

ABSTRACT

PARKER, MEGAN NICOLE. Comparison of Natural Sweeteners in Protein Beverages using Time-Intensity, Temporal Dominance of Sensations, and Temporal Check-All-That-Apply. (Under the direction of Dr. Mary Drake).

Protein beverage consumption by Americans has increased in recent years. Coupled with this increased consumption is an interest in natural sweeteners. Each sweetener has unique temporal properties that can influence beverage sensory properties and consumer acceptability. Iso-sweet concentrations of sweeteners (sucralose, sucrose, fructose, stevia, monk fruit) in ready-to-mix (RTM) vanilla whey protein beverages (25 g protein/12 oz. water) were established using magnitude estimation scaling and 2-alternative forced choice testing. Temporal sensory profiling was then conducted on each beverage by a trained panel using Time Intensity (TI), Temporal Dominance of Sensations (TDS), and Temporal Check-All-That-Apply (TCATA). These findings were used to formulate seven natural sweetener blends that more closely matched the temporality of sucrose sweetened RTM vanilla protein beverages. These blends were also subjected to temporal sensory profiling in RTM beverages. One sugar free blend (25% stevia/75% monk fruit) and one reduced sugar blend (25% stevia/25% monk fruit/25% fructose) were then selected for consumer testing, in addition to 3 control RTM beverages containing sucralose, stevia, or monk fruit. A two-day crossover design study (n=150 consumers) was conducted to investigate the impact of priming statements about sweetener as well as sweetener source on consumer acceptance of RTM protein beverages. Two distinct consumer clusters were identified. The 'label conscious' segment of consumers preferred beverages sweetened with natural blends when primed. The 'flavor driven' segment of consumers conceptually preferred naturally sweetened beverages, but preferred sucralose sweetened beverages when primed. An "all natural" label claim was most preferred across all consumers. Application of these findings to

commercially produced RTM protein beverages aids in development of naturally sweetened protein beverages with reduced calories and desirable sensory properties, and highlights the importance of label claims to consumers overall, but in particular to a 'label conscious' segment of consumers.

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Comparison of Natural Sweeteners in Protein Beverages using Time-Intensity, Temporal
Dominance of Sensations, and Temporal Check-All-That-Apply

by
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DEDICATION

This thesis is dedicated to my families; both my biological family and my crazy MAD Lab family. Both have offered unconditional support, advice, and encouragement throughout this journey. Thank you to my parents; both Jane and Randy and my ‘lab mother’ Dr. Drake for teaching me to work hard, persevere, and never give up. I could not have done it without you.

BIOGRAPHY

Megan Parker was born in Des Moines, Iowa to Randy and Jane Parker, and is the oldest of five children. Growing up, she had a passion for food and cooking and an interest in math and science. When a college counselor suggested she study Food Science, it seemed like the perfect fit. Megan graduated in 2014 with a B.S. in Food Science from the University of Minnesota in Minneapolis. During her time as an undergraduate she was involved with the University cheerleading team, a sorority, worked as a tutor to high school students, and completed several independent research projects. She moved to North Carolina in August 2014 to pursue her M.S. degree in Food Science at North Carolina State University under Dr. MaryAnne Drake. Megan worked in the lab for 2 ½ years while completing her degree, and graduated in December 2016. When she's not studying or working, Megan enjoys teaching yoga, reading, cooking, and traveling.

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CHAPTER 1:
LITERATURE REVIEW. COMPARISON OF NATURAL SWEETENERS IN
PROTEIN BEVERAGES USING TIME-INTENSITY, TEMPORAL DOMINANCE
OF SENSATIONS, AND TEMPORAL CHECK-ALL-THAT-APPLY.

ABSTRACT

The use of natural, alternative sweeteners in WPI protein beverages is currently a topic of interest. Consumers indicate preference for the use of natural over artificial alternative sweeteners in these products, and the replacement of sugar also has the effect of reducing calorie consumption. To make successful substitutions for sucrose, the iso-sweet intensities of natural alternative sweeteners compared with sucrose must first be determined through the application of Magnitude Estimation Scaling and power curve generation. The temporal effects of the alternative sweeteners must also be considered, as off-flavors such as bitterness may be more common. The evaluation techniques of time-intensity, temporal dominance of sensations, and temporal check-all-that-apply are useful to meet this objective.

INTRODUCTION

Protein beverages have seen an increase in popularity as more Americans are trying to increase protein content in their diets (Gerdes, 2012; Jacobson, 2015). Protein beverages may serve as a meal replacement, recovery drink, weight management tool, or nutrition supplement. Protein sources for protein beverages are often whey protein in the form of whey protein isolate (WPI) or whey protein concentrate (WPC), byproducts of cheese manufacture that are produced from the concentration of liquid whey by membrane filtration techniques (Tunick, 2008; Foegeding and Luck, 2011). Whey protein isolate has the higher protein content of the two most common whey protein products with at least 90% protein. It is also soluble across a wide range of pH values, making it a great candidate for use in both neutral and acidic protein beverages (Horne, 1990).

Along with the increase in protein beverage popularity, has been the increase in demand for protein beverages that are naturally sweetened with a low carbohydrate content (Gerdes, 2012; Jacobson, 2015; Oltman et al., 2015). A recent conjoint survey conducted on the topic showed that type of sweetener was a key attribute for all consumers surveyed, and that natural sweeteners (over artificial alternative sweeteners) were the most attractive type of sweeteners to health conscious, sweetener aware consumers. Reducing the sugar content through replacement of calorie-containing sweeteners with natural, non-nutritive sweeteners in protein beverages and other food products could reduce energy intake for Americans to help with weight management, and prevention of obesity and other complication conditions such as Type 2 diabetes, hypertension, and dental caries (Malik et al., 2006). The replacement with non-nutritive sweeteners would still maintain the sweet taste desired by consumers, preventing a negative impact to consumer liking (Drewnowski et al., 2012).

Natural non-nutritive sweeteners applicable for use in protein beverages include monk fruit (mogroside V from the fruit *Siraitia grosvenorii*) and stevia (Rebaudioside A/Stevioside), glycoside isolates from the leaves of the Stevia rebaudian herb (Kim and Kinghorn, 2002; Savita et al., 2004). Depending on their concentrations, stevioside is about 300 times sweeter than sucrose, Rebaudioside A up to 450 times sweeter, and mogroside V about 250-425 times sweeter (Kinghorn et al., 2001). Crystalline fructose is also a natural sweetener that contains fewer calories than sucrose, and is a nutritive sweetener. It is found naturally in fruits, honey, and vegetables, and has a higher relative sweetness than other carbohydrates (White and Osberger, 2001; Lima et al., 2011).

