

10-2018

## **Desensitization but not Sensitization from Commercial Chemesthetic Beverages**

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### **Recommended Citation**

Running, Cordelia, "Desensitization but not Sensitization from Commercial Chemesthetic Beverages"  
(2018). *Department of Food Science Faculty Publications*. Paper 17.  
<https://docs.lib.purdue.edu/foodscipubs/17>

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Food Quality and Preference 69, 21-27

<https://doi.org/10.1016/j.foodqual.2018.05.001>

1 Desensitization but not sensitization from commercial chemesthetic beverages

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8

9 **Abstract**

10 Sensations such as spiciness or stinging are particularly challenging to assess in sensory evaluation tests,  
11 as sensitization (increase in intensity with repeated tasting) and desensitization (decrease in intensity  
12 with repeated tasting) phenomena can confound intensity ratings. However, much of the published  
13 work on these phenomena are with model solutions or complex meals rather than commercial  
14 beverages. Thus, we tested whether we could observe sensitization or desensitization using canned  
15 spicy ginger beer (contained chili extract) and seltzer water. Samples were presented in pairs, with a 20 s  
16 wait and no rinse within a pair, but a 4 min wait with rinsing between pairs. Pairs of samples were:  
17 ginger beer followed by ginger beer, ginger beer followed by seltzer, seltzer followed by ginger beer, and  
18 seltzer followed by seltzer. These pairs were intended to allow us to also test for cross-  
19 sensitization/desensitization between the two beverages. Tests were conducted both in open cups and  
20 capped vials to observe how loss of carbonation influenced sample ratings. Participants tasted all pairs  
21 of samples in counterbalanced order and rated samples for intensity of “Spiciness, burning, or stinging  
22 sensation,” bitterness, sweetness, sourness, overall flavor, and liking/disliking. Results indicate no  
23 sensitization effects. Desensitization, however, likely occurred for both beverages. Further, tasting  
24 seltzer and ginger beer together in a pair amplified the “bitterness” of the seltzer water, a likely contrast  
25 effect. Overall, while sensitization may not interfere with the sensory ratings for these beverages,  
26 contrast effects and desensitization should be considered carefully when planning sensory evaluation  
27 tests.

28

29 Keywords: spiciness, carbonation, chemesthesis, sensitization, desensitization

30

31

32 **1. Introduction**

33 Many flavors can be challenging to evaluate in sensory tests due to order effects when tasting. Some  
34 compounds linger, leading to difficulty clearing the sensation before tasting other samples and  
35 confounding results. Other compounds, capsaicin and other chemesthetic compounds in particular, can  
36 have sensitization and desensitization effects. Chemesthesis is the chemical induction of thermal and  
37 irritating sensations, such as spiciness from peppers, cooling from menthol, and stinging or biting from  
38 carbonation (Green, 1996, 2003). Sensitization occurs when re-tasting of the sample leads to increased  
39 intensity compared to the first taste, where desensitization occurs when re-tasting leads to decreased  
40 intensity. For spiciness from capsaicin, the inter-stimulus interval (time between samplings) directly  
41 influences whether sensitization or desensitization should be expected. Prior work indicates that up  
42 through approximately 2.5-3.5 minutes between tastes, sensitization occurs, and after 5.5 minutes  
43 between tastes desensitization occurs (Green, 1989, 1991). The desensitization can even last several  
44 days (Karrer & Bartoshuk, 1991; McBurney, Balaban, Christopher, & Harvey, 1997).

45

46 Some of the alterations in sensitivity to chemesthetic compounds with repeated exposure can certainly  
47 be psychological, as the exposures increase the familiarity and/or could change the affective response to  
48 a flavor; however, sensitization and desensitization are mechanistically driven through peripheral cells  
49 as well (Bevan, Quallo, & Andersson, 2014; Szallasi & Blumberg, 1999). The phenomenon is perhaps best  
50 studied for the transient receptor potential channels (TRP), in particular the subfamily V member 1 or  
51 vanilloid receptor 1 (TRPV1). TRPV1 is activated by capsaicin, as well as temperatures above 42°C,  
52 acidity, and additional chemical compounds such as allyl isothiocyanate (found in mustard and wasabi)  
53 (Bevan et al., 2014; Nagy, Friston, Valente, Torres Perez, & Andreou, 2014). Cellular phosphorylation of  
54 specific residues of TRPV1 lead to increased reactivity of the protein to stimuli, while dephosphorylation

55 leads to desensitization (Tominaga, 2006). The dephosphorylation can be driven by calcium flux into the  
56 cell, which occurs when TRPV1 is stimulated (see (Bevan et al., 2014) for a detailed discussion of these  
57 processes).

