Positive allosteric modulators of the human sweet taste receptor enhance sweet taste

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To identify molecules that could enhance sweetness perception, we undertook the screening of a compound library using a cell-based assay for the human sweet taste receptor and a panel of selected sweeteners. In one of these screens we found a hit, SE-1, which significantly enhanced the activity of sucralose in the assay. At 50 μM, SE-1 increased the sucralose potency by >20-fold. On the other hand, SE-1 exhibited little or no agonist activity on its own. SE-1 effects were strikingly selective for sucralose. Other popular sweeteners such as aspartame, cyclamate, and saccharin were not enhanced by SE-1 whereas sucrose and neotame potency were increased only by 1.3- to 2.5-fold at 50 μM. Further assay-guided chemical optimization of the initial hit SE-1 led to the discovery of SE-2 and SE-3, selective enhancers of sucralose and sucrose, respectively. SE-2 (50 μM) and SE-3 (200 μM) increased sucralose and sucrose potencies in the assay by 24- and 4.7-fold, respectively. In human test cases, 100 μM of SE-1 and SE-2 allowed for a reduction of 50% to >80% in the concentration of sucralose, respectively, while maintaining the sweetness intensity, and 100 μM SE-3 allowed for a reduction of 33% in the concentration of sucrose while maintaining the sweetness intensity. These enhancers did not exhibit any sweetness when tasted on their own. Positive allosteric modulators of the human sweet taste receptor could help reduce the caloric content in food and beverages while maintaining the desired taste.

enhancer | sweetness | perception | sucrose

The steady increase of the daily consumption of dietary sugar over the last decades may have contributed to the obesity crisis and the early onset of type-II diabetes observed in many developed countries (1, 2). As a result, food and beverage companies have launched a plethora of diet brands where sugar has been partly or fully replaced by noncaloric sweeteners to decrease caloric intake. Currently, some of the commonly used noncaloric sweeteners include saccharin, aspartame, cyclamate, sucralose, and acesulfame K (3, 4). However, none of these substances can completely reproduce the taste of sugar. These sweeteners all suffer from one or more shortcomings including a bitter or metallic aftertaste at high concentrations, limiting their use to lower concentrations, or temporal issues such as a delayed sweet taste onset, a lingering sweet aftertaste, or a limited maximum sweetness intensity (4, 5). Another appealing approach to address the problem, in addition to looking for novel noncaloric sweeteners, would be to find molecules capable of enhancing sweetness perception. Ideally, such an enhancer molecule would not elicit sweetness on its own but it would boost the sweetness intensity of a lower amount of sweetener or sugar. Such enhancers could therefore allow for a reduction in the amount of sugar, and calories, in food and beverages while maintaining the desired taste. Similarly, a sweet taste enhancer could allow for a reduction in the amount of noncaloric sweeteners used in “0”-calorie diet brands, decreasing the associated bitterness, lingering tastes, and other off-tastes observed at high concentrations (4, 5) and therefore potentially improving the palatability and flavor profile of several consumer products.

Sweet taste is mediated by an obligate heterodimeric receptor composed of two distinct subunits (G protein-coupled receptors, GPCRs), T1R2 and T1R3, located at the surface of taste receptor cells in the taste buds (6, 7). These subunits, members of family C GPCRs, possess a large extracellular N-terminal domain, the Venus flytrap domain (VFT), linked to the seven-transmembrane C-terminal domain (TMD) by a shorter cysteine-rich domain (6–8). Every one of these three domains contains agonist binding sites, explaining sweet receptor activation by a vast repertoire of chemically distinct molecules. Sucrose and noncaloric sweeteners such as aspartame and neotame interact within the VFT of T1R2 (9, 10), other noncaloric sweeteners such as cyclamate and neohesperidin dihydrochalcone (NHDC) interact within the TMD of T1R3 (10–12), S819, a synthetic sweet agonist, interacts with the TMD of T1R2 (10), and the sweet-tasting protein brazzein requires the cysteine-rich domain of human T1R3 to activate the receptor (13). Family C GPCRs seem ideal targets for allosteric modulation. The savory (umami) taste receptor, a close relative to the human sweet taste receptor, is significantly enhanced by 5′-ribonucleotides (6, 14). Positive allosteric modulators (PAMs) have also been identified for the GABAR receptor, the calcium sensing receptor, and several subtypes of the metabotropic glutamate receptor (mGluR) family (15, 16). In all cases, the PAMs show little or no agonist activity on their own but significantly enhance the activity of the agonist on the receptor and, in functional assays, this behavior is depicted by a leftward shift of the agonist dose–response in the presence of the PAM (16–18). We report here the identification of a unique chemical class of PAMs for the human sweet taste receptor. These PAMs considerably increase the sucralose and sucrose potencies in a sweet taste receptor cell-based assay, are not sweet on their own, and significantly enhance the sweetness of sucralose or sucrose in taste tests.

