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Dose-response functions and methodological insights for sensory tests with astringent stimuli

Running title: Methodological insights for astringency

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Abstract

Sensations such as bitterness and astringency can limit the acceptance of many purportedly healthy foods. The purpose of this study was to investigate dose-response relationships of various astringent and bitter stimuli in a beverage, and to simultaneously gain additional methodological insight for the effects of wording, repeated tasting, and beverage matrix on these sensations. Untrained participants were presented with samples of a “flavored beverage” or water containing various concentrations of four stimuli (alum, malic acid, tannic acid, and quinine) and were asked to rate intensities of tastes (bitterness, sourness, and sweetness) and astringency sub-qualities (roughing, drying, and constricting or puckering) using a generalized visual analog scale. Using constricting in place of puckering had no effect on ratings. The effects of repeated tasting and beverage matrix on astringency perception were stimulus-dependent. This study informs future investigations to understand the psychophysics of tastes and astringency.

Practical Applications

This study provides stimulus- and quality-specific data to improve astringency research. Furthermore, dose response functions will aid researchers when selecting appropriate concentrations of astringent stimuli. We also provide recommendations for a variety of testing contexts, such as beverage matrix and the number of samples, to optimize the design of astringency studies, especially for naïve participants. This study further demonstrates how affective responses influence evaluation of astringent samples among untrained participants.

Keywords: Astringency, beverage matrix, alum, tannic acid, astringent sub-qualities

1. Introduction

Astringency is a commonly misunderstood sensation (Bajec & Pickering, 2008). By definition, astringency is "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins," (ASTM, 1991), and so encompasses multiple sensations and various classes of compounds. Although alum is commonly recommended as an astringent standard (Lee & Lawless, 1991), tannins are much more common dietary sources of astringency. However, astringent compounds exhibit different sensory profiles at different concentrations for both astringent sub-qualities (e.g. drying, roughing, and puckering) and side tastes (bitterness, sweetness, and sourness) (Fleming, Ziegler, & Hayes, 2015, 2016). In addition to complexities introduced by multiple classes of astringent stimuli and diverse sensory characteristics, divergent food and beverage matrix interactions also complicate definition of a single astringent standard. For instance, the presence of acid increases astringency perception in polyphenols while decreasing that of alum (Peleg, Bodine, & Noble, 1998). Furthermore, confusion identifying astringency and its sub-qualities, especially among naïve participants, presents additional challenges: similar ratings for sourness, astringency, and puckering (a common astringency descriptor), by untrained assessors suggest possible confusion identifying and differentiating astringent sub-qualities and side tastes (Duffy et al., 2016; Fleming et al., 2016). The fatiguing nature of astringent samples introduces additional challenges for astringency research. Due to such intricacies, some have suggested the study of individual sub-qualities, rather than astringency as a whole, as a more appropriate research approach (Lawless & Corrigan, 1994).

As bitterness and astringency are characteristic sensations of polyphenols and other bioactive plant compounds (reviewed in Bajec & Pickering, 2008), study of these sensations may inform strategies to promote consumption of functional foods. Indeed, polyphenols and polyphenol-enriched products have numerous reported health benefits (Auger et al., 2005; Landrault et al., 2003; Pandey & Rizvi, 2009). Despite their health-promoting properties, polyphenol acceptance is limited by characteristic bitterness and astringency (Duffy et al., 2016; Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009; Lesschaeve & Noble, 2005).

Given the complexities of astringency research, the objectives of this study were to, 1) establish dose-response functions for various classes of astringent stimuli in a model beverage, 2) determine the influence of replacing the astringent sub-quality descriptor "puckering" with "constricting", 3) observe the effect of repeated tastings of bitter and/or astringent stimuli on participant responses, and 4) determine the effect of the beverage matrix on perception of astringency for selected stimuli.

2. Methods

2.1 Study participants and procedures

Healthy participants (n=57, 30 female, 27 male, 0 other, age range 19-42, average age 26) were recruited from Purdue University and the surrounding community. Participant exclusion criteria included known smell or taste issues; tongue, lip, and/or check piercings; over age 45; and smoking within the last 30 days. Purdue University's Institutional Review Board for Human Subjects Research approved all recruiting and testing procedures; this review board approved the study as exempt under category 6, testing of foods and food ingredients. Participants were compensated for their time. Using iPad mini 2s (Apple, Cupertino, CA) with RedJade software (Curion, Redwood City, CA), participants viewed and accepted an electronic informed consent, provided demographic information, and completed a warm-up exercise to familiarize them with the generalized visual analog scale (gVAS). The inset scale (entire range from -10 to 110) was anchored by "none" (defined on the initial instructions screen as, "you did not experience any of this sensation at all from the product") at 0 and "strongest ever" (defined as "strongest sensation you have ever experienced") at 100. The warm-up exercise asked participants to rate remembered or imagined sensation intensity for the brightness of this room, the brightness of the sun on a clear day, the loudness of a shout, the loudness of a whisper, the sweetness of pure sugar, and the bitterness of black coffee. To verify that participants were reading directions and understood how to use the scale, responses were checked to ensure "the brightness of this room" was rated lower than "the brightness of the sun on a clear day" and "the loudness of a whisper" was rated lower than "the loudness of a shout." Two participants failed this check both days, and so were removed from the dataset (final n=55, 29 female, 26 male, 0 other). Three additional participants failed this check only one day, thus only a single day of responses from these participants were removed.

