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TASTE-AROMA INTERACTIONS AS A SUGAR REDUCTION STRATEGY IN FLAVORED MILK

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Gloria Wang

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The thesis of Gloria Wang was reviewed and approved* by the following:

Helene Hopfer
Assistant Professor
Thesis Advisor

Gregory Ziegler
Professor of Food Science

Robert F. Roberts
Professor of Food Science
Head of the Department of Food Science

*Signatures are on file with the Graduate School

ABSTRACT

Food manufacturers are actively seeking methods for reducing sugar in products without compromising flavor, functionality, and consumer acceptability. The use of alternative sweeteners is the most common strategy; however, consumers have become wary of these for various reasons. Cross-modal interactions, in particular taste-aroma interactions, have been proposed as another strategy for sugar reduction, as it has been shown that the addition of a congruent aroma to a sweetened matrix may enhance the matrix's perceived sweetness and vice versa. Currently there are two underlying factors that make this phenomenon difficult to accept as a real occurrence: 1) most studies have been done in model solutions or model foods and not real foods, and 2) enhancement is either present or disappears depending on the sensory testing method and consequently the perceptual strategy used. Enhancements thus have been attributed to what is known as the "dumping effect" i.e., when panelists are given a limited ballot of attributes to rate, they will "dump" any similar perceptions into one category, causing what seems to be an enhancement. When panelists are given an extended ballot of salient product attributes, they are then able to separate these perceptions into individual components, causing enhancements to disappear. However, this method of rating is not representative of a typical eating situation for the average consumer. Instead of this analytical mindset that is employed when rating scales are used, consumers' perception of food is more often holistic and hedonic. This integration of perceptions is known as the synthetic strategy and methods that encourage such behavior have been proposed as an alternative approach when testing for enhancements in taste-aroma interaction studies. The following chapters outline studies to answer the following questions:

1) Can enhancements of either aroma or taste be elucidated in a typical rating study of vanilla flavored milk when controlling for response biases?

2) Can these enhancements be assessed by testing mathematically for synergistic, antagonistic, or no interaction effects with the isobole method?

3) Can sweetness enhancement be found in vanilla flavored milk using a non-scaling testing approach, i.e., a modified ABX test?

First, a dose-response study was conducted to induce panelists to use the analytical mindset when rating sucrose milk and vanilla flavored milk samples. Here, interaction effects of taste and aroma were found as measured by the isobole approach. Vanilla flavor and liking scores were best fit by a second-order model, while sweetness, milk flavor, and thickness were best modeled by a first-order model (both as a function of sucrose and vanilla extract concentration). The addition of vanilla had significant effects on perceived sweetness ($p < 0.05$), and similarly, sugar had a significant effect on vanilla flavor perception ($p < 0.05$). Based on the isobole method, skim milk samples containing both vanilla and sugar were found to act synergistically for perceived sweetness at the low to medium sugar concentrations ($I < 1$), demonstrating that taste aroma enhancement occurs even in a rating setting when using an extended ballot and encouraging an analytical mindset.

To further test the feasibility of a non-scaling method for assessing sweetness enhancement, the ABX test was modified to create a set of matching experiments (ABCX and ABCDX).

All matching experiments demonstrated greater interaction between vanilla flavor and sugar in skim milk samples than was found in the dose-response study: a greater proportion of panelists matched a vanilla-sucrose milk to a sucrose-milk reference higher in sugar than that of the aroma-spiked sample. All observed proportions of matched samples were significantly above the expected probabilities ($p < 0.05$). This enhancement was repeatedly observed for both a congruent vanilla flavor and incongruent beef flavor. Further, it appears that congruency is proportional to the degree of enhancement, as more panelists matched the congruent vanilla-sugar milk to a higher sugar reference than in the case of the beef-sugar milk.

Overall, synergistic interactions between vanilla flavor and sugar were found with both scaling (i.e., analytical strategy) and non-scaling (i.e., synthetic strategy) testing methods in skim milk, with the effect being greater when using a non-scaling method. The effect was small but was still found in the analytical rating task even after controlling for the dumping effect, over a range of concentrations, and using a real food. Thus, taste-aroma interactions seem to be robust and should be further investigated in other real food applications.

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Note, the following will be riddled with grammatical errors and not enough transitions (but to the best of my ability, the rest of this thesis will not be!) in order to fit all those who had a hand in my project: So I waited until the very end to write this section because I follow "save the best for last" principles with almost everything I do (in addition to GMPs, of course). Although I've thoroughly enjoyed every moment and learning opportunity that my research has given me, the best part about graduate school was still, without any hesitation, the people I've met while being here. First and foremost, thank you to my advisor, Dr. Helene Hopfer, for digging through all those graduate school applications to find mine and taking me on as your first master's student. You believed in me every day from day one and your genuine confidence in me was what kept me going until the very end, especially on the days I couldn't believe in myself. Next, thank you to my committee members, Dr. Bob Roberts and Dr. Greg Ziegler for making sure I wasn't diving head first too fast into anything and for helping me through parts of my thesis that I should have known way before I came to ask you. I also thank my pseudo-committee members, Dr. John Hayes and Dr. Federico Harte, for your help in refining my research on the sidelines. A huge thank you also goes to Dr. Alyssa Bakke, Tiffany Murray, and the undergrads at the SEC for helping us design our tests and for making sure everything related to sensory evaluation was in place in Compusense and in the SEC. Special shout-out to Tiffany on Valentine's Day 2018 for helping me problem solve through what was probably the craziest day of my graduate school career so we could go ahead with testing to make this thesis possible and in time.

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Chapter 1: Literature Review

1.1 Sugar and Health

Sugar has long played a critical role in food, from providing structure and preservation properties, to enhancing palatability and flavor. However, the ongoing obesity epidemic and increased prevalence of diabetes and cardiometabolic problems have caused sugar to become a major topic of concern, resulting in heightened consumer awareness of sugars in the diet. Accordingly, food manufacturers have also felt the push to decrease sugar in their products, with some of the world's major food companies (e.g., Nestle, Pepsico, Kellogg's, Mars, and Unilever) updating their policies to reduce sugar by various amounts in the coming years (see Table 3 in Hutchings, Low, and Keast 2018 for more information). Additionally, Kraft Heinz has already been reducing sugar in many of its products in the UK since 1985, and reformulations are underway in Australia, New Zealand, and parts of Europe ("Kraft Heinz 2017 Corporate Social Responsibility Report" 2017). As of 2017, General Mills has already reduced sugar by 5 to 30% in more than 300 of their products ("General Mills, Inc. 2018 Global Responsibility Report" 2018).

Despite the literature suggesting that excessive sugar intake should be minimized, there still has yet to be a definitive link of sugar's direct effects on our health, with most studies reporting only observational data (Stanhope 2016; Te Morenga et al. 2014; Yang et al. 2014; WHO 2015). Scientific evidence from randomized controlled trials with systematic reviews of these are the best quality for informing policy; however, these are not often available. As such, lower quality evidence that is more prone to bias is relied upon, which only contributes confusion to the health literature (Evans 2017). What also makes the relation confusing are the grey areas between the definitions of different sugar sources and the ambiguity regarding which term is the most relevant to health outcomes. “Total sugars” refers to all mono- and disaccharides present in food, from any source; “added sugars” are those added to foods during processing, and include naturally occurring sugars from a whole food that has been concentrated to the point where sugar is the primary component; “free sugars” are defined as all mono- and disaccharides present, except those that are naturally occurring in whole fruit and vegetables or dairy products, and include “added sugars” and naturally occurring sugars from nonintact fruits and vegetables (refer to Table 2 in Mela and Woolner 2018 for full definitions). This blurring has likely led to the inconsistencies in the usage of each type for defining health policies around the world. Globally, most labelling uses total sugars for informing consumers about the sugar content of foods, whereas the World Health Organization (WHO) and the Scientific Advisory Committee on Nutrition (SACN) make their recommendations based on free sugars, and the Dietary Guidelines Advisor Committee (DGAC) of the United States uses added sugars (WHO 2015; SACN 2015; DGAC 2015). Although sugar molecules are biologically and chemically indistinguishable by source, it is likely these institutions do not use total sugars in their recommendations because of the assumption that a physiologic difference exists between these classes that is based on the matrix in which these sugars are found (Mela and Woolner 2018). As such, the totality of evidence suggests that free sugars, which includes added sugars, should be the main focus of attention for public health intervention (Mela and Woolner 2018). Thus, both will be considered in the following work as much of the current research uses these terms interchangeably.

To date, there are no systematic reviews of free sugar intake on cardiovascular disease (CVD), and although there is strong evidence of a causal relationship on type II diabetes, the risk is more elucidated for sugar sweetened beverages and less so for other products outside of this category (Evans 2017; Moore and Fielding 2016). Even so, the uncertainty surrounding the impact of added sugars on our health is likely what has led to the trend that consumers are continuing to reduce their added sugar consumption. Based on a 2016 Mintel survey on sugars and sweeteners, 84% of consumers are looking to limit the amount of sugar in their diet, with a third of these indicating they will be reducing their sugar intake more than the previous year (Welsh, Sharma, Grellinger, et al. 2011; Mintel 2016). As a result, 80% of the consumers surveyed also reported they engage more with their food and drink packaging by checking labels for the amount of sugar and sweetener, with 79% checking for the type of sugar or sweetener used (Mintel 2016). The mandate by the FDA for food companies to update their nutrition facts panel to include added sugars by 2020 will likely lead to improved transparency with consumers.

Though added sugar consumption has been on the decline, mean intakes of sugar continue to exceed the WHO recommendations (Welsh, Sharma, Grellinger, et al. 2011). The WHO recommends that free sugars should constitute less than 10% of one's total energy intake (roughly 50 g or 12 teaspoons) per day, based on a 2000 calorie diet (WHO 2015). The WHO also suggests that a further sugar reduction to 5% of total energy or 25 g per day would provide additional health benefits (WHO 2015). It appears that more attention needs to be placed on these guidelines, as a more recent US study published in 2014 on added sugars and CVD found that added sugar consumption, which is associated with significantly increased risk of CVD mortality, is still exceeding the recommended amounts (Yang et al. 2014). Overall, the average percentage of daily calories from added sugars increased from 15.7% from 1988-1994 (n=11 733), to 16.8% in 1999-2004 (n= 8786), then decreased to

14.9% from 2005-2010 (n = 10 628), which still translates to most adults on average consuming approximately 5% more than the recommendations (Yang et al. 2014). One tenth of these people also consumed 25% or more of total calories as added sugar in 2005-2010 (Yang et al. 2014). This high intake of added sugars is likely attributed to the widespread, and sometimes unassuming, availability of sugars on the market. The average availability of added sugars, based on the Economic Research Service (ERS) Food Availability Data System, increased by 19% in the US from 1970 to 2005, with sugar-sweetened beverages such as sodas, sport drinks, and sweetened coffees and teas being the primary source of added sugars in the diet (R. K. Johnson et al. 2009; USDA 2015b; ERS 2018). For the US population, sugar sweetened beverages make up almost half of all added sugar consumption (USDA 2015b). It should be noted that these are not the only sources of added sugars, as many other foods such as sweetened grain desserts, dairy products and alternatives, and other sugars "hidden" in processed foods, too contribute to overall added sugar consumption (Welsh, Sharma, Grellinger, et al. 2011; USDA 2015a; WHO 2015). This is especially relevant to the dairy industry as products such as yogurt and flavored milk beverages, having seen an increase in consumption for their purported health benefits, can also contain large quantities of added sugars. For example, flavored milk, though nutrient dense and an effective way of promoting overall milk consumption, can easily contribute half of a child's daily added sugar allowance (R. K. Johnson, Frary, and Wang 2002), and is a major contributor of sugar intake in schools (Poti, Slining, and Popkin 2014). As a result, Americans are becoming wary of flavored milk drinks as they start to perceive them as being unhealthy due to their high sugar content (Euromonitor International 2017).

1.2 Dairy

To address the issue of added sugars in some dairy products, manufacturers have been actively seeking ways to reduce sugar in their formulations. The main strategy has been the use of sugar substitutes to replace the sweetness of sucrose, in combination with fibers or sugar alcohols to make up for the bulk that sucrose provides for structure (Hutchings, Low, and Keast 2018). However, 40% of consumers from a 2016 Mintel survey reported that they would rather avoid artificial sweeteners, with some consumer segments reporting they are moving towards using natural non-nutritive sweeteners like stevia and monk fruit (Mintel 2016; Hutchings, Low, and Keast 2018). The negative perception is likely due to these sweeteners' inherent bitter and other side tastes, as well as differences in the temporal sweetness profile from sugar, regardless of whether they are natural or artificially produced (Azad et al. 2017; Zorn et al. 2014; Reyes, Castura, and Hayes 2017; Antenucci and Hayes 2015; Ayya and Lawless 1992; Dubois and Prakash 2012). Additionally, consumers may also be wary of the uncertainty of the long-term effects of non-nutritive sweeteners on their health; though already generally regarded as safe (GRAS) by the FDA, it has been suggested that further research is still needed (Shankar, Ahuja, and Sriram 2013; Azad et al. 2017; Lohner, Toews, and Meerpohl 2017). Overcoming these challenges while still maintaining the same flavor, one of the top purchasing drivers of food (Mintel 2017b), as well as the functionality of sugar, leaves manufacturers with few alternatives for sugar reduction.

1.3 Alternative Sugar Reduction Strategies

Aside from non-nutritive sweeteners, there are a few other sugar reduction strategies available to the food industry, including the use of multisensory integration, experimentation with food structure, and the gradual reduction of sugar (Hutchings, Low, and Keast 2018). Of these strategies, the use of multisensory integration principles (i.e., the enhancement of other sensations which can lead to an increase in perceived sweetness) seems to be the most promising, as this just implies reformulating based on already existing ingredients in the product (Hutchings, Low, and Keast 2018). This will be discussed in more detail in section 1.4.

Adjustments via food texture, which involves the discontinuous stimulation of taste receptors to induce a perceived sweetness enhancement, may be more complicated to carry out. For example, pulsated delivery of tastant solutions into the mouth causes an irregular stimulation that delivers an increase in tastant intensity compared to the same quantity of tastant at a constant flow rate (Burseg et al. 2010). For solid food structures, an inhomogeneous distribution of sucrose has been shown to produce a similar sweetness enhancing effect from stimulating sweet taste receptors in a discontinuous manner (Mosca et al. 2015; Mosca et al. 2010). A sugar reduction of at least 20% appears to be feasible and could potentially be even higher if both sweetness and aroma are modified (Burseg et al. 2010). However, producing an inhomogeneous tastant distribution in foods may be difficult to achieve on an industrial scale and may require process redesign for manufacturers (Hutchings, Low, and Keast 2018). Other food structure modification strategies include experimenting with serum release from solid foods (Sala, Stieger, and van de Velde 2010) and reducing particle size during mastication (Sala and Stieger 2013). It should be noted these techniques have only been evaluated on a lab scale and may not be feasible for industry.

Further, within the category of sugar reduction via food structure, experimenting with different hydrocolloids to reduce product viscosity has also shown promise to increase perceived sweetness (and flavor in some cases) as a result of cognitive and physical and chemical interactions (Delwiche 2004; Arabie and Moskowitz 1971; Moskowitz and Arabie 1970; Pangborn and Szczesniak 1974; Pangborn, Trabue, and Szczesniak 1973). However, changing the mouthfeel of a food could have a major negative influence on consumer acceptability and applications may be limited with drier products (Hutchings, Low, and Keast 2018). Aside from structure modifications, manufacturers could also consider gradually reducing sugar levels in food over time, a technique that has shown success in a lab setting, though its long-term effectiveness is uncertain in terms of repeated acceptance and/or purchase (Hutchings, Low, and Keast 2018; Isogai and Wise 2016).

1.4 Cross Modal Interaction Studies for Sugar Reduction

Given the challenges of implementing the other sugar reduction strategies, as described in 1.3., the use of multisensory integration principles, i.e., cross-modal interactions, has been investigated as a more practical alternative sugar reduction strategy to non-nutritive sweeteners for foods. It is known that different modalities influence each other and integrate to form what we think of as flavor. For example, sensations such as color and aroma may enhance the perception of sweetness. Whereas color's effect on taste and flavor intensity is still unclear (Spence et al. 2010), investigating aroma's impact on taste has been suggested to be a more viable option for industrial use (Hutchings, Low, and Keast 2018).

The mechanism behind these findings—known as taste-aroma interactions in particular—is based on the confusability between odor and taste. Taste, technically speaking, is the perceptual description for the pure gustatory properties, i.e., sweet, sour, salty, bitter, and umami, whereas olfaction is a dual sensory modality that senses objects from the external environment (orthonasal olfaction), as well as from volatiles originating from the mouth (retronasal olfaction) (Rozin 1982). Although we often use the word “taste” in the English language to describe what we perceive as the flavor of foods, i.e., the combination of gustatory and olfactory sensations, taste is actually the chemical sense that detects non-volatile components in the taste buds via taste receptor cells (TRCs) which are present throughout the oral cavity (Knoop 2011). G-protein coupled receptors (GPCRs), specifically T1R and T2R, are those responsible for sweet, bitter, and umami tastes, while salty and sour tastes are perceived through ion-channels (Bachmanov and Beauchamp 2007; DeSimone and Lyall 2006). Sweetness in particular is elicited by sugars (sucrose, fructose, glucose, maltose, lactose), artificial sweeteners (e.g., saccharin, acesulphame-K, aspartame), D-amino acids (e.g., D-Phenylalanine, D-alanine, D-Serine), and sweet proteins (e.g., monellin, thaumatin, curculin) binding to receptors T1R2 and T1R3 (Chandrashekar et al. 2006). These then activate a G-coupled protein, subsequently leading to neurotransmitter release and a sweet taste signal in the brain (Chandrashekar et al. 2006; Guichard et al. 2017).

1.5 Mechanisms of Taste and Olfaction

While taste has been thoroughly defined in humans, olfaction is slightly more ambiguous and yet is what constitutes most of what we think of as “taste”. Two kinds of olfaction have been defined: orthonasal olfaction refers to the volatiles experienced during sniffing or the sense used for identifying objects from a distance, whereas retronasal olfaction occurs starting from the mouth (Rozin 1982). In the latter case, volatiles release from food during eating, rise into the nasal cavity, bind to olfactory receptors, and are then referred to the mouth (see **Figure 1-1**) (Small 2012; Rozin 1982; Shepherd 2006). Though olfactory receptors are found in the olfactory epithelium and are far away from any taste and tactile receptors, we often think of these volatiles as the “taste” of foods, even though there are no taste receptors in the nasal cavity (Harper, Land, and Griffiths 1968; Dravnieks 1985; Voirol and Daget 1986). Anecdotally, we find that we lose our sense of “taste” when ill, even though this is actually due to our blocked nasal passages that are preventing volatiles from binding to the olfactory receptors. The general confusion between taste and smell has partly led to the findings of taste qualities being attributed to odors and consequently the apparent enhancement of taste by odor and vice versa compared to when either are presented alone (Stevenson, Prescott, and Boakes 1999; Frank and Byram 1988; Kuo, Pangborn, and Noble 1993; Frank, Ducheny, and Mize 1989). A number of studies involving various combinations of odors and tastes have since been done to elucidate the mechanism behind their interaction.

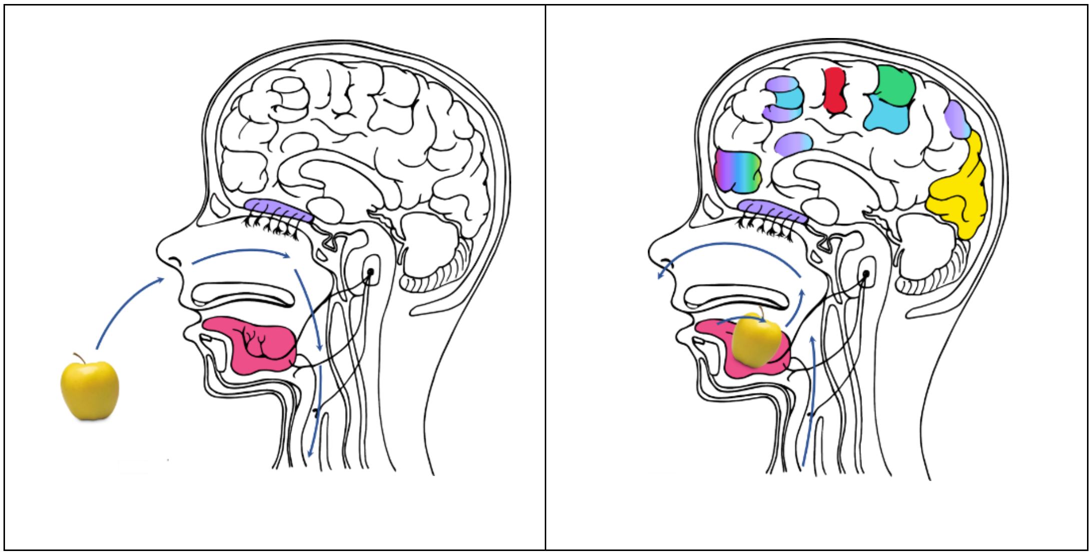


***Figure 1-1** Brain systems involved in smell perception during orthonasal olfaction (left) vs. retronasal olfaction (right). Air flows are indicated by the solid arrows. Smell = purple, taste = blue, motor = red, texture = green, yellow = vision. Adapted from Shepherd (2006).*

1.6 Taste-Aroma Interactions

1.6.1 Location of Taste-Aroma Interactions: Cognitive or Receptor Level?

To date, much of the taste-aroma interaction literature has looked at the impact of aroma on sweet perception, possibly due to the numerous odors that we perceive as "sweet" (Dravnieks 1985). Aside from sweetness, other interactions have also been reported for saltiness and certain aromas for salt reduction purposes. For instance, soy sauce odor (Djordjevic, Zatorre, and Jones-Gotman 2004b), various aromas associated with salty food (e.g. bacon, anchovy) (Lawrence et al. 2009), savory aromas (e.g., chicken, beef) (Batenburg and van der Velden 2011), and sardine (Nasri et al. 2013) were all able to enhance perceived saltiness in solution. Similar abilities were also found in model cheeses with added Comte cheese and sardine flavors, but not with carrot flavors (Lawrence et al. 2011). The finding that only certain odors can enhance tastes, in addition to the fact that gustation and olfaction do not share receptor cells or peripheral transduction mechanisms, suggests that taste-aroma interactions occur at the cognitive level, rather than the receptor level (Noble 1996; Murphy and Cain 1980; Bult, Wijk, and Hummel 2007; Lim, Fujimaru, and Linscott 2014; Dalton et al. 2000). Further evidence that this occurs at the central processing level is that taste enhancement can also be observed even in situations where the odorant and tastant are presented separately (Djordjevic, Zatorre, and Jones-Gotman 2004a; Valentin, Chrea, and Nguyen 2006; Sakai 2001). Additionally, imagined odors, especially ones that are congruent to the taste (e.g., strawberry), were shown to enhance sucrose detection more than incongruent odors, such as ham (Djordjevic, Zatorre, and Jones-Gotman 2004a). Lastly, perhaps even physicochemical interactions may have less of an effect than thought: it was shown that perceived flavor intensity increased when presented in combination with high amounts of sucrose and/or acid, even though the release of volatiles remained constant during drinking (as measured by APCI-MS) (Pfeiffer et al. 2006; Lethuaut et al. 2004). In short, taste-aroma interactions seem to occur at the cognitive level rather than at the physicochemical or receptor level.