58

59 These phenomena surrounding the response to chemesthetic stimuli exhibit themselves in human  
60 behavior. Sensitization is anecdotally reported when consuming spicy meals, and cross-sensitization  
61 between stimuli through events such as experiencing stronger burning sensations when taking a drink of  
62 a carbonated beverage immediately after eating a spicy food (“Mouth on Fire?,” 2014; “The Dos and  
63 Dents of Eating Spicy Foods,” 2014). However, observing the sensitization effect for real foods in the  
64 laboratory has proven challenging, though desensitization has been observed (Prescott, 1999). Indeed,  
65 chronic desensitization is thought to drive the differences in reported spiciness intensity of consumers  
66 and non-consumers of spicy chili peppers, as consumers consistently report lesser intensity of spiciness  
67 compared to non-consumers (Nolden, 2016; Nolden & Hayes, 2017; Prescott & Stevenson, 1995;  
68 Stevenson & Prescott, 1994).

69

70 Consequently, gaining accurate comparative estimates of sensory intensity for products containing  
71 capsaicin, and potentially other chemesthetic stimuli, is challenging. Using actual foods and beverages  
72 can complicate these phenomena further, as context, mixture suppression, matrix effects, and a number  
73 of other possible factors in actual foods could influence outcomes. Thus, we designed the following  
74 experiment to test whether sensitization and desensitization could be observed for two commercially  
75 available chemesthetic beverages: a spicy ginger beer and a carbonated water. We also designed the  
76 experiment to test for possible cross-sensitization; i.e., to test whether sensitization from spiciness  
77 crossed over to enhance stinging from carbonation and vice versa.

78

## 79 **2. Methods**

### 80 **2.1 Samples**

81 Samples for this study included a non-alcoholic ginger beer (Q drinks Spectacular Ginger Beer, packaged  
82 in 12 oz aluminum cans; ingredient list: carbonated water, agave, ginger extract, lime extract, coriander  
83 extract, cardamom extract, orange extract, chili extract, citric acid) and a carbonated water (Kroger  
84 brand Seltzer water, packaged in 12 oz aluminum cans; ingredient list: carbonated water). The  
85 carbonated water will be referred to as “seltzer” for brevity.

86

### 87 **2.2 Tests**

88 Two tests were conducted: the first with approximately 15 mL of sample poured into 4 oz cups with sip-  
89 through lids prior to tasting (referred to as the “Open” test hereafter), and the second with  
90 approximately 15 mL samples served in 0.5 oz amber vials with PTFE-cone lined caps to help prevent  
91 loss of carbonation (referred to as the “Capped” test hereafter). The sip-through design of the lids for  
92 the Open test cups allowed air to escape through the hole for drinking, but participants could not see  
93 the color of the samples. We attempted to keep samples for no longer than 15 minutes after opening  
94 the canned beverages. All samples (cups or vials) were kept a refrigerator at approximate 4°C until  
95 participants arrived for their scheduled tests. As the initial results from the Open test indicated  
96 substantial carbonation loss over the course of the experiment (much lower ratings for seltzer over the  
97 course of the tests for each participant), we reran the test with the capped amber vials.

98

### 99 **2.3 Tasting procedure**

100 Other than the open or capped containers, the procedure for sensory evaluation was the same for both  
101 experiments. Participants were recruited from Purdue University's campus and surrounding area. All  
102 testing methods were approved by the Purdue University Human Subjects Biomedical Review Board as  
103 exempt under exemption 6 for tasting of whole foods and food ingredients. Participant screening  
104 information, scheduling, demographic, and sensory data were collected using RedJade Sensory Software  
105 (Curion, Redwood City, CA). Eligible participants reported no known problems with their sense of taste  
106 and smell, no tongue/lip/cheek piercings, were over 18 years of age, and were willing to drink  
107 carbonated beverages such as "sparkling water, ginger ale, non-alcoholic ginger beer, cinnamon flavored  
108 beverages, and others." The generalized visual analog scale used to collect intensity data was a  
109 horizontal 606 pixel length scale (presented on an iPad mini 2 in landscape orientation), programmed to  
110 collect with ratings from -10 to 110, with inset anchors of "None" and "Strongest ever" at 0 and 100. On  
111 screen instructions told participants that "None" meant they did not experience any of this sensation at  
112 all, and "Strongest ever" meant the strongest sensation they have ever experienced. For warm-up  
113 questions, participants were told to rate the intensity of the sensation based on remembered intensity,  
114 or imagined intensity if they had never experienced the sensation. A liking scale was also used, which  
115 was the same size as the intensity scale, but had the anchors "Worst ever," "Neutral," and "Best ever" at  
116 0, 50, and 100 on the scale (end anchors for worst and best were inset by 10 pts as before).

