

2018

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

Jonathan C. Kershaw

Cordelia Running

Follow this and additional works at: <https://docs.lib.purdue.edu/foodscipubs>

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries.
Please contact epubs@purdue.edu for additional information.

This is the author copy of an accepted manuscript, posted to the Purdue University Repository after an embargo period as permitted by the publisher.

The published copy can be found at:

[Dose–response functions and methodological insights for sensory tests with astringent stimuli](#)

JC Kershaw, CA Running

Journal of sensory studies 34 (1), e12480

<https://doi.org/10.1111/joss.12480>

1 Dose-response functions and methodological
2 insights for sensory tests with astringent stimuli
3

4 **Running title:** Methodological insights for astringency

5

6 Jonathan C. Kershaw^a

7 Cordelia A. Running^{a*}

8

9 ^aDepartment of Nutrition Science and Department of Food Science, 700 W State St, Purdue
10 University, West Lafayette IN USA

11

12 *Corresponding author: crunning@purdue.edu

13

14

15

16

17

18

19 **Abstract**

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

31

32 **Practical Applications**

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

39

40

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

42 **1. Introduction**

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

62

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

70

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

76

77 **2. Methods**

78 *2.1 Study participants and procedures*

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

99

100 *2.2 Stimuli*

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

115

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

137

138 *2.3 Statistical analysis*

139 Data was analyzed using SAS 9.4 using the mixed procedure to generate linear mixed models. Participant
140 was identified as a repeated measure using the autoregressive covariance structure and the Kenward-
141 Roger approximation for denominator degrees of freedom. Data was sorted in the following order:
142 quality, stimuli, participant ID, day, order. Analyses were run for each stimuli/quality pair for a total of
143 24 analyses. Terms where $p < 0.05$ using Type 3 tests of fixed effects were considered significant.

144

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

156 and tannic acid against the respective water control (Model 4). A summary of the models is shown in
157 Table 2.

158

159 **3. Results and discussion**

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

170

171 *3.1 Effect of stimuli concentration on sensory ratings*

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

184

185 Increasing stimuli concentration significantly increased bitterness and sourness perception and
186 decreased sweetness perception in all tested stimuli. Although the increase in bitterness ratings for
187 quinine and tannic acid samples is in harmony with observations in pure solutions (Fleming et al., 2016;
188 Keast & Roper, 2007), the association of bitterness with alum is inconsistent. Using untrained
189 participants, others have detected a dose-dependent increase in bitterness with alum concentration,
190 bitterness clustering closer to astringency relative to other side tastes, and frequent (46%) endorsement
191 of “bitter” for alum samples in a CATA design (Fleming et al., 2015, 2016). The lack of participant training
192 both in our study and others’ may partially explain observations of bitterness-alum associations, as

193 bitterness and astringency are often confused (Lea & Arnold, 1978; Lee & Lawless, 1991). When trained
194 or semi-trained participants evaluate samples, bitterness is less frequently associated with alum
195 (Brannan, Setser, & Kemp, 2001; Lim & Lawless, 2005). Because the association of alum and bitterness
196 occurs more often in untrained participants, a similar affective response (i.e., dislike) rather than
197 increased stimulation likely explains the correlation, as suggested by others (Fleming et al., 2016). As
198 further support of affective influence among untrained participants, we observed that astringency
199 ratings increased with quinine concentration, despite the lack of known quinine astringency. Similarly,
200 sourness perception increased with stimuli concentration. Confusion among untrained participants
201 regarding sourness and other unpleasant sensations such as bitterness and astringency has been
202 observed by others (Melis et al., 2017). Due to potential misunderstanding of sensory descriptors, non-
203 verbal methods, such as sorting or polarized-sensory position (Varela & Ares, 2012), may be better
204 suited to distinguish astringency and bitterness when using untrained participants. Such methods allow
205 participants to evaluate similarity of samples and standards without the potential biasing effect of
206 descriptors.

