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The Influence of Acid on Astringency of Alum and Phenolic Compounds

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Abstract

Astringency of aqueous solutions of phenolic compounds (grape seed tannins, tannic acid, catechin and gallic acid) increased upon addition of citric acid, whereas the astringency of alum was reduced. Astringency of alum was decreased equivalently by addition of equi-sour levels of lactic acid, citric acid or hydrochloric acid. The difference between alum and the phenolic compounds is speculated to result from chemical modifications affecting binding of the astringents with oral proteins rather than cognitive differences. Chelation of the aluminum ion in alum by acids reduces its availability for interacting with salivary proteins or epithelial proteins. In contrast, the increased astringency produced upon acidification of phenolic compounds is speculated to result from the pH driven increase in the affinity of the phenols for binding with proteins. These results suggest that alum cannot be used interchangeably with phenolic astringents in psychophysical studies.

Introduction

Astringency is an important flavor attribute of fruit products and beverages, including wine, cider, beer, cranberry juice and tea. Thus investigating factors affecting perception of this sensation is potentially economically valuable in beverage formulation in addition to contributing to our understanding of the mechanism of astringency perception. Astringency is a complex phenomenon: it elicits a range of sensations, different types of compounds evoke it and several mechanisms have been suggested to explain it. Bate-Smith (1954, 1973) and more recently Naish *et al.* (1993) and Smith *et al.* (1996) proposed that the drying sensation of astringency may be a consequence of the decrease in salivary lubrication caused by precipitation of salivary proteins by astringents. Green (1993) speculated that the drying sensation associated with astringency may be directly attributable to the increase in friction between mucosal surfaces. Alum swabbed between the upper lip and gum elicited astringency, implying that mechanoreceptors rather than taste receptors on the tongue are involved in perception of astringency (Breslin *et al.*, 1993). Lawless and Corrigan (1994) proposed that astringency is caused by drying of the oral cavity, sensations of increased roughness of the oral tissues and a puckering or drawing sensation felt in the buccal musculature. They noted that drying and roughing require mouth movements to be clearly perceived. Thorngate and Noble (1995) suggested that astringents are perceived by mechanoreceptors and that salivary protein precipitation may not be the only astringency mechanism. In contrast, Jellinek (1985) proposed that astringent substances change the cell layers at the mouth's surface which then feels rough. Alternatively, astringent stimuli may cross-link with proteins in the epithelium, thus causing tightness and constriction similar to astringency (Green, 1993).

Astringency occurring in wine, cider and other plant-derived products has been for many years related to the presence of phenolic compounds and more specifically to polyphenols. The perceived astringency caused by phenols as well as the nature of their complexation with proteins has been extensively studied. The mechanism of the protein–tannin interaction has been variously attributed to hydrophobic interactions (Hagerman and Butler, 1980a,b; Oh *et al.*, 1980) and/or hydrogen bonding (Haslam, 1974). The structure of both the phenol and the protein as well as their concentration determine the specific mode of interaction. Astringency, defined chemically as the ability of the astringent to precipitate proteins or sensorially by intensity of perceived astringent sensation, increases as molecular weight increases (Lea and Arnold, 1978; Arnold *et al.*, 1980; Robichaud and Noble, 1990). Astringency of phenolic compounds has been generally shown to increase in the presence of added acid or lowered pH (Guinard *et al.*, 1986; Fischer and Noble, 1994; Kallithraka *et al.*, 1997). The effect of added acid or decrease in pH on perception of astringency has been speculated to result from the shift to the undissociated phenolic species as pH is decreased, hence

increasing the affinity for binding with salivary proteins (Fischer, 1990). However, the sensation of astringency has also been reported to be elicited by acids (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995). In fact, some acids at lower concentrations were reported to be more astringent than sour.

