

# Comparison of the Effects of Concentration, pH and Anion Species on Astringency and Sourness of Organic Acids

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#### Abstract

The separate effects of concentration, pH and anion species on intensity of sourness and astringency of organic acids were evaluated. Judges rated sourness and astringency intensity of lactic, malic, tartaric and citric acid solutions at three levels of constant pH varying in normality and at three levels of constant concentration varying in pH. To assess the comparative sourness and astringency of the organic acid anions of study, binary acid solutions matched in pH and titratable acidity were also rated. As pH was decreased in equinormal solutions, both sourness and astringency in- creased significantly (P < 0.001). By contrast, as the normality of the equi-pH solutions was increased, only sourness demonstrated significant increases (P < 0.001) while astringency remained constant or decreased slightly. At the lowest normality tested, all solutions were more astringent than sour (P < 0.05). Although lactic acid was found to be significantly more sour than citric acid (P < 0.05), no other sourness or astringency differences among the organic acid anions were noted. This study demonstrates for the first time that astringency elicited by acids is a function of pH and not concentration or anion species, and confirms that sourness is independently influenced by concentration, pH and anion species of the acid.

# Introduction

Until very recently, the primary sensory property studied in acid solutions was that of sour taste. Sourness has been shown to vary independently with pH (Richards, 1898), total acid concentration (Harvey, 1920) and specific anion. Early studies of the relative sourness of organic acid anions yielded conflicting results because of the confounding effect of pH variations in solutions of equal normality (Richards, 1898) or equimolarity (Ough, 1963; Buechsenstein and Ough, 1978). To isolate the effect of the nature of the anion on sourness, binary acid solutions with equal pH and titratable acidity were evaluated (Norris et al., 1984; Noble et al., 1986). Using time-intensity methods, maximum sourness intensity of citric acid was found to be lower than that of fumaric acid and tartaric acids (Norris et al., 1984). In pair tests, citric acid was significantly less sour than tartaric, malic, succinic, fumaric or lactic acids. Lactic acid was signficantly more sour than both citric and fumaric acids (Noble et al., 1986).

Astringency has been defined as 'the complex of [oral] sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alum or tannins' (ASTM, 1989). Unlike sourness, a primary taste, astringency is tactile oral sensation (Breslin *et al.*, 1993; Green, 1993), although direct stimulation of taste afferents by astringent compounds has been reported

(Schiffman et al., 1991). Kahlenberg (1900) observed that highly diluted acid solutions lost perceptible sourness while remaining astringent. However, a 1992 sensory study of organic acids (Rubico and McDaniel, 1992) was the first investigation in which astringency of either organic or inorganic acids had been addressed. In evaluations of several organic acids varying in wide ranges of pH (3.5, 4.5 and 6.5: Hartwig and McDaniel, 1995; 3.0, 5.0 and 7.0: Lawless et al., 1996), astringency was found to vary inversely with pH. However, the contribution of total or titratable acidity which varied in the samples was not assessed in either study.

Although there is preliminary evidence that the astringency of acid solutions might be a function of pH, an appropriately controlled direct examination of those aspects of acid chemistry which might influence the astringency of organic acid solutions has not yet been attempted. The aim of the present investigation was to determine the independent effects of concentration, pH and anion species on the perceived astringency of aqueous solutions of organic acids. In addition, the effect of gender, PROP status and salivary flow rate was explored.

#### Materials and methods

#### Design

In experiment 1, the effect of pH and acid normality on sourness and astringency intensity was evaluated for lactic, malic, tartaric and citric acids. In the second experiment, the role of the organic acid anion on sourness and astringency was evaluated using binary acid solutions matched in pH and titratable acidity.

#### **Subjects**

For both studies, the sensory panel was composed of students from the University of California at Davis who were selected based on their availability and interest. Fourteen judges, including 10 males and four females, ranging in age from 19 to 49 years, completed all phases of training and testing; seven of these judges had participated in previous sensory experiments. Judges were classified on the basis of proplylthiouracil (PROP) and salivary flow status prior to the experiments.