2.2 Stimuli

Stimuli representing both bitterness (quinine monohydrochloride dihydrate, "quinine", Sigma-Aldrich, St. Louis, MO; and tannic acid, Sigma-Aldrich) and the three broad classes of astringent compounds (aluminum sulfate, "alum"; malic acid, Milliard Brands, Lakewood, NJ; and tannic acid) were chosen and evaluated at three concentrations in a flavored beverage (Table 1). Flavored beverage background included sucrose (6.0 % w/w), imitation almond flavor (0.2 mL/1000g, approximately 0.02 % w/w; McCormick & Company, Hunt Valley, MD), and food coloring (red 0.227%, blue 0.026 % w/w; General Mills Inc., Minneapolis, MN). High and low stimuli concentrations were determined based on existing literature and extensive benchtop testing in an effort to match sensory intensity across the high and low concentrations of each compound. Intermediate concentrations were then determined as the logarithmic midpoint between high and low concentrations for each stimuli. To assess the influence of the beverage flavors on astringency perception, alum and tannic acid in water alone were included in the sample set (only two water-based comparisons were included to minimize the number of tested samples; tannic acid and alum were selected as commonly studied astringents). The "flavored beverage" solution with no stimuli was also included.

As the term “puckering” could be confused with sour taste, we tested the hypothesis that “constricting” could be used in place of “puckering.” The entire sample set was thus evaluated on two testing days, where the only difference was the descriptor name (see Supplemental Table 1 for group sample sizes and characteristics across days). The order of these two days was randomly assigned to participants. Fifteen participants attended only one day or failed the warm-up exercise on a single day; as the statistical code can account for missing values without any further adjustments, their data remains in the final analysis. During check-in, participants were given a verbal overview of the study procedures, namely to pour the entire sample (10 mL) in their mouth, hold and swish it for 10 seconds, swallow the sample, and then rinse with water. Participants were told they could swallow or spit the rinse water. These instructions were also provided on-screen for each sample. A two-minute inter-stimulus interval was enforced using an on-screen timer. Participants evaluated samples in a counter-balanced order using the gVAS for three side-tastes (sweetness, sourness, and bitterness, presented in a randomized order between subjects) and three astringent sub-qualities (drying, roughing, and puckering/constricting, presented in a randomized order between subjects). Each screen contained a reminder of scale usage: “Remember, 'Strongest Ever' is the strongest sensation of any kind that you have ever experienced.” Descriptions for each of the astringent sub-qualities were provided on-screen for every sample, based on existing definitions (Lawless & Corrigan, 1994; Lee & Lawless, 1991) but slightly modified to simplify wording. Drying was defined as, “A lack of moistness or lubrication that causes a feeling of friction between mouth surfaces;” roughing as, “An un-smooth or bumpy texture comparable to sandpaper;” and puckering or constricting as, “A tightening, shrinking, or pulling feeling in the mouth, lips, and/or cheeks.”

2.3 Statistical analysis

Data was analyzed using SAS 9.4 using the mixed procedure to generate linear mixed models. Participant was identified as a repeated measure using the autoregressive covariance structure and the Kenward-Roger approximation for denominator degrees of freedom. Data was sorted in the following order: quality, stimuli, participant ID, day, order. Analyses were run for each stimuli/quality pair for a total of 24 analyses. Terms where $p < 0.05$ using Type 3 tests of fixed effects were considered significant.

The initial dose-response model included Concentration, Wording (puckering vs. constricting), Day, and Order of tasting as predictors of sensory rating (Model 1). Residuals were analyzed and observed to be not identically distributed, so data were transformed by square root of each response and $\log_{10}$ of concentration. Negative values were replaced by zero to accommodate the square root transformation. Wording was found to be not significant, so it was dropped from the model, and puckering/constricting ratings were combined for all analyses. Statistically significant two-way interactions were retained in the model, resulting in Model 2 for final analyses. To determine differences among the three astringent sub-qualities within each sample, additional post-hoc analyses were conducted by adding sub-quality as an additional term in the model (Model 3). Sample means for each sub-quality were compared following a Tukey-Kramer adjustment. Comparisons where $p < 0.05$ were considered significant. To understand the effect of the flavored beverage on ratings, a similar model was used to compare sample means of alum and tannic acid against the respective water control (Model 4). A summary of the models is shown in Table 2.