117

118 Participants provided information on their age, gender identity, biological sex, and ethnicity. Next,  
119 participants completed a warm-up questionnaire to familiarize them with the visual analog scales our  
120 laboratory uses to collect data. The warm-up asked the subject to rate the intensity of the brightness of  
121 the sun, the brightness of this room, the loudness of a shout, the loudness of a whisper, the bitterness  
122 of black coffee, and the sweetness of pure sugar. Questions were presented in randomized order.  
123 Participants were told that we use this scale to verify they understand the scale, and were asked to

124 please rate the items as accurately as possible even if they had attended sessions in our lab in the past  
125 (this helps reduce the number of participants who simply click through all the warm-up screens without  
126 giving actual ratings). Ratings from the warm-up were used as a check on whether participants  
127 understood the directions and used the scale as instructed. This was done by verifying that participants  
128 rated the brightness of the sun as greater than the brightness of this room, and the loudness of a shout  
129 as greater than the loudness of a whisper. Participants who failed this check were excluded from the  
130 final analysis.

131

132 After completing the demographic questionnaire and warm-up, participants began rating samples.  
133 Samples were presented as pairs and organized onto a tray template to aid in the tasting process (see  
134 supplemental files, available through Purdue Repository). The details of the questionnaire are included  
135 in supplemental file 2 . The iPads led the participants through the tasting procedure, explaining that they  
136 would be tasting several pairs of samples in a timed fashion, with very specific times for rinsing with  
137 water or not. Each participant received 4 pairs of samples: seltzer water followed by seltzer water,  
138 seltzer water followed by ginger beer, ginger beer followed by seltzer water, and ginger beer followed  
139 by ginger beer. The pairs were presented in counterbalanced order. Participants were instructed to  
140 drink the entire sample, hold it in their mouth for 10 seconds, swallow, then rate the intensity of the  
141 “Spiciness, burning, or stinging sensation,” “Sweetness,” “Sourness,” “Bitterness,” “Overall flavor  
142 intensity,” and then “Overall liking.” After 20 seconds, participants repeated this tasting process for the  
143 second sample of the pair (no water rinse in between). After tasting and rating the second sample, a 4  
144 minute wait was enforced during which the participant was instructed to rinse with water (room  
145 temperature spring water, Hickory Springs, purchased locally in 6 gallon containers for a water cooler).  
146 After the 4 minute wait, the participant moved on to the next pair of samples, and the process repeated.  
147 An overview of this tasting procedure is shown in figure 1.

148

## 149 **2.4 Participants**

150 In both tests there were 47 participants (Open: 16 male, 31 female; Capped: 15 male, 32 female). Details  
151 on age ranges and ethnic distribution of the participants is provided in supplemental file 3. Notably, the  
152 participants in the tests were not all the same individuals. Some may be repeats between the Open and  
153 Capped tests, but we did not collect identifiable information during the sensory tests so we cannot be  
154 certain who the repeated participants are. Thus, all participants in both tests are treated as unique  
155 individuals in the statistical analysis. After removing the participants who failed the warm-up check  
156 (sun>room, shout>whisper), 45 participants remained for analysis in the Open experiment (31 female,  
157 14 male) and 43 participants remained in for analysis in the Capped experiment (30 female, 13 male).  
158 No participants selected the “Other” gender category in either test.

159

## 160 **2.5 Analysis**

161 Data were analyzed using SAS 9.4. For all tests, data were analyzed separately for each rated quality  
162 (i.e., spiciness/burning/stinging, bitterness, sourness, sweetness, overall flavor, liking). Additionally,  
163 residuals indicated no transformation of the data was necessary for the qualities of main interest. Some  
164 patterns were evident in the residuals for sourness, bitterness, and sweetness, but the patterns indicate  
165 this is due to many participants giving the samples ratings at or near 0 for these qualities, which was  
166 expected (seltzer water is not sweet, neither beverage was sour, etc.). These data were not of primary  
167 interest for the study, so no further analysis or transformations were conducted.

168

169 First, data were analyzed to evaluate the effect of open cups compared to capped vials on sensory  
170 ratings. The mixed procedure with subject as a repeated measure was used to run the following linear  
171 mixed model, using the Kenward-Roger approximation for degrees of freedom:

172 Rating = Test, Gender, Beverage, PairOrder, Test\*Beverage, Test\*Gender

173 “Beverage” was seltzer or ginger beer, “Test” was Open or Capped, and “PairOrder” was the first,  
174 second, third, or fourth pair within the sample set (i.e., tasting order for the pairs, which was  
175 counterbalanced). Participant was entered as a repeated factor, with the autoregressive covariance  
176 structure (data sorted by test, sample, participant, then order of tasting). All interaction terms were  
177 tested, but only Test\*Beverage and Test\*Gender showed any significant effects; thus, other interaction  
178 terms were removed for clarity.

