Effect of Zero-and-Low Calorie Sweeteners on Retronasal Odor Enhancement

by
Erin E. Schenk

A PROJECT

submitted to
Oregon State University
University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Food Science and Technology
(Honors Scholar)

Presented May 18th, 2015
Commencement June 2015
This study aimed to investigate the relationship between taste and odor through the phenomenon called retronasal odor enhancement by sweet taste. The specific sweet tastants used were zero or low calorie sweeteners. Experiment 1 used an aqueous model system where subjects sampled of vanilla solutions with and without five different sweeteners (sucrose, tagatose, sucralose, saccharin, and rebaudioside-a). Subjects rated perceived intensities of all relevant qualities on the generalized version of the Labeled Magnitude Scale. Experiment 2 used custard samples with addition of vanillin and the same five sweeteners. In addition to the perceived intensity ratings, subjects in experiment 2 also rated degree of liking and disliking of the samples using the labeled hedonic scale. The results from both experiments showed significant enhancement of vanilla ratings by all five sweeteners with no significant difference across the mean values. Hedonic data showed similar ratings across all sweeteners, however, rebaudioside-a showed the largest hedonic rating range potentially due to an associated bitter taste. This study demonstrates that zero and low calorie sweeteners could enhance a retronasally perceived food odor at about the same degree as sucrose, but that the degree of liking may differ across samples due to specific characteristics of each sweeteners.

Key Words: odor enhancement, sweeteners, non-nutritive, sensory, psychophysics

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Erin E. Schenk, Author
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CHAPTER ONE: LITERATURE REVIEW

Food scientists serve a pinnacle role in many food companies and organizations. Sensory studies provide information to food scientists that compliment other analytical tools in order to bridge information in the design and production of food items (for review, see Tuorila and Monteleone 2009). Understanding taste and odor relationships allows people in the field of food science to gain sources of information for research and development, as well as maintain and control the quality of products. Taste and odor represent the most fundamental attributes of human’s relationship to food (for review, see Small and Prescott 2005). With the knowledge of these interactions between sensory and analytical data/information, the food system can be integrated and be enhanced through concurrently improved research.

Sensory research also contributes to and supports the fields of nutrition and medicine. With increased awareness of where our food comes from, what is in the food we eat, and the implications for our overall health, sensory studies serve as an important tool. Measurements in sensory studies are gathered directly from those individuals, whose health and eating habits are influenced by the information gained, showing direct applicability between research and the consumer. The design of food products that can reduce the rates of obesity is one of the many examples where food science along with sensory science can integrate food science, nutrition, and healthy living (Shankar and others 2013).

Historically taste and smell have been areas of research that have gained the attention of researchers in the fields of both medicine and food science. In the beginning the focus primarily was on taste. However, this topic has broadened into
the research on smell and the interactions between taste and smell. Current research ranges from work on specific taste attributes, nutrient value, taste receptor mechanisms, to the broader sense of the connections between genetic profiles and taste and odor interactions (Reed and others 2006)(for review, see Bartoshuk 2000).

Taste serves as a very important component in many food systems, specifically in knowing if a taste is nutritive or toxic. Sweet and umami tastes are considered to be signals for energy sources for the body, and are classified as attractive tastes (Zhao and others 2003). Saltiness is critical for the body in signaling salt regulation, hormone balance, and osmoregulation (Lawless and Heymann 2010). Bitterness is associated with toxic compounds. Taste’s essential attribute in identifying sources of functional nutrients for the body exemplifies one of the many ways in which our bodies rely on sensory organs. It is this uniting element between sensory research, health, medicine, and food systems that established my interest in this area of research.

**Sensory Science Background and Fundamentals:**

**Taste and Smell:**

Taste: Scientific discovery in the field of sensory science began with these questions: what is taste, what is its fundamental function, and what is its dimensional complexity in the formation of a relationship between food and sensory organs in the body. There are five fundamental taste qualities; sweet, salty, sour, bitter, and umami (Lawless and Heymann 2010). Because taste is one of the primary components describing a food product, understanding concurrent taste attributes in a food can be very useful for food scientists. An example of a concurrent taste descriptor can be seen through
the example of the artificial sweetener acesulfame-K. This sweetener has been occasionally described to have a metallic taste, although not classified as one of the five primary tastes, this consumer description represents an important data point that food producers can use when making decisions regarding the use of this sweetener in their product (Lawless and Heyman 2010). Using these attributes, food scientists are able bridge qualities of specific food elements, and knowledge of consumer opinions to produce a product that is a unique part of existing food cultures and systems.

**Smell:** Retronasal delivery of smell is caused by the ascent of odorants through the posterior nares of the nasopharynx, and typically occurs during respiratory exhalation or after swallowing (Pierce and Halpern 1996). It is in this route of delivery that odor can be studied and applied most easily to food applications/consumption, as it most closely resembles the natural habits of human consumers. The other form of smell is known as orthonasal delivery. In this delivery method, odor molecules enter the nose from the front and the smell is comprehended by the brain (Lawless and Heymann 2010). The varieties of volatile airborne molecules that enter either retronasally or orthonasally become interpreted as the precept of flavor (Lawless and Heymann 2010). Understanding these two routes, and the origin of flavor is a key element of current sensory research.

