Astringent Subqualities in Acids

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Abstract

Astringency, astringent subqualities (drying, roughing and puckering) and sourness were compared among six acids: hydrochloric, lactic, citric, acetic, fumaric and malic acids. The attribute profiles of organic acids were similar to each other but different from hydrochloric acid, the only inorganic acid, which was the most astringent and the least sour. In a second experiment, two inorganic acids (hydrochloric and phosphoric) and two organic acids (citric and malic) were tested at three concentration levels. At approximately equal levels of overall sensory impact, the inorganic acids were alike in astringency and sourness, receiving higher ratings for roughing and drying, and lower ratings for sourness than the organic acids. Interactions with concentration (differences in psychophysical functions) for the subquality of drying were noted, in addition to the differences in the astringent subqualities of roughing and drying seen across acids in both experiments. The higher level of astringency for inorganic acids suggests that the current model for tannin binding to salivary proteins as an explanation of astringency needs to be extended to include a direct pH-dependent effect. Chem. Senses 20: 593–600, 1995

Introduction

The term astringency denotes sensations of tightening and drying. More than 40 years ago, Bate-Smith (1954) labeled astringency a tactile sensation, stating, '... we are dealing with the sense of touch, and the sensation of dryness or puckeriness has to do with feeling, not with taste.' Astringency is perceived through motions of the tongue against other surfaces of the mouth (Breslin *et al.*, 1993). Several percepts or cues may contribute to astringency. Lee and Lawless (1991) proposed that several subqualities comprise overall astringency, including drying of the mouth, roughing of oral tissues, and a puckery or drawing sensation felt in the cheeks and muscles of the face. Subjects may attend to one or more of these sensations when asked to rate the astringency of a stimulus. The first goal of these experiments was to see whether acids are generally similar or different in their astringent profiles on these subqualities. Differences would provide further support for the theory that these are independent sensory experiences contributing to the overall perception of astringency (Lawless and Corrigan, 1994).

Generally regarded as a binding reaction (Haslam and Lilley, 1988), astringency has been thought to occur through the precipitation of combined salivary proteins and tannins (Bate-Smith, 1954). Tannins are polyphenols that bind with salivary or epithelial proteins (Haslam and Lilley, 1988). Binding is hypothesized to occur when the hydroxyl groups of the tannin molecule form hydrogen bonds with sites such as the carbonyl groups at the keto-imide linkages of the salivary proteins (McManus *et al.*, 1981; Hagerman and

Butler, 1981). The bound proteins precipitate, resulting in dryness and roughness since saliva no longer lubricates effectively (Bate-Smith, 1954).

Some acids may act by this classical mechanism. Chlorogenic acids, components of coffee, bind with proline-rich proteins found in saliva and produce low levels of astringency (Naish *et al.*, 1989, 1993). Nevertheless, other acids may contribute to astringency without tannins present or without the anions containing suitable -OH groups for salivary protein hydrogen bonding (Straub and McDaniel, 1989; Lee and Lawless, 1991; Rubico and McDaniel, 1992). Straub and McDaniel (1989) found that acids differed in astringency and sourness. Astringency/mouthfeel was the most important discriminating characteristic in acids as determined from generalized procrustes analysis of free-choice profiling data (Rubico and McDaniel, 1992). Acids with the lowest pH values, phosphoric and hydrochloric acids, were more astringent than sour, suggesting that pH may determine astringency. Whether astringency arises from a pH-dependent effect (i.e. from acidity directly) or from salivary protein binding, as occurs with tannins and chlorogenic acids, remains to be determined. Inorganic acids would be expected to participate in the first, but not the second mechanism. So, a second goal of these experiments was to extend the findings of Rubico and McDaniel in examining the astringency of inorganic acids by using subquality profiles. If astringency of acids is more closely tied to a direct effect of acidity than to salivary protein binding, one would predict that the inorganic acids should again evoke a stronger astringent response than the organic acids.

