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# Dose-Response Relationships for Vanilla Flavor and Sucrose in Skim Milk: Evidence of Synergy 

Gloria Wang ${ }^{1,2}$, John E. Hayes ${ }^{1,2}{ }^{(D}$, Gregory R. Ziegler ${ }^{1}$, Robert F. Roberts ${ }^{1}$ and Helene Hopfer ${ }^{1,2, *}$<br>1 Department of Food Science, The Pennsylvania State University, University Park, PA 16802, USA; gzw19@psu.edu (G.W.); jeh40@psu.edu (J.E.H.); grz1@psu.edu (G.R.Z.); rfr3@psu.edu (R.F.R.)<br>2 The Sensory Evaluation Center, The Pennsylvania State University, University Park, PA 16802, USA<br>* Correspondence: hopfer@psu.edu; Tel.: +1-814-863-5572

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#### Abstract

Regarding cross-modality research, taste-aroma interaction is one of the most studied areas of research. Some studies have reported enhancement of sweetness by aroma, although it is unclear as to whether these effects actually occur: depending on the cognitive strategy employed by panelists, the effects may disappear, e.g., forcing panelists into an analytical strategy to control for dumping may not be able to reveal perceptual interactions. Previous studies have largely focused on solutions and model foods, and did not test stimuli or concentrations relevant to real food applications. This study addresses these gaps: 18 vanilla flavored sucrose milks, varying between $0-0.75 \%(w / w)$ two-fold vanilla, and $0-5 \%(w / w)$ sucrose, were rated by 108 panelists for liking and perceived sweetness, vanilla flavor, milk flavor, and thickness. Interactions between vanilla and sucrose were measured using deviations of real mixtures from additive models (via the isobole method), indicating vanilla aroma does enhance perceived sweetness. However, the sweetness enhancing effect of vanilla aroma was not as pronounced as that of sucrose on vanilla flavor. Measurable cross-modal interactions occur despite using an analytical cognitive strategy. More work is needed to investigate the influence of perceptual strategy on the degree of taste-aroma interactions in real foods.


Keywords: cross-modality; taste-aroma interactions; sweetness enhancement; vanilla flavor; flavored milk; sugar; isoboles; synergy

## 1. Introduction

Flavor perception is the result of chemical and physical food properties, and how they interact with our senses [1]. Flavor involves the integration of multiple modalities, including smell, taste, and touch, and studying each in isolation does not reflect what humans experience during eating. As a result, cross-modal interactions (i.e., the interaction of taste, aroma, vision, texture, and chemesthesis) are a popular area of study in flavor research. Taste-aroma interactions are the most commonly described interaction between sensory modalities, and occur as a result of physical, physiological, cognitive, and psychological effects [2,3]. Research on multisensory processes, including taste-aroma interactions, has been used to better explain the processes humans use to assess food flavor [4-6]. Due to the common confusion between smell and taste, taste perception is influenced by odor and vice versa [7-11]. In order for perceived taste intensity to be modified by an odor (or vice versa), not only are the method of stimulation and the instructions given important, but also the perceptual similarity between a tastant and odorant [3,10]. As defined by Schifferstein and Verlegh [12], the extent to which two stimuli interact in combination in a food is called congruency. Some work on taste-aroma interactions suggested that sweetness intensity can be enhanced by a congruent odor, although many of these studies have only been done in model sucrose solutions [7,9-11,13-21] or model foods [22-26].

Due to the complexity of food products, only a few taste-aroma interaction studies have been conducted in real foods, such as whipped cream, milk, fruit juices, ciders, custards and cherry drinks [7,27-30]. Although these studies moved taste-aroma interaction research into a more realistic matrix, the concentration ranges for both the odor and the taste component used in these studies was not always commercially relevant. This may be important since odor compounds have been shown to impact flavor perception at both subthreshold and suprathreshold concentrations, i.e., taste-aroma interactions have also been shown to be concentration dependent [29,31-33]. The lack of studies in real food products over a concentration range comparable to those used in commercial products prevents a comprehensive understanding and utilization of such cross-modal interaction phenomena in real foods.

Fluid milk is a relatively simple food well suited for studying taste-aroma interactions. While plain milk consumption has been on the decline, flavored milk consumption has been increasing, and is expected to continue to grow as flavor becomes more important to consumers, notably in the US [34]. A familiar congruent aroma-taste pair in Western cultures used in numerous dairy products is vanilla and sucrose. Vanilla is the most popular flavor for dairy applications such as yogurt and ice cream, and is a complementary ingredient in flavored milks [35]. The combination of vanilla and sucrose is also commonly studied in cross-modal research [36-38]; therefore, using vanilla and sucrose in a flavored milk application increases the ecological validity of studying cross-modal interactions in foods.

