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Desensitization but not Sensitization from Commercial Chemesthetic Beverages

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Abstract

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

Keywords: spiciness, carbonation, chemesthesis, sensitization, desensitization

1. Introduction

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

Some of the alterations in sensitivity to chemesthetic compounds with repeated exposure can certainly be psychological, as the exposures increase the familiarity and/or could change the affective response to a flavor; however, sensitization and desensitization are mechanistically driven through peripheral cells as well (Bevan, Quallo, & Andersson, 2014; Szallasi & Blumberg, 1999). The phenomenon is perhaps best studied for the transient receptor potential channels (TRP), in particular the subfamily V member 1 or vanniloid receptor 1 (TRPV1). TRPV1 is activated by capsaicin, as well as temperatures above 42°C, acidity, and additional chemical compounds such as allyl isothiocyanate (found in mustard and wasabi) (Bevan et al., 2014; Nagy, Friston, Valente, Torres Perez, & Andreou, 2014). Cellular phosphorylation of specific residues of TRPV1 lead to increased reactivity of the protein to stimuli, while dephosphorylation leads to desensitization (Tominaga, 2006). The dephosphorylation can be driven by calcium flux into the cell, which occurs when TRPV1 is stimulated (see (Bevan et al., 2014) for a detailed discussion of these processes).

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

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

2. Methods

2.1 Samples

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

2.2 Tests

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

2.3 Tasting procedure

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

Participants provided information on their age, gender identity, biological sex, and ethnicity. Next, participants completed a warm-up questionnaire to familiarize them with the visual analog scales our laboratory uses to collect data. The warm-up asked the subject to rate the intensity of the brightness of the sun, the brightness of this room, the loudness of a shout, the loudness of a whisper, the bitterness of black coffee, and the sweetness of pure sugar. Questions were presented in randomized order. Participants were told that we use this scale to verify they understand the scale, and were asked to please rate the items as accurately as possible even if they had attended sessions in our lab in the past (this helps reduce the number of participants who simply click through all the warm-up screens without giving actual ratings). Ratings from the warm-up were used as a check on whether participants understood the directions and used the scale as instructed. This was done by verifying that participants rated the brightness of the sun as greater than the brightness of this room, and the loudness of a shout as greater than the loudness of a whisper. Participants who failed this check were excluded from the final analysis.

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

2.4 Participants

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

2.5 Analysis

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

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

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

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

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

Rating = Sample, Gender, PairOrder

In this model, "Sample" specifically referred to individual samples within the full tasting paradigm (there were eight, see Table 1). "PairOrder" again referred to counterbalanced order of tasting. See Table 1 for the details of the model factors. Participant was entered as a repeated factor, and the covariance structure was set as autoregressive (data were sorted by test, beverage, participant, then order of tasting). After evaluating the overall effects from the factors listed above, least squared means estimates were calculated for specific comparisons, as shown in Table 1. The model was primarily used to interpret effects for "Spiciness, burning, or stinging sensation" (hereafter referred to as "burning"), but since data were collected on bitterness, sweetness, sourness, overall flavor, and liking the results for those are also included (mostly in supplemental file 4). Specific comparisons analyzed to determine sensitization or desensitization are listed in Table 1. From prior work, we expected to observe sensitization most strongly when a sample was tasted immediately after itself within a pair, while we expected to see desensitization through decreasing ratings with increasing tasting order (PairOrder, in our analysis). Note that we used "PairOrder" to observe these effects rather than actual order (i.e, first through eighth), as actual order was confounded with the sample (i.e., "Ginger beer after Ginger beer" could never be tasted first, but it could have been tasted within the first pair). Bonferroni adjustments were used for post hoc analyses involving multiple comparisons. Interaction terms were tested but none were found to be significant, so they were removed for clarity.