The judges were assessed for their PROP taster status by the method of Bonnans (1991). Judges were presented with two replicate triangle tests each consisting of two 15 ml deionized water and one 0.0001 M (0.017 g/l) PROP samples presented in a randomized order in randomly numbered cups. A PROP taster was defined as a panelist who correctly identified the sample containing PROP in both of the replications and who specifically ascribed the differences among the samples to bitterness.

The mean induced salivary flow rate for each panelist was determined by a modification of the method of Ishikawa and Noble (1995). Judges rinsed with 10 ml of a 4 g/l citric acid solution for 10 s prior to expectoration of the rinse solution. Next, the judges expectorated into a pre-weighed plastic cup for 1 min. The cup was then weighed to determine salivary flow rate in units of g/min. Four replications (two tests on each of 2 days) per panelist were averaged to determine their mean salivary flow rate.

#### Stimuli

In experiment 1, appropriate amounts of DL-malic (Eastman Kodak Co., Rochester, NY), L-tartaric and citric acids (Fisher Scientific, Fair Lawn, NJ) were weighed out by analytical balance, while DL-lactic acid syrup (synthetic, 85% w/w syrup, 98% pure; Sigma Chemical Co., St Louis, MO) was measured volumetrically. Acids were then diluted volumetrically with deionized water. Samples requiring pH adjustment were titrated to within 0.001 unit of the desired pH endpoint with a 20.0% w/v sodium hydroxide solution (LabChem, Inc., Pittsburgh, PA). Three concentrations of each acid (0.02 N, 0.06 N and 0.10 N) were tested at each of three pH levels (2.8, 3.4 and 4.0), resulting in nine test solutions for each acid. High sourness and astringency standards consisted of 0.150 N citric acid, pH 3.4, and 2.80 g/l aluminum sulfate (alum), respectively, while deionized water was used as the low intensity standard for both modalities.

For experiment 2, binary acid solutions matched both in pH and titratable acidity based on the same two organic acids were formulated for all possible pairwise combination of the four acids from experiment 1 using PHACTGS, a BASIC program developed by Boulton (personal communication). All binary pair solutions were prepared as in experiment 1, and identical sourness and astringency standards were used. The composition of samples used in experiment 2 is shown in Table 1. The actual solution pH was measured with a standard pH meter (Corning Model 130), while titratable acidity was determined by a standard titration method (Ough and Amerine, 1988).

Protocol

Panelists were initially trained to rate sourness on a 10.2 cm unstructured line scale anchored by deionized water and high sourness standard, and then to rate astringency on the same scale anchored by deionized water and the high astringency standard. Astringency was defined as the friction or drying sensation felt in the (alum) astringency standard. Judges were then trained to rate both sourness and astringency of single samples on paired line scales. Panelists were instructed to ingest the sample, swirl it in the mouth until sourness attained maximum intensity, score the sourness and expectorate. Astringency was then rated post-expectoration, when maximum intensity had been 🤶 reached. All samples were presented at room temperature in  $\varsigma$ randomly coded 2 oz plastic cups. Judges were instructed to rinse three times with deionized water between all standards and samples.

Test samples were rated for sourness and astringency intensity during the final 2 days of the training session. For experiment 1, a set of nine samples plus the accompanying > standards presented in a single session represented one complete replication for a particular organic acid. The first replications for each of the four acids assayed were presented during the first week of testing, while the second replications were presented during the following week; the presentation order of the different acids was varied between the replications. All orders of presentation of samples within a session were balanced for the first order carry-over effect using Williams Latin Squares (Schlich, 1993). For experiment 2, a set of all 12 binary acid samples plus the accompanying standards presented in a single session represented one complete replication. The first replication was presented on the first day of testing, while the second replication was presented the following day; the presentation order was varied between the replications for each judge. All testing was performed in individual tasting booths under red lighting to eliminate distractions.