Differences between the sensations of puckering, drying and roughing support the notion that these are independent qualities, but other associations are possible. Puckering may co-occur in many stimuli that are both astringent and sour. Overlap of the concepts of puckering and sourness seems to exist in the minds of subjects (Bertino and Lawless, 1993). In sortings of oral sensation terms, males associated astringency with puckery sensations, but females associated puckery with sour. At least in such conceptual tasks, puckery is related to both sourness and astringency. It is possible that astringent acids may be rated as puckery due to their sour taste as opposed to their astringency. The definition of astringency should then be altered to include puckering as partially related to both astringency and sourness. A third goal of these experiments was to assess correlations between the astringent subqualities and the sour taste.

Experiment 1

Materials and methods

Subjects

Volunteers were recruited from the Cornell University community and were paid to participate in the experiment. Nine males and six females between the ages of 20 and 40 were divided into three groups of five subjects.

Stimuli

The acids were selected from previous research (Lee and Lawless, 1991; Straub, 1992) and are common food components. Observations of three group discussions helped to narrow the possibilities down to six acids. Bitter acids were avoided. Aluminum ammonium sulfate (ammonium alum, referred to as alum here), was included for comparison and as a reference for astringency since it has little taste but is a potent astringent (Lee and Lawless, 1991).

Matched levels of astringency are necessary to compare subquality intensities, so concentrations from prior research (Lee and Lawless, 1991; Straub, 1992) were evaluated to equalize the overall astringent impact of the stimuli. Eight tasters experienced in rating astringency tasted several concentrations of each acid. Adjustments were made; the final concentrations were alum, 1.0 g/l; acetic acid, 1.98 g/l; citric acid, 2.49 g/l; fumaric acid, 2.28 g/l; hydrochloric acid, 0.92 g/l; lactic acid, 1.68 g/l; malic acid, 2.25 g/l.

Procedure

Three group discussions were conducted with naive volunteers before the experiment to confirm ballot terms and to gather impressions for subject training. Details may be found in Corrigan (1994). The following terms were selected: sourness, overall astringency, puckering, roughing and drying. Subjects attended a 1-h training session in which the ballot terms were defined and references were presented to align their concepts of the terms (O'Mahony, 1991). The rating procedure was also practiced at this time. Alum was the astringent reference and citric acid was the sour reference (subjects were warned not to overlook its astringency). Hydrochloric, fumaric and malic acids were used as practice samples in varied concentrations for experience with different sourness and astringency intensities. Ratings and subquality profiles for the latter were not suggested in any way by the experimenter.

For the intensity ratings, 20-ml samples were served at room temperature in 120-ml plastic cups. Testing was limited to three stimuli per day. Alum was presented in first, second or third position to test for sequential effects. The serving order for the remaining samples was counterbalanced. The five attributes were rated over a four-minute period to discrete points using a 15-point category scale. Subjects were cued at each interval by an experimenter.

Before testing, subjects rinsed with spring water. When cued at the start of the rating period, subjects at once put the entire 20-ml sample into their mouths and swished vigorously to ensure all oral surfaces were contacted. At the 15-s point, subjects expectorated and immediately rated the attributes. The next rating was at the 30-s point, then subsequent ratings occurred every 30 s for seven intervals. A 5-minute break was given between samples, when plain crackers and spring water were used to help clear the mouth of residual sensations.