3. Results and discussion

In this study, we established dose response functions for three astringent stimuli and quinine in a model flavored beverage (Table 3, Supplemental Tables 1 and 2). Astringency perception, as measured by drying, roughing, and puckering/constricting, increased with concentration in each tested stimuli. Perception of side-tastes was also altered by increasing concentration of astringent stimuli: bitterness and sourness perception increased, while sweetness perception decreased with concentration of astringent. Furthermore, we found that the use of “constricting” in place of “puckering,” when paired with the same definition, did not affect participant ratings (Figure 1). Repeated tasting of the samples influenced astringency ratings in alum and malic acid, but not tannic acid. Compared to water, the use of a flavored beverage blunted astringency ratings in tannic acid, but not alum (Figure 2). These findings are described in detail below.

3.1 Effect of stimuli concentration on sensory ratings

The effect of each factor on participant response (Model 2) is shown in Table 3. As expected, ratings for all astringent sub-qualities increased with concentration for alum, malic acid, and tannic acid. Interestingly, perception of astringency increased with quinine concentration as well. We detected a significant difference between each sub-quality for each astringent stimuli, contrasting others’ conclusions that the terms “drying” and “roughing” are redundant (Fleming, Ziegler, & Hayes, 2016). Whether the size of the difference is relevant to participant perception is an area for further research. For both alum and tannic acid samples, drying was rated as the most intense sub-quality, while puckering/constricting followed by drying was the most intense for malic acid samples. Others have documented similar relative intensity of astringent sub-qualities among the same astringent compounds (Fleming, Ziegler, & Hayes, 2015; Fleming et al., 2016). Differences in characteristic side tastes associated with classes of astringent stimuli, such as the bitterness of polyphenols or sourness of acids, may partially explain variation in sub-quality perception.

Increasing stimuli concentration significantly increased bitterness and sourness perception and decreased sweetness perception in all tested stimuli. Although the increase in bitterness ratings for quinine and tannic acid samples is in harmony with observations in pure solutions (Fleming et al., 2016; Keast & Roper, 2007), the association of bitterness with alum is inconsistent. Using untrained participants, others have detected a dose-dependent increase in bitterness with alum concentration, bitterness clustering closer to astringency relative to other side tastes, and frequent (46%) endorsement of “bitter” for alum samples in a CATA design (Fleming et al., 2015, 2016). The lack of participant training both in our study and others’ may partially explain observations of bitterness-alum associations, as bitterness and astringency are often confused (Lea & Arnold, 1978; Lee & Lawless, 1991). When trained or semi-trained participants evaluate samples, bitterness is less frequently associated with alum (Brannan, Setser, & Kemp, 2001; Lim & Lawless, 2005). Because the association of alum and bitterness occurs more often in untrained participants, a similar affective response (i.e., dislike) rather than increased stimulation likely explains the correlation, as suggested by others (Fleming et al., 2016). As further support of affective influence among untrained participants, we observed that astringency ratings increased with quinine concentration, despite the lack of known quinine astringency. Similarly, sourness perception increased with stimuli concentration. Confusion among untrained participants regarding sourness and other unpleasant sensations such as bitterness and astringency has been observed by others (Melis et al., 2017). Due to potential misunderstanding of sensory descriptors, non-verbal methods, such as sorting or polarized-sensory position (Varela & Ares, 2012), may be better suited to distinguish astringency and bitterness when using untrained participants. Such methods allow participants to evaluate similarity of samples and standards without the potential biasing effect of descriptors.

Our observation of decreased sweetness perception with increasing concentration of bitter (tannic acid, quinine) and sour stimuli (malic acid) is consistent with the well-established phenomenon of mixture suppression (Keast & Breslin, 2003; Mennella, Reed, Mathew, Roberts, & Mansfield, 2015). We also observed a decrease in sweetness perception with increasing alum concentration; while some researchers have associated a subtle sweet taste with alum (Breslin, Gilmore, Beauchamp, & Green, 1993; Fleming et al., 2016), others have not (Brannan et al., 2001). Given the limitations of this study, such as untrained participants and fatiguing samples, our results are insufficient to support conclusions regarding the sweet taste of alum.

Participant responses were generally lower on the second day of testing than on the first. The difference in ratings may be partially explained by the high number of participants that had no previous experience in sensory evaluation, or perhaps more specifically, no experience in evaluation of astringent samples like the ones in our study. After experiencing the full range of intensities of the sample set, it is possible that participants adjusted their use of the scale, as they had now experienced these sensations and thus the context of "strongest ever" had shifted. Dose response equations from Day 1 may be more appropriate when predicting responses from participants with no prior sample experience, whereas blunted responses may be expected from more experienced or repeat participants. The linear relationships between the $\log_{10}$ of stimuli concentration and the square root for each response (three side-tastes and three sub-qualities) for each day of testing are displayed in Supplemental Tables 1 and 2.