3. Results

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

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

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

4. Discussion

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

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

The decreased ratings as the order of tasting pairs increased is likely evidence of desensitization to chemesthesis from carbonation and/or spiciness. We actually expected this effect to be stronger for the spiciness of ginger beer compared to the sting from carbonation, but no such interaction effect was evident. Prior work indicates desensitization occurs for zingerone (from ginger), capsaicin (from chilis), and piperine (from black pepper) (Affeltranger et al., 2007; Dessirier, Nguyen, Sieffermann, Carstens, & O’Mahony, 1999; Prescott & Stevenson, 1996a), which could all plausibly be found in spicy beverage formulations such as the ginger beer used in this study. However, desensitization for carbonation stinging is relatively unexplored. Cross-desensitization has been reported for capsaicin to carbonation sting (Dessirier, Simons, O'Mahony, & Carstens, 2001), which could certainly be the source of desensitization for carbonation observed in our study. In a study separating the sensation of bubbles from carbonation "bite," significant time effects were observed for carbonation bite, and decreases in ratings over time can be seen in the data from that work (Wise, Wolf, Thom, & Bryant, 2013). Potentially, the decreases in ratings could also be due to a habituation type phenomenon instead of true desensitization, where participants become accustomed to the sensation and so lower the associated sensory ratings. However, cellular evidence confirms sensitization/desensitization of transient receptor potential channels, such as for capsaicin and TRPV1 (Gordon-Shaag, Zagotta, & Gordon, 2008; Joseph, Wang, Lee, Ro, & Chung, 2013; Leamy, Shukla, McAlexander, Carr, & Ghatta, 2011; Lennertz, Kossyreva, Smith, & Stucky, 2012; Numata, Kiyonaka, Kato, Takahashi, & Mori, 2011; Zhu et al., 2005). While carbonation has not been extensively tested for desensitization at the cellular level, the TRPA1 receptor responds to acidification from carbonic acid that is created from carbonic anhydrase IV acting on carbon dioxide (Wang, Chang, & Liman, 2010), and so the mechanisms for desensitization similar to other transient reception potential channels may be plausible for carbonation "sting" or "bite."

Given the higher ratings for the first pair of samples compared to other pairs, we considered that the decrease in ratings for both ginger beer and seltzer was due to a "first sample effect" rather than actual desensitization (Lawless & Heymann, 2010). In this case, participants would have initially rated the sample as high due to lack of context, familiarity, or perhaps surprise, but the latter ratings would be stable. To check for this, we re-analyzed the data removing the first time each beverage was tasted (not the entire first pair, just the first rating for each beverage, which may or may not have been in the first pair due to the counterbalancing). In that analysis, the patterns of responses were not substantively different, though the main effect for PairOrder was lost (results included in the supplemental file 4). We suspect this is due to loss of power from excluding some of the sample size, as the means across the PairOrders still followed the same general downward pattern. Thus, while the first sample may indeed be rated differently from the others, desensitization should still be considered as well.

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

Finally, we were not surprised to find differences between ratings for open compared to capped containers when serving carbonated beverages. However, the finding that the ginger beer had a trend toward being less intense for burn when capped compared to open was unexpected. As many of our participants were likely the same in both tests, this could have been due to increased familiarity with the product (we have found in many tests that ratings tend to decrease over time, especially for “bad” sensations; unpublished data). However, the phenomenon of initial elevation bias has been observed in psychological surveys of thoughts, feelings, and behaviors (Shrout et al., 2018). Notably, this group also found that this initial elevation of ratings/values was higher for negative than positive affect. Nonetheless, without knowing for certain how many and which subjects were repeated in our own experiments, we cannot state for certain that the potential decline in ginger beer burn was due to this elevation bias effect or due to another factor, such as interaction with other sensory active ingredients.