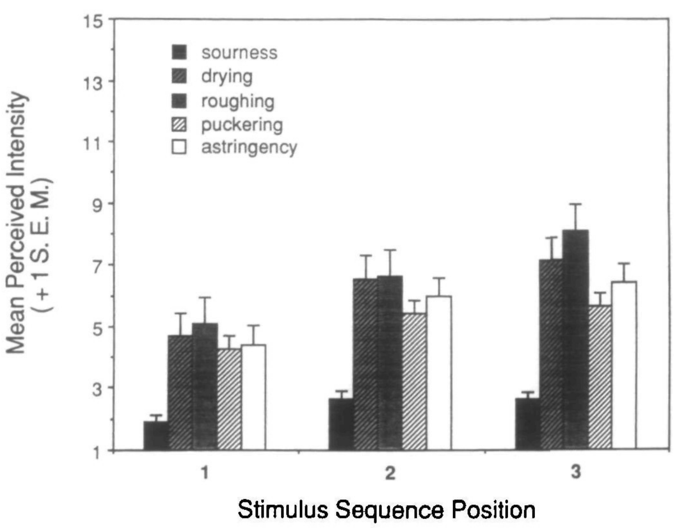

Figure 1 Mean attribute intensity ratings (+ 1 SEM) of alum for each position in the stimulus sequence in Experiment 1, collapsed across time

Statistical analysis

Repeated measures analyses of variance (ANOVA), with time and stimulus (time and position for the alum samples) as factors, were applied using Systat 5.2 (Wilkinson, 1989). Acids and alum were analysed separately. Means were collapsed across time to compare acids using Duncan's test. Principal components analysis (PCA) was conducted to determine relationships between attributes. Fisher's z transformation (Guilford, 1956) was used to compare the size of correlations between attributes.

Results and discussion

As expected the ANOVA showed significant decrements over time ($P < 0.001$) for all samples in all attributes. The plots of the time-intensity curves did not clearly represent the differences among the nine samples and they are not pictured here. Acids differed in sourness, roughing and drying [all $F(5,70) > 2.7$, $P < 0.05$], but not in astringency or puckering. In the alum reference samples, drying, roughing, puckering and astringency increased across the three positions of testing [all $F(2,28) > 3.5$, $P < 0.05$], but there was no significant change for sourness (Figure 1). This buildup has been seen in earlier studies of astringency (Guinard *et al.*, 1986).

Although the concentrations were adjusted for equal overall astringency, the subjects could detect differences in other attribute intensities and thus discriminate between acids in the statistical sense. The organic acids were more similar to each other than hydrochloric acid, which was rated the highest for roughing and drying. Hydrochloric and lactic acids were significantly lower than the rest of the acids in sourness, especially citric acid. Malic acid most closely paralleled the high astringent intensity of hydrochloric acid, especially for feelings of roughness. These effects are seen across rows in Table 1, as shown by the

Table 1 Differences among means of acids for astringency, astringency subqualities and sourness: Experiment 1

	Hydrochloric	Acetic	Lactic	Citric	Fumaric	Malic
Astringency	5.85	4.76	4.55	5.57	5.33	5.79
Puckering	5.88	4.83	4.84	5.73	5.64	5.57
Roughing	6.38^{b}	4.39^{a}	5.09^{a,b}	5.71^{a,b}	5.52^{a,b}	6.06^{b}
Drying	5.96^{b}	4.36^{a}	4.39^{a}	5.40^{a,b}	5.07^{a,b}	5.31^{a,b}
Sourness	3.80^{a}	4.96^{b}	3.86^{a}	5.70^{b}	5.45^{b}	5.19^{b}

Means are collapsed across time. Samples sharing letters within an attribute row were not significantly different from each other. No letters within an attribute row mean that no significant difference was found for that attribute.

Table 2 Factor loadings from principal component analysis—rotated solution

	Factor I	Factor II	Factor III	Factor IV	Factor V
Astringent	—	—	—	0.80	—
Puckering	—	—	0.85	—	—
Roughing	—	—	—	—	0.85
Drying	0.83	—	—	—	—
Sourness	—	0.97	—	—	—
(variance explained	20%	22%	20%	18%	20%)

Values less than |0.4| have been omitted for clarity

Correlation matrix

	Sourness	Drying	Roughing	Puckering
Drying	0.26			
Roughing	0.10	0.76		
Puckering	**0.51**	0.62	0.58	
Astringent	0 39	0.74	0.72	**0.69**

letters for Duncan's tests indicating statistically significant differences. An alternate view is to examine the profiles of the acids by comparing values down columns. In this approach, hydrochloric and lactic acids are more astringent than sour, while the other acids show about equal perceived intensities, i.e. a more 'balanced' profile across attributes.