207

208 Our observation of decreased sweetness perception with increasing concentration of bitter (tannic acid,
209 quinine) and sour stimuli (malic acid) is consistent with the well-established phenomenon of mixture
210 suppression (Keast & Breslin, 2003; Mennella, Reed, Mathew, Roberts, & Mansfield, 2015). We also
211 observed a decrease in sweetness perception with increasing alum concentration; while some
212 researchers have associated a subtle sweet taste with alum (Breslin, Gilmore, Beauchamp, & Green,
213 1993; Fleming et al., 2016), others have not (Brannan et al., 2001). Given the limitations of this study,
214 such as untrained participants and fatiguing samples, our results are insufficient to support conclusions
215 regarding the sweet taste of alum.

216

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

227

228 *3.2 No effect of “constricting” in place of “puckering” on sensory ratings.*

229 To clarify potential misunderstanding and misreporting of astringent sensations, we tested whether
230 “constricting” could be used in place of “puckering” to describe the same sub-quality. Untrained
231 participants may confuse sourness with astringency, as suggested by similar ratings given in aronia berry

232 juice samples (Duffy et al., 2016). Using “puckering” to describe astringency may add further confusion,
233 as untrained participants rate puckering intermediate to sourness and astringency (Fleming et al., 2016).
234 Although lexicons have been developed to describe wine astringency, naïve consumers have difficulty
235 relating to complex definitions (Vidal, Gimenez, Medina, Boido, & Ares, 2015).

236

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

246

247 *3.3 Effect of repeated tasting on sensory ratings*

248 Because testing fatigue influences astringency perception, we investigated the effect of repeat tastings
249 on sub-quality and side taste ratings. Although others have noted that the duration of astringency
250 perception increases with repeated ingestion (Guinard, Pangborn, & Lewis, 1986), specific evidence
251 regarding sub-qualities and side tastes is sparse. Additionally, reports of astringency duration are varied,
252 as some studies report astringency six minutes post ingestion (Lee & Lawless, 1991), while others show a
253 return close to basal levels in less than two minutes (Fischer, Boulton, & Noble, 1994; Guinard et al.,
254 1986; Valentova, Skrovankova, Panovska, & Pokorny, 2002).

255

256 In this study, repeated tasting of astringent and/or bitter samples (tested through the factor “order”;
257 Table 3) significantly increased astringency ratings in alum and malic acid samples, but not in tannic acid
258 samples. Repeated tasting also decreased bitterness and sweetness perception in tannic acid and malic
259 acid, respectively, and increased sourness perception in malic acid samples. Our failure to detect an
260 order effect among astringency qualities in tannic acid was unexpected, as increased astringency
261 intensity following repeated tasting has been observed by others (Guinard et al., 1986; Lyman & Green,
262 1990). Although some have observed that sucrose decreases tannic-acid induced astringency order
263 effects (Lyman & Green, 1990), others have detected similar rates of order-induced astringency in soy
264 milk samples with and without sucrose (polyphenol content is thought to contribute to soy milk
265 astringency) (Courregelongue, Schlich, & Noble, 1999). Due to limited data specific to order effects, the
266 influence of sucrose on overall astringency perception may further explain observed differences among
267 tested stimuli, as discussed in the subsequent paragraph. Taken together, these results demonstrate
268 that the effect of repeated tastings on astringency perception is quality- and stimulus-dependent.