179

180 As effects were observed due to open compared to capped vials, all further analysis was conducted only  
181 on the data from the capped vials, in order to disentangle potential desensitization effects from loss of  
182 carbonation effects. For these analyses, the following model was used:

183 Rating = Sample, Gender, PairOrder

184 In this model, “Sample” specifically referred to individual samples within the full tasting paradigm (there  
185 were eight, see Table 1). “PairOrder” again referred to counterbalanced order of tasting. See Table 1 for  
186 the details of the model factors. Participant was entered as a repeated factor, and the covariance  
187 structure was set as autoregressive (data were sorted by test, beverage, participant, then order of  
188 tasting). After evaluating the overall effects from the factors listed above, least squared means  
189 estimates were calculated for specific comparisons, as shown in Table 1. The model was primarily used  
190 to interpret effects for “Spiciness, burning, or stinging sensation” (hereafter referred to as “burning”),

191 but since data were collected on bitterness, sweetness, sourness, overall flavor, and liking the results for  
192 those are also included (mostly in supplemental file 4). Specific comparisons analyzed to determine  
193 sensitization or desensitization are listed in Table 1. From prior work, we expected to observe  
194 sensitization most strongly when a sample was tasted immediately after itself within a pair, while we  
195 expected to see desensitization through decreasing ratings with increasing tasting order (PairOrder, in  
196 our analysis). Note that we used “PairOrder” to observe these effects rather than actual order (i.e, first  
197 through eighth), as actual order was confounded with the sample (i.e., “Ginger beer after Ginger beer”  
198 could never be tasted first, but it could have been tasted within the first pair). Bonferroni adjustments  
199 were used for post hoc analyses involving multiple comparisons. Interaction terms were tested but none  
200 were found to be significant, so they were removed for clarity.

201

### 202 **3. Results**

203 Results of statistical tests are shown in Figures 2 and 3 as well as Table 2 (the table is included in  
204 addition to the figures in order to provide the specific means and standard errors). For the first model,  
205 used to assess the effect of tasting when using the capped vials compared to open cups, significant  
206 effects are apparent for the test type, beverage, order of tasting, gender, interaction of test type with  
207 beverage, and interaction of test type with gender. As expected, ratings for burning (“Spiciness, burning,  
208 or stinging sensation”) were lower in the open cups compared to the capped vials. Lower ratings were  
209 given over the course of the test (as indicated by PairOrder). Post-hoc comparisons show that samples  
210 tasted in the first pair of the testing order were rated as higher than subsequent samples. Regarding the  
211 interaction of test type and beverage, seltzer was significantly more intense for burning when tasted  
212 from capped vials compared to open; a trend may be apparent for ginger beer ( $p=0.061$ ) for slightly  
213 lower burning ratings when presented in vials compared to open, which was not expected. Significant

214 effects are also present for the interaction of test and gender, however the small sample size for males  
215 make interpretation of those effects unreliable.

216

217 Both models indicate that females rated the beverages are more burning than males. Again, this should  
218 be interpreted with caution due to the small number of males in these tests.

219

220 The second model was run only on data from the capped vials test and was primarily intended to check  
221 for sensitization/desensitization effects for burning. The results do not support sensitization, but do  
222 support desensitization. When ginger beer was tasted immediately after itself, burning had a tendency  
223 to decrease (drop of 5 pts,  $p=0.075$ ). More convincingly, burning ratings decreased with tasting order as  
224 indicated by lower ratings with increasing PairOrder. However, after Bonferroni adjustments,  
225 differences are only marginally significant and only for samples tasted in the first pair compared to in  
226 the third or fourth pairs. Also of note, seltzer was rated as more intense for burning after tasting ginger  
227 beer, which is likely due to carryover of the burning from the ginger beverage. Finally, some effects for  
228 bitterness, overall flavor, and liking ratings are also noted. For bitterness, ratings decreased over the  
229 course of the experiment as evidenced by PairOrder. Additionally, when ginger beer was tasted after  
230 seltzer it was rated as less bitter, and when seltzer was tasted after ginger beer it was rated as more  
231 bitter. Regarding overall flavor, seltzer was rated as more intense after tasting ginger beer, again likely  
232 due to carryover from the ginger beer spices. Finally, ginger beer was rated lower for liking when tasted  
233 after itself.

234

235 **4. Discussion**

236 Our data do not support sensitization or cross-sensitization effects for the “Spiciness, burning, or  
237 stinging sensation” from spicy ginger beer or seltzer in an acute sensory test. However, desensitization is  
238 apparent in the lower burning-type ratings over the course of the experiment. Our data support the  
239 concept of contrast effects for bitterness. Finally, our data confirm that careful attention should be given  
240 to how carbonated beverages are served for sensory tests, as differences were observed between the  
241 capped and open containers.