In order to correctly formulate sucrose replacement with alternative sweeteners, the sweetness equivalence/iso-sweetness, or the amount of alternative sweetener that produces

the same sweetness intensity when substituted for another sugar must be determined to maintain sensory properties of the product (Cardello et al., 1999). This is done by conducting magnitude estimation scaling followed by descriptive analysis and 2-AFC (Lawless and Heymann, 2010; Li et al., 2015). This technique has been previously used to determine iso-sweetness of many alternative sweeteners in a variety of simple and complex food matrices (Lisak et al., 2012; Reis et al., 2011; Souza et al., 2013; Azevedo et al., 2015; Li et al., 2015).

Alternative sweetener temporality must also be considered when using these products as sucrose replacements. Temporal methods of descriptive analysis examine how the sensations and flavors from products change over time (Lawless and Heymann, 2010). This approach is especially useful to evaluate alternative sweeteners, as they have a very different timing of sweetness perception and may also contain undesirable off flavors such as bitter or metallic tastes (Souza et al., 2013; Morais et al., 2014; Zorn et al., 2014). Temporal methods of evaluation include time-intensity (TI), temporal dominance of sensations (TDS), and temporal check-all-that-apply (TCATA). TI involves trained panelist evaluation of a single flavor attribute, and how its intensity changes throughout the evaluation period (Lawless and Heymann, 2010). TDS involves evaluation of multiple attributes at once (Pineau et al., 2009). All relevant product attributes are presented on a computer screen at the beginning of the evaluation period, and panelists select the attribute they feel is dominant at any given time, with the ability to select only one attribute at a given time (Labbe et al., 2009). TCATA is the most novel of the temporal methods, allowing panelists to select multiple attributes at one time (Castura et al., 2015). Throughout the test, panelists may select or de-select as many attributes as they like, based on what they feel is relevant. The use of temporal evaluation techniques allows for the identification of flavor profiles of alternative sweeteners, which

helps in the creation of mixtures in order to produce the best combination of sweetness without off-flavors in order to be an effective substitute for sucrose.

PROTEIN BEVERAGES

Protein beverage popularity is on the rise in the United States. According to a recent online consumer survey conducted by *Beverage Industry*, the attribute of ‘high protein’ moved from No. 10 to No. 1 on the list of latest beverage trends (Jacobson, 2015).

Consumption of high-protein beverages aligns with the desire of approximately 54% of Americans to add more protein to their diets (Gerdes, 2012). The desire for additional protein may be for a variety of reasons, including sports nutrition, weight management, meal replacement, and/or athletic recovery. Protein beverages fall into the functional foods category, a category defined as ‘foods and food components that provide a health benefit beyond basic nutrition’ with aims to promote health or reduce the risk of disease (Institute of Medicine, Food and Nutrition Board, 1994; Serafani et al., 2012). Functional foods are not clearly defined and definitions vary between countries (Corbo et al., 2014). In the United States, functional foods are regulated by the same rules used for conventional foods. The protein sources for protein beverages include casein proteins, milk proteins, and plant proteins such as soy (Oltman et al., 2015).

Whey Protein

Liquid whey is the liquid produced from casein precipitation of liquid milk during cheese production (Horne, 1990; Tunick, 2008). Approximately 9L of whey is produced from each kilogram of cheese made. Some properties of whey depend on the cheese source it comes from, and how the curd was formed during the cheese-make. Whey from a rennet-coagulated cheese such as Cheddar cheese is considered sweet whey, and has a pH of at least

5.6 (Tunick, 2008). When the curd is precipitated using rennet, the casein-derived protein glycomacropeptide (GMP) is also released into the whey due to the cleavage of κ -casein (Foegeding and Luck, 2011). Acid whey has a lower pH (5.1 or below) and results from acid-coagulated cheese production, such as Cottage cheese (Tunick, 2008). The structure of whey proteins is tight and globular, consisting of several proteins: Beta-lactoglobulin, alpha-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, and lactoperoxidase (Horne, 1990; Foegeding and Luck, 2011). Beta-lactoglobulin and alpha-lactalbumin make up the majority of whey protein (approximately 80% by weight) (Horne, 1990).

Whey Protein Processing

Liquid whey is approximately 93% water and 7% total solids, with the solids composed of approximately 8-10% protein (total protein content is approximately 0.6%), 70-72% lactose, and 8-10% minerals (Jelen, 2011). Due to the low percent solids as well as fat and lactose components, whey protein must be processed to isolate and purify the protein fraction. Many methods and combinations of methods are used for this concentration and purification, most often to produce WPC and WPI (Figure 1). These products are considered the 'edible protein' components of liquid whey, making them desirable for use in food products, including high-protein beverages, soups, dressings, bakery products, and processed cheese (Horne, 1990; Bylund, 2003). To isolate these fractions, liquid whey must be fractionated to concentrate proteins and then spray dried. The type of membrane filtration used varies depending on the desired end product.

Ultrafiltration is used to fractionate liquid whey to produce whey protein concentrate, which has 20-80% protein (Table 1) (Tunick, 2008; Kelly, 2011). Ultrafiltration membranes separate milk components based on molecular size, preventing larger components such as protein and fat from passing through the membrane, while allowing smaller components such as water, lactose and minerals to permeate the membrane (Kelly, 2011). Most WPCs contain either 34-35% or 80% protein, known as WPC34 or WPC80, respectively (Foegeding and Luck, 2011). Ultrafiltration alone is sufficient to concentrate liquid whey to WPC34 or WPC35 (Kelly, 2011). To further concentrate WPC34 to WPC80, diafiltration in combination with UF must be used. Diafiltration uses water to wash the concentrated retentate and further remove lactose and minerals (Kelly, 2011).