Phenolic compounds elicit bitterness and sourness in addition to astringency, and this interferes with studies of astringency. Thus, aluminum sulfate (alum), although characterized by low levels of sweetness and sourness, has been extensively used as a model astringent in many studies. Aluminum sulfate, aluminum ammonium sulfate and aluminum potassium sulfate have been used to define perceptual astringency (Jellinek, 1985; Lee and Lawless, 1991; Breslin *et al.*, 1993) The mechanism by which alum elicits astringency is not understood. However, aluminum has been shown to bind proteins, including those from blood plasma (Trapp, 1985; Khalil-Manesh *et al.*, 1989). Aluminum salts also complex strongly with phenols and carboxylates (Trapp, 1985; Haslam *et al.*, 1992).

In informal evaluations of solutions of alum and acids (H. Peleg, unpublished data), acids appeared to decrease the astringency of alum, in contrast to the enhancing effect of acid on astringency reported previously (Guinard *et al.*, 1986; Fisher, 1990). This research was thus initiated with two goals: to compare the effect of acid on the perceived astringency of alum and selected phenolic compounds and to compare the effect of different acids on the astringency of alum.

Materials and methods

Experiment 1

Design

To study the effect of acid on the sourness and astringency of several astringents, both attributes were rated in aqueous solutions of alum, gallic acid, tannic acid, catechin and grape seed tannin (GST) with and without the addition of citric acid. Concentrations of the astringents were tested which were equi-astringent in water. In addition citric acid was evaluated alone. Gallic and tannic acids were chosen to represent the monomeric and polymeric forms, respectively, of the nonflavanoid, hydrolyzable tannins. Monomeric and polymeric forms of condensed tannins were similarly represented by the catechin and grape seed tannin.

Sample preparation

The following reagents were used: aluminum sulfate (alum), citric acid, hydrochloric acid (HCl), quinine sulfate, sodium chloride (Fisher Scientific, Fair Lawn, NJ), catechin, gallic acid, tannic acid (Sigma Chemical Co., St Louis, MO) and lactic acid (Mallinckrodt Inc., Paris, KY). Grape seed tannin (GST) was extracted from seeds with ethanol as described elsewhere (Thorngate and Singleton, 1994).

To determine the levels of each astringent compound which produced astringency equal to that of 0.5 g/l alum, astringency pair tests were conducted using ascending concentrations of the astringents versus 0.5 g/l alum. The equi-astringent level was selected as that at which 50% of the subjects selected alum as more astringent. The compositions of the 12 experimental stimuli are shown in Table 1. Samples and reference standards were prepared weekly in de-ionized water from a Millipore filtration system with a conductance of at least 16 mΩcm and stored at 2°C until brought to room temperature (~23°C) the night before each session. Sample pH values were measured using a combination electrode on a Corning pH meter, at 20 ± 2°C.

Experiment 2

Design

To compare the effect of different acids on perceived astringency of alum and evaluate perceived astringency of various acids, lactic acid and citric acid were selected, respectively, for reported differences in chelation. Citric acid was reported to have a higher affinity for aluminum in soils than lactic acid (Sillen, 1964; Martell and Smith, 1989) and hydrochloric acid was chosen to examine the effect of an inorganic acid. Astringency and sourness were rated in aqueous solutions of the acids and of alum in combination and individually.

Sample preparation

Two levels of each acid were evaluated. Equi-sour intensity levels of each acid were chosen in pair tests as described in Experiment 1, in which ascending concentrations of lactic or HCl versus 3.9 mM citric acid (low concentration) and versus 7.8 mM citric acid (high concentration) were evaluated. To provide an optimal number of samples for the design of the experiment, distilled water was included as a test stimulus, while three stimuli containing alum in water were presented to assess within-session reproducibility. The composition and pH of the 16 experimental samples and the reference standards are shown in Table 1.

Sensory protocol

Students and faculty members of UC Davis were selected as judges based on availability and interest. Eighteen unpaid volunteers, eight female and 10 male, aged 19–34 years, participated in both experiments and in the initial pair tests for determining equi-astringent stimuli (Experiment 1) and equi-sour stimuli (Experiment 2). The terms 'sourness' and 'astringency' were defined to each judge verbally and using reference standards for both terms and for bitterness (Table 1). Two practice sessions were conducted prior to the formal tests to familiarize the judges with the computerized system and in the use of the intensity scales for these terms.