242

243 The lack of a sensitization phenomenon for either carbonation sting or ginger beer spice has precedent.  
244 While sensitization to capsaicin is reasonably well established when capsaicin is applied with filter discs  
245 (Affeltranger, McBurney, & Balaban, 2007; Balaban, McBurney, & Stoulis, 1999; Cliff & Green, 1996;  
246 Green, 1989, 1991), the phenomenon is often inconsistent or absent when using oral rinses or actual  
247 foods (Cliff & Green, 1996; Dessirier, O’Mahony, Iodi-Carstens, & Carstens, 2000; Nasrawi & Pangborn,  
248 1990; Prescott, 1999). Notably, the ginger extract ingredient in our ginger beer likely contributed  
249 zingerone as a spicy compound, and zingerone has not been demonstrated to cause sensitization in  
250 general (Prescott & Stevenson, 1996b), though isolated participants may show sensitization (Prescott &  
251 Stevenson, 1996a).

252

253 The decreased ratings as the order of tasting pairs increased is likely evidence of desensitization to  
254 chemesthesis from carbonation and/or spiciness. We actually expected this effect to be stronger for the  
255 spiciness of ginger beer compared to the sting from carbonation, but no such interaction effect was  
256 evident. Prior work indicates desensitization occurs for zingerone (from ginger), capsaicin (from chilis),  
257 and piperine (from black pepper) (Affeltranger et al., 2007; Dessirier, Nguyen, Sieffermann, Carstens, &  
258 O’Mahony, 1999; Prescott & Stevenson, 1996a), which could all plausibly be found in spicy beverage

259 formulations such as the ginger beer used in this study. However, desensitization for carbonation  
260 stinging is relatively unexplored. Cross-desensitization has been reported for capsaicin to carbonation  
261 sting (Dessirier, Simons, O'Mahony, & Carstens, 2001), which could certainly be the source of  
262 desensitization for carbonation observed in our study. In a study separating the sensation of bubbles  
263 from carbonation "bite," significant time effects were observed for carbonation bite, and decreases in  
264 ratings over time can be seen in the data from that work (Wise, Wolf, Thom, & Bryant, 2013).  
265 Potentially, the decreases in ratings could also be due to a habituation type phenomenon instead of true  
266 desensitization, where participants become accustomed to the sensation and so lower the associated  
267 sensory ratings. However, cellular evidence confirms sensitization/desensitization of transient receptor  
268 potential channels, such as for capsaicin and TRPV1 (Gordon-Shaag, Zagotta, & Gordon, 2008; Joseph,  
269 Wang, Lee, Ro, & Chung, 2013; Leamy, Shukla, McAlexander, Carr, & Ghatta, 2011; Lennertz, Kossyрева,  
270 Smith, & Stucky, 2012; Numata, Kiyonaka, Kato, Takahashi, & Mori, 2011; Zhu et al., 2005). While  
271 carbonation has not been extensively tested for desensitization at the cellular level, the TRPA1 receptor  
272 responds to acidification from carbonic acid that is created from carbonic anhydrase IV acting on carbon  
273 dioxide (Wang, Chang, & Liman, 2010), and so the mechanisms for desensitization similar to other  
274 transient reception potential channels may be plausible for carbonation "sting" or "bite."

275

276 Given the higher ratings for the first pair of samples compared to other pairs, we considered that the  
277 decrease in ratings for both ginger beer and seltzer was due to a "first sample effect" rather than actual  
278 desensitization (Lawless & Heymann, 2010). In this case, participants would have initially rated the  
279 sample as high due to lack of context, familiarity, or perhaps surprise, but the latter ratings would be  
280 stable. To check for this, we re-analyzed the data removing the first time each beverage was tasted (not  
281 the entire first pair, just the first rating for each beverage, which may or may not have been in the first  
282 pair due to the counterbalancing). In that analysis, the patterns of responses were not substantively

283 different, though the main effect for PairOrder was lost (results included in the supplemental file 4). We  
284 suspect this is due to loss of power from excluding some of the sample size, as the means across the  
285 PairOrders still followed the same general downward pattern. Thus, while the first sample may indeed  
286 be rated differently from the others, desensitization should still be considered as well.

287

288 Regarding the effects observed for bitterness, we theorize this is mostly due to contrast effects between  
289 the two beverages (Lawless, 1983; Lawless & Heymann, 2010). It is also possible that seltzer tasted after  
290 ginger beer may have been rated as more bitter due to carryover of some of the ginger beer taste, but if  
291 these were only carry over effects then it is unclear why ginger beer after seltzer would be rated as less  
292 bitter than ginger beer first. Alternatively, these ratings may not have been for true bitterness, but for  
293 the unpleasant quality of the plain seltzer water in comparison to the ginger beer. We suspect the ginger  
294 beer likely emphasized the “bitterness” in the seltzer water, and the seltzer water emphasized less  
295 “bitterness” in the ginger beer. However, such contrast effects should be more specifically targeted in a  
296 separate study to give conclusive results. At very least, our data confirm the need to consider contrast  
297 when tasting very different flavors in a single experiment.