The first interval (the 15-s point, after expectoration) was the point of highest intensity, so it was used in the PCA. In the rotated PCA, attributes loaded on separate factors (Table 2) suggesting that they are different tactile sensations and also differ from the taste property of sourness. Fisher's z transformation revealed a stronger correlation between puckering and astringency than between puckering and sourness ($P < 0.05$).

Taken together, these results show small but significant differences across the astringent subqualities and differences in acid type. As in previous studies, the inorganic acid, hydrochloric acid, was higher in astringent impact, especially when viewed relative to its sourness, and in comparison to the organic acids. The present study, however, shows that the effect may be stronger for the astringent subqualities of dryness and perceptions of rough surfaces in the oral cavity, as compared to the overall ratings of astringency or to the puckery/tightening sensations that are felt.

Experiment 2

Astringent profiles of organic and inorganic acids were compared, using phosphoric acid and three acids selected from Experiment 1: hydrochloric (most astringent, least sour), citric (most sour), and malic (second most astringent) acids. Three concentrations of each acid were tested to see if astringency changes with concentration and as a function of the acid. Correlations of puckering with astringency and sourness were again examined. A primary question was whether the pattern for hydrochloric acid would hold for another inorganic acid.

Materials and methods

Subjects

Twelve subjects from the first experiment and four additional volunteers were recruited from the Cornell University community. Twelve males and four females between the ages of 21 and 40 were paid for testing.

Stimuli

The stimuli consisted of the following compounds and concentrations: citric acid, 1.25, 2.49 and 3.74 g/l; hydrochloric acid, 0.46, 0.92 and 1.38 g/l; malic acid, 1.13, 2.25 and 3.38 g/l; phosphoric acid, 0.5, 1.0 and 1.5 g/l. The concentrations were 50, 100 and 150% of levels in Experiment 1. Levels of phosphoric acid were determined as in the preliminary evaluations of Experiment 1.

Procedure

Experienced subjects reviewed procedures, tasted reference samples and learned the revised procedure in a 30-min training session. The new subjects were trained in a 1-h session as in Experiment 1. After they mastered the original procedure, the revised procedure was taught in another 30-min session.

The tasting protocol from Experiment 1 was used, except that subjects made the first interval rating at the 5-s point while swishing the sample, then at the second interval rating after expectoration at the 15-s point. The third rating was made at the 30-s point, and the remaining seven interval ratings were cued every 30 s after that. All concentrations of an acid were tasted in one session and assigned randomly to subjects. Subgroups of four subjects tested acids in different sequences.