Results and Discussion

Identification and Characterization of a PAM for the Human Sweet Taste Receptor. We have developed a very sensitive cell-based assay for the detection of human sweet taste receptor modulators. In this assay system, the human sweet taste receptor couples to the promiscuous G protein Gα15 to induce PLC


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activation, causing a net increase in calcium mobilization inside cells (10, 19). We have now carefully characterized and quantified the effects of >50 different known sweeteners to validate the assay (19). The human T1R2/T1R3 receptor is activated by every sweetener that we have tested and their relative potency in the assay correlates tightly with their relative sweetness intensity in human taste tests (19). Using this assay, we undertook screening of our corporate collection of compounds against carbohydrate sweeteners such as sucrose and fructose and several commercially relevant noncaloric sweeteners including aspartame, rebuadisu-
decta, and sucralose. Sucralose is highly related to sucrose and is currently sold under the brand Splenda (see structure in Table 1). Our sucralose screen generated the first hit, SE-1 (Fig. 1A), exhibiting the typical behavior of a PAM. Application of SE-1, on its own, did not produce any agonist activity but significantly enhanced the activity produced by the subsequent addition of a suboptimal concentration of sucralose (Fig. 1B). The SE-1 effect was dose dependent, with an EC50 of 3.0 ± 1.1 μM (average ± SD; n = 6) and could be observed only in the presence of sucralose (Fig. 1C). Indeed, SE-1 at concentrations as high as 75 μM produced little or no agonist activity when tested on its own (Fig. 1C). The enhancement effect of SE-1 could be explained, for the most part, by a considerable increase in the sucralose potency in the assay. Fig. 1D shows a representative experiment where sucralose dose–response effects were measured in the presence of various concentrations of SE-1. At a final concentration of 1 μM (after sweetener addition), SE-1 shifted the sucralose dose–response curve to the left and produced an EC50 ratio of 1.5 (Fig. 1D). SE-1 concentrations of 3, 10, and 30 μM further shifted the sucralose dose–response curve to the left and produced EC50 ratios (or fold enhancement) of 2.5, 6.3, and 19, respectively (Fig. 1D). The enhancement effect of SE-1 was also very reproducible. A fixed concentration of 50 μM SE-1 produced an EC50 ratio of 23 ± 3 (average ± SD; n = 13) (Fig. S1). Similar results were obtained when using a coapplication protocol instead of a preincubation protocol (Materials and Methods), showing that a preincubation of the receptor with SE-1 is not necessary to reveal its enhancement properties (Fig. S2). In addition, SE-1 effects were specific to the human sweet taste receptor because it did not enhance the activity of isoproterenol on the β2-adrenergic receptor or the effect of L-glutamate (MSG) on the umami taste receptor (Fig. S3). A robust binding assay for the human sweet receptor is currently not available. So, we do not know at this time if the increase in sucralose potency, observed in the assay, can be exclusively explained by an increase of the sucralose affinity for the receptor. However, mutagenesis and modeling data suggest that the sweet taste enhancers bind in proximity to the sucralose orthosteric binding site in the VFT of hT1R2 (see Zhang et al., in this issue of PNAS) (20). We therefore propose that these enhancers stabilize a VFT conformation with a higher affinity for sucralose.