To produce WPI, which contains at least 90% protein, nearly all the moisture, lactose, fat, and ash must be removed from the liquid whey stream (Foegeding and Luck, 2011). The membrane separation process used for this objective is microfiltration (MF). MF has a larger pore cutoff size than UF, and therefore allows larger particles into the permeate (Figure 2) (Kelly, 2011). This is desirable to remove any residual fat content and allow for a protein content above 90%. The vast majority of WPI is produced using MF, although a small percentage is produced using anion exchange followed by MF. Anion exchange is a technique used to demineralize whey by separating particles based on charge (Gernigon et al., 2011). Negatively charged ions are retained by the positively-charged exchange resin, while positive and neutral molecules are not. Anion exchange is not as practical for WPI production due to its high cost (Gernigon et al., 2011).

Due to its high protein content WPI has excellent water-binding, gelling, emulsifying, and foaming abilities, making it useful in a wide variety of food and beverage products

(Foegeding and Luck, 2011). However, the protein amount and fractions present may vary depending on both the starting material and processing techniques used. Most WPI contains GMP, except for WPI manufactured by anion exchange. The presence of GMP may affect the functional performance of WPI in food and beverage applications. If desired, GMP may also be removed using fractionation techniques.

Use of Whey Protein in Protein Beverages

The disposal of whey is viewed as an environmental burden due to its high biological oxygen demand (Jelen, 2011). Given the high nutritional content of whey proteins, their incorporation into health-aimed beverages was a logical connection. WPC and WPI are complete sources of essential amino acids and are also high in branched-chain amino acids such as valine, isoleucine, and leucine, which may aid in muscle recovery after exercise by increasing protein synthesis and decreasing protein degradation (Blomstrand and Saltin, 2001; Hazen, 2006). In addition to their nutritional benefits, whey proteins are also desirable for use in protein beverages due to their solubility in water over a wide range of pH values (Pelegri and Gasparetto, 2005).

There are two categories of whey protein beverages: acidic whey protein beverages and neutral pH whey protein beverages. Acidic whey beverages (pH < 3.5) are typically clear due to increased protein solubility at low pH levels, while neutral pH whey beverages (pH approx. 6.8) are opaque due to decreased protein solubility (Pelegri and Gasparetto, 2005; Beecher et al., 2008). While increased protein solubility is a benefit of low pH beverages, there is also the concern of high astringency with whey protein beverages that contain >3% protein at a low pH (Sano et al., 2005). It is hypothesized that the increased astringency is related to protein aggregation caused by interactions between positive whey proteins and

negative saliva proteins (Beecher et al., 2008). However, astringency does not necessarily negatively impact protein beverage acceptance. Childs and Drake (2010) investigated consumer perception and acceptance of astringency in clear acidic whey protein beverages. Consumers correctly identified astringency in beverages, but astringency was not related directly to dislike of WPI beverages. Acceptance of WPI beverages was lower when consumers were not wearing nose clips, which suggests that WPI flavors in the beverages negatively impacted acceptance more than the astringent mouth feel.

In addition to preferences about the protein flavor, previous research suggests that consumers also have preferences for the type of sweetener used in protein beverages. A recent consumer survey showed an increased desire for ‘natural’ beverages (Jacobson, 2015). There is also a desire among consumers for low carbohydrate content and use of natural sweeteners (Gerdes, 2012; Oltman et al., 2015).

SUGAR AND ALTERNATIVE SWEETENERS

The 2015 U.S. dietary guidelines recommends that Americans decrease their consumption of sugar-sweetened beverages, desserts, and sweet snacks in order to decrease overall dietary intake from added sugars (USDA, 2015). The largest contributors to this ‘added sugars’ category are beverages, making up 47% of the average added sugars in the American diet. Additionally, the beverage category makes up 19% of total energy intake. According to the World Health Organization (2003), added sugars should only compose 10% or less of total dietary energy. Reducing calories consumed from sugar sweetened beverages could reduce overall energy intake and help with weight control as well as prevention of obesity and the risk of obesity and other health problems (Type 2 diabetes, hypertension, dental caries) (Malik et al., 2006). Sugar consumption from sugar-sweetened beverages is

especially of concern in children and adolescents. Bigornia et al. (2014) found that higher consumption of sugar-sweetened beverages in children ages 10-13 y was associated with a larger waist circumference, which can be indicative of risk for obesity as well as obesity-related diseases.

Sweet taste is considered desirable by consumers and sugar reduction in food and beverage products may negatively impact consumer liking. Sweet taste reception results in a pleasure response in the brain, thus producing a positive hedonic response to sugar-sweetened foods and beverages (Drewnowski et al., 2012). This response developed evolutionarily, as sweetness is linked to energy consumption (Drewnowski et al., 2012). The use of low-calorie sweeteners may be able to eliminate this link; providing the positive physiological response to sweetness without the added calories. Several previous studies have looked at the impact of sugar reduction on consumer acceptance in food products, and in particular, dairy products. Chollet et al. (2013) found that while consumers preferred flavored yogurt with 10% sugar, yogurts with 7% sugar were also acceptable. Hoppert et al. (2013) also investigated sugar reduction in yogurt, finding that a 30% reduction of sugar (from 16% to 11%) was acceptable to consumers, and that increasing flavoring content may be useful to increase acceptance of reduced sugar yogurts. Li et al. (2015) explored sugar reduction in skim chocolate milk, demonstrating a 30% sugar reduction (from 205 mM) was still accepted by young adults and children. Oliveira et al. (2015) found similar results in probiotic chocolate milk; that sugar reduction up to 40% did not affect consumer liking of preference. In vanilla ice cream, Cadena et al. (2012) reported that a 25% sugar reduction was not linked to decreased acceptance, and that more important was the quality of starting materials and use of an appropriate sweetener or sweetener system.

Non-nutritive Alternative Sweeteners

Non-nutritive alternative sweeteners can be used to replace sucrose in order to maintain sweet taste while reducing calories. Many non-nutritive sweeteners are synthetic, chemically derived products such as saccharin, cyclamate, aspartame, acesulfame, sucralose, and advantame (DuBois and Prakash, 2012). Many consumers reject these products seeing them as ‘unnatural,’ ‘unhealthy,’ and a ‘public risk’ (Beath et al., 2014). Alternatively, consumer perception of natural products was positive. Oltman et al. (2015) found similar results for consumer acceptance of artificial sweeteners in protein beverages. Results from a conjoint analysis survey showed that sweetener type was a key attribute for protein beverages across all consumers surveyed. The study also investigated protein beverage attributes using Kano analysis, and found that natural nonnutritive sweetener was an attractive attribute, while artificial sweetener was a reverse attribute (unattractive) to the group of health conscious, sweetener aware consumers.