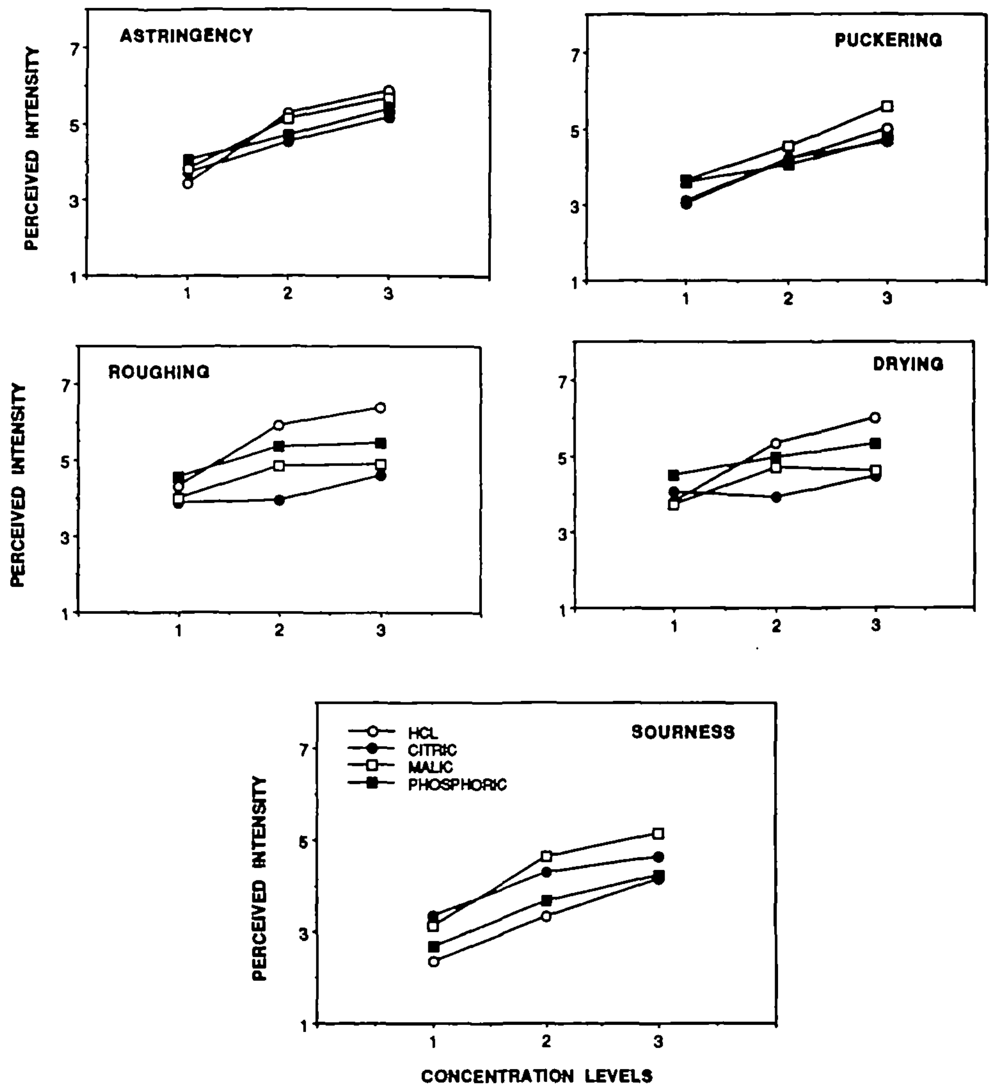

Figure 2 Intensity changes of astringency, for subqualities of drying, roughing, puckering and for sourness with increases in concentration. Acids are represented by the different symbols: hydrochloric acid (open circle), citric acid (filled circle), malic acid (open square) and phosphoric acid (filled square).

Statistical analysis

Data were analysed using PROC GLM in SAS (SAS Institute Inc., 1985). Repeated measures analysis of variance (ANOVA) was conducted with time, acid and concentration as factors. Duncan's test compared the means of the acids. Principal components analysis was used to determine relationships between attributes. Correlation coefficients were compared with Fisher's z transformation (Guilford, 1956).

Results and discussion

Again, the ANOVA on the time-intensity data revealed significant decreases over time ($P < 0.001$) for all acids and concentrations in all attributes. Concentration was a significant source of variance in all attributes as well ($P < 0.002$). Overall astringency levels were comparable among acids and no significant differences or interactions with concentration were found (see Figure 2, astringency panel).

Once again, the organic acids were relatively more sour than astringent as compared to the inorganic acids. Separate examinations of the astringent subqualities of each concentration showed differences only at the middle and high concentrations (Table 3). For both astringent subqualities and sourness, the differences among acids were concentration-dependent with acid profiles changing at different rates with concentration. To illustrate this effect, the means for each acid collapsed across time were plotted as a function of

Table 3 Differences among means of acids for astringency, astringent subqualities and sourness: Experiment 2