3.2 No effect of "constricting" in place of "puckering" on sensory ratings.

To clarify potential misunderstanding and misreporting of astringent sensations, we tested whether "constricting" could be used in place of "puckering" to describe the same sub-quality. Untrained participants may confuse sourness with astringency, as suggested by similar ratings given in aronia berry juice samples (Duffy et al., 2016). Using “puckering” to describe astringency may add further confusion, as untrained participants rate puckering intermediate to sourness and astringency (Fleming et al., 2016). Although lexicons have been developed to describe wine astringency, naïve consumers have difficulty relating to complex definitions (Vidal, Gimenez, Medina, Boido, & Ares, 2015).

In the current work, using “constricting” in place of “puckering” had no effect on participant ratings (Figure 1). Due to the similarity of the means, we suspect that higher-powered analyses would also fail to detect a difference. However, in our study the definitions for astringent sub-qualities were given on every screen. It is possible that different behavior could be observed if the definition were not always available to participants. Because puckering is considered a primary descriptor of astringency (Fleming et al., 2016), evaluating this sub-quality is important for future astringency research. Whether the use of constricting in place of puckering clarifies potential confusion between astringency and sourness remains to be determined, as this study was not designed to determine the effect of wording on sourness ratings.

3.3 Effect of repeated tasting on sensory ratings

Because testing fatigue influences astringency perception, we investigated the effect of repeat tastings on sub-quality and side taste ratings. Although others have noted that the duration of astringency perception increases with repeated ingestion (Guinard, Pangborn, & Lewis, 1986), specific evidence regarding sub-qualities and side tastes is sparse. Additionally, reports of astringency duration are varied, as some studies report astringency six minutes post ingestion (Lee & Lawless, 1991), while others show a return close to basal levels in less than two minutes (Fischer, Boulton, & Noble, 1994; Guinard et al., 1986; Valentova, Skrovankova, Panovska, & Pokorny, 2002).

In this study, repeated tasting of astringent and/or bitter samples (tested through the factor “order”; Table 3) significantly increased astringency ratings in alum and malic acid samples, but not in tannic acid samples. Repeated tasting also decreased bitterness and sweetness perception in tannic acid and malic acid, respectively, and increased sourness perception in malic acid samples. Our failure to detect an order effect among astringency qualities in tannic acid was unexpected, as increased astringency intensity following repeated tasting has been observed by others (Guinard et al., 1986; Lyman & Green, 1990). Although some have observed that sucrose decreases tannic-acid induced astringency order effects (Lyman & Green, 1990), others have detected similar rates of order-induced astringency in soy milk samples with and without sucrose (polyphenol content is thought to contribute to soy milk astringency) (Courregelongue, Schlich, & Noble, 1999). Due to limited data specific to order effects, the influence of sucrose on overall astringency perception may further explain observed differences among tested stimuli, as discussed in the subsequent paragraph. Taken together, these results demonstrate that the effect of repeated tastings on astringency perception is quality- and stimulus-dependent.

3.4 Influence of beverage matrix on sensory ratings

Various beverage matrix components, such as sweetness, polysaccharides, ethanol, and polyphenols, influence astringency perception (reviewed in Ma et al., 2014; Soares, Brandao, Mateus, & de Freitas, 2017). However, beverage matrix components do not influence astringency equally among different classes of astringent stimuli, as acid increases the potency of tannic acid while decreasing that of alum (Peleg, Bodine, & Noble, 1998). In our study, we assessed the influence of beverage matrix on astringency perception by comparing alum and tannic acid samples with their respective water-only controls (Figure 2, Model 4). In both alum and tannic acid, the presence of the beverage matrix increased sweetness ratings, as expected. Compared to water, the flavored beverage matrix lowered astringency and bitterness ratings in tannic acid, but did not reach statistical significance in alum. The lack of statistical difference in bitterness of alum samples is likely explained by lower initial ratings. Similarly, differences in astringency ratings in tannic acid, but not alum, may be explained by the greater change in affective response due to differences in bitterness perception. Although sucrose can decrease astringency perception of tannic acid and other polyphenol-containing beverages (Courregelongue et al., 1999; Duffy et al., 2016; Ishikawa & Noble, 1995; Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009), further research is needed to understand whether the phenomenon is specific to polyphenols or pertains to astringency in general, as other classes of astringent compounds were not evaluated in these studies. Different effects of alum and tannic acid on salivary flow and viscosity may also account for our observed differences, as both factors have documented effects on astringency perception (Lyman & Green, 1990; Smith, June, & Noble, 1996). Furthermore, whether sucrose alters the well-studied tannin-salivary protein interaction, a common hypothesis to explain astringency perception (reviewed in (Soares, Brandao, Mateus, & de Freitas, 2017), also remains to be determined. Whether altered sensory perception or differences in hedonic response play a greater role in altering matrix-induced changes in astringency perception is an area for further research. These observations highlight that the effect of the food matrix on astringency perception is stimulus-dependent, in agreement with others' conclusions (Peleg et al., 1998).