Before each training and formal session, judges tasted the low and high intensity standards for sourness and

Table 1 Composition and pH of stimuli and reference standards for Experiments 1 and 2

A. Experiment 1. Astringent compounds in water and in 1.5 g/l citric acid (7.8 mM)

Astringent compound	Concentration, g/l (mM)	pH (water)	pH (citric acid)
Alum	0.5 (1.046)	4.00	2.37
Gallic acid	3.5 (20)	3.05	2.62
Tannic acid	2.8 (na)	2.97	2.57
Catechin	2.0 (6.89)	5.10	2.65
GST	3.25 (na)	4.21	2.68
None	–	6.5	2.64

B. Experiment 2. Acids in water and in 0.5 g/l alum (1.05mM)[a]

Acid	Level	Concentration, g/l (mM)	pH of Solutions	
			Water	Alum
None	none	–	6.5	4.06
Citric	low	0.75 (3.9)	2.80	2.44
	high	1.5 (7.8)	2.59	2.41
Lactic	low	0.78 (8.6)	2.97	3.13
	high	1.57 (17.4)	2.88	2.90
HCl	low	0.17 (4.7)	2.32	2.38
	high	0.34 (9.4)	2.05	2.17

C. Composition of reference standards for Experiments 1 and 2

Attribute	Compound	Low		High	
		g/l (mM)	pH	g/l (mM)	pH
Sourness	citric acid	0.20 (1.04)	3.22	2.0 (10.4)	2.55
Astringency	alum	0.20 (0.59)	4.11	1.5 (4.39)	3.98
Bitterness	quinine	0.002 (0.0053)	5.80	0.015 (0.04)	5.62

[a]Triplicate alum solutions were presented, to bring the number of stimuli to 16.

astringency (Table 1). In both experiments, judges rated intensity of all samples in each session to provide a complete replicate for a single attribute. Sourness was rated in two sessions, and astringency was rated in two subsequent sessions. Subjects rated the maximum perceived intensities of sourness or astringency on an unstructured rating scale anchored by the low and high terms (corresponding to ratings of 0 to 1000) using a computerized data acquisition system described elsewhere (Matysiak and Noble, 1991). These anchors were defined by the intensity of the previously tasted standards. Subjects rinsed three times with water and waited 30 s between samples. All 20 ml samples were presented in a different randomized order to each judge for each session and term, in coded, odor-free plastic cups. All evaluations were conducted in isolated booths under red light.

Data analysis

All statistical analyses were performed using SAS version 6.09 (SAS Institute Inc., Cary, NC). To examine effects of treatments, mixed model analyses of variance (ANOVA) using PROC GLM were performed on the intensity ratings

