C(S)

Astringency of Organic Acids is Related to pH

Harry T. Lawless, John Horne and Paul Giasi

Department of Food Science, Cornell University, Ithaca, NY 14853, USA

Correspondence to be sent to: Harry Lawless, Department of Food Science, Cornell University, Ithaca, NY 14853, USA

Abstract

Astringency and sourness of lactic, acetic and citric acids, each adjusted to pH 3, 5 and 7, were evaluated in two experiments, one starting at equal concentrations in wt/vol before neutralization and the second starting at equal molarity. Astringency and sourness decreased with increasing pH. However, acids were differentially sour at equal pH, consistent with previous findings. In contrast, the tactile attributes associated with astringency (drying, roughing of oral tissues and puckery/tightening sensations) were similar across acids; pH was the major influence on astringency. Strong dependence on pH suggests that astringency of these acids is a direct result of their acidic properties, and not solely due to the hydrogen bonding mechanisms previously suggested as an explanation of astringency in tannin interactions with salivary proteins. **Chem. Senses 21: 397–403, 1996.**

Introduction

In addition to sour and bitter tastes, acids and polyphenols are capable of inducing astringency in the oral cavity. Astringency arises from chemically induced tactile sensations (Bate Smith, 1954; Lyman and Green, 1990; Breslin *et al.*, 1993; Green, 1993; Corrigan and Lawless, 1994). From the perceptual viewpoint, astringency is complex, and includes the tightening and drawing sensations felt in the buccal musculature, and sensations of drying and roughness of oral surfaces felt when contact and movement is made (Lawless and Corrigan, 1994). Astringency is also a complex phenomenon when viewed from the stimulus side. A growing body of literature has studied the astringency induced by acids, once commonly thought of as prototypically sour stimuli, but whose tactile properties are now more widely recognized (Straub, 1992; Corrigan, 1993; Rubico and McDaniel, 1992).

A popular theory holds that astringency arises from hydrogen bonding of -OH groups on polyphenols or acids to salivary proteins, aggregating or precipitating them and thus delubricating the oral cavity. This delubrication subsequently leads to the sensations of tightness, dryness and rough surfaces (McManus *et al.*, 1981; Clifford, 1986). For acids that have adjacent hydroxyl groups (e.g. tartaric), this mechanism is plausible. For other organic acids, involvement of other potential hydrogen bonding sites such as carbonyl groups needs to be invoked.

The hydrogen bonding that occurs in protein–polyphenol interactions is only one potential mechanism for inducing astringency, especially given the astringency of acids. Based on ^{1}H-NMR analysis, Murray *et al.* (1994) found that hydrophobic associations between hydrolyzable tannins and synthetic peptide sequences similar to mouse proline-rich

proteins (PRPs) could act as another potential mechanism. Proline provides a flat, rigid, open, hydrophobic surface favorable for association with other flat, rigid, hydrophobic structures such as the aromatic rings of gallic acid in galloyl-glucose or other hydrolyzable types of tannins. A combination of hydrogen bonding and hydrophobic interactions might also occur. In this respect, Murray *et al.* noted the participation of an arginine residue, with its hydrogen-bond-donating, terminal guanidino group. Their data implied that a hydrophobic association was made between the tannin and the PRPs, followed by the formation of stabilizing hydrogen bonds. Cross-linking occurs between the tannin–PRP complexes, facilitated by the strong tannin–tannin interactions and high probability of multiple interactions produced by the repeat sequences in the salivary PRPs. These higher orders of complexation would eventually lead to precipitation.

Other mechanisms are also possible. Guinard *et al.* (1986) suggested the possibility of multiple mechanisms, with the polyphenols directly attacking epithelial tissues after stripping away the salivary coatings from the epithelium. Dawes (1964) proposed that acid precipitation of salivary proteins could play a role in dental plaque formation. Precipitation of salivary proteins after different buffer conditions was consistent with a mechanism of local denaturation of protein structure, even under conditions near physiological pH. Studies of the astringency of acids have shown a special potency of HCl in inducing astringency (relative to its sour taste) when compared with organic acids (Straub, 1992; Thomas and Lawless, 1995). Since HCl has no -OH groups to participate in the hydrogen bonding mechanism, a second mechanism, perhaps one directly related to the acid properties of HCl, is probably responsible for its astringency. If so, one would expect that increases in pH due to buffering or neutralization should decrease the astringent impact of acids. Recently, evidence for pH dependence was noted in a free-choice profiling study by Hartwig and McDaniel (1995). However, interpretation of the principal axes from the analysis of free-choice experiments is not clear cut. The following experiments tested this possible effect of pH on astringency using a more conventional psychophysical profiling method.