269

270 *3.4 Influence of beverage matrix on sensory ratings*

271 Various beverage matrix components, such as sweetness, polysaccharides, ethanol, and polyphenols,
272 influence astringency perception (reviewed in Ma et al., 2014; Soares, Brandao, Mateus, & de Freitas,
273 2017). However, beverage matrix components do not influence astringency equally among different
274 classes of astringent stimuli, as acid increases the potency of tannic acid while decreasing that of alum
275 (Peleg, Bodine, & Noble, 1998). In our study, we assessed the influence of beverage matrix on
276 astringency perception by comparing alum and tannic acid samples with their respective water-only
277 controls (Figure 2, Model 4). In both alum and tannic acid, the presence of the beverage matrix
278 increased sweetness ratings, as expected. Compared to water, the flavored beverage matrix lowered
279 astringency and bitterness ratings in tannic acid, but did not reach statistical significance in alum. The
280 lack of statistical difference in bitterness of alum samples is likely explained by lower initial ratings.
281 Similarly, differences in astringency ratings in tannic acid, but not alum, may be explained by the greater
282 change in affective response due to differences in bitterness perception. Although sucrose can decrease
283 astringency perception of tannic acid and other polyphenol-containing beverages (Courregelongue et al.,
284 1999; Duffy et al., 2016; Ishikawa & Noble, 1995; Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009),
285 further research is needed to understand whether the phenomenon is specific to polyphenols or
286 pertains to astringency in general, as other classes of astringent compounds were not evaluated in these
287 studies. Different effects of alum and tannic acid on salivary flow and viscosity may also account for our
288 observed differences, as both factors have documented effects on astringency perception (Lyman &
289 Green, 1990; Smith, June, & Noble, 1996). Furthermore, whether sucrose alters the well-studied tannin-
290 salivary protein interaction, a common hypothesis to explain astringency perception (reviewed in
291 (Soares, Brandao, Mateus, & de Freitas, 2017), also remains to be determined. Whether altered sensory
292 perception or differences in hedonic response play a greater role in altering matrix-induced changes in
293 astringency perception is an area for further research. These observations highlight that the effect of the
294 food matrix on astringency perception is stimulus-dependent, in agreement with others' conclusions
295 (Peleg et al., 1998).

296

297 **4. Conclusion**

298 In this study, we found that the relative perceived intensity of astringent sub-qualities and the effect of
299 beverage matrix on astringency ratings were stimulus-dependent. Additionally, we provide stimuli- and
300 quality-specific measures of how repeated tastings of bitter and astringent samples influences untrained
301 participant responses. Although the use of untrained participants limits interpretation of results, such as
302 whether observed effects were due to changes in actual sensory perception or biased by hedonics, it
303 also provides meaningful context for application of the findings. However, conclusions regarding order
304 effects have greater implications for future sensory testing rather than the consumer experience;
305 although people often taste beverages through multiple sips, the requirement to rinse, wait, and
306 evaluate a different beverage is not representative of most consumption experiences. Furthermore,
307 whether similar order effects would be observed with an alternate number of tastings cannot be
308 determined with the present data, as the study was not powered to prescribe the ideal sample set size.
309 Additional studies are needed to determine whether differences induced by repeated sampling and

310 beverage ingredients among tested stimuli are observed in other food matrices. Given our observed
311 differences among stimuli, we advise against the use of single astringent standard if attempting to
312 introduce a naïve participant to the concept of “astringency.” Product developers and sensory
313 researchers should consider the class of the astringent compound, the sensation of interest, and the
314 food matrix when studying astringency perception. Taken together, these data agree with prior work
315 supporting stimuli- and sub-quality specific aspects of astringency.

316

317

318 **Acknowledgments**

319 The authors thank Miguel Odrón for his assistance conducting this study. This study was supported by
320 the USDA National Institute of Food and Agriculture, Hatch project 1013624. The authors declare no
321 conflicts of interest.