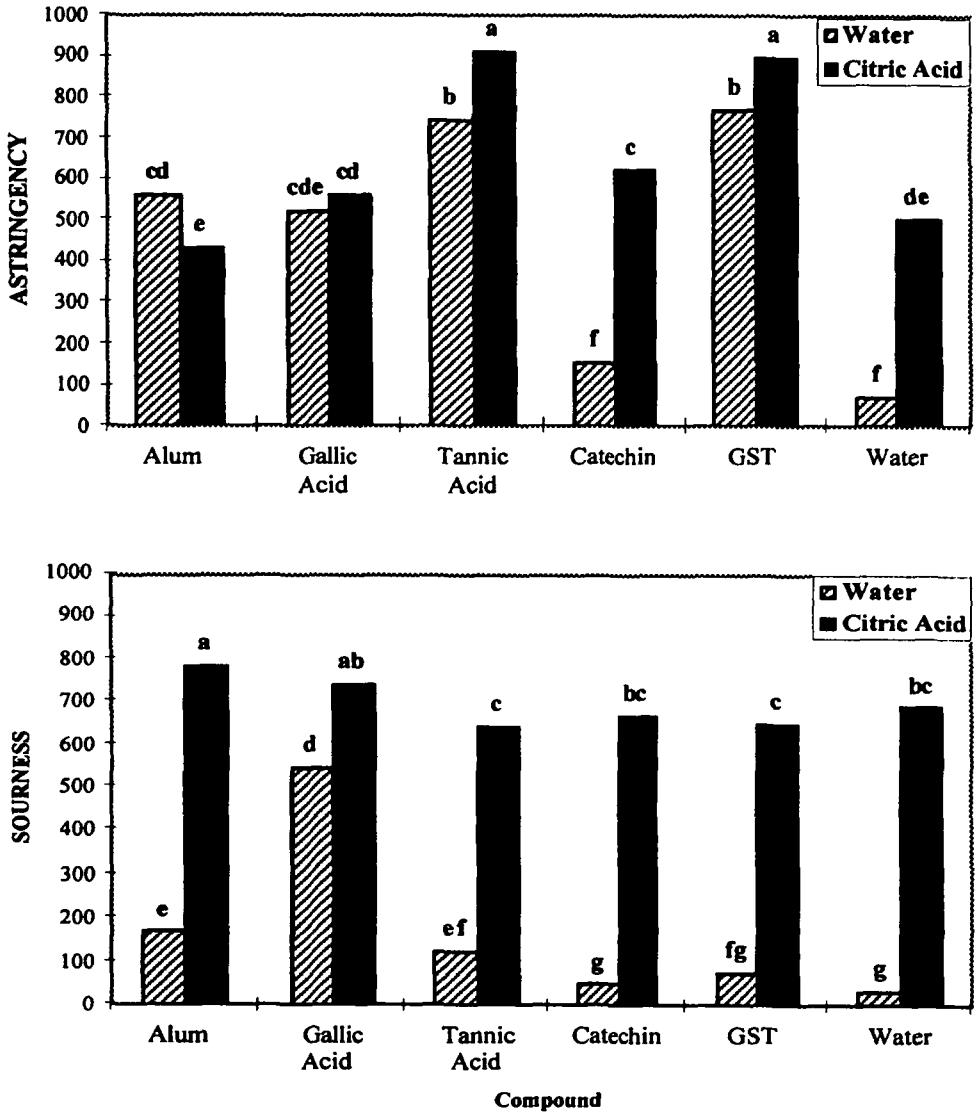

Figure 1 Mean astringency (top) and sourness (bottom) ratings of water, citric acid (7.08 mM) and astringents in water and mixed with citric acid (7.08 mM). For each attribute bars with different letters differ significantly at $P < 0.05$. $LSD_{0.05}$ = 104.54 (astringency) and 78.05 (sourness) (n = 18 subjects × two replications).

with judges treated as a random factor. Comparisons among treatment means were made using Fischer's least significant difference (LSD) or by calculation of the appropriate contrasts.

Results

Experiment 1

Astringency differed significantly across the stimuli (F = 48.82, df = 11/187, $P < 0.001$). As displayed in Figure 1, all phenolic compounds increased in astringency upon the addition of acid, whereas astringency of alum decreased. For all astringent compounds except gallic acid, the effect of acid addition on astringency was shown to be significant by application of LSD (Figure 1) and by testing contrasts for each astringent between water and acid solutions. In a previous experiment astringency of a lower concentration of gallic acid (1.5g/l) was significantly increased by citric acid (Bodine, 1996), suggesting that the insignificant increase in astringency of gallic acid observed here may be attributed to the higher initial level of astringency. The very low astringency observed for catechin cannot be explained. Catechin is more bitter than astringent (Robichaud and Noble, 1990; Thorngate and Noble, 1995), hence this low rating is in contrast to the inflated value that might have been expected due to dumping the intensity of unrated bitterness onto the astringency rating (Clark and Lawless, 1994).

Sourness varied highly significantly across the solutions (F = 121.12, df = 11/187, $P < 0.0001$). The mean sourness