Two experiments were conducted, one starting with acids at equal wt/vol concentrations and the second starting with equimolar concentrations. In both studies, pH was altered by neutralization with NaOH from pH 3 to pH 5 and 7.

Experiment 1

Materials and methods

Subjects

Twenty paid subjects served as panel members (13 male, 7 female). All had previous experience in evaluating astringency.

Stimuli

Nine different stimuli were used consisting of three different organic acids neutralized to three different pH. The acids used were citric acid monohydrate (mol. wt 210.14), lactic acid (85% wt/wt syrup; mol. wt 90.08) and acetic acid glacial (mol. wt 60.05). Acid concentrations were 0.2% wt/vol. Solutions were neutralized to pH 3.00, 5.00 and 7.00 using 1.0 N NaOH (mol. wt 40). The pH of each solution was determined using an Accumet portable pH meter (model no. 955) and a standard-sized, polypropylene body, gel-filled KCl/AgCl electrode. Solutions were mixed at 23°C and stored at 4°C. The pH of each solution was checked every 3 days during the experiment, and again at the conclusion of the experiment. Test solutions were moved from their storage temperature of 4°C, decanted into 20 ml samples in glass test tubes and placed in a 35°C water bath at least 1 h prior to each experimental session.

Procedure

Attributes on the ballot were sourness, drying, roughing, puckering and astringency. Each attribute was rated on a 15-point category scale anchored with the words 'none' at the left-hand side (box no. 1) and 'strong' at the right-hand side (box no. 15). Subjects attended an orientation session wherein the procedure and attributes were discussed. Informed consent was obtained. Examples of astringency and sourness were provided at 1 g/l alum and 1.68 g/l citric acid respectively. Three other acids from the study were presented for practice. Subjects were instructed to swish each sample in their mouths for 15 s, expectorate the sample and simultaneously make an initial rating of five attributes (0 s). They then made subsequent ratings of the same five attributes at 30, 60 and 120 s following expectoration. The experimenter kept the time on a stopwatch and instructed the subjects to make the ratings at the appropriate intervals. Ratings of the five attributes for each time interval were made on separate pages on the ballot. All stimuli were presented in random orders. All nine stimuli were tasted by

