evaluated at different pH values (Kallithraka et al., 1997b) using the time−intensity (T−I) method. The results showed that the maximum intensity (I_{max}) and the total duration (T_{tot}) of this attribute were increased significantly with decreasing pH in both model solutions and red wine, but no significant differences were found between malic and lactic acids. It was suggested that a possible change in the charge of salivary proteins might have affected the binding and dissociation of the phenolic compounds or that the precipitation of the salivary proteins was increased as their p*I* was approached.

Astringency is a very important sensory characteristic of wine produced by procyanidins, and it can be affected by the organic acids that are naturally present in wine (Guinard et al., 1986; Kallithraka et al., 1997b). The aim of this study was to assess the possible action of acids on the perception of astringency of model solutions and red wine. Time−intensity methodology was employed to evaluate astringency over time. An evaluation procedure was developed involving the tasting of an acid solution (malic acid), an astringent model wine solution, and a wine, in a number of orders and mixtures, while the concentration of the phenolic compounds in each sample set was kept constant. For example, the acid was presented simultaneously with the astringent stimulus as a mixture in one of the protocols, and on separate occasions either the acid or the astringent stimulus was given first. From these different protocols the following outcomes were anticipated. If astringency became more intense when the acid was presented *before* an astringent stimulus was used, this would imply either that a conformational change in the proteins had occurred or that subsequent binding/precipitation was enhanced. If astringency became more intense when the acid was presented *after* the phenolic compound, it would imply that there was some effect on the protein−phenol complex once formed or that the effect on saliva proline-rich proteins (PRP) resulted in greater astringency only if phenols were already present in the mouth.

MATERIALS AND METHODS

Subjects. Twelve healthy subjects (all female) were paid to participate in the experiment as part of a trained taste panel. All were experienced in sensory assessments of a range of foods including wines and model wine solutions and had used the T−I method for the evaluation of the sensory attributes.

Preparation of the Samples. The two model wines were solutions of 300 mg/L potassium bitartrate and 10% ethanol containing grape seed extract and (+)-catechin, respectively.

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Table 1. Description of the Samples

code	sample
C	control (pH 3.7,[a] pH 3.8[b,c])
C+A	control mixed with acid (pH 3.2,[a,b] pH 3.5[c])
A→C	aqueous acid mouth rinse (pH 3.2), then control[a−c] sample
C→A	control[a−c] sample mouth rinse, then aqueous acid sample (pH 3.2)
A	aqueous acid sample (pH 3.2)

[a] Catechin model. [b] Grape seed model. [c] Wine.

The phenolic compounds were extracted from grape seeds of *Vitis vinifera* cv. Roriz grapes (Douro valley, Portugal). An amount of 25 g of seeds was crushed and immediately extracted for 5 min with 266.7 mL of 75% ethanol (Hayman Ltd). After 10 min, the seeds were removed using a Büchner funnel (filter paper Whatman No. 4). The filtrate was stored at −20 °C. The morning before the experiment, it was thawed and diluted to 2 L with a solution of 346.2 mg/L potassium bitartrate (Peter Whiting Chemicals) in deionized water. For sample C+A, the pH of 400 mL of that model solution was reduced from 3.8 to 3.2 by the addition of 302 mg of malic acid, corresponding to 755 mg/L.

The solution containing catechin was prepared the morning before the experiment by adding 1500 mg/L (+)-catechin (Peter Whiting Chemicals) to 2 L of 300 mg/L potassium bitartrate and 10% ethanol solution in deionized water. For sample C+A the pH of 400 mL of that solution was also reduced from 3.7 to 3.2 by the addition of 382 mg of malic acid, corresponding to 955 mg/L. This method of preparation ensured that only the pH values of the solutions were adjusted while the concentration of phenols was kept constant.

The acid solution with a pH value of 3.2 was prepared by adding 1600 mg/L malic acid to deionized water.