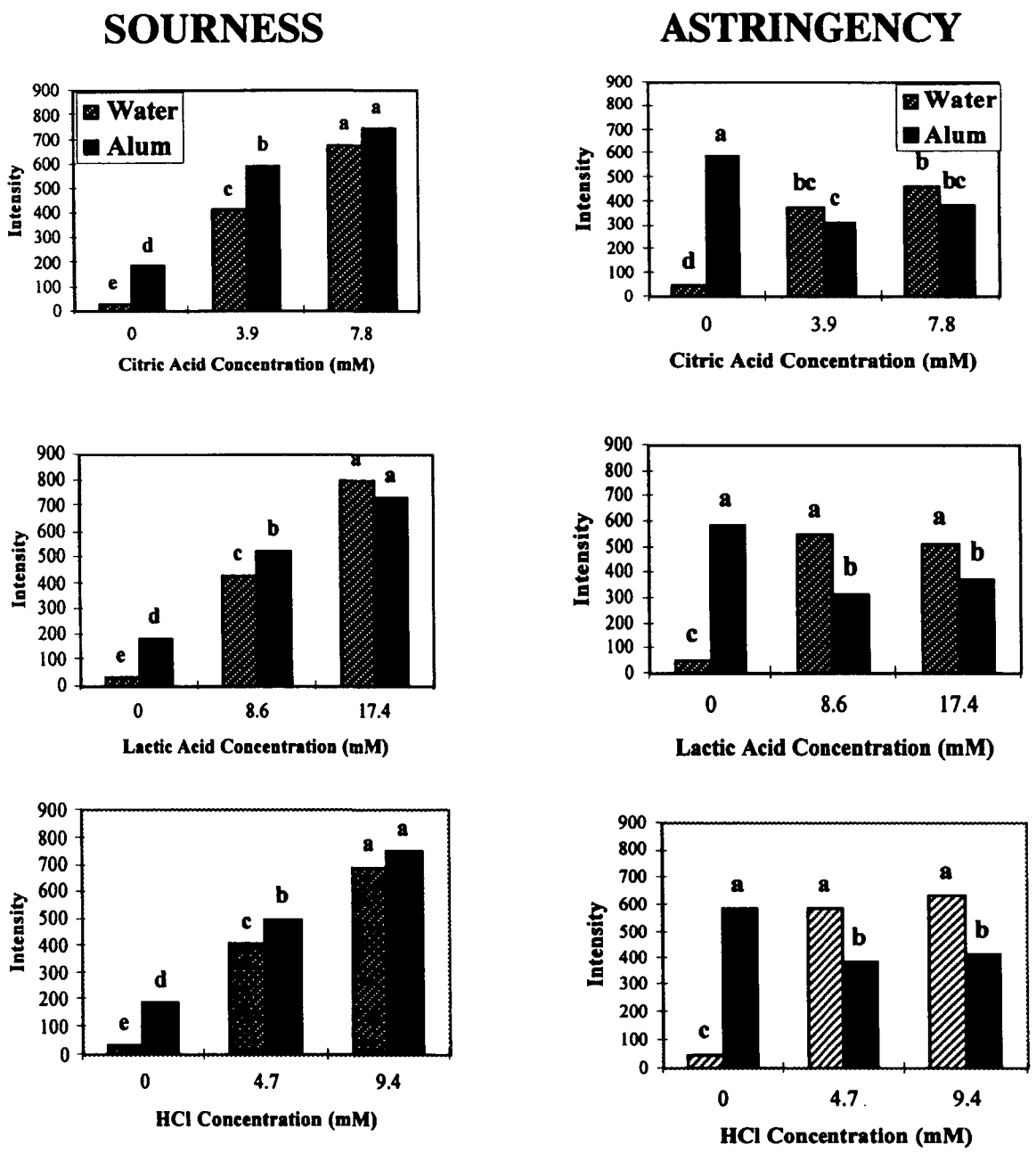

Figure 2 Mean sourness (left) and astringency (right) ratings of citric, lactic and hydrochloric acids in water and mixed with 1.05 mM alum. $LSD_{0.05}$ = 80.62 (sourness) and 105.64 (astringency). For each attribute, bars with different letters differ significantly at $P < 0.05$ (n = 18 subjects × two replications).

ratings for each sample with and without citric acid are presented in Figure 1. As expected, addition of citric acid to each compound produced a highly significant increase in sourness and decrease in pH (Table 1). Excluding gallic acid, which has a low pH in water, the decrease in pH upon addition of citric acid showed a linear relationship with increase in sourness intensity rating (r = 0.92, df = 3, $P < 0.05$).