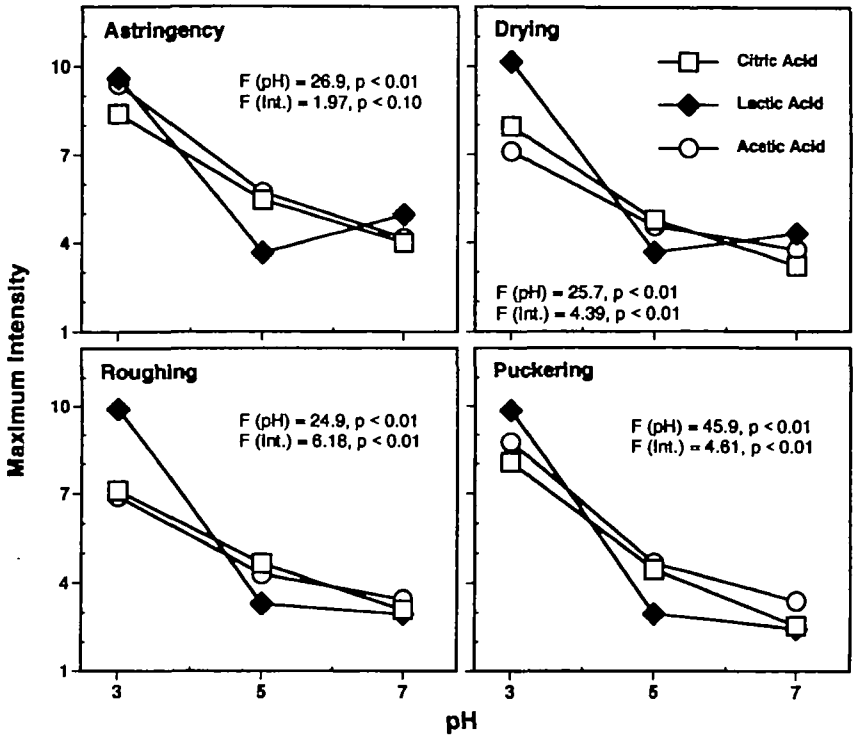

Figure 1 Mean maximum intensity (I_{max}) for three acids at three pH levels is shown from experiment 1, along with ANOVA statistics for the pH and acid by pH interaction in analysis I_{max} data, for astringency and related tactile attributes of drying, roughing of oral tissues and puckery sensations.

each subject for a total of three experimental sessions plus orientation. The interval between stimuli was 5 min to allow the mouth to recover from the effects of the previous stimulus. During the time between each sample, subjects were instructed to rinse their mouths with spring water. Unsalted table water crackers were provided for cleansing the palate between samples.

Analysis

Data were analyzed using SYSTAT v. 5.2. Each attribute was subjected to a repeated measures mixed model ANOVA with acids, pH, time intervals and subjects (a random effect) as factors. Due to the possible violations in the assumptions of repeated measures ANOVA (mainly heterogeneity of covariance), Greenhouse–Geisser adjusted *P*-values were used and data were also analyzed by MANOVA using Wilks' lambda. ANOVAs and MANOVAs were also conducted on the intensity maxima (I_{max} values) with acids, pH and subjects (random) as factors. All effects reported below for ANOVA were also significant in MANOVA, with *P*-values <0.05. Complete time–intensity curves may be found in Giasi (1995).

Results

All ANOVAs showed strong effects of time as sensations decreased over the 2 min evaluation interval [all $F(3,57)$ 9.88, $P < 0.001$]. A strong effect of pH was seen for all attributes, with decreasing sensation intensity at increasing pH [all $F(2,38)$ 13.9, $P < 0.001$]. All attributes showed time by pH interactions, due to the convergence near baseline at later time intervals and high pH (all $P < 0.001$).

Figure 1 shows the decrease in astringency intensity and astringent subqualities of drying, roughing and puckering feelings as a function of increasing pH. Mean I_{max} values are used as the data points. *F*-ratios for the pH effect on I_{max} and *F*-ratios for the acid by pH interactions are shown. The common pattern was that lactic acid decreased most rapidly between pH 3 and 5, while acetic and citric acids are more linear. With the exception of sourness, there were no significant main effects for differences among acids [sourness $F(2,38) = 16.3$, $P < 0.01$ for the raw data, and $F(2,38) = 19.5$, $P < 0.01$ for the I_{max} values]. Acetic acid was the most sour—a result that was expected as acetic acid has the lowest molecular weight of the three acids.