Although taste and odor have traditionally been considered two independent senses, understanding how taste and odor, in particular retronasal odor, interact to form a flavor percept has recently become of great interest.

**Taste Odor Interactions:** Sensory perception has been an area of interest for scientists since the early investigations of Hollingworth and Poffenberger in 1917.
From this early work, the emergence of how taste and retronasal odor interact to produce sensations prompted further exploration. Beginning with a study done by Murphy and others in 1977, taste-odor mixtures where used to investigate confusion that might occur between the two sensations. Confusion for subjects was evident when trying to distinguish the differences between taste and odor. It appeared that because of their confusion, subjects turned to taste as the source of sensations. This study also found that when addressing overall intensity of the taste/odor mixture, the mixture appeared to provide on average somewhat less than that of the sum of taste and odor (Murphy and others 1977). It was concluded that taste and odor must be independent from one another (Murphy and others 1977). Later studies (Murphy and Cain 1980, Hornung and Enns 1984, 1986) also concluded that taste and odor were independent. Research then shifted to investigate the concept of enhancement. Enhancement is categorized as an increase in a given rating due to a given attribute and was investigated by looking specifically at the role of taste.

**Enhancement of Taste by Odor:** The focus of sensory studies eventually shifted to looking at taste enhancement by various odors. One study observed that strawberry odor enhanced sweetness for all sucrose levels tested (Frank and Byram 1988). In contrast, Frank and Byram (1988) showed that peanut butter odor had no influence on sweetness ratings for sucrose. They concluded that there was some aspect of strawberry odor that lead to enhanced sweetness (Frank and Byram 1988). In the same study it was also found that strawberry odor does not enhance saltiness of NaCl. In addition, when subjects were asked to pinch their noses there was a reduction in sweetness of 85%. The significant reduction in sweetness, led researchers to believe
there was an olfactory process occurring. A later study confirmed both strawberry odor enhancement of sweetness, and observed again there was odorant dependency (Frank and others 1989).

**Halo-Dumping:** Setting up a proper experimental design for sensory studies has been shown to be important to obtain accurate results. The separate functions of taste and odor were classified in a study conducted by Hornung and Enns (1986). While this finding of separate functionality was a large portion of their investigation, they also looked into the importance of experimental design. They concluded that the design of an experiment is influential, and could pose negatively if not conducted in a correct manner.

Dumping is a response bias resulting from a lack of appropriate rating categories (e.g., rating sweetness only or vanilla odor only while both attributes are prominent). One result of dumping can be the misrepresentation of attributes in intensity ratings. An area of concern is studies where sweetness has been rated alone for stimuli that are mixtures of sucrose and “sweet” odors (Clark and Lawless 1994). Concern arose from Clark and Lawless (1994) on the accuracy of research conducted with procedures where ‘dumping’ could have occurred.

**Odor enhancement by taste:** Giving that taste enhancement is the consequence of dumping, new studies began using inclusive categorical rating. Green and others (2012) completed a defining study where all subjects were asked to rate both odor and taste intensities on every trial. The first experiment in this study had subjects taste aqueous samples (0.56 M sucrose, 0.32 M NaCl, 10mM citric acid, 0.56 M sucrose, and 10mM citric acid), odors (0.56mM furaneol, 0.00025% citral, and 1.8mM
vanillin), and their 12 taste-odor mixtures. Using the general version of Labeled Magnitude Scale (Green and others 1993, 1996), subjects rated taste categories and an “other” category. Results of this study found that “other” sensations produced by citral, vanillin, and furaneol in a mixture were rated significantly higher than those by odor alone. The second component of this study was to apply the concept used in the aqueous samples to a food system. Using a cherry flavored drink and vanilla pudding as the food systems, it was found that with more sugar, cherry flavor was rated higher. However, by adding more vanillin (0.2%) to the custard samples, the “other” rating representative of vanillin was not more than that of the value with added sucrose. With the addition of sucrose to the custard, “other” representative of vanilla flavor increased nearly 10 fold (Green and others 2012). This result was an important finding as the addition of sucrose enhanced the perception of the vanillin better than the addition of greater amounts of vanillin. This study concluded that retronasal odor is enhanced specifically by the sweetness of sucrose when the experimental design avoids halo dumping.

**Factors affecting taste-odor interactions:**

**Congruency:** Congruency is a term used to describe relationships between two or more objects. Two things (tastes and odors) are said to be congruent if they are commonly experienced together in foods and are therefore associated with each other (Lim and others 2014). Lim and others (2014) looked at the effects of congruency on degree of odor enhancement and odor referral. They confirmed that congruency plays an essential role in retronasal odor enhancement by taste in showing that sucrose, a known congruent tastant to citral and coffee odors, enhanced the intensity of both
odors (Lim and others 2014). Sucrose was the only tastant that caused retronasal odor enhancement, suggesting that the nutritive classification of a taste is required for such phenomena to occur (Lim and others 2014).