1.6.2 Taste-Aroma Interactions for Sweet Matrices

For achieving sugar reduction in foods, previous research studying the effect of aroma on sweet taste has suggested that the addition of a certain type of odor to a sweetened medium may enhance the perceived sweetness of the system, similar to the effects seen with salty media above (Frank, van der Klaauw, and Schifferstein 1993; Schifferstein and Verlegh 1996; Frank and Byram 1988; Cliff and Noble 1990; de Araujo et al. 2003; Stevenson 2001; van der Klaauw and Frank 1994; C. C. Clark and Lawless 1994; Lavin and Lawless 1998;

Bingham et al. 1990; Prescott 1999). To explain why only certain odors are able to enhance particular tastes, it is thought that odor-induced enhancements depend on the perceptual similarity between a pairing, as a result of one's prior experience with particular retronasal odors with a taste (Small et al. 2004; Delwiche 2004; Stevenson, Prescott, and Boakes 1999). The extent to which two stimuli are appropriate to combine in a food is known as "congruency", as termed by Schifferstein and Verlegh (1996). Based on previous literature—in addition to Schifferstein and Verlegh's study—congruency or harmony between a taste and odor pair seems to be a necessary condition for aroma-induced taste enhancement. For instance, chocolate, vanilla, and strawberry aromas are often thought of as "sweet", at least within Western culture (Dravnieks 1985). One of the first studies on congruency was shown by Frank and Byram (1988), who found sweetness enhancement from strawberry but not peanut butter odor in sugar solutions, and no enhancement from strawberry odor on the saltiness of salt solutions. Later, Frank and colleagues (1993) could not find an enhancement of bitterness when pairing quinine with sucrose, and Djordjevic et al. (2004) were able to induce a saltiness but not sweetness enhancement when using soy sauce odor. These findings suggest that the degree of association between a taste and an odor is important for enhancement. Such associations occur after experiencing specific combinations found in one's own cultural context and/or during controlled flavor learning studies (Small et al. 2004; Stevenson, Prescott, and Boakes 1995; Stevenson, Prescott, and Boakes 1999; Prescott, Johnstone, and Francis 2004; Valentin, Chrea, and Nguyen 2006).

Evidence of the importance of the congruency condition is further supported by neural processing studies in both animals and humans. At the neuronal level, taste and olfactory pathways are brought together in the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) to form flavor (Rolls and Baylis 1994; Rolls 1996). Rolls and Bayliss (1994) were the first to study this when researching the caudo-lateral orbito-frontal cortex of monkeys. These researchers found multimodal neurons in the brain that responded to many taste and olfactory inputs that occur together in flavors. Later, Small et al. (2004) observed in human subjects that when a congruent vanilla-sucrose solution was consumed, significantly greater activation was seen in the OFC, insula, and ACC—all regions implicated as key components responsible for flavor perception and taste-smell integration—versus when an incongruent pairing was sampled.

However, it should be noted that even when congruent pairings are tested, it is unclear whether these perceived sweetness enhancements can actually occur in real eating situations. Much of the literature has only reported effects in sucrose solutions (Stevenson, Prescott, and Boakes 1999; Labbe et al. 2007; Frank and Byram 1988; Prescott 1999; Frank, Ducheny, and Mize 1989; Frank, van der Klaauw, and Schifferstein 1993; Stevenson 2001; Lawless and Schlegel 1984; Prescott, Johnstone, and Francis 2004; Stevenson, Prescott, and Boakes 1995; Bingham et al. 1990; Djordjevic, Zatorre, and Jones-Gotman 2004b; Pfeiffer et al. 2006; Boakes and Hemberger 2012) or model systems (Arvisenet, Guichard, and Ballester 2016; Hewson et al. 2008; Jones et al. 2008; Lethuaut et al. 2004; Bult, Wijk, and Hummel 2007). Enhancement effects may not be reflected or seen as extensively in real foods due to the inherent complexity of other components that contribute to flavor perception (see also Table 1 in Poinot et al. (2013) for a categorization of taste-aroma interaction studies organized by sensory methodology). As only a few studies have been carried out in real foods (e.g., whipped cream, milk, fruit juices, ciders, custards, and cherry drinks) (Frank and

Byram 1988; Lavin and Lawless 1998; von Sydow et al. 1974; Symoneaux et al. 2015; Green et al. 2012), taste-aroma interactions warrant further exploration in real foods.

1.6.3 Sweet Taste-Aroma Interactions- Matrix Considerations

One reason for the limited number of taste-aroma interaction studies conducted in real foods is due to their complexity. When choosing a food to study, it is important to start simple to better isolate and understand the effects of the modalities of interest. The use of a food with as few components as possible is important for preliminary testing, as physicochemical interactions have been found to affect our perception due to their effects on aroma and flavor release. While eating, the food matrix is broken down and interacts with our saliva, causing the release of volatiles into the oral cavity headspace. This release is affected by multiple events, including changes in structure, surface area, and composition, as food is sheared and mixed with saliva and its components (e.g., enzymes) (Roberts and Acree 1995; Taylor 1996; Burdach and Doty 1987; Van Ruth and Roozen 2000). Retronasal olfaction then occurs as the volatiles transfer from the back of the mouth to the nasal passages where the olfactory receptors are located (Noble 1996; Shepherd 2006).

At its simplest, the release of volatiles from foods during eating is dependent on the partition coefficient between the water phase (saliva) and the food matrix (Miettinen 2004). However, foods are rarely well-defined two-phase systems. Instead, individual volatile compounds may be bound to polysaccharide, protein, and lipid components in food to different extents, and so various models have been proposed to measure and model flavor release (Taylor 2002). Further, since eating is dynamic, equilibrium concentrations in the headspace of the oral cavity are never actually achieved, and so the release of volatiles is determined more by the rate of release (Baek et al. 1999), while partition coefficients are better for estimating the maximum potential extent of release (Miettinen 2004). The rate of release in the mouth is thus determined by the resistance to mass transport, which is not only a function of physical factors such as food texture (e.g., viscosity) and surface area, but also the interaction between aroma compounds and macro components (proteins, carbohydrates, and lipids) as well as minor food constituents (tastants) (Miettinen 2004).

Therefore, non-volatile solutes and other components in the food matrix must also be considered during taste-aroma interaction studies. Notably, salt and acid have been found to increase the headspace concentration of nonpolar volatiles due to their influence on saliva-air partitioning (Nawar 1971; Taylor 2002), potentially increasing the intensity of certain aroma attributes (Noble 1996). For products that contain a lipid phase, the aroma is distributed between lipid, water, and air according to:

$$P_{ow} = \frac{Co}{Cw} \qquad \textit{Equation 1-1}$$

Where P_{ow} is the oil-water partition coefficient, and C_o and C_w are the concentrations of the aroma compound in oil and water (de Roos 2000).

Of all the food components, the amount and type of fat generally has been found to have the most influence on aroma compound release (Rabe, Krings, and Berger 2003; Etievant et al. 2016). As most aroma compounds are more lipophilic, hydrophobicity of the matrix plays an important role in the thermodynamic behavior of flavor compounds (Guichard 2002). The phase in which flavor compounds partition into, i.e., the liquid or the headspace, is dependent on the affinity of volatiles for the lipid phase and solute-solvent interactions (Guichard 2002). If a volatile favors the lipid phase, a lower rate of release and subsequent odor intensity is experienced. Fundamentally, flavor perception is therefore more determined by the aroma molecules released from the product versus those that stay in it (see **Figure 1-2**). Additionally, while carbohydrates and proteins interact less with aroma compounds as they do not have the same dissolving capability as lipids, they are able to bind, adsorb and entrap aroma compounds (Etievant et al. 2016). With increasing carbohydrate and protein content, viscosity may also play a role in flavor perception. Flavor release tends to decrease with increasing resistance to mass transport as a result of more components in the system for the volatiles to diffuse through, as well as through binding mechanisms (de Roos 2000; Roberts and Acree 1995). Subsequently, taste and aroma intensities generally decrease with increasing viscosity (Vaisey, Brunon, and Cooper 1969; Moskowitz and Arabie 1970; Pangborn, Trabue, and Szczesniak 1973; Christensen 1980). At the same time, there have also been reports stating that viscosity does not necessarily affect diffusivity and therefore flavor release as some theoretical models would predict (Taylor 2002; Hollowood, Linforth, and Taylor 2002). Considering these studies all together, it is very important to keep the food matrix to be studied as simple as possible, that is, to keep viscosity as well as the amount of fat, protein, and carbohydrates low, or at least constant.

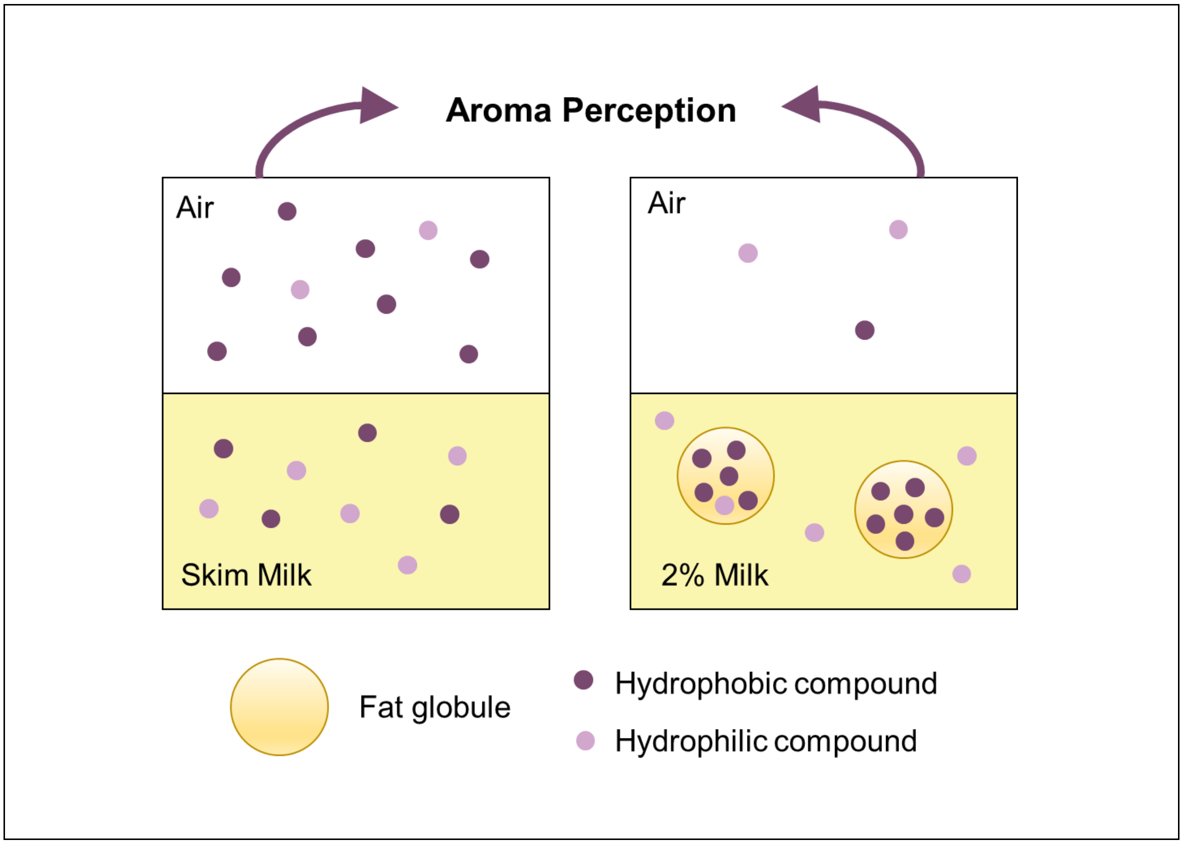


Figure 1-2 *Aroma release and perception as affected by the presence of fat within the matrix of study. Given that most odor molecules are hydrophobic, increasing hydrophobicity of the matrix increases volatile retention, delaying the release into the headspace.*

1.7 Taste-Aroma Interaction Media Choice

1.7.1 Food Choice- Milk

Milk is a particularly relevant food to study taste-aroma interactions. Although plain fluid milk consumption has been declining in recent years, flavored milk consumption has been increasing and is expected to continue growing as taste and flavor become more important to consumers (Mintel 2017a). Additionally, with almost all parents buying dairy milk for their children, there is an opportunity to make this milk category appealing for adults to drink as well, since it speaks to desires for a healthy indulgence and a sense of nostalgia (Mintel 2017a). The choice of a liquid food for taste-aroma interaction studies also removes the effect of mastication and physiological oral behavior (e.g., strength, time, and speed of chewing), which have been shown to impact the kinetics of aroma release and perception (Baek et al. 1999; Bult, Wijk, and Hummel 2007; Saint-Eve et al. 2011; Gierczynski, Laboure, and Guichard 2008; Hollowood, Linforth, and Taylor 2002). Furthermore, milk does not contain extraneous ingredients that could alter flavor perception and has a mild flavor profile with no apparent aftertaste (S. Clark et al. 2009). Any inherent flavor is imparted by the following components: fat, milk solids non-fat (MSNF, such as proteins, lactose, and ash), and water. In general, the contribution of total solids in milk from a Holstein cow, the most popular breed from which fluid milk is obtained, is on average around 12% (3.3% fat, 3.3% protein, 4.7% lactose, 0.7% ash) (FAO 2013). Though the amount of fat in fluid drinking milk rarely exceeds 4%, even small changes in oil content have been shown to have significant effects on the vapor pressure of fat soluble flavor compounds and subsequently flavor perception (Schirle-Keller, Reineccius, and Hatchwell 1994; Hatchwell 1996). When studying flavor release from model dairy custards, González-Tomás et al. (2007) found that the type of milk had a significant effect on the partitioning of aroma compounds, as the lipophilic aroma compounds had a higher affinity for the matrix due to the presence of fat. As such, skim milk custards resulted in a higher volatile release than whole milk samples. Thus, to mitigate the effects of fat on volatile release, the following studies will use skim milk, which should have less than 0.5% of fat ("U.S. FDA CFR - Code of Federal Regulations Title 21" 2017a), but often contains less than this amount as most commercial milk separation operations are more efficient than what is required legally. Though milk also contains a small amount of proteins and carbohydrates, the assumption is that these components will have a negligible effect on flavor perception.

1.7.2 Flavor Choice- Vanilla

As for selecting a flavor to study, vanilla is one of the most used flavors in dairy applications such as yogurt, ice cream, and flavored milk (Havkin-Frenkel and Belanger 2010). Many previous aroma-taste studies have also used vanilla or vanillin in combination with sugar for studying taste-aroma interactions, as this is a common pairing seen in foods within the North American context (Kuo, Pangborn, and Noble 1993; C. C. Clark and Lawless 1994; Lavin and Lawless 1998; Small et al. 2004). One recent cross-modal interaction study by Alcaire et al. (2017) used vanilla milk desserts made of skim milk, though they also varied starch levels, which may dilute the effects of sugar and vanilla on flavor and make it more difficult to draw conclusions based on these components alone.

In addition to keeping the food to be studied simple, the studies in the current work will use vanilla extract as the aroma component as it does not add sufficient solids to the matrix that would contribute significant effects on viscosity (see vanilla extract nutrient composition, (USDA 2018)). This approach allows any effects on flavor to be attributed solely to the vanilla aroma compounds, in addition to those in the milk, and their possible interaction with sucrose.

1.7.2.1 Vanilla

Vanilla flavor comes from the cured pods of different species of the *Vanilla* genus, a type of orchid. Of the 110 *Vanilla* species, only three are commercially important: *Vanillus planifolia*, *Vanillus tahitensis,* and *Vanillus pompona* (Ramachandra Rao and Ravishankar 2000; Sinha, Sharma, and Sharma 2008). The green seed pods that are harvested require a curing step to induce enzymatic changes that lead to the characteristic vanilla flavor. The volatile constituents responsible for vanilla flavor include acids, ethers, alcohols, acetals, phenolics, hydrocarbons, esters, carbonyls, and heterocyclics (Klimes and Lamparsky 1976). More than 200 compounds have been identified in the flavor profile of vanilla, with only 26 occurring in concentrations greater than 1 mg/kg. The familiar aroma and flavor note of vanilla is mostly attributed to the presence of vanillin (or 4-hydroxy-3-methoxybenzaldehyde), which is present in trace amounts in the green vanilla beans, but increases to 1-2% (w/w) after curing (Bettazzi et al. 2006; Westcott, Cheetham, and Barraclough 1994; Sharma et al. 2006). During curing, β-glucosidase acts on the glucoside, glucovanillin, to cleave off the vanillin from the glucose. Additionally, this enzymatic hydrolysis produces other flavor compounds such as *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-anisic acid, *p*-anisaldehyde, piperonal, vanillyl alcohol, and vanillic acid. The levels of these compounds vary depending on the species, curing technique, storage conditions, and geographical origin (**Figure 1-3**) (Ranadive 1992; de Guzman 2004). Other non-volatile constituents such as polyphenols, tannins, free amino acids, and resins, also contribute to the flavor of vanilla (Ramachandra Rao and Ravishankar 2000).

To acquire these flavor compounds for use, typically macerated vanilla pods are solvent extracted with ethanol and water. In the US, single-fold vanilla contains 100 g of extractable material per liter at no less than 35% alcohol by volume ("U.S. FDA CFR - Code of Federal Regulations Title 21" 2017b). Given the high price of vanilla extract, synthetic vanillin is also available for use at one hundredth of the price of extract derived from vanilla beans. Vanilla flavoring blends are also available commercially for more customized applications. Although chemically complex, vanilla extract will be used to provide the flavor for all studies in the current work. Specifically, two-fold vanilla extract will be used so that less volume of material is used, which could otherwise contribute effects to the samples.

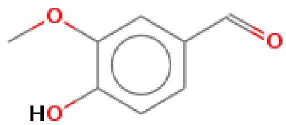
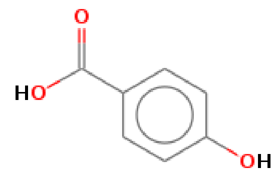
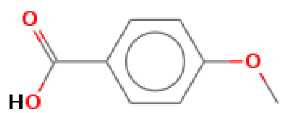
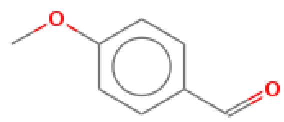
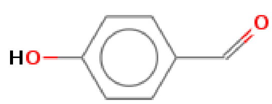
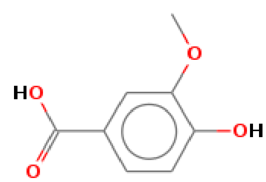
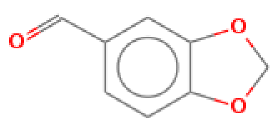
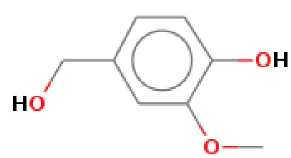
Vanillin 	*p*-hydroxybenzoic acid 
p-anisic acid 	*p*-anisaldehyde 
p-hydroxybenzaldehyde 	Vanillic Acid 
Piperonal 	Vanillyl alcohol 

Figure 1-3 *Common flavor compounds found in vanilla extracts (Adapted from webbook.nist.gov and de Guzman 2004).*

1.8 Testing Methods

1.8.1 Flavor Perception: Analytic or Synthetic Strategy?

While it is important to consider all aspects of the medium of study, it is equally important to consider the method used to test for taste-aroma interactions. Amidst all the literature on sweetness enhancement by an aroma, whether it is a real effect is still unclear: not only are interactions influenced by the compounds that are used, but also the instructions given to panelists and consequently the cognitive task have shown to impact the results (Noble 1996). Generally, the various sensory methods, as well as the different systems used to study flavor perception, have made it difficult to conclude if taste enhancements are real.

Much of the uncertainty is due to the method of assessing flavor perception. During sensory tests, typically the researcher is interested in different aspects of the sample through the use of scaling methods, where participants are asked particular questions and rate perceived intensities of various attributes. This induces what is known as an analytical mindset, which involves separating a food's properties into multiple attributes (Lockhead 1979; Schifferstein and Verlegh 1996). Although useful when it comes to evaluating the magnitude of these attributes, when thinking analytically, another complication arises: the number of relevant attributes to be rated either allows or prevents enhancements from being detected (Frank, van der Klaauw, and Schifferstein 1993; C. C. Clark and Lawless 1994; van der Klaauw and Frank 1996). Frank and colleagues first noted this when giving panelists both limited and extended ballots to rate the aromas of strawberry-sucrose solutions (Frank, van der Klaauw, and Schifferstein 1993). When asked to rate only sweetness intensity, panelists used broad definitions of this concept in that collectively, they included sweetness and other relevant product attributes, such as strawberry flavor, into the perceived sweetness category, causing what was thought to be an enhancement. On the other hand, when given multiple attributes to rate, such as sweetness and strawberry flavor intensity, no increase in sweetness was observed. It was hypothesized that panelists were able to separate the attributes when multiple appropriate response categories were provided, whereas when the categories were limited, they were more prone to integrate the dimensions. This can occur if a stimulus evokes a sensation (e.g., vanilla flavor) that is to some degree similar (or congruent) to the target sensation (e.g., sweetness). If the congruent stimulus is included in the target sensation, an increase in the attribute intensity rating occurs (Schifferstein and Verlegh 1996).

Thus, it appears that mixture-induced taste enhancement is a result of a "dumping effect", as coined by Clark and Lawless (1994). This would imply that any observed enhancement is more of a measurement artefact rather than a true perceptual phenomenon. Given the observation of the dumping effect, it seems that perhaps any taste enhancements observed do not actually exist, especially since much of the previous literature that has reported enhancements used limited ballots and did not provide all the relevant attributes to rate (see Frank and Byram 1988; Frank, Ducheny, and Mize 1989; Lavin and Lawless 1998; Boakes and Hemberger 2012; Labbe et al. 2007; Djordjevic, Zatorre, and Jones-Gotman 2004b; Sakai 2001). However, it should be noted that the analytical strategy that is encouraged by scaling different product attributes may not be representative of the mindset adopted during a real eating situation. As stated previously, a significant portion of what we consider as flavor stems from the odor of foods, mainly experienced retronasally as well as orthonasally to some extent (Rozin 1982). Since both kinds of olfaction provide different types of information, it naturally follows that we also combine a food's retronasally induced odors and taste into one flavor perception in order to recognize what it is (Prescott 1999). To further support that this is how sensory properties are perceived, people's initial responses to foods are usually only hedonic and holistic while eating (Prescott 1999). Upon the first bite, the average consumer is not typically thinking about every individual attribute of a food, but more about whether it tastes good or bad. It is also known that when combining different odorants in a mixture, subjects are not able to identify each individual component, suggesting odor-odor interactions are occurring (Saint-Eve, Paci Kora, and Martin 2004; Laing and Francis 1989; Laing et al. 2002; Livermore and Laing 1998). Similarly, mixtures of different tastants are known to interact, leading typically to a suppression of each mixture component's intensity compared to the pure tastant's intensity (Pangborn 1960; Kroeze and Bartoshuk 1985; Keast and Breslin 2002; Lawless 1979; Laing et al. 2002; Schifferstein and Frijters 1990). Thus, although flavor perception occurs thanks to multiple senses, these individual sensations are often integrated into a single percept. This integration is known as the synthetic mindset, opposite to the analytical evaluation of a food (Lockhead 1979; Prescott 1999; Schifferstein and Verlegh 1996). While it may seem that the synthetic mindset and the dumping effect are indistinguishable in that perceptual integration is involved, the dumping effect is more of a measurement bias that is a result from using scales, rather than a theory to explain how people switch between the analytic and synthetic strategy. We propose that the synthetic mindset is the more appropriate way to assess taste-aroma interactions, as opposed to the analytical strategy that is encouraged by the use of direct scaling techniques. Therefore, taste-aroma interaction effects should be tested by non-scaling methods.