To evaluate SE-1 selectivity we screened several additional sweeteners at suboptimal concentrations (producing 10–20% receptor activity) and using a high concentration of SE-1 (50 μM). Under these conditions, SE-1 did not enhance the effect of the peptide sweeteners alitame and aspartame or the effects of saccharin, dulcin, stevioside, cyclamate, mogroside, perillartine, glycyrrhizic acid, NHDC, and the sweet protein thaumatin (Fig. IE). Among all of the sweeteners evaluated, SE-1 enhanced only neotame and sucrose activity, albeit to a much lower extent than what was observed with sucralose. SE-1 increased the neotame potency by 2.5 ± 0.5-fold (average ± SD; n = 7) (Fig. S1) and the sucrose potency by 1.3 ± 0.1-fold (n = 3) (Table 1). The lack of major enhancement of sucrose by SE-1 is intriguing because sucralose is, in fact, a trichloro derivative of sucrose (1′,4′,6′-trichlorosucrose; see Table 1 for structure). To understand the structure–activity relationship between sucralose and SE-1, mono- and disubstituted chlorosucrose derivatives were synthesized and tested for enhancement in the assay. Addition of 1′-chloro or 6′-chloro to the fructose moiety of sucrose or of 4-chloro to the glucose moiety of sucrose increased the agonist potency (over that of sucrose) by 22–45%, and 6.5-fold, respectively (Table 1 and Fig. S4). The magnitude of SE-1 enhancement of 1′-chlorosucrose remained comparable to what was seen with sucrose. 4-Chlorosucrose and 6′-chlorosucrose achieved a significant improvement of enhancement over that of sucrose by ∼2-fold (Table 1 and Fig. S4). 1′,4-Dichlorosucrose and 1′,6′-dichlorosucrose had improved agonist potencies over the monosubstituted sucrose derivatives, being 150- and 87-fold more potent than sucrose. SE-1 had a more pronounced effect on these disubstituted derivatives relative to the monosubstituted derivatives, significantly improving the enhancement by ∼3-fold over that of sucrose. However, none of these molecules could be enhanced to the same extent as sucralose (Table 1 and Fig. S4). Strikingly, the 4,6′-dichlorosucrose derivative was enhanced by SE-1 to the same extent as sucralose, even if it was 18-fold less potent as an agonist (Table 1 and Fig. S4). Taken together, these data suggest that the 1′-chloro position mainly contributes to the binding affinity of sucralose whereas the 4-chloro and 6′-chloro positions contribute to the binding affinity of sucralose and, in combination, are also essential and sufficient for the enhancement effect of SE-1.

Table 1. Evaluation of SE-1 effects on mono- and dichlorosucrose derivatives

<table>
<thead>
<tr>
<th>Sweeteners</th>
<th>Y</th>
<th>Z</th>
<th>R1</th>
<th>R2</th>
<th>EC50 (μM)</th>
<th>EC50 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>41,300 ± 1,550</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Sucralose</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
<td>39 ± 3.0</td>
<td>23 ± 3.8</td>
</tr>
<tr>
<td>1-Chlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>1,880 ± 305</td>
<td>1.7 ± 0.2 ns</td>
</tr>
<tr>
<td>4-Chlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>7,320 ± 1,830</td>
<td>2.8 ± 0.8***</td>
</tr>
<tr>
<td>6-Chlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>9,100 ± 2,910</td>
<td>2.8 ± 0.9***</td>
</tr>
<tr>
<td>1,4-Dichlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>275 ± 49</td>
<td>3.6 ± 0.3***</td>
</tr>
<tr>
<td>1,6′-Dichlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>473 ± 59</td>
<td>4.2 ± 0.6***</td>
</tr>
<tr>
<td>4,6-Dichlorosucrose</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>720 ± 100</td>
<td>23 ± 3.8*, NS</td>
</tr>
</tbody>
</table>

Dose–response effects of depicted sweeteners were monitored after the cells had been preexposed to D-PBS or 75 μM SE-1 (50 μM final concentra-
tion). EC50′s and EC50 ratios produced by SE-1 were calculated for each sweetener (see Fig. S4 for representative results). Values correspond to an average ± SD of a least three independent experiments performed in trip-
llicate. †, P < 0.0001 vs. EC50 ratio obtained with sucrose. **, P < 0.01 vs. EC50 ratio obtained with sucrose. ‡, P < 0.05 vs. EC50 ratio obtained with sucrose. ****, P < 0.0001 vs. EC50 ratio obtained with sucralose; ns, no signi-
nificant difference vs. EC50 ratio obtained with sucrose; NS, no significant difference vs. EC50 ratio obtained with sucralose, Student’s t test.