**Nutritive Tastes:** In Green and others (2012), it was observed that sucrose was the only taste source that caused retronasal odor enhancement. From this observation it was then hypothesized that it is only nutritive tastes that causes retronasal odor enhancement. Sweetness in the form of sucrose is categorized as a nutritive taste as it serves as a signal for the presence of simple carbohydrates, which provide necessary calories for survival (Breslin 2013). In contrast, some other tastes such as bitterness or sourness signal the presence of toxic compounds (Breslin 2013). Studies by Lim and others (2014), investigated the phenomena by testing the roles of nutritive tastes and the congruency between taste and odor. Based on the findings, they concluded that both congruency and a nutritive taste might be necessary conditions for retronasal odor enhancement to occur.

**Sweeteners:** Research on sweet taste and sugar consumption has been of great interest in the areas of food science, nutrition, and public health. With an increased awareness of obesity and diabetes, and their prevention, the impact of caloric consumption from sugar has lead to greater interest in the use of alternative sweeteners in food products (Bellisle and Drewnowski 2007). Alternative sweeteners that are currently on the market have a range of attributes and characteristics. Some major differences include caloric content, synthetic vs. natural, known side tastes (e.g., bitter, sour, metallic, salty), cost, and physical attributes (bulk vs. high potency). Understanding how alternative sweeteners affect retronasal odor enhancement is useful in the
development of low calorie food products that are accepted by wide variety of consumers.

Sucrose, the most common and traditional sweetener provides essential calories to the body and signals the presence of carbohydrates. Sweeteners are used in products ranging from carbonated beverages to baked goods, and are commonly found in candy as well as household products. Alternative forms of sweeteners have been highlighted for their zero or low calorie content and ability to lower the overall caloric content of food products. While much is known about the physicochemical structure of various alternative sweeteners and their behaviors in food systems, it is unknown whether they can enhance retronasally perceived food odors as sucrose does (Kroger and others 2006).

Though artificial sweeteners have been in production for almost a hundred years, there has been, and continues to be an interest in research looking at the safety and functionality of their use. Choosing a wide range of non-nutritive sweeteners is essential in this experiment. All sweeteners chosen for this experiment are either FDA approved or have achieved GRAS status. In addition to a sweetener’s status, the four sweeteners were selected based on bulk vs. high-potency status, zero vs. low calorie content, and presence or absence of side-tastes identified by prior research.

**Tagatose:** Tagatose, is naturally low calorie bulk sweetener that achieved GRAS status in 2001. Tagatose is unique in the sweetener market for its natural occurrence in some fruits and dairy products, and is commercially produced from D-lactose (Fujimaru and others 2012). Tagatose is 0.9 to 0.92 times as sweet as sucrose, and has a clean taste profile lacking significant side tastes (Fujimaru and others 2012).
Tagatose was chosen as a sweetener in the category “bulk sweetener” with natural origins, no significant side tastes, and low calorie content.

**Saccharin:** The oldest of alternative sweeteners, saccharin was discovered in 1879 by Remsen and Fahlberg at John Hopkins University. Saccharin is currently approved for use in the United States as an interim food additive, allowing its use for special dietary and technological purposes (Bakal and Nabors 2012). Commonly sold under the name Sweet’N Low®, saccharin is reported to be from 200 to 700 times sweeter than sucrose (Shankar and others 2013). Saccharin was chosen as it is reported to provide zero calories, and has a taste profile described as containing both bitter and metallic components (Delwiche and Warnock 2008; Horne and others 2002; Kamerud and Delwiche 2007; Sediva and others 2006; Wiet and Beyts 1992).

**Sucralose:** Sold under the commercial name of Splenda®, sucralose was discovered in 1976, and was first approved by the FDA in 1998 for 15 types of foods and beverages (Kroger and others 2006). Sucralose contains zero calories, is stable when subject to high heat, and is used in over 4000 products in the United States (Shankar and others 2013). Its taste is said to contain low bitterness as a function of sweetness intensity (Weit and Beyts 1992). With little identified after taste, zero calories and high potency, sucralose is a good sample choice to investigate enhancement of retronasal odor.

**Rebaudioside A:** Rebaudioside A is a steviol glycoside originating from the Stevia plant. It gained GRAS status in 2008 (Kinghorn and others 2011). Classified as a high potency sweetener, rebaudioside A contains zero calories (Wölwer-Rieck and others 2010). Rebaudioside A has been observed to have evident bitter tastes in prior
sensory experiments (Fujimaru and others 2012). Due to the characteristics of rebaudioside-a, its inclusion balanced the set of sweeteners being used.

**Conclusion:** The evolving use of non-nutritive sweeteners in everyday foods highlights the importance of further investigations of these products. In determining the optimal use of these sweeteners, many avenues must be assessed. This study aims to determine the effects of selected zero and low calorie sweeteners on retronasal odor detection. The experimental procedure has been designed to eliminate the phenomenon of “dumping”. The broader aim of this study is to increase knowledge of taste-odor interactions, specifically the role of low or zero calorie sweeteners and their use in food products.
CHAPTER TWO: EFFECT OF ZERO-AND-LOW CALORIE SWEETENERS ON RETRONASAL ODOR ENHANCEMENT

EXPERIMENT I.