Despite an abundance of literature suggesting an enhancement of perceived sweetness by a congruent aroma (including vanilla), there are other contradictory studies as well. This discrepancy exists because assessment of taste-aroma interactions strongly depends on the cognitive task (i.e., test questions and instructions) used by assessors when evaluating samples. Previous studies using rating scales have shown reduced or no mixture-induced taste enhancement when assessors are asked to evaluate perceived sweetness as well as perceived aroma intensity $[7,10]$. Thus, some researchers have attributed any sweetness enhancement to a "dumping effect" [37]. That is, when a scale for a pertinent flavor attribute is not provided in the test (e.g., vanilla flavor), assessors will "dump" their perceptions into another similar category (e.g., sweetness) instead, leading to an increase in attribute intensity [37]. In contrast to this analytical mindset, adopting a synthetic mindset is more in-line with real eating behavior where the food is experienced as a whole and a hedonic and holistic evaluation of the food occurs. In the analytical mindset, enhancement effects have been found [7,13,15,17,18,27,39], but in many of these early studies participants did not rate the intensity of all salient product attributes (taste, aroma, and texture), which may have resulted in dumping. This makes them somewhat inconclusive, raising the question of whether enhancements or any interactions truly occur.

To address these unresolved questions, additional experiments that provide a full range of relevant product attributes are needed. Several studies have found strong evidence for odor (but not taste) enhancements in aqueous solutions and food matrices, even when controlling for dumping. For example, Green and colleagues [30] found enhanced odor perception in solutions, vanilla custard, and a cherry drink, by the addition of sucrose. Lim's group [40] found a similar effect for citral and sucrose solutions, and later [41] for citral and coffee solutions. Although these studies demonstrated that an analytical strategy did not prevent taste-aroma interactions, their use of a sip and spit procedure is not representative of normal eating behavior, which has been shown to influence the sensory profile of samples, depending on the taste and flavor characteristics [42,43].

Separately, testing for interaction between mixture components also requires a different model approach above and beyond simple significance testing of mean attribute ratings. Instead, one needs to test whether the degree of interaction between two stimuli is above (or below) what would be predicted in an additive model. A common approach for testing drug interactions is the isobole method [44-48]. The isobole approach uses concentrations, effects, and empirical concentration-effect relationships, and is independent of the mechanism of interaction; instead, it is based on the concept of concentration addition. The points on an isobole indicate the mixing values of multiple components at which a specific quantitative effect is produced that is either synergistic, antagonistic, or simply additive.

Isoboles have been used in a few studies to measure the interaction between food ingredients on chemosensation [49-51]. As the isobole approach is a useful concept for evaluating the type and degree of interactions between substances, regardless of their mechanisms of action [48], cross-modality of a sweet tastant and a congruent aroma can thus also be described and tested with the isobole method.

This study aimed to address the gaps outlined above by: (i) conducting a dose-response experiment in fluid milk to measure the cross-modal interactions between a sweet tastant and a congruent aroma; (ii) using a complete concentration design space relevant for flavored milks; (iii) controlling for potential dumping effects; and (iv) using the isobole method to measure the type and degree of interaction.

## 2. Materials and Methods

### 2.1. Experimental Design and Sample Production

Eighteen sucrose-vanilla combinations were generated to model human sensory responses to the various mixtures with higher-order models (Table A1). Part of the design was composed of four levels across each of the two ingredients (in half-log steps) to generate dose-response functions for sucrose (granulated cane sugar) in milk and vanilla in milk. Additionally, a $3 \times 3$ factorial design was overlaid to create sucrose-vanilla mixtures in milk to generate the response surfaces for each sensory attribute. Collectively, this provides a total of 17 milk samples at systematically varied combinations of vanilla and sucrose. An additional control (plain milk) was added to bring the total number to 18. Milks were formulated with sucrose ranging $0-5 \%(w / w)$ and two-fold vanilla extract (i.e., twice the quantity of vanilla beans extracted in water and ethanol) ranging $0-0.75 \%(w / w)$, spanning a wide range of sucrose and vanilla concentrations, including those used industrially [52]. Two-fold vanilla extract as opposed to single-fold was used to minimize the amount of extractives added to the samples.

All milks were mixed with varying levels of sucrose in eight 32 kg -batches for pasteurization. Following pasteurization, 10 kg batches of each sucrose-vanilla combination were produced for sensory testing. Two-fold vanilla extract (David Michael \& Co, now Tastepoint by International Flavors and Fragrances; Philadelphia, PA, USA) was used as the vanilla flavor. Pasteurized skim milk ( $0.18 \%$ fat, $8.91 \%$ solids) and sucrose (Golden Barrel, Honey Brook, PA, USA) were provided by the Berkey Creamery (University Park, PA, USA). Prior to pasteurization, all amounts of milk and sugar were pre-weighed into stainless steel milk cans and plastic tubs, respectively. On the day of pasteurization, sugar was dissolved into one third of the milk from each milk can and then transferred back to the milk can for further mixing with metal agitators. Sucrose-milk premixes were then blended into each milk can by mixing with metal agitators on high speed for 10 min for complete dispersion and dissolution of the sugar. All mixes were pasteurized (high temperature short time (HTST), APV Junior Pasteurizer, APV Invensys, Woodstock, GA, USA) at $75^{\circ} \mathrm{C}$ for 25 s , and homogenized (Gaulin, Lake Mills, WI, USA) in a two-stage process at 10.3 and 3.5 mPa ( 2000 and 500 psi ), cooled ( $<7^{\circ} \mathrm{C}$ ), and collected into milk cans. From there, 13 mixes were flavored with pre-weighed vanilla extract and divided into 17 different sucrose-vanilla combinations. Each milk was packaged into $\frac{1}{2}$-gallon opaque plastic milk jugs and stored at refrigeration temperature $\left(<5^{\circ} \mathrm{C}\right)$ for $5-7$ days prior to sensory testing. All 18 samples were collected for physical analysis (percent total solids and percent fat; SMART Trac, CEM Corporation, Matthews, NC, USA) to ensure sucrose concentrations were within the required ranges. All 18 samples were also tested for coliforms (high-sensitivity Petrifilm; 3M, Maplewood, MN, USA) to ensure samples were suitable for human consumption. Viscosity of all milk samples was tested to account for potential physicochemical interactions that may lead to differences in flavor perception.