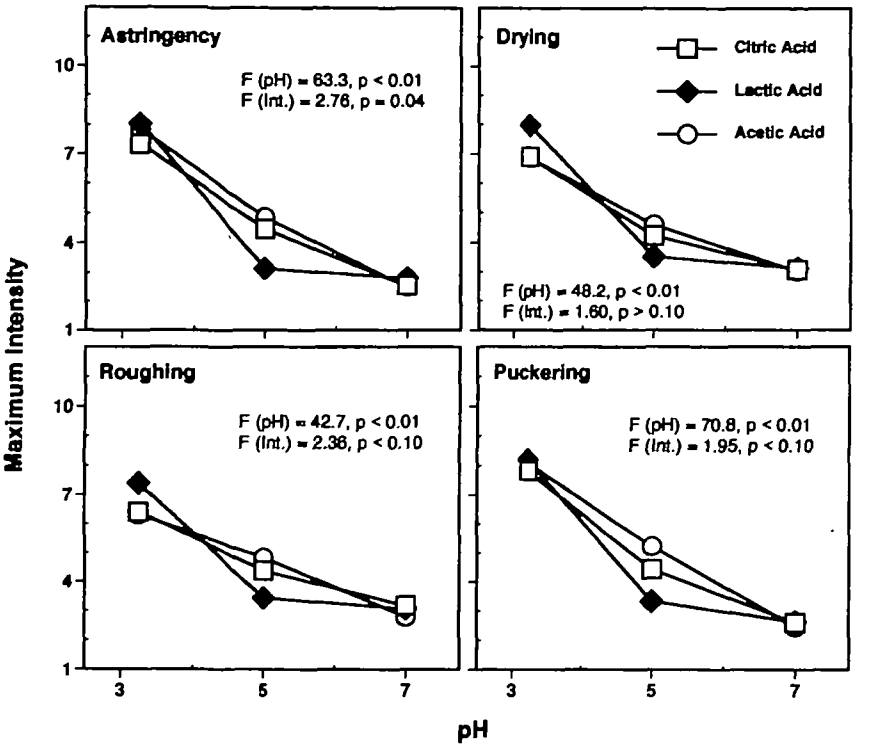

Figure 2 Mean maximum intensity (I_{max}) for three acids at three pH levels is shown from experiment 2, along with ANOVA statistics for the pH and acid by pH interaction in analysis of I_{max} data, for astringency and related tactile attributes of drying, roughing of oral tissues and puckery sensations.

Experiment 2

Materials and methods

Fifteen subjects (9 male, 6 female) from experiment 1 were paid for participation.

Acids were all in equimolar concentrations of 0.024 M. The same acid by pH combinations were used as in experiment 1, except that the lowest pH was 3.25. Neutralization was achieved with 1 N NaOH, as in experiment 1, using the same equipment and procedures described above. Solutions were stored at 4°C; pH was checked every 3 days during the experiment and again at the conclusion of the experiment. The pH was found to vary by $<\pm 0.02$. Samples of 20 ml were warmed to 37°C and presented in 60 ml plastic cups that were labeled with three-digit random codes. Due to some cooling (~2°C during the pouring process) detected in pilot work, the higher bath temperature was used instead of the 35°C used in experiment 1.

Attributes were the same as those used in experiment 1, except that bitterness was added. Ratings were again made on 15-point category scales, anchored at either end with the phrases 'not ____' at the leftmost box (box no. 1) and 'extremely ____' at the rightmost box (box no. 15) (the appropriate attribute name was repeated in each blank).

No orientation was given since subjects had recently participated in a similar experiment. Subjects were given verbal examples of the attributes to be rated, e.g. citrus fruits having sourness; caffeine for bitterness; drying as a lack of moisture; formation of ridges in the mouth for roughness; puckering as a drawing together or tightening. Astringency was described as a sensation that results from the complex of the three attributes: drying, roughing and puckering. Each subject rated each of the nine stimuli twice for a total of 18, receiving either four or five stimuli in each session for a total of four sessions.

Due to the smaller subject pool used in experiment 2, a replicate was conducted in a separate randomized block. Analyses by ANOVA (repeated measures, subjects random) and MANOVA were conducted on both the raw data and I_{max} values as in experiment 1, except that data were collapsed across replicates prior to analysis.

Results

All ANOVAs showed strong effects of time, as sensations decreased over the 2 min [all $F(3,87)$ 15.8, $P < 0.001$]. A

strong effect of pH was also seen for all attributes, with decreasing sensation intensity at increasing pH [all $F(2,54)$ 39, $P < 0.001$], except for bitterness [$F(2,52) = 4.92$, $P < 0.05$]. Bitterness ratings were fairly low in intensity compared with the other attributes and showed no other significant effects.

Figure 2 shows the mean I_{max} values for the four tactile attributes and the F-ratios for the pH effect on I_{max} and the acid by pH interactions. While lactic acid again showed a tendency to decrease most steeply between pH 3 and 5, the acid by pH interaction was now significant for only the overall astringency attribute.

Figure 3 shows the sourness rating means for both experiments (I_{max} values). The main effect of acid was once again significant [$F(2,54) = 14.9$, $P < 0.01$ for the raw data, and $F(2,58) = 18.7$, $P < 0.01$ for the I_{max} values]. Citric acid and acetic acid behaved similarly when equated on a molar basis, while lactic acid was less sour at pH 5.