A 3-year-old experimental wine was used, made from Tinta Roriz grapes from the demarcated area of the Douro valley (Portugal). The normal red wine vinification procedure was used, using 75 mg/L SO_2 when the grapes were crushed prior to fermentation. To enhance the astringency, 600 mg/L (+)-catechin was added to the wine immediately prior to the sensory experiment. For sample C+A the pH (3.8) of 400 mL was reduced to 3.5 by the addition of 820 mg of malic acid, corresponding to 2050 mg/L. Although red wine has its own astringency, it was fortified with (+)-catechin to ensure an adequate stimulus for this experiment. This addition of (+)-catechin to the wine prevented the assessment of the actual wine astringency but allowed the investigation of the effect of pH on astringency.

Training and Procedure. Two training sessions were conducted to familiarize participants with the different tasting procedures that they would be instructed to use and to give them experience of the stimuli they would encounter during this experiment. During the first session, they were presented with an example of a standard astringent compound (1500 mg/L tannic acid), and the assessment of the standard was discussed to refresh previous training in the use of astringency, bitterness, and sourness terms. In a recent previous study (Kallithraka et al., 1997b), they had been extensively trained to differentiate astringency from bitterness and sourness using 1500 mg/L tannic acid as an example of astringency and 1000 mg/L caffeine and 1500 mg/L citric acid as examples of bitterness and sourness, respectively. All panelists had shown their ability to discriminate between these stimuli before starting the experiment.

During the second session, they were given written instructions describing the three different tasting procedures they had to follow and the four samples they had to use during the first three sets of the experiment to practice the procedures. They were already trained in the use of T−I methology in numerous previous experiments. The instructions given were the following (for sample codes see Table 1).

Procedure 1 (for Samples C, C+A, and A Tasted Individually): At 0 s place the entire sample into the mouth, swirl it for 15 s, and expectorate. The scoring starts at 0 s and continues until the attribute is no longer present.

Procedure 2 (for Sample Sequence A→C): Two samples with the same code are given, the first (A) and the second (C). Place the first one into the mouth, swirl it for 15 s, and expectorate. Immediately after expectoration, place the second sample in the mouth (0 s), swirl it for 15 s, and expectorate. The scoring starts at 0 s (the moment you place the second sample in the mouth) and continues until the attribute is no longer present.

Procedure 3 (for Sample Sequence C→A): Two samples with the same code are given, the first (C) and the second (A). Place the first one into the mouth (at 0 s), swirl it for 15 s, and expectorate. The scoring starts at 0 s when the first sample was put in the mouth and continues until the attribute is no longer present. Immediately after expectoration, place the second sample in the mouth, swirl it for 15 s, and expectorate. Thus, for each experimental sample set (red wine, grape seed model, and (+)-catechin model solution) the concentration of the astringent stimulus due to the phenolic compounds remained constant; the order of presenting the acid was varied.

Experimental Design. Nine sets [three replications for model solution with grape seed extracts, three for model solution with (+)-catechin, and three for wine with added (+)-catechin] were assessed during the study, which lasted for 1 month. During each set, four samples were rated for perceived astringency. One additional sample, the acid solution in water, was evaluated in triplicate during the first three sets. The 12 judges were divided into two groups of 6, the maximum that could be served at any one time, but the data were treated as from one panel. To overcome a buildup of the astringent sensation over time and to balance the effect of order of presentation, the samples were served using a balanced block design (MacFie et al., 1989).

Sample Presentation and Assessment. Judges were presented with 10 mL samples at room temperature in 30 mL plastic cups, randomly coded with three-digit numbers. A description of the samples is presented at Table 1. For the model wines and the wines, their mixtures with acid (pH 3.2), and the acid solution (only in the first three sets), the assessors were asked to follow tasting procedure 1 (see Training and Procedures). For tasting procedure 2 the first sample was the acid solution in water (pH 3.2) and the second one was model wine or wine, whereas for procedure 3, model wine or wine samples were presented first and the acid solution in water was the second sample. A 4 min break was taken between the samples, during which time the panelists were asked to eat a cracker and wash their mouths thoroughly with spring water. The selection of this time interval between the samples was based on the existing literature, and it is believed to be adequate to prevent fatigue of the judges (Kallithraka et al., 1997a). A computerized T−I method was used to rate astringency by manipulating a marker using a mouse on an unstructured line scale of 150 mm length, anchored at either end by 0 = none and 100 = extreme. The collection of the data was performed via TASTE software. Judges were asked to rate the intensity of astringency continuously over the time from taking the sample into the mouth until the attribute had disappeared. The end of the assessment was after 2 min.