The viscosity was calculated as the slope of the shear stress vs. shear strain rate flow curve with shear strain rate ranging from 0 to $100 \mathrm{~s}^{-1}$ at $5^{\circ} \mathrm{C}$ to mimic sample serving temperature. The flow curves were plotted with Trios Software (TA Instruments, New Castle, DE, USA), omitting stress overshoot/noise at low shear strain rate ( 0 to $15 \mathrm{~s}^{-1}$ ). Two measurements for each sample were taken on a Discovery H3 Hybrid rheometer (TA Instruments, New Castle, DE, USA), equipped with a double
wall concentric cylinder (inner diameter 40.77 mm , outer diameter 43.88 mm , cup diameter 30.21 mm ; Table A1).

### 2.2. Consumer Acceptability and Intensity Ratings

A central location test was conducted with 108 participants (women $=76$, age $=19-71$ ) over three days; panelists tasted six milk samples per day in complete block design. Sample presentation was counterbalanced across panelists using a modified Williams-Latin Square design, presenting each sample at each position 6-7 times, and all 18 samples were served on each day to avoid potential confounding between samples and test days. Approximately $45-50 \mathrm{~mL}$ of milk were poured into 3.25-oz. ( $\sim 96 \mathrm{~mL}$ ) plastic cups (Fabri-Kal, Kalamazoo, MI, USA) and lidded; cups were labeled with random three-digit blinding codes. All data were collected with Compusense Cloud (Compusense Inc., Guelph, ON, Canada).

Participants were screened for dietary restrictions, food allergies and product use (i.e., those who consumed skim or $1 \%$ milk at least once a week, and indicated they would be interested in tasting vanilla flavored milk). Procedures were exempted from institutional review board review by professional staff in the Penn State Office of Research Protections under the wholesome foods exemption in 45 CFR 46.101(b) (protocol number 33164). Participants provided informed consent prior to testing and were compensated for their time. All samples were served at $5^{\circ} \mathrm{C}$ and tasted in individual tasting booths under red light and at ambient temperature ( $\sim 21-22^{\circ} \mathrm{C}$ ). Panelists were given deionized water at room temperature $\left(20^{\circ} \mathrm{C}\right)$ to rinse before and in between each sample. Degree of liking for each sample was measured first on a nine-point hedonic scale, with labels for each value ranging from "like extremely" to "dislike extremely" [53]. Panelists then rated the perceived intensities for sweetness, vanilla flavor, milk flavor, and thickness on an unstructured line scale (valued from 0 to 100), anchored with "very weak" on the left and "very strong" on the right.

### 2.3. Statistical Analysis

Data were analyzed using R (version 3.3.3) [54] and RStudio (version 1.1.453, Boston, MA, USA). A two-way Analysis of Variance (ANOVA) $(p<0.05)$ with all two-way interactions for sucrose and vanilla as fixed effects on viscosity and all sensory attributes was conducted, and followed by calculation of Tukey's honestly significant differences (HSD) with the agricolae package (version 1.2-8) [55]. The rsm package (version 2.8) [56] was used to create 3D response-surface plots of percent sucrose, percent vanilla, and each sensory attribute, modelling up to a second-order regression model by multiple linear regression, and using unaveraged responses from each assessor. Final equations were chosen based on lack of fit testing ( $p>0.05$ ), variance explained and overall fit [57]. Using the isobole approach [58], the degree of interaction between sucrose and vanilla was calculated for perceived sweetness ratings. According to the criteria set by Suhnel, $I=1$ indicates no interaction, $I<1$ indicates synergism, and $I>1$ indicates antagonism. In the equation, " $c_{\mathrm{i}}$ " denotes the concentration of component " i " in the mixture, and " $C_{i}$ " represents the concentration of " i " that would individually produce the same intensity as the mixture, calculated from the corresponding dose-response functions of vanilla-only and sucrose-only milks as found by multiple linear regression of the non-mixture samples. Since the milk samples consist of sucrose and vanilla, the general equation can be rewritten as:

$$
\frac{[\% \text { Sucrose in mixture }]}{\left[\begin{array}{c}
\% \text { Sucrose to achieve same sweetness }  \tag{1}\\
\text { as in mixture }
\end{array}\right]}+\frac{[\% \text { Vanilla in mixture }]}{\left[\begin{array}{c}
\% \text { Vanilla to achieve same sweetness } \\
\text { as in mixture }
\end{array}\right]}=I
$$

Three-dimensional isobolograms were generated for perceived sweetness using OriginPro (Origin Lab Corporation, version 2017 64-bit 94E, Northampton, MA, USA). To generate these plots, linear regression on the sweetness ratings found for the vanilla-only and sucrose-only samples was applied to generate a 3D sweetness plane. The mean attribute ratings for each of the sucrose-vanilla mixtures
were then plotted onto the plane. Any points above the plane indicate synergism, while any values that fall below the plane are indicative of antagonism, and any that contact the plane indicate no interaction. Variation within the $I$ values was accounted for by establishing a range of $0.9<I<1.1$ (= $\pm 10 \%)$ as the zero-interaction criterion, similar to Fleming et al. [49].