Discussion

These two experiments demonstrate a strong relationship between pH and astringency and its related tactile subqualities of dryness, roughing of oral tissues and puckery/tightening sensations in the mouth. Neutralization had a strong effect on sourness, but the taste and tactile effects were not entirely parallel, as can be seen from the figures. Acids differed in sourness at equal pH, an effect that has been shown in the previous literature (e.g. Noble *et al.*, 1986). However, differences among acids were much smaller in their tactile effects and pH dominated as the main variable affecting astringency. The difference between the effects of sourness and the tactile attributes attests to the fact that our subjects could differentiate among these words used to describe oral sensations. Furthermore, previous factor analyses of sourness and tactile attributes has shown that the sourness and astringent attributes load on different factors (Corrigan and Lawless, 1994; Lawless and Corrigan, 1994; Thomas and Lawless, 1995).

Among the tactile attributes, the pattern of results was very similar, suggesting that the perceptual categories were not well differentiated by our subjects or that all the tactile sensations arose from the same or related mechanisms. We favor the latter interpretation since previous work has shown that people will respond differently to the astringent subqualities across substances. For example, when alum and tartaric acid are approximately equal in their puckery/tightening effect, alum will be more intensely drying in the mouth (Lee and Lawless, 1991). In another study, factor analysis showed the tactile subattributes loading on different factors (Lawless and Corrigan, 1994). Unfortunately, the present studies do not supply additional support for this differentiation. A reviewer has suggested that the simultaneous rating of all attributes might have contributed to this perceived equivalence, and that having subjects rate the attributes in different sessions might yield different results. The methodology used herein was primarily for cost-efficiency in data collection. A rating method wherein subjects rate the tactile attributes in

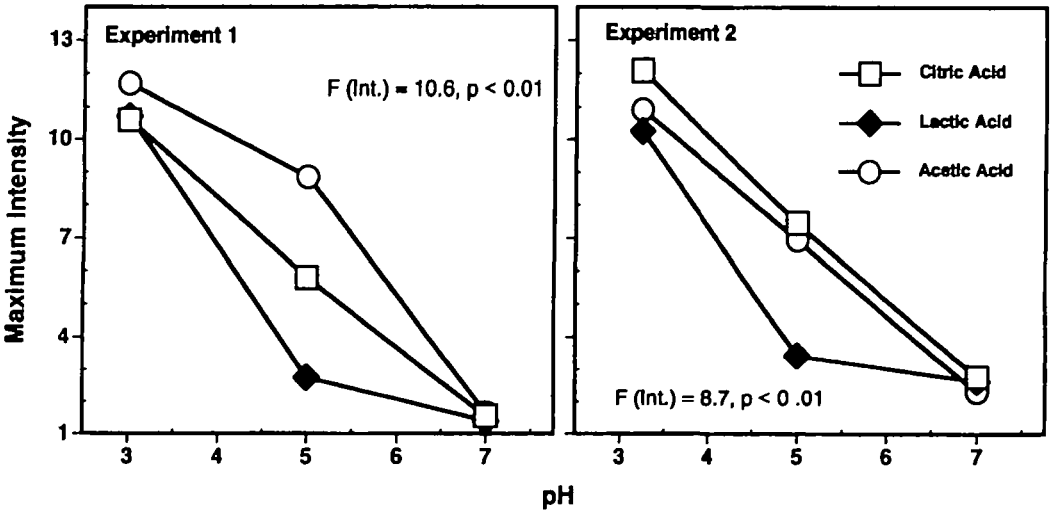

Figure 3 Mean maximum intensity (I_{max}) for three acids at three pH levels is shown from experiment 1 (left panel) and experiment 2 (right panel), along with ANOVA statistics for the acid by pH interaction in analysis of I_{max} data, for sourness.

separate sessions from astringency might very well better reveal differences between the tactile effects.