SE-1 Enhances the Sweet Taste of Sucralose. Human panelists were trained over several weeks to precisely score, on a 15-point linear scale, the sweetness intensity of sucralose solutions using a per-
centage of sucrose (weight/volume) equivalence scale (Materials and Methods). A solution of 100 ppm sucralose solution (251 µM) had a similar sweetness intensity to a 6% (wt/vol) sucrose solution (175 mM) (Fig. 2B), a concentration producing roughly half the maximum sweetness intensity. Addition of 100 µM SE-1 to the 100-ppm sucralose solution enhanced the sweetness intensity to levels produced by 200 ppm (502 µM) to 300 ppm (753 µM) sucralose solutions (Fig. 2B). SE-1 therefore allowed for a reduction of the sucralose concentration by 2- to 3-fold while maintaining the sweetness intensity. SE-1 behaved as a pure enhancer in taste tests because it was not sweet when evaluated on its own (Fig. 2A), in agreement with the cell-based assay results (Fig. 1C). However, SE-1 did exhibit a bitter off-taste when tasted on its own (Fig. S5). Contrary to the marked effect on sucralose, SE-1 could not significantly enhance the effect of sucrose in taste tests (Fig. 2C) even though we detected a small but reproducible enhancement effect in the cell assay (Table 1). Our in vitro assay is more sensitive than taste tests and we have observed that ≥3-fold enhancement is typically necessary to detect effects in taste tests.

**Identification of Other PAMs by Lead Optimization.** During the course of these studies, we discovered that concentrated stocks (>10 mM) of SE-1 in DMSO produced a dimer by reduction of the thiol group. Also, we felt that this thiol group could potentially contribute to the bitter off-taste of SE-1 (21). We therefore replaced the thiol of SE-1 by a ketone, producing SE-2 (Fig. 3A). SE-2 was also a potent enhancer of sucralose, with an EC₅₀ of 22 ± 4.5 µM (average ± SD; n = 3) (Fig. 3B), and it increased the sucralose potency by 11 ± 1.7-fold and 23 ± 1.5-fold at 25 and 50 µM, respectively (average ± SD; n = 3) (Fig. 3C). SE-2 showed little or no agonist activity (Fig. 3B). Surprisingly, in taste tests, SE-2 was noticeably more effective than SE-1 (Fig. 4B). SE-2 (100 µM) significantly enhanced the sweetness intensity of a 100-ppm sucralose solution (251 µM) to levels produced by 400 ppm (1.004 mM) to 600 ppm (1.506 mM) sucralose solutions (Fig. 4B). The enhancement effect of SE-2 was also dose dependent. Concentrations as low as 3.12 µM enhanced the sucralose sweetness intensity and increasing concentrations of SE-2 further enhanced the sweet taste (Fig. 4C). We also evaluated lower concentrations of sucralose with a fixed concentration of enhancer. At 100 µM, SE-2 significantly enhanced the sweetness intensity of a 12.5-ppm sucralose solution (31 µM) to levels produced by a 100-ppm sucralose solution (251 µM) (Fig. 4D). Thus, 100 µM SE-2 allowed for a ≥80% reduction of the
The close structural similarity between sucralose and sucrose (Table 1) prompted us to investigate the structure-activity relationship of SE-1 to see if we could find sweet taste enhancers for sucrose. We synthesized several analogs of SE-1 and identified SE-3 as a proof-of-concept sucrose enhancer (Fig. 3D). At 50, 100, and 200 μM, SE-3 increased the potency of sucrose in the assay by 2 ± 0.3-fold, 3 ± 0.7-fold, and 5.1 ± 0.4-fold (average ± SD; n = 3-6), respectively (Fig. 3E). On the contrary, at 200 μM SE-1 increased the potency of sucrose only by 2.0 ± 0.1-fold (average ± SD; n = 3) (Fig. 3F), showing that these molecules are indeed different in their abilities to enhance the sucrose-induced receptor activation in the cell-based assay. SE-3 was less potent than SE-1 and SE-2 as a sucrose enhancer. At 50, 100, and 200 μM, SE-3 increased the potency of sucrose by 4 ± 0.3-fold, 7.8 ± 0.9-fold, and 19 ± 0.7-fold (average ± SD; n = 3), respectively (Fig. 3F). In taste tests, 100 μM SE-3 enhanced the sweetness intensity of a 6% sucrose solution (175 mM) to levels corresponding to 8% (233 mM) to 10% (291 mM) sucrose solutions (Fig. 4F). Similarly to SE-1 and SE-2, SE-3 did not exhibit a sweet taste on its own (Fig. 4E) and it was not bitter (Fig. S5). In agreement with in vitro data, SE-3 could also enhance sucralose sweetness intensity albeit to a lower extent than what was observed with SE-2 (Fig. S7).

