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Perception and Acceptance of Sweeteners

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This study examined the possibility that variation in acceptability of sweeteners is due more to variation across individuals in sensitivity to non-sweet tastes rather than sensitivity to sweetness *per se*. Thirty individuals assessed 13 sweeteners, rating sweet, sour, salty, bitter and metallic intensities, as well as liking and acceptance. Results indicated that bitter intensity and sweetener type were the two largest factors contributing to liking and acceptance. Sensitivity to PROP did not contribute significantly to liking or acceptance.

Until recently, cumulative biochemical and electrophysiological research suggested two models of sweet taste transduction[1]. Several such studies with a variety of rodents indicated that while carbohydrate sweeteners were transduced by a cAMP second messenger system, artificial sweeteners and amino acids were transduced by an IP3 second messenger system[2-9]. In contrast, more recent findings indicate there is only one receptor[10, 11] and one signaling pathway [12] involved in the perception of sweetness. These most recent findings contradict psychophysical research findings that routinely suggest more than a single mechanism is involved in the perception of sweeteners[13-17].

This apparent contradiction between the recent neurophysiological findings and human perception can be explained by hypothesizing that some sweeteners are activating other taste receptors in addition to the sweet receptors. Finding suggest that there are 20-30 bitter receptors[18-21] and extreme variability in the perception of bitter compounds across individuals is well documented (see [22] for

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a review). It is possible that individuals have similar variability in their sensitivity to the non-sweet tastes associated with certain sweeteners.

Of course, differences in liking and acceptance of particular sweeteners are driven by more than differences in perceived intensity alone. Acceptance of any taste or flavor (or any other perceptual experience for that matter) is logically driven by a minimum of three factors. The first is ***sensitivity*** because in order to accept or reject something, one must first be able to perceive it. The second is ***familiarity***. It is well-known that the valence of the affect (positive or negative) of previous exposure(s) will shape the affect elicited by subsequent exposure. In other words, if prior exposure to the sensation is associated with a pleasant experience, such as a subsequent feeling of satiety, it will be more likely to elicit a positive affect, and in turn liking of the sensation, when it is experienced later. If instead the prior exposure to the sensation is associated with an unpleasant experience, such as a feeling of nausea following the sensation, it will be more likely to elicit negative affect, and in turn dislike of the sensation, when it is experienced later. Of course, the culture in which one lives will determine which experiences will become familiar. A third factor that impacts liking and acceptance is ***personality***. While some individuals actively seek out new experiences and new sensations, others prefer to limit their contact with the unknown and prefer to limit their exposure to new sensations. Such traits will impact an individual's liking and acceptance of sensations.

The perceptual differences between various sweeteners are obvious when used by consumers. A brief search of the internet on November 5, 2006 revealed half a dozen non-commercial sites where individuals expressed a preference for the taste of one artificial sweetener over others. This study examined the hypothesis that individual differences in liking and acceptance of sweeteners is due to variation across individuals in sensitivity to the taste qualities of non-sweet tastes (bitter, sour, and metallic) of some sweeteners. Thirty individuals assessed thirteen sweeteners for perceived intensity, liking, and acceptance.

Materials and Methods

Stimuli

Aqueous solutions were made from thirteen types of sweeteners, selected from several chemical categories (*carbohydrates*: sucrose, glucose, and fructose; *proteins/amino acids*: thaumatin, aspartame, d-tryptophan, and glycine; *terpenoids*: stevioside; *N-sulfonylamides*: acesulfame-K and sodium saccharin; *halogenated suga*r: sucralose; *sugar alcohol*: xylitol; and *sulfamate*:

sodium cyclamate). Concentrations of each sweetener was set to be the same intensity as 200 mM sodium chloride as determined by Guinard et al.[23] for most (aspartame, acesulfame-K, cyclamate, d-tryptophan, sucrose, glucose, thaumatin, xylitol, glycine and saccharin) and bench top testing for the compounds not included in that study (sucralose, fructose, stevioside). Specifically, the concentrations were as follows: 401 mM sucrose, 1120 mM glucose, 15 mM d-tryptophan, 0.0023 mM thaumatin, 930 mM xylitol, 5.21 mM sodium saccharin, 2.89 mM aspartame, 2380 mM glycine, 0.745 mM stevioside, 29.1 mM sodium cyclamate, 1.21 mM sucralose, 600 mM fructose and 0.038 mM acesulfame potassium. All solutions were made with Millipore™ polished water (Millipore RiOs™ 16 and Milli-QR Gradient, Millipore Corporation, Bedford, MA) between 24 hours and 5 days prior to testing.