322 **References**

- 323 ASTM. (1991). Standard terminology relating to sensory evaluation of materials and products. In
324 *Annual Book of ASTM Standards* (pp. 1-3). Philadelphia, PA: American Society for Testing
325 Materials.
- 326 Auger, C., Rouanet, J. M., Vanderlinde, R., Bornet, A., Decorde, K., Lequeux, N., . . . Teissedre, P.
327 L. (2005). Polyphenols-enriched Chardonnay white wine and sparkling Pinot Noir red wine
328 identically prevent early atherosclerosis in hamsters. *Journal of Agricultural and Food Chemistry*,
329 *53*, 9823-9829. doi:10.1021/jf050988m
- 330 Bajec, M. R., & Pickering, G. J. (2008). Astringency: Mechanisms and perception. *Critical Reviews*
331 *in Food Science and Nutrition*, *48*, 858-875. doi:10.1080/10408390701724223
- 332 Brannan, G. D., Setser, C. S., & Kemp, K. E. (2001). Interaction of astringency and taste
333 characteristics. *Journal of Sensory Studies*, *16*, 179-197. doi:10.1111/j.1745-
334 459X.2001.tb00295.x
- 335 Breslin, P. A. S., Gilmore, M. M., Beauchamp, G. K., & Green, B. G. (1993). Psychophysical
336 evidence that oral astringency is a tactile sensation. *Chemical Senses*, *18*, 405-417.
337 doi:10.1093/chemse/18.4.405
- 338 Courregelongue, S., Schlich, P., & Noble, A. C. (1999). Using repeated ingestion to determine the
339 effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food*
340 *Quality and Preference*, *10*(4), 273–279. [https://doi.org/10.1016/S0950-3293\(98\)00055-X](https://doi.org/10.1016/S0950-3293(98)00055-X)
- 341 Duffy, V. B., Rawal, S., Park, J., Brand, M. H., Sharafi, M., & Bolling, B. W. (2016). Characterizing
342 and improving the sensory and hedonic responses to polyphenol-rich aronia berry juice.
343 *Appetite*, *107*, 116-125. doi:<https://doi.org/10.1016/j.appet.2016.07.026>
- 344 Fischer, U., Boulton, R. B., & Noble, A. C. (1994). Physiological factors contributing to the
345 variability of sensory assessments: Relationship between salivary flow rate and temporal
346 perception of gustatory stimuli. *Food Quality and Preference*, *5*, 55-64.
347 doi:[http://dx.doi.org/10.1016/0950-3293\(94\)90008-6](http://dx.doi.org/10.1016/0950-3293(94)90008-6)
- 348 Fleming, E. E., Ziegler, G. R., & Hayes, J. E. (2015). Check-all-that-apply (CATA), sorting, and
349 polarized sensory positioning (PSP) with astringent stimuli. *Food Quality and Preference*, *45*, 41-
350 49. doi:10.1016/j.foodqual.2015.05.004
- 351 Fleming, E. E., Ziegler, G. R., & Hayes, J. E. (2016). Investigating mixture interactions of astringent
352 stimuli using the isobole approach. *Chemical Senses*, *41*, 601-610. doi:10.1093/chemse/bjw064
- 353 Guinard, J.-X., Pangborn, R. M., & Lewis, M. J. (1986). The time-course of astringency in wine
354 upon repeated ingestion. *American Journal of Enology and Viticulture*, *37*, 184.
- 355 Ishikawa, T., & Noble, A. C. (1995). Temporal perception of astringency and sweetness in red
356 wine. *Food Quality and Preference*, *6*(1), 27–33. [https://doi.org/10.1016/0950-3293\(94\)p4209-o](https://doi.org/10.1016/0950-3293(94)p4209-o)
- 357 Jaeger, S. R., Axten, L. G., Wohlers, M. W., & Sun-Waterhouse, D. (2009). Polyphenol-rich
358 beverages: insights from sensory and consumer science. *Journal of the Science of Food and*
359 *Agriculture*, *89*, 2356-2363. doi:10.1002/jsfa.3721
- 360 Keast, R. S. J., & Breslin, P. A. S. (2003). An overview of binary taste–taste interactions. *Food*
361 *Quality and Preference*, *14*, 111-124. doi:[https://doi.org/10.1016/S0950-3293\(02\)00110-6](https://doi.org/10.1016/S0950-3293(02)00110-6)
- 362 Keast, R. S. J., & Roper, J. (2007). A complex relationship among chemical concentration,
363 detection threshold, and suprathreshold intensity of bitter compounds. *Chemical Senses*, *32*,
364 245-253. doi:10.1093/chemse/bjl052