Experiment 2

The mean astringency and sourness ratings of the aqueous acid solutions (with no alum) are compared in Figure 2. Acids were equi-sour at the low level, but at the high level lactic acid was most sour, even though its pH was 0.5 pH units higher than the less sour HCl. Citric acid solutions had the lowest astringency ratings at both acid concentrations. Hydrochloric was the most astringent acid, in agreement with the results of Settle *et al.* (1986) and Rubico and McDaniel (1992). Although perceived sourness of all acids increased significantly with increase in concentration and decrease in pH, no significant difference in astringency was observed.

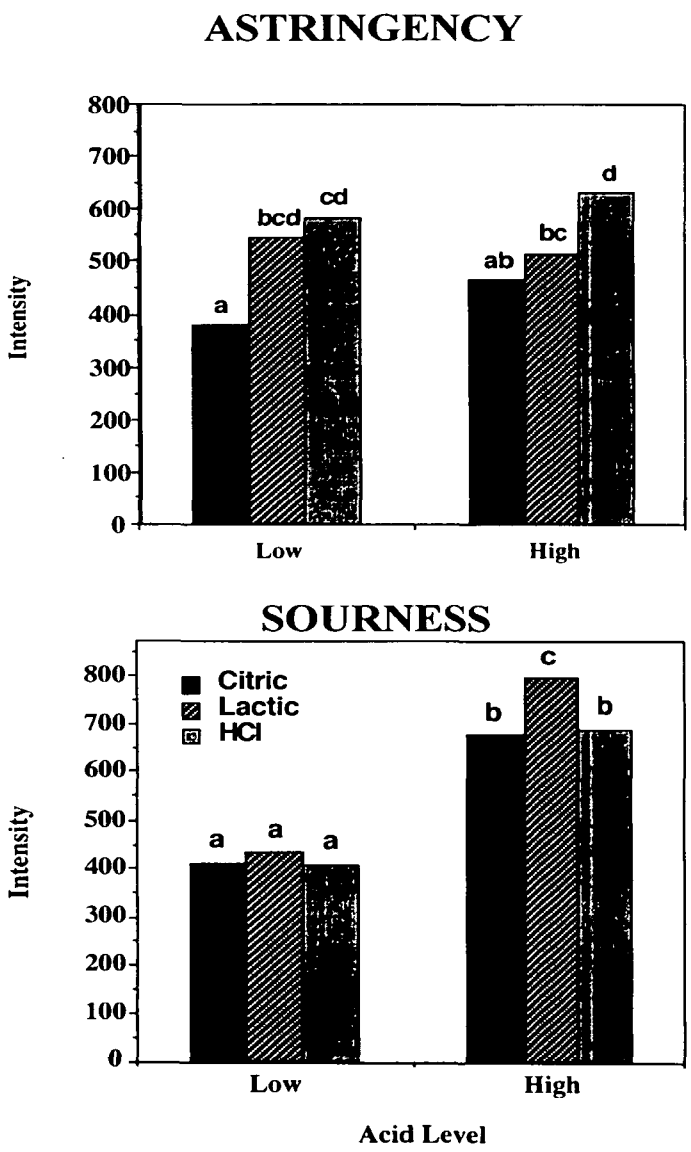

Figure 3 Mean astringency (top) and sourness (bottom) ratings for acid level. $LSD_{0.05}$ = 105.64 (astringency) and 80.619 (sourness). For each attribute, bars with different letters differ significantly at $P < 0.05$ (n = 18 subjects × two replications).

The effect of addition of alum to these acid solutions is displayed in Figure 3. Astringency, which varied highly significantly among samples (F = 15.7, $P < 0.001$, df = 15,255), was highest in the alum in water, and for the aqueous solutions of lactic acid and HCl. In all cases, contrast tests showed that addition of acid to alum significantly lowered astringency from that of alum in water (Figure 3). Addition of alum to the acid solutions reduced astringency below that elicited by acid alone (Figure 3). However, the decrease in astringency of the acids by addition of alum was not significant for either level of citric acid when tested across the entire set of samples, but was significant at the high level of citric acid by contrast tests. In contrast to predicted results based on the reported greater chelating power of citric acid, alum astringency was reduced more by lactic acid than citric acid.

