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Characterizing Dynamic Sensory Properties of Nutritive and Nonnutritive Sweeteners with Temporal Check-All-That-Apply

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Abstract

Temporal Check-All-That-Apply (TCATA) is a rapid method where attributes are actively checked and unchecked to track sensations over time, so that the checked words fully describe the sample in any given moment. Here, we characterize the temporal profiles of sweeteners using TCATA. In two experiments, sweeteners were tested in water at concentrations known to elicit weak to moderate sweetness. Before evaluating the sweeteners, participants ($n = \sim 125$ per experiment) were familiarized with the TCATA method with a guided example using imaginary samples of Kool-Aid. Participants then received 10 ml of each sweetener solution and were instructed to click “start” after he or she finished swallowing the sample. Incidence of 6 attributes obtained from prior literature – sweet, bitter, metallic, licorice, cooling, and drying – was obtained for 60s. We observed similarities across temporal profiles of various sweeteners: sweet and bitter occurred first (~ 10 s), then metallic and cooling (~ 10 – 25 s), followed by drying much later (30–50s depending on the stimulus). The presence of side tastes influenced how often ‘sweet’ was endorsed: stimuli with fewer endorsements of side tastes showed more prominent sweetness. Finally, unfamiliar side tastes may lead to a smearing bias where participants check every attribute in an attempt to characterize those unfamiliar sensations.

Keywords

fructose; rebaudioside; thaumatin; neohesperidin dihydrochalcone; sucralose

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Conflict of interest disclosure

M.M.R. has no conflicts to disclose.

Author Contributions

J.E.H conceived and designed experiments, interpreted data, and revised the manuscript. M.M.R. designed and performed the experiments, analyzed and interpreted data, and prepared the manuscript. J.C.C. designed experiments, interpreted data, and edited the manuscript.

Introduction

Taste is a dynamic process (Lawless and Heymann 2010) where physical and physiological interactions in the oral cavity may cause swift changes in perception. Rapidly evolving sensations make characterizing the sensory profile of products challenging. Although many temporal methods have been developed to document the sensory profile of products over time, many of these methods do not allow for continuous tracking of a sensation, or are relatively inefficient regarding the breadth of data they can provide. For example, continuous time intensity (TI) scaling only allows for either one or possibly two attributes to be scaled at a time (Lee and Pangborn 1986, Duizer, Bloom et al. 1997, Pineau, Schlich et al. 2009). Kroeze (1982) defined side tastes as ‘taste sensations that are qualitatively distinct from the main taste of a stimulus, and have a lower intensity than the main taste’; existence of such side tastes is widely reported for many non-nutritive sweeteners (e.g., DuBois and Prakash 2012; Antenucci and Hayes 2015). Accordingly, to fully characterize the sensory profile of a sweetener over time, an assessor may be required to evaluate the product in numerous sessions, with a specific scale for the attribute being tested in that particular session. On the other hand, sensory methods like Multi-Attribute Time Intensity (MATI) (e.g. (Green and Hayes 2004, Nolden, McGeary et al. 2016)) require evaluation of the sample at discrete time points and may therefore miss quick changes in sensory profile (Kuesten, Bi et al. 2013).

Temporal Check-All-That-Apply (TCATA) is a recently described rapid sensory method that documents multiple sensory qualities of a sample dynamically over time (Boinbaser, Parente et al. 2015, Oliveira, Antúnez et al. 2015, Castura, Antúnez et al. 2016). TCATA involves continuous checking and unchecking words to track changes in the sample, such that at any given moment, the collection of words that are checked fully describe the sample. During sample evaluation, participants check attributes at the times when the attribute is applicable (e.g., check “sweet” when the sample is sweet) and uncheck those attributes when they are no longer present. Critically, TCATA allows participants to check multiple attributes simultaneously. This use of multiple attributes in TCATA allows for tracking of side tastes that are not the most intense / dominant sensation (Oliveira, Antúnez et al. 2015), potentially making it a useful technique to study the differing temporal profiles of nonnutritive sweeteners. Because many nonnutritive sweeteners evoke side tastes other than sweet, TCATA is able to not only identify their occurrence, but also *when* they occur, providing a more holistic taste profile of the sweeteners studied.

The importance of the temporal profile of nonnutritive sweeteners has become increasingly apparent in product development as sweet intensity is not the only relevant attribute in selecting lower calorie alternatives to sucrose (DuBois and Prakash 2012). Previous work suggests sweeteners differ in sweetness onset, linger, and rate of intensity (DuBois and Lee 1983, Shamil, Birch et al. 1988, Ayya and Lawless 1992, Temple, Laing et al. 2002, Schiffman, Sattely-Miller et al. 2007, Palazzo, Carvalho et al. 2011), as well as presence or absence of side tastes such as bitter, metallic, and sour (DuBois, Walters et al. 1991, Ayya and Lawless 1992, Kinghorn 2010, Antenucci and Hayes 2015). However, to the best of our knowledge, previous work has only investigated side tastes of sweeteners at a single time point (DuBois, Walters et al. 1991), with the exception of bitter side tastes (Swartz 1980, Ayya and Lawless 1992). Recent work has used temporal dominance of sensations (TDS) to

investigate the temporal profiles of sweetener side tastes, but due to the nature of the TDS (where only the most dominant attributes are tracked), many of the weaker side tastes were not documented (Zorn, Alcaire et al. 2014). Many side tastes of sweeteners such as metallic, licorice, and cooling remain uninvestigated temporally. Accordingly, the objective of the present study was to comprehensively characterize and compare the dynamic properties of nonnutritive sweeteners using TCATA.

Methods

Overview

Participants were given 10 mL aliquots of sweetener solutions, consisting of a single compound in water, and asked to track the attributes perceived over 60 s using TCATA. In two separate experiments, ~125 adults participated in each (total n = 248).

Participants

Participants were recruited using an existing opt-in database maintained by the Sensory Evaluation Center at Penn State; this pool consists of 1,000+ individuals who are diverse in age and gender. To qualify for participation, individuals were prescreened on the following criteria: aged 18 to 50, non-smoker, no tongue/lip/cheek piercings, no history of choking or difficulty swallowing, no pregnant or breastfeeding women, and no known food allergies. This study was exempted from IRB review by the Penn State Office of Research Protections under the wholesome foods exemption in 45 CFR 46.101(b)(6). Participants provided informed consent and were compensated for their time.