298

299 Finally, we were not surprised to find differences between ratings for open compared to capped  
300 containers when serving carbonated beverages. However, the finding that the ginger beer had a trend  
301 toward being less intense for burn when capped compared to open was unexpected. As many of our  
302 participants were likely the same in both tests, this could have been due to increased familiarity with the  
303 product (we have found in many tests that ratings tend to decrease over time, especially for “bad”  
304 sensations; unpublished data). However, the phenomenon of initial elevation bias has been observed in  
305 psychological surveys of thoughts, feelings, and behaviors (Shrout et al., 2018). Notably, this group also

306 found that this initial elevation of ratings/values was higher for negative than positive affect.  
307 Nonetheless, without knowing for certain how many and which subjects were repeated in our own  
308 experiments, we cannot state for certain that the potential decline in ginger beer burn was due to this  
309 elevation bias effect or due to another factor, such as interaction with other sensory active ingredients.

310

## 311 **5. Conclusions**

312 Neither sensitization nor cross-sensitization to “spiciness, burning, or stinging sensation” were observed  
313 using a commercially available spicy ginger beer and seltzer water. However, lower ratings for these  
314 sensations over the course the experiment point to desensitization during the experiment. Such order  
315 effects, which may have a physiological as well as psychological basis, should be considered when  
316 planning sensory evaluations.

317

## 318 **Funding**

319 This work was supported by USDA Hatch Project Accession No 1013624. The author has no financial  
320 conflicts of interest in this work.