Sourness varied highly significantly among samples (F = 70.9, $P < 0.0001$, df = 15,255). The mean sourness ratings for acid with and without alum are presented in Figure 3. Addition of acid to alum as well as increasing the acid concentration significantly increased perceived sourness in all cases. With two exceptions (high levels of HCl and of lactic acid), contrast tests confirmed that addition of alum to acid significantly increased perceived sourness intensity. There was no consistent relation between pH decrease with alum addition and increased sourness. Addition of alum to citric acid caused a decrease in pH but the opposite was found for the lactic and hydrochloric acid samples (Table 1).

Discussion

Three different explanations for the effects on sourness and astringency observed in these mixtures may be considered. First, the alteration in sensations may result from chemical reactions or interactions occurring in the mixtures before the test solutions reach the oral cavity. Secondly, the results could be attributable to differences in binding with salivary proteins and/or epithelial tissue in the oral cavity. Thirdly, suppression or addition may have occurred during neural processing.

Addition of acid to flavonoid and nonflavonoid phenols increased intensity of astringency, consistent with previous studies (Guinard *et al.*, 1986; Fischer, 1990; Kallithraka *et al.*, 1997). The increase in astringency upon acidification with citric acid could be a result of cognitive summation of perceived astringency of citric acid and the phenolic compounds. Alternatively, the role of acid in increasing the binding between phenolics and salivary proteins may be the important factor. Astringency has been speculated to result from decreased salivary lubrication resulting from precipitation of salivary proteins upon binding with phenolic compounds (Lyman and Green, 1990; Smith *et al.*, 1996). Given that binding of phenolics to proteins occurs primarily through hydrogen bonding, the increase in astringency of phenolic astringents upon acid addition may result from the pH-driven shift in equilibrium lowering the concentration of charged phenolate ions, which cannot participate in hydrogen bonding (Fischer, 1990), and equivalently increasing the number of uncharged phenolic molecules, which do participate in protein binding.

In contrast to the acid enhancement of astringency observed for phenolic astringents, astringency of alum decreased upon acidification. Lawless *et al.* (1994) reported mixture suppression when alum was presented with gallic or citric acid. Both alum–acid mixtures were less intense in astringency and drying, roughing and puckering/drawing sensations than the unmixed components. Aluminum salts complex strongly with phenolic compounds and carboxylic

acids, including lactic and citric (Sillen, 1964; Martell and Smith, 1989; Haslam *et al.*, 1992). Thus, in contrast to suppression, we speculate that chelation of the aluminum ion upon acid addition reduces the availability of aluminum ions for interaction with salivary proteins or oral epithelial tissues. Aluminum ions may act as Lewis bases, binding with atoms with unshared pairs of electrons, such as carboxylate ions, as proposed previously (Lawless *et al.*, 1994). Both ionic interaction and chelation of aluminum ions with the acids are consistent with the observed reduction in astringency in acid–alum mixtures.

From these data it is obvious that studies using alum cannot be generalized to predict factors influencing astringency of phenolic compounds in model systems or naturally occurring foods or beverages. However, the difference in the responses provides a basis for speculation on the chemical basis of these observations. Acid chelation of aluminum ions reduces the ability of alum to bind or change the conformation of salivary proteins, whereas acid addition to phenolic compounds is speculated to increase the affinity for binding between phenolics and salivary and/or epithelial proteins.

Conclusions

When acid was added to monomeric (catechin and gallic acid) and polymeric phenolic compounds (grape seed tannin and tannic acid) astringency increased. In contrast, addition of monocarboxylic and tricarboxylic organic acids and an inorganic acid reduced the astringency of alum. The striking difference in behavior between alum and the phenolic compounds may be due to chelation of the aluminum ion by the acids, which reduces the effectiveness of alum in interacting with salivary proteins or epithelial proteins. In contrast, the increased astringency produced upon acidification of phenolic compounds may be a function of the stronger protein binding resulting from the pH-driven increase in uncharged phenolic compounds which can undergo hydrogen bonding with the salivary proteins.

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