This experiment was designed to investigate the odor enhancement power of alternative sweeteners in a model system. It is hypothesized that all zero and low calorie sweeteners will enhance congruent food odors due to their “sweet” taste. It is also hypothesized that alternative sweeteners that elicit side-tastes (e.g., bitter taste), may display odor enhancement to a lesser degree than sweeteners without side tastes.

Materials and methods

Subjects

A total of 21 subjects (17 F, 4 M) between 18 and 45 years of age (mean= 23 years, SD 5.7 years) were recruited from the Oregon State University campus and were paid to participate. All subjects were healthy, non-pregnant, nonsmokers, who self-reported no deficits in taste or smell functions. No subjects had known lip or tongue piercings, oral lesions or known food allergies. Subjects who were on prescription medication were excluded from this study. When participating in this study subjects were asked to refrain from eating or using menthol products for a minimum of one hour prior to their scheduled session. The Oregon State University Institutional Review Board approved the experimental protocol, and subjects gave written informed consent.

Stimuli:

A total of 11 samples were used for test stimuli: 5 taste stimuli, 1 food odor, and 5 taste-odor mixtures. The taste stimuli consisted of sucrose (>99% reagent
grade, Macron Fine Chemicals), rebaudioside A (97% food grade, Pure Circle Ltd.,
Malaysia), sucralose (>99%, food grade, Tate and Lyle Singapore Pte. Ltd.,
Singapore), saccharin (reagent grade, ≥99%, Sigma Aldrich), and tagatose (>99%,
food grade, CJ Corp., South Korea). The concentrations of taste stimuli (Table 1)
were chosen to produce approximately equi-intense sweetness values based on
previous study concentrations and pilot testing. All taste stimuli were prepared
weekly with deionized water and were stored in the refrigerator in airtight glass
containers. Odor and taste-odor mixture stimuli were prepared daily, also using
deionized water. The odorant vanillin was prepared at a concentration of 1mM, by
measuring a calculated value of solid vanillin and dissolving the product in deionized
water. The test stimuli were allowed to come to room temperature (22-24°C) prior to
testing.

Table 1-Concentration of sweeteners used.

<table>
<thead>
<tr>
<th>Sweetener (Molecular weight)</th>
<th>Concentration in molarity and in % (w/v)</th>
<th>Calorie Composition (kcal/g)</th>
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</thead>
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<tr>
<td>Sucrose (MW= 342.30)</td>
<td>0.25 M (8.56%)</td>
<td>3.94</td>
</tr>
<tr>
<td>Rebaudioside A (MW= 967.03)</td>
<td>1 mM (0.097%)</td>
<td>0</td>
</tr>
<tr>
<td>Saccharin (MW= 183.18)</td>
<td>1 mM (0.018%)</td>
<td>0</td>
</tr>
<tr>
<td>Sucralose (MW=397.14)</td>
<td>0.3 mM (0.012%)</td>
<td>0</td>
</tr>
<tr>
<td>Tagatose (MW=180.16)</td>
<td>0.60 M (10.81%)</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Procedure:

Each subject attended a single session. The beginning of the session was as a
training period where each subject learned how to use the scale used in the
experiment. After the structure of the generalized version of the Labeled Magnitude
Scale (gLMS; Green et al. 1993, 1996) (Appendix 1) was introduced or reviewed, the subject used the scale to rate 15 practice remembered or imagined sensations (e.g., sourness of a lemon, the pain from stubbing your toe). Instructions were given to the subject to then recognize the qualities of sweetness, bitterness, and vanilla flavor using three practice stimuli (0.18 M sucrose, 0.056M quinine, and 1mM vanillin). Subjects were told that in each stimulus could elicit one, or multiple sensation(s), and the label “no sensation” should be used to demonstrate an absence of a certain quality.

Subjects were given a total of 11 samples. They were asked to taste each 5mL sample with a gentle smacking motion for three seconds, expectorate it, and rate the perceived intensities of sweetness, bitterness, and vanilla flavor. Subjects were asked to make their perceived intensity ratings based upon the maximum intensity experienced from the samples. All 11 samples were evaluated in a pseudorandom order, with 15 different orders that were counterbalanced across subjects. The subject rinsed his or her mouth at least three times with deionized water during a one-minute break between stimuli. A container of deionized water for rinsing between stimuli was kept in a heated circulated water bath at a controlled 37°C to avoid cooling of the mouth during rinsing. The experiment was conducted on a one-to-one basis various times of day in the psychophysical testing laboratory in the Department of Food Science and Technology at Oregon State University.