## 3. Results

### 3.1. Physical Characterization

Overall, milk samples differed significantly in instrumental viscosity ( $p<0.05$ ), but only between the highest ( $5 \%$ ) and lowest ( $0 \%$ ) sucrose concentrations (Table A1). The observed viscosity differences were minor compared to the other factors, and were reflected in the sensory measurement of thickness, which also only differed significantly between the same high and low sucrose samples (Table A1).

### 3.2. Response Surface Models for Vanilla-Sucrose Milks

Three-dimensional response surface models were created for overall liking and perceived sweetness, vanilla flavor, milk flavor, and thickness (Figure 1). In general, the experimental design space used here was effective in quantifying potential interactions between sucrose and vanilla and their effects on sweet taste and liking in milk across a wide range of concentrations. Sweet taste, milk flavor, and thickness were all sufficiently modeled by a first-order model, indicating a linear relationship, while for overall liking and perceived vanilla flavor intensity a second-order model best represented the observed responses. Second-order models for liking and vanilla flavor perception were expected as both often follow an inverted U-shaped optimum: at too high sugar concentrations a product becomes too sweet for consumers. Likewise, vanilla dosing can have a strong effect on its flavor profile. Prior reports suggest that at low levels, vanilla extract adds slight modification notes, regular dosages impart the characteristic and expected flavor profile, while at high levels, off-notes (e.g., woody, phenolic, and alcoholic notes) may negatively influence liking [59].

For the three linear models for sweetness, milk flavor, and thickness, an increase in sucrose was accompanied by a significant increase in sweetness $\left(\mathrm{R}^{2}=0.37, \mathrm{~F}(2,1941)=567.5, p<0.05 ; \mathrm{m}=10.54\right.$ for sucrose, $\mathrm{m}=5.11$ for vanilla) and thickness $\left(\mathrm{R}^{2}=0.022, \mathrm{~F}(2,1941)=23.2, p<0.05 ; \mathrm{m}=1.97\right.$ for sucrose, $\mathrm{m}=-2.42$ for vanilla), and a decrease in milk flavor $\left(\mathrm{R}^{2}=0.015, \mathrm{~F}(2,1941)=16.24 ; \mathrm{m}=-1.04\right.$ for sucrose, $\mathrm{m}=-10.37$ for vanilla) (Figure $1 \mathrm{~b}, \mathrm{~d}, \mathrm{e}$ ). These findings were all expected and are similar to those found for coffee-flavored milks [60], where both thickness and milk flavor were influenced by sucrose and coffee extract concentration, respectively. That is, increasing sugar concentration led to higher viscosities, and both vanilla and coffee flavor extracts decreased/masked milk flavor.

For the two second-order models, both liking and vanilla flavor increased up to a concentration after which ratings for both decreased. For liking, the design space covered the preferred concentration range, as the optimal liking, i.e., the maximal point on the liking surface, was found at $3.79 \%$ sucrose and $0.53 \%$ vanilla (Figure 1a). When compared to the maximum liked sample served to participants, this optimum was quite close in terms of sucrose concentration ( $3.82 \%$ ), but was slightly lower than the tasted sample's vanilla concentration of $0.625 \%$. The maximal point for the second-order model for vanilla flavor was found to be at $4.98 \%$ sucrose and $0.52 \%$ vanilla (Figure 1c), which is above the highest tested sucrose-vanilla mixture in terms of sucrose concentration $(3.82 \%)$ and just below the highest tested sucrose concentration of $5 \%$, but within the range of tested vanilla concentrations of $0.3 \%, 0.435 \%$ and $0.625 \%$.

Inspecting the response surfaces, it becomes apparent from the first-order model for sweetness that increasing the vanilla concentration leads to a slight but statistically significant enhancement of sweetness (coefficient $=5.11 ; p<0.05$ ). This can be seen in Figure 1b as the bottom and top edges of the plane are tilted slightly upward as vanilla concentration increases). Conversely, the addition of sucrose increased the perception of vanilla significantly and substantially in the regression model for vanilla flavor (coefficient $=11.25 ; p<0.05)$ (Figure 1c). These findings are in agreement with Green
et al. [30] and Welge-Lussen et al. [61] who found that the perception of retronasal vanilla odor was driven to a larger degree by the addition of sucrose than the addition of more vanilla flavor. Similarly, Alcaire et al. [62] found a decrease in vanilla flavor perception when a vanilla flavored milk dessert was reduced in sugar by $20 \%$. The same pattern has been observed for other tastes: Linscott and Lim [63] found that the tastes impacted odor perception more than the odors affected tastes when pairing NaCl and monosodium glutamate (MSG) with chicken and soy sauce odors, respectively. Earlier, Pfeiffer et al. [14] reported synergistic effects of sucrose and acid on strawberry flavor, and that strawberry flavor was perceived more intensely when sucrose and acid were used together. Furthermore, it seems unlikely that these findings could be explained by a physiochemical "salting out" effect from the addition of sugar, as three different studies found that instrumentally measured volatile concentration and release profiles remained constant even when tastants concentrations (e.g., sucrose or acids) were varied $[14,23,64]$. Generally, our findings agree with others who reported significant odor enhancement by taste [40,41,65], and seem to indicate a cognitive basis for the observed flavor enhancement rather than physicochemical interactions that influence to the solubility of components in the vanilla milk mixtures.