4. Conclusion

In this study, we found that the relative perceived intensity of astringent sub-qualities and the effect of beverage matrix on astringency ratings were stimulus-dependent. Additionally, we provide stimuli- and quality-specific measures of how repeated tastings of bitter and astringent samples influences untrained participant responses. Although the use of untrained participants limits interpretation of results, such as whether observed effects were due to changes in actual sensory perception or biased by hedonics, it also provides meaningful context for application of the findings. However, conclusions regarding order effects have greater implications for future sensory testing rather than the consumer experience; although people often taste beverages through multiple sips, the requirement to rinse, wait, and evaluate a different beverage is not representative of most consumption experiences. Furthermore, whether similar order effects would be observed with an alternate number of tastings cannot be determined with the present data, as the study was not powered to prescribe the ideal sample set size. Additional studies are needed to determine whether differences induced by repeated sampling and beverage ingredients among tested stimuli are observed in other food matrices. Given our observed differences among stimuli, we advise against the use of single astringent standard if attempting to introduce a naïve participant to the concept of “astringency.” Product developers and sensory researchers should consider the class of the astringent compound, the sensation of interest, and the food matrix when studying astringency perception. Taken together, these data agree with prior work supporting stimuli- and sub-quality specific aspects of astringency.

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Tables

Table 1. Concentration of test stimuli at low, medium, and high concentrations.

Stimuli	% w/w	Background
Alum	0.0268	6.0% sucrose, flavor extract, color
Alum	0.0847	
Alum	0.2676	
Malic acid	0.0865	
Malic acid	0.2019	
Malic acid	0.4808	
Tannic acid	0.0488	
Tannic acid	0.1073	
Tannic acid	0.2439	
Quinine	0.0007	
Quinine	0.0024	
Quinine	0.0075	
None	N/A	
Alum	0.2676	Water
Tannic acid	0.2439	

Table 2. Statistical models.

Model	Response variable	Predictor variables
Model. 1: Original model	Rating	Wording, Concentration, Day, Order
Model. 2: Final model	sqrt(Rating)	$\log_{10}$(Concentration), Order, Day, $\log_{10}$(Concentration)*Day, Order*Day
Model. 3: Comparison of astringent sub-qualities	sqrt(Rating)	Quality, $\log_{10}$(Concentration), Order, Day, $\log_{10}$(Concentration)*Day, Order*Day
Model. 4: Effect of beverage flavors	sqrt(Rating)	Sample, Order, Day, Sample*Order, Day*Order

Table 3. Effects (p-values below) of each factor on participant response.