Data Analysis. Time to maximum intensity (T_{max}), maximum intensity (I_{max}), and total duration (T_{tot}) of astringency were extracted from T−I curves, which were plotted using Genstat Software. Each variable was analyzed using Genstat by analysis of variance in which the judges were treated as a random effect. The least significant differences (lsd) were calculated at 5% significance level.

Chemical Analysis of the Wine and the Model Wine Solutions. The chemical analysis of the wine and the model solutions included pH, titrable acidity, total phenols, organic acids, and noncolored phenols. Determination of total pigments took place only for the wine. The pH was measured with a glass electrode of a Beckman digital pH meter, Model 3500, that had been standardized to pH 4.00 and 7.00 with standard buffer solutions. The acidity was determined according to the method of Ough and Amerine (1988) and expressed as grams per100 mL of tartaric acid. The total phenols and total pigments were determined by spectrophotometer at 280 and 520 nm, by measuring the absorbance on a 100× dilution in 0.1 M HCl in a 10 mm cell (Bakker et al.,

Table 2. Chemical Analysis of Wine and Model Solutions

		model wine solutions	
	red wine	grape seed	(+)-catechin
pH	3.8	3.8	3.7
titratable acidity (g/100 mL as tartaric acid)	0.85	0.15	0.12
total phenols (absorbance units at 280 nm)	48.5	13.0	26.0
total phenols (mg/L (+)-catechin)[a]	3112	843	1668
total pigments (absorbance units at 520 mn)	9.1		
organic acid analysis (mg/L)			
tartaric	1978		
malic	128		
lactic	1500		
acetic	361		
analysis of noncolored phenols			
gallic acid (mg/L)	9.2	0.8	0.0
3,4-dihydroxybenzoic acid (mg/L gallic acid)	0.5	0.0	0.0
4-hydroxyphenethyl alcohol (mg/L gallic acid)	6.7	0.0	0.0
trans-caftaric acid (mg/L gallic acid)	0.8	0.0	0.0
(+)-catechin (mg/L)	4.3	6.4	1538.0
vanillic acid (mg/L gallic acid)	3.3	0.0	0.0
caffeic acid (mg/L gallic acid)	2.4	0.0	0.0
syringic acid (mg/L gallic acid)	3.0	0.0	0.0
p-coumaric acid (mg/L gallic acid)	3.5	0.0	0.0
(−)-epicatechin (mg/L catechin)	30.8	6.9	0.0
myricetin (mg/L catechin)	22.2	0.0	0.0
quercetin (mg/L catechin)	7.7	0.0	0.0
kaempferol (mg/L catechin)	12.4	0.0	0.0
isorhamnetin (mg/L catechin)	4.3	0.0	0.0
procyanidin B1 (mg/L)	*b*	1.7	0.0
procyanidin B2 (mg/L)	*b*	1.3	0.0
procyanidin C1 (mg/L)	*b*	2.1	0.0
(−)-epicatechin gallate (mg/L catechin)	*b*	5.5	0.0

[a] A value of 3753 [as mg/L (+)-catechin] was determined after the addition of 600 mg/L (+)-catechin in the wine. [b] Not detected.

1986). The concentrations of the organic acids were measured by ion chromatography (Dionex Model 4500) with chemical eluent suppression (2 mM octanesulfonic acid in 2% 2-propanol as eluent, anion micromembrane as suppressor, and 5 mM tetra-*n*-butylammonium hydroxide as regenerator) and conductivity detection (conductivity cell). The samples were membrane filtered (0.45 μm), the phenolics were adsorbed on a C_{18} Sep-Pak cartridge, to avoid interference with the organic acid analysis, and the eluted sample containing the organic acids was diluted 25-fold in water. A 50 μL prepared sample was injected on a single ion-exclusion column (AS1 Dionex) and eluted using a flow rate of 0.8 mL/min.