Figure 1. 3D response surfaces established from the 18 samples evaluated by 108 consumers, for all sensory response variables and sucrose ( S ) and vanilla ( V ) concentration. Dose-response functions are included for each surface: (a) overall liking OL $=4.40+0.669 \cdot \mathrm{~S}+1.42 \cdot \mathrm{~V}+0.198 \cdot \mathrm{~S} \cdot \mathrm{~V}-0.102 \cdot \mathrm{~S}^{2}-$ $2.05 \cdot \mathrm{~V}^{2} ; \mathrm{R}^{2}=0.11, \mathrm{~F}(5,1938)=47.92, p<0.05 ;(\mathbf{b})$ perceived sweetness $\mathrm{SW}=18.1+10.5 \cdot \mathrm{~S}+5.11 \cdot \mathrm{~V} ; \mathrm{R}^{2}$ $=0.37, \mathrm{~F}(2,1941)=567.5, p<0.05$; (c) perceived vanilla flavor $\mathrm{VF}=13.8+11.3 \cdot \mathrm{~S}+50.6 \cdot \mathrm{~V}-1.17 . \mathrm{S}-$ $1.07 \cdot \mathrm{~S}^{2}-42.6 \cdot \mathrm{~V}^{2} ; \mathrm{R}^{2}=0.21, \mathrm{~F}(5,1938)=104.7, p<0.05$; (d) perceived milk flavor $\mathrm{MF}=46.9-1.04 \cdot \mathrm{~S}-$ $10.4 \cdot \mathrm{~V} ; \mathrm{R}^{2}=0.02, \mathrm{~F}(2,1941)=16.24, p<0.05$; and $(\mathbf{e})$ perceived thickness $\mathrm{TH}=29.4+1.97 \cdot \mathrm{~S}-2.42 \cdot \mathrm{~V}$; $R^{2}=0.02, F(2,1941)=23.2, p<0.05$.

### 3.3. Testing for Interactions with the Isobole Approach

The response surface models indicated deviations from simple additive effects when sucrose and vanilla are combined; however, the degree of such interactions need to be formally tested with a method that accounts for the shape of the zero-interaction response surface (i.e., the isobole method). In Table 1, the indices of interaction (I) for sweetness, as calculated by Equation (1), are shown for all nine sucrose-vanilla mixtures. If using Suhnel's [58] liberal criterion for interaction (i.e., any $I \neq 1$ ), every mixture except for two ( S 3.82 V 0.3 and S 3.82 V 0.625 ) exhibited synergism for perceived sweetness $(I<1)$, with the other two showing antagonism $(I>1)$ and no interaction $(I \sim 1)$. However, applying our more realistic cut-off criterion, four samples at the low to medium sugar concentrations ( $1.31 \%$ and $2.24 \%$ sucrose at both $0.3 \%$ and $0.435 \%$ vanilla) showed evidence of synergy, with $I$ values between 0.776 and 0.886 . Further, interactions between sucrose and vanilla were stronger at lower vanilla concentrations, as observed for both sucrose concentrations. This indicates that lower levels of vanilla induce larger cross-modal interactions with sugar.

Table 1. Indices of interaction $(I)$ for perceived sweetness in the nine sucrose-vanilla mixtures. $S=$ sugar, $\mathrm{V}=$ vanilla, Cs and Cv represent the concentration of sugar and vanilla, respectively, that would individually produce the same intensity as the vanilla-milk mixture. $C_{S}$ and $C_{V}$ were calculated using the dose-response functions for sweetness with the sucrose-only mixtures (Sweetness $S W_{\text {sugar }}=$ $\left.17.35+10.52 \cdot \mathrm{~S} ; \mathrm{R}^{2}=0.43, \mathrm{~F}(1,538)=406.24, p<0.05\right)$ and the vanilla-only mixtures (Sweetness $\left.S W_{\text {vanilla }}=15.71+6.41 \cdot \mathrm{~V} ; \mathrm{R}^{2}=0.007, \mathrm{~F}(1,538)=4.92, p<0.05\right) ; I$ was calculated using Equation (1).