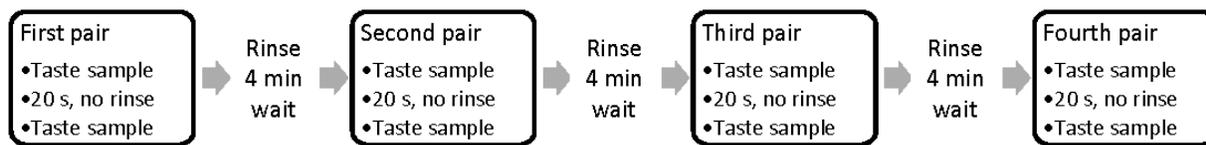
321

## 322 **Acknowledgements**

323 The author thanks Mr. Miguel Odrón for helping administer the sensory tests.

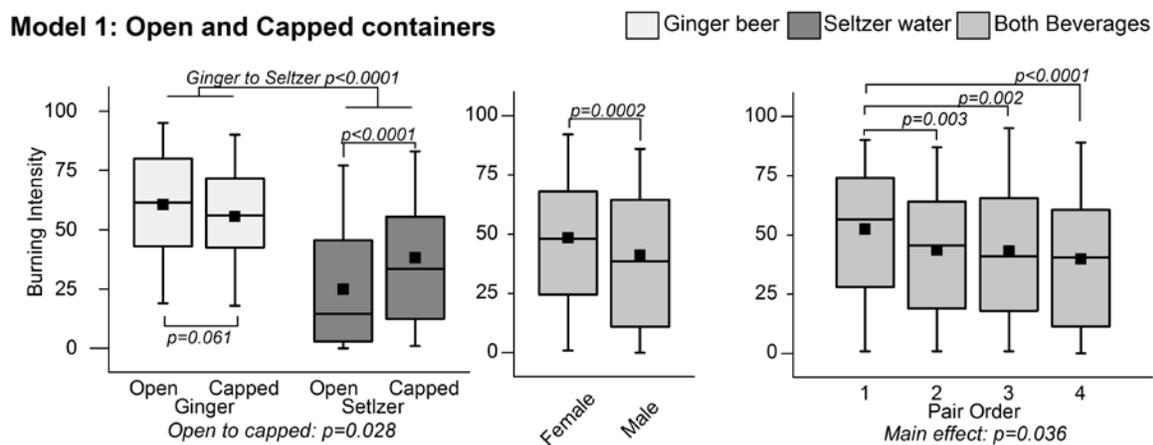
324 Figures

325



326 Figure 1: Tasting paradigm. Order of pairs was counterbalanced.

327



328

329 Figure 2: Results of Model 1 for “Spiciness, burning, or stinging sensation”, analyzing data from open

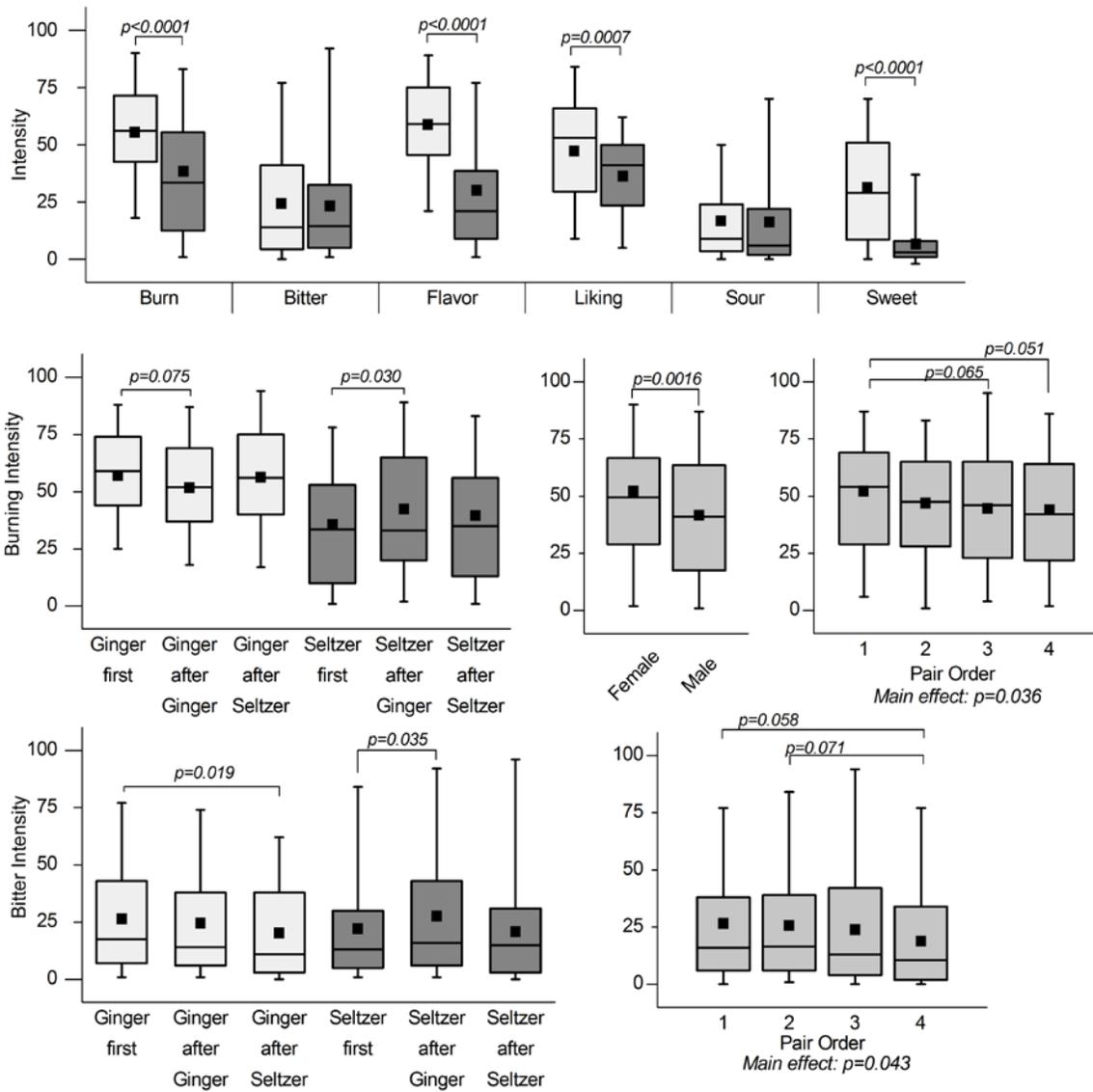
330 cups and capped vials together. Details on interaction effects can be found in supplemental files.

331 Significant or marginal effects are noted with their p-values, all other comparisons were  $p > 0.1$

332

**Model 2: Capped vials only**

□ Ginger beer ■ Seltzer water ▒ Both Beverages



333

334 Figure 3: Significant results of Model 2 for all qualities (top), “Spiciness, burning, or stinging sensation”

335 referred to as Burning (center), and Bitterness (bottom). Significant or marginal effects are noted with

336 their p-values, all other comparisons were  $p > 0.1$

337

**Table 1: Linear Mixed Model details****Model:** Rating = Test, Sample, Gender, PairOrder, Test\*Sample, Test\*Gender, Test\*PairOrder**Explanation of factors:**