The noncolored phenolic compounds of the wine and the model wine solutions were analyzed by HPLC (HP Model 1090M) by direct injection (autoinjector, 25 μL injection volume) and detected by a diode array detector at 280 nm. A reversed-phase ODS Hypersil column (100 mm × 2.1 mm; particle size 5 μm) at 40 °C was used, with a flow rate of 0.3 mL/min. Using 0.6% aqueous perchloric acid and methanol as eluants, the following linear gradient was used: in 30 min (for the model solution) and 50 min (for the wine) from 20% to 50% methanol, in 1 min to 98% methanol, hold for 3 min at 98% methanol to wash the column, and then return to the initial conditions to re-equilibrate for 10 min. The concentrations of the phenolic acids are expressed as milligrams per liter gallic acid. The concentrations of (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, myricetin, quercetin, kaempferol, isorhamnetin, and procyanidins B1, B2, and C1 are expressed as milligrams per liter (+)-catechin. The concentrations were calculated using external standards of gallic acid (60 mg/L) (Sigma) and (+)-catechin (63 mg/L) (Sigma) in 95% aqueous methanol containing 0.6% perchloric acid.

RESULTS AND DISCUSSION

The results of the chemical analysis of the red wine and the model solutions are presented in Table 2. The grape seed extract contained less gallic acid and (−)-epicatechin than the wine. (+)-Catechin concentrations were about the same in both media before the addition of 600 mg/L to the wine. Procyanidin dimers B1 and B2 and trimer C1 were detected only in the grape seed extract. The total phenol measurement was also expressed as milligrams per liter (+)-catechin, using the molar absorptivity value (4520) of (+)-catechin at 280 nm (Santos-Buelga, personal communication). The polymeric fraction that was detected by the HPLC analysis as a late eluting broad peak in both wine and grape seed model wine and the pigments in wine contributed to the total phenol measurement, whereas the value obtained for the (+)-catechin model wine is attributed to (+)-catechin only. The astringency of (+)-catechin model solution is due to (+)-catechin only, while in both red wine and grape seed model solution, a considerable contribution to astringency may well be made by the polymerized compounds.

Model Solution with Grape Seed Phenols. The *F* ratios after the analysis of variance are presented in Table 3 and show that significant differences among the samples were found for T_{max}, T_{tot}, and I_{max} of astringency.

Tasting the acid solution after the control sample significantly increased I_{max} of astringency (Table 4), whereas rinsing the mouth with the acid before did not significantly alter the astringency of the control sample. In agreement with our previous study (Kallithraka et al., 1997b), the sample containing the acid in the model grape seed solution (pH 3.2) was significantly more astringent than the control, but it was not significantly different from the sample when the acid was tasted after the control. T_{tot} and T_{max} of astringency were significantly increased when the acid was tasted after the control, but no significant differences were found between the control and the rest of the samples that contained grape seed extracts.

In agreement with Hartwig and McDaniel (1995) and Thomas and Lawless (1995) the solution of malic acid in water (pH 3.2) was found to elicit astringency even

Table 3. *F* Ratios and Significant Levels (sig) for the Time−Intensity Parameters, Time to Maximum Intensity (T_{max}), Maximum Intensity (I_{max}), and Total Duration (T_{tot}) of Astringency in the Model Solutions and the Wine[a]

	model solutions					
	grape seed		(+)-cateichin		wine	
T−I parameter	*F*	sig	*F*	sig	*F*	sig
T_{max}	21.92	<0.001	18.70	<0.001	0.79	0.508
T_{tot}	15.40	<0.001	5.38	<0.05	0.28	0.837
I_{max}	11.42	<0.001	4.48	<0.01	3.42	<0.05

[a] Degrees of freedom = 4.44 for the grape seed model solution and 3.33 for the (+)-catechin model soltion and the wine.