| Sample | Sucrose (\% w/w) | Vanilla (\% w/w) | Sweetness (-) | Cs | Cv | $\boldsymbol{I}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| S1.31V0.300 | 1.31 | 0.300 | 37.4 | 1.91 | 3.38 | $0.776^{+}$ |
| S1.31V0.435 | 1.31 | 0.435 | 37.2 | 1.89 | 3.35 | $0.823^{+}$ |
| S1.31V0.625 | 1.31 | 0.625 | 36.3 | 1.80 | 3.21 | $0.922^{0}$ |
| S2.24V0.300 | 2.24 | 0.300 | 46.8 | 2.80 | 4.85 | $0.862^{+}$ |
| S2.24V0.435 | 2.24 | 0.435 | 46.9 | 2.81 | 4.87 | $0.886^{+}$ |
| S2.24V0.625 | 2.24 | 0.625 | 44.8 | 2.61 | 4.54 | $0.997^{0}$ |
| S3.82V0.300 | 3.82 | 0.300 | 58.8 | 3.94 | 6.73 | $1.01^{0}$ |
| S3.82V0.435 | 3.82 | 0.435 | 60.9 | 4.14 | 7.06 | $0.984^{0}$ |
| S3.82V0.625 | 3.82 | 0.625 | 58.1 | 3.88 | 6.62 | $1.088^{-}$ |

${ }^{+}$synergistic interaction; ${ }^{0}$ zero interaction, ${ }^{-}$antagonistic interaction.

A more intuitive, graphical representation of these interactions is provided in Figure 2. Here, a zero-interaction response surface (the two-dimensional plane depicted in blue) was produced from the individual dose-response functions of the vanilla-only (Sweetness $S W_{\text {vanilla }}=15.71+6.41 \cdot \mathrm{~V}$ ) and sucrose-only (Sweetness $S W_{\text {sugar }}=17.35+10.52 \cdot$ ) samples. Observed responses for specific vanilla-sucrose mixtures are visualized by individual dots; any mixtures that are located above the plane indicate synergism $(I<1)$, any mixtures below the plane are indicative of antagonism $(I>1)$, and any that lay on the zero-interaction surface demonstrate no interaction. Similar to the $I$-values shown in Table 1, all but two mixtures are above the additive plane, in line with Suhnel's criteria, and four of these also satisfied our working criterion (mixtures circled in black).

Overall, synergism seems to occur at the lower to medium sucrose and vanilla concentration ranges as suggested by the lower interaction values, indicating a concentration dependence effect of the vanilla and sugar on sweetness perception.


Figure 2. Isobologram for the vanilla-sucrose milks determined from their respective dose-response functions. Deviations above the zero-interaction surface correspond to $I$-values below 1 (synergy). Deviations below the surface correspond to $I$-values above 1 (antagonism). Those on or near the surface indicate no interaction $(I=1)$. Mixtures that satisfied the working criterion are circled in black.

## 4. Discussion

Overall, this study demonstrates in a real food product how closely sweet taste is associated with vanilla, i.e., the combination of vanilla and sugar creates a stronger vanilla flavor than vanilla alone. Vanilla also increases the perceived sweet taste of sugar, but the effect is smaller. It has consistently been reported that vanilla odor smells "sweet", at least by western participants, even though the olfactory system does not contain any receptors for sweet tastants [16,66]. This induction of sweetness by odors, even when they cannot be detected by taste receptors, seems related to previous combined exposure to vanilla aroma and a sweet tastant, such as those that could occur during eating [16,20,67].

Even when panelists were given a presumably complete list of attributes to rate (in an attempt to account for potential dumping effects), we still found evidence for interaction between the taste and the aroma. That is, adopting an analytical perceptual strategy did not appear to prevent taste enhancements from occurring. Aroma had a significant impact on taste and vice versa, though taste induced-odor enhancement appeared larger than odor induced-taste enhancement. Our findings are similar to other studies in that increasing sucrose concentration resulted in higher ratings of flavor [32,33,36,68]. Further, it should be noted that although increasing vanilla led to a significant sweetness enhancement, the magnitude of this taste enhancement by a congruent odor may be small in comparison to the use of a non-nutritive sweetener.

Despite results from other literature suggesting that any sweetness enhancement by an odor may be the result of a dumping artifact, our findings do not support that view. Rather, we were able to measure a small, but significant effect of vanilla aroma on sweetness perception. Valentin and Nguyen [69], when studying the effect of vanilla and lemon aroma on sourness ratings using two different tasks, came to a similar conclusion: providing more appropriate scales decreases odor-induced taste enhancement, but does not completely suppress it. Tasks that do not depend on intensity ratings have similarly found evidence of cross-modal interactions: in a sweet taste detection task, sucrose detection was increased when combined with a congruent strawberry odor versus an incongruent ham
odor [70]. Similarly, in a categorization task, participants' categorization performance was poorer when the concentration of vanillin changed along with the sucrose concentration, implying that participants were unable to ignore the olfactory component when trying to categorize solutions according to the tastants [71]. Collectively, these studies and present results indicate that taste-odor interactions cannot be explained solely by response biases [72].

We also provide novel evidence of synergistic effects of mixture components by testing for interactions effects that go beyond the simple additivity by using the isobole method. Of the vanilla-sucrose milk mixtures we tested, four had indices of interaction (I-values) of less than 0.9 (a conservative cutoff), indicating a synergism when the two components were mixed together. As the isobole method was adapted from pharmacology, it would be important to determine where the index of interaction (I) provides a meaningful sensory effect. Future experiments with mixtures of varying $I$ values are needed to determine differences that are perceivable by consumers. As Suhnel's criterion is calculated based on empirical data collected separately for each food matrix, it would be important to test whether the cut-off criteria used here generalizes across products. Based on our data, taste-aroma interactions are more likely to occur at low to medium sucrose-vanilla concentrations, which is in line with others (e.g., [73]) who similarly have found a concentration dependence.