Table 1 Composition, pH, titratable acidity (expressed as q/l tartaric acid), buffer capacity (mM/l/pH), and mean intensity of sourness and astringency of anion pair solutions

Anion pair (HIGH/low)	High acid (g/l)	Low acid (g/l)	рН	Titratable acidity	Buffer capacity	Sourness $(n = 14Ss \times$	Astringency 2 r)
LAC/mal	1.000	0.600	2.720	1.50	8.11	4.9 <sup>a</sup>	6.9ª
MAL/lac	1.005	0.455	2.733	1.50	8.07	4.9 <sup>a</sup>	6.2 <sup>a</sup>
LAC/tar	1.095	0.595	2.655	1.60	9.66	5.1 <sup>a</sup>	6.7 <sup>a</sup>
TAR/lac	1.095	0.495	2.623	1.50	10.93	4.5 <sup>a</sup>	6.4 <sup>a</sup>
LAC/cit	1.100	0.503	2.695	1.50	8.35	5.0ª	7.0 <sup>a</sup>
CIT/lac	1.000	0.400	2.716	1.50	8.31	4.0 <sup>b</sup>	6.3ª
MAL/tar	0.850	0.550	2.698	1.50	9.33	4.3 <sup>a</sup>	6.1ª
TAR/mal	0.975	0.470	2.652	1.50	10.51	4.5 <sup>a</sup>	6.7ª
MAL/cit	0.990	0.340	2.740	1.40	8.10	4.1 <sup>a</sup>	6.0 <sup>a</sup>
CIT/mal	0.970	0.330	2.740	1.50	8.17	3.9 <sup>a</sup>	6.0 <sup>a</sup>
TAR/cit	0.800	0.600	2.650	1.60	10.35	4.6 <sup>a</sup>	6.1 <sup>a</sup>
CIT/tar	0.985	0.349	2.688	1.50	9.22	3.9 <sup>a</sup>	6.3 <sup>a</sup>

Significant differences in intensity within pairs (P < 0.05) are denoted by different letters. The higher concentration acid is indicated in capital letters; the lower concentration acid in lower case letters, where lac = lactic, mal = malic, tar = tartaric and cit = citric acid.

#### Data analysis

Statistical analyses were performed using the SAS® System for Windows™, Version 6.10 (SAS Institute Inc., Cary, NC). Mixed-model analyses of variance (ANOVAs) with judges treated as random effects were performed on sourness and astringency ratings for experiment 1; for experiment 2, a modification of this analysis which nested samples in matched anion pair groups was employed. Mean sourness and astringency ratings of the samples were compared using Fisher's Least Significant Difference (LSD) test in experiment 1, while for experiment 2 a contrast analysis was employed to test differences in sourness and astringency between matched anion pairs.

For both studies, induced salivary flow values were analyzed by fixed-model ANOVAs to permit classification into three salivary flow groups (low, medium and high) such that all judges within a flow group differed significantly from those in other salivary classes. To examine the influence of judge gender, salivary flow rate and PROP taster status on the perception of sourness and astringency in the samples, a mixed-model ANOVA with judges nested in groups was used.

#### Results

# Effect of pH and normality (experiment 1)

For each acid, highly significant differences in sourness [F(8,104) = 80.02-90.73] and astringency [F(8,104) =19.46–34.02] were found among the samples (P < 0.001).

The results for each acid showed the same trends, as illustrated in Figure 1. At each concentration level sourness intensity increased with decreasing pH, while at each pH level sourness increased with increasing acid concentration (expressed as normality) (Figure 1, left), consistent with all previous studies.

For each of the four acids studied, decreasing the pH of the solutions resulted in significant increases in astringency intensity at each of three concentration levels (Figure 1, right). In contrast, increasing the organic acid concentration under conditions of constant pH had no effect on astringency intensity for any of the acids tested.

# Effect of anion (experiment 2)

From a mixed-model ANOVA of sourness and astringency ratings for the binary solutions with equal pH and titratable acidity no significant differences in either attribute were found among the samples nested as binary pairs [sourness, F(6,78) = 2.11; astringency, F(6,78) = 1.68]. Using contrast statements to directly compare sourness and astringency differences for each binary pair, only one significant difference was found (P < 0.01) (Table 1). For the lacticcitric pair, the sample with lactic acid as the major anion and citric as the minor one was rated 20% more sour than the sample in which citric acid was the dominant anion, consistent with previous results (Noble et al., 1986). For the anions compared in experiment 2, no differences in astringency due to the specific anion was observed.