Table 4. Least Significant Differences (lsd)[a] $P < 0.05$ and Mean Values for the T−I Parameters Obtained for Astringency of Grape Seed Model Solution, (+)-Catechin Model Solution, and Red Wine

T−I parameter	sample C[b]	sample C+A[b]	sample A→C[b]	sample C→A[b]	sample A[b]	lsd
			Grape Seed Model Solution			
T_{max}	21.33 b	18.94 ab	21.31 b	33.50 c	16.36 a	4.0
T_{tot}	41.81 b	47.44 b	42.69 b	56.10 c	33.69 a	6.0
I_{max}	37.31 a	61.08 b	43.64 a	63.86 b	41.47 a	10.2
			(+)-Catechin Model Solution			
T_{max}	22.03 a	21.00 a	19.99 a	32.42 b		3.9
T_{tot}	47.08 ab	51.83 bc	42.04 a	57.08 c		8.0
I_{max}	45.69 a	65.19 b	47.46 a	65.18 b		14.7
			Red Wine			
T_{max}	20.14	21.64	21.37	23.47		
T_{tot}	52.19	51.25	50.75	49.17		
I_{max}	69.64 b	68.69 b	65.72 ab	58.31 a		8.0

[a] The acid solution was evaluated only during the three replicates of the grape seed model wine session (degrees of freedom = 4.44 for the grape seed model and 3.33 for the (+)-catechin model solution and the wine). [b] Means with the same letter at each row are not significantly different at 5% significance level.

when it was tasted on its own (Table 3). Since the panel was extensively trained, in previous experiments, to discriminate between sourness and astringency, it is believed that the sensation detected after the tasting of the malic acid sample was astringency. I_{max} of malic acid was not significantly different from the control, but T_{tot} and T_{max} were significantly lower compared to the control. According to Thomas and Lawless (1995), the astringency of acids could be attributed to their hydroxyl groups binding to form a complementary hydrogen bond pair with the protein keto−imide linkages or to a second mechanism involving a direct attack on the mucous layer and oral epithelium as well as denaturation of salivary proteins.

Model Solution with (+)-Catechin. In a previous study (Kallithraka et al., 1997a) it was shown that flavan-3-ols possessing only one 1,2-dihydroxyphenyl moiety, e.g. (+)-catechin and (−)-epicatechin, were astringent when assessed in a model wine solution. This observation was in agreement with results from Thorngate and Noble (1995), who assessed these compounds in aqueous solutions. Further evidence that even small phenolic compounds can elicit astringency was reported by Naish et al. (1993), who found that 5-*O*-caffeoylquinic acid (5-CQA), which also possesses only one dihydroxyphenyl residue, was astringent. Hence, it appears that multiple 1,2-dihydroxyphenyl groups are not required for sensory astringency.

This was also confirmed during this experiment by the relatively high scores obtained for the astringency of the control sample. I_{max} of astringency was significantly increased (Table 4) when the pH of the model solution was reduced to 3.2 by the addition of malic acid. The same significant increase was observed when the acid solution was tasted after the control. Rinsing the mouth with the acid solution before the tasting of the model solution had no effect on that parameter. T_{max} and T_{tot} of astringency followed the same pattern as I_{max}, although the increase in T_{max} and T_{tot} for the sample mixed with the acid was not statistically significant.

Wine. From Table 3, it can be shown that significant differences were only found for I_{max} of astringency at the 5% significance level. The control sample was rated as significantly more astringent (Table 4) than the sample when the acid solution was tasted after the control. The wine sample that contained the acid (pH 3.5) was not rated significantly different from the control, in contrast with the results obtained from our recent study (Kallithraka et al., 1997b) in which wine samples at pH 3.5 containing the same acid (malic) were found to be significantly more astringent than the control at pH 4.0.