1.8.2 Scale-Free Techniques

To avoid the response bias that can occur during scaling, a scale-free task should be used as an alternative method for testing taste-aroma interactions. To date, only a few cross-modal interaction studies have used methods that encourage the synthetic strategy to elucidate sweetness enhancements. Lawless and Schlegel (1984), when comparing direct and indirect scaling of sucrose and citral solutions, found evidence for interactions when indirect scaling was used. Since no specific attribute was prompted, participants were thought to integrate the mixtures into a single flavor percept instead of analyzing the pairs of attributes (Kuznicki, Hayward, and Schultz 1983). Alternatively, a study involving a scale-free matching task by van der Klaauw and Frank (1994; personal correspondence 2017) was conducted on sucrose solutions with added strawberry and coffee odors. Here, panelists were asked to match the flavored solutions to sucrose solutions that came closest to the perceived sweetness of the test sample. The authors concluded that enhancements could be found using both scaling and matching tasks, with subjects judging the mixture to be more than 1 concentration step (in the matching task) or two units (in the scaling task) sweeter than sucrose alone. Due to its ease of use with untrained consumers, a matching test was therefore explored in the study described in Chapter 3.

1.8.3 Current Taste-Aroma Study Limitations

The following section briefly summarizes limitations of taste-aroma interaction studies to date, namely, with regards to the ingredient concentrations, and testing methods used.

1.8.3.1 Effect of Concentration Range

Many of previous studies only tested a limited number of stimuli concentrations, so it is unclear whether their findings could generalize across a broader range of concentration combinations. Some authors have reported that perceived sweetness enhancement can occur only at certain concentrations. One of the earliest studies on the effect of sucrose on flavor intensity was done in both aqueous solutions and fruit nectars; both experiments found an optimum level of sucrose (up to 35% in aqueous solutions and up to 15% in fruit nectars) at which sucrose enhances apparent flavor intensity (Valdes, Hinreiner, and Simon 1956; Valdes, Simone, and Hinreiner 1956). Cliff and Noble (1990) concluded that at higher levels of glucose (above 9%), sweetness may enhance fruitiness in sweetened peach flavored solutions. Schifferstein and Verlegh (1996) too found that any sweetness enhancement decreased with decreasing sucrose concentration. On the opposite end of the concentration range, Brossard et al. (2006), when studying sweet custards flavored with four different aroma compounds, observed that sweetness had an effect on *aroma* intensity only at low to medium level sucrose concentrations (24-45 g/kg or 2.4-4.5%); however, no modification of sweetness perception was found from changes in aroma compound concentrations. A recent study in apple ciders was able to observe the reverse, in that cider aromas were able to modify *sweetness* only in products with medium levels of sugar used (35-40 g/L or 3.4-4.0%) (Symoneaux et al. 2015).

With all these findings reporting sweetness enhancements at different concentration levels tested, the effect of tastant concentration is difficult to elucidate. Further, another study (Hewson et al. 2008) on citrus perception and fructose found that an increase in flavor perception can also occur at all levels of tastant. In the studies described in the present work, the sucrose concentration range covered commercially relevant levels for flavored milk (between 0-5% (w/w)) to ensure that a complete spectrum of interactions can be analyzed.

1.8.2.2 Effect of Response Categories

As stated previously, much of the literature has only used limited response ballots (thus not controlling for potential dumping effects), making these findings inconclusive. More experiments that provide a full range of salient attributes to rate should be conducted to confirm whether dumping occurs. After investigating 16 different ciders with an extended list of attributes, Symoneaux et al. (2015), when providing a full ballot of attributes, were able to rule out dumping effects after observing a sweetness intensity modification, though only for certain sugar concentrations. Lim and colleagues (2012; 2013; 2014) have too recognized this gap in the literature, and have found some degree of taste enhancement in aqueous solutions and food matrices, even when controlling for dumping. At the same time, this group found more enhancement for odors by the tastant compared to taste enhancement by odors. First, an enhanced odor perception induced by taste was seen in solution, in a cherry flavored beverage, and a vanilla custard (Green et al. 2012). Similar effects were found in citral and sucrose solutions (Fujimaru and Lim 2013), as well as for citral and coffee solutions (Lim, Fujimaru, and Linscott 2014). Overall it was concluded that odor induced enhancement by taste is a stronger effect than taste enhancement by odor. It is promising that adopting an analytical strategy may not actually prevent interactions between odors and tastes; however, the use of a sip and spit protocol during these experiments (to elucidate odor referral), is not representative of normal eating behavior, and so findings are difficult to generalize to a typical eating experience. It has also been shown that whether one expectorates or swallows impacts certain tastes and flavor attributes differently, which could result in an altered sensory character of the samples (Pizarek and Vickers 2017; Running and Hayes 2017). Overall, there is still a lack of understanding of taste-aroma interactions and dumping; therefore, a modified full-factorial dose-response experiment in connection with an extended attribute ballot is used in Chapter 2, to elucidate taste-aroma interaction effects in a skim milk application.

1.9 Interaction Evaluation

1.9.1 Isobole Approach

Out of all the literature evaluated, it appears that only a few studies have found *significant* effects of aroma on perceived sweetness and vice versa. While perhaps a valid observation, this could be due to the lack of testing for interaction effects. Instead of significance testing of just the mean attribute ratings from sensory evaluation that most authors use to interpret their results, a test should be used that specifically evaluates whether the interaction between components in a mixture is synergistic (= enhancement), antagonistic (= masking), or zero (= additive). According to Lawless (1998), this lack of interaction testing might be due to the fact that demonstrating synergy is not straightforward, and depends on the model one adopts to make comparisons. When measuring taste-olfaction mixtures, researchers must carefully consider the model and test to demonstrate synergy, and whether these assumptions are reasonable (Lawless 1998).

A multitude of methods for testing interaction effects have been proposed in other fields such as pharmacology. One of these, the isobole approach (Suhnel 1993), seems well suited for testing aroma and taste mixtures, as it does not require making any assumptions about the concentration-response relationship, nor about the shapes of relations between the concentrations and the effects, or the underlying mechanism (Berenbaum 1985). First used by Fraser (1872) to describe the antagonism of the drugs physostigmine and atropine, and later coined the 'isobole method' by pharmacologists Loewe and Muischnek (1926) when studying the joint action of drugs, this method uses concentration effects and empirical concentration-effect relationships, allowing it to be independent of the mechanism of interaction (Suhnel 1993).

Previously, this approach has mostly been used to predict interactions of drug combinations by comparing the observed effects to the linear isobole (Suhnel 1990; Suhnel 1992; Tallarida 2012; Berenbaum 1977; Berenbaum 1989). Its usefulness for measuring the interaction between food ingredients and their effects on taste perception has been demonstrated in only three studies to date (Fleming, Ziegler, and Hayes 2016; Wolf, Bridges, and Wicklund 2010; Reyes 2017).

On the basis of concentration addition, the points on an isobole are indicative of the mixture values of multiple components at which a specific quantitative effect is produced, i.e., the set of pairs in the mixture that give a specified effect size. This can all be plotted on an isobologram generated from the following equation:

$$\sum_{i-1}^{n} \frac{c_i}{C_i} = I \qquad \textit{Equation 2}$$

Where c_i represents the concentration of component “I” in the mixture and “Ci” denotes the concentration of “I” that would individually produce the same intensity as that of the mixture, calculated from their corresponding dose response functions.

If looking at the interaction of two components A and B, Eq. 2 then becomes:

$$\frac{Ca}{CA} + \frac{Cb}{CB} = I \qquad \textit{Equation 3}$$

When components interact synergistically, the sum is less than 1, whereas if the interaction is antagonistic, the sum is greater than 1. If the equation is equal to 1, this is indicative of no interaction between the components in the mixture. Though this standard cut-off has been set by Suhnel, interaction criteria may be product dependent and should be adjusted depending on the matrix to be studied. For example, Fleming and colleagues (2016) used this method with a more conservative interaction criteria of synergism (when the I-value is less than 0.9), to study the sensory attributes of astringent compounds alone and in mixtures while allowing for some variation around 1—given the success of this technique outside of drug studies, the isobole approach will be used to investigate taste-aroma mixture interactions in sucrose-vanilla skim milk samples (Chapter 2).

1.10 Aims and Hypotheses

It is **hypothesized that the addition of a congruent aroma, i.e., vanilla, to sweetened skim milk will cause an enhancement in perceived sweetness of the milk as determined by a sucrose-matching exercise**. This will be tested via the following specific aims (**Figure 1-4**):

1. **Investigate the interaction of vanilla flavor and sucrose when added to skim milk and how they change perceived sweetness and hedonic ratings in an analytical scaling sensory evaluation**. Intensity of the overall liking, sweetness, vanilla flavor, milk flavor, and thickness will all be asked to control for potential dumping effects. This aim will also inform the ideal range of sugar and vanilla concentrations to use in Aim 2 based on liking and sweetness ratings. By controlling for dumping and spanning a wide concentration range, the occurrence of interaction using a scaling technique can also be confirmed.

2. With the optimum sucrose and vanilla concentration range from the dose-response experiments in Aim 1 (as found by the sucrose-vanilla combination with the most synergism based on the Isobole approach), **the ability of vanilla flavor to induce a sweetness enhancement effect in sweetened milk will be determined** using a sucrose concentration-matching experiment. The condition that sufficient perceptual similarity between the aroma and taste is a necessary condition for enhancement will also be investigated with an incongruent aroma (beef).

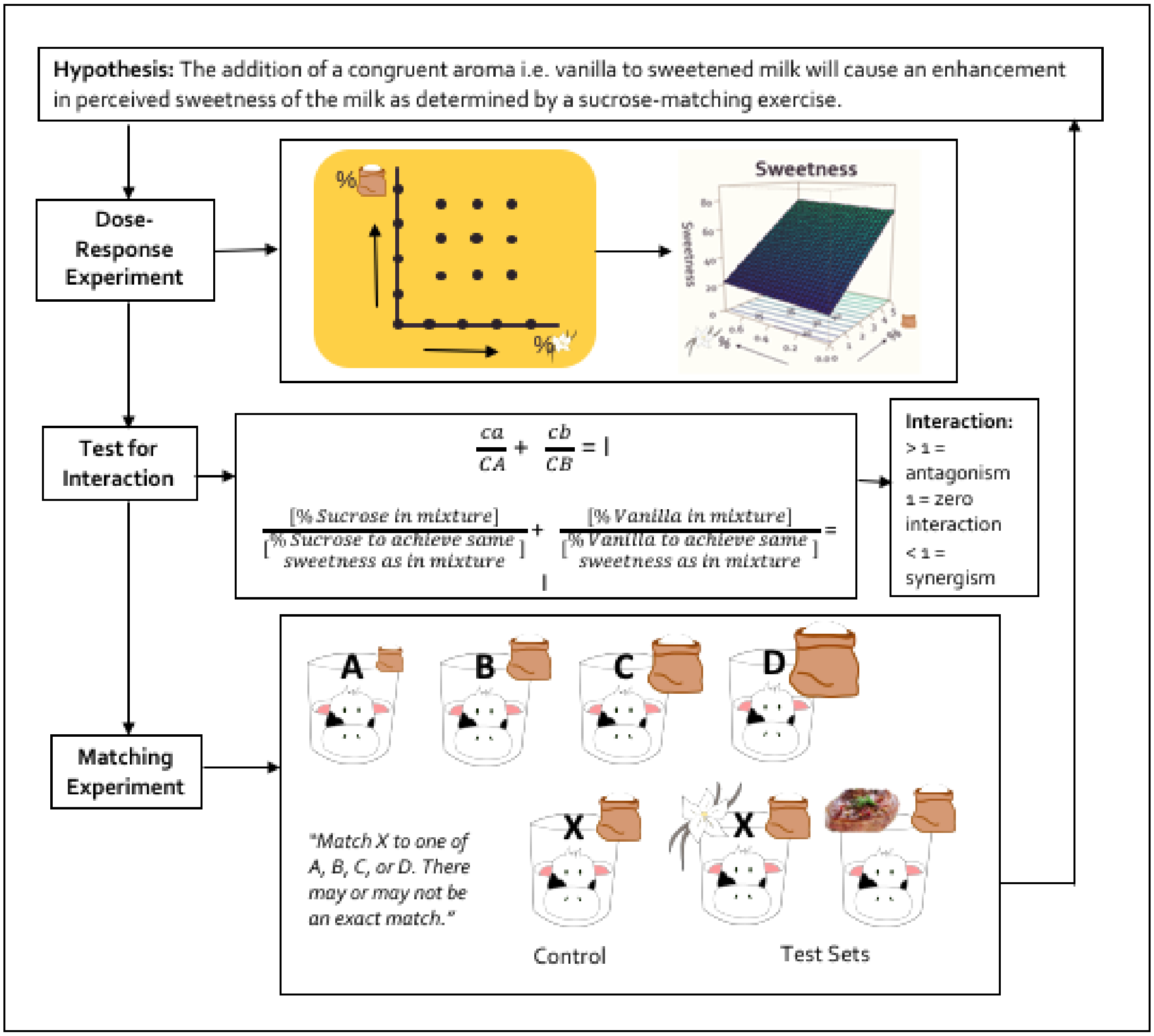


Figure 1-4- *Experimental design of the proposed taste-aroma interaction study.*

Chapter 2: Dose-response relationship of vanilla flavor and sucrose in skim milk

2.1 Introduction

Flavor perception is the result of both chemical and physical properties of a food and how they interact with our senses (Delwiche 2004). With flavor involving the integration of multiple modalities, including smell, taste, and touch, studying just one modality and its interaction with food does not reflect what humans experience during eating. As such, 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 in particular are the most commonly described interaction between sensory modalities, and occur as a result of physical, physiological, cognitive, and psychological effects (Knoop 2011; Noble 1996). Research on multisensory processes, including taste-aroma interactions, has been used to better explain the processes humans use to assess the flavor of products (Blake 2004; Gilbert and Firestein 2002; Shepherd 2006). Due to the common confusion between smell and taste, taste perception is influenced by odor and vice versa (Frank and Byram 1988; Köster, Prescott, and Köster 2004; Prescott 1999; Frank, van der Klaauw, and Schifferstein 1993; Lawless and Schlegel 1984). In order for perceived taste intensity to be modified when an odor is present (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, all seem to be a prerequisite for taste intensity changes by odors (Frank, van der Klaauw, and Schifferstein 1993; Noble 1996). This interaction arises as odors can acquire a taste quality when a certain odor and taste combination is commonly experienced in a food (Labbe et al. 2007). As defined by Schifferstein and Verlegh (1996), 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, though many of these studies have only been done in model sucrose solutions (Stevenson, Prescott, and Boakes 1999; Labbe et al. 2007; Frank and Byram 1988; Prescott 1999; Frank, Ducheny, and Mize 1989; Frank, van der Klaauw, and Schifferstein 1993; Stevenson 2001; Lawless and Schlegel 1984; Prescott, Johnstone, and Francis 2004; Bingham et al. 1990; Djordjevic, Zatorre, and Jones-Gotman 2004b; Pfeiffer et al. 2006; Boakes and Hemberger 2012) or model foods (Arvisenet, Guichard, and Ballester 2016; Hewson et al. 2008; Jones et al. 2008; Lethuaut et al. 2004; Bult, Wijk, and Hummel 2007).

Due to the complexity of food products, only a few taste-aroma interaction studies have been done in real foods, such as whipped cream, milk, fruit juices, ciders, custards and cherry drinks (Frank and Byram 1988; Lavin and Lawless 1998; von Sydow et al. 1974; Symoneaux et al. 2015; Green et al. 2012). Although these studies move the taste-aroma interaction research into a more realistic matrix, the concentration ranges for both the odor and the taste used in these studies were not always broad. This factor may be important as 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 (Dalton et al. 2000; Symoneaux et al. 2015; Valdes, Simone, and Hinreiner 1956; Valdes, Hinreiner, and Simon 1956). The lack of studies in real food products that cover a concentration range comparable to those used in industry, prevents a comprehensive understanding and utilization of such cross-modal interaction phenomena in food.

Fluid milk is a real food system with a relatively simple component makeup. While plain milk consumption has been on the decline since 2012, flavored milk consumption has been increasing and is expected to continue growing as taste and flavor become more important to consumers (Mintel 2017a). A familiar congruent aroma-taste pair used in dairy products is vanilla and sucrose, at least in the Western diet; in fact, vanilla is the most popular flavor for dairy applications such as yogurt and ice cream, and is a complementary ingredient in flavored milks (Havkin-Frenkel and Belanger 2010). Additionally, the combination of vanilla and sucrose is commonly studied in cross-modal research (Kuo, Pangborn, and Noble 1993; C. C. Clark and Lawless 1994; Small et al. 2004).

Although there is an abundance of literature favoring an enhancement of perceived sweetness in the presence of a congruent aroma, such as vanilla, it is still not clear if this effect really occurs in any matrix at all. This discrepancy exists because results from taste-aroma interaction studies depend on the questions and instructions given to the judges and thus the cognitive task. Previous studies using rating scales have shown that mixture-induced taste enhancement is reduced or eliminated completely when judges are asked to evaluate not just perceived sweetness but also perceived aroma intensity (Frank and Byram 1988; Frank, van der Klaauw, and Schifferstein 1993). Thus, some researchers have attributed any sweetness enhancement to a “dumping effect” (Clark and Lawless 1994). That is, when a scale for a flavor attribute is not provided in the test (e.g., vanilla flavor), assessors will “dump” their perceptions into another similar category (for example, sweetness) instead, leading to an increase in attribute intensity (Clark and Lawless 1994). It becomes apparent that the attentional strategy taken during the evaluation of foods plays a role in flavor perception (Prescott, Johnstone, and Francis 2004). Whether one adopts a synthetic mindset, i.e., experiencing the food as a whole, or takes an analytical approach, i.e., describing the food with separate attributes, may determine whether an enhancement effect occurs.

In addition to not employing a full range of concentrations, many early studies required panelists to rate taste attributes (as well as provide hedonic scores and flavor or odor intensity ratings in some cases), but not necessarily the perceived intensity of specific aroma and texture attributes (e.g., fruity flavor, thickness) (see Frank and Byram 1988; Frank, Ducheny, and Mize 1989; Lavin and Lawless 1998; Boakes and Hemberger 2012; Labbe et al. 2007; Djordjevic, Zatorre, and Jones-Gotman 2004b; Sakai 2001). Not providing a full list of salient product attributes to rate may have resulted in dumping, making these early findings inconclusive and raising the question of whether enhancements or any interactions truly occur at all.

In order to confirm the existence of a dumping effect, more experiments that provide a full range of relevant product attributes to score are needed. Several researchers recognizing this gap performed experiments using solutions and 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 (2012) found the addition of sucrose enhanced the perception of odor intensities in solutions, vanilla custard, and a cherry flavored beverage; Fujimaru and Lim (2013) found a similar effect for citral and sucrose solutions, as did Lim et al. (2014) with citral and coffee solutions. Although these studies demonstrated that adopting an analytical strategy does not necessarily prevent the interaction between congruent tastes and odors, their use of a sip and spit procedure was not representative of normal eating behavior. This tasting method can also impact the sensory profile of samples, depending on the taste and flavor characteristics (Running and Hayes 2017; Pizarek and Vickers 2017).

Lastly, perhaps the reason the literature on taste aroma interactions is unclear could be explained by the lack of knowledge on how to test for the presence of interactions between components in a mixture: the studies above used statistical significance testing of mean attribute ratings to make their conclusions, rather than testing whether the degree of interaction between two stimuli was above a pure additive level. Partly this is because "*the demonstration of synergy is not straightforward and depends on the model one adopts*", as stated by Lawless (1998). Testing for interaction between stimuli is commonly done in dose-response studies for drugs, but less so for foods. One common approach is the isobole method (Suhnel 1993), a technique that was first developed by Fraser (1872) and Loewe and Muischnek (1926) in the field of pharmacology to describe antagonism and synergism of drug combinations. 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 are primarily used to predict the effect of drug combinations (Suhnel 1990; Suhnel 1992; Tallarida 2012; Berenbaum 1977; Berenbaum 1989), but a few studies have used this method to measure the interaction between food ingredients on taste perception (Fleming, Ziegler, and Hayes 2016; Wolf, Bridges, and Wicklund 2010; Reyes 2017). As the isobole approach is a useful concept for evaluating the type and degree of interactions between substances, regardless of their mechanisms of action (Berenbaum 1989), cross-modal interactions of a sweet tastant and a congruent aroma can thus also be described and tested by the isobole method.

This study aims 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 while (iii) controlling for potential dumping effects, and (iv) using a dedicated test (i.e. isobole method) to measure the type and degree of interaction.

2. Materials and Methods

2.1 Experimental Design

In order to model human sensory responses to the various sucrose-vanilla mixtures with higher-order models, 18 sucrose-vanilla combinations were generated using two approaches (**Table 2-1**). Part of the design was composed of four levels across each of the two ingredient factors, separated by half-log steps, to generate the dose-response functions of sucrose in milk and vanilla in milk. Additionally, a 3 x 3 factorial design was followed to create the sucrose-vanilla mixtures in milk to generate the response surfaces for each sensory attribute. Collectively this provides a total of 17 milk samples at different combinations of vanilla and sucrose. An additional plain milk as a control was added to bring the total number of samples to 18. Thus, the sample space included 1 plain milk, 4 sucrose-only, 4 vanilla-only, and 9 sucrose-vanilla combinations. Milks were formulated with sucrose content ranging from 0-5% (w/w) and vanilla content ranging from 0-0.75% (w/w), spanning a wide range of sucrose and vanilla extract concentrations, including those used in industry (Chandan 2006).

2.2 Sample Production

All milks were mixed with varying levels of sucrose in eight 32 kg-batches for pasteurization; from these, 10 kg batches of each sucrose-vanilla combination were formulated for sensory analysis. Two-fold vanilla extract (David Michael & Co, now Tastepoint by International Flavors and Fragrances; Philadelphia, PA) was used as the vanilla flavor. Pasteurized skim milk (0.18% fat, 8.91% solids) and sucrose (Golden Barrel, Honey Brook, PA) were provided by the Berkey Creamery (University Park, PA). Prior to pasteurization, all milk and sugar amounts were pre-weighed into stainless steel milk cans and plastic tubs. 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 on high speed for 10 minutes, using metal agitators to allow for complete dispersion and dissolution of the sugar. All mixes were then pasteurized (high temperature short time (HTST), APV Junior Pasteurizer, APV Invensys, Woodstock, GA) at 75°C for 25 s, and homogenized (Gaulin, Lake Mills, WI) in a 2-stage process at 10.3 and 3.5 mPa (2,000 and 500 psi), cooled (< 7°C), and collected into milk cans.

Once all processing was complete, 13 mixes were flavored with pre-weighed vanilla extract amounts and divided into 18 different sucrose-vanilla combinations. Each milk was packaged into ½-gallon opaque plastic milk jugs and stored at refrigeration temperature (< 5°C) for one week prior to sensory testing. The eight sucrose-milk samples were collected for physical analysis (% total solids and % fat) and the 18 finished vanilla milk samples were collected for microbiological analysis. Fat and total solid contents were measured using the SMART Trac (CEM Corporation, Matthews, NC) to confirm that the sucrose concentrations requirements were met in the final samples (see **Table A-1** in Appendix). High-sensitivity coliform counts were conducted (Petrifilm; 3M, Maplewood, MN) to ensure samples were suitable for human consumption.

2.3 Physical Characterization

Since total solid contents were not kept constant between the samples, and to account for potential physicochemical interactions that may lead to differences in flavor perception, the viscosity of each sample was measured with a Discovery H3 Hybrid Rheometer (TA Instruments, New Castle, DE), equipped with a double wall concentric cylinder geometry (inside diameter 40.77 mm, outside diameter 43.88 mm, cup diameter 30.32 mm). The viscosity was calculated as the slope of a flow curve (shear stress vs. shear strain rate) with shear strain rate ranging from 0 to 100 s^{-1} at 5°C to mimic sample serving temperature. The flow curves were plotted with Trios Software (TA Instruments, New Castle, DE), omitting stress overshot/noise at low shear strain rate (0 to 15 s^{-1}). Two measurements for each sample were taken. All viscosities are reported in **Table A-2**.