Since taste and smell involve distinct mechanisms and do not share receptors, any interactions that take place would seem to be cognitive and not peripheral [3]. Neuroimaging studies have supported this view, in that certain brain regions are subsequently activated by taste-aroma combinations. For example, presenting a taste-odor combination activates the anterior region of the orbitofrontal cortex (OFC), a region that is not activated by taste or smell alone [38]. Similarly, blood oxygen level demand showed superadditivity in the OFC and amygdala for taste-odor combinations, compared to the individual components [74], and superadditivity of neural activity was also found in the OFC, insula, and anterior cingulate cortex (ACC) regions when presented with bimodal stimulation [38]. Additionally, congruency between tastants and odor further increases activation of these brain areas, suggesting that prior exposure is a necessary prerequisite for taste-aroma interactions [75]. These results all indicate that the combination of taste and smell leads to effects that are larger than the sum of their perceptual parts [1]. Even when the mathematical determination of synergism might be unclear, synergistic effects of tastants and odors are supported by neuroimaging research.

While the enhancement of taste by a congruent odor in this study was not strong, this may also be attributed to the cognitive task used. Early evidence of the dependence on cognition was found in 1984, when Lawless and Schlegel came to very different conclusions, depending on whether their panelists were given a direct or indirect scaling task to evaluate taste-odor mixtures. It was thought that panelists treat flavor mixtures as integral sensations during discrimination tasks, rather than as an analyzable pair of attributes during attribute rating [76]. This implies that the testing method induces different perceptual strategies: the discrimination task may reveal interactions between flavor notes, which would otherwise not be apparent when ratings are made of individual characteristics [11]. McBurney [77] stated that whether a taste-odor mixture is perceived analytically or synthetically can be determined by the method of evaluation required from the subject. The resulting elimination of or at least reduction in taste enhancement by the aroma occurs when one is forced to adopt an analytical approach once ratings of multiple attributes of a sample are required (thereby supporting the dumping phenomenon), whereas an overall evaluation of a food product encourages the synthesis of the common quality from both sensory modalities of odor and taste, causing enhancement [20].

Because the occurrence of an enhancement may result from scaling artifacts, a non-scaling method may be better suited for confirming the enhancement effects observed here. Using a more holistic (synthetic) testing method, consumers may perform more similarly to their "natural" behavior and, in doing so, reveal a greater taste enhancement. Such tests should be further explored, as the cognitive task during rating of various attributes does not represent how we perceive flavor when we eat food outside of such a controlled setting. To our knowledge, only a single study [77] has employed such a synthetic tactic to test for sweetness enhancement by using a matching task to control for possible
scaling biases. More non-scaling studies are needed in cross-modal interaction research, as flavor is experienced as a singular percept rather than a series of individual components.

## 5. Conclusions

Even when controlling for potential dumping artifacts by providing all salient response categories to participants, significant interactions between vanilla and sugar were found for sweetness perception in a real food (skim milk). Use of a superadditive model, the isobole approach, provided further evidence for synergism in sweetness perception between a congruent taste-aroma pair, although these effects appear to be rather small in magnitude. Potential applications for sugar reduction in foods could be accomplished with these findings, although it probably requires combination with other current techniques, such as the use of non-nutritive sweeteners. Future work should test the most synergistic combinations with a holistic, non-scaling method, to better represent how we experience flavor when eating.

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## Appendix A

Table A1. Mean sensory attributes ( $n=108$ ) and measured instrumental viscosities ( $n=2$ ) (Pa.s) of final vanilla sucrose milks used in the dose-response study. $\mathrm{S}=$ sugar, $\mathrm{V}=$ vanilla, both in percent $(w / w)$.