The reason that a significant increase in astringency was not observed during the present experiment might be that this wine had a higher astringency compared with the wine used in the previous study (Kallithraka et al., 1997b), as suggested by the higher score for the control I_{max}, that masked any changes induced by acidity adjustments. Alternatively, the limiting factor to tannin−protein complex formation was no longer the tannin concentration but the quantity of proteins in the mouth. This higher astringency of the wine used in the present study could be attributed to the lower pH of the control in this experiment (3.8) compared with the pH (4.0) for the wine used in the previous study.

It is known that increasing the acidity of a given wine may increase its astringency but that a progressive increase does not necessarily increase the astringency indefinitely. Presumably, subsequent increases in astringency become less than the threshold needed for detection and/or the sensory mechanism becomes saturated. In the previous experiment, a significant increase in astringency was found between the wine at pH 4.0 and the wine adjusted to pH 3.7 with malic acid, but the increase in astringency between the wines assessed at pH 3.7 and 3.5 (Kallithraka et al., 1997b) was not significant. Similarly, in the present experiment the pH reduction from 3.8 to 3.5 did not result in detectable increase in astringency, possibly because the

low pH of the control wine had already enhanced its astringency so that any further changes were not detectable.

In addition, when comparing different wines, one should note that the effect on astringency of increased acidity is likely to be a function also of the phenol and tannin content of the wines. Although the wines had similar total phenolic contents as defined by A_{280} (48.5 absorbance units for the wine used in this experiment compared with 46.4 absorbance units for the wine used in the previous experiment), the relative amounts of individual phenols and polymerized phenolic material might differ considerably.

General Results. From Table 4, it can be seen that malic acid is astringent when tasted on its own (A). However, total duration of astringency elicited by the acid (A) was significantly less than the same T−I parameter of the model solution (C), although their I_{max} values did not differ significantly. Since previous T−I studies (Robichaud and Noble, 1990; Peleg and Noble, 1995) have shown that the I_{max} of an attribute is correlated with the T_{tot}, the lack of such a correlation in this study would suggest that astringency of acids could be explained by a mechanism different from that for astringency of the phenolic compounds. One possible explanation is that the ingestion of the acid in the mouth decreases the pH value in the mouth, thus changing the PRP net charge and affecting their configuration and possibly influencing the viscosity of the saliva or enhancing precipitation of PRP.

Astringency I_{max} and T_{tot} increased in the grape seed model solution when the pH decreased from 3.8 to 3.2 (as can be seen from the comparison of C with C+A), consistent with the results of Fisher et al. (1994) and Guinard et al. (1986). This indicates that the procyanidins and the polymeric phenols as well as (+)-catechin and (−)-epicatechin are perceived as more astringent at lower pH values. The same parameters of astringency elicited by the monomeric phenolic compound (+)-catechin were also increased when the pH was reduced from 3.7 to 3.2. Although the monomeric phenolic compounds possess only one 1,2-dihydroxyphenyl in their molecules, it is now clear that they are astringent in wines and model wine solutions (Kallithraka et al., 1997a) and that their astringency increases with decreasing pH values in the same way as the astringency of tannic acid is enhanced.

The lack of a significant difference in the astringency of the model solutions when the acid was given to the panelists before (sequence A→C compared with sample C), might be due to the lack of difference between the astringency of the control (C) and the acid (A) (Table 4). Hence, it is likely that while assessing sequence A→C for the first 15 s the panelists were rating the astringency of the acid and after they expectorated the acid solution, including most of the saliva present in the mouth, they started to rate the astringency of the model solutions and the wine. It is possible that as soon as they placed the astringent solutions in their mouth, new saliva was produced that had not been affected by the acid, and hence there was no lasting influence of the acid on saliva. Additionally, the PRP content and/or the amount of saliva elicited by malic acid may be the same as that elicited by the astringent model solutions.