	Hydrochloric	Citric	Malic	Phosphoric
Low concentration				
Astringency	3.41	3.71	3.81	4 04
Puckering	3.03	3.10	3.64	3.59
Roughing	4.31	3.88	3.99	4 56
Drying	3.81	4.08	3.73	4.52
Sourness	2 35[a]	3.34[b]	3.13[a,b]	2.67[a,b]
Medium concentration				
Astringency	5.28	4.53	5.14	4.71
Puckering	4.19	4.21	4.53	4.06
Roughing	**5.49[b]**	**3.96[a]**	**4.86[a,b]**	**5.36[b]**
Drying	**5.34[b]**	**3.93[a]**	**4.72[a,b]**	**4.98[a,b]**
Sourness	3.32[a]	4.29[a,b]	4.65[b]	3.68[a,b]
High concentration				
Astringency	5.87	5.16	5.68	5.38
Puckering	4.99[a,b]	4.66[a]	5.58[b]	4.73[a]
Roughing	**6.40[b]**	**4.60[a]**	**4.91[a]**	**5.46[a,b]**
Drying	**6.01[b]**	**4.48[a]**	**4.62[a]**	**5.31[a,b]**
Sourness	4.15[a]	4.64[a,b]	5.14[b]	4.24[a]

Means are collapsed across time. The significant differences at the middle and highest concentrations for astringent subqualities are shown in bold type. Samples sharing letters within an attribute row were not significantly different from each other Where letters are omitted from an entire row, no significant difference was found for that attribute among acids at that concentration.

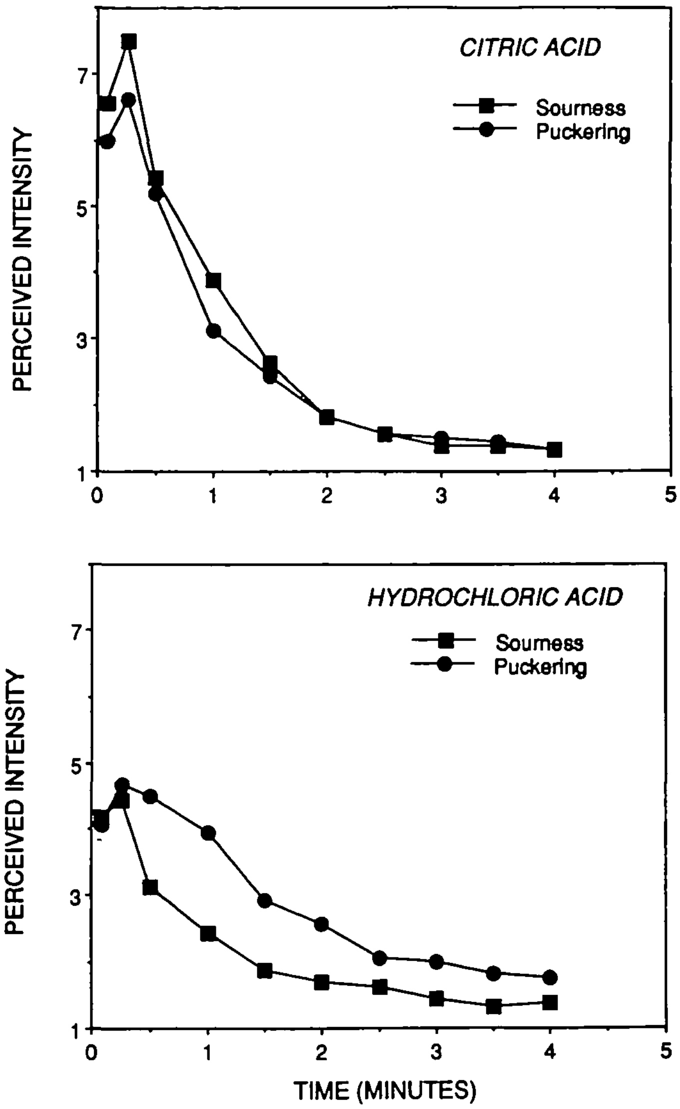

Figure 3 Comparison of sourness and puckering intensity ratings over time for the low concentrations of citric and hydrochloric acids, highlighting the similarities (citric acid) and the differences (hydrochloric acid) in these sensations.

concentration for each attribute (Figure 2). Again, acids did not differ in overall astringency nor was there a concentration interaction. The same was true for the puckering attribute. Drying, however, showed a significant concentration by acid interaction [$F(6,90) = 2.72$, $P < 0.02$]. Hydrochloric acid showed a steep psychophysical function for tactile properties—it became the most drying as concentration increased. A similar pattern is seen for the roughing attribute. On the other hand, hydrochloric acid always remained the least sour. Increasing concentration allowed differences between the acids to become more clear, but the astringent subqualities did not always increase equally.