Stimuli	Quality[1]	Intercept (β0)	LogConc (β1)	Order (β2)	Day (β3)	LogConc* Day (β4)	Order* Day (β5)
Alum	Drying[a]	3.92	**2.88***	**0.12***	**1.93***	0.58	**-0.14***
			<.0001	***0.0450***	***0.0003***	*0.2180*	***0.0135***
Alum	Roughing[b]	3.04	**2.53***	**0.11***	0.41	-0.12	-0.05
			<.0001	***0.0032***	*0.4755*	*0.8011*	*0.3573*
Alum	Puckering/Constricting[c]	3.61	**2.43***	0.07	**1.14***	**1.12***	-0.06
			<.0001	*0.0792*	***0.0429***	***0.0215***	*0.3264*
Alum	Bitterness	3.04	**3.35***	0.06	0.57	-0.08	-0.06
			<.0001	*0.3061*	*0.2805*	*0.8836*	*0.2573*
Alum	Sweetness	5.12	**-1.14***	0.02	0.69	-0.11	-0.03
			<.0001	*0.9185*	*0.1267*	*0.7859*	*0.5231*
Alum	Sourness	2.87	**2.79***	0.05	0.87	-0.19	-0.07
			<.0001	*0.4115*	*0.0976*	*0.6704*	*0.2306*
Malic acid	Drying[a]	2.26	**1.72***	0.10	**2.28***	0.24	**-0.14***
			<.0001	*0.3413*	***0.0004***	*0.7309*	***0.0259***
Malic acid	Roughing[b]	1.88	**1.63***	**0.08***	0.81	-0.49	-0.02
			<.0001	***0.0098***	*0.1624*	*0.3938*	*0.7116*
Malic acid	Puckering/Constricting[c]	1.9	**2.34***	**0.18***	**2.28***	**1.42***	**-0.20***
			<.0001	***0.0019***	***<.0001***	***0.0160***	***0.0003***
Malic acid	Bitterness	1.93	**0.68***	0	**1.03***	-0.09	-0.03
			0.0094	*0.4607*	***0.0313***	*0.8533*	*0.5219*
Malic acid	Sweetness	5.24	**-1.35***	**-0.01***	**1.29***	-0.29	-0.09
			<.0001	***0.0096***	***0.0098***	*0.5641*	*0.0518*
Malic acid	Sourness	4.65	**2.89***	**0.04***	-0.05	1.03	0.02
			<.0001	***0.0299***	*0.9251*	*0.0896*	*0.6912*
Tannic acid	Drying[a]	4.51	**3.82***	0.05	**0.82**	0.88	-0.06
			<.0001	*0.6367*	*0.2244*	*0.2762*	*0.4160*
Tannic acid	Roughing[b]	3.66	**3.20***	0.01	-0.17	0.26	0.01
			<.0001	*0.6872*	*0.8234*	*0.7207*	*0.8748*
Tannic acid	Puckering/Constricting[c]	3.45	**3.70***	0.05	**1.69***	**1.59***	-0.11
			<.0001	*0.8218*	***0.0152***	***0.0234***	*0.1524*
Tannic acid	Bitterness	4.08	**5.92***	**-0.05***	0.96	0.93	-0.05
			<.0001	***0.0176***	*0.1003*	*0.1817*	*0.4643*
Tannic acid	Sweetness	5.04	**-2.27***	-0.01	0.52	-0.22	0.01
			<.0001	*0.6548*	*0.3301*	*0.6716*	*0.9239*
Tannic acid	Sourness	2.47	**2.49***	-0.02	0.65	0.4	0
			<.0001	*0.6263*	*0.2664*	*0.5150*	*0.9735*
Quinine	Drying[a]	3.55	**0.56***	0.04	**2.07***	0.67	0
			<.0001	*0.1359*	***0.0240***	*0.1340*	*0.9888*
Quinine	Roughing[b]	3.41	**0.78***	0.03	0.78	0.04	-0.01
			0.0002	*0.2499*	*0.3809*	*0.9296*	*0.8628*
Quinine	Puckering/Constricting[ac]	4.73	**1.54***	0.07	0.48	-0.49	-0.04
			<.0001	*0.0908*	*0.6378*	*0.3310*	*0.5753*
Quinine	Bitterness	12.33	**4.57***	0.04	0.83	0.07	0.02
			<.0001	*0.0829*	*0.3511*	*0.8704*	*0.7876*
Quinine	Sweetness	-0.24	**-2.21***	0.09	0.48	-0.57	**-0.14***
			<.0001	*0.4917*	*0.5972*	*0.1952*	***0.0183***
Quinine	Sourness	3.76	**1.08***	0.04	0.88	-0.22	-0.07
			<.0001	*0.7197*	*0.3055*	*0.5959*	*0.1928*

[1]Means of astringent sub-qualities within each stimuli were compared using Model 3; different superscript letters indicate significant differences ($p < 0.05$). Other significant terms are indicated by boldface and *.

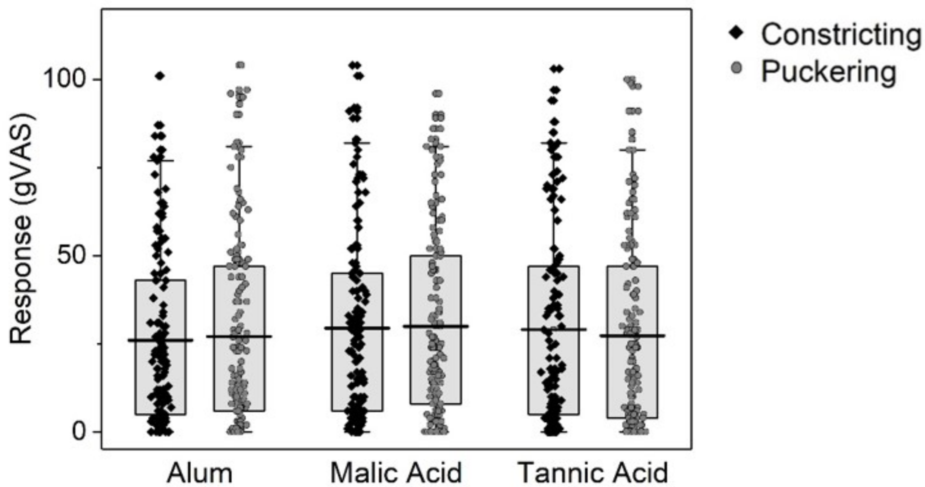


Figure 1. Individual participant ratings for “puckering” and “constricting” for all three concentrations of the three evaluated astringent stimuli. The box represents 50% of responses, whiskers represent 5th and 95th percentiles, and the central line represents the mean.