Our prototypical sweet taste enhancers SE-1, SE-2, and SE-3 are unique examples of PAMs for the human sweet taste receptor. Up to now, sweetness enhancement has been reported only for sweetener combinations apparently showing modest synergistic effects in taste tests (25, 26). However, results from studies measuring the effect of sweetener combinations are difficult to interpret because of the inherent sweetness of each of the mixture components and their different psychophysical functions (their relative sweetness intensity at increasing concentrations) (27). For this reason, such studies have sometimes led to contradictory results raising questions about the reality of synergism between sweeteners or the mechanism of action of synergy (26, 28, 29). In contrast, the PAMs reported in this study are not agonists and do not taste sweet on their own, strikingly simplifying the measurement of enhancement properties and interpretation of the results. SE-2 enhances the sucralose sweetness in human taste tests with an unparalleled magnitude (by up to 8-fold) whereas our proof-of-concept sucrose enhancer, SE-3, enhances the sucrose sweetness by 1.3- to 1.5-fold. So, PAMs for the sweet taste receptor may offer an alternative or complementary approach to lower the caloric content of food and beverages while maintaining the desired taste. Of interest, efficacious enhancers such as SE-2 or its derivatives could potentially improve the palatability of sucralose-sweetened products. By decreasing the amount of sucralose, the associated bitterness, dominating licorice note, dryness, and lingering could be attenuated or even be eliminated (4, 5, 30). Perhaps of even greater importance, further optimization of SE-3 could lead to more efficacious enhancers allowing for a drastic reduction in the caloric content of sucrose-sweetened consumer products. SE-2 and SE-3 did not exhibit a bitter taste or any other off-taste at the concentrations used in this study. More importantly, no change in the sweet taste quality has been observed. Neither SE-2 nor SE-3 makes the sweet taste of sucralose or sucrose last longer.

The astonishing sweetener selectivity for these enhancers is at the core of their mechanism of action within the VFT (20). Multiple PAMs have been identified for the mGluRs, the GABA_A receptor, and the calcium sensing receptor (15, 16), and they all bind to the TMD. In contrast, hits that interact with the TMD of sweet taste receptor are agonists (10, 18). This could be due to the significant level of constitutive activity of the sweet taste receptor (31), which would enable the active molecules interacting within the TMD to elicit a significant agonist activity and perceptible level of sweet taste (17). A continuing research and screening effort should allow us to discover additional selective enhancers.

![Fig. 3](image-url)
Materials and Methods

Sweeteners. The following sweeteners and antagonists were purchased from Sigma-Aldrich: aspartame,acesulfame K, sucrose, cyclamate (sodium salt), and thaumatin. Steviolide was from Emperors Herbologists. Perillartine was from Pfaltz & Bauer. Dulcin was from Maybridge. NHDC was from Indofine. Sucralose was from Toronto Research Chemicals. The following sweeteners were kindly provided by Grant DuBois at The Coca-Cola Company: glycyr rhizic acid monoammonium salt, neotame, and mogroside. Aclame (Aclame) was a generous gift from The Coca-Cola Company and Danisco.

Fluorometric Imaging Plate Reader (FLIPR) Assays. hT1R2/R3-HEK293 Gα15 cells (6) were seeded in 384-well clear bottom plates (Fisher) at a density of ~32,000 cells/well and grown overnight. Where indicated, to test the specificity on enhancers, cells expressing the umami taste receptor, hT1R1/R3-HEK293 Gα15 cells, were used (Fig. 53). In these cells, expression of the T1R1 and T1R3 subunits is under the control of a mifepristone-inducible mammalian expression system where mifepristone acts as an agonist to activate gene transcription by binding to a truncated human progesterone receptor (GeneSwitch System; Invitrogen). On the day of the experiment, hT1R2/R3-HEK293 Gα15 cells or mifepristone-induced hT1R1/R3-HEK293 Gα15 cells were loaded with the calcium indicator Fluor3 AM (4 μM) (Invitrogen) in D-PBS (Invitrogen), using a Multiprop. Cells were incubated for 1 h at room temperature and excess dye was washed out with D-PBS using an EMBLA cell washer ( Molecular Devices), leaving a residual volume of 25 μL/well. After 30 min of rest time at room temperature, Fluor3 AM-loaded cell plates, compound plates, and sweetener plates were loaded into a FLIPR (Molecular Devices). The 384-well compound plates and sweetener plates (Greiner) were prepared at 3x final concentration in D-PBS. Imaging was performed using a 480-nm excitation and a 535-nm emission and was initiated with the acquisition of the baseline fluorescence for a period of 7 sec. The cells were stimulated on line with addition of 25 μL of solution from the compound plate and the resulting change in intracellular calcium concentration ([Ca2+]i) was monitored over the next 90 sec postaddition. At 220 sec, 25 μL of solution from the sweetener plate was added to the cells and the resulting change in [Ca2+]i was monitored for an additional 100 sec. Where indicated, a coap lication protocol was used instead of the preincubation protocol described above. Sweeteners and test compounds were prepared at 4x final concentration and mixed 1:1 in a 384-well Greiner plate ( bring the sweeteners and test compounds concentrations down to 2x final concentration). Imaging was initiated with the acquisition of the baseline fluorescence for a period of 7 sec and then cells were stimulated on line with addition of 25 μL stimul/well. Subsequent images were acquired every other second for a period of 2 min. Raw fluorescence counts were then normalized in each well (using custom-made data import software) by calculating delta F/F values (maximum fluorescent count obtained after stimulation – minimal fluorescent count obtained before stimulation/minimal fluorescent count obtained before stimulation). EC50's were determined using a nonlinear regression algorithm ( GraphPad PRISM), where the Hill slope, bottom asymptotes, and top asymptotes were allowed to vary. Enhancement properties of test compounds were quantified by determining the magnitude of the leftward shift in the sweeteners’ EC50 values (or an EC90 ratio): the value of the EC50 measured in the absence of the enhancer divided by the value of the EC50 measured in the presence of the enhancer.

Human Taste Tests. All solutions for tasting were prepared in a low sodium buffer solution (pH 7.1). The buffer solution was prepared with 0.952 g KCl, 5.444 g NaH2PO4, and 0.952 g KH2PO4 in 40 L of deionized ultra-filtered water. Reference samples prepared included buffer alone, 100–600 ppm sucrose, and 6–15% sucrose. Test samples containing sweetness enhancers SE-1, SE-2, or SE-3 in for other commercially relevant sweeteners acting within the VFT of the sweet taste receptor.
buffer, 100 ppm sucralose, or 6% sucrose were also evaluated. Enhancers were first prepared as 1,000-fold concentrated stock solutions in ethanol to ensure dissolution of the ingredient. The concentrated stock solutions were then diluted 1,000-fold in buffer, sucralose, or sucrose solutions, resulting in a final concentration of 0.1% ethanol. Samples without enhancers were also balanced to contain 0.1% ethanol. A 0.1% ethanol solution did not exhibit sweetness on its own.

Trained panelists from the San Diego area were used for the taste tests. Subjects were instructed not to eat or drink anything (except water) for at least 1 h before the test. The exact number of panelists evaluating the samples is reported with each test. Panelists rinsed their mouths with water before starting any test. Samples were presented monadically to panelists in a randomized, counterbalanced order. Panelists rinsed with water and had up to a 1-min delay to clean the mouth of any tastes after each evaluation. The samples were rated on a 15-point scale for sweetness intensity using a percentage of sucrose equivalence scale, where 0 = 0% sucrose and 15 = 15% sucrose. Data were analyzed using analysis of variance followed by a Tukey’s honestly significant difference test to determine statistical significance (α = 0.05).

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