Test Stimuli

Sucrose (Domino, Yonkers, NY, USA), glucose (Dot Scientific, Burton, MI, USA) acesulfame-potassium (AceK) (donated by PepsiCo), aspartame (Spectrum, New Brunswick, NJ, USA), sucralose (Tate & Lyle, Decatur, IL, USA), saccharin (Spectrum, New Brunswick, NJ, USA), fructose (Alfa Aesar, Ward Hill, Massachusetts, USA), thaumatin (Alfa Aesar, Ward Hill, Massachusetts, USA), neohesperidin dihydrochalcone (NHDC) (Tokyo Chemical Industry Co., Tokyo, Japan), and rebaudioside A (RebA; 98% pure) (Tokyo Chemical Industry Co., Tokyo, Japan) were tested in water at concentrations previously shown to elicit weak to moderate sweetness on a general labeled magnitude scale (gLMS). Stimuli were chosen to represent a broad array of commercially relevant sweeteners: carbohydrate sweeteners (sucrose, glucose, and fructose), synthetic nonnutritive sweeteners (AceK, aspartame, sucralose, saccharin, and NHDC), and natural nonnutritive sweeteners (thaumatin and RebA). Concentrations were determined from previous studies conducted by our team with a different set of participants (under review). Here, each sweetener was tested at two concentrations: a lower concentration that roughly corresponds to a weak ('6') rating on a gLMS and a higher concentration that should elicit a rating near moderate ('17'); see Tables 1 & 2. No intensity ratings were collected here. An additional sample of the higher sucrose concentration (i.e., sucrose moderate) was included as a blind replicate for test day one, while an additional sample of the higher fructose concentration (i.e., fructose moderate) was used for day two. All solutions were prepared at least 24 hours

prior to testing with reverse osmosis (RO) water. Within one session, a total of 11 test samples were presented in counterbalanced order using a Williams design (Williams 1949).

TCATA Orientation

Testing was conducted in individual, computerized booths under a northern daylight illuminant (5000K daylight LED) located directly overhead and the data were collected using Compusense Cloud 5.6 (Compusense Inc., Guelph, Ontario, Canada). Prior to testing, participants were given reference stimuli and definitions for the following attributes: sweetness, bitterness, drying, licorice, metallic, and cooling (Table 3). Inclusion of these specific attributes was based on prior literature (DuBois and Prakash 2012).

After attribute orientation, participants familiarized themselves with the TCATA protocol by following a brief guided example in Compusense Cloud using imaginary samples of Kool-Aid. In the orientation, participants were told to imagine changes over time in the flavor of Kool-Aid by checking and unchecking words in a list as prompted by pop-up directions on the computer screen. Participants were given the opportunity to repeat the guided example until they understood the evaluation method. The participants then repeated the procedure with a sample of Kool-Aid without pop-up instructions. For most participants, the total process took under 5 minutes.

Sample Evaluation

After orientation, participants received 10 ml aliquots of each sweetener solution at room temperature in plastic medicine cups labeled with a three-digit blinding code. Before tasting each sample, participants were told to thoroughly rinse their mouths with RO water and to familiarize themselves with the attribute list on the screen. Next, participants were instructed to place the entire sample in his or her mouth, swirl in mouth for 5 s (with a countdown timer provided on the screen), and then click “start” once he or she finished swallowing. To prevent potential inconsistencies in timing by the participants, evaluation occurred after swallowing. Each evaluation consisted of 6 attributes (sweetness, bitterness, drying, licorice, metallic, and cooling) and lasted for 60 seconds (Figure 1). Attribute order was counterbalanced across assessors to minimize the effect of position (Bennett and Hayes 2012, Ares and Jaeger 2013), but the order remained fixed within a single assessor to reduce confusion. The participants were then instructed to wait 60 seconds before proceeding to the next sample and inter-stimulus interval was enforced with a countdown at the bottom of the screen. Participants rinsed and expectorated room temperature RO water between samples.

Statistical Analysis

TCATA curves and difference curves were obtained using the approach described previously (Castura, Antúnez et al. 2016). Smoothed trajectories were obtained for the samples using principal component analysis (PCA) on the TCATA citation rates, as described elsewhere (Castura, Baker et al. 2016). Code from the R package *tempR* (Castura 2016) was used to construct the biplots, where trajectories were smoothed along each dimension independently. Briefly, TCATA curves graphically summarize citation rates for each attribute in a sample at each moment of the evaluation, while TCATA difference curves represent pairwise comparisons of individual pairs of samples. Attributes checked at a proportion greater than

chance probability (1/6) were reviewed for TCATA curves. For each attribute and for each stimulus, three summary parameters were used to evaluate the TCATA curves. First, area under the curve (AUC) was calculated from TCATA curves. Second, maximum citation proportions (MaxCite) were calculated as the maximum proportion of times an attribute was selected at each moment of evaluation. MaxCite values correspond to the peak of the TCATA curves. Lastly, the time when maximum citations (MaxTime) occurred was determined.

The TCATA difference curves are plotted by subtracting the citation proportions for each attribute in the pair of samples at each time point, and Fisher's exact test is used to determine significant differences. All data were analyzed using R software version 3.3.1 (R Core Team 2016).

Results

TCATA curves

TCATA curves were used to visualize the dynamic profiles of the sweeteners studied here; see Figure 2, Supplemental Figure 1 and Supplemental Figure 2. AUCs for each attribute are summarized and presented in Figure 3 and Supplemental Figure 3. The attributes endorsed for each sweetener are summarized in Table 4. Dots in Table 4 indicate attributes checked at a proportion greater than 1/6 (i.e., chance with six attributes) for at least 30% of consecutive time points for the moderate concentration samples. This value (30%) was selected following visual inspection of the curves; while somewhat arbitrary, we felt it was appropriate to have a fixed decision rule for consistency. MaxTime values for each attribute were plotted and presented in Figure 4.

From the TCATA curves, we found carbohydrate sweeteners to have the fewest side tastes and were characterized as sweet, drying, cooling. Non-nutritive sweeteners (both synthetic and natural) had more side tastes than the carbohydrate sweeteners. The synthetic sweeteners were perceived as sweet, bitter, metallic, drying, and cooling. While the natural sweeteners were also perceived as sweet, bitter, metallic, and cooling, they were also the only samples to be perceived as having the licorice attribute.

As would be expected, the AUC values were generally the highest for sweetness for almost all of the stimuli (Figure 3a), with thaumatin, sucralose, and aspartame having the highest AUC values. Both concentration levels of NHDC had markedly lower AUC values than the rest of the sweeteners tested. Synthetic and natural sweeteners also had greater AUC values for bitterness than the carbohydrate sweeteners (Figure 3b). Saccharin, RebA, and AceK had the highest AUC values in the moderate level, while AUC values for the weak level samples were not as markedly different. For metallic, RebA and AceK had the highest AUC values for the moderate level, while thaumatin had the highest AUC values for the weak level, followed by NHDC, saccharin, and AceK (Figure 3c). Lastly, in the moderate concentration thaumatin and RebA had the highest AUC values for licorice, but weak concentration samples of all sweeteners did not have notably different licorice AUC values (Figure 3d).