*Data Analysis:*

Before statistical analyses were conducted, the intensity ratings were log transformed as gLMS data is log-normally distributed (Green et al., 1993, 1996).
After log transformation, the data was analyzed using repeated-measures Analysis of Variance (ANOVA) followed by Tukey’s honestly significant difference (HSD) test in order to examine the effect of various sweeteners on perceived intensities of sweetness, bitterness, and vanilla flavor. Ratings of the taste stimuli samples were compared to its same sweetener in a taste-odor pair, and statistically evaluated using dependent T-tests. As a statistical tool the T-tests served to investigate if taste enhancement occurred with the addition of the odorant. All statistical analysis was performed using Statistica 12 (StatSoft Inc., Tulsa, Okla., U.S.A.).

Results

Figure 1 shows the log mean perceived intensity ratings of sweetness for each of the sweeteners tested. The sweetness intensities of the five sweeteners were not statistically different from each other. Repeated-measures ANOVA with stimulus as the factor confirmed that there was no significant main effect of the stimulus [F (4, 80)=2.1104, p=0.09].
Figure 1: Log mean ratings of the perceived intensity of sweetness of five sweeteners in aqueous solutions. Perceived intensity of sweetness was not statistically different between sweeteners (Tukey’s HSD test, p>0.05). Labels in the right y axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean.

Perceived intensity of bitterness was an additional attribute recorded by each subject for each sample. As seen in Figure 2, repeated measures ANOVA showed that there was a significant difference between bitterness intensity ratings of the five different sweeteners \( [F (4.80)=12.64489, p<0.0001] \). Further analysis using Tukey’s HSD test revealed that rebaudioside-a and saccharin were similar in bitterness ratings, which were significantly more bitter than the sweeteners sucrose, sacralose, and tagatose.
Figure 2: Log mean ratings of the perceived intensity of bitterness of five sweeteners in aqueous solutions. Perceived intensity of bitterness was not statistically different between the rebaudioside-a and saccharin samples, but these two bitterness ratings were significantly different from sucrose, sacralose, and tagatose (Tukey’s HSD test, p<0.001). Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean. Different letters indicate significant differences in the perceived intensity of bitterness across different sweeteners (Tukey’s HSD test, p<0.001).

Results of vanilla flavor ratings of taste-odor mixtures of the five different sweeteners in comparison to odorant alone are presented in Figure 3. Across all sweeteners an increase in perceived intensity of vanilla flavor was evident with the addition of the sweeteners to the vanillin sample. Repeated-measures ANOVA with Tukey HSD confirmed that there was no significant main effects of vanilla flavor intensity with changing sweetener, however there was a statistically significant difference between vanillin alone and the other samples \[F(5,100)=4.49, p<0.001]\. Overall findings conclude that the retronasal odor enhancement occurred with the addition sucrose, and all four zero or low calorie sweeteners.
Figure 3: Log mean ratings of the perceived intensity of vanilla flavor of the binary taste odorant mixture stimuli with five different sweeteners. Perceived intensity of vanilla flavor was not statistically different between sweeteners, but statistically significant from vanillin alone. Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S strong. Vertical bars represent the standard errors of the mean. Different letters indicate significant differences in the perceived intensity across different sweeteners when added to the vanillin odorant (Tukey’s HSD test, p<0.001).

Sweetness levels of binary mixtures (sweetener plus vanilla odorant) were recorded by each subject. These values were compared to the individual sweetness ratings of each given sweetener using dependent t-tests. For each of the five sweeteners there was no significant difference between sweetness ratings of the sweetener alone and the addition of the vanilla odorant; [sucrose p=0.075, tagatose p=0.740, sucralose p=0.648, saccharin p=0.533, rebaudioside a p=0.183] In some cases the value of sweetness ratings on average was found to be less with the odorant included. Figure 4 below shows the dependent t-tests for the five sweeteners (sucrose, tagatose, sucralose, saccharin, and rebaudioside a) displaying a lack of taste enhancement with the introduction of vanillin odorant.
Figure 4: Log mean ratings of the perceived intensity of sweetness of five sweeteners presented alone (dark bar) or with the retronasal odorant vanillin (light bar) is shown above. Perceived intensity of sweetness was not statistically different between the sweetener alone and its binary mixture with vanillin odorant (Dependent T-tests p>0.05). Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean.

Bitterness levels of the taste-odorant mixtures were also analyzed, investigating the effect of vanillin on the perceived intensity of bitterness. Intensity levels of the sweetener with vanillin odorant were compared to the sweetener alone using dependent t-tests. All sweeteners except rebaudioside-a, and its complimentary binary mixture showed no significant difference in bitterness intensity [sucrose p=0.076, tagatose p=0.300, sucralose p=0.419 and saccharin p=0.131]. The bitterness of rebaudioside-a was significantly reduced by the addition of vanillin p<0.05, (Figure 5).
Figure 5: Log mean ratings of the perceived intensity of bitterness of five sweeteners presented alone (dark bars), and with retronasal odorant vanillin (light bars). Perceived intensity of bitterness was found to be only statistically significant between the samples of rebaudioside-a, and rebaudioside-a plus vanillin (Dependent t-tests, p<0.05, p=0.041). Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean.