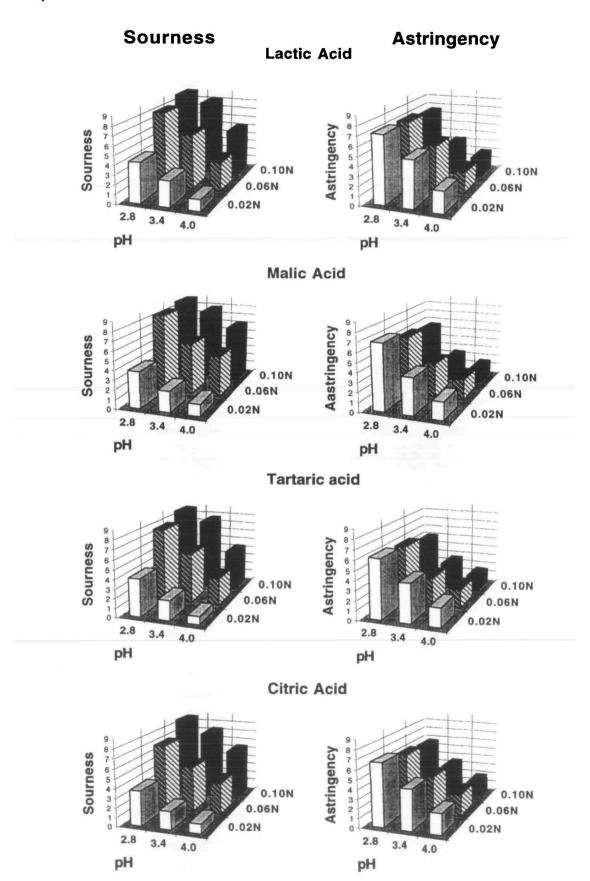


Figure 1 Mean sourness (left) and astringency (right) intensity ratings for acid solutions. Least significant difference for sourness and astringency (P < 0.05), respectively for lactic = 0.86 and 1.02; malic = 0.85 and 1.01; tartaric = 0.81 and 0.96; citric = 0.78 and 1.11 (n = 14 judges  $\times$  2 replications).

### Effect of gender, PROP and salivary flow status (experiments 1 and 2)

Based on an ANOVA of their saliva flow weights, five judges were assigned to each salivary flow group. In experiment 1, for each acid the intensity ratings of sourness did not differ significantly among flow groups [sourness, F(2,11) = 0.33-2.131. A significant difference in astringency ratings among flow groups was observed for tartaric acid only [F(2,11) = 4.19, P < 0.05). Medium-flow judges scored tartaric acid astringency lower (3.06) than the high- (4.42) or low-flow (3.21) judges, suggesting that this significant effect arose from differences in scale usage by judges in these groups, rather than as a function of salivary flow rate. Further, no differences in astringency perception due to salivary flow status were found for the other acids [F(2,11) =0.60 - 0.91].

In addition, no significant differences were found between the nine PROP tasters and five non-tasters for sourness [F(1,12) = 0.01-3.06] or astringency [F(1,12) = 0.01-1.28]. Similarly, no effect of gender was found: sourness, F(1,12) =0.13-0.49; astringency, F(1,12) = 0.03-4.56.

In experiment 2, no significant differences in sourness or astringency were found as a function of salivary flow rate, PROP taster status or gender of the panelists.

#### Discussion

For each acid, there was a significant decrease in the mean sourness intensity of the samples as pH was raised or normality (N) decreased, consistent with previous studies (Harvey 1920; Norris et al., 1984; Ganzevles and Kroeze, 1987, 1988). More significantly, the current investigation is the first to demonstrate that astringency of organic acids is solely a function of pH. Neither the specific anion nor acid concentration, whether expressed as total acidity (N) or titratable acidity (potentially dissociable protons), affects astringency intensity. In previous studies, pH was decreased by addition of acid, resulting in a simultaneous change in total and titratable acid concentration (Guinard et al., 1986; Fischer et al., 1993; Corrigan Thomas and Lawless, 1995; Kallithraka et al., 1997). In previous reports of the association of pH and astringency (Hartwig and McDaniel, 1995; Lawless et al., 1996), although organic acid solutions were varied in pH under conditions of constant acid concentration, the role of variation in acid level per se was not examined