365 Landrault, N., Poucheret, P., Azay, J., Krosniak, M., Gasc, F., Jenin, C., . . . Teissedre, P. L. (2003).
366 Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. *Journal of Agricultural*
367 *and Food Chemistry*, *51*, 311-318. doi:10.1021/jf020219s
368 Lawless, H. T., & Corrigan, C. J. (1994). Semantics of astringency. In H. Ogawa (Ed.), *Olfaction and*
369 *Taste XI* (pp. 288-292). Tokyo: Springer.
370 Lea, A. G. H., & Arnold, G. M. (1978). Phenolics of ciders – bitterness and astringency. *Journal of*
371 *the Science of Food and Agriculture*, *29*, 478-483. doi:10.1002/jsfa.2740290512
372 Lee, C. B., & Lawless, H. T. (1991). Time-course of astringent sensations. *Chemical Senses*, *16*,
373 225-238. doi:10.1093/chemse/16.3.225
374 Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties
375 and their effects on food and beverage preferences. *American Journal of Clinical Nutrition*, *81*,
376 330S-335S.
377 Lim, J., & Lawless, H. T. (2005). Qualitative differences of divalent salts: multidimensional scaling
378 and cluster analysis. *Chemical Senses*, *30*, 719-726. doi:10.1093/chemse/bji064
379 Lyman, B. J., & Green, B. G. (1990). Oral astringency – effects of repeated exposure and
380 interactions with sweeteners. *Chemical Senses*, *15*, 151-164. doi:10.1093/chemse/15.2.151
381 Ma, W., Guo, A. Q., Zhang, Y. L., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and
382 bitterness perception of tannins in wine. *Trends in Food Science & Technology*, *40*, 6-19.
383 doi:10.1016/j.tifs.2014.08.001
384 Melis, M., Yousaf, N. Y., Mattes, M. Z., Cabras, T., Messana, I., Crnjar, R., . . . Tepper, B. J. (2017).
385 Sensory perception of and salivary protein response to astringency as a function of the 6-n-
386 propylthioural (PROP) bitter-taste phenotype. *Physiology & Behavior*, *173*, 163-173.
387 doi:10.1016/j.physbeh.2017.01.031
388 Mennella, J. A., Reed, D. R., Mathew, P. S., Roberts, K. M., & Mansfield, C. J. (2015). "A spoonful
389 of sugar helps the medicine go down": bitter masking by sucrose among children and adults.
390 *Chemical Senses*, *40*, 17-25. doi:10.1093/chemse/bju053
391 Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and
392 disease. *Oxidative Medicine and Cellular Longevity*, *2*, 270-278. doi:10.4161/oxim.2.5.9498
393 Peleg, H., Bodine, K. K., & Noble, A. C. (1998). The influence of acid on astringency of alum and
394 phenolic compounds. *Chemical Senses*, *23*, 371-378.
395 Smith, A. K., June, H., & Noble, A. C. (1996). Effects of viscosity on the bitterness and astringency
396 of grape seed tannin. *Food Quality and Preference*, *7*(3), 161–166.
397 [https://doi.org/10.1016/S0950-3293\(96\)00028-6](https://doi.org/10.1016/S0950-3293(96)00028-6)
398 Soares, S., Brandao, E., Mateus, N., & de Freitas, V. (2017). Sensorial properties of red wine
399 polyphenols: Astringency and bitterness. *Critical Reviews in Food Science and Nutrition*, *57*, 937-
400 948. doi:10.1080/10408398.2014.946468
401 Valentova, H., Skrovankova, S., Panovska, Z., & Pokorny, J. (2002). Time-intensity studies of
402 astringent taste. *Food Chemistry*, *78*, 29-37. doi:10.1016/s0308-8146(01)00330-2
403 Varela, P., & Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer
404 science. A review of novel methods for product characterization. *Food Research International*,
405 *48*(2), 893–908. <https://doi.org/10.1016/j.foodres.2012.06.037>
406 Vidal, L., Gimenez, A., Medina, K., Boido, E., & Ares, G. (2015). How do consumers describe wine
407 astringency? *Food Research International*, *78*, 321-326. doi:10.1016/j.foodres.2015.09.025