5. Conclusions

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

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Figures

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

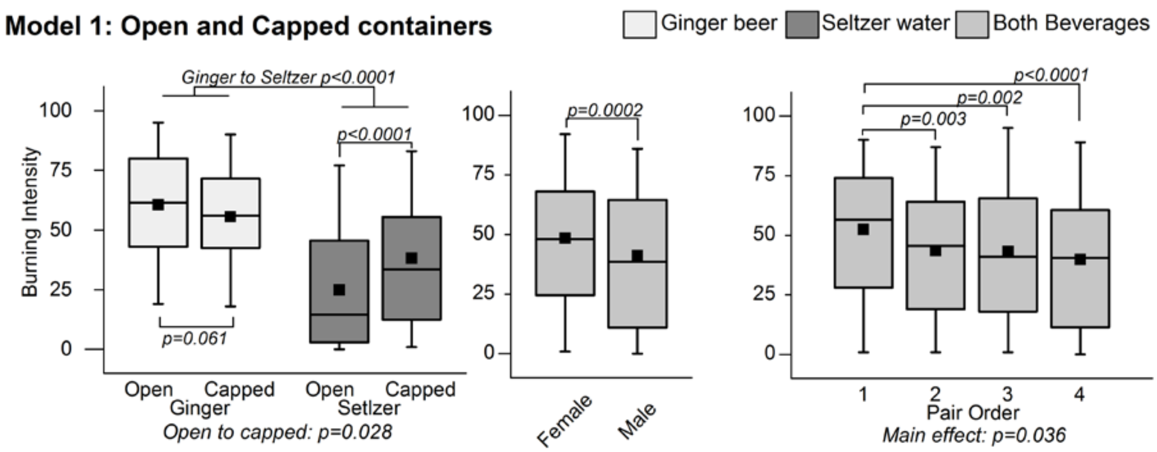

Figure 2: Results of Model 1 for "Spiciness, burning, or stinging sensation", analyzing data from open cups and capped vials together. Details on interaction effects can be found in supplemental files. Significant or marginal effects are noted with their p-values, all other comparisons were $p>0.1$