The lack of significant increase in astringency when the acid solution at pH 3.2 was tasted before the model solutions (sequence A→C) and the significant increase in the astringency of the model solutions when the acid was placed in the mouth after the astringent solution (sequence C→A), suggests that pH affects salivary proteins and enhances astringency of phenols if the latter compounds are present in the mouth simultaneously with the acid. One possibility is that the low pH enhances the precipitation of the formed phenol−protein complexes. The pH value of 3.2 might be close to their p*I* but may not be low enough to precipitate the PRP. However, if these proteins are bound to phenols, it may be that they are precipitated more easily. Thus, the complexes formed in the mouth are possibly more prone to precipitation, and a decrease in the pH value due to the acid addition may then enhance precipitation. This would also explain why a mixture of astringent and acid compounds with a lower pH value than the astringent solution alone would be perceived as more astringent. The complexes would precipitate more easily at the low pH value.

T_{max} of astringency of the samples when the acid was tasted after the model solutions (sequence C→A) was significantly increased compared with the control and the sample that contained the acid. This parameter is reported to be more strongly affected by the specific compound, medium, or attribute being rated than by stimulus concentration, which has a very small effect (Noble, 1995). Since all of the above parameters remained the same during the experiment, the significant increase of T_{max} could be because the time at which the panelists started scoring (0 s) was when the astringent solution was placed in the mouth, while the acid solution was placed in the mouth 15 s later.

However, the results obtained for the wine showed a different pattern. When the acid solution was tasted after the wine sample (sequence C→A), the perceived astringency I_{max} was significantly lower than the control. One possible explanation for this result, which is in contrast with the results of the model solutions, is that the wine was already so astringent that the judges could not detect any further increase. Another explanation might be that the wine had a higher titratable acidity and higher concentrations of naturally present organic acids compared with the model solutions, and thus the acids that were present were adequate to act on the protein−tannin complexes and to precipitate them. The acid solution introduced in the mouth after the wine did not cause any further precipitation of such complexes, but could possibly have helped in rinsing the mouth of the remaining phenols (assuming that phenols were present in excess compared with the salivary proteins) and eliminate them from the mouth upon expectoration together with the phenol−protein insoluble complexes. In addition, it is possible that the aqueous acid solution together with the new saliva produced in the mouth—it is known that acids increase the saliva volume output (Norris et al., 1984; Fischer et al., 1994)—lubricated the mouth and thus reduced the sensation of astringency. Since it has not been demonstrated that increase in saliva volume is necessarily accompanied by increased secretion of salivary proteins (Clifford, 1997), no assumptions about the production of more PRP can be made.

Another difference between the wine and the model solutions was that the wine sample that contained the acid (C+A) was not rated as significantly more astringent than the control wine (C), whereas the model wine samples with the added acid (C+A) were significantly more astringent than the control model wine samples

(C). The higher pH values of the wine samples (3.5) compared with the pH values of the model solutions (3.2) might be responsible for this different astringency perception of the wine. This higher pH might have influenced the interaction of phenols with saliva PRP, resulting in the observed lack of significant difference in astringency of the (C+A) wine samples. To lower the pH of the wine to the value of 3.2, a considerable amount of malic acid would be required due to the high wine buffering capacity, and such a high malic acid concentration may be expected to contribute to astringency, as Lawless et al. (1996) have shown that organic acids are perceived to be astringent. Finally, the organic acid composition of the wine was different from that of the model solution, which contained mainly tartaric acid, and could have contributed to perceptual differences in astringency. However, according to Lawless et al. (1996) and Kallithraka et al. (1997b) organic acids of similar pH value are expected to be equally astringent and to elicit similar mouth drying, roughing, and puckering sensations. Hence, this difference in the organic acid content of the wines is expected to have less influence on perception than the pH value.

Conclusion. The perceived I_{max} and T_{tot} of astringency were significantly increased when a solution of malic acid at pH 3.2 was given to the panelists 15 s after the grape seed and (+)-catechin model solutions. This increase was similar to that observed when the acid was mixed with the controls in a solution with a pH value of 3.2. However, no significant increase of astringency was observed when the same malic acid solution was tasted just before the model solutions, suggesting that one possible action of the acid is on the formed protein−tannin complexes in the mouth increasing their precipitation. One possible explanation is that malic acid causes conformational changes of the salivary proteins, induced by a decrease in the mouth pH.

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