To explore and quantify the temporal nature of the results, we plotted MaxTime values for all attributes over time, except licorice. Licorice was not plotted as only the moderate concentration samples for RebA and thaumatin were cited above 1/6. We found that attributes generally peaked at similar times across the different sweeteners and followed a similar order: sweetness peaked first, followed by bitterness, metallic, cooling, and finally drying. For sweet, the average MaxTime value was 8.8 ± 3.4 s (Figure 4, top left). With the exception of weak NHDC, most sweeteners peaked around the average time. For bitter, the average MaxTime value was 12.5 ± 2.9 s (Figure 4, top right). Unlike sweet, the bitter MaxTime values were more spread out, ranging from 9 s to 19 s. For metallic, the average MaxTime value was 14.9 ± 5.0 s (Figure 4, middle left). Like bitter, the metallic MaxTime values had a larger spread, ranging from 9 s to 24 s. For cooling, the average MaxTime value was 15.5 ± 11.3 s (Figure 4, middle right). Both levels of sucrose were outliers in the cooling plot and when their values are removed, the mean MaxTime became 11.75 ± 2.5 s for cooling. Lastly, for drying, the mean MaxTime value was 29.3 ± 7.4 s (Figure 4, bottom). As with bitter and metallic, the drying MaxTime values had a large spread, ranging from 15 s to 45 s.

Smoothed Trajectories

The temporal results found in the MaxTime summary graphs are further illustrated by the smoothed trajectories. PCA was performed to graphically represent the association between samples and temporal attributes. These biplots are read as product trajectories. The far end of a line away from the sample number is the start of the trajectory. As you move along the trajectory, that indicates the attribute perception over time. An example of this would be moderate AceK (sample 4 in Figure 5): the trajectory starts in the middle where it is near nothing, then it moves towards sweet, then pulled down towards bitter, then it loops around cooling and licorice, and ends near drying.

Two principal components explained most of the variation in sensory attributes among the nonnutritive sweeteners: 80% for test day 1 and 87% for test day 2. For both test days, the first component (horizontal axes in Figures 5 & 6) was correlated highly with sweetness, while the second component correlated highly with side tastes (bitterness, cooling, licorice, metallic, and drying). From the test day 1 biplot (Figure 5), we can see that the sucrose moderate and its blind replicate have similar trajectories (stimuli 2 and 11). Additionally, the fructose moderate and blind replicate samples from the test day 2 biplot (Figure 6) also have similar trajectories (products 2 and 11). These results suggest samples were consistently evaluated by the panel. In both biplots, we also see that the moderate level stimuli (2, 4, 6, 8, 10, and 11 in each) were more frequently endorsed as sweet than the weak level stimuli (products 1, 3, 5, 7, and 9). Instead, trajectories for weak concentration stimuli are aligned more closely with the side tastes than for the stimuli at the moderate concentration.

Finally, the trajectory for weak NHDC was markedly different from the other sweeteners (sample 3 in Figure 6), suggesting that participants perceive it as having a quality mainly characterized by drying. The moderate concentration of NHDC (product 4 in Figure 6) was also characterized by other attributes like licorice, metallic, and cooling, but its trajectory did not approach sweet, in contrast to the moderate concentrations for the other sweeteners.

These associated side flavors found in the smoothed trajectory are consistent with the results from the TCATA curves.

Difference Curves

The results from the smoothed trajectories are consistent with the difference curve results showing that the moderate levels had fewer side tastes than the weak levels. Looking at the difference curves for the same sweetener at two concentrations, side tastes were cited more frequently for the weak concentration than for the moderate concentration. For example, for aspartame, bitter, metallic, drying, and cooling are cited significantly more for the weak concentration than for the moderate concentration (Figure 7, top). Meanwhile, the moderate concentration was only cited significantly more for sweetness (as would be expected). A comparison of weak and moderate sucralose found similar results: participants found the weak concentration bitter, metallic, drying, and cooling significantly more often than for the moderate concentration (Figure 7, bottom).

We also compared different sweeteners with the same expected sweet intensity using difference curves, finding that sweeteners with fewer side tastes were perceived sweet more often than those with more side tastes. For example, Figure 8 (top) shows weak sucrose compared to weak AceK; sucrose was endorsed as being sweet more frequently than AceK, while AceK was endorsed as bitter, metallic, drying, and cooling significantly more frequently than sucrose. A similar pattern was seen when moderate fructose was compared to moderate NHDC (Figure 8, bottom). Sweet was endorsed significantly more often for fructose than for NHDC. Also, while fructose was also seen as cooling more than NHDC, NHDC had more side tastes that lasted longer, such as bitterness and drying.

Lastly, from the TCATA difference curves, we found that the blind replicates used in each study day was not significantly different from their counterparts, suggesting participants were able to perform the task consistently (Supplemental Figure 4).

Discussion

From the TCATA curves, difference curves, and product trajectories, we find similarities in temporal profiles among the different sweeteners. Sweet is cited most frequently in the earlier time points, with maximum sweet citations ranging in the time points of 7 s to 11 s for the carbohydrate sweeteners, natural nonnutritive sweeteners, and for most synthetic nonnutritive sweeteners. The one exception was NHDC, which had a very low sweet maximum citation (0.09) at 22 s to 22.5 s for the weak concentration. For those sweeteners perceived as bitter – AceK, RebA, NHDC, and saccharin – that attribute is also experienced earlier in the product evaluation, generally after sweet has peaked. For the synthetic sweeteners, time to bitter maximum citations ranged from 9 s to 14 s, similar to the natural sweeteners time to maximum values of 9 s to 19.5 s. Metallic also peaked after sweet and bitter. For metallic, peak citation times ranged from 13.5 s to 15.5 s for NHDC and saccharin and slightly later at 17.5 s to 21.5 s for thaumatin. After sweet, bitter, and metallic attributes pass their peaks, participants perceive cooling more often than in earlier time points. For 10.5 s to 13.5 s for most of the carbohydrate sweetener samples, 9 s to 18.5 s for the synthetic sweetener samples, and 10 s to 16 s for the natural sweeteners. Sucrose had time to

maximum cooling citation values that were later than for the other sugars: 35 s for the weak concentration and 56.5–58.5 s for the moderate concentration. Lastly, drying was perceived most often at the end of the evaluation for all sweeteners, with maximum drying citations occurring from 21.5 s to 43 s.

Interestingly, all samples were rated as both drying and cooling, even the sugars which are usually found to be only sweet (Ayya and Lawless 1992, DuBois and Prakash 2012). A potential cause of all the samples being perceived as drying may arise from the total number of samples tasted and rinses between them. Each participant tasted 6 samples during attribute orientation, one sample for TCATA procedure orientation, and 11 product samples, for a total of 18 samples (with rinsing in between). The extensive number of samples and rinses could have depleted salivary proteins involved in lubricity and thereby increasing the perception of drying. While all the samples were also perceived as cooling, the ratings were inconsistent within the samples. For example, fructose, a stimuli not typically considered to elicit cooling, had a maximum cooling citation of 0.3 in the moderate level and participants experienced it at both 13–13.5 s and 21.5–22.5 s. This leads us to conclude that the participants were not properly identifying cooling. It is possible that participants perceived the difference in temperature between room temperature samples and body temperature as cooling. Another possibility is that participants might have thought the cooling related to refreshing or thirst quenching properties. Additional work is needed to clarify this finding.