Natural, non-nutritive sweeteners are derived from plants and made up of natural compounds including mogroside V, rebaudioside A, and stevioside (Kim and Kinghorn, 2002). Mogroside V is a glycoside found in the fruit *Siraitia grosvenorii* (alternatively known as luo han kuo and monk fruit) (Pawar et al., 2013). *S. grosvenorii* also contains the sweet glycosides mogroside IV and mogroside VI, but mogroside V is the most abundant in the fruit. *S. grosvenorii* concentrate with up to 55% mogroside V is GRAS (Kinghorn et al., 2001). Monk fruit has a long history of use in southern China to treat medical issues including cold, sore throat, and stomach problems (Kinghorn et al., 1986). Depending on concentration, mogroside V is approximately 250-425 times sweeter than sucrose (Kinghorn and Compadre, 1991). The use of monk fruit as a natural sweetener in the United States is

low compared with other natural sweeteners, but popularity and usage is increasing; up to 10% in 2015 from 8% in 2014 (Mintel, 2015).

Rebaudioside A and stevioside are glycosides isolated from the leaves of *Stevia rebaudian* (Bertoni), a sweet herb native to northern Paraguay (Savita et al., 2004). Stevioside is the more abundant sweet compound in *S. rebaudian*, but is less sweet than rebaudioside A (Kim and Kinghorn, 2002). Depending on concentration, stevioside is approximately 300 times sweeter than sucrose (0.4% w/v), while rebaudioside A is 250-450 times sweeter than sucrose (0.4% w/v) (Kinghorn et al., 2001). Both purified compounds are GRAS as sweeteners in the United States, while whole leaf *Stevia* or crude extracts are not (Pawar et al., 2013). Rebaudioside A is often better suited for use in food and beverage products due to its water solubility and more pleasant taste, as stevioside has undesirable sensory properties such as bitterness and unpleasant aftertaste (Bakal and Nabors, 1986; Kim and Kinghorn, 2002). However, stevioside does have redeeming properties such as heat stability, salty taste suppression, and resistance to precipitation at low pH levels (Kinghorn et al., 2001).

Crystalline fructose is an additional sweetener option that has seen increased popularity in the past few decades. Fructose is a nutritive sweetener naturally found in many fruits, honey, and some vegetables (Lima et al., 2011). Compared to other nutritive carbohydrates, fructose has a high relative sweetness, as much as 1.8 times that of sucrose for crystalline fructose (White and Osberger, 2001). For this reason, crystalline fructose can be used to replace sucrose to provide sweetness while reducing calories and sugar. Sweet taste in fructose is also perceived more quickly than in sucrose, with a sharper intensity and peak followed by a faster dissipation, making fructose a good flavor enhancing sweetener (Zamora

et al., 1998). Additionally, fructose has a high solubility in water, low glycemic index (compared to other sugars), high freezing point depression, and high humectancy (Lima et al. 2011).

The Chemistry of Sweet Taste Perception

Taste is the detection and discrimination of chemical stimuli via chemoreceptive events that occur in the mouth (Lindemann, 2001). Taste perception occurs in clusters of modified epithelial cells known as taste receptors which are arranged in taste buds (Lindemann, 2001). The cell membranes of these taste buds bind taste stimuli to receive a chemical signal and subsequently send a synaptic signal to the brain (Lawless and Heymann, 2010). Humans can detect sweet, salty, sour, bitter, or umami tastes, and the mechanism of reception varies depending on the taste perceived (Lindemann, 2001). Salty and sour tastes are detected via ion channels and membrane potential created by the influx of cations H^+ and Na^+ (Sugita, 2006; Bachmanov and Beauchamp, 2007). Bitter, sweet, and umami tastes are perceived through the interaction of taste stimuli with G-protein-coupled receptors in the membranes of taste buds (Lindemann, 2001; Margolskee, 2002; Scott, 2005).

Many different molecules included in the categories of natural sugars, artificial sugars, and proteins can be perceived as sweet. In order to elicit this response, the compound must contain an acidic proton, an electronegative atom or center, and a hydrophobic group (Shallenberger and Acree, 1969; Mathlouthi and Portmann, 1990). These structures allow for intermolecular hydrogen bond formation, which is a necessary chemical reaction in the perception of sweet taste. In natural sugars, the glycol group contains the acidic proton and electronegative center, thus allowing interaction with the sweet taste receptors (Shallenberger

and Acree, 1969). Artificial sweeteners contain alternate proton donor/receptor structures, such as $\text{NH}_3^+/\text{COO}^-$ in aspartame and $\text{NH}/\text{carbonyl}$ groups in Acesulfame-K (Mathlouthi and Portmann, 1990).

Two ‘families’ of receptor proteins function for bitter, sweet, and umami taste perceptions; T1Rs and T2Rs for sweet/umami and bitter tastes respectively (Bachmanov and Beauchamp, 2007). Each family of receptor proteins is composed of many amino acid subunits, 850 amino acids make up the T1Rs and 300-330 amino acids make up the T2Rs (Bachmanov and Beauchamp, 2007). In order to produce a taste stimulus, the sweet, bitter, or umami taste compound binds the G-protein-coupled receptor, which causes separation of the G-protein subunits, activating intracellular enzyme systems. This results in changes in ionic potential that release neurotransmitters to stimulate the associated taste nerves in the brain (Akabas et al., 1988; Miyoshi et al., 2001; Lawless and Heymann, 2010).