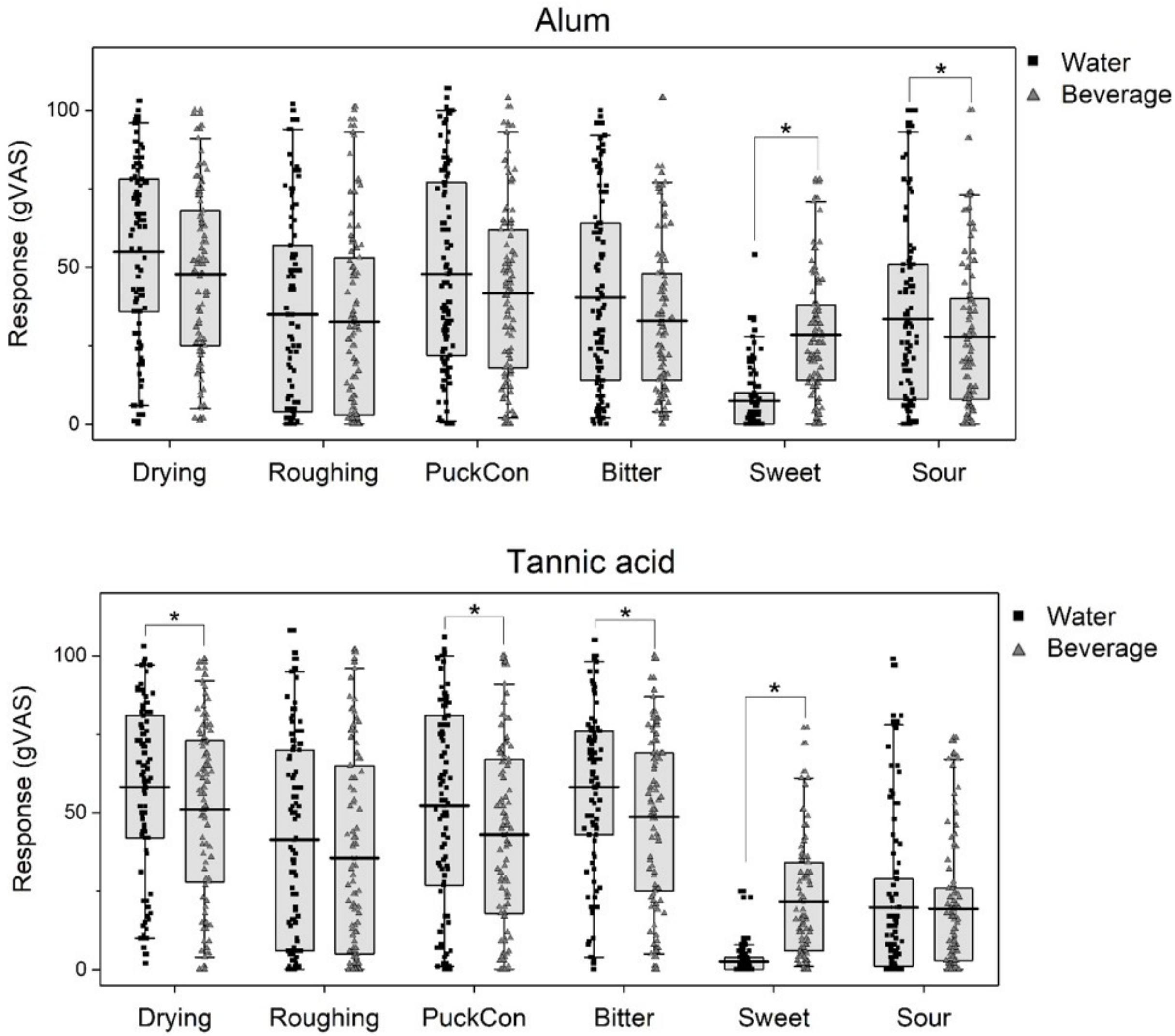
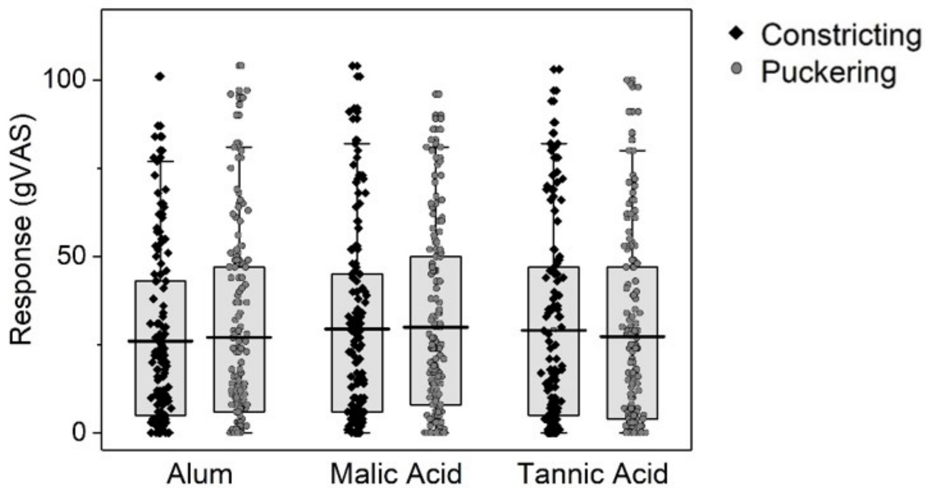