2.4 Consumer Acceptability and Intensity Ratings

For all 18 vanilla-sucrose samples, a central location test was conducted with 106 participants (women = 76, age = 19-71) over three days, where each day panelists tasted six milk samples. Participants were screened for dietary restrictions, food allergies and product use (i.e., those who consumed skim and 1% milk at least once a week and indicated they would be interested in tasting vanilla flavored milk) (see **Appendix A1.1** for recruitment screener). Procedures were exempt from institutional review board review by 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 via computer in the testing booths and were compensated for their time. All samples were tasted in individual tasting booths under red light and at ambient temperature (25°C). Panelists were given deionized water at room temperature (20°C) to rinse before and in between each sample. Degree of liking for each sample was measured first on a 9-point hedonic scale, after which panelists rated the perceived intensities for sweetness, vanilla flavor, milk flavor, and thickness on a 100-point line scale, anchored with "very weak" on the left and "very strong" on the right (see **Appendix A1.2** for ballots). All 18 milks were sampled each day, and sample presentation order was counterbalanced across panelists using a modified Williams-Latin Square design. Each sample was shown at each presentation position either six or seven times. Approximately 45-50 mL of milk were poured and lidded into 3.25-oz. plastic cups (Fabri-Kal, Kalamazoo, MI) and labeled with random 3-digit blinding codes. Data were collected using Compusense Cloud (Compusense Inc., Guelph, ON, Canada).

2.5 Statistical Analysis

An initial two-way Analysis of Variance (ANOVA) ($p < 0.05$) with sucrose and vanilla as fixed effects on viscosity values as well as on all the attributes and with all two-way interactions (% sucrose and % vanilla concentrations) was performed using RStudio (version 1.1.419, Boston, MA). If significant differences for each attribute were found, Tukey's honestly significant differences (HSD) were calculated for all the samples using the agricolae package (de Mendiburu 2017). Data were then analyzed with the rsm package

(Lenth 2017) to create 3D response-surface plots of % sucrose, % vanilla, and each sensory attribute, modelling up to a second-order regression model by multiple linear regression.

Using the isobole approach (Suhnel 1993), the degree of interaction between sucrose and vanilla was calculated for perceived sweetness ratings (Equation 2-1). 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_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.

$$\sum_{i-1}^{n} \frac{c_i}{C_i} = I, \qquad \textit{Equation 2-1}$$

Where n = 2, for component A (sugar) and B (vanilla extract).

Since the vanilla milk samples consist of sucrose and vanilla, Eq. 2-1 can be rewritten as:

$$\frac{[\%\ Sucrose\ in\ mixture]}{\begin{bmatrix}\%Sucrose\ to\ achieve\ same\ sweetness\\ as\ in\ mixture\end{bmatrix}} + \frac{[\%\ Vanilla\ in\ mixture]}{\begin{bmatrix}\%Vanilla\ to\ achieve\ same\ sweetness\\ as\ in\ mixture\end{bmatrix}} = I \qquad \textit{Equation 2-2}$$

Three-dimensional isobolograms were generated for perceived sweetness using OriginPro (version 2017 64-bit 94E). 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, any values that fall below the plane are indicative of antagonism, and any that contact the plane indicate no interaction.

Similar to the method used by Fleming et al. 2016, we chose not to use inferential statistics on the I values reported, as this approach would require an estimation of I values at the individual level. It would be too computationally intensive to estimate the dose response functions for each stimulus and percept, and instead acknowledge that variation in I should exist. This variation was accounted for by establishing a range of $0.9 < I < 1.1$ as the zero-interaction criterion.

3. Results

3.1 Physical Characterization

Overall, milk samples did show significant differences in instrumental viscosity measurements, but only between the highest (5%) and lowest (0%) sucrose concentrations ($p < 0.05$; **Table 2-1**). Similar to the sensory evaluation of thickness (see section 3.2), which also only differed significantly between the same high and low sucrose samples, viscosity differences were minor compared to the other factors.

Measurement of total solids and fat in the formulated sample indicated final sugar concentrations were as formulated: % error of the final estimated % sugar values deviated only up to 3% (**Table A-1**).

3.2 Sensory Attribute Differences Among Samples

As expected, varying sucrose and vanilla extract concentrations led to statistical significant differences between samples in overall liking and all sensory attributes ($p < 0.05$; **Table 2-1**). In general, attribute means differed significantly between the samples with the highest concentration and the lowest concentrations of sugar. The smallest differences between samples were seen for perceived thickness and milk flavor; the highest amount of variability was seen with sweet taste and vanilla flavor, which was expected, given that those two attributes are directly impacted by varying sucrose and vanilla flavor concentrations. The individual sucrose concentrations generally led to significant sweetness differences, confirming that the half-logarithmic spreading of the concentration intervals was sufficient to span the complete design space and for estimating perceivable sweetness differences.

Overall, the samples with sucrose were more liked on average than those without and liking was significantly higher for the samples containing both sucrose and vanilla compared to those with just vanilla. Liking between samples did not differ significantly between most of the different sugar levels, implying that sucrose concentrations could be reduced while still maintaining consumer acceptability. The maximum overall liking score of 6.04 was found for the sample containing 3.82% sucrose and 0.625% vanilla, though this liking score did not differ significantly from samples at lower sugar concentrations. Apart from samples without any sugar, all samples showed similar overall liking scores, even though the perceived sweetness differed significantly between the different sucrose concentrations ($p < 0.05$). This is potentially meaningful for food companies who are looking to reduce sugar in products; although differences in sweetness are perceivable to a consumer, this does not necessarily indicate changes in liking.

The few significant differences between samples in milk thickness were also reflected by the instrumental viscosity measurements (**Table 2-1**); generally, an increase in sugar led to an increase in perceived thickness ratings and instrumental viscosity measurements and the effect of sucrose on perceived thickness was also significant ($p < 0.05$). No effect on perceived thickness was found due to the addition of vanilla, which is in contrast to findings by Lavin and Lawless (1998) who found samples with vanilla were rated higher in creaminess, a term that has connotations of thickness perception among other texture attributes (Frøst and Janhøj 2007; Tournier et al. 2007). The full meaning of creaminess has been attempted to quite some degree as it has implications of many other terms and is interpreted differently depending on one's cultural background—perhaps because we only asked for thickness alone, we did not see any effect of vanilla on the thickness of samples. Alternatively, as Lavin and Lawless did not provide a vanilla flavor category to rate, the increase in perceived creaminess due to added vanilla aroma could also be the result of a dumping effect.

Overall, the samples showed significant differences in all sensory attributes and spanned a product space of sucrose and vanilla concentrations that is well liked and should translate well to industry applications. These findings also suggest that sugar reduction in foods can be possible simply by manipulating tastants and odors already existing in the matrix while maintaining consumer acceptance.

Table 2-1 *List of the 18 samples used in the study. Samples are shown with their sucrose (S) and Vanilla Extract (V) concentrations in % (w/w), as well as means and Tukey's honestly significant differences (HSD) for overall liking, (measured on a 9-point hedonic scale), and perceived sweet taste, vanilla flavor, milk flavor, and thickness (all measured on an unlabeled 100-point scale). Instrumental viscosity measurements are shown as the average of two replicate measurements ± standard deviation and HSDs. Within a column, means that do not share a common letter are significantly different from each other ($p < 0.05$).*

Label	**S (% w/w)**	**V (% w/w)**	**Overall Liking**	**Sweet Taste**	**Vanilla Flavor**	**Milk Flavor**	**Thickness**	**Viscosity (Pa.s)**
S0V0	0.00	0.00	4.49^{fg}	14.8^{g}	12.9^{i}	50.5^{a}	30.3^{bcde}	$2.77E\text{-}03 \pm 2.62E\text{-}05^{bcde}$
S0V0.25	0.00	0.25	4.81^{defg}	18.2^{g}	25.5^{gh}	44.9^{abc}	28.8^{cde}	$2.71E\text{-}03 \pm 6.29E\text{-}05^{bcde}$
S0V0.36	0.00	0.36	4.49^{fg}	18.7^{g}	27.0^{gh}	41.8^{bc}	28.7^{cde}	$2.66E\text{-}03 \pm 2.62E\text{-}05^{de}$
S0V0.52	0.00	0.52	4.44^{g}	19.0^{g}	28.2^{gh}	38.7^{c}	26.5^{de}	$2.68E\text{-}03 \pm 6.36E\text{-}06^{cde}$
S0V0.75	0.00	0.75	4.39^{g}	20.0^{fg}	27.9^{gh}	38.1^{c}	26.2^{e}	$2.61E\text{-}03 \pm 2.76E\text{-}05^{e}$
S1V0	1.00	0.00	4.74^{efg}	27.1^{f}	22.8^{h}	47.9^{ab}	28.8^{cde}	$2.84E\text{-}03 \pm 6.93E\text{-}05^{abcde}$
S1.31V0.3	1.31	0.30	5.52^{abcd}	37.4^{e}	38.0^{ef}	40.3^{bc}	32.9^{abcd}	$2.74E\text{-}03 \pm 2.69E\text{-}05^{bcde}$
S1.31V0.435	1.31	0.435	5.50^{abcd}	37.2^{e}	38.9^{de}	39.5^{c}	30.6^{bcde}	$2.70E\text{-}03 \pm 3.61E\text{-}05^{bcde}$
S1.31V0.625	1.31	0.625	5.22^{cdef}	36.3^{e}	39.2^{de}	39.7^{c}	29.9^{bcde}	$2.83E\text{-}03 \pm 2.83E\text{-}06^{abcde}$
S1.71V0	1.71	0.00	5.31^{abcde}	38.8^{de}	30.6^{fg}	44.8^{abc}	34.6^{abc}	$2.82E\text{-}03 \pm 9.40E\text{-}05^{abcde}$
S2.24V0.3	2.24	0.30	5.86^{abc}	46.8^{c}	44.9^{bcde}	40.7^{bc}	35.1^{abc}	$2.88E\text{-}03 \pm 1.89E\text{-}04^{abcd}$
S2.24V0.435	2.24	0.435	5.91^{abc}	46.9^{c}	46.6^{bcd}	42.3^{bc}	34.2^{abc}	$2.83E\text{-}03 \pm 9.76E\text{-}05^{abcde}$
S2.24V0.625	2.24	0.625	5.81^{abc}	44.8^{cd}	47.8^{abc}	39.5^{c}	33.1^{abcd}	$2.77E\text{-}03 \pm 2.40E\text{-}05^{bcde}$
S2.92V0	2.92	0.00	5.43^{abcde}	50.2^{c}	38.4^{e}	41.8^{bc}	35.2^{abc}	$2.85E\text{-}03 \pm 1.41E\text{-}06^{abcde}$
S3.82V0.3	3.82	0.30	5.77^{abc}	58.8^{b}	50.3^{abc}	40.4^{bc}	35.4^{ab}	$2.93E\text{-}03 \pm 7.64E\text{-}05^{abc}$
S3.82V0.435	3.82	0.435	6.00^{ab}	61.0^{ab}	51.9^{ab}	39.9^{bc}	35.4^{ab}	$2.96E\text{-}03 \pm 4.95E\text{-}05^{ab}$
S3.82V0.625	3.82	0.625	6.04^{a}	58.1^{b}	54.7^{a}	37.8^{c}	36.4^{ab}	$2.94E\text{-}03 \pm 1.41E\text{-}06^{ab}$
S5V0	5.00	0.00	5.27^{bcde}	67.7^{a}	43.4^{cde}	39.4^{c}	37.4^{a}	$3.06E\text{-}03 \pm 9.90E\text{-}06^{a}$

3.3 Creating Response Surface Models for Vanilla-Sucrose Milks

Dose-response models were created based on the consumer data to study the interaction between sucrose and vanilla. Three-dimensional response surface plots for overall liking and perceived sweetness, vanilla flavor, milk flavor, and thickness, based on multiple regression models, are shown in **Figure 2-1**. In general, the employed experimental design was an effective way of finding the ideal sucrose-vanilla combination for studying interactions effects in sweet taste and liking in milk products across a wide range of concentrations. Perception of 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 sufficiently represented the observed responses. Second order models were expected for these attributes as liking tends to decrease as a product becomes too sweet to consumers and flavoring dosages in food strongly influence its flavor profile—low dosages add slight body modification notes, regular dosages impart the characteristic/expected flavor profile, and high dosages can add some chemical off notes which may reflect negatively in acceptance scores (Ziegler 2007). For example, other characteristics found in vanilla extract, such as woody, phenolic, and alcoholic notes, are more pronounced at higher concentrations.

As for the three linear models for sweetness, milk flavor, and thickness, as expected, an increase in sucrose concentration was accompanied by a significant increase in sweetness ($R^2 = 0.37$, $F(2,1941) = 567.5$, $p < 0.05$; m = 10.54 for sucrose, m = 5.11 for vanilla) and thickness ($R^2 = 0.022$, $F(2,1941) = 23.2$, $p < 0.05$; m = 1.97 for sucrose, m = -2.42 for vanilla), while a decrease in milk flavor was observed ($R^2 = 0.015$, $F(2,1941) = 16.24$; m = -1.04 for sucrose, m = -10.37 for vanilla) (see **Figure 2-1 B, D, E**). These findings are similar to (Li, Hayes, and Ziegler 2014) for coffee-flavored milks, 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 were able to decrease or mask the dairy notes in milk.

For the two second-order models, both liking and vanilla flavor increased up to a concentration after which both attributes decreased. For liking, the design space covered the preferred concentration range, as the optimal liking contour, i.e., the stationary point on the liking surface, was found within the sample space at 3.79% sucrose and 0.53% vanilla (**Figure 2-1 A**). This combination was close to the maximum liked sample that was served to the consumers in terms of sucrose concentration (3.82%), but was slightly lower than the sample's vanilla concentration of 0.625%. The stationary point for the second-order model for vanilla flavor was found to be at 4.98% sucrose and 0.52% vanilla (**Figure 2-1 C**), 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 in the range of tested vanilla concentrations of 0.3, 0.435 and 0.625%. These all showed similar vanilla flavor intensities between 50-55 on a 100-point scale (**Table 2-1**).

Inspecting the response surfaces, it becomes apparent for the first-order sweetness model that increasing the vanilla concentration leads to a slight, but statistically significant, enhancement of sweetness perception (coefficient = 5.11; $p < 0.05$) (**Figure 2-1 B**). However, the magnitude of this taste enhancement by an odor may be small in comparison to the use of non-nutritive sweeteners. Conversely, the addition of sucrose increased the perception of vanilla also to a significant degree in the regression model for vanilla flavor (coefficient = 11.25; $p < 0.05$) (**Figure 2-1 C**). These findings are in agreement with Green et al. (2012) and Welge-Lüssen et al. (2009) 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. When studying milk desserts, Alcaire et al. (2017) too found that a 20% reduction in sugar caused a decrease in vanilla flavor perception. The same pattern can be seen for other tastes: Linscott and Lim (2016), when studying sodium chloride and monosodium glutamate (MSG), among other tastes, in combination with chicken and soy sauce odors, found that the tastes impacted odor perception more than the odors affected tastes; earlier, Pfeiffer et al. (2006) even 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. Generally, our findings agree with others who reported significant odor enhancement by taste (Isogai and Wise 2016; Lim, Fujimaru, and Linscott 2014; Fujimaru and Lim 2013). Although it is possible that these findings may be also affected by a physiochemical "salting out" effect from the addition of sugar, Pfeiffer et al. (2006) found that volatile concentration remained constant even when increasing sucrose and acid concentration, as measured by in-nose volatile release using atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). Similarly, volatile release profiles of citrus flavored model beverages did not change when varying tastant levels (Hewson et al. 2008). Measuring the volatile release profiles of solutions with and without acid, Cayeux and Mercier (2003) too ruled out physicochemical interactions as they did not find any significant differences in the release of volatiles. Based on these results, we believe that our flavor enhancement findings are too cognitive in origin rather than due to the solubility of components in the vanilla milk mixtures.

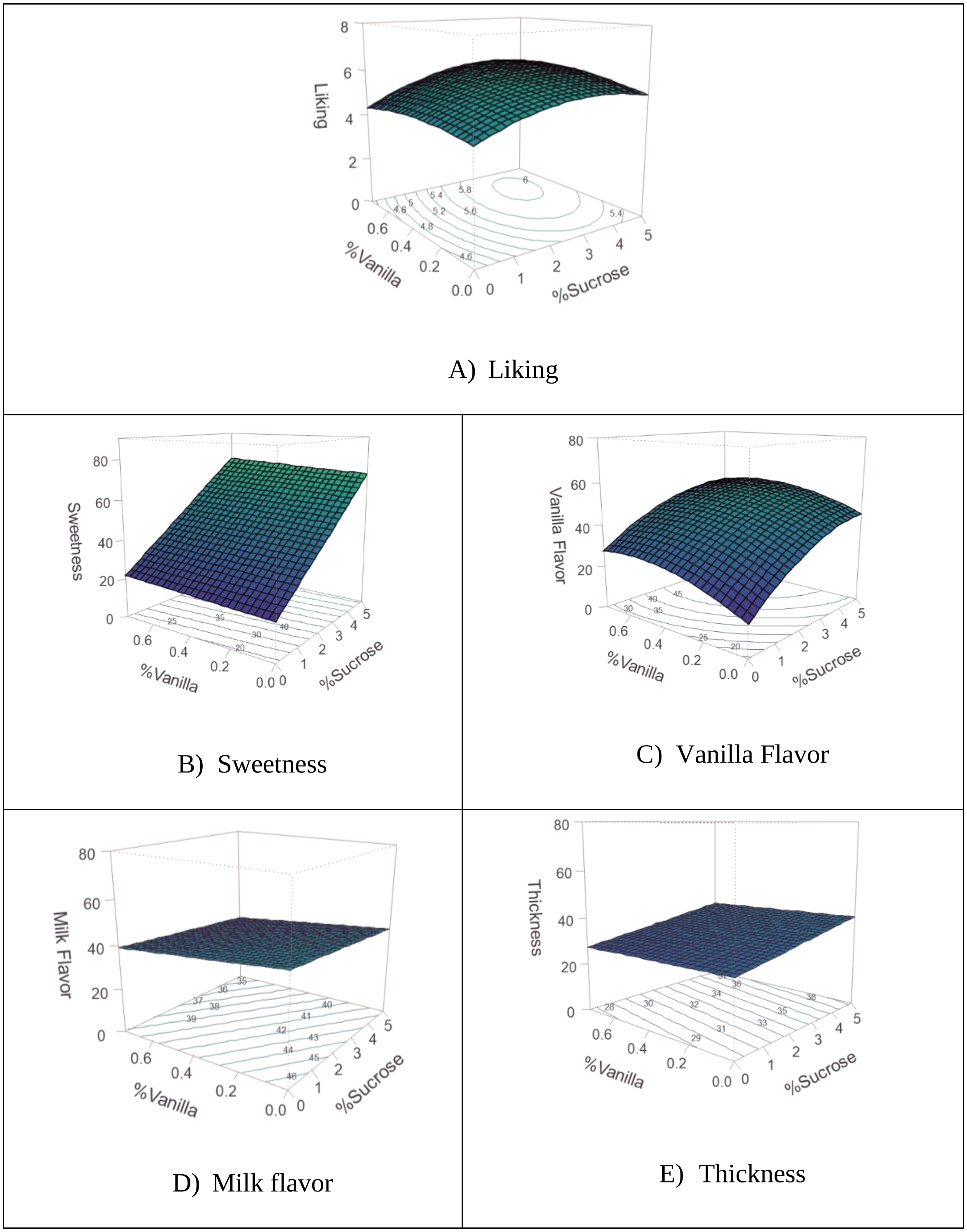


Figure 2-1 *Response surface plots established from the 18 samples evaluated by 106 consumers, for each of the sensory response variables and the sucrose (S) and vanilla (V) concentrations. Regression equations are included for each surface. (a) overall liking* $OL=4.40+0.669\cdot S+1.42\cdot V+0.198\cdot S\cdot V-0.102\cdot S^2-2.05\cdot V^2$ *(*$R^2 = 0.11$*,* $F(5,1938) = 47.92$*,* $p < 0.05$*), (b) perceived sweetness* $SW=18.1+10.5\cdot S+5.11\cdot V$ *(*$R^2 = 0.37$*,* $F(2,1941) = 567.5$*,* $p < 0.05$*), (c) perceived vanilla flavor* $VF=13.8+11.3\cdot S+50.6\cdot V-1.17\cdot S\cdot V-1.07\cdot S^2-42.6\cdot V^2$ *(*$R^2 = 0.21$*,* $F(5,1938) = 104.7$*,* $p < 0.05$*), (d) perceived milk flavor* $MF=46.9-1.04\cdot S-10.4\cdot V$ *(*$R^2 = 0.02$*,* $F(2,1941) = 16.24$*,* $p < 0.05$*), and (e) perceived thickness* $TH=29.4+1.97\cdot S-2.42\cdot V$ *(*$R^2 = 0.02$*,* $F(2,1941) = 23.2$*,* $p < 0.05$*)*

3.4 Testing for Interactions with the Isobole Approach

Both individual ANOVAs and response surface models indicated the combination of sucrose and vanilla induced cross-modal interactions; however, the degree of such interactions can be tested using the isobole method. In **Table 2-2** the index of interaction (I) for sweetness, as calculated by equation (2), are shown for all nine sucrose-vanilla mixtures. If using Suhnel's (1993) criterion for interaction (i.e., any $I \neq 0$), every mixture except for two (S3.82V0.3 and S3.82V0.625) exhibited evidence of synergism for perceived sweetness ($I < 1$), with the other two showing antagonism ($I > 1$) and no interaction ($I \sim 1$). Similar to Fleming and colleagues (2016), a working cut-off criterion for interaction of $0.9 < I < 1.1$ was defined instead of using Suhnel's definition, which may be too liberal. When using this cut-off point for sweetness interactions, 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 0.78 and 0.89. Further, interactions between sucrose and vanilla were stronger at lower concentrations of vanilla at both sucrose concentrations, indicating that lower levels of vanilla induce higher cross-modal interactions in combination with sugar.

A more intuitive, graphical representation of the interaction between sucrose and vanilla in milk drinks is shown in **Figure 2-2**. Here, a zero-interaction response surface (the two-dimensional plane depicted in blue) can be produced from the individual dose-response functions of the vanilla-only and sucrose-only samples. Observed responses for specific vanilla-sucrose mixtures can be visualized by the nine individual dots plotted; 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 2, most mixtures are above the additive plane, except for the two antagonistic mixtures that either fall below or touch the plane, in line with Suhnel's criteria.