Before subjects began assessing intensities, the subjects were presented with reference solutions for sweet (292 mM sucrose), sour (5.2 mM citric acid), salty (125 mM sodium chloride), bitter (0.032 mM quinine sulfate) and metallic (250 mg ferrous sulfate tablets per liter). In addition, to encourage the subjects to rate a single solution with more than one taste quality, a “sweet + bitter” reference containing 292 mM sucrose and 0.032 mM quinine sulfate was included. In the final session, individuals’ sensitivity to 6-n-propyl-3-thiouracil (PROP) was determined by panelists’ ratings of PROP bitterness intensity at several concentrations: 0.055 mM, 0.174 mM, 0.55 mM, 1.74 mM and 5.50 mM (6-n-propyl-3-thiouracil, Sigma Chemical, St. Louis, MO).

Panelists

Thirty paid volunteer subjects (19 female, 11 male; 18 - 45 years of age) were recruited in accordance with the approval of procedures by The Ohio State University Office of Responsible Research Practices. All subjects gave informed consent before participating. No qualifying criteria were used.

Procedure

Each panelist attended four sessions. All sessions were conducted in computer equipped sensory testing booths and data was collected using Compusense ® five version 4.6 software (Compusense Inc, Guelph, Ontario, Canada). In all sessions, samples were presented as 20 mL aliquots in 1 oz plastic cups (Solo Plastic Souffles, P100, Solo Cup Company, Baltimore MD) labeled with random 3-digit codes. Samples were counterbalanced across panelists and blocked so that each panelist received all solutions once before receiving any of the solutions a second time. In sessions 2 and 3 only, panelists were instructed to rinse for 30 seconds between assessments while a 30-second countdown appeared on-screen. Depending on the particular session (see details below), the rinse was either water (Millipore™ polished) or concentrated sucrose (814 mM).

In the first session, panelists rated both overall liking and overall acceptability of each of the thirteen sweeteners in duplicate, for a total of 54 assessments. Overall liking was rated on the 9-point hedonic scale[24], ranging from “1 = dislike extremely” to “9 = like extremely.” Overall acceptability was rated on a 7-point scale that ranged from “not acceptable at all” to “completely acceptable.” Half of the subjects rated all stimuli for acceptability before proceeding to rate the stimuli for liking, while the remaining half rated all stimuli for liking before proceeding to rate the stimuli for acceptability. As they were hedonic assessments, the participants did not receive any specific training before beginning.

In the sessions 2 and 3, the panelists rated, in replicate, perceived intensity of sweet, sour, salty, bitter, and metallic taste of the thirteen sweeteners and a water blank on the generalized LMS scale[25] for a total of 28 assessments. Before assessing the sweeteners, panelists were familiarized with the taste qualities by sampling and rating labeled reference solutions (described above). The difference between session 2 and session 3 was the rinse solution. In session 2 the rinse solution was Millipore™-polished water while in session 3 the rinse was a concentrated sucrose solution (814 mM). It is well documented that the intensities of taste qualities are often perceived as less intense when present in a mixture. For example, a mixture of quinine and sucrose is less sweet than an equal concentration of sucrose tasted alone, and less bitter than an equal concentration of quinine tasted alone[26]. However, after adaptation to one of these, the perceived intensity of the other mixture component will return to its unmixed intensity level, a phenomenon known as release from suppression [27]. As the focus of this research was non-sweet tastes, this condition was intended to accentuate non-sweet taste intensities, the premise being that increasing the intensity of these non-sweet tastes would allow for more accurate assessment. Half of the panelists completed session 2 (water rinse) before completing session 3 (concentrated sucrose rinse) while the remaining panelists completed the sessions in the reverse order.

In the fourth and final session, panelist sensitivity to PROP was determined following the protocol of Delwiche et al.[22]. Panelists rated the perceived bitterness of 5 concentrations of PROP in duplicate on the generalized LMS scale. Also in this final session, panelists rated the perceived loudness intensity of a series of tones (0, 20, 35, 50, 65 and 80 decibels) twice on the generalized LMS scale[25]. The tones were played for 1 second at 4000 Hz. Tones were presented to the right ear of each panelist via a headset attached to the AS208 audiometer from Interacoustics (Denmark). These tone intensity ratings were used to account for differences in scale usage, as described below.

Statistical Analysis

The loudness ratings of the tones collected in the last session were used following the protocols developed in Delwiche et al[22] to reduce discrepancies due to differences in scale usage. Using natural breaks in the bitterness intensity ratings of 1.74 mM PROP, subjects were broken into three groups, with 10 hypo-tasters, 17 tasters, and 3 hyper-tasters (often called non-tasters, tasters, and super tasters, as in [28, 29, 30]).