410 **Tables**411 **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	

412

413

414 **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

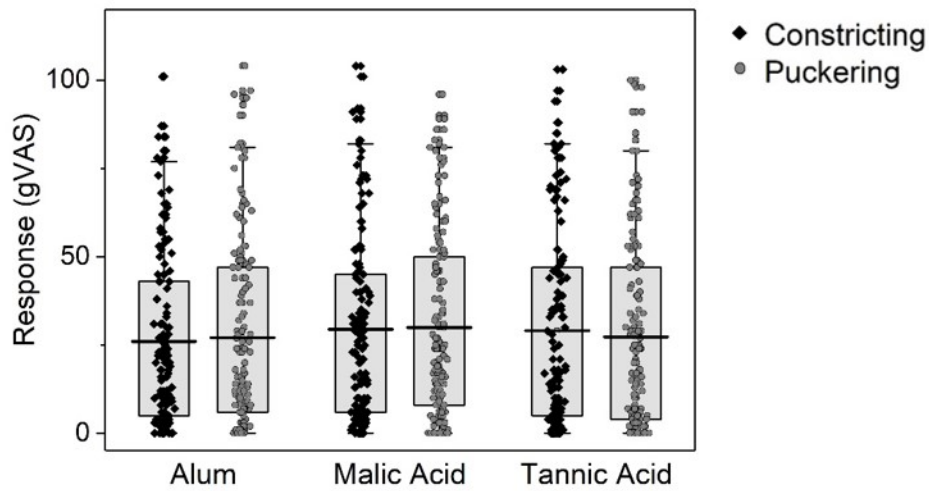
415

416

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

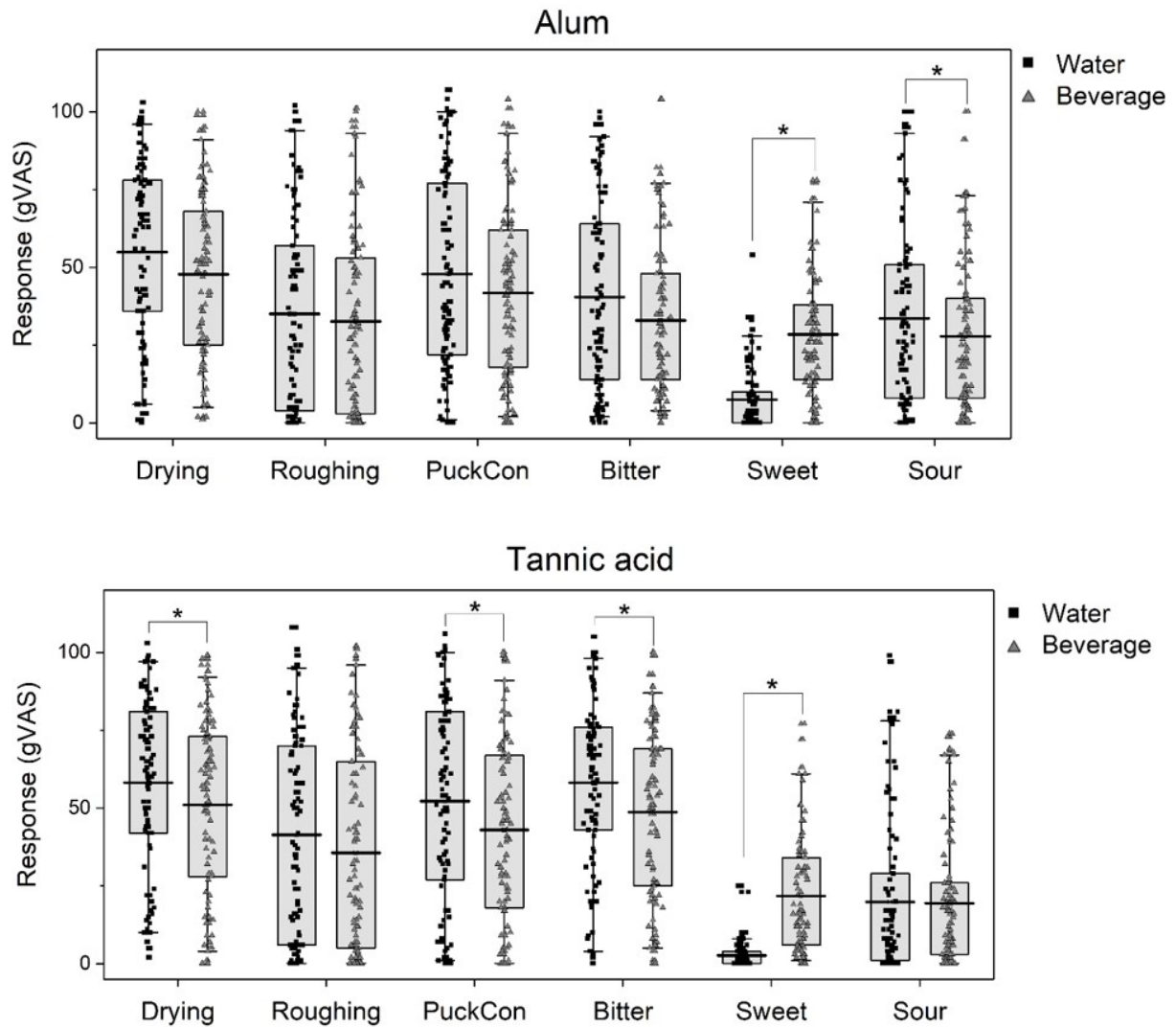
Stimuli	Quality ¹	Intercept (β0)	LogConc (β1)	Order (β2)	Day (β3)	LogConc* Day (β4)	Order* Day (β5)
Alum	Drying ^a	3.92	2.88*	0.12*	1.93*	0.58	-0.14*
			<.0001	0.0450	0.0003	0.2180	0.0135
Alum	Roughing ^b	3.04	2.53*	0.11*	0.41	-0.12	-0.05
			<.0001	0.0032	0.4755	0.8011	0.3573
Alum	Puckering/Constricting ^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