Table 2-2 *Indices of interaction (I) for perceived sweetness in the nine sucrose-vanilla mixtures. S = sugar, V = vanilla.* C_S *and* C_V *were calculated using the linear regression for sweetness with the sucrose-only mixtures (Sweetness = 10.52 · % sucrose + 17.35;* $R^2 = 0.43$*,* $F(1,438) = 406.24$*,* $p < 0.05$*) and the vanilla-only mixtures (Sweetness = 6.41 · % vanilla + 15.71;* $R^2 = 0.007$*,* $F(1,538) = 4.92$*,* $p < 0.05$*), and I was calculated using equation (2).*

Label	Sucrose (% w/w)	Vanilla (% w/w)	Sweetness	C_S	C_V	I
S1.31V0.3	1.31	0.30	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
S2.24V0.3	2.24	0.30	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
S3.82V0.3	3.82	0.30	58.8	3.94	6.73	1.01
S3.82V0.435	3.82	0.435	60.9	4.14	7.06	0.984
S3.82V0.625	3.82	0.625	58.1	3.88	6.62	1.08

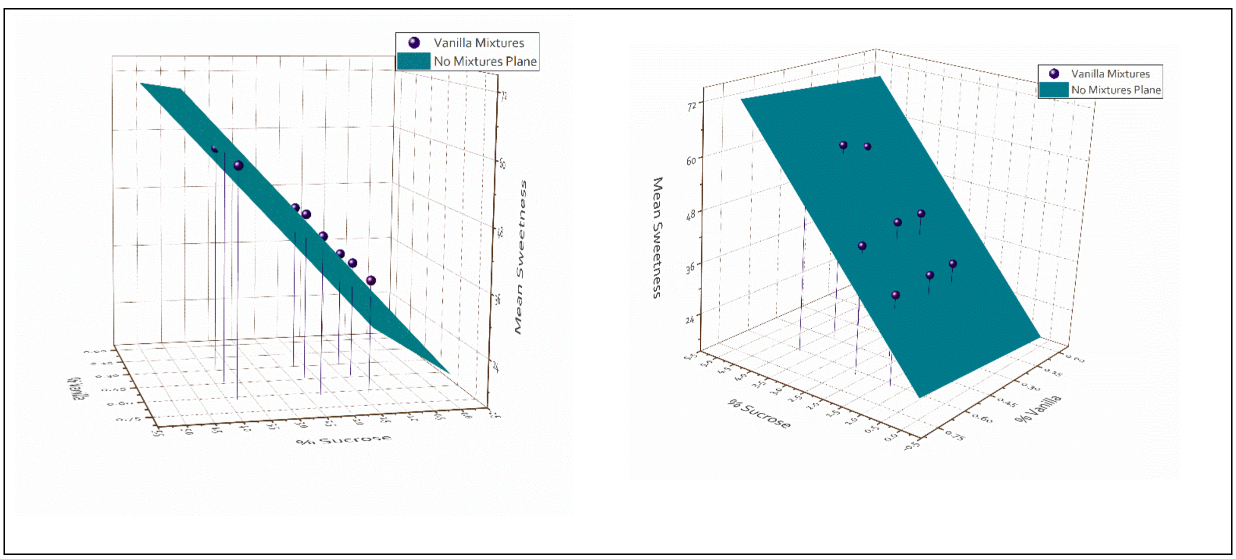


Figure 2-2 *Isobolograms for the vanilla-sucrose milks determined from their respective dose-response functions. Deviations above the zero-interaction surface correspond to I-values below 1 (synergy). Deviations below the surface correspond to I-values above 1 (antagonism). Those on or near the surface indicate no interaction (I=1).*

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 on its own. It should be noted that these results were found for consumers who were predominantly of western descent, and so these results may not necessarily apply to cultures where the combination of vanilla and sugar is not as familiar or as present in their food culture. 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 (Stevenson, Prescott, and Boakes 1999; Dravnieks 1985). 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 (Prescott, Johnstone, and Francis 2004; Stevenson, Prescott, and Boakes 1995; Stevenson, Prescott, and Boakes 1999). Nguyen et al. (2002) found that vanilla, caramel, and strawberry odors induce sweetness enhancement in western countries where these odors are commonly experienced with sucrose. In contrast, non-western participants did not describe some of these odors as sweet, which could be due to the infrequency of these odor pairings with sweetness in their culture. Thus, prior exposure to and/or learning of the taste-aroma combination seems to be a necessary factor for any enhancements and/or interactions to occur. Future dose-response studies with different cultural groups could be conducted to test how culture-dependent our findings are.

In this study, even when panelists were given a full list of attributes to rate to account for dumping effects, we still found evidence for interaction between the taste and the aroma. That is, adopting an analytical perceptual strategy does not prevent taste enhancements from occurring. Aroma had a significant impact on taste and vice versa, though taste induced-odor enhancement may be larger than that of odor induced-taste enhancement. Our findings are similar to other studies in that increasing the concentration of sucrose resulted in higher flavor ratings (Valdes, Simone, and Hinreiner 1956; Valdes, Hinreiner, and Simon 1956; Bonnans and Noble 1993; Kuo, Pangborn, and Noble 1993).

Despite the results from other literature suggesting that any sweetness enhancement disappears due to a dumping effect, our findings indicate there still is some effect of aroma on taste perception, though these effects may not be as large as what could be achieved with non-nutritive sweeteners (i.e., supplementing with these while removing sugar provides a greater reduction than using findings from taste-aroma interactions). Valentin and Nguyen (2001), when studying the effect of vanilla and lemon aroma on sourness ratings using two different tasks, made a similar conclusion in that although providing more scales decreases odor-induced taste enhancement, they do not suppress it. Other studies that used non-intensity rating strategies have also hinted at the presence of interactions. Djordjevic, Zatorre, and Jones-Gotman (2004a) experimented with a sweet taste detection task, and asked participants to indicate which of two solutions (either a weak sucrose solution or blank solution) was stronger. They found that sucrose detection was better when combined with strawberry odor versus ham odor. Nguyen, Dacremont, and Valentin (2000) used a categorization task free of response biases to study sweetness perception as affected by changes in vanilla aroma. Here they found participants' categorization performance was poorer when the concentration of vanillin changed along with the sucrose concentration, inferring they were unable to ignore the olfactory component when trying to categorize solutions according to the tastant. Given these studies, it seems that taste-odor interactions cannot be described solely by response biases (Valentin, Chrea, and Nguyen 2006).

It would be worth exploring the isobole method to determine at what point the interaction criterion set by Suhnel (1993) becomes relevant to human sensory perception, that is, where the interaction index I provides a meaningful sensory effect. Future studies could look at a standard range for taste mixtures involving perceivable differences by the consumer. Different media should also be tested to determine whether the cut-off criteria can be generalizable across products. Given the current criteria, it seems that taste-aroma interactions are more likely to occur at low to medium sucrose-vanilla concentrations, in line with others who have found a concentration dependence. For instance, Brossard et al. (2006) observed that sweetness had an effect on aroma intensity at low to medium sucrose concentrations (24-45 g/kg) in custards flavored with different aroma compounds, though no modification of sweetness was found from changes in fruity aroma intensity.

To further argue in favor of interaction between smell and taste, different neural responses have been seen when taste and aroma components are consumed in combination versus when presented separately. Since gustation and olfaction are distinct mechanisms and do not share receptors, any interactions that take place are thus cognitive and not peripheral (Noble 1996). Several neuroimaging studies have supported this hypothesis in that certain brain regions are subsequently activated by taste-aroma combinations. For example, activation has been found in the anterior region of the orbitofrontal cortex (OFC) when a tastant and odor were presented in combination, a region not activated by taste or smell alone, suggesting that this area is important for the integration of taste and smell (de Araujo et al. 2003). Small and Jones-Gotman (2001) found an increase in blood oxygen level demand in the OFC and amygdala when taste and smell were given together, at a higher amount than the sum of the taste and smell activity alone. Small and colleagues (2004) also found that when taste is perceived simultaneously with a retronasally induced odor, the OFC, insula, and anterior cingulate cortex (ACC) regions showed superadditive responses (meaning that neural activity evoked during bimodal stimulation is greater than the summed activity from the single presentation of its unimodal components). Furthermore, de Araujo et al. (2003) investigated congruent odor-taste pairings and found that activation of certain brain areas by these was correlated with increasing congruence of the two stimuli, suggesting that prior exposure is a necessary prerequisite for taste-aroma interactions. These results all indicate that when taste and smell compounds are combined, something more than the sum of their perceptual parts occurs (Delwiche 2004). Even when the mathematical determination of synergism might be unclear, these effects are physically manifested, as supported by neuroimaging research.

While the taste enhancement effects found were not strong, this could also be attributed to the cognitive task used to study such interactions. 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 (Kuznicki, Hayward, and Schultz 1983). This implies that each 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 (Lawless and Schlegel 1984). Thus, the current question regarding sweetness enhancements becomes: does this interaction effect reflect a real perceptual phenomenon, or is it simply a consequence of the strategy panelists use? (Auvray and Spence 2008). McBurney (1986) 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 effect), whereas a single rating of a sensory quality encourages the synthesis of the common quality from both sensory modalities of odor and taste, causing an enhancement effect (Prescott, Johnstone, and Francis 2004).

Because the occurrence of an enhancement may be a result of scaling biases, a non-scaling method would be better suited for confirming enhancement effects. Using a more holistic testing method, i.e., a non-scaling method, consumers may perform more similar to their “natural” behavior and be able to reveal a “true” 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. McBurney (1986) argues that flavors more so reflect the combination of tastes and odors into a single perception, in line with our initial reactions to foods when eating which are hedonic and holistic (Prescott 1999). To our knowledge, only one study (van der Klaauw and Frank 1994) 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 should be conducted in cross-modal interaction research as flavor is experienced as a singular percept rather than split into individual components.

5. Conclusions

Even when controlling for the dumping effect by providing numerous response categories or attributes to scale, significant interactions between vanilla and sugar were found on sweetness perception when added to skim milk. A mathematical regression, the isobole approach, provided evidence that synergism for sweetness enhancement occurs between a congruent taste-aroma pair, though these effects appear to be small. Potential applications for sugar reduction in foods could be accomplished with these findings, though the magnitude of sweetness induction may be less in comparison to other current techniques, such as the use of non-nutritive sweeteners. Future work could evaluate what a working criterion for interaction effects should be, as the narrow criteria established by Suhnel needs to be modified for dose-response studies in food. Additionally, testing the most synergistic combinations for sweet taste enhancement by a congruent vanilla aroma should be conducted using a holistic rather than an analytical approach, to better represent how we experience flavor when eating.

Chapter 3: A modified ABX matching test demonstrates taste-aroma enhancement in skim milk

3.1 Introduction

The current literature on sugar so far has not found any direct causal links of its consequences to our health, yet it still has become regarded as something to avoid (Te Morenga, Mallard, and Mann 2013; Te Morenga et al. 2014; Yang et al. 2014). As a result of the numerous, albeit mostly observational, reports that surround the health effects of sugar, consumers have become more aware of added sugars in their diets, causing a decrease in sugar consumption over the years (Welsh, Sharma, Grellinger, et al. 2011). A recent Mintel survey on sugars and sweeteners found that 84% of consumers are limiting the amount of sugar in their diet, and a third of these consumers indicated they will be limiting their sugar intake more than the previous year (Mintel 2016). Although sugar consumption has been on the decline, mean intakes of added sugar continue to exceed recommendations given by the World Health Organization (WHO), the Dietary Guidelines Advisor Committee (DGAC), and the Scientific Advisory Committee on Nutrition (SACN) (Welsh, Sharma, Grellinger, et al. 2011; WHO 2015; USDA 2015a; SACN 2015). A more recent US study published in 2014 on added sugars and cardiovascular disease found the average percentage of daily calories from added sugars increased from 15.7% from 1988-1994 (n = 11 733), to 16.8% in 1999-2004 (n = 8786), then decreased to 14.9% from 2005-2010 (n =10628), which translates to most adults on average consuming approximately 5% more than the recommendations set by the WHO, DGAC, and SACN (Yang et al. 2014). This steady intake of a large amount of added sugars is likely due to the increasing availability of sugars on the market. From 1970 to 2005, the average availability of added sugars increased by 19% in the US, with soft drinks and other sugar-sweetened beverages being the primary source of added sugars in the diet (R. K. Johnson et al. 2009). However, these products are not the only culprits for added sugars, as many other foods such as sweetened grain desserts, dairy products, and other sugars “hidden” in processed foods, can contribute to the amount of added sugars consumed (Welsh, Sharma, Argeseanu, et al. 2011; WHO 2015). This is particularly relevant to the dairy industry as products like yogurt and flavored milk beverages, having seen an increase in consumption due to their associated health benefits, often contain high amounts of added sugars which not only offsets any nutritional value but is also misleading to consumers. A recent systematic review on flavored milk consumption and its association with energy intake and obesity revealed that although flavored milk does have many nutritional benefits and contributes to overall milk intake, many of the studies reviewed suggested it may also lead to a higher overall calorie consumption (Noel et al. 2013; Wilson 1991; Wilson 2000; Patel et al. 2018).

To meet consumer demand, many of the leading food manufacturers have been actively seeking ways to reduce the amount of sugar in their products (Hutchings, Low, and Keast 2018; Mintel 2018). Currently, most of the sugar reduction strategies in foods have involved the use of artificial and alternative sweeteners; however, a 2016 Mintel report suggests that 40% of consumers think that artificial sweeteners should be avoided (Mintel 2016). This negative perception may be due to sweeteners’ long-term effects on our health for which there is still ongoing uncertainty, regardless of their GRAS status, as well as unappealing, sometimes bitter and other side tastes, and differing temporal/flavor profiles from sucrose (Zorn et al. 2014; Reyes, Castura, and Hayes 2017; Antenucci and Hayes 2015; Ayya and Lawless 1992; Dubois and Prakash 2012; Azad et al. 2017). At the same time, maintaining an acceptable flavor while using less sugar and artificial sweeteners leaves manufacturers with few options.

Cross-modal interactions, in particular taste-aroma interactions, may present an alternative or complementary approach to sugar reduction as it has been found that the addition of particular aromas to a food may enhance sweetness perception. That is, the combination of a certain taste and aroma together may enhance the perception of the other more than when either modality is presented independently (e.g., Stevenson, Prescott, and Boakes 1995; Hewson et al. 2008). This effect has been shown when there is enough perceptual similarity between an odorant and tastant as a result of frequently encountering particular pairings in foods (Stevenson, Prescott, and Boakes 1999; Small et al. 2004; Delwiche 2004). Some examples of harmonious odor taste pairs include vanilla with sugar, lemon with citric acid, and broth with salt (Dravnieks 1985; Sakai 2001; Noble 1996). These are known as "congruent" pairings, which Schifferstein and Verlegh define as the extent to which two stimuli are appropriate for combination in a food product (1996), and may be a necessary condition for enhancements to occur. However, most research on taste-aroma interaction has been done using sucrose solutions or model systems (Stevenson, Prescott, and Boakes 1999; Labbe et al. 2007; Frank and Byram 1988; Prescott 1999; Frank, Ducheny, and Mize 1989; Frank, van der Klaauw, and Schifferstein 1993; Stevenson 2001; Lawless and Schlegel 1984; Prescott, Johnstone, and Francis 2004; Boakes and Hemberger 2012; Arvisenet, Guichard, and Ballester 2016; Hewson et al. 2008; Jones et al. 2008; Lethuaut et al. 2004; Bult, Wijk, and Hummel 2007). It is unclear if these effects can also be observed in real foods, since only a few studies have been done in these due to their inherent complexity that can make elucidating the contribution of specific compounds difficult (Frank and Byram 1988; Lavin and Lawless 1998; von Sydow et al. 1974; Symoneaux et al. 2015; Green et al. 2012; Alcaire et al. 2017).

The choice of food to study cross-modal interactions should be determined by the degree of its complexity, as foods contain multiple components which all play a role in flavor perception. When conducting cross-modal interaction studies in foods, the medium should be relatively simple to allow isolation of the effects originating from different modalities (which are known to interact perceptually) i.e., taste and odor in this case. The rate of release of volatiles into the nasal cavity to cause flavor perception is a function of physical factors such as viscosity, as well as the interaction between aroma compounds and components in food like fat, protein, and carbohydrates (Miettinen 2004). Fat has been found to have the most influence on volatile release out of all the food components (Rabe, Krings, and Berger 2003); therefore, a food that is low in fat, such as skim milk (which contains mostly water, less than 0.5% fat and has on average 9% milk solids (FAO 2013)) would be an ideal medium to study. Within the dairy category, flavored milk (a product that is generally sweetened) would be a relevant medium for studying taste-aroma interactions as its consumption has been increasing in recent years, with flavor becoming a more important purchasing factor to consumers (Mintel 2017a).

A common combination used in aroma-taste research—and relevant to the dairy industry—is vanilla and sugar, though mostly studied in aqueous solutions (Kuo, Pangborn, and Noble 1993; C. C. Clark and Lawless 1994; Small et al. 2004). One of the first studies of this pairing in milk was when Pangborn (1988) researched liking and attribute responses of vanilla milk drinks varying in milk fat and sucrose; however, interaction effects between the ingredients were not the focus and thus not studied. Lavin and Lawless (1998), similar to our experiments, studied vanilla milk (1% milk and vanilla extract at 0.015%) but did not include an extended ballot of attributes to panelists to account for potential ‘dumping’ that could affect whether an enhancement is observed (see also further below). Recently, a taste-aroma interaction study (Alcaire et al. 2017) used vanilla milk desserts made with skim milk. However, in addition to vanilla and sucrose concentration, the starch levels were also varied in this study, which may dilute the effects of sugar and vanilla on flavor and make it more difficult to draw conclusions about cross-modal interaction effects between these ingredients. In the present study, we limit the factors that contribute to perceived viscosity (e.g., starch), and use vanilla extract as it does not add sufficient solids to significantly affect viscosity and thus perhaps flavor perception. This allows our results to be attributed solely to the tastant and the aromas in the milk and their possible perceptual interactions.

Despite the magnitude of literature on taste-aroma interaction and a potential odor and/or taste enhancement, there are still open questions regarding the actual occurrence of this effect. Previous studies have found that enhancement effects will differ based on the questions given, and the cognitive process panelists use to evaluate products. Flavor perception seems to happen in one of two ways: analytically or synthetically, the former involving separating the properties of samples into their individual flavor components (i.e., taste vs. aroma), the latter requiring the sample properties to be thought of together as one flavor entity (Lockhead 1979; Schifferstein and Verlegh 1996). When an analytical mindset is adopted—and this typically occurs during sensory evaluation tests with intensity or rating scales—another complication arises, i.e., the number of relevant attributes to be rated determines whether an enhancement occurs (Frank, van der Klaauw, and Schifferstein 1993; C. C. Clark and Lawless 1994; van der Klaauw and Frank 1996). This was first noted by

Frank and van der Klaauw (1993), when researching solutions with added strawberry and other aromas. They found that when given a limited response ballot with only a few of the salient flavor attributes of the product listed (e.g., only sweetness but not vanilla flavor), panelists will "dump" (a term coined by Clark and Lawless 1994) any perceptually similar attributes into the same category. As a result, the category increases, showing what is thought to be an enhancement. In contrast, when asked to rate multiple relevant attributes (e.g., sweetness and vanilla flavor), no increase or enhancement was observed. It was hypothesized that providing appropriate responses encourages panelists to separate the attributes of a sample, while with a limited response ballot, panelists are prone to integrating these dimensions (van der Klaauw and Frank 1996). The authors concluded that mixture-induced taste enhancement is a result of a "dumping effect", making it seem as though any observed enhancement is more of a measurement artefact rather than a true perceptual phenomenon. Several other authors also observed that enhancement of taste by odors is weak or not present at all when using an extended ballot (Green et al. 2012; Lim, Fujimaru, and Linscott 2014; Fujimaru and Lim 2013; Linscott and Lim 2016; Wang et al., unpublished.)

Though this dumping effect may explain perceived taste enhancements when using intensity rating scales, the analytical strategy taken during these studies may not be representative of what is experienced during a real eating situation. Much of what is perceived as flavor is in fact from the odors of foods, which are experienced by two kinds of olfaction: orthonasal, where objects are smelled from a distance, and retronasal, where food identification occurs in the mouth (Rozin 1982). As both of these deliver different information, it makes sense that we combine a food's tastes and retronasal odors, in addition to chemesthetic and tactile senses which can also be stimulated, into one flavor perception in order to recognize food (Prescott 1999). Further, when it comes to eating, people's initial responses to foods are usually holistic and hedonic (Prescott 1999). Hence, although perception arises from multiple senses, sensory information is often integrated into one unitary percept, which evokes a synthetic mindset (Prescott 1999). This idea that gustatory mixtures are synthetic, i.e., the mixing of stimuli is a unique flavor on its own, has been suggested by several authors. For example, Kuznicki and Ashbaugh (1979; 1982) demonstrated participants were unable to attend to individual tastes in heterogeneous taste mixtures and that the presence of irrelevant tastes caused a shift in the quality of the target sensations. Subjects were only able to provide an unaffected response when the components were separated by space and time. This supports the contention that subjects perceive mixtures as a whole instead of analytically during flavor perception (Schifferstein and Frijters 1990).

Therefore, using a direct scaling approach that evokes an analytical mindset might not be the best way to assess taste-aroma interactions. To avoid this response bias, a scale-free matching task presents an appropriate alternative which can be used as a cross-check on the response scale to give relative information on sweetness while maintaining a synthetic mindset (McBride 1983). To date, only a few taste-aroma interaction studies have explored methods that encourage the use of a synthetic strategy. van der Klaauw and Frank (1994; personal correspondence 2017) asked panelists to match varying sucrose solutions to sucrose solutions with added strawberry and coffee odors that came closest to the **sweetness** of the test sample. Lawless and Shlegel (1984) compared direct and indirect scaling of sucrose and citral solutions and found evidence for interactions when using indirect scaling. In the latter study, since no specific attribute was identified, subjects chose to integrate flavor mixtures instead of as analyzable pairs of attributes (Kuznicki, Hayward, and Schultz 1983).

In the current study, we build upon the matching experiment of van der Klaauw and Frank (1994) to mitigate possible response biases that could arise from using rating scales, but modify the task to mimic an ABX test. The ABX test (Munson and Gardner 1950) involves the matching of a sample (X) to either a reference A or B (usually a control and a treatment). In theory, providing two references allows the panelists to inspect the samples and determine for themselves the nature of the sensory differences, if any exist (Lawless and Heymann 2010). In contrast to the van der Klaauw & Frank study (1994), the nature of the difference is not specified; therefore, the panelist is not directed to any specific attribute, which could otherwise evoke an analytical mindset. Further, by including several sucrose concentrations as references A, B, etc. and asking panelists to match a sucrose-vanilla mixture X, one of the major differences of our test to the conventional ABX is that X may or may not have an exact match (i.e., none of the references will have added flavor). The testable hypothesis here then becomes: if vanilla aroma has a sweetness enhancing effect, panelists will match a vanilla-sucrose mixture X to a reference **higher** in sucrose than without added vanilla.

Additionally, this adapted test allows us to assess the assumption that a necessary condition for taste enhancement by an aroma is congruency or harmony between the taste and odor. For example, Frank and Byram 1988 found that sweetness could be enhanced by a strawberry odor but not peanut butter, and strawberry odor did not enhance saltiness; other findings include that bitterness of quinine could not be enhanced by sucrose (Frank, van der Klaauw, and Schifferstein 1993) and soy sauce odor can enhance perceived saltiness but not sweetness (Djordjevic, Zatorre, and Jones-Gotman 2004b). This suggests that the degree of association between the two is crucial for taste enhancement, which can only occur when they have previously been experienced together. This can be explained by the learning of certain combinations through their repeated pairing in one's cultural context, as well as during controlled flavor learning studies (Small et al. 2004; Stevenson, Prescott, and Boakes 1995; Stevenson, Prescott, and Boakes 1999; Valentin, Chrea, and Nguyen 2006; Prescott 2012). de Araujo et al. (2003) found increased orbitofrontal cortex (OFC) activity when participants were presented with congruent combinations of olfactory and gustatory stimuli compared to incongruent combinations. Given all the evidence supporting the idea of congruency as a prerequisite, the present work also tested the impact of congruency in a parallel matching experiment by comparing a congruent aroma to an incongruent one when paired with sucrose. Thus, we further hypothesize that a sweetness enhancement may be found when encouraging a synthetic mindset, but only if there is enough perceptual similarity, or congruency, between the odor and taste.

The current study outlines two experiments that attempt to answer the following questions: (1) Can a sweetness enhancement be elucidated when panelists are tasked with a non-scaling method that encourages a synthetic strategy?, and (2) Given a range of sucrose concentrations above and below that of the actual sugar concentration, can a sweetness enhancement still be induced by a congruent aroma? Additionally, (3) does the congruency between an odor and taste affect the occurrence of a sweetness enhancement?

2. Materials and Methods

2.1 Experimental Design

The following experiments both use a modification of the ABX test. For experiment 1, an ABCX test was used, while an ABCDX test was used in experiment 2 (see **Figure 3-1**). In both experiments, panelists are required to match an unknown sample (X) to one of three (A, B, C) or four (A, B, C, D) references. One of the major differences of our test compared to the classical ABX test is that X may or may not have an exact match depending on the set: for each experiment, panelists are asked to match two sets of samples, a control and a test set (**see Table 3-1**). The control set uses a sucrose concentration as sample X that matches the concentration of one of the references. This set is used to check that panelists are able to correctly conduct the test and to show there are perceivable differences between the sucrose concentrations. The test set uses an aroma-sucrose milk as sample X, and so has no exact match in the references given, to determine the potential sweetness enhancing effect of the aroma.