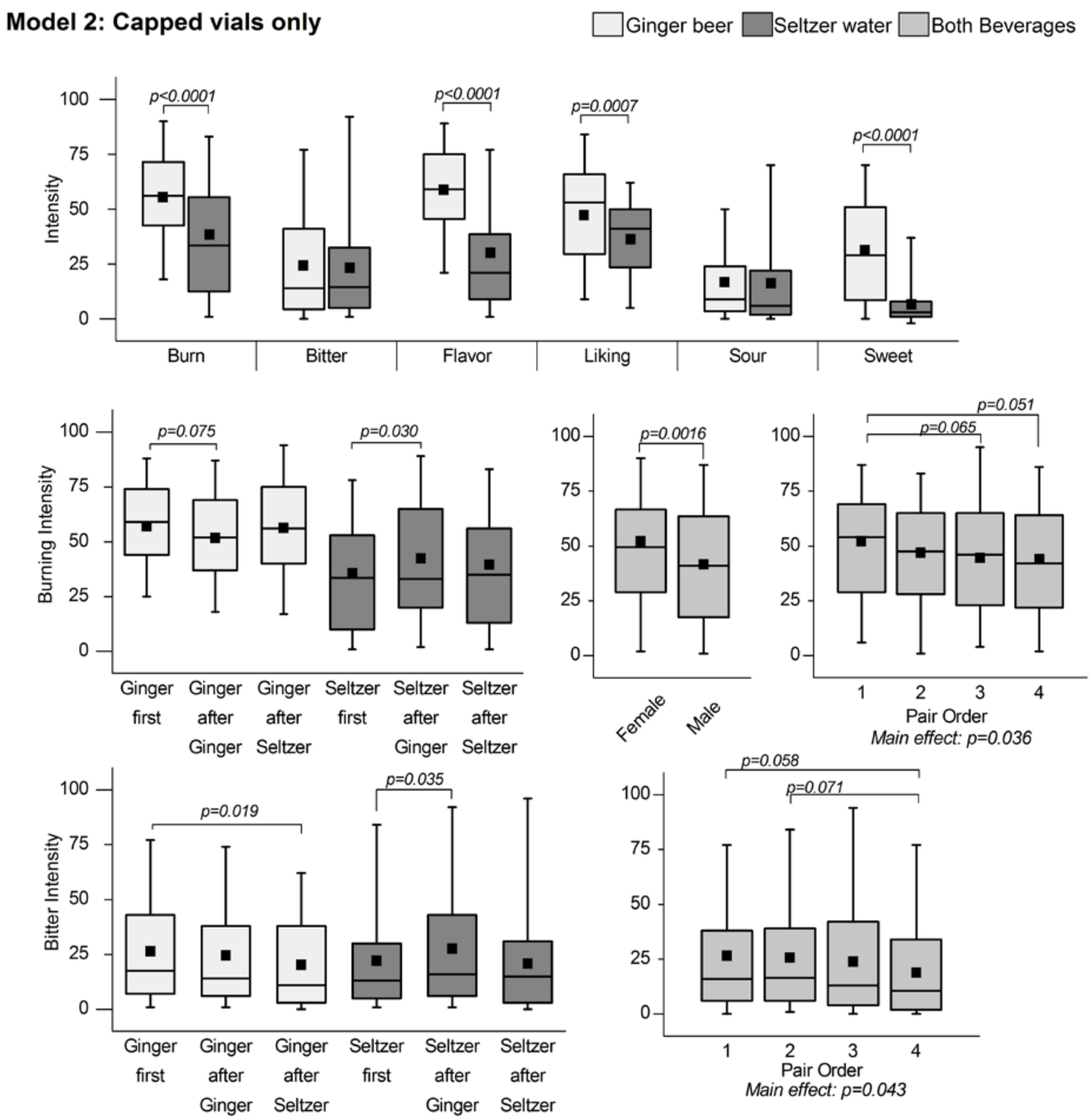

Figure 3: Significant results of Model 2 for all qualities (top), "Spiciness, burning, or stinging sensation" referred to as Burning (center), and Bitterness (bottom). Significant or marginal effects are noted with their p-values, all other comparisons were p>0.1

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

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
p-value

	Test	Gender	Beverage	PairOrder	Test*Beverage
Burning	Capped: 47±1 Open: 43±1 *p=0.028*	Male: 41±2 Female: 48±1 *p*=0.0002	Ginger beer: 58±1 Seltzer: 32±1 *p<.0001*	1: 52±2 2: 44±2 3: 43±2 4: 40±2 *p<.0001* *Bonferroni adjusted:* *1 to 2: p=0.003* *1 to 3: p=0.002* *1 to 4: p<0.0001* *All other p>0.1*	Capped Ginger beer: 56±2 Open Ginger beer: 61±2 *p=0.061* Capped Seltzer: 38±2 Open Seltzer: 25±2 *p<0.0001*

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

Rating = Sample Gender PairOrder

Mean ± SEM
p-value

	Gender	PairOrder	Beverage type[1]	Sample[2]
Burning	Male: 42±4 Female: 52±2 *p=0.016*	1: 52±3 2: 47±3 3: 45±3 4: 44±3 *p=0.036* *Bonferroni adjusted:* *1 to 3 : p=0.065* *1 to 4 : p=0.051* *All other p>0.1*	Ginger beer: 56±3 Seltzer: 37±3 *p<0.0001*	Ginger beer first: 57±3 Ginger beer after ginger beer: 52±3 *p=0.075* Ginger beer after seltzer: 56±3 *p=0.787* Seltzer first: 36±3 Seltzer after seltzer: 40±4 *p=0.195* Seltzer after ginger beer: 42±3 *p=0.030*

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

	PairOrder	Beverage type[1]	Sample*
Bitterness	1: 27±2 2: 26±2 3: 24±2 4: 19±2 *p=0.043* *Post hoc (Bonferroni)* *1 to 4: p=0.058* *2 to 4: p=0.071* *All other p>0.1*	No effect	Ginger beer first: 26±4 Ginger beer after seltzer: 20±4 *p=0.019* Seltzer first: 22±4 Seltzer after ginger beer: 28±4 *p=0.035*
Flavor	No effect	Ginger beer: 56±3 Seltzer: 29±3 *p<0.0001*	Seltzer first: 26±3 Seltzer after ginger beer: 38±3 *p<0.0001*
Liking	No effect	Ginger beer: 47±3 Seltzer: 37±3 *p=0.0007*	Ginger beer first: 48±3 Ginger beer after ginger beer: 44±3 *p=0.039*
Sweetness	No effect	Ginger beer: 31±3 Seltzer: 6±3 *p<0.0001*	No effect

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

[2]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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