Aside from drying and cooling, the other side tastes elicited by sweeteners varied among the individual sweeteners and between the different sweet intensity levels. From the difference curves of the same sweetener at different intensities, we found that more side tastes appear in the weak concentration than in the moderate concentration, while only sweet is perceived more often in the moderate level (Figure 8). As sweet was endorsed more frequently at the moderate concentration, sweet may be more prominent making the other side tastes less pronounced than in weak concentration. This was initially surprising and generally contrary to what we would have expected, as the off tastes of nonnutritive sweeteners tends to increase with concentration (Antenucci and Hayes 2015). Also, there were some exceptions to this pattern, such as NHDC, RebA and thaumatin having a licorice side taste and AceK and saccharin having more bitter side tastes in the moderate level, in accordance with the expected side tastes for these stimuli (Ayya and Lawless 1992, DuBois and Prakash 2012, Antenucci and Hayes 2015). That would lead us to believe that in those instances, the side tastes were not masked by the prominence of sweet, and increased with concentration as expected. These exceptions also argue against simple mixture suppression as an explanation. Collectively, this suggests the overall increase in endorsement of side tastes in the weaker samples may represent a non-specific response bias when participants are struggling to describe the sensations.

Also, our data suggest sweeteners with unfamiliar side tastes, such as NHDC and thaumatin, may show a “smearing bias” wherein some participants flail about, endorsing many attributes in an attempt to characterize unfamiliar side tastes. Previously, we discussed a potential “smearing bias” when untrained assessors were given stimuli with unfamiliar sensations and lots of response options (Bennett and Hayes 2012). That is, when given seven rating scales for various chemesthetic sensations (i.e., itch, tickle, burn, etc.), participants

appeared to use *all* of the scales (relatively indiscriminately) to rate the overall intensity of the stimulus, such that high ratings for one quality was strongly correlated with a high rating for all the other qualities (Bennett and Hayes, AChemS 2011 poster). We speculated smearing may be the inverse of a dumping bias (Bennett and Hayes 2012), which occurs when a participant *lacks* the opportunity to rate a salient quality due to the absence of an appropriate scale, and then dumps into an inappropriate attribute (Lawless and Heymann 2010). One potential strategy to mitigate smearing bias may be to have a catch-all attribute, such as “indescribable” or “other” to describe sensations that could not be characterized due to lack of clarity. Kawasaki and colleagues speculated such a category might be used for sensations above detection threshold but below identification threshold (e.g., Kawasaki, Sekizaki et al. 2016). Also, as noted by an anonymous reviewer, TCATA tasks might also be susceptible to another related bias, over-partitioning, which describes the depression of intensity scores when too many scales are provided (Lawless and Heymann 2010). On its face, it seems like over-partitioning might be less relevant in a TCATA task, given that TCATA looks at incidence, and not intensity. Still, as TCATA becomes more widely adopted, it would be very prudent to confirm that adding checkboxes for irrelevant and/or distractor attributes does not influence (i.e., depress) the willingness of participants to check boxes for the salient characteristics of a product or stimulus.

Looking at the different sweeteners at the same intensity level (i.e., sucrose moderate compared with RebA moderate), we found that sweeteners with fewer side tastes were perceived sweet more often (Figure 7). Conversely, we could view this as sweeteners with more side tastes were characterized less as being sweet. The various side tastes may be suppressing the perception of sweet in these samples, possibly via mixture suppression (Pelletier, Lawless et al. 2004, Lawless and Heymann 2010). Generally, the difference curve comparisons suggest that the simple sugars were endorsed as sweet more often than both the natural and synthetic nonnutritive sweeteners. Comparison of difference curves also found that glucose and fructose were not perceived as significantly different at both weak and moderate levels (Supplementary Figure 2). This finding is consistent with the concept of monogeusia, which is the inability to discriminate between simple sugars that are evaluated at concentrations of comparable intensity (Kemp, Beauchamp et al. 1994). However, we should also note that TCATA asks participants to characterize products in terms of presence or absence of an attribute without scaling, so differences in level rather than in kind could be missed.

Several potential limitations of this work should be mentioned. First, the evaluations began after the participants swallowed; whether different results might occur with in-mouth evaluation is unknown. Second, we did not include a water blank within our sample set – in hindsight, this would have been helpful to help assess the likelihood of participants selecting attributes randomly (i.e., a demand characteristic of the task). Third, our study did not account for well known individual differences in the perception of nonnutritive sweeteners which are due, in part, to genetic variability. Specifically, AceK and saccharin are known to possess bitter side tastes that increase with concentration (Horne, Lawless et al. 2002, Antenucci and Hayes 2015), and perception of this bitterness varies with polymorphisms in the bitter taste receptor gene *TAS2R31* (Allen, McGeary et al. 2013, Allen, McGeary et al. 2013). As the participants in this study were not segmented or stratified by genotype, the

group proportions shown here may not accurately represent the bitterness from these two sweeteners within various subgroups. Even without genetic information, it would be interesting to segment participants on the basis of phenotype (i.e., differences in perceived intensity) to see how this may influence TCATA results; we were unable to do so here, as we did not collect intensity ratings within these participants. This should be revisited in future work. Lastly, no attribute extinction times were found for any of the samples. This may be due to the consumers being more attentive with identifying onset than offset of sensations (Ares, Castura et al. 2016), or simply an artifact arising from the relatively short time frame of the study (60 s). Future work should be conducted with a longer time frame using TCATA fading. In TCATA fading, the selected attributes gradually fade so that it is obvious to assessors that the attribute is no longer present (Ares, Castura et al. 2016). To confirm that the attribute is still present, the participant must actively click on the attribute again before it fades into the same shade as the unselected attributes. Future work should use this approach to explore the offset time of these sweeteners.

Conclusion

Results from our experiments found similarities among temporal profiles of sweeteners. Sweet and bitter occur around the first 10 s, then metallic and cooling around 10–25 s, and then drying much later, 30–50 s depending on the stimulus. We also found that side tastes influence how often sweetness was endorsed: more side tastes make sweetness less pronounced (i.e., fewer citations), and stimuli with fewer endorsements of less side tastes are perceived with more prominent sweetness (taking care to note that prominence is not synonymous with intensity, as TCATA does not measure intensity). This does not seem to be due to mixture suppression, and may instead reflect demands of the task on the participant. Additionally, less familiar side tastes may lead a smearing bias where participants check every attribute in an attempt to characterize those unfamiliar sensations. Lastly, extinction time was not found here, suggesting studies with TCATA fading may be appropriate future direction for this research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Practical applications

Traditional continuous time intensity methods only allow one or two attributes to be assessed at a time, while discrete multi-attribute time intensity (MATI) methods may miss important changes in sensation, depending on the interval between ratings. The inclusion of multiple attributes in TCATA allows continuous tracking of side tastes that are not the most intense / dominant sensation; this may be especially important for stimuli where a secondary attribute is still a strong driver of acceptability in spite of not being the most intense sensation, as with nonnutritive sweeteners.

Sample: 976



Figure 1.
Example TCATA evaluation.