The low concentrations of hydrochloric and citric acids best displayed the dissociation of the tactile effect of puckering from the taste effect of sourness (Figure 3). Hydrochloric acid had different temporal properties for sourness and puckering; sourness disappeared more rapidly than puckering. For citric acid, the time courses of puckering and sourness were almost superimposed. A similar effect is evident in Table 3, where the inorganic acids had low sourness and high ratings for puckering while citric acid had almost equal means for those two sensations.

Correlation analysis also showed differentiation among the astringent subqualities and sourness. PCA was again conducted on the peak intensity interval at 15 s (Table 4). Drying and roughing loaded on one factor, sourness on a separate factor and astringency and puckering on a third factor. Roughing also loaded on a fourth factor, showing some independent variance from drying. The correlation

Table 4 Factor loadings from principal component analysis—rotated solution

	Factor I	Factor II	Factor III	Factor IV
Astringent	—	—	0.60	—
Puckering	—	—	0.86	—
Roughing	0.54	—	—	0.72
Drying	0.87	—	—	—
Sourness	—	0.93	—	—
(variance explained	27%	24%	31%	16%)

Values less than |0.4| have been omitted for clarity.

Correlation Matrix

	Sourness	Drying	Roughing	Puckering
Drying	0.51			
Roughing	0.47	0.84		
Puckering	**0.65**	0.67	0.74	
Astringent	0 65	0.77	0.85	**0.91**

between puckering and astringency was once again significantly stronger than the correlation between puckering and sourness ($P < 0.05$).

General discussion

A common anecdote among wine experts is that the organic acids produce qualitatively different sensations. In this study, the acids differed in their sensory profiles, but the differences found here were small. The most robust effect was that inorganic acids produced more roughing and drying than the organic acids, with hydrochloric acid being the most potent tactile stimulus relative to sourness, confirming earlier work (Straub and McDaniel, 1989; Rubico and McDaniel, 1992).

The accepted astringency mechanism is that proteins in saliva combine with tannins and precipitate, producing roughness and dryness of oral tissues (Bate-Smith, 1954). The hydroxyl groups on a tannin molecule bind to form a complementary hydrogen bond pair with an electronegative site such as the keto-imide linkages of the protein (McManus *et al.*, 1981). This theory explains the astringency caused by tannins, but not the astringency caused by acids without adjacent -OH groups. Some acids, such as tartaric acid, a potent astringent, contain adjacent hydroxyl groups and will fit the theory. The potency of inorganic acids shown here requires revision or expansion of proposed mechanisms to encompass acid stimuli without obvious hydroxyl pairs, such as hydrochloric acid. For these inorganic acids, a second mechanism such as denaturation of salivary proteins or direct attack on the mucous layer and oral epithelium may be required. The direct effect of acidity on induction of astringency sensations could be tested using acid solutions with constant concentrations but pH adjustment.

Puckering and sourness were not strongly associated in these results. Thus, the common semantic association of these two terms may be somewhat misleading. While lemons can evoke both sensations, careful analysis of the patterns of correlation in these studies shows the tightening, drawing or puckering effect to be more closely related to the tactile experiences associated with astringency than to sour taste.

Acids are capable of producing multiple sensations in the mouth and they should not be conceived of as purely 'sour' stimuli. Each acid may be defined by its own unique profile. Inorganic acids or alum elicit strong tactile sensations and cause intense roughing and drying. Organic acids are effective sour stimuli, but their ability to induce astringency should not be overlooked.

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