Figure 2. Individual participant ratings for the same concentration of stimuli evaluated in either water or flavored beverage. The box represents 50% of responses, whiskers represent 5th and 95th percentiles, and the central line represents the mean. Significant differences between means (P <0.05) are indicated by *.

Supplemental

Supplemental table 1. Participants



tested, by day and wording presentation.

	Day 1	Day 2
Puckering	27	23
Constricting	23	22

Supplemental table 2. Day 1 dose-response equations.

Stimuli	Quality	Intercept (β0 + β3)	Log_{10}Conc (β1 + β4)	Order (β2 + β5)
Alum	Drying	5.85	3.46	-0.02
Alum	Roughing	3.45	2.42	0.05
Alum	PuckCon	4.75	3.55	0.02
Alum	Bitterness	3.61	3.27	-0.01
Alum	Sweetness	5.81	-1.25	-0.01
Alum	Sourness	3.75	2.60	-0.01
Malic acid	Drying	4.54	1.96	-0.04
Malic acid	Roughing	2.69	1.13	0.05
Malic acid	PuckCon	4.18	3.76	-0.02
Malic acid	Bitterness	2.96	0.59	-0.03
Malic acid	Sweetness	6.53	-1.64	-0.10
Malic acid	Sourness	4.60	3.92	0.06
Tannic acid	Drying	5.32	4.70	-0.01
Tannic acid	Roughing	3.49	3.45	0.02
Tannic acid	PuckCon	5.14	5.29	-0.06
Tannic acid	Bitterness	5.04	6.85	-0.09
Tannic acid	Sweetness	5.56	-2.49	-0.01
Tannic acid	Sourness	3.13	2.89	-0.01
Quinine	Drying	5.63	1.23	0.04
Quinine	Roughing	4.19	0.82	0.02
Quinine	PuckCon	5.21	1.05	0.03
Quinine	Bitterness	13.15	4.64	0.05
Quinine	Sweetness	0.24	-2.78	-0.05
Quinine	Sourness	4.64	0.86	-0.03

Log_{10}Conc = coefficient for log_{10} of concentration, and order = coefficient for sample testing order. Effects for each term were derived from Table 3, where Day = 0 indicates Day 1.

Supplemental table 3. Day 2 dose-response equations.

Stimuli	Quality	Intercept (β0)	LogConc (β1)	Order (β2)
Alum	Drying	3.92	2.88	0.12
Alum	Roughing	3.04	2.53	0.11
Alum	PuckCon	3.61	2.43	0.07
Alum	Bitterness	3.04	3.35	0.06
Alum	Sweetness	5.12	-1.14	0.02
Alum	Sourness	2.87	2.79	0.05
Malic acid	Drying	2.26	1.72	0.10
Malic acid	Roughing	1.88	1.63	0.08
Malic acid	PuckCon	1.90	2.34	0.18
Malic acid	Bitterness	1.93	0.68	0.00
Malic acid	Sweetness	5.24	-1.35	-0.01
Malic acid	Sourness	4.65	2.89	0.04
Tannic acid	Drying	4.51	3.82	0.05
Tannic acid	Roughing	3.66	3.20	0.01
Tannic acid	PuckCon	3.45	3.70	0.05
Tannic acid	Bitterness	4.08	5.92	-0.05
Tannic acid	Sweetness	5.04	-2.27	-0.01
Tannic acid	Sourness	2.47	2.49	-0.02
Quinine	Drying	3.55	0.56	0.04
Quinine	Roughing	3.41	0.78	0.03
Quinine	PuckCon	4.73	1.54	0.07
Quinine	Bitterness	12.33	4.57	0.04
Quinine	Sweetness	-0.24	-2.21	0.09
Quinine	Sourness	3.76	1.08	0.04

$Log_{10}Conc$ = coefficient for log_{10} of concentration, and order = coefficient for sample testing order. Effects for each term were derived from Table 3, where Day = 1 indicates Day 2.