| Sample | S | V | Overall <br> Liking | Sweet Taste | Vanilla Flavor | Milk <br> Flavor | Thickness | Viscosity (Pa.s) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| S0V0 | 0.000 | 0.000 | 4.49 fg | $14.8{ }^{\text {g }}$ | $12.9{ }^{\text {i }}$ | $50.5{ }^{\text {a }}$ | 30.3 bcde | $2.77 \times 10^{-3} \pm 2.62 \times 10^{-5 ~ b c d e}$ |
| S0V0.25 | 0.000 | 0.250 | $4.81{ }^{\text {defg }}$ | $18.2{ }^{\text {g }}$ | $25.5{ }^{\text {gh }}$ | 44.9 abc | $28.8{ }^{\text {cde }}$ | $2.71 \times 10^{-3} \pm 6.29 \times 10^{-5 ~ b c d e}$ |
| S0V0.36 | 0.000 | 0.360 | 4.49 fg | $18.7{ }^{\text {g }}$ | $27.0{ }^{\text {gh }}$ | $41.8{ }^{\text {bc }}$ | $28.7{ }^{\text {cde }}$ | $2.66 \times 10^{-3} \pm 2.62 \times 10^{-5} \mathrm{de}$ |
| SOV0.52 | 0.000 | 0.520 | 4.44 g | $19.0{ }^{\text {g }}$ | 28.2 gh | $38.7{ }^{\text {c }}$ | 26.5 de | $2.68 \times 10^{-3} \pm 6.3 \times 10^{-6}$ cde |
| S0V0.75 | 0.000 | 0.750 | 4.39 g | 20.0 fg | 27.9 gh | $38.1{ }^{\text {c }}$ | $26.2{ }^{\text {e }}$ | $2.61 \times 10^{-3} \pm 2.76 \times 10^{-5} \mathrm{e}$ |
| S1V0 | 1.00 | 0.000 | $4.74{ }^{\text {efg }}$ | $27.1{ }^{\text {f }}$ | $22.8{ }^{\text {h }}$ | $47.9{ }^{\text {ab }}$ | $28.8{ }^{\text {cde }}$ | $2.84 \times 10^{-3} \pm 6.93 \times 10^{-5}$ abcde |
| S1.31V0.3 | 1.31 | 0.300 | $5.52{ }^{\text {abcd }}$ | $37.4{ }^{\text {e }}$ | $38.0{ }^{\text {ef }}$ | $40.3{ }^{\text {bc }}$ | $32.9{ }^{\text {abcd }}$ | $2.74 \times 10^{-3} \pm 2.69 \times 10^{-5}$ bcde |
| S1.31V0.435 | 1.31 | 0.435 | 5.50 abcd | $37.2{ }^{\text {e }}$ | 38.9 de | $39.5{ }^{\text {c }}$ | 30.6 bcde | $2.70 \times 10^{-3} \pm 3.61 \times 10^{-5}$ bcde |
| S1.31V0.625 | 1.31 | 0.625 | 5.22 cdef | $36.3{ }^{\text {e }}$ | 39.2 de | $39.7{ }^{\text {c }}$ | 29.9 bcde | $2.83 \times 10^{-3} \pm 2.83 \times 10^{-6}$ abcde |
| S1.71V0 | 1.71 | 0.000 | 5.31 abcde | 38.8 de | $30.6{ }^{\text {fg }}$ | 44.8 abc | $34.6{ }^{\text {abc }}$ | $2.82 \times 10^{-3} \pm 9.40 \times 10^{-5}$ abcde |
| S2.24V0.3 | 2.24 | 0.300 | 5.86 abc | $46.8{ }^{\text {c }}$ | 44.9 bcde | $40.7{ }^{\text {bc }}$ | 35.1 abc | $2.88 \times 10^{-3} \pm 1.89 \times 10^{-4} \mathrm{abcd}$ |
| S2.24V0.435 | 2.24 | 0.435 | 5.91 abc | $46.9{ }^{\text {c }}$ | $46.6{ }^{\text {bcd }}$ | $42.3{ }^{\text {bc }}$ | 34.2 abc | $2.83 \times 10^{-3} \pm 9.7 \times 10^{-5}$ abcde |
| S2.24V0.625 | 2.24 | 0.625 | $5.81{ }^{\text {abc }}$ | $44.8{ }^{\text {cd }}$ | $47.8{ }^{\text {abc }}$ | $39.5{ }^{\text {c }}$ | 33.1 abcd | $2.77 \times 10^{-3} \pm 2.40 \times 10^{-5}$ bcde |
| S2.92V0 | 2.92 | 0.000 | 5.43 abcde | $50.2{ }^{\text {c }}$ | $38.4{ }^{\text {e }}$ | $41.8{ }^{\text {bc }}$ | $35.2{ }^{\text {abc }}$ | $2.85 \times 10^{-3} \pm 1.41 \times 10^{-6}$ abcde |
| S3.82V0.3 | 3.82 | 0.300 | 5.77 abc | $58.8{ }^{\text {b }}$ | 50.3 abc | $40.4{ }^{\text {bc }}$ | $35.4{ }^{\text {ab }}$ | $2.93 \times 10^{-3} \pm 7.64 \times 10^{-5} \mathrm{abc}$ |
| S3.82V0.435 | 3.82 | 0.435 | $6.00{ }^{\text {ab }}$ | $61.0{ }^{\text {ab }}$ | $51.9{ }^{\text {ab }}$ | $39.9{ }^{\text {bc }}$ | $35.4{ }^{\text {ab }}$ | $2.96 \times 10^{-3} \pm 4.95 \times 10^{-5} \mathrm{ab}$ |
| S3.82V0.625 | 3.82 | 0.625 | $6.04{ }^{\text {a }}$ | $58.1{ }^{\text {b }}$ | $54.7{ }^{\text {a }}$ | $37.8{ }^{\text {c }}$ | $36.4{ }^{\text {ab }}$ | $2.94 \times 10^{-3} \pm 1.41 \times 10^{-6} \mathrm{ab}$ |
| S5V0 | 5.00 | 0.000 | 5.27 bcde | $67.7{ }^{\text {a }}$ | $43.4{ }^{\text {cde }}$ | $39.4{ }^{\text {c }}$ | $37.4{ }^{\text {a }}$ | $3.06 \times 10^{-3} \pm 9.90 \times 10^{-6 ~ a}$ |

Same superscript letters in columns do not differ by Tukey's HSD ( $p<0.05$ ).

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