Two-way repeated measures ANOVAs on sweeteners (all 13) and rinse (water vs. sucrose) were performed on ratings for each taste quality (sweet, sour, salty, bitter, metallic). In addition, a one-way repeated measures ANOVA across compounds (13 sweeteners and the water blank) was conducted on ratings of each taste quality from the water-rinse session. Scheffé's post-hoc tests were used when appropriate. Repeated measures ANOVAs were conducted with Statistica 7 (Statsoft Inc. Tulsa, OK).

Additionally, two linear models of the data were created, the dependent variables being ratings of overall liking in one and ratings of acceptability in the other. The independent variables in both were the intensity ratings of the sweet, sour, salty, bitter and metallic taste qualities from the water rinse condition, the PROP status (non-taster, taster, or hyper-taster) and the sweetener. The categorical variables (PROP status and sweetener) were included in the model by means of dummy coding [31]. Linear models were created with SPSS 14.0 (SPSS, Inc. Chicago, IL).

Results

Despite the fact that concentrations were selected so that all sweeteners would have the same sweet intensity, a significant difference in sweetness was found across compounds (one-way ANOVA, $p<0.001$ – see Table I). In fact, when sweeteners were compared to water (with one-way ANOVA), no significant difference in sweetness was found between water, acesulfame-K, glycine, and stevioside (Scheffé's, $p < 0.05$ – see Table I). However, these differences in the means do not make clear the huge individual differences in perceived sweetness of the compounds. For example, ratings for acesulfame K showed the greatest variation, ranging from barely detectable to strongest imaginable while glucose, which showed the least variation, ranged from weak to strong. As expected, sweetness ratings were lower after the sucrose rinse than after the water rinse (two-way ANOVA, $p<0.001$). While the concentrated sucrose rinse significantly reduced sweetness for fructose, sucralose and sucrose (Scheffé's, $p <0.05$), it did not for the other sweeteners (Scheffé's, $p > 0.05$), resulting in a significant interaction between rinse and sweeteners (two-way

ANOVA, $p<0.001$). Since the average sweetness of acesulfame K, glycine, and stevioside was not rated as sweeter than water (Scheffé's, $p > 0.05$), it is not surprising that the concentrated sucrose rinse did not significantly lower their ratings. However, it is more difficult to explain why the concentrated sucrose rinse did not significantly suppress the sweetness of aspartame, glucose, xylitol, saccharin, d-tryptophan, sodium cyclamate and thaumatin without hypothesizing the existence of more than one perceptual mechanism for sweetness. These findings do, nevertheless, correspond with earlier findings[15-17] indicating that sweeteners do not cross-adapt symmetrically or uniformly.

Table I. Mean Intensity of Sweetener Taste Qualities

Compound	*Sweet*	*Sour*	*Salty*	*Bitter*	*Metallic*
Water	0.33[a]	0.96[a]	0.43[a]	2.07[a]	2.54[a]
AceK	0.43[a]	0.42[a]	0.29[a]	2.16[a]	2.88[a]
Aspartame	18.93[bcd]	0.91[a]	0.72[a]	1.45[a]	0.42[a]
Cyclamate	25.12[cd]	2.51[a]	4.11[ab]	3.14[a]	1.56[a]
D-tryptophan	19.18[bcd]	1.52[a]	0.87[a]	20.05[c]	2.97[a]
Fructose	28.04[cd]	3.69[a]	0.98[ab]	2.54[a]	1.63[a]
Glucose	24.26[cd]	2.37[a]	0.56[a]	1.35[a]	2.30[a]
Glycine	6.78[ab]	24.14[b]	5.82[b]	1.97[a]	3.00[a]
Saccharin	24.91[cd]	2.39[a]	0.79[a]	14.53[bc]	1.39[a]
Stevioside	14.00[abc]	2.07[a]	1.63[ab]	10.12[abc]	0.18[a]
Sucralose	30.98[d]	0.98[a]	0.32[a]	1.96[a]	0.74[a]
Sucrose	27.48[cd]	0.71[a]	0.22[a]	0.84[a]	0.88[a]
Thaumatin	25.70[cd]	1.59[a]	2.44[ab]	8.51[ab]	4.69[a]
Xylitol	25.48[cd]	4.95[a]	1.03[ab]	0.94[a]	0.86[a]
AOV p-values	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.411

NOTE: Means in a column with the same superscript are not significantly different (Scheffé's, $p > 0.05$). Significant p-values are in **bold**.