Test	Open or Capped	
Pairs / Samples	Pair: Ginger beer – Ginger beer	Ginger beer first
		Ginger beer after Ginger beer
	Pair: Ginger beer – Seltzer	Ginger beer first
		Seltzer after Ginger beer
Pair: Seltzer – Seltzer	Seltzer first	
	Seltzer after Seltzer	
Pair: Seltzer – Ginger beer	Seltzer first	
	Ginger beer after Seltzer	
Gender	Male or Female	
PairOrder	First, Second, Third, or Forth pair of samples in the tasting order	

**Least Squares Means and Estimates:**

Comparison	Purpose
(Ginger beer after Ginger beer) compared to (Ginger beer first)	Sensitization with Ginger beer
(Ginger beer after Seltzer) compared to (Ginger beer first)	Cross sensitization of Ginger beer to Seltzer
(Seltzer after Seltzer) compared to (Seltzer first)	Sensitization to Seltzer
(Seltzer after Ginger beer) compared to (Seltzer first)	Cross-sensitization of Seltzer to Ginger beer
PairOrder	Overall sensitization/desensitization

338

339

**Table 2:** Results from statistical models

**Model for effect of open cups compared to closed vials:**

Rating = Test, Beverage, PairOrder, Test\*Beverage, Test\*Gender

	Mean ± SEM				
	<i>p-value</i>				
	Test	Gender	Beverage	PairOrder	Test*Beverage
<b>Burning</b>	Capped: 47±1	Male: 41±2	Ginger beer: 58±1	1: 52±2	Capped Ginger beer: 56±2
	Open: 43±1	Female: 48±1	Seltzer: 32±1	2: 44±2	Open Ginger beer: 61±2
	<i>p</i> =0.028	<i>p</i> =0.0002	<i>p</i> <.0001	3: 43±2	<i>p</i> =0.061
				4: 40±2	
				<i>p</i> <.0001	Capped Seltzer: 38±2
				<i>Bonferroni adjusted:</i>	Open Seltzer: 25±2
				1 to 2: <i>p</i> =0.003	<i>p</i> <.0001
				1 to 3: <i>p</i> =0.002	
				1 to 4: <i>p</i> <.0001	
				All other <i>p</i> >0.1	

**Model for test of sensitization/desensitization (from capped vials data only):**

Rating = Sample Gender PairOrder

	Mean ± SEM			
	<i>p-value</i>			
	Gender	PairOrder	Beverage type <sup>1</sup>	Sample <sup>2</sup>
<b>Burning</b>	Male: 42±4	1: 52±3	Ginger beer: 56±3	Ginger beer first: 57±3
	Female: 52±2	2: 47±3	Seltzer: 37±3	Ginger beer after ginger beer: 52±3
	<i>p</i> =0.016	3: 45±3	<i>p</i> <.0001	<i>p</i> =0.075
		4: 44±3		Ginger beer after seltzer: 56±3
		<i>p</i> =0.036		<i>p</i> =0.787
		<i>Bonferroni adjusted:</i>		
		1 to 3 : <i>p</i> =0.065		Seltzer first: 36±3
		1 to 4 : <i>p</i> =0.051		Seltzer after seltzer: 40±4
		All other <i>p</i> >0.1		<i>p</i> =0.195
				Seltzer after ginger beer: 42±3
				<i>p</i> =0.030

**Other qualities (only significant effects shown; full results in supplemental files)**

	PairOrder	Beverage type <sup>1</sup>	Sample*
<b>Bitterness</b>	1: 27±2	No effect	Ginger beer first: 26±4
	2: 26±2		Ginger beer after seltzer: 20±4
	3: 24±2		<i>p</i> =0.019
	4: 19±2		Seltzer first: 22±4
	<i>p</i> =0.043		Seltzer after ginger beer: 28±4
	<i>Post hoc (Bonferroni)</i>		<i>p</i> =0.035
	1 to 4: <i>p</i> =0.058		
	2 to 4: <i>p</i> =0.071		
	All other <i>p</i> >0.1		
<b>Flavor</b>	No effect	Ginger beer: 56±3	Seltzer first: 26±3
		Seltzer: 29±3	Seltzer after ginger beer: 38±3
		<i>p</i> <.0001	<i>p</i> <.0001
<b>Liking</b>	No effect	Ginger beer: 47±3	Ginger beer first: 48±3
		Seltzer: 37±3	Ginger beer after ginger beer: 44±3
		<i>p</i> =0.0007	<i>p</i> =0.039
<b>Sweetness</b>	No effect	Ginger beer: 31±3	No effect
		Seltzer: 6±3	
		<i>p</i> <.0001	

<sup>1</sup>Comparison generated using least squared means estimate statement using sample codes (i.e., all ginger beers compared to all seltzer waters)

<sup>2</sup>All sample comparisons are made between when the beverage was tasted first compared to when it was tasted second (either after itself or the other beverages)

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435 Supplemental

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