In mammals, multiple types of T1R sweet taste receptor genes have been identified in this family, notably T1R1, T1R2 and T1R3 receptors (Nelson et al., 2001). Each receptor has the ability to respond to multiple types of structurally different sweet molecules (Nelson et al., 2001). Additionally, previous research has demonstrated that sweet taste perception of both natural and artificial sweeteners (despite their structural differences) can occur via the T1R2 and T1R3 receptors (Chandrashekar et al., 2006). However, further research suggests that T1R3 is the only receptor that responds to sweet taste in artificial sweeteners, while multiple receptors respond to sweet taste in nutritive natural sugars in conjunction with the T1R3 receptor (Damak et al., 2003). A study by Frank et al. (2008) provided supporting results, demonstrating that consumption of sucrose activated 10 regions of the brain, while consumption of sucralose only activated 3 regions of the brain. These results show that

although natural and artificial sweeteners both result in the perception of sweet taste, the pathways and mechanisms behind these perceptions are different. Dubois and Lee (1983) suggested this difference in perception results from differences in diffusion and binding of sweet tasting compounds to receptors between natural and artificial sweeteners. Sucrose (and other similar sweeteners) rapidly diffuse to the receptor site prior to binding the sweet taste receptor, while non-sucrose sweeteners initially diffuse to and bind with non-receptor sites on the receptor protein, followed by secondary diffusion and binding to the receptor sites (Dubois and Lee, 1983).

Additionally, the taste perception of artificial sweeteners is influenced by their concentrations. At low concentrations, artificial sweeteners are perceived as sweet due to the activation of T1R2 and T1R3 receptors as previously discussed (Li et al., 2002). At higher concentrations, artificial sweeteners such as saccharin, acesulfame-K, and aspartame produce bitter and metallic tastes, in part due to activation of the bitter sensing T2R receptors (Kuhn et al., 2004; Riera et al., 2007).

Flavor Interaction with Sweet Taste Perception

Past research has shown that sweetness perception can be influenced by interacting factors, such as taste-aroma interactions or presence of flavorings and/or volatile compounds. Hornung and Enns (1994) found that simultaneously sniffing volatile compounds (ethyl butyrate) increased the sweet taste perception of sucrose solutions. Djordjevic et al. (2004) reported strawberry odor increased sweetness perception of sucrose and water solutions. Clark and Lawless (1994) reported the addition of both vanilla and strawberry volatiles to sweet sucrose solutions increased sweetness perception. Hoppert et al. (2013) reported an

interaction between sweetness perception and flavor concentration in low sugar vanilla yogurts, and suggested increased flavoring concentration as a way to boost acceptance of low and reduced sugar products.

Not all odor compounds enhance sweet perception, however. Frank and Byram (1988) reported that strawberry volatiles had a sweetness-enhancing effect, while peanut butter volatiles did not enhance sweetness, suggesting that taste-smell interactions such as sweetness enhancement are dependent on the odor/taste combination. Similar results were reported by Stevenson et al. (1999). In the study, odors were added to sweet solutions and sweetness was measured. Some odors (caramel, strawberry) enhanced sweetness while other odors (maltol, eucalyptol) depressed sweetness. It was suggested that odors that smelled sweeter were likely to enhance sweetness while odors that did not smell sweet were likely to depress sweetness. According to a model reported by Small and Prescott (2005), odor/taste integration depends on the odor and taste compounds being “perceptually congruent” or that they are perceived similarly (ex. fruity smells are usually associated with sweet taste and not with sour taste). Odor/taste interactions also depend on the person’s prior experience with the odor and taste (Small and Prescott, 2005).

DETERMINATION OF ISO-SWEETNESS

Iso-sweetness, or sweetness equivalence, is the amount of an alternative sugar that will produce the same sweetness intensity response when substituted for another sugar in a given product at the same pH and temperature. Determination of sweetness equivalence is very important when making sweetener substitutions while trying to maintain the same sensory characteristics of the product (Cardello et al. 1999). Often, the sugar to be replaced is sucrose and the objective will be to determine the iso-sweetness of an alternative sugar

compared with sucrose. In order to correctly make a sweetener substitution, the sweetness equivalence of the alternative sugar compared with sucrose must first be determined (Reis et al., 2011). This is often first done in a model system such as water before working with a more complex food matrix, in which sweetness may be affected by pH, protein, and fat. To determine sweetness equivalence, magnitude estimation scaling (MES) is one of the most widely used techniques and has been successfully used in many previous studies involving a wide variety of food matrices. Magnitude estimation is a basic scaling technique in which a standard stimulus is assigned as the reference with a known fixed value, and subsequent samples are assigned a value depending on their ratio to the reference sensation (Lawless and Heymann, 2010). The assigned numbers do not have a numerical value; they simply represent the ratio of sensation between samples (Land and Shepard, 1984). The geometric mean for each sample is plotted against sweetness concentration (or other known concentration) and log transformed to give a straight line for analysis (Figure 3). MES results can then be used to generate power functions using the formula $S=kl^n$ where S is the sensation magnitude, I is the intensity of the sensation given by the stimulus, k is a constant and n is an exponent representing growth or decay (Land and Shepard, 2010; Lawless and Heymann, 2010). Power function curves demonstrate the relationship between increase of a stimulus and the response, in this case specifically the relationship between increase in sweetener concentration and perceived sweetness intensity. Power functions with an exponent less than one represent a system in which larger and larger stimulus increases are needed to maintain a proportional increase in response, while power functions with an exponent greater than one represent a system in which constant stimulus increases produce exponential response increases.

Iso-sweetness of Alternative Sweeteners in Food Matrices

Redlinger and Setser (1987) explored the sweetness and other sensory characteristics of sucrose, fructose, aspartame, acesulfame-K, sodium saccharin, and calcium cyclamate in model aqueous and lipid systems. The liquid model system consisted of deionized water mixed with the appropriate amount of sweetener to match a 5.0% w/v sucrose reference (4.1% fructose, 0.028% aspartame, 0.026% acesulfame-K, 0.014% sodium saccharin, 0.140% calcium cyclamate) and then flavored with lemon or vanilla flavoring. The model lipid systems consisted of lemon or vanilla flavoring, hydrogenated shortening, deionized water and the appropriate amount of sweetener to match a 25.0% w/v sucrose reference solution (20.63% fructose, 0.22% aspartame, 0.22% acesulfame-K, 0.22% sodium saccharin, 0.50% calcium cyclamate). In the model aqueous solution, sucrose had the greatest maximum sweetness intensity followed by aspartame, fructose, saccharin/cyclamate, and acesulfame-K. Results for the model lipid systems differed, with saccharin/aspartame perceived to have the greatest maximum sweetness intensities followed by sucrose/acesulfame-K/fructose and then cyclamate, demonstrating that components of the food system influence sweetness perception depending on the type of sweetener used.