The result observed here is in partial agreement with findings of Hartwig and McDaniel (1995) that increasing pH will decrease astringency. However, direct comparison of the two studies is not straightforward. They employed a free-choice profiling method in which each subject is free to use his or her own words to describe the stimuli. The data set is then subjected to generalized procrustes analysis in order to extract common dimensions and optimally align subject's configurations. As in other multivariate techniques, interpretation of the dimensions (or principal axes, as they are formally referred to) is necessarily subjective. Hartwig and McDaniel interpreted the third principal axis of their analysis as being related to astringency, and values on this dimension generally decreased from pH 3.5 to 4.5. However, this comparison hinges on the accuracy of the interpretation of their principal axes.

Other interpretations of these data are possible in addition to the simple hypothesis that pH itself was a causal factor. Two other components increased as the acids were neutralized—notably sodium cation concentration and anion concentration. One possibility is that the addition of NaOH to neutralize the acids interfered with hydrogen bonding or with hydrophobic interactions with salivary proteins (Murray et al., 1994). However, if increasing sodium were a causal factor, different results might be expected. In experiment 2, the amount of sodium needed to alter the pH of tricarboxylic citric acid to pH 5 was roughly twice that needed to alter the pH of both acetic and lactic acids to the same pH. At pH 7, the difference was >3 times. If sodium alone were responsible for the inhibition of tactile effects, then citric acid should diverge considerably from the other two acids. Instead, our data show the acids to be fairly similar to one another, with only lactic acid as a potential outlier. Sodium is known to be an important inhibitor of bitterness (Kroeze and Bartoshuk, 1985) and this must still be entertained as a potential explanation of the small reduction in the already low bitterness ratings given to the acids.

Another possible explanation for these findings is that increasing anion concentration could cause decreasing astringency. Once again, the increased number of anionic groups on citric acid might predict very different behavior for citric acid if anions were an important contributor to this mechanism. In addition, having a decreased sensory response in the face of an increasing concentration requires the addition of a second, presumably inhibitory, process. On the grounds of parsimony, further evidence is needed before such a mechanism can be adopted. Further research is also needed to rule out other chemical parameters that could be invoked as explanations. Nonetheless, our observations from simple direct scaling methods stand to support the indirect evidence from Hartwig and McDaniel that a change in pH can influence the astringency of acids.

Ionic interactions would seem to be a likely mechanism for the astringency of acids. Since proteins may be denatured (i.e. lose their usual conformation) at low pH, it seems reasonable that the lubricating and protective functions of salivary proteins would be compromised by strongly acidic stimuli. Dawes (1964) found that low pH caused precipitation of salivary proteins and that some local conformational change was possible even when pH-buffers were present. While stimulation with acids is known to cause an increase in salivary flow (e.g. Froehlich and Pangborn, 1986), the effects of acids on the proteins may defeat this protective reflex to a certain degree.

As dietary influences on salivary PRP production have been shown in rodent species (Mehansho *et al.*, 1983; Glendinning, 1992), it would be interesting to know whether high tannin content in the human diet could influence PRP production and modulate the astringency response. In some studies, salivary flow rates have shown a small to moderate relationship to astringency responses (Fischer, 1990; Fischer *et al.*, 1994; Ishikawa and Noble, 1995). It may be that salivary composition as well as flow rate are modulating factors. For the astringency induced by acids, this might include buffering capacity from bicarbonate as well as protein production.

ACKNOWLEDGEMENTS

This research was partially supported by a grant from the National Institutes of Health, no. 5-RO1-DC-00902.

REFERENCES

Bate Smith, E.C. (1954) Astringency in foods. *Food Proc. Packag.*, **23**, 124–127.

Breslin, P.A.S., Gilmore, M.M., Beauchamp, G.K. and Green, B.G. (1993) Psychophysical evidence that oral astringency is a tactile sensation. *Chem. Senses*, **18**, 405–417.

Clifford, M.N. (1986) Phenol–protein interactions and their possible significance for astringency. In Birch, G.G. and Lindley, M.G. (eds), *Interactions of Food Components*. Elsevier, London, pp. 143–163.

Corrigan, C.J. (1993) Time–intensity measurements of astringent subqualities in selected organic and inorganic acids. MSc Thesis, Cornell University.