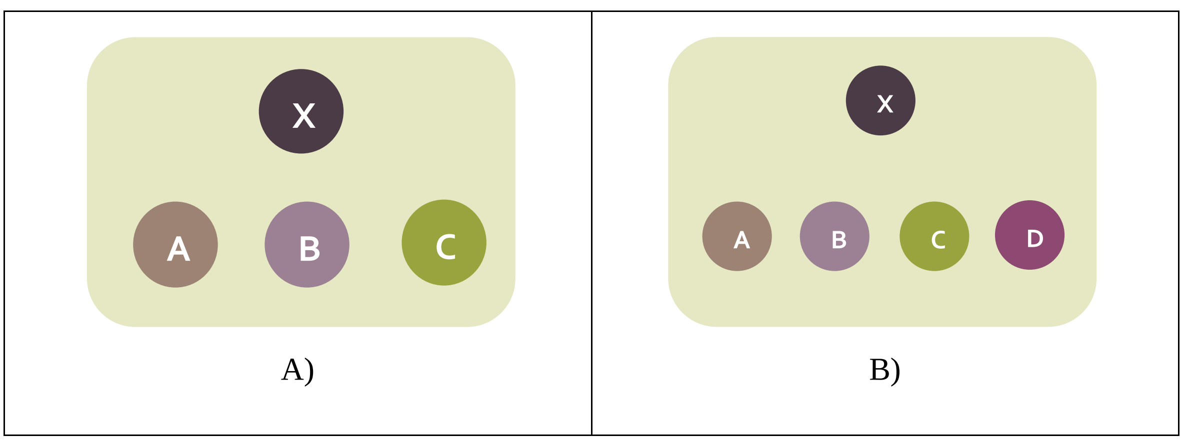


Figure 3-1 *The general design for experiment 1 (ABCX) (Fig. 3-1 A) and experiment 2 (ABCDX) (Figure 3-1 B). In experiment 1, X is either a sucrose-milk or sucrose-aroma milk, both at the same sucrose concentrations as A. In experiment 2, X is again either a sucrose-milk or sucrose-aroma milk, at the same sucrose concentration as B.*

2.2 Materials and Sample Production

Thirty-two kg batches of the various sucrose-milk mixtures were formulated for pasteurization, resulting in 10 kg batches of sucrose-aroma milk samples for sensory evaluation. Six (experiment 1) and four (experiment 2) different sucrose-milk concentrations (**Table 3-1**) were prepared in the Penn State pilot plant, after which aroma was dosed to achieve the required sucrose-aroma milk concentrations. Two-fold vanilla extract and beef filet mignon flavor (both from David Michael & Co, now TastePoint by International Flavors and Fragrances; Philadelphia, PA) were used as the congruent and incongruent aromas respectively. Pasteurized skim milk (TS = 8.88%, TF = 0.11% for ABCX test processing; TS: 9.03%, TF 0.15% for ABCDX test processing) and granulated cane sugar (Golden Barrel, Honey Brook, PA and Michigan Sugar Company, Bay City, MI) were provided by the Berkey Creamery (University Park, PA). The day before pasteurization, all amounts of milk and sugar were pre-weighed into stainless steel milk cans and plastic tubs. During the day of pasteurization, all sugar was dissolved into a third of the milk from each can by mixing and then transferred back to the milk can for further mixing with metal agitators. The milk and sucrose in these cans were then further blended with metal agitators on high speed for 10 minutes to ensure even distribution of the sucrose using a steam table. All mixes were then high temperature short time (HTST) pasteurized (APV Junior Pasteurizer; APV Invensys, Woodstock, GA) at 75°C for 25 s and homogenized (Gaulin, Lake Mills, WI) in a 2-stage process at 10.3 and 3.5 MPa (2000 and 500 psi). Once all processed milks were collected, mixes were dispersed into 6-kg aliquots and flavored with pre-weighed vanilla or beef flavor extract, packaged into half-gallon milk jugs and stored at refrigeration temperature (<°5C) for no more than one week until sensory evaluation. Samples were collected for physical analysis and microbiological analysis. Fat and total solid contents were measured with the Smart Trac (CEM Corporation, Matthews, NC) to confirm the sucrose concentration requirements were sufficiently met in the final samples (see **Tables B-1 and B-3**). High-sensitivity coliform counts were conducted (Petrifilm; 3M, Maplewood, MN) to ensure samples were suitable for human consumption.

Concentrations for the sucrose-vanilla milks for both experiments were chosen based on results from a previous dose-response experiment (Wang et al., unpublished), where sucrose-vanilla interaction scores according to Suhnel (1993) were calculated for perceived sweetness. The sucrose reference concentrations were determined in the following manner: linear regression equations for sweetness ratings were determined for the vanilla-sucrose milk mixtures as well as the sucrose-milk samples. Mean sweetness of the vanilla-sucrose mixture was then used in the sucrose-milk sample equation to find the amount of sucrose needed to achieve the same sweetness level as the mixture. The difference between the baseline sugar concentration in the mixture and that from the equation was then used as the spacing between the individual reference sucrose concentrations.

The concentration sets for experiment 1 (1.31% sucrose/0.625% vanilla and 2.24% sucrose/0.435% vanilla) were chosen based on significant differences of the attributes and liking scores from the dose-response experiment of vanilla milk (Wang et al., unpublished). As for experiment 2, the concentration of 2.24% sugar and 0.435% vanilla was chosen based on the same dose-response study results which indicated that this sample had a lower index of interaction (i.e., higher level of synergism) and a higher average liking score than the 1.31% sugar/0.625% vanilla combination (Wang et al., unpublished). The sugar concentration for the beef milk was kept constant to that of the vanilla sample and the concentration of beef flavor was intensity-matched to that of the vanilla flavor in a small-scale pilot study, where seven different incongruent aromas (Tastepoint by IFF, Philadelphia, PA) as well as the congruent vanilla aroma were tested. Thirteen panelists (8 women; 22-36 years) rated odor and flavor intensity (on a line scale from 0-100, with anchors from not extremely to extremely) and appropriateness (on a line scale from 0-100 with anchors from not very appropriate to very appropriate) for the various sweet skim milk samples, served in randomized order for each panelist. Scores for odor and flavor intensity and appropriateness were averaged and plotted (see **Figure B-1**). The beef aroma was chosen as it was not significantly different from the vanilla milk sample in terms of flavor and odor intensity and was also rated significantly inappropriate ($p < 0.05$).

***Table 3-1** Stimuli/concentrations for experiments 1 and 2 (sucrose S, vanilla extract V, beef flavor B, all in skim milk). All concentration percentages are in % (w/w).*

Experiment	**Set**	**Tray**	**Sample**	**References**			
			X	**A**	**B**	**C**	**D**
EXPERIMENT 1	Set 1	Control	1.31% S	1.31% S	1.80% S	2.29% S	-
		Test	1.31% S + 0.625% V				
	Set 2	Control	2.24% S	2.24% S	2.81% S	3.38% S	-
		Test	2.24% S + 0.435% V				
EXPERIMENT 2	Set 1	Control	2.24% S	1.67% S	2.24% S	2.81% S	3.38% S
		Test	2.24% S + 0.435% V				
	Set 2	Control	2.24% S	1.67% S	2.24% S	2.81% S	3.38% S
		Test	2.24% S + 0.400% B				

2.3 Physical Characterization

To account for physicochemical interactions that could contribute to differences in flavor perception, and since total solids content was not kept constant between samples, the viscosity of each sample was determined using a Discovery H3 Hybrid Rheometer (TA Instruments, New Castle, DE), equipped with a double wall concentric cylinder geometry (inside diameter 40.77 mm, outside diameter 43.88 mm, cup diameter 30.32 mm). The viscosity was calculated as the slope of a flow curve (shear stress vs. shear strain rate) with shear strain rate ranging from 0 to 100 s^{-1} at 5°C to mimic sample serving temperature. The flow curves were plotted with Trios Software (TA Instruments, New Castle, DE), omitting stress overshot/noise at low shear strain rate (0 to 15 s^{-1}). Three measurements for each sample were taken. A one-way ANOVA was performed on all the viscosity values and significance was determined at $p < 0.05$ (RStudio, (version 1.1.419, Boston, MA). If significant differences were found, a Tukey's HSD test was performed. All viscosity values can be found in **Tables B-2 and B-4**.

2.4 Sensory Evaluation

For both experiments, panelists were tasked with tasting the sucrose-milk references (A-C or A-D) in the order given followed by the sample X. They were then asked the following question: *Which sample is the most similar to X? There may or may not be an exact match* (see **Appendix B1.1 and B1.2** for ballots). Panelists were provided with deionized water at room temperature (20°C) and were asked to rinse their mouths in between sample sets. Data were collected using Compusense Cloud (Compusense Inc., Guelph, ON, Canada). Participants for both tests were screened for dietary restrictions, food allergies, and product use (i.e., those who consumed skim and 1% milk at least once a week or more and indicated they would be interested in tasting vanilla flavored milk). Procedures were exempted from institutional review board (IRB) review by the Penn State Office of Research Protections under the wholesome foods exemption in 45 CFR 46.101(b). Participants provided informed consent via computer in the testing booths and were compensated for their time.

For Experiment 1, 91 participants from the State College, PA, area were recruited (women = 70, aged 19-68). All 16 samples were tested within the same day/sitting, with a 5-minute break in between the two sets. Sensory evaluation took place under red light and at ambient temperature (25°C). Four samples were placed on each tray: one sample X of either sucrose-milk or vanilla-sucrose milk, and three reference sucrose-milks A-C. The sets were counterbalanced between panelists, and samples within each set were randomized so that each sample was presented at each position the same number of times. Approximately 45-50 mL of milk were poured and lidded into 3.25-oz. plastic cups (Fabri-Kal, Kalamazoo, MI) and labeled with random 3-digit blinding codes.

Experiment 2 was designed to include an additional reference at a sucrose concentration lower than that of sample X. This was to confirm the reliability of our data from experiment 1 and that our panelists were not simply guessing—given our hypothesis that aroma has a taste enhancement effect, panelists should not be selecting the sample with a lower sucrose concentration. Experiment 2 also included an incongruent aroma set to test the congruency hypothesis. Central location tests were conducted using 92 participants (women = 71, aged 18-64). Out of both days, only one panelist did not return on the second day of testing and so n = 91 for the beef milk set. Sensory evaluation took place under white light and at ambient temperature (25°C). Due to the large number of samples, testing took place over two days, with panelists testing ten samples per day. Five samples were placed on each tray: one sample X of either sucrose-milk or aroma-sucrose milk and four reference sucrose-milks A-D. The vanilla milk and beef milk sets were randomized across days, i.e., half of the panelists received the vanilla milk set first and the beef milk set the second day. Approximately 45-50 mL of milk were poured and lidded into 4-oz. plastic cups (Fabri-Kal, Kalamazoo, MI) and labeled with random 3-digit blinding codes.

2.5 Data Analysis

Samples that were indicated as the match were tallied and plotted in RStudio with the ggplot2 package (Wickham and Chang 2016). The proportions of each of these responses on the whole were then determined.

Binomial tests were conducted on two independent proportions for the samples using a two-tailed test (RStudio). For the control tests, whether the panelists were able to match to the correct sample above chance was verified; the same was done for the test sets to determine if an enhancement could be seen based on the references that were matched to the aroma-spiked milks. For experiment 1, an expected probability (or probability of guessing) of 50% was assumed, to test whether panelists were able to experience an enhancement (= matching to either the higher sucrose references B or C) or not (= matching to the exact sucrose reference A). The same approach was used for experiment 2, using an expected probability of 33%: enhancement when the sample X was either matched to the higher sucrose references C or D, no enhancement when X was matched to the exact sucrose match B, and negative enhancement when X was matched to the lower sucrose concentration reference A. Ninety-five percent confidence intervals as found by the modified Wald method were then calculated for each of the proportions, and compared to the guessing probabilities. If the confidence interval did not overlap with the probability under the null hypothesis, it was concluded that the experimental proportion was significantly above chance ($p < 0.05$).

Perceived sweetness levels were then estimated for the reference sucrose samples, using a first-order model determined in a previous dose-response study on vanilla-sucrose milk samples (Sweetness = 18.1 + 10.5 * % sucrose + 5.11 * % vanilla; $R^2 = 0.368$). These values were then compared to those found in the dose-response study (Wang et al., unpublished), to determine whether a non-scaling matching task led to a larger degree of enhancement than when using a rating task.

3. Results and Discussion

3.1 Physical Characterization

Based on the measured % total solids and % fat results, % sugar in the final products was estimated using the % total solids of the plain skim milk used for processing (8.88% for the ABCX test and 9.03% for the ABCDX test). All final sugar contents were approximately the same to those formulated: % error of the final estimated % sugar values deviated up to 2% for the ABCX test and 3% for the ABCDX test (see **Table B-1 and B-3** for all values). For both experiments, no significant differences in viscosities were found for any of the samples ($p > 0.05$), therefore, effects of viscosity on flavor perceptions were expected to be negligible.

3.2 Experiment 1 – ABCX

In both sets of concentrations (**Table 3-3**), a significant proportion of panelists was able to correctly identify the exact match in the control tests (where the test sample X contained only sucrose): 68% of the panelists (= 0.68; CI [0.58,0.77], $p = 0.0007$) for the 1.31% sucrose reference test, and 63% of panelists (= 0.63; CI [0.52,0.72], $p = 0.0206$) for the 2.24% sucrose reference test. This indicates that the task was feasible for the panelists to perform and that the sugar concentrations differed perceivably.

When it came to matching the sucrose-vanilla milk samples X to the exact sucrose concentration match (= reference A), the proportion of matches decreased to 0.33 (33%) and 0.32 (32%) for the 1.31% and 2.24% sucrose concentration sets respectively (**Table 3-2**). Both of these proportions are significantly lower than the expected proportion of 0.5 (50%), as indicated by the binomial test (p = 0.0015, CI [0.24, 0.43] for the 1.31% sucrose test and p = 0.0007, CI [0.23, 0.42] for the 2.24% sucrose test, 2-sided). This shows that panelists performed significantly worse than expected for the vanilla-sucrose mixtures when there was not an exact reference match. Instead, the matches for both concentrations of the vanilla-sucrose mixtures distributed to higher sugar concentration references: a significant proportion of panelists matched the vanilla-sucrose mixture to the sucrose references B and C, both containing higher sugar levels than in the sample X. Combining the responses for B and C, the proportion totaled to 0.67 (67% of panelists) (CI [0.57,0.76], p = 0.0015, 2-sided) and 0.68 (68% of panelists) (95% CI [0.58,0.77], p = 0.0007, 2-sided) for the lower (1.31%) and higher (2.24%) concentration sets respectively. These results indicate that a significantly higher proportion of panelists perceived the vanilla-sugar mixture as sweeter since they matched the sample to the higher sucrose concentrations. From these results it seems that the addition of an aroma leads to a taste-enhancement effect in a matching experiment, though it is unclear whether this effect could have also been due to confusion as to how to match a sample with added aroma, given that no exact match was provided.

3.3 Experiment 2 – ABCDX

To validate results from Experiment 1, Experiment 2 was conducted with an additional lower sucrose concentration as another reference, expanding the test to an ABCDX.

Just as in Experiment 1, a significant proportion of panelists, i.e., more than the expected 33%, was able to match the sucrose-only sample X to the exact sucrose reference sample B (**Table 3-3**). Half of the panelists (proportion = 0.5) correctly matched the 2.24% sucrose milk reference in the vanilla control test (CI [0.4,0.6], p = 0.0012), and nearly a half of the panelists (proportion = 0.46) correctly matched it in the beef control test (CI [0.36,0.56], p = 0.014). This indicates the task was still feasible for the panelists to perform and that sugar concentration differences were perceivable. However, the ABCDX test may have been more difficult to perform than the ABCX test, since there was an additional sample to choose from (i.e., the probability of choosing the correct match based on chance alone decreased from 50% (experiment 1) to 33% (experiment 2)).

Similar to experiment 1, once the vanilla aroma was added, again a significantly higher proportion of panelists than the expected 33% (0.652 or 65.2% of panelists, $p < 0.05$, CI [0.55,0.74]) matched the vanilla-sucrose sample X to sucrose references C or D that contained higher sucrose concentrations than the sample X. In contrast, only 17.4% of panelists each matched the vanilla-sucrose milk to the sucrose reference B (= same sucrose concentration as in the sample), or the lowest sucrose reference A (= sucrose concentration below that of the sample), a result that is below the guessing probability of 33.3% (p = 0.0008, CI [0.1,0.27]).

When looking at the samples that contained the incongruent beef aroma, similar trends to the vanilla milk test were found but to a lesser degree: for the beef-sucrose milk samples, a significant proportion of panelists matched the sample to the higher sucrose concentrations C or D (0.6 or 60% of panelists, CI [0.50,0.71], $p < 0.05$). This proportion is slightly lower than what was found in the vanilla milk experiment, though still significantly above the expected probability of 33%. Similarly, the panelist proportions that matched the sample to either the lowest concentration of sugar (reference A) or the baseline sucrose concentration (reference B) was below the expected proportion of 0.333 (0.165 or 16.5% of panelists for A, p = 0.0005, CI [0.10,0.26], and 0.231 (23.1%) for B, p = 0.044, CI [0.16,0.33]).

Overall, for both the congruent and incongruent aroma condition, a significant proportion of panelists selected sucrose concentrations that were above the actual sucrose concentration in sample X as the closest match. Based on these results, although congruency may have a role in taste enhancement, it is not the only determinant for whether these interactions can occur, as beef aroma also showed enhancing properties.

When inspecting the proportions more closely (**Figure 3-2**), differences in response behavior between the congruent and incongruent aroma become apparent: for the vanilla-sucrose milk, a higher proportion of panelists (34 of 92 or 0.37) chose the **highest** sucrose concentration (reference D) as the closest match, while for the beef-sucrose milk, a higher proportion of panelists (33 out of 91 or 0.36) chose the **second highest** sucrose concentration (reference C). This result indicates that though either additional aroma might be able to enhance taste perception, the added layer of congruency between the tastant and the aroma may lead to a greater enhancement than an incongruent aroma.

To further compare our results, and demonstrate the impact of using a matching study compared to a scaling method, the matching results were compared to our previous dose-response work in the same matrix (Wang et al., unpublished): using the equation model to describe perceived sweetness as a function of sucrose and vanilla concentration, in the scaling condition (which is expected to encourage an analytical assessment mindset), the vanilla-sucrose milk with 2.24% sucrose and 0.435% vanilla was rated as 43.85 on a 100-point scale in sweetness, a value that is just slightly above that of the 2.24% sucrose-only milk (41.62). Further, the sweetness of the vanilla-milk mixture is below the sweetness level achieved by the two higher sucrose concentrations of 2.81% and 3.38% (references C and D) of 47.61 and 53.59. It should be noted that the vanilla mixture sweetness rating was not significantly below the sweetness of 2.81% sucrose but was significantly different from the 3.38% sucrose milk (HSD = 7.13).

These comparatively small enhancement effects in the dose-response study are in contrast to the results obtained from the matching study, a scaling-free task likely to elicit a synthetic strategy: here, a significant proportion of panelists selected one of the higher sucrose concentration references as the closest match to the vanilla-sucrose mixture: In the ABCX test, a significant proportion of panelists matched the vanilla-sugar milk sample with 1.31% (w/w) sucrose to higher sucrose concentrations of 1.80% and 2.29% (w/w). These two sucrose concentrations would lead to a perceived sweetness rating of 37.1 and 42.26, compared to the sweetness ratings of 35.1 for the mixture in the dose-response rating study. The 1.80% sucrose milk is not significantly different in sweetness from the mixture, though the sweetness of 2.29% sucrose milk is just above the HSD for sweetness of 7.13 (7.16). Similarly, for the higher vanilla-sucrose mixture containing 2.24% (w/w) sucrose: a significant proportion of panelists matched the mixture to the two higher sucrose references of 2.81 and 3.38% (w/w). These two sucrose concentrations would result in a perceived sweetness of 47.8 and 53.7, compared to the mixture's sweetness rating of 44.0 in the dose-response study. Lastly, for the ABCDX test, the same pattern is seen: the 2.24% sucrose and 0.435% vanilla milk led to a sweetness perception of 44.0 on a 100-point scale in the dose-response study, just slightly higher than the same sample without vanilla (41.7). The same sample when tested in the matching experiment, suggest a higher sweetness enhancement, as the majority of panelists matched the vanilla-milk sample to a sucrose concentration that was at least 25.4% (or 0.57% w/w) above the actual sucrose concentration in the sample.