Li et al. (2015) used MES to create power function curves to calculate iso-sweetness of sucrose to stevia and monk fruit in both water (a model system) and skim chocolate milk (Figure 3). Sucrose in water at a concentration of 150 mM was used as a reference solution to compare with monk fruit concentrations of 100, 400, 700, 1000, 1300, 1600, and 2000 mg/L and stevia concentrations of 50, 150, 350, 450, 550, 650, and 750 mg/L. Results from power function generation and subsequent descriptive analysis were that the iso-sweetness concentrations of the sweeteners in skim chocolate milk were 350 mg/L and 200 mg/L for

monk fruit and stevia respectively. Additionally, stevia had a greater sweetness response than monk fruit, which aligned with expected results, as stevia is a more potent sweetener than monk fruit (Kinghorn et al., 2001).

Reis et al. (2011) used MES and power curve generation to determine the sweetness equivalence of aspartame, aspartame/acesulfame-K (2:1), cyclamate/saccharin (2:1), cyclamate/saccharin/stevia (1:8:1) with 11.5% (w/w) sucrose in strawberry flavored yogurt. Sucralose was added as the smallest mass percentage to reach equivalent sweetness, while aspartame was added as the largest mass percentage. The aspartame/acesulfame-K (2:1) mixture had the highest sweetening power, while aspartame alone had the least sweetening power. These results are consistent with those found by Cardoso and Bolini (2007). Lisak et al. (2012) also explored the use of alternative sweeteners in strawberry yogurt through the comparison of sweetness of yogurt sweetened with stevia, sucrose, and a mixture of the two stored for 7 days. Target sweetener amounts were 3%, 4.5%, and 5% (w/w). Six grams of stevia had equivalent sweetness to 1000 g of sucrose in the strawberry yogurt samples (about 167 times sweeter). After seven days, the sucrose and stevia only samples decreased in sweetness, while the sucrose/stevia samples had not. These results are likely due to a pH drop of approximately 0.1 pH units.

Azevedo et al. (2015) investigated the equivalent sweetness of sucralose, aspartame, neotame, stevia, and cyclamate/saccharin (2:1) with 12.5% (w/v) sucrose-sweetened espresso coffee. Neotame was the most potent sweetener (7812 times sweeter) and stevia was the least potent sweetener (125 times sweeter). In mixed fruit jam (40% w/w sucrose), Souza et al. (2013) reported that sucralose had the highest sweetening power (1033.59 times sweeter), followed by sucralose/acesulfame-K/neotame (5:3:0.1) (982.80 times sweeter),

sucralose/stevia (2:1) (862.67 times sweeter), sucralose/acesulfame-K (3:1) (847.45 times sweeter), and sucralose/thaumatococin (1:0.6), which was 284.29 times sweeter than sucrose. Sweetening power was determined by first finding the equivalent sweetness of each sweetener mixture compared with 40% sucrose, then determining how many times sweeter the mixture was based on its iso-sweetness. Based on the various evaluations of alternative sweeteners in food products, results have demonstrated that each alternative sweetener behaves slightly differently in each food system. However, the sweetening power (or relative sweetness) of each alternative sweetener will stay within a general range. For these reasons, past literature provides a good starting point for finding equivalent sweetness in a food product, but this should be determined individually for each study.

TEMPORAL SENSORY METHODS

Descriptive Analysis

Descriptive analysis is one of the most sophisticated, widely applicable, and useful sensory techniques, in that it can provide a complete, detailed sensory description of a product (Lawless and Heymann, 2010). Several different techniques are used under the general umbrella of descriptive analysis, but they are all similar in that they are used to provide a detailed description of the sensory attributes of a product or to make a detailed comparison and subsequent discrimination between several products. The major descriptive analysis approaches are Flavor Profile, Quantitative Descriptive Analysis (QDA), and the Spectrum™ method. The Flavor Profile method is a consensus technique in which a product's perceived flavors, intensities, aftertastes, and overall impression are considered and rated. QDA uses product-specific scales to evaluate the intensity of an attribute. A product specific scale is used and the use of physical references is not as common as with the

Spectrum method. The Spectrum method (developed by Gail Civille) relies heavily on references and input from the panel leader. This method uses a universal scale to rate the intensity of all attributes.

In order to successfully conduct descriptive analysis, regardless of the specific approach used, similar steps must be followed in order to produce reliable data. These steps consist of selecting a panel, selecting a language to describe product attribute, and panel calibration (Murray et al., 2001). To be successful, panelists must have experience and personalities that fit well with the group, as well as dedication. A new panel will then need to either adapt or develop a lexicon that is descriptive, non-redundant, and clear (providing written and/or physical references) that will be used to differentiate between products. Once the lexicon has been selected, panelists will be trained on these terms, often using references until the panel is discriminating and reproducible. At this point in time, the panel is ready to evaluate products using the specific approach selected.

Often, the sensation and/or intensity or sensations of a food change over time. Flavor release doesn't happen in a single instant, and also doesn't even occur at a constant rate (Dijksterhuis and Piggott, 2000). Several factors (physical, chemical, and psychological) may affect the perception of flavor and how it changes over time. Flavor release occurs in a series of several steps, starting with the release of flavor molecules as chewing breaks down the food matrix and releases flavors and texture. Saliva flow may change the pH and provide an aqueous phase in which non-volatile compounds may be released. Swallowing, tongue movement, and breathing can also influence perception. As time goes on, lubrication in the mouth decreases which can result in increased perception of astringency. Additional psychological processes that can influence perception over time include adaptation (short-

term reduced sensitivity from repeated exposure), cognition (memory/recognition), fatigue (long-term reduction in sensitivity from prolonged exposure), and sensitization (increased sensitivity) (Dijksterhuis and Piggott, 2000).

Temporal sensory methods allow for panelists to track perceived sensations and how these sensations and intensities of these sensations change over time (Lawless and Heymann, 2010). Temporal responses to food were first tracked and quantified beginning in the 1950s and 1960s (Cliff and Heymann, 1993). Sjoström (1954) and Jellinek (1964) conducted early work involving temporal responses. Panelists judged bitterness intensity at timed 1-second intervals. Responses were recorded on ballots and plotted against time. Nielson (1957) also explored bitterness intensity as it developed over time, but asked panelists to mark responses directly on graph paper every 2 seconds. Beginning in the late 1970s and early 1980s, time-intensity methods began to include more dynamic methods of recording signal intensity over time, as opposed to the static methods previously used. Larson-Powers and Pangborn (1978) made a large improvement to the current temporal methods with the use of a moving chart recorder and foot pedal to record sweetness intensity in beverages. Lawless and Skinner (1979) developed a time-intensity methodology that included turning a dial attached to a strip chart recorder to record sweetness intensity. Birch and Munton (1981) also used a dial system called SMURF (Sensory Measuring Unit for Recording Flux) to record signal intensity over time.