Corrigan, C.J. and Lawless, H.T. (1994) Influence of oral volume capacity on perception of astringent intensity. In *Sixteenth Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL*, pp. 455–456.

Dawes, C. (1964) Is acid-precipitation of salivary proteins a factor in plaque formation? *Arch. Oral Biol.*, **9**, 375–376.

Fischer, U. (1990) The influence of ethanol, pH and phenolic composition on the temporal perception of bitterness and astringency, and parotid salivation. MSc Thesis, University of California, Davis, CA.

Fischer, U., Boulton, R.B. and Noble, A.C. (1994) Physiological factors contributing to the variability of sensory assessments: relationships between salivary flow rate and temporal perception of gustatory stimuli. *Food Qual. Pref.*, **5**, 55–64.

Froehlich, D.A. and Pangborn, R.M. (1986) Induction of human parotid salivary alpha amylase sretion by oral stimulation. In *IX Meeting of ISOT*, vol. 11, p. 602.

Giasi, P. (1995) Astringency and sourness of acids with variation of duration of oral exposure and pH. MSc Thesis, Cornell University.

Glendinning, J.I. (1992) Effect of salivary proline-rich proteins on ingestive responses to tannic acid in mice. *Chem. Senses*, **17**, 1–12.

Green, B.G. (1993) Oral astringency: a tactile component of flavor. *Acta Psychol.*, **84**, 199–125.

Guinard, J.-X., Pangborn, R.M. and Lewis, M.J. (1986) The time-course of astringency in wine upon repeated ingestions. *Am. J. Enol. Viticult.*, **37**, 184–189.

Hartwig, P. and McDaniel, M.R. (1995) Flavor characteristics of lactic, malic, citric, and acetic acids at various pH levels. *J. Food Sci.*, **60**, 384–388.

Kroeze, J.H.A. and Bartoshuk, L.M. (1985) Bitterness suppression as revealed by split-tongue taste stimulation in humans. *Physiol. Behav.*, **35**, 779–783.

Ishikawa, T. and Noble, A.C. (1995) Temporal perception of astringency and sweetness in red wine. *Food Qual. Pref.*, **6**, 27–33

Lawless, H.T. and Corrigan, C.J. (1994) Semantics of astringency. In Kurihara, K. (ed.), *Olfaction and Taste XVI*. Springer-Verlag, Tokyo, pp. 288–292.

Lee, C.B. and Lawless, H.T. (1991) Time-course of astringent sensations. *Chem. Senses*, **16**, 225–238.

Lyman, B.J. and Green, B.G. (1990) Oral astringency: effects of repeated exposure and interactions with sweeteners. *Chem. Senses*, **15**, 151–164.

McManus, J.P., Davis, K.G., Lilley, T.H. and Halsam, E. (1981) Polyphenol interactions. *J. Chem. Soc. Chem. Commun.*, 309–311.

Mehansho, H., Hagerman, A.E., Clements, S., Butler, L.G., Rogler, J. and Carlson, D.M. (1983) Modulation of proline-rich protein biosynthesis in rat parotid glands by sorghum with high tannin levels. *Proc. Natl Acad. Sci. USA*, **80**, 3948–3952.

Murray, N.J., Williamson, M.P., Lilley, T.H. and Haslam, E. (1994) Study of the interaction between salivary proline-rich proteins and a polyphenol by ^{1}H-NMR spectroscopy. *Eur. J. Biochem.*, **219**, 923–935.

Noble, A.C., Philbrick, K.C. and Boulton, R.B. (1986) Comparison of sourness of organic acid anions at equal pH and equal titratable acidity. *J. Sensory Stud.*, **1**, 1–8.

Rubico, S.M. and McDaniel, M.R. (1992) Sensory evaluation of acids by free-choice profiling. *Chem. Senses*, **17**, 273–289.

Straub, A. (1992) Power function determination of sourness and time–intensity measurements of sourness and astringency for selected acids. MSc Thesis, Oregon State University.

Thomas, C.J.C. and Lawless, H.T. (1995) Astringent subqualities in acids. *Chem. Senses*, **20**, 593 – 600.

Received August 11, 1995; accepted April 4, 1996