**EXPERIMENT II.**

The second experiment was designed to investigate retronasal odor enhancement by sweet taste, as well as the degree to which subjects liked samples in a food system. Through the use of custard as a model system, retronasal odor enhancement by four, zero and low-calorie sweeteners was investigated. It is hypothesized that all zero and low-calorie sweeteners will enhance retronasal odor to the same degree as sucrose, a result observed in Experiment 1. It is also hypothesized that some samples with saccharin and rebaudioside-a may be liked less than others due to their side-tastes (e.g., bitterness).
Materials and methods

Subjects

A total of 20 subjects (15F, 5M) between 19 and 48 years of age (mean= 24 years SD=7 years) were recruited from the Oregon State University campus and were paid to participate. The same restrictions and guidelines for participation were followed as in Experiment 1. The Oregon State University Institutional Review Board approved the experimental protocol, and subjects gave written informed consent.

Stimuli

A total of six custard samples were used as test stimuli. The custard was made using custard powder (Bird’s Custard Powder, Premier Ambient Products Ltd.; Ingredients: cornflower, salt, color: annatto, and flavoring) with varying sweeteners as listed in Table 1, and vanillin (1mM; Sigma-Aldrich). The concentrations of taste stimuli (Table 1) were chosen to produce approximately equi-intense sweetness values. The custard was formulated using 35 grams of the custard powder and appropriate calculated concentrations of sweetener according to molarities in Table 1 (51.345 g for sucrose, 64.858 g for tagatose, 0.071g for sucralose, 0.110g for saccharin, and 0.580g for rebaudioside-a). The dry ingredients were then combined in 600mL of whole milk, and stirred until well mixed. The mixture was then microwaved for three minutes before being stirred, heating was continued with occasional stirring for a total cook time of six minutes. After the custard was cooled, vanillin (0.091g) was added and the custard was mixed again. All custard samples were kept for no more than three days, and were stored in the refrigerator in airtight
containers. The custard samples were allowed to come to room temperature (22-24°C) prior to testing.

Procedure:

Each subject attended a single session. Each session was divided into two parts; the first part measuring degree of liking and disliking of the samples, and the second part measuring intensity of flavor qualities. The beginning of each session was a training period where the subject learned how to use the appropriate scale for that each part.

The first part of the experiment aimed to assess the degree to which subjects liked or disliked the six different custard samples using the labeled hedonic scale (LHS) (Appendix 2). After the structure of the LHS scale was introduced or reviewed, the subject used the scale to rate 15 remembered or imagined sensations (e.g. taste of plain bread, the smell of a rose) to gain experience and a context for sensations represented in the scale. Subjects were then given six different custard samples. They were asked to taste a small spoonful of each sample (approximately 3 g) with a gentle smacking motion for three seconds, expectorate it, and rate the degree to which they liked or disliked the sample. Samples were expectorated to prevent residual taste in subjects’ mouth. All six samples were evaluated in a random order, with 12 different orders that were counterbalanced across subjects. During a one-minute break between stimuli the subject rinsed his or her mouth at least three times with deionized water kept under the same conditions as in Experiment 1.

The second part of the session involved the use of the gLMS and began with either an introduction or revision of the scale and the 15 practice sensations as done in
Experiment 1. Subjects were given the same six custard samples as in the previous part and sampled them in the same manner. Subjects then rated the perceived intensities of sweetness, bitterness, and vanilla flavor for each sample. Subjects were asked to make their perceived intensity ratings based upon the maximum intensity experienced from the samples. All six samples were evaluated in a random order, with 12 different orders that were counterbalanced across subjects and not the same order as used in the previous hedonic ratings. The subject rinsed his or her mouth at least three times with deionized water during a one-minute break between stimuli. The experiment session was conducted on a one-to-one basis in the psychophysical testing laboratory in the Department of Food Science and Technology at Oregon State University.

Data Analysis:

Before statistical analyses were conducted, the intensity ratings were log transformed as gLMS data is log-normally distributed (Green et al., 1993, 1996). Using the log transformed gLMS data, and the original hedonic data, a repeated-measures ANOVA followed by Tukey’s HSD test were performed. These statistical analyses were used in order to examine the effect of various sweeteners on perceived intensities of sweetness, bitterness, and vanilla flavor in addition to the degree of liking across samples. All statistical analysis was performed using Statistica 12 StatSoft Inc., Tulsa, Okla., U.S.A.).
Results

Figure 6 shows the log mean intensity ratings for sweetness of each custard sample containing a different sweetener. Repeated measures ANOVA showed that there were statistically significant differences in sweetness intensity between the six custard samples [F (5,95)=24.23, p<0.0001]. However, Tukey’s HSD test confirmed that between the five different sweetener custard samples there was no statistically significant difference in sweetness.