A possible explanation for the inverse relationship between pH and astringency may be the reduction in salivary viscosity (Nordbö et al., 1984b) upon rapid alteration of salivary proteins under conditions of reduced pH (Nordbö et al., 1984a). Flavanoid phenols or tannins, the astringent compounds in wine, fruit and tea, have a strong affinity for the salivary proline-rich proteins (PRP) (Hagerman and Butler, 1981), resulting in precipitation of polyphenol-PRP complexes. As salivary PRPs bind with

phenolics or are modified at lower pH, the rheological properties of saliva are altered and the viscosity decreases (Nordbö et al., 1984b; Luck et al., 1994). The increase in astringency intensity perceived in response to polyphenolic compounds or decreases in pH may thus directly result from the decrease in effectiveness of lubrication and corresponding increase in oral friction or astringency, as speculated by Green (1993) and Smith (1996). Consistent with this hypothesis, astringency intensity of tannin solutions was significantly reduced when viscosity was increased (Smith et al., 1996), perhaps as a result of restoration of lubrication.

The role of acid in increasing the astringency of phenols may also occur by the same mechanism. Astringency of tannins or tannic acid (a polymer of gallic acid) in model solutions or wines was increased upon addition of acid (Guinard et al., 1986; Fischer et al., 1993; Kallithraka et al., 1997. Fischer (1990) proposed that the enhancement of astringency upon the addition of acid was a function of the decrease in pH. The concentrations of the charged phenolate ions, which are unable to hydrogen bond with proteins, are reduced at lower pH values.

A different mechanism for astringency may be involved for aluminum sulfate. Astringency of alum was recently demonstrated to decrease upon the addition of acid and the lowering of the pH, in contrast to the increase in astringency of acids at lower pH and of phenolics upon acidification (Peleg et al., 1997). It was speculated that the observed reduction in astringency of alum-acid mixtures results from the complexation of the charged aluminum ions by the acid anions, although no difference in suppression of astringency was observed when acids varying in chelation effectiveness were tested. Thus, although it is possible that reduction in salivary viscosity could be affected by the sequestration of free salivary Ca2+ cations by the organic acid anions (Nordbö et al., 1984b), the absence of an anion effect suggests this is not likely.

As salivary flow rate increases in response to stimulation, total protein levels, bicarbonate content, buffer capacity and pH rise (Funakoshi and Kawamura, 1967; Dawes, 1967; Benedek-Spät, 1973; Norris et al., 1984; Inomata and Kurahashi, 1987; Watanabe and Dawes, 1988). Hence it has been speculated that individuals with low salivary flows may perceive sourness or astringency differently than subjects with high flow rates. In the present study there was no effect of salivary flow status on sourness and astringency perception. Although these findings contradict previous studies which demonstrated an inverse relationship between salivary flow rate and perceived intensity of sourness (Norris et al., 1984; Christensen et al., 1987) and astringency (Fischer et al., 1994; Ishikawa and Noble, 1995), they are in agreement with other investigations which found no association between salivary flow rate and sourness (Bonnans and Noble, 1995; Bodine, 1996; Peleg et al., 1997) or astringency (Smith et al., 1996; Peleg et al., 1997). The

inconsistent reports of the effect of salivary flow on sensory perception of sourness or astringency may arise from lack of power in previous experiments. To detect an effect with a small size, the power of the test must be improved by testing a larger number of subjects.

Several studies have noted that PROP supertasters perceive increased intensity for several modalities, including irritation relative to non-tasters (Karrer and Bartoshuk, 1991; Bartoshuk et al., 1994). In the current investigation, no significant difference between PROP tasters and non-tasters was found for either sourness or astringency, consistent with previous reports (Fischer et al., 1994; Bonnans and Noble, 1995; Smith et al., 1996; Peleg et al., 1997). The failure of the present study to classify PROP tasters into super-tasters and 'just tasters' may account for the lack of a PROP effect on either sensation.

#### Conclusion

Lowering pH, while holding the acid concentration constant, resulted in significant increases in intensity of astringency and sourness for each of the organic acids tested. Differential responses were observed for sourness and astringency in response to varying total acidity. Increasing the acid level failed to affect astringency intensity, although it produced a significant increase in sourness. Therefore, astringency of aqueous acid solutions is a function solely of the hydrogen ion concentration.

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