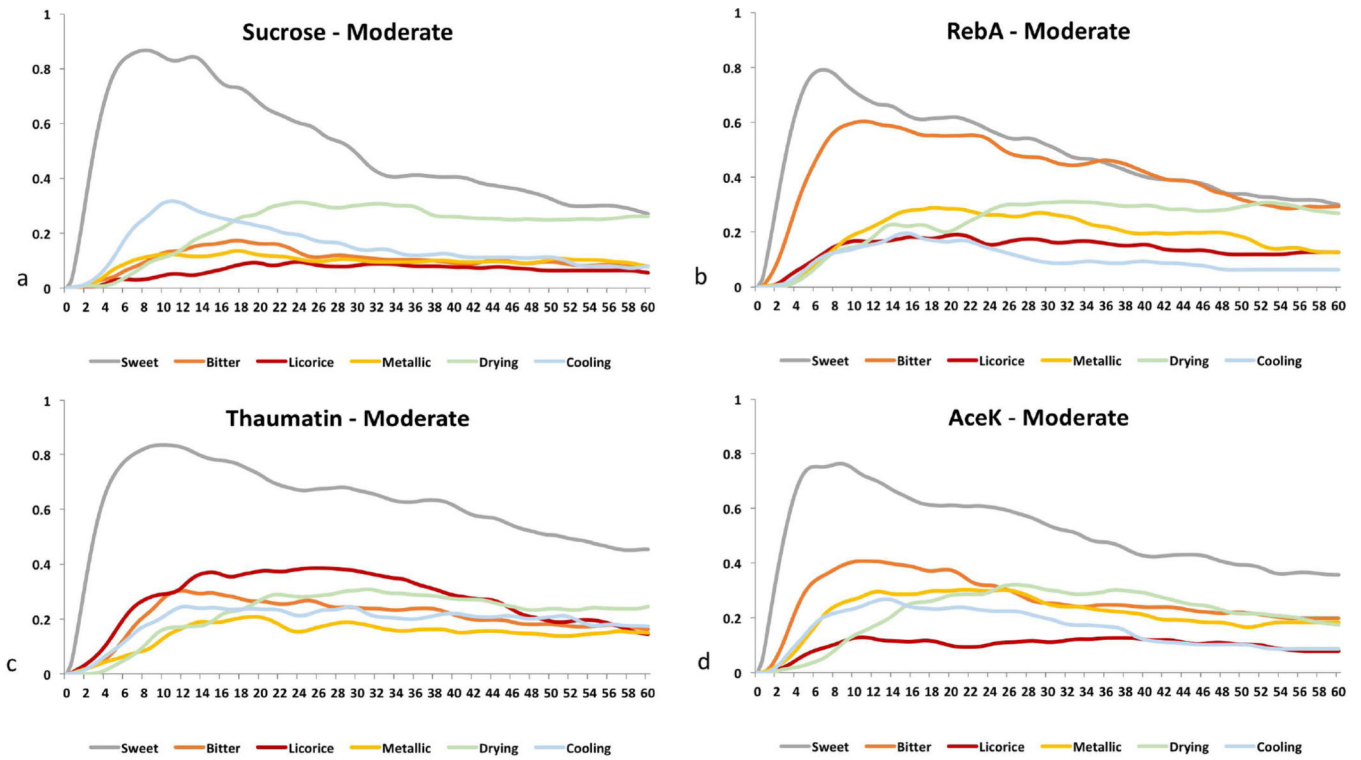


Figure 2. TCATA curves for (a) sucrose, (B) RebA, (c) thaumatin, and (d) AceK at concentrations found to elicit moderate sweetness intensities on a gLMS. TCATA curves graphically represent citation proportions for each attribute in a sample.

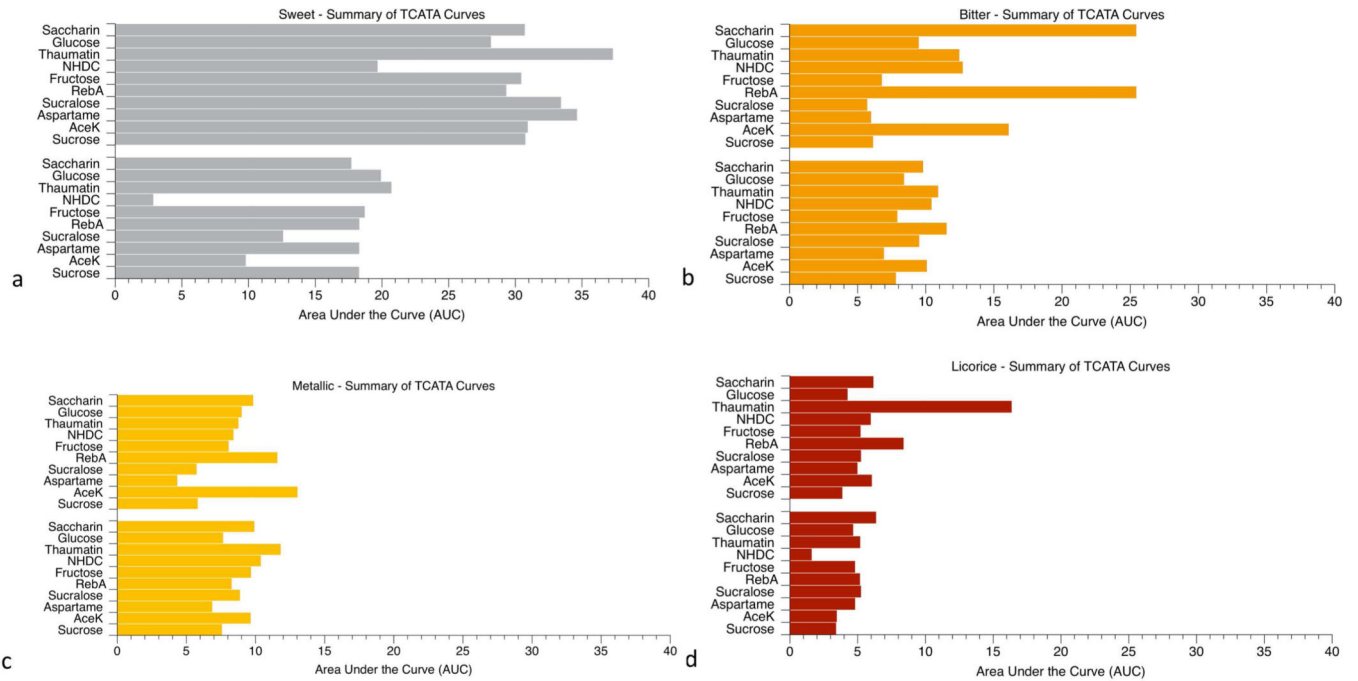


Figure 3. Area under the curve (AUC) summaries for (a) sweet, (b) bitter, (c) metallic, and (d) licorice. Within each panel, summary AUC values are shown for the moderate level samples on the top, with the weak level samples on the bottom are presented.