Time Intensity

Today, time-intensity analysis is performed and analyzed with the help of computers and specialized programs. Panelists use the computer interface to track the intensity of a *single* flavor attribute continuously over time, often using a slider on the screen to record

intensity from minimum to maximum (Dijksterhuis and Piggott, 2000; Lawless and Heymann, 2010). The result of this analysis is a time-intensity curve that includes the common features maximum intensity (I_{\max}), lag time (T_{lag}), time to maximum intensity (T_{\max}), total time (T_{end}), and area under the curve (Cliff and Heymann, 1993; Dijksterhuis and Piggott, 2000). Figure 4 shows an example of a typical time-intensity curve and common parameters measured.

Training of time-intensity (TI) panelists and panel preparation is similar in many aspects to training/preparing for a descriptive analysis panel, as TI panelists are also trained and calibrated on sensory attributes and intensities (Lawless and Heymann, 2010). Peyvieux and Dijksterhuis (2001) outlined procedure steps for training a sensory panel to conduct time-intensity. The three basic steps proposed were introduction to the method, training with the use of a simple product assessment such as basic tastes, and running a TI pilot experiment. Introduction to the method consisted of first verbally explaining the method to potential panelists and introducing panelists to the computer system. Next, panelists used basic taste solutions to practice with the computer system and train with tracking a single attribute over time. The third step involved training panelists with the actual product. Attributes for the product were previously established, and consistently used attributes were pre-determined by the panel. Panelists then trained on single attributes until they could produce consistent data (replicate two out of three TI curves for the product).

Following data collection, data needs to be analyzed and interpreted. Several different methods have been previously used to analyze time-intensity data. ANOVA can be performed on raw data using the time intervals as input values (Lawless and Heymann, 2010). Another technique involves extracting the curve parameters of interest (i.e. I_{\max} , etc.)

and performing ANOVA individually across each group of parameters. This method can also involve averaging the curves of all panelists, and then recording the parameters of interest from the curve (Dijksterhuis and Piggott, 2000). Traditional analysis methods have disadvantages, however, as each panelist has a unique response and curve shape, known as the panelist's "signature," which is consistently reproduced by the panelists, but differs from the curve shapes of other panelists (Lawless and Heymann, 2010). This results from individual differences in salivary flow rate and response, which can cause differences in stimulus perception (Fischer et al., 1992). To help correct for these problems, analysis methods involving averaging TI data or fitting data to a model has also been proposed.

Lallemand et al. (1999) developed a new way to model TI results using a trapezoid model (Figure 5). Five data points were extracted from each TI curve: I_{max} , t_{i5} (time at 5% I_{max} in the increasing segment), t_{i90} (time at 90% I_{max} in the increasing segment), t_{d5} (time at 5% I_{max} in the decreasing segment), and t_{d90} (time at 90% I_{max} in the decreasing segment). Secondary parameters of duration, rate, and area of increasing and decreasing parts of the curve were also calculated to help construct the trapezoidal curves. Construction of trapezoid models is useful in discriminating between products and easily displaying this difference.

McGowan and Lee (2005) compared two TI analysis methods: grouping panelists with similar individual curves and an enhanced method (Liu and MacFie, 1990). For the first method, grouping panelists with similar individual curves, panelists' curves were grouped via curve style, accounting for ascending slope, I_{max} plateau, descending slope, and presence of an extinction point (McGowan and Lee, 2005). After grouping similar curves, a representative curve for each group was created. The enhanced method procedure involved determining average values for I_{max} , t_{start} , t_{max} , t_{dec} , and t_{end} followed by normalizing each

curve so the values of the previously listed attributes were consistent across all curves (Liu and MacFie, 1990). Next, individual curves were segmented into equal time intervals and the average intensity values for each interval were found in order to construct an overall average curve. It is important to note for the enhanced method that if all panelists did not return to zero-intensity towards the end of the time interval, it was not possible to divide the curves into equal time intervals and complete the enhanced method; the data from panelists who did not return to zero was discarded. Overall, this study found that analyzing TI data by grouping similar individual curves was accurate and representative of the individual curves, regardless if panelists returned to zero intensity. This method was more representative of curves than the enhanced method if the data from panelists that did not return to zero was not discarded.

Another method to analyze TI curves was proposed by Eilers and Dijksterhuis (2004). A parametric model was used, developed by splitting the TI curve into two parts in the middle of the plateau, the first part consisting of the rising slope and the second consisting of the falling slope. Each part was modeled as an S-shaped curve using a logistic growth function and decay function, containing five parameters related to the shape of the curve (rising slope, falling slope, start and end of the plateau, and plateau height). This model fits well with normally shaped TI curves that return to zero and don't have multiple plateaus.

Dual-Attribute Time Intensity

Dual-Attribute Time-Intensity (DATI) is another temporal method that evolved from TI, in which two attributes can be evaluated simultaneously over time (Duizer et al., 2007). This allows for evaluation of the relationship between two attributes as they evolve, and takes half the time as TI. DATI follows the same procedure as TI, with the exception of the screen interface used for evaluation. Two intensity scales (one vertical and one horizontal)

are used during the evaluation period, and both attributes are scored by moving the mouse cursor to the appropriate intersection point (Duizer et al., 1997). The curves produced are very similar to those produced from single attribute TI, and the same parameters can be extracted and analyzed.