¹Means 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 *.



418

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

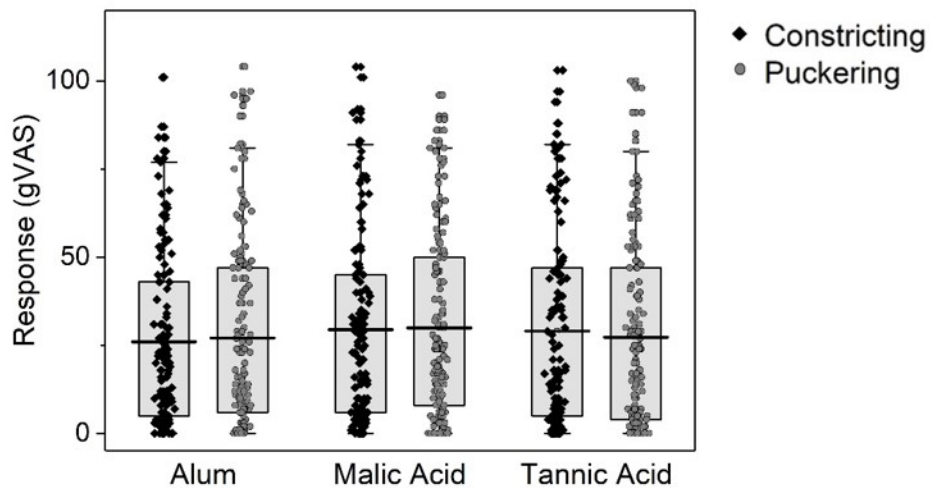


422

423 **Figure 2.** Individual participant ratings for the same concentration of stimuli evaluated in either
 424 water or flavored beverage. The box represents 50% of responses, whiskers represent 5th and
 425 95th percentiles, and the central line represents the mean. Significant differences between means
 426 ($P < 0.05$) are indicated by *.

427

428 Supplemental
 429
 430 **Supplemental table 1. Participants**



431
 432 and wording presentation.
 433

tested, by day

	Day 1	Day 2
Puckering	27	23
Constricting	23	22

434
 435

436 **Supplemental table 2.** Day 1 dose-response equations.

Stimuli	Quality	Intercept ($\beta_0 + \beta_3$)	Log ₁₀ Conc ($\beta_1 + \beta_4$)	Order ($\beta_2 + \beta_5$)
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

Log₁₀Conc = coefficient for log₁₀ of concentration, and order = coefficient for sample testing order. Effects for each term were derived from Table 3, where Day = 0 indicates Day 1.

437

438

439 **Supplemental table 3.** Day 2 dose-response equations.

Stimuli	Quality	Intercept (β_0)	LogConc (β_1)	Order (β_2)
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

Log₁₀Conc = coefficient for log₁₀ of concentration, and order = coefficient for sample testing order. Effects for each term were derived from Table 3, where Day = 1 indicates Day 2.

440

441

442