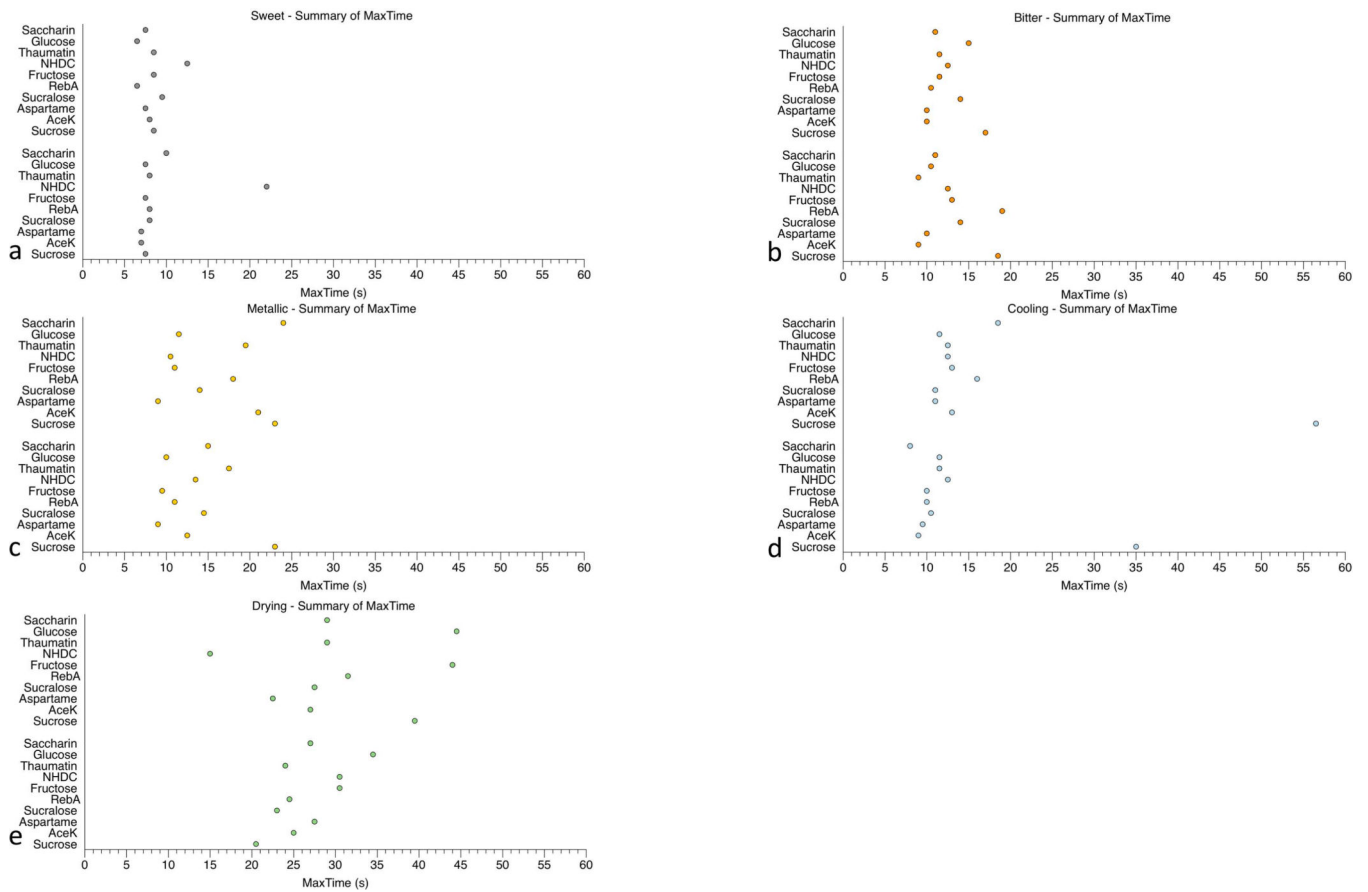


Figure 4. MaxTime summaries for (a) sweet, (b) bitter, (c) metallic, (d) cooling, and (e) drying.

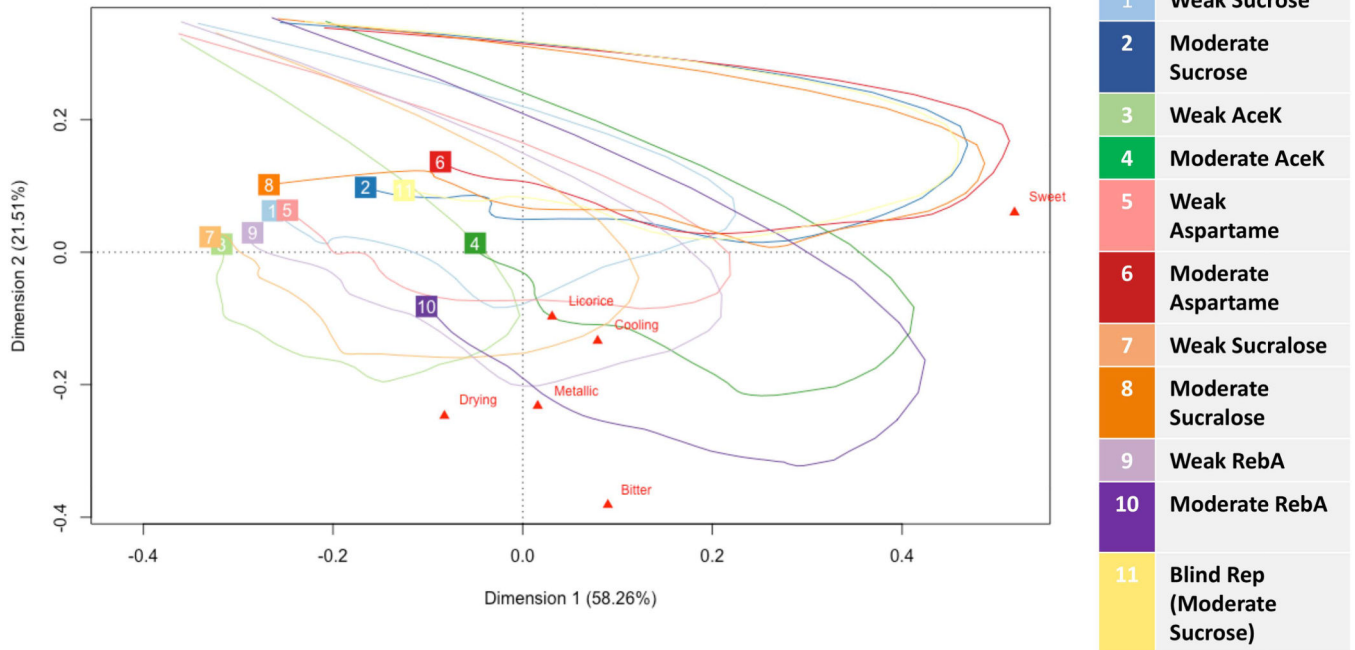


Figure 5. Smoothed trajectories for stimuli based on data from test day 1. Each trajectory terminates in a label indicating the sample number.