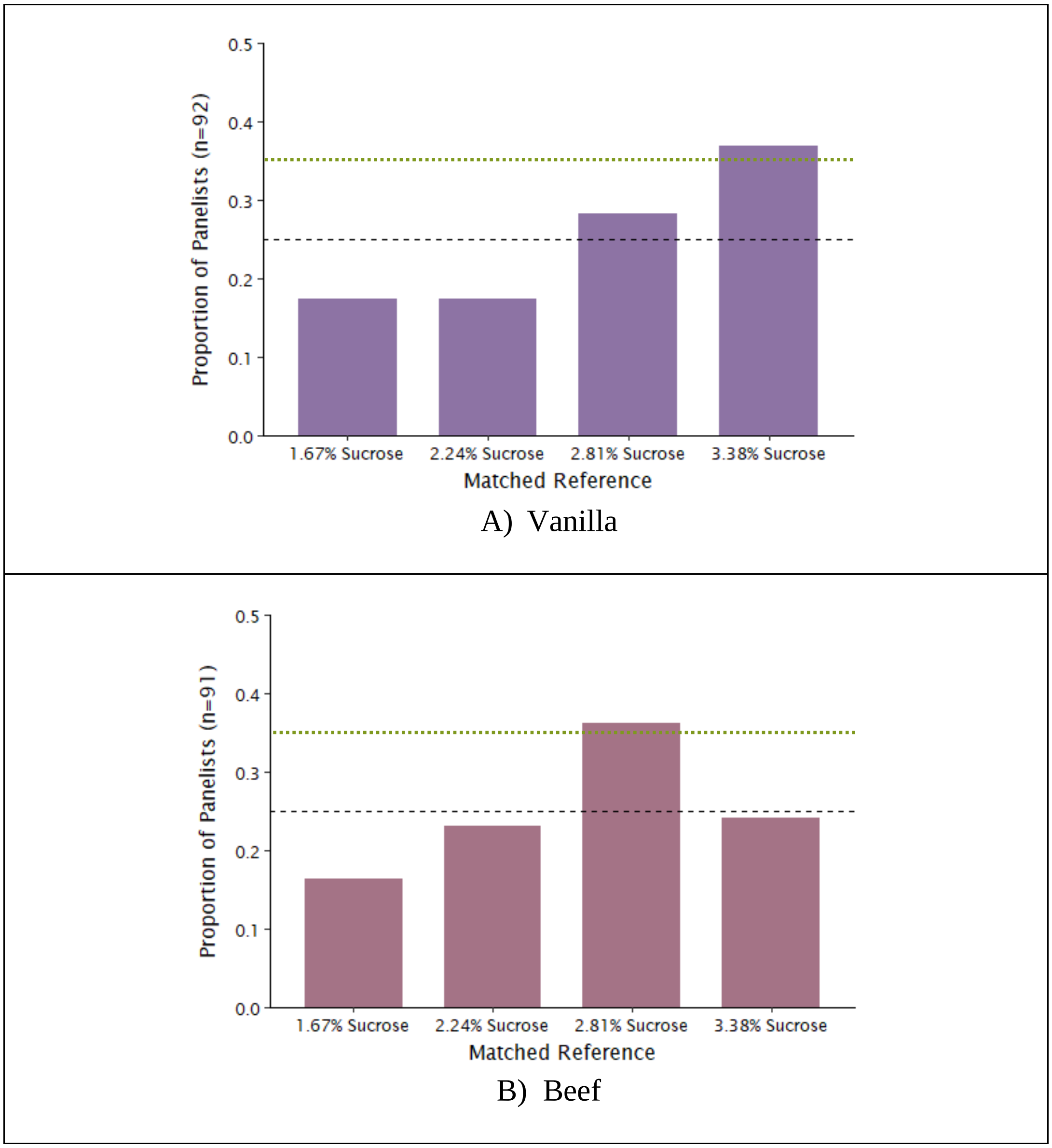


Figure 3-2 *Proportions of all responses where Fig 3-2 A shows the number of responses for the vanilla matching test and Fig 3-2 B gives responses for the beef matching test. The unknown vanilla milk and beef milk samples were both at 2.24% sucrose (w/w). --- =* p_{chance} *(random guessing) at 0.25* **…** *= binomial significance test (proportion = 0.35 for both tests; p<0.05).*

Table 3-2 *Proportions and confidence intervals of all responses for the ABCX and ABCDX matching tests. Expected proportion for Experiment 1 = 0.5; expected proportion for Experiment 2 = 0.333. Effects are as follows: negative = sucrose below sample X, reference A only in ABCDX test; no effect = sucrose same as in sample, reference A in ABCX Test and reference B in ABCDX test; enhancement = sucrose above sample X, reference B and C combined in ABCX test and reference C and D combined in ABCDX test. n = 91 for all tests except for the Vanilla Set (n=92).*

Effect	**Experiment 1 (95% CI)**		**Experiment 2 (95% CI)**	
	Lower Concentration Set (Sample A = 1.31% sucrose)	**Higher Concentration Set (Sample A = 2.24% sucrose)**	**Vanilla Set (Sample B = 2.24% sucrose)**	**Beef Set (Sample B = 2.24% Sucrose)**
Negative	-	-	0.174 (0.10,0.27)	0.165 (0.10,0.26)
No effect	0.330 (0.24,0.43)	0.319 (0.23,0.42)	0.174 (0.10,0.27)	0.231 (0.15,0.33)
Enhancement	0.670 (0.57,0.76)	0.681 (0.58,0.77)	0.652 (0.55,0.74)	0.604 (0.50,0.71)

Table 3-3 *Proportions and confidence intervals of all correct sucrose reference matches in the control sets for Experiment 1 (ABCX) and Experiment 2 (ABCDX) matching tests. n = 91 for all sets except the vanilla (n = 92).*

Set	**Experiment 1**		**Set**	**Experiment 2**	
	Sample X	**Proportion Correct (95% CI)**		**Sample X**	**Proportion Correct (95% CI)**
Low Concentration	1.31% Sucrose	0.681 (0.58,0.77)	Vanilla	2.24% Sucrose	0.500 (0.4,0.6)
High Concentration	2.24% Sucrose	0.626 (0.52,0.72)	Beef	2.24% Sucrose	0.462 (0.36,0.56)

3.4 - General Discussion

Overall, our modified ABX method—the ABCX and ABCDX matching test—was shown to be successful for testing taste-aroma interactions while encouraging a synthetic evaluation strategy. Compared to a direct scaling exercise that evokes an analytical evaluation mindset, the ABC(D)X matching test reveals larger interaction effects when assessing both congruent and incongruent aroma-tastant mixtures in a real food application. However, one downside of the matching test is that it is unclear as to what the panelists were basing their matches on, as no directions were provided. We like to think that panelists were using perceived sweetness as their criteria, but it could also be that they were matching on the basis of total flavor in the sample. It is possible that by tasting the sample containing both the aroma and the tastant, a stronger overall flavor intensity was induced, encouraging panelists to choose the sucrose reference sample that induced a similar intensity of perception overall. This would be in agreement with an early study by (Valdes, Hinreiner, and Simon 1956), where for sweetened aqueous solutions it was shown that panelists tend to associate sweetness with flavor by attributing higher sweetness to the more flavorful sample. At the same time, if a similar percept is induced and the end goal of using less sugar is met, it may not matter what basis panelists are using to match their samples. In other words, if a similar percept is induced using less sugar, then an acceptance test could be performed in future studies with these concentrations to determine whether reduced sweetness also results in reduced liking of the product or not. It should be noted that when looking at the results from the dose-response study outlined in Chapter 2, this was not the case.

Furthermore, we showed that in both experiments, a significant proportion of panelists correctly matched the sucrose-only sample to the corresponding sucrose reference sample, providing evidence that consumers could taste differences in the samples, thus providing reliable data. Additionally, adding an aroma to a sucrose-sweetened milk affects the perception of what we believe is sweetness, as a significantly greater proportion of panelists matched the aroma-spiked samples to sucrose concentrations at least 37.4% (or (0.49% (w/w)) and 25% (or 0.57% (w/w)) higher than the sample's actual sucrose concentration.

Despite many reports that have suggested congruency is necessary for taste-aroma enhancement (de Araujo et al. 2003; Green et al. 2012; Lim and Johnson 2012; Symoneaux et al 2015), our results also show an effect with an incongruent aroma. Thus, whether the aroma needs to be congruent, i.e., "appropriate" for the food it is used in is unclear, as increased matching to higher sucrose references were seen for both the congruent vanilla and the incongruent beef aroma. Concurrently, the congruent aroma led to higher enhancement than the incongruent one (i.e., more people matched the sample X to the highest sucrose concentration reference). It seems that though congruency between the aroma and the taste may not be an absolute factor for enhancement, congruent odors are potentially able to induce a greater effect than incongruent odors.

The harmony between taste and aroma was first studied by Murphy and Cain (1980). Note that these authors used the term "harmony" in the same sense as the words, "familiar", "congruous", and "common". Similar to our beef-milk results, the researchers did not see an effect on interaction under a congruent condition when comparing sucrose with citral, a compound with a lemon-like aroma (Pubchem 2018), to a combination of citral with sodium chloride, both in aqueous solutions. Though the patterns of enhancement for the harmonious and inharmonious mixtures were approximately similar in that both showed additive effects, it should be noted that the degree of congruency between the tastant and odorant were based on the authors' assumptions and were not tested or validated by the participants. On the other hand, Schifferstein and Verlegh (1996) determined taste-aroma enhancements for congruent mixtures (strawberry and sucrose, lemon and sucrose), but not for incongruent combinations (sucrose and ham). However, they also reported that the degree of congruency was not linearly correlated with the degree of taste enhancement induced by an odor, i.e., the congruency ratings did not contribute to the predictive power of the regression equation for sweetness enhancement. This is in contrast to what was found for the current study, where the degree of congruency may be related to the degree of enhancement as seen in the ABCDX test. Instead, the authors concluded that congruency and subsequent enhancement could be more of a reflection of how much a panelist likes the combination of the two components, rather than the extent of association. This explanation is possible but does not quite explain how the beef milk was still able to suggest a sweetness enhancement, given its low appropriateness score for sweet milk found in our preliminary studies. We do however acknowledge that level of appropriateness and hedonism may not reflect the same concepts as similarity and congruency, two terms with an ambiguous distinction that have been used interchangeably in the literature (Linscott and Lim 2016). Although they may seem to be invoking the same ideas, they actually suggest two different concepts: congruency being the "degree to which taste and odor components are reminiscent of a flavor object", and similarity being the "degree to which one component shares the quality of another" (Linscott and Lim 2016). For example, although citrus odor and sweetness may be congruent (in the case of lemonade), they are not perceptually similar (Linscott and Lim 2016). This should be further tested with other incongruent aromas; future work might benefit from requiring panelists to indicate the quality or factor they based their matches on.

It is also possible that sweetness or flavor enhancement may depend on the amount of sugar in a food and thus its degree of sweetness (Valdes, Hinreiner, and Simon 1956; Cliff and Noble 1990; Symoneaux et al. 2015; Brossard et al. 2006). A previous dose-response study on the same vanilla-sucrose milk formulations also found a concentration dependence, with greater synergistic interactions seen at low to medium sucrose concentrations (Wang et al., unpublished). Further, another predictor of sweetness enhancement, suggested by Stevenson et al. (1999), could be the degree of "smelled taste" of odorants (which was not collected in the current study). Here, subjects rated the smelled sweetness of odorants which was found to be a strong predictor for the extent an odor could modify taste intensity (R^2 = 0.67). A similar hypothesis was tested by Nguyen, Dacremont, and Valentin (2000), who evaluated the cognitive association between tastes and odors by asking subjects to rate harmony, congruency, similarity, and smelled taste of several mixtures of sucrose, vanilla, citric acid, and lemon. Although ratings were all positively correlated, only "smelled taste" (40%) and similarity (29%) were able to predict perceived odor-induced taste enhancement, but not congruency nor harmony. As these two scales—smelled taste and similarity—were highly correlated, it may have been the case that they were just measuring the same aspect of the odor-taste relationship, as a result of potential odor-taste confusion (Stevenson 2001; Stevenson, Prescott, and Boakes 1999). With these findings in mind, it appears that the relationship between tastants and odorants is more difficult to define than thought; though smelled taste could be a reason for enhancement in the vanilla milk, it does not quite explain the results for beef milk. More factors other than congruency are necessary for enhancements to occur and need to be further explored. For instance, another explanation for the similar results of vanilla and beef aroma, is the fact that umami and sweet tastants both utilize the T1R3 receptor. It could be that similar sensations may have been generated, which could lead to higher congruency for the beef-sucrose combination than initially thought. This hypothesis would need to be tested in a future study.

The finding that congruency is not the only determining factor for sweetness enhancement was also demonstrated in sour and bitter taste studies. When looking at coffee and citral solutions, citric acid and caffeine were not able to enhance citral and coffee odor respectively, and there were no effects of retronasal odor on perceived taste intensity, even though these pairings were rated as highly congruent (Lim, Fujimaru, and Linscott 2014). Though it may seem that sour and bitter taste perception cannot be enhanced by congruent aromas per se, interestingly an odor-induced taste enhancement was found for the combination of citric acid and vanilla, a rather incongruent, inharmonious, and dissimilar mixture (Nguyen, Dacremont, and Valentin 2000). Neither vanilla flavor nor aroma has any reported association with sourness, and so it is possible that vanilla could have enhancing effects on multiple taste properties, which cannot simply be explained by cognitive association or perceptual similarity alone (Sauvageot, Nguyen, and Valentin 2000; Valentin, Chrea, and Nguyen 2006). Adding to this, more recently, it was found the aroma compound vanillin was able to suppress the bitterness of sucrose octaacetate (SOA) in solution (Isogai and Wise 2016). Given all this, it seems that the relationship between tastants and odorants are more prone to individual panelist variation than smelled similarity or congruency (Valentin, Chrea, and Nguyen 2006), a relationship we observed in this study as well when tracking each panelist's performance (data not shown). During their scaling and matching study, van der Klaauw and Frank (1994) recognized that individual differences between panelists were consistent across both tasks, indicating that some panelists are better than others at separating their perception into different components, i.e., some panelists were found to adopt an analytical strategy easier than others.

One other possibility to explain the enhancement seen with the beef milk has been suggested by Lim's group: in order for there to be an interaction, the taste has to be nutritive (i.e., those that signal the presence of macronutrients), e.g., sweet, salty, or savory (Lim, Fujimaru, and Linscott 2014). Though their research has overall found that taste enhancement by odor is a weak effect, odor enhancement by taste is stronger, similar to findings in the vanilla milk dose-response study (Wang et al., unpublished). To explain this, Lim's group proposed that nutritive tastes increase the salience of a flavor, causing an odor enhancement that may help us recognize potential sources of nutrients. While their taste-aroma interaction studies on citric acid and citrus odors and caffeine and coffee odors did not find any enhancements (Lim, Fujimaru, and Linscott 2014), one of their more recent studies found savory odor enhancements by congruent tastes, i.e., chicken and soy sauce odors were enhanced by salty and umami tastes respectively (Linscott and Lim 2016). Whereas sourness is typically an indicator of spoilage and unripe fruits, and bitterness is indicative of toxins, saltiness, umami, and sweetness all signal the presence of nutrients (sodium, L-glutamate, and sugars), which all benefit the body as sources of energy and electrolytes (Linscott and Lim 2016). These authors speculate that odor enhancement could be a mechanism by which the association between the odor of a food and its metabolic consequence is increased. As beef aroma would be considered a nutritive smell, this hypothesis could explain the enhancement effect seen for the beef aroma-sucrose milk samples.

4. Conclusions

A modified ABX test, the ABC(D)X matching test, was found to be a robust method for elucidating a possible sweetness enhancement by an aroma. The test allowed panelists to adopt a synthetic strategy that is more reflective of eating, and so potential dumping effects could be ruled out. With these findings in mind, it is promising that even without directing the panelists' attention to any specific attribute, and when there was no exact match to the reference, they were still able to match samples based on perceived sweetness (or possibly overall flavor intensity).

As such, the ABC(D)X test was able to discover interaction effects, i.e., taste-aroma enhancement in a non-scaling testing environment, whereas studies involving scaling of attributes have found such interactions to be present but small, most likely due to the analytical strategy that is induced. However, whereas dose-response or other scaling studies of taste-aroma interactions are able to estimate an effect size of the enhancement, the ABC(D)X test does not allow a comparison of proportions between tests. Using the ABC(D)X matching test in our study, we are able to conclude that taste-aroma interaction may be occurring, but do not know directly how strong this effect actually is. Future work should determine methods to measure this effect size.

Based on our results, taste-aroma interaction appears to be a robust effect as panelists matched the aroma-tastant mixtures to references containing higher sucrose concentrations in two separate experiments, under slightly different conditions (ABCX vs. ABCDX, red light vs. white light). The same pattern of enhancement was seen for the beef-sucrose milk sample, suggesting that factors other than congruency may be underlying an enhancement, such as the nutritive response and individual panelist variability. Future studies could verify these findings by testing other congruent and incongruent aromas. Overall, the relationship between taste and aroma is complex and perhaps effects should be considered on a case by case basis. It is our hope that this study provides further insight into taste enhancement by an aroma, information that food manufacturers could utilize to optimize liking while minimizing added sugar when reformulating their products.

Chapter 4: Conclusions and Future Work

4.1 Significance and Implications

The results of the current work imply that sweet taste-aroma interactions do exist perceptually, even when controlling for the dumping effect during a scaling study, and more evidently through a matching study. Overall the dumping effect cannot account for taste-aroma interactions, as this work plus several others have found evidence for interactions even when providing an extended ballot during scaling and/or when using methods free of response biases (Green et al. 2012; Fujimaru and Lim 2013; Lim, Fujimaru, and Linscott 2014; Dalton et al. 2000; van der Klaauw and Frank 1994; Lawless and Schlegel 1984). According to the dose-response experiment, the interaction may be stronger for taste-induced odor enhancement than it is for odor-induced taste enhancement, although both effects are significant. Perhaps this is the case as some flavors are more commonly perceived in combination with certain other components. In our case, anecdotally, when vanilla extract is consumed on its own, it can be unpleasant, but when combined with sugar, this is what we more often associate with vanilla flavor. At the same time, adding more sugar does not equate to more vanilla flavor either, particularly at higher concentrations. Furthermore, similar to observations by other researchers, taste enhancement does seem to be concentration-dependent—instead of interactions occurring across all sucrose concentrations, low to medium sucrose concentrations showed evidence for more interaction according to the isobole calculations. On the other hand, when considering the matching study, there was stronger evidence of a larger taste-enhancement by an aroma given that a significant proportion of panelists matched the aroma-containing samples to sucrose references higher than the baseline sucrose concentration.

In addition, congruency seems to have an effect on the degree of enhancement, though is by no means the only criterion for it to occur. It may also be the case that the degree of congruency is proportional to the amount of enhancement that can be induced. In general, it appears experimenting with sugar and aroma may be a feasible strategy for dairy food manufacturers to consider when it comes to reducing sugar in products, though the magnitude of the reduction that can be accomplished may not be as large as the effect non-nutritive sweeteners would have. The combination of both approaches would be worth looking into in future studies.

4.2 Study Limitations

4.2.1 Overall Limitations

One limitation for both experiments was the control over the processing variables to reach the target sucrose and vanilla extract values. Although sample ingredients were all weighed out accurately, after HTST processing, samples were lower in concentration than what was formulated for. Timing for collection and waiting between samples was set to the same standard even though there may have been slight viscosity changes between mixes which could have affected the rate of processing. Nonetheless, all samples were close to the target concentrations (% error within 2-3% based on calculation estimates from % total solids and % fat values—see **Tables A-2, B-2, B-4**). Additionally, significant attribute differences between the samples, covering the entire range of concentrations we aimed for, were still seen by sensory evaluation as well as through instrumental measurements of viscosity and total solids.

We also acknowledge that in addition to the sucrose and vanilla extract, dairy-related volatiles, constituents in milk, as well as the inherent lactose in the milk have contributed to the flavor perception. To manage that effect, a plain milk sample was included as a control in the dose-response experiment to allow for attribute comparisons (in addition to it acting as a control for panelists). Lactose can also impart a mild sweet taste to milk (Parrish, Talley, and Phillips 1981; Etievant et al. 2016), which was confirmed in our dose-response experiment where plain milk was on average still rated sweet to some degree by our consumer panelists. The milk samples with only added vanilla were rated slightly higher in sweetness and vanilla flavor scores on average; though still not significantly different from the plain milk, this might be indicative of an additive effect from the vanilla in combination with the inherent lactose rather than a sole effect from adding vanilla. It is also possible for vanillin, the character-impact compound in vanilla extract, to react with the proteins in milk and cause an altered flavor release, though the concentrations of casein and whey proteins naturally in milk may not be large enough to cause significant interactions (Hansen and Heinis 1991; Hansen and Booker 1996; Chobpattana et al. 2002). Lastly, the volatile composition of the milk may have interacted with the volatiles of the vanilla extract. Fluid milk contains many aroma and flavor compounds such as aldehydes, ketones, sulphur compounds, and short chain fatty acids. This profile can be manipulated by changing the cow's diet (Forss 1979) as well as from oxidation reactions, heat treatment, and non-enzymatic browning reactions (Scanlan et al. 1968; Chugh et al. 2014). In this case, since pasteurized skim milk that had already been pasteurized was used, more Maillard reaction products as a result of the interaction of milk proteins and lactose may have occurred. More cooked aromas and flavors especially could have been imparted from the double pasteurization, especially since we pasteurized at a temperature well over the minimum requirement for milk with added sweeteners set by the CFR (75°C for 15 s; CFR 2017) to ensure the product was safe to consume. An analysis of the volatile fraction of the samples may be worth conducting to determine which flavor compounds are present to provide a complete flavor profile analysis (see **Section 4.3.3** below).

4.2.2 Dose-Response Study Limitations

We chose to use consumers, who are typically much more variable, instead of a trained panel. Although training the panelists could provide more consistent results, using a trained panel would remove any applicability of the results to the average, untrained consumer. Studies have revealed that trained judges are less sensitive to instructional manipulations, likely due to their concepts of certain attributes being more clearly defined (van der Klaauw and Frank 1996). In the case of taste-aroma interaction studies, taste enhancements often disappear once a panel is trained (Bingham et al. 1990; Stevenson 2001). More recently, during a study on model wines, it was found that training and expertise reduced enhancement effects by directing the panel to the salient attributes in the samples (Arvisenet, Guichard, and Ballester 2016). At the same time, it should be acknowledged that no differences in judgements have been observed between experienced and untrained panelists. In a study using sucrose/citric acid mixtures, trained and novice assessors evaluated taste mixtures in the same way, with only a slight indication that experienced panelists are less prone to taste suppression (McBride and Finlay 1989). Overall, we understand the variability that comes with untrained panelists and accounted for it by recruiting a large number of participants for all tests.

4.2.3 Matching Test Limitations

One of the biggest limitations for the matching studies was the comparability between the two matching experiments. Lighting for the first test was red whereas the second experiment used white light; given these different conditions, it may seem that the results cannot be compared due to possible effects from color, which may have been more apparent during the second matching study. For instance, studies in wine have shown that when coloring a white wine with red dye, olfactory information is discounted when provided visual information (Morrot, Brochet, and Dubourdieu 2001). The lighting of the room where the wine is sampled is also known to affect the flavor (Oberfeld et al. 2009). One of the first to research the effects of color were (J. Johnson and Clydesdale 1982) who showed that increasing the color of a drink had a significant effect on sweetness perception in that they were rated higher than the lighter samples, despite the sucrose concentration actually being lower. However, since we did see similar effects in both matching experiments, independent of the lighting conditions, lighting may not have been as big a variable for the flavored milk samples as previous research might indicate. It should be noted that the literature on color is still up for discussion as a number of studies have also reported no effects of color on human flavor perception (Alley and Alley 1998; Pangborn, Berg, and Hansen 1963; Frank, Ducheny, and Mize 1989). Further, panelists' attention was not directed to sweetness, whereas many of the color-flavor studies did, so it is possible that the interaction or the effect of color on the results may not even apply to this study at all.

The preliminary intensity matching study for the ABCDX matching experiment also could have included a liking evaluation in addition to the appropriateness scale. It was unclear whether these could be correlated and/or if panelists were choosing the least appropriate flavor based on their hedonic evaluation of the sugar and aromas in combination. Previously, congruency and subsequent enhancement may be more of an indication of how much someone likes a particular combination of two components, instead of the extent of association (Schifferstein and Verlegh 1996). It is possible that though the panelists indicated low appropriateness for the beef flavor, it cannot be postulated that this combination was also disliked without directly asking for liking. The liking theory also does not quite explain why enhancement was still seen, and so more work is needed with other incongruent flavors for sweetened milk to elucidate whether similar patterns occur.

Furthermore, when tracking panelists individually, it was observed that panelists were not consistent between sets of samples. Generally, although a panelist could correctly match a sample in one of the sets of concentrations, it was not necessarily the case that they could match the sample correctly in the other set. Again, similar to the dose-response study, the variability of untrained consumers is acknowledged; however, using a trained panel would not be appropriate for these experiments. For future studies, panelists could be specifically asked on what basis they matched their sample, whether it was sweetness, or some other attribute, to confirm our findings.

Lastly, the ABC(D)X test, an entirely new test, was analyzed with a binomial test approach. For our experiments, multinomial confidence intervals might provide more appropriate analysis, especially for the ABCDX test where each experiment had more than two possible outcomes (with there being essentially three samples to choose from). However, the analysis of multinomial data is more complicated and different methods have been devised, each with its strengths and limitations. Future studies should consider different multinomial data analysis approaches. Although using a binomial analysis was a more conservative choice, it was still able to provide some preliminary insight into the strength of taste-aroma enhancement with a non-scaling, synergistic testing concept.

4.3 Future Directions

4.3.1 Testing with Other Products

Given that effects could be seen in skim milk, it would be worth conducting similar experiments with other food matrices. The simplest step from here would be to test if the same effects can be seen in milk at different levels of fat. A pilot study conducted prior to those listed in this work used 2% milk and showed similar results, though, was not included in this body of work due to a missing sample. Qualitatively, these samples using the same sucrose and vanilla concentrations had a different flavor profile, most likely due to the effect of the fat retaining more of the vanilla volatiles in the milk matrix as well as from different processing variables. It has been shown that fattiness and sweetness estimates are independent in that fat may have more of an effect on flavor release rather than sweetness perception (Drewnowski and Greenwood 1983). When fat is present in the matrix, vanilla volatiles are released slower in comparison to the immediate impact of vanilla's smoky, alcoholic, beany, and woody notes one might experience in a fat-free product (Hatchwell 1996).