![Sweetness](image)

**Figure 6:** Log mean ratings of perceived intensity of sweetness in custard stimuli alone and with the addition of five different sweeteners. Perceived intensity of sweetness was not statistically different between sweeteners, but significantly different from the sample with no sweetener (Tukey’s HSD test, p<0.0001). Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean. *Different letters* indicate significant differences in the perceived intensity across different sweeteners when added to the custard sample (Tukey’s HSD test, p<0.0001)
Subjects also rated perceived bitterness of each custard sample, represented as log mean values in Figure 7. As seen in Figure 7, there was a significant difference between bitterness intensity ratings of the custard with five different sweeteners [F(5,95)=6.47 p<0.0001]. Further analysis using Tukey’s HSD test revealed that rebaudioside-a and the custard sample with no sweetener were similar in bitterness ratings. Further, the rebaudioside-a sample was similar in bitterness to the samples with tagatose and saccharin. However, tagatose and saccharin had mean log intensity values of bitterness also not significantly sucrose and sucrrose. Bitterness levels of the custard samples with sweeteners sucrose, sucrrose, tagatose, and saccharin all fell below the levels of “barely detectable”, with rebaudioside-a and no sweetener falling slightly above.

**Figure 7:** Log mean ratings of perceived intensity of bitterness in custard stimuli with no sweetener and the addition of five different sweeteners. Perceived intensity of bitterness was not statistically different between the no sweetener sample and the custard with rebaudioside-a. Bitterness was also not statistically different between
rebaudioside-a, tagatose, and saccharin samples, with tagatose and saccharin statistically insignificant from sucrose and sucralose (Tukey’s HSD test, p<0.01). Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S, strong. Vertical bars represent the standard errors of the mean. Different letters indicate significant differences in the perceived intensity across different sweeteners when added to the custard sample (Tukey’s HSD test, p<0.0001)

Vanillin served as the odorant used in the custard samples of this experiment. Results of vanilla flavor ratings of custard samples with the five different sweeteners in comparison to odorant alone are presented in Figure 8. Across all sweeteners, an increase in perceived intensity of vanilla flavor was evident with the addition of the sweeteners to the vanillin custard sample. Repeated-measures ANOVA with Tukey’s HSD confirmed that there were no significant main effects of vanilla flavor intensity across various sweeteners used, although there was a statistically significant difference between the custard with vanillin alone and the other samples [F(5,95)=14.49, p<0.0001]. Overall findings conclude that the retronasal odor enhancement occurred with the addition sucrose, and all four zero or low calorie sweeteners.
Figure 8: Log mean ratings of perceived intensity of vanilla flavor in custard stimuli alone and with the addition of five different sweeteners. Perceived intensity of vanilla flavor was not statistically different between sweeteners, but statistically significant from custard and vanillin with no added sugar. Labels in the right y-axis represent labels in the gLMS: BD, barely detectable; W, weak; M, moderate, and S strong. Vertical bars represent the standard errors of the mean. Different letters indicate significant differences in the perceived intensity across different sweeteners when added to the custard sample (Tukey’s HSD test, p<0.0001).

Hedonic ratings were also recorded to determine the degree to which subjects liked or disliked custard samples with different sweeteners. The mean hedonic ratings for custard with sweeteners were statistically greater than the custard sample without sweetener [F (5,95)=8.59, p<0.0001] as determined by repeated measures ANOVA followed by Tukey’s HSD post-hoc test. As displayed in Figure 9, there were statistically insignificant differences in mean hedonic rating values between all custard samples with sweetener added. However, the high potency sweetener samples (sucralose, saccharin, and rebaudioside-a) were all liked slightly less than the samples with bulk sugars (sucrose and tagatose). The custard sample with tagatose had the highest average hedonic rating, followed by sucrose, and sucralose. The lowest hedonic ratings were with the sweeteners saccharin and rebaudioside-a.
Figure 9: Mean hedonic ratings of custard stimuli with no sweetener, and addition of five different sweeteners. Hedonic rating of the custard samples was statistically insignificant between sweeteners, but statistically significant from custard and vanillin with no added sweetener. Labels in the right y-axis represent labels in the LHS: LS, like slightly; LM, like moderately; DS, dislike slightly, and DM, dislike moderately. Vertical bars represent the standard errors of the mean. Different letters indicate significant differences in the perceived intensity across different sweeteners when added to the custard sample (Tukey’s HSD test, p<0.0001).

Figure 10 displays the mean hedonic rating for the given sweetener addition bound by the range of ratings obtained from all subjects. The range of hedonic ratings for the samples with no sweetener, sucralose and rebaudioside-a show the largest individual differences in liking and disliking of the samples. The broad range of hedonic ratings displays the variability of subjects.
Figure 10: Hedonic ratings of custard stimuli with no sweetener, and the addition of five different sweeteners. Central dot represents mean value, top bar bounded by highest hedonic rating, and bottom bar bounded by lowest hedonic rating for that given sample. Labels in the right y-axis represent labels in the LHS: LS, like slightly; LM, like moderately; DS, dislike slightly, and DM, dislike moderately.