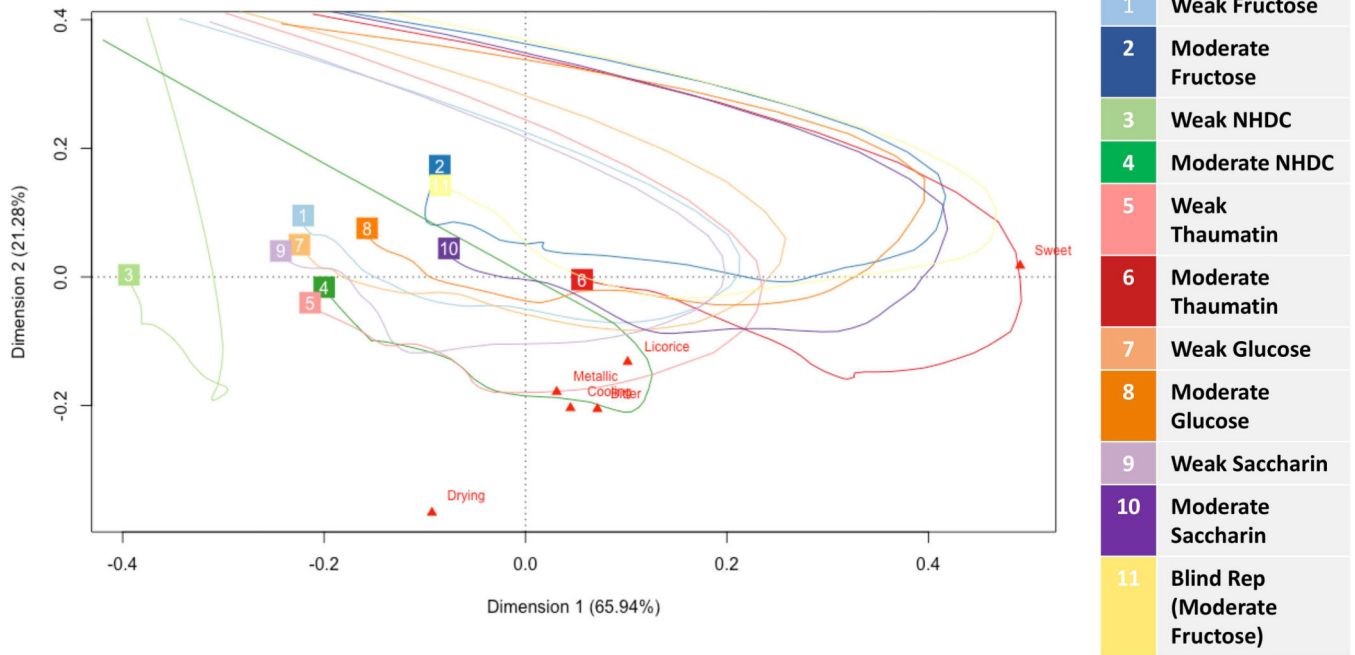
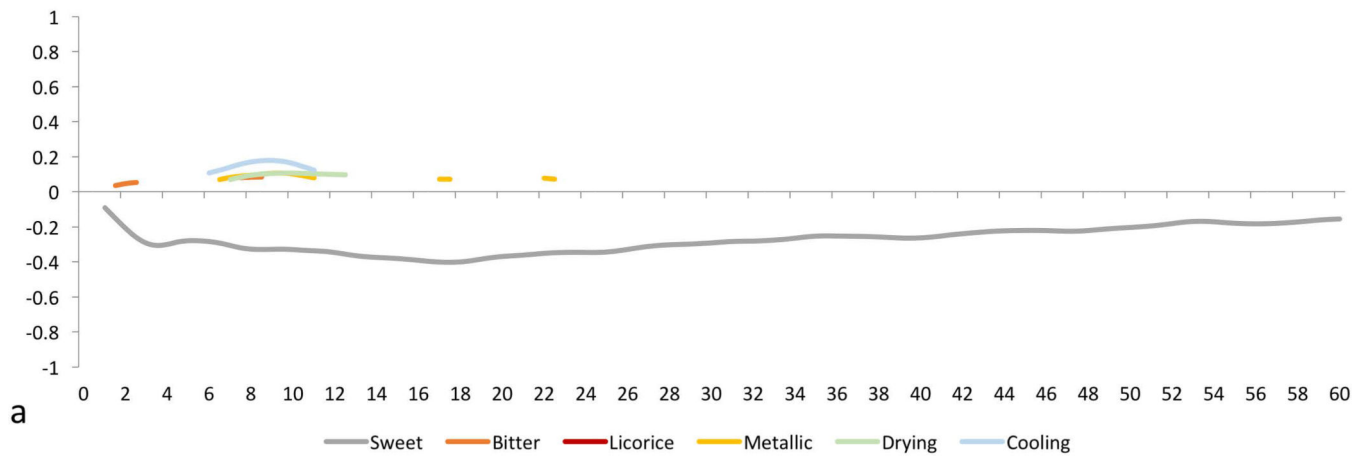


Figure 6. Smoothed trajectories for stimuli based on data from test day 2. Each trajectory terminates in a label indicating the sample number.