Further studies could look into other more complex dairy products such as yogurt. Yogurt is a complex gel produced by the fermentation of milk with a symbiotic culture of bacteria that is made up of a more concentrated mixture of polysaccharides, proteins, and lipids (Cheng 2010). It would be interesting to see if the same patterns hold with this medium as it provides a more diverse volatile composition and flavor due to the lactic acid and volatiles naturally present in milk and from fermentation (Tamime and Deeth 1980; Ott, Fay, and Chaintreau 1997). One could hypothesize that the interaction of the volatiles and vanilla would cause different patterns, especially with the inherent sourness of the yogurt now being a factor. In addition, the semi-solid texture and Non-Newtonian behavior of yoghurt would add another variable to the matrix that is expected to impact flavor perception. On a similar notion, different aromas, both congruent and incongruent, could also be tested in both the dose-response and matching experiments, first in skim milk and afterwards in different foods. The effect of color (e.g., yellow) could also be further explored in a taste-aroma-color interaction study of vanilla-flavored milk.

4.3.2 Complexity

While investigating aroma-taste interactions, it has also been found that not only does the addition of a congruent aroma possibly enhance sweetness, but the complexity of the aroma could also play a role in our perception. For example, yogurts flavored with a mixture of aroma compounds led to a perceived smoother texture than those flavored with a single aroma compound (Saint-Eve, Paci Kora, and Martin 2004). On the other hand, this same study also found yogurts flavored with single aroma compounds were perceived as thicker than those flavored with multiple components.

Another study investigated multicomponent versus single-component strawberry aromas in yogurts and found that the multicomponent aroma was able to enhance perceived satiation (Ruijschop et al. 2010). Whether this increasing chemical aroma complexity translates to more perceived sweetness would be interesting to investigate as well, given the hypothesis from Lim's group that nutritive tastes may be necessary for enhancements to occur (Lim, Fujimaru, and Linscott 2014). We believe vanilla flavor is ideal for studying the impact of complexity as vanilla extract itself can contain over 200 compounds. Its character impact compound, vanillin, contributes to most of what we perceive as vanilla, and is used widely commercially as a cheaper alternative to vanilla extract. Vanilla flavor blends incorporating different extractives from vanilla are also available on the market for different applications (Sinha, Sharma, and Sharma 2008). This would provide a wide range of component combinations to explore the flavor complexity's role in taste-aroma interactions for future studies.

4.3.3 Instrumental Measures

Finally, instrumental testing would be worth investigating to elucidate flavor perception in humans, to better understand how aroma compounds are perceived in the absence and presence of sucrose.

Throughout all this work on taste-aroma interactions, few studies have attempted to link the perceptual experience to an instrumental measure of the aromatic volatiles. Although *in vitro* sampling and analysis of the samples is an option, it may not be as perceptually meaningful as human perception is not always proportional to the amount of volatiles present in a sample. Most of what is perceived as flavor is a result of aroma molecules volatizing from the food once in the mouth and traveling into the nasal cavity and stimulating receptors in the olfactory cleft (Rozin 1982; Murphy, Cain, and Bartoshuk 1977). As mentioned earlier, this pathway, known as retronasal olfaction, is a different experience from orthonasal olfaction, the other pathway aroma stimuli can take to reach the olfactory epithelium (Ruijschop et al. 2009; Shepherd 2006). Orthonasal perception involves the intake of stimuli from the external environment—mimicking the sampling of products *in vitro,* which is what is more commonly done in flavor profiling studies—the aroma molecules of which travel through the olfactory mucosa while smelling (Ruijschop et al. 2009). Overall it appears that retronasal stimuli are perceived as less intense than orthonasal stimuli, suggesting that the thresholds for orthonasal odorants are lower than that of retronasal (Goldberg et al. 2018). Furthermore, since the concentration of aroma that reaches the olfactory cleft here is usually much lower than that of the retronasal pathway (due to the effects of warming, mastication, and salivation in the latter), when measuring instrumentally, it becomes important to use a method and sampling technique that is an accurate representation of what is actually occurring when we experience flavor *in vivo*.

Currently, there are only a few techniques that have been applied to elucidate the volatiles responsible for the perception: Solid Phase Microextraction (SPME) Gas Chromatography Mass Spectroscopy (GC-MS), which involves static analysis of volatiles in the headspace of a sample, as well as Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS) and Proton Transfer Reaction MS (PTR-MS), which both look at the type and concentration of volatiles reaching the olfactory epithelium during eating (Poinot et al. 2013). However, the former technique has mostly only been used to measure volatile release *in vitro*, while the latter techniques, though more representative of the eating experience, can be quite invasive. Recently, the use of SPME GC-MS for sampling in-nose has been shown to be successful for understanding volatile metabolites in the nasal cavity

(Robert-Hazotte et al. 2018); however, the concentration of volatiles that can be sampled with the SPME fiber can be quite low.

A third technique to help explain retronasal aroma-related interactions would be Stir-Bar Sorptive Extraction (SBSE), a solvent-free extraction technique, similar to SPME but with a higher extraction capacity. A glass-coated, magnetic stir-bar is coated with a thick layer of polydimethylsiloxane (PDMS) as the extraction medium (commercially available under the name of Twister ® by Gerstel) and is said to be 1000 x more sensitive than SPME due to its higher sorbent volume. Using this tool to sample exhaled volatiles directly from the human nasal cavity would provide a way to measure retronasal volatiles in a less invasive manner than PTR-MS and APCI-MS. The model for such a set-up can be seen in **Figure 4-1**.

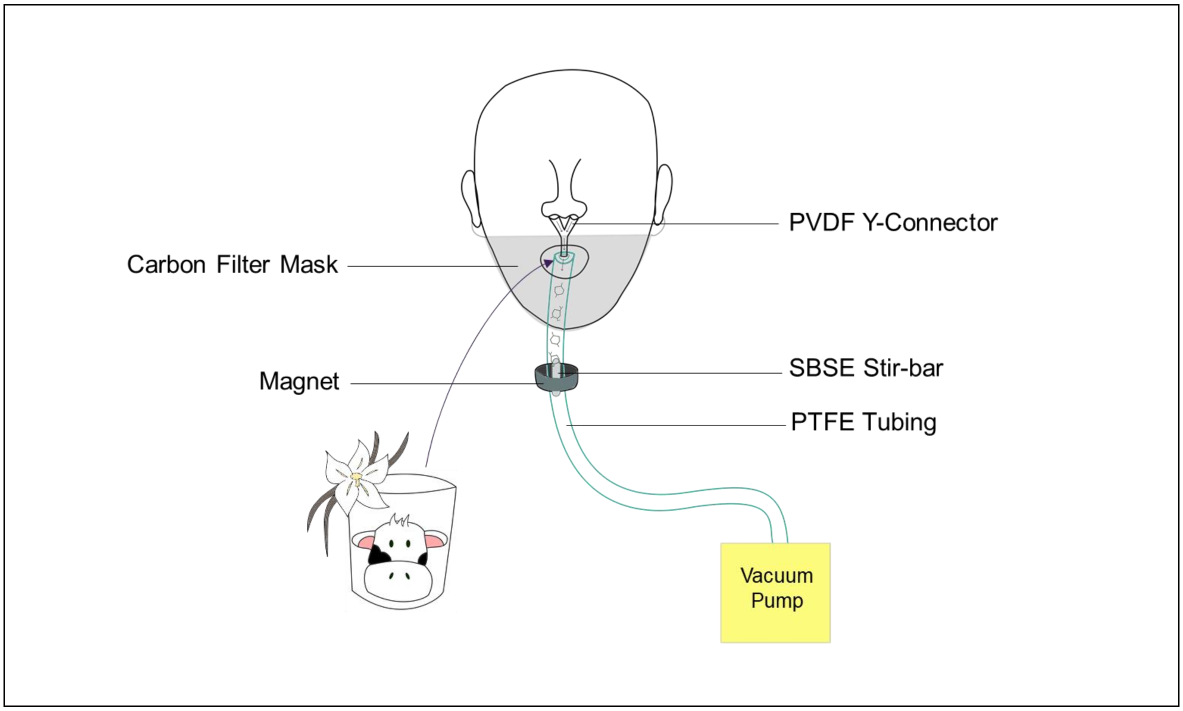


Figure 4- 1 *Overview of proposed instrumental study.*

4.4 Overall Conclusions

In summary, taste-aroma interaction literature can be confusing due to the variety of testing approaches used that make comparisons between studies difficult. Within each study, the degree to which conclusions are valid must also be carefully considered. Factors such as the matrix used, sample concentration range, evaluation method and subsequently perceptual strategy, and degree of panelist experience all contribute different effects to flavor perception and so perhaps any interaction conclusions should be taken on a case by case basis. We expect that by controlling for some of this variability, our work provides some clearer insight into taste-aroma interactions and hope the findings can be used by food manufacturers to reformulate products to ultimately make them healthier for consumers.

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APPENDIX A:

Chapter 2 Supplemental Data

A1 Dose-Response Test

A1.1 Screener for Panelist Recruitment

Note: the same screener was also used for all experiments below (Appendix B).

Please indicate your gender:

- ○ Female
- ○ Male

Do you have any known food allergies?

- ○ No
- ○ Yes ______

Do you have any known dietary restrictions (eg. vegetarian, lactose intolerance)?

- ○ No
- ○ Yes ______

Please indicate your age.

- < 18
- 18-21
- 22-25
- 26-29
- 30-35
- 36-40
- 41-44
- 45-49
- 50-54
- 55-60
- 61-65
- 66+

Please select all of the packaged products below you have purchased and/or consumed in the last 6 months.
(Choose as many that apply)

- Water
- Milk
- Soda
- Juice
- Coffee

How often do you consume milk as a beverage? (Please consider only cow's milk for this study).

- ○ Once every 6 months
- ○ Once every 3 months
- ○ Once a month
- ○ More than once a month
- ○ Every week
- ○ Daily

Which type(s) of milk do you regularly consume?
(You may choose up to three)

- ☐ Skim
- ☐ 1%
- ☐ 2%
- ☐ Whole

Please indicate which flavors of milk you currently consume and/or are willing to try.
(You may choose up to three.)

- ☐ Plain
- ☐ Vanilla
- ☐ Chocolate
- ☐ Strawberry
- ☐ Coffee

A1.2 Sensory Testing Ballot

Welcome to the Vanilla Milk Study!

As part of this study you will be participating in a total of **3 tests** this week. It is really important to us that you come to all 3 sessions. You will receive $5/session for your participation and a $5 bonus on the last day **if you attended the previous two sessions**. **You will be paid on the last day of testing.**

You will be given 6 samples per day for a total of 18 samples. The test will ask you questions about **how much you like the samples** as well as the **intensity for different attributes**. There will be a 30-second break between each sample, so please use this time to cleanse your palate with the water provided.

Once you are done with your set of samples, you may exit the test. When you return for the next session, you will be prompted to resume with the rest of the study when you log in.

If you have any further questions, feel free to pass a sticky note through the hatch for assistance.

Please click "next" to proceed.

Please take a sip of the milk and indicate how much you **like** the sample overall.

Sample: BC111

Liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Now, please take another sip of the sample and indicate the **intensities** of the following attributes.

Sample: BC111

Sweetness

Very Weak   Very Strong

Vanilla Flavor

Very Weak   Very Strong

Milk Flavor

Very Weak   Very Strong

Thickness

Very Thin   Very Thick

A1.3 Composition of Final Samples

Table A- 1- *% Total solids, % fat, % estimated sugar of sucrose-milk samples for the Dose-Response test.*

Product	Can	% Sugar	% Milk	% TS Skim	% Total Solids Product	% Fat Product	% TS From Milk	% Estimated TS	% TS Absolute Error	% TS Error	% Sugar in Solids	% Estimated Sugar	% Sugar Error
1	1a	0	100.0	8.91	8.66	0.14	8.66	8.91	0.250	2.89	0.0	0.00	0.00
1	1b	0	100.0	8.91	8.7	0.12	8.70	8.91	0.210	2.41	0.0	0.00	0.00
2	2	1	99.0	8.91	9.53	0.14	8.82	9.82	0.291	3.05	10.2	0.97	2.96
3	3a	1.31	98.7	8.91	9.82	0.12	8.79	10.10	0.283	2.88	13.0	1.27	2.80
3	3b	1.31	98.7	8.91	9.88	0.13	8.79	10.10	0.223	2.26	13.0	1.28	2.21
4	4	1.71	98.3	8.91	10.25	0.12	8.76	10.47	0.218	2.12	16.3	1.67	2.08
5	5a	2.24	97.8	8.91	10.65	0.14	8.71	10.95	0.300	2.82	20.5	2.18	2.74
5	5b	2.24	97.8	8.91	10.69	0.12	8.71	10.95	0.260	2.44	20.5	2.19	2.38
6	6	2.92	97.1	8.91	11.24	0.11	8.65	11.57	0.330	2.93	25.2	2.84	2.85
7	7a	3.82	96.2	8.91	12.24	0.14	8.57	12.39	0.150	1.22	30.8	3.77	1.21
7	7b	3.82	96.2	8.91	12.16	0.13	8.57	12.39	0.230	1.89	30.8	3.75	1.85
8	8a	5	95.0	8.91	13.38	0.13	8.46	13.46	0.085	0.63	37.1	4.97	0.63
8	8b	5	95.0	8.91	13.29	0.12	8.46	13.46	0.175	1.31	37.1	4.94	1.30

A1.4 Viscosities

***Table A- 2** Viscosities (Pa.s) of final vanilla sucrose milks for the Dose-Response test.*

Sample	% Sugar	% Vanilla	% Milk	Trial 1	Trial 2	Avg	Std Dev
1	1	0	99.0	0.00276	0.00272	0.00274	2.69E-05
2	1.71	0	98.3	0.00289	0.00279	0.00284	6.93E-05
3	2.92	0	97.1	0.00285	0.00285	0.00285	1.41E-06
4	5	0	95.0	0.00306	0.00305	0.00306	9.90E-06
5	0	0.25	99.8	0.00275	0.00266	0.00271	6.29E-05
6	0	0.36	99.6	0.00264	0.00267	0.00265	2.62E-05
7	0	0.52	99.5	0.00269	0.00268	0.00268	6.36E-06
8	0	0.75	99.3	0.00259	0.00262	0.00260	2.76E-05
9	1.31	0.3	98.4	0.00273	0.00268	0.00270	3.61E-05
10	1.31	0.433	98.3	0.00283	0.00283	0.00283	2.83E-06
11	1.31	0.625	98.1	0.00289	0.00275	0.00282	9.40E-05
12	2.24	0.3	97.5	0.00275	0.00302	0.00288	1.89E-04
13	2.24	0.433	97.3	0.00276	0.00289	0.00283	9.76E-05
14	2.24	0.625	97.1	0.00275	0.00279	0.00277	2.40E-05
15	3.82	0.3	95.9	0.00288	0.00299	0.00293	7.64E-05
16	3.82	0.433	95.7	0.00293	0.00300	0.00296	4.95E-05
17	3.82	0.625	95.6	0.00295	0.00294	0.00294	1.41E-06
18	0	0	100.0	0.00275	0.00279	0.00277	2.62E-05

APPENDIX B:

Chapter 3 Supplemental Data

B1 Matching Test

B1.1 ABCX Test

B1.1.1 Sensory Testing Ballots

Welcome to the Flavored Milk Study! Instructions for the test are below:

There are **four sections** in this study. You will be tasting a total of **16 samples** during this test (four samples each section).

In each section, you will be required to taste three milk samples from left to right, and then taste a fourth sample. You will be asked to **match** this last sample to **one** of the first three milk samples. Match this sample based on the one that is the **most similar to you**. There **may or may not be exact matches,** so choose the closest match.

There will be a 2 minute break between each session. Please use this time to rinse your mouth with water.

Please click "next" to continue.

Please taste the three samples in the first row from left to right.

Now taste sample **112**.

Which sample in the first row is **most similar to sample 112**?

There may or may not be an exact match, so choose the closest match.
You may re-taste the samples before making a selection.

○ BC111 ○ BC222 ○ BC333

B1.1.2 Composition of Sucrose Milk Samples

Table B- 1 *% Total solids, % fat, and % estimated sugar of sucrose-milk samples for the ABCX Matching Test.*

Product	% Sugar	% Milk	% TS Skim	% Total Solids Product	% Fat Product	% TS From Milk	% Estimated TS	% Absolute Error	% Error	% Sugar in Solids	% Estimated Sugar	%Sugar Error
1	1.31	98.7	8.88	9.91	0.12	8.76	10.1	0.164	1.65	13.0	1.29	1.625
2	1.8	98.2	8.88	10.45	0.12	8.72	10.5	0.070	0.67	17.1	1.79	0.667
3	2.24	97.8	8.88	10.85	0.11	8.68	10.9	0.071	0.66	20.5	2.23	0.651
4	2.29	97.7	8.88	10.91	0.12	8.68	11.0	0.057	0.52	20.9	2.28	0.517
5	2.81	97.2	8.88	11.37	0.12	8.63	11.4	0.070	0.62	24.6	2.79	0.616
6	3.38	96.6	8.88	11.84	0.11	8.58	12.0	0.120	1.01	28.3	3.35	1.002

B1.1.3 Viscosities

Table B- 2 *Viscosities (Pa.s) of final vanilla-milk samples for the ABCX Matching Test.*

% Sugar	% Vanilla	% Milk	Trial 1	Trial 2	Trial 3	Avg	Std dev
1.31	0	98.7	0.00276	0.00245	0.00271	0.002640	0.000164
1.31	0.625	98.1	0.00269	0.00270	0.00265	0.002678	0.000027
1.8	0	98.2	0.00277	0.00278	0.00287	0.002805	0.000052
2.29	0	97.7	0.00271	0.00282	0.00285	0.002797	0.000075
2.24	0	97.8	0.00280	0.00270	0.00282	0.002771	0.000066
2.24	0.435	97.3	0.00278	0.00276	0.00278	0.002773	0.000009
2.81	0	97.2	0.00276	0.00275	0.00303	0.002846	0.000160
3.38	0	96.6	0.00286	0.00276	0.00274	0.002786	0.000063

B1.2 ABCDX Test

B1.2.1 Intensity Matching

B1.2.1.1 Sensory Ballot

Please smell sample 450 and rate the **intensity** of the **odour.**

Low High

Now please take a sip of 450 and rate the **intensity** of the **overall flavour.**

Low High

Please rate the **appropriateness** of the flavour in sample 450 for sweetened milk.

Not Appropriate Extremely Appropriate

B1.2.1.2 Data

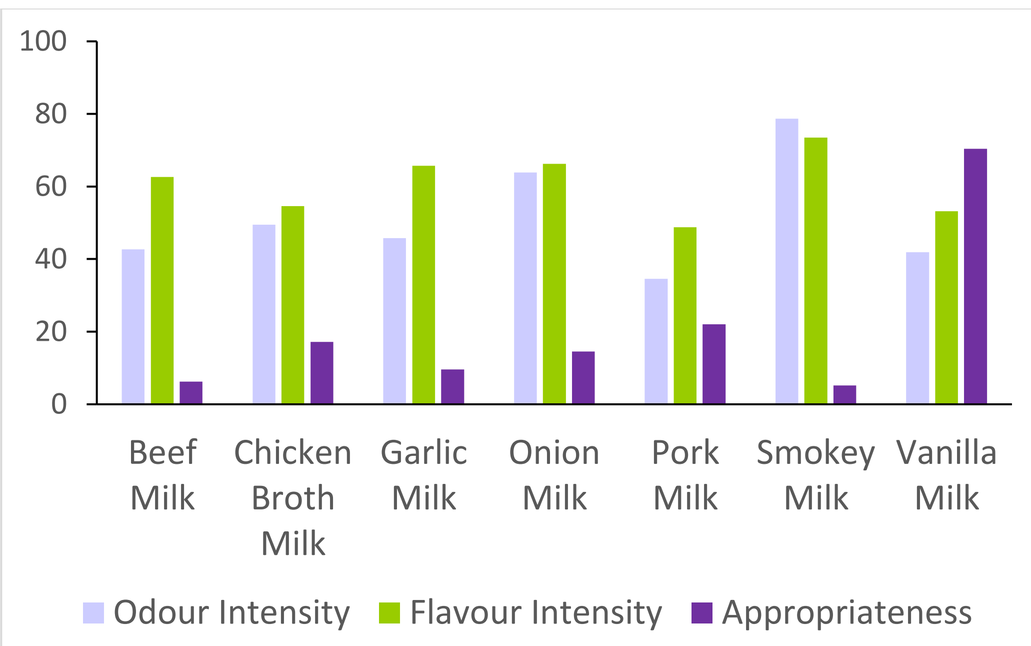

Figure B- 1 *Testing different incongruent flavors for intensity matching. Beef filet mignon 0.4% (w/w), chicken broth 0.35% (w/w), roasted garlic 0.45% (w/w), onion 0.4% (w/w), pork fat 0.35% (w/w), hickory smoke 0.05% (w/w), vanilla (2-fold) 0.435% (w/w).*

B1.2.2 ABCDX Sensory Test

B1.2.2.1 ABCDX Testing Ballots

Welcome to the Flavored Milk Study! Instructions for the test are below:

This is a **two-day study**. Each day you will be tasting through two sections, so there are **four sections** in total**.** You will be tasting a total of **20 samples** during this test (five samples each section, which is **ten samples each day**). You will see a screen to tell you to pause after the second section so that you can come back the next day to finish the study.

In each section, you will be required to taste four milk samples from left to right, and then taste a fifth sample. You will be asked to **match** this last sample to **one** of the first four milk samples. Match this sample based on the one that is the **most similar to you**. There **may or may not be exact matches,** so **choose the closest match.**

There will be a 2 minute break between each session. Please use this time to rinse your mouth with water.

Please click "next" to continue.

Please taste the four samples in the
first row from left to right.

Now taste sample **452**.

Which sample in the first row is **most similar to sample 452**?

There may or may not be an exact match, so choose the closest match.
You may re-taste the samples before making a selection.

○ BC111

○ BC222

○ BC333

○ BC444

B1.2.2.2 Composition of Final Samples

Table B- 3 *% Total solids, % fat, and % estimated sugar of final sucrose-milk samples for the ABCDX Matching Test.*

Sample	% Sugar	% Vanilla Extract	% Beef Flavor	% Milk	% TS Skim	% TS Product	%Fat Product	% TS From Milk	% Estimated TS	% Absolute Error	% Error	% Sugar in Solids	% Estimated Sugar	% Sugar Error
1	1.67	0	0	98.3	9.03	10.3	0.11	8.88	10.55	0.249	2.42	15.8	1.63	2.36
2	2.24	0	0	97.8	9.03	11.07	0.12	8.83	11.07	-0.002	-0.02	20.2	2.24	-0.02
3	2.24	0.435	0	97.3	9.03	10.96	0.12	8.79	11.03	0.068	0.62	20.3	2.23	0.62
4	2.24	0	0.4	97.4	9.03	11.09	0.12	8.79	11.03	-0.058	-0.53	20.3	2.25	-0.53
5	2.81	0	0	97.2	9.03	11.92	0.4	8.78	11.59	-0.334	-2.80	24.3	2.89	-2.88
6	3.38	0	0	96.6	9.03	12.04	0.13	8.72	12.10	0.065	0.54	27.9	3.36	0.54

B1.2.2.3 Viscosities and significance tables

Table B- 4 *Viscosities (Pa.s) of final aroma-milk samples for the ABCDX Matching Test.*

Sample	% Sugar	% Vanilla	% Beef	% Milk	Trial 1	Trial 2	Trial 3	Avg	Std dev
1	1.67	0	0	98.3	0.00301	0.00291	0.00284	0.00292	8.61E-05
2	2.24	0	0	97.8	0.00304	0.00293	0.00296	0.00298	5.59E-05
3	2.24	0.435	0	97.3	0.00278	0.00291	0.00293	0.00287	7.83E-05
4	2.24	0	0.4	97.4	0.00292	0.00294	0.00291	0.00292	1.75E-05
5	2.81	0	0	97.2	0.00292	0.00299	0.00302	0.00298	5.45E-05
6	3.38	0	0	96.6	0.00297	0.00298	0.00298	0.00298	4.16E-06