One-way ANOVAs also found significant differences across sweeteners for sourness, saltiness and bitterness ($p < 0.05$, Table I), although none was found for metallic taste ($p > 0.05$, Table I). As with the sweetness intensities, tremendous variation across panelists was found in assessments of the other attributes as well. For sour, salty, bitter, and metallic taste qualities, the rinse condition (water vs. concentrated sucrose) did not significantly alter ratings (two-way ANOVAs, $p>0.05$), nor were interactions between rinses and sweeteners significant (two-way ANOVAs, $p>0.05$). The non-sweet tastes of the sweeteners did seem to be somewhat increased by rinsing with concentrated sucrose, which may have become significant with the testing of additional panelists or at other sweetener concentration, suggesting there was some release from suppression. However, rinsing with concentrated sucrose certainly did not clarify assessments of non-sweet tastes. Due to its differential impact on the perception of sweetness across sweeteners, it added an unwarranted level of complexity to the dataset; these assessments were not used in any additional analyses.

Table II. Significance of Variables in Linear Models

Variable	*Liking (p-values)*	*Acceptance (p-values)*
Sweet	0.997	0.214
Sour	0.138	0.128
Salty	0.866	0.474
Bitter	**0.007**	**0.013**
Metallic	0.839	0.098
Sweetener	**<0.001**	**<0.001**
PROP Status	0.678	**0.030**
Adjusted R-squared	**0.327**	**0.350**

Values in bold were significant (p<0.05).

The first linear model showed that two variables significantly contributed to ratings of overall liking (see Table II): sweetener compound (p<0.001) and bitter intensity rating (p=0.007). The second model, using ratings of overall acceptability as the dependent variable, showed three independent variables made significant contributions (see Table II): sweetener compound (p<0.001), bitter intensity rating (p=0.013) and PROP status (p=0.030). Individuals who were PROP hypo-tasters had a larger negative regression coefficient than either tasters or hyper-tasters, suggesting that hypo-tasters are more likely to rate sweetener acceptability as low than tasters or hyper-tasters. This finding is fairly surprising as one might predict that since hypo-tasters are generally less sensitive to bitter tastes, they would also be less sensitive to the bitterness of certain sweeteners and thus more accepting of them. However, it is not entirely clear how the panelists interpreted “Acceptability,” as there were a few panelists who paradoxically gave ratings for a particular sweetener of both “completely acceptable” and “dislike extremely.” Perhaps they assumed as all sweeteners were safe and therefore acceptable. Or perhaps they recognized certain sweeteners and, while they personally disliked it, recognized that others found it acceptable. However, as the rinsing protocol was much more lax during session

1, the most likely explanation is that the presentation order had a profound impact on these hedonic assessments.

Discussion

The large individual differences in intensity ratings for the sweeteners that remained even after centralization of the means for all rated attributes were striking. On average, the acesulfame K solution was rated so low so as to be found not significantly different in sweetness than water, while one individual rated the solution as being the strongest imaginable sweetness. While there was also large variability across individuals in the perceived intensities of the non-sweet attributes, they were not as large as the variations in sweetness. In addition, it is important to note that adaptation to a high concentration sucrose solution did not significantly reduce the perceived sweetness of several sweeteners. Thus, it still remains difficult to reconcile the most recent neurophysiological findings on sweetness with the human perception of sweetness.

The results of the general linear models suggest the perception of bitterness and the sweetener type were the two largest factors contributing to overall liking of a sweetener. Since the concentration levels of the sweeteners were selected to be similar, greater variation in non-sweet tastes was expected, which may explain why sweetness did not contribute significantly to the models of overall liking or acceptance. Despite the fact that metallic taste is a common complaint associated with certain sweeteners, it also failed to contribute significantly to the models of overall liking or acceptance.

While PROP status did not contribute significantly to the linear model of overall liking, it did contribute significantly to the model of acceptability. As mentioned above, the hypo-tasters were less accepting of the sweeteners they rated higher in bitterness than were tasters and hyper-tasters. It is possible that hyper-tasters not only perceive more bitterness than do the hypo-tasters, but also more sweetness, which in turn suppressed the additional bitterness. In fact, several studies have shown that hyper-tasters are more sensitive to all tastes, not just bitterness (e.g., [28, 29, 36, 38-40]).

The adjusted R-squared values of both models are relatively low. Clearly, the variables considered do not fully account for the variability across individuals in the liking and acceptance of sweeteners. One variable not measured which has been shown both to differ across sweeteners[32-36] and to impact liking[37] is the onset and off-times of each sweetener. In addition, as mentioned earlier, liking and acceptance is also influenced by familiarity and personality factors, which also went unmeasured. Thus, it is not surprising that the adjusted R-squared values were not higher. Nonetheless, it is striking that bitterness and type of sweetener are as effective as they are at predicting liking. Also worth noting is that consumer acceptance of sweeteners are better predicted from perceived bitterness than from perceived sweetness, as was hypothesized.

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