Aspartame - Weak - Aspartame - Moderate



Sucralose - Weak - Sucralose - Moderate

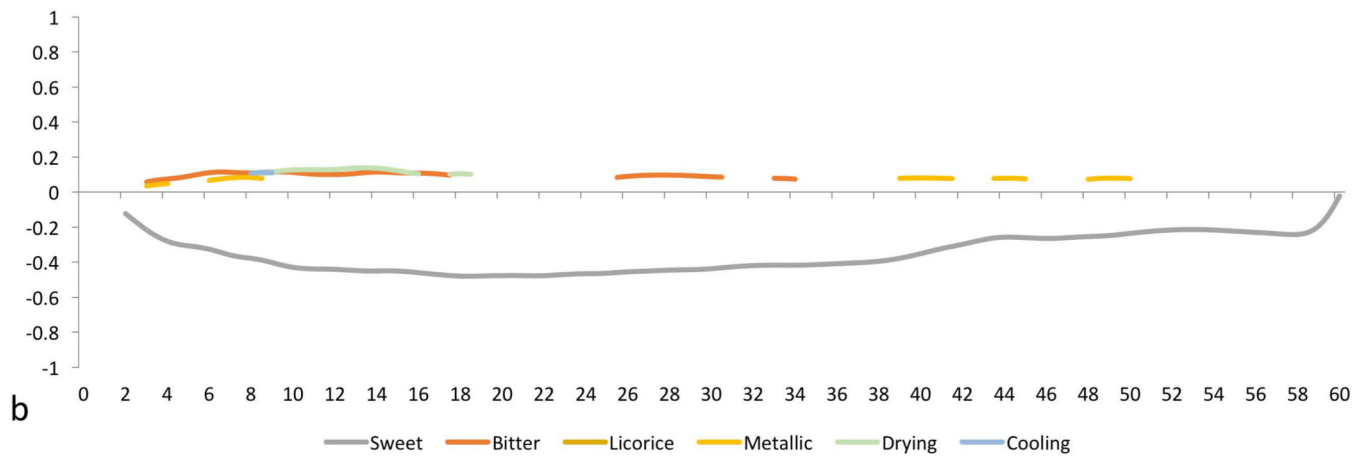
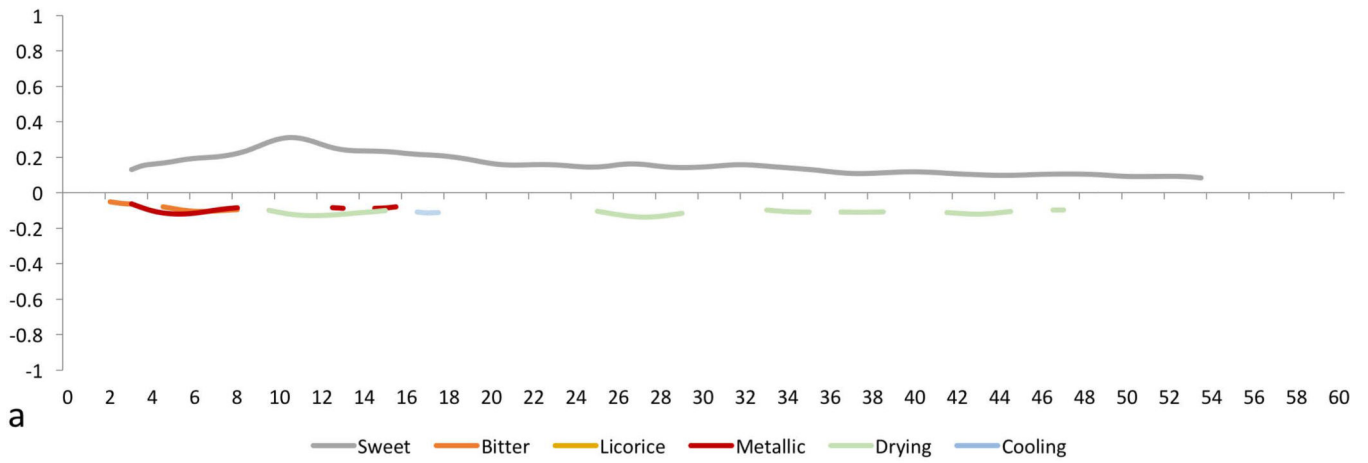


Figure 7. TCATA difference curves for (a) weak aspartame compared to moderate aspartame and (b) weak sucralose compared to moderate sucralose. TCATA difference curves compare pairs of samples and are plotted by subtracting the citation proportions for each attribute in two samples at each time point.

Sucrose - Weak - AceK - Weak



Fructose - Moderate - NHDC - Moderate

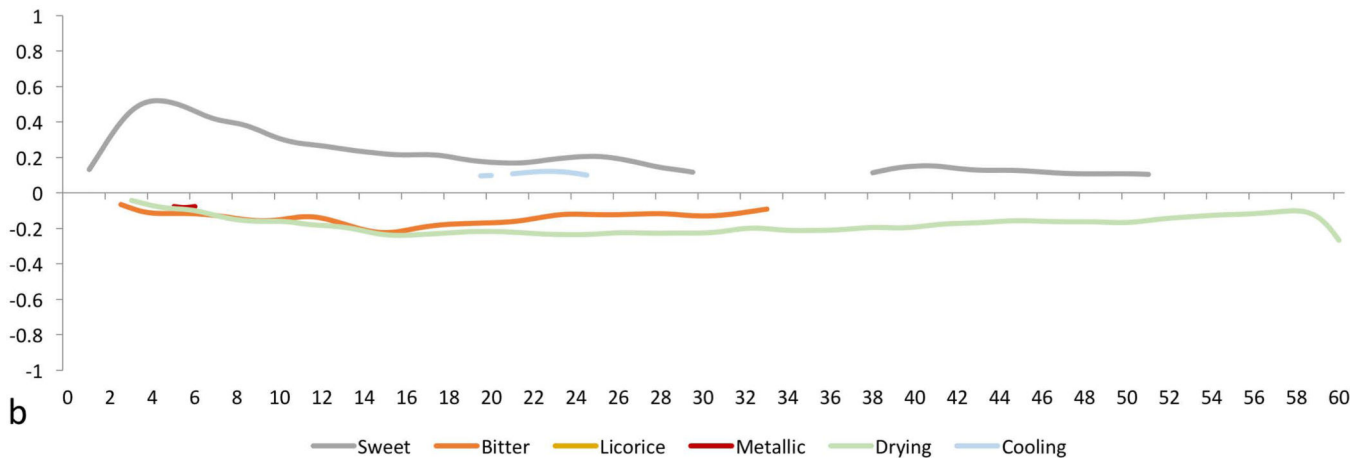


Figure 8. Same as Figure 7, but for (a) weak sucrose compared to weak AceK and (b) moderate fructose compared to moderate NHDC.

Table 1

Stimuli and concentrations used on Day 1.

Stimuli	Level	Conc. (mmol/L)
Sucrose	Weak	130.35
Sucrose	Moderate	248.91
Sucrose	(Blind Rep)	248.91
Aspartame	Weak	0.5346
Aspartame	Moderate	1.6485
AceK	Weak	0.6809
AceK	Moderate	5.2267
Sucralose	Weak	0.1193
Sucralose	Moderate	0.366
RebA	Weak	0.1275
RebA	Moderate	1.0389

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Table 2

Stimuli and concentrations used on Day 2.

Stimuli	Level	Conc. (mmol/L)
Fructose	Weak	181.96
Fructose	Moderate	438.07
Fructose	(Blind Rep)	438.07
NHDC	Weak	0.0628
NHDC	Moderate	0.5783
Thaumatococcus	Weak	0.0003
Thaumatococcus	Moderate	0.0017
Glucose	Weak	467.19
Glucose	Moderate	771.55
Saccharin	Weak	0.5578
Saccharin	Moderate	2.0673

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Table 3

References used in Attribute Orientation.

TCATA Term	Compound	Conc. (g/L)	Definition
Sweetness	Sucrose	100	The taste characteristic of table sugar
Bitterness	Quinine Monochloride Dehydrate	0.5	The taste characteristic of substances like caffeine or quinine
Drying	Alum	2.75	The lack of lubrication or moistness resulting in friction in your mouth
Licorice	Essential Anise Oil	~0.12	Characteristic of black licorice or anise
Metallic	Ferrous Sulfate	0.272	Characteristic flavor of blood, copper pennies, and metal spoons
Cooling	Menthol	~0.15	The sensation of cold in your mouth

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Table 4

Summary of results for moderate concentration samples. Dot indicates attribute was checked proportion greater than 1/6 for at least 30% of timepoints. The first 3 sweeteners are carbohydrates, the next 4 are synthetic, and the last 3 are natural sweeteners.

Stimulus	Sweet	Bitter	Licorice	Metallic	Drying	Cooling
Sucrose	•				•	•
Fructose	•			•	•	•
Glucose	•				•	•
AceK	•	•		•	•	•
Aspartame	•				•	•
Sucralose	•				•	•
Saccharin	•	•		•	•	•
NHDC	•	•		•	•	•
RebA	•	•	•		•	•
Thaumatococin	•		•	•	•	•