DATI has previously been used to characterize a wide variety of products, including chewing gum, meat, and sorghum (Duizer et al., 1996; Duizer et al., 1997; Zimoch and Findlay, 1998; Kobue-Lekalake et al., 2012). In chewing gum, the relationship between the duration and intensities of sweetness and peppermint flavor was explored (Duizer et al., 1996; 1997). The results demonstrated that DATI was effective in differentiating between four chewing gum samples that differed in sweetness and flavor release speed. Gum with a fast sweetness release had a higher intensity and longer length of sweet perception, while gum with slower sweet release had a lower maximum sweet intensity and lower maximum peppermint intensity (Duizer et al., 1996). When compared to results from single attribute TI, DATI produced consistent results with the exception of one test parameter, the angle at which perception increased. This parameter was equal for single-attribute TI, but was not equal for DATI (Duizer et al., 1997). Zimoch and Findlay (1998) used DATI to evaluate toughness and juiciness of beef samples. Panelists were first trained on single attribute TI before starting DATI evaluation. Results showed that DATI gave results as good as those obtained from single attribute TI and that DATI gave a good separation of the two measured attributes. It was also concluded that DATI helped control for variability within samples, yielding more accurate and reproducible data. Kobue-Lekalake et al. (2012) explored the relationship between bitterness and astringency in sorghum with varying tannin content using DATI. The study demonstrated that bitterness and astringency were related attributes in the

samples and also that the panelist effect was significant (perception and intensity of the attributes differed from one panelist to the next).

Temporal Dominance of Sensations

Temporal Dominance of Sensations (TDS) is another recently developed temporal method in which multiple attributes (previously determined by descriptive analysis) are presented to the panelists at once using a computer screen (Pineau et al., 2009). Panelists then select which attribute is dominant at any given time. A dominant attribute can be thought of as the dominant sensation or as a new sensation appearing at a given time (Labbe et al., 2009; Pineau et al., 2009). Panelists may select a new attribute at any time during the duration of the test. Throughout the test, one attribute may be selected several times while another attribute is not selected at all.

TDS provides similar results to TI, but is a more dynamic technique that can demonstrate the evolution of several attributes over time and show the sequence of dominant sensations rather than their intensities (Le Reverend et al., 2008). Results from TDS can be graphed by calculating the proportion of runs for which an attribute was dominant and graphing these proportions against time, without consideration for the intensity of the attributes (Pineau et al. 2009). Multiple attributes can be superimposed on the same graph in order to compare the evolution of dominant attributes in a product (Figure 6).

One of the most common statistical analysis techniques used for TDS is the calculation of ‘chance level’ and ‘significance level’ of attribute dominance rates using a binomial proportion (Pineau et al., 2009). Chance level (P_0) is the dominance rate obtained by chance, while significance level is significantly higher than P_0 . Chance and significance level calculations have been previously used in several studies (Meillon et al., 2009; Morais

et al., 2014; Zorn et al., 2014). The equations for these calculations can be seen in Figure 7. Chance and significance levels are displayed on the TDS curves to indicate which attributes were truly dominant. Multiple Factorial Analysis (MFA) has been used to analyze TDS results as well (Le Reverend et al., 2008). However, MFA is not ideal for TDS analysis as data is not continuously recorded, and many curves are not bell-shaped, so the necessary parameters cannot be extracted. Le Reverend et al. (2008) were only able to analyze 25% of TDS curves using MFA due to discontinuity of the curves.

To directly compare TDS results between two products, TDS difference curves are a useful technique (Pineau et al., 2009). Attribute curves are superimposed on the same plot, but only the areas of the curve that are significantly different than zero appear on the graph ($p=0.05$). The limit of significant difference is also calculated using a binomial proportions test and plotted on the graph. Difference curves highlight differences over time between two specific products at once, and show which differences are actually statistically significant (Figure 8).

TI vs. TDS

Previous studies have compared the results between TI and TDS evaluations of the same products (Le Reverend et al., 2008; Pineau et al. 2009). Le Reverend et al. (2008) characterized samples of hot beverages using both temporal techniques. Data was analyzed using parametric modeling and MFA. ‘Score over time curves’ and related correlation coefficient values showed similar, highly correlated curves for different products were generated with both methods, although it was easier to distinguish between products using the TI data. Pineau et al. (2009) compared TI and TDS results for the sensory evaluation of five different types of dairy products. The specific types of products were not identified.

Results showed a similar pattern of attribute sensations between TI and TDS, and that TDS more effectively illustrated the sequence of attributes.

TI curves were produced by averaging the responses for each time point across all subjects and replications. TDS curves were constructed by computing the proportion of runs for which an attribute was seen as dominant and plotting this against time. Results showed many similarities between TI and TDS curves for the same products. V_{\max} values (maximum intensity score for TI and maximum dominance rate for TDS) were significantly correlated between the products, but T_{\max} values (time to reach V_{\max}) were not. This demonstrates that TI and TDS serve different purposes and one may be more useful over the other depending on the objective. TI is best to follow specific attribute intensities over time, and TDS is best when comparing temporal dominance of multiple attributes over time, but doesn't provide a full time-intensity profile for each attribute.

Comparisons between TDS and standard sensory profiling have also been made. Labbe et al. (2009) investigated the differences in product characterization of gels formulated to have different levels of flavorings, citric acid, cooling agent, and gums with standard sensory profiling and TDS. Sensory profiling was conducted by scoring attribute intensity on a 10-cm unstructured line scale based on comparison to external references. The scale was anchored with 'not at all intense' and 'very intense.' TDS also used the same 10-cm unstructured line scale, and presented all attributes at once for the test duration of five minutes. TDS scores were calculated to compare the results from sensory profiling and TDS, and a two-way analysis of variance was conducted on TDS scores (Figure 9). TDS scores account for the average of each attribute's intensity and duration during the TDS evaluation period. Results showed that both methods effectively discriminated the products, and

attribute correlations on PCA biplots were very similar (RVD coefficient=0.84).

Additionally, TDS provided information about attribute perception after consumption, which was not available using classic sensory profiling.

TI and TDS Evaluation of Sweeteners

TI and TDS have been used in many previous studies to evaluate the temporal nature of high-intensity sweeteners in water and various food matrices such as coffee, chocolate, fruit jam, and dairy desserts. Azevedo et al. (2015) evaluated samples of espresso coffee sweetened with sucrose, sucralose, aspartame, neotame, stevia, and cyclamate/saccharin (2:1) using TI analysis. There was no significant difference between alternative sweetener samples and sucrose for time to reach I_{\max} . Stevia had the highest maximum sweetness intensity (I_{\max}), with aspartame, sucralose, and cyclamate/saccharin having significantly lower I_{\max} values. Sucralose