Discussion

The results of these studies confirm the enhancement of retronasal odor by sweeteners. There was no statistically significant difference in perceived intensity of sweetness across all sweetener stimuli in either experiment. Therefore enhancement values can be examined in relation to the sweetener in use and not sweetness level differences (Figure 1 & Figure 6). Results from both the aqueous and food system models indicated that all five sweeteners (sucrose, tagatose, sucralose, saccharin, and rebaudioside-a) had an enhancing power on vanilla flavor in the base product (Figure 3 & Figure 8). In Experiment 1 the sweeteners rebaudioside-a and saccharin did not have statistically different enhancement levels from the other sweeteners although
they evoked side-tastes. This finding suggests that even sweeteners with side-tastes could provide a similar degree of flavor enhancement in a food product. This knowledge could directly impact food design, and nutritional composition of various food products through the lowering of overall caloric content.

In addition to intensity of vanilla flavor, subjects were asked to rate sweetness and bitterness during Experiment 1 and Experiment 2. One observation made during Experiment 1 was the absence of “sweet” taste enhancement with the addition of the vanillin odorants. Observable in Figure 4, all binary mixtures (sweetener + vanillin odorant) had no difference in mean log intensity of sweetness from the sweetener alone. This trend supports previous studies disproving the phenomena of taste enhancement by odor (Green and others 2012).

We saw statistically significant bitterness ratings between sweeteners in each experiment. Experiment 1 yielded two groups that were significantly different from each other: group one (sucrose, tagatose, sucralose) and group two (saccharin & rebaudioside-a) (Figure 2). Comparing bitterness levels when factoring in the presence of the vanillin odorant, there was no significant difference between bitterness except for rebaudioside-a whose bitterness was significantly lower in the binary-mixture sample (Figure 5). Experiment 2 results also yielded high levels of bitterness for the custard sample containing the sweetener rebaudioside-a. Though high levels of bitterness were confirmed for the sweeteners saccharin and rebaudioside-a (Figure 2) and then evident in the food system (rebaudioside-a sample) vanilla flavor ratings yielded a different trend. There was still equivalent, statistically insignificant difference in mean log intensity values of vanilla flavor in
both model systems. From these results it is evident that the use of sweeteners that are identified to be more bitter can still provide the same flavor quality as those that don’t.

The hedonic data obtained in Experiment 2 yielded results on the subjects’ liking and disliking of the custard samples with the various sweeteners. The three sweeteners (sucrose, tagatose, and sucralose) were the most liked, though saccharin and rebaudioside-a were less liked, there was no significant difference in ratings between all five sweeteners. All custard samples yielded mean values that were between “like slightly”, and “like moderately” (Figure 9). The custard sample with no sweetener was significantly different from the other samples and was disliked with a mean value between “disliked slightly” and “disliked moderately”.

Though the mean hedonic values for all five sweeteners were not statistically different from each other there was evident difference in the range of ratings for each sweetener. As displayed in Figure 10, the range of ratings for a given sample can be quite extensive. Such range in values highlights the differences in liking of the samples by the 20 subjects in this study. The rebaudioside-a custard sample with significant bitter taste (Figure 7) had the lowest hedonic rating of the five sweeteners and largest range of all custard samples (maximum hedonic value of 73.17; minimum hedonic value of -16.77; range value of 89.94). This observation highlights differences in individuals in perception of taste, specifically bitter tastes. From one direction this observation supports prior research on the topic of genetic factors accounting for differences in bitter and sweet perception (Reed and others 2006). The hedonic range for the most bitter sample was the largest, highlighting the differences
in ratings between subjects specifically related to bitter taste. However, there are several other components that could have contributed to the observed ranges of liking. Other than genetics factors pre-exposure to a given sweetener, and general availability and presence in the food market could have been factors in the observed rating differences.
CHAPTER THREE: SUMMARY & CONCLUSIONS

In summary, the phenomenon of retronasal odor enhancement occurred in both the aqueous and food systems with addition of all five sweeteners. Though some of these sweeteners are known to have side tastes (e.g., bitterness), the degrees of retronasal odor enhancement were similar across sweeteners with or without such recognized tastes. Results gathered from these experiments did not support the phenomenon of taste enhancement by vanillin. Hedonic results displayed no significant difference in liking of custard samples with the five different sweeteners. However, the range in ratings suggests that genetics, pre-exposure, and availability of the sweeteners may affect the degree to which some of the zero or low calorie sweeteners were liked.

The overall results of these experiments were substantial in clarifying sensory principles and highlighting important attributes of using alternative zero and low calorie sweeteners. These results can be implemented by food scientists to further explore the use of zero and low calorie sweeteners in their development of new food products. In conclusion, the occurrence of retronasal odor enhancement by all five sweeteners suggests zero or low calorie sweeteners can be used in food products negating the concern of change in flavor to lower caloric composition. Beyond food science these results can serve as the forefront for nutritional development in tackling health and obesity worldwide.
gLMS scale

Appendix 1:

- Strongest Imaginable Sensation of Any Kind
- Very Strong
- Strong
- Moderate
- Weak
- Barely Detectable
- No Sensation
Appendix 2:

Labeled Hedonic Scale (LHS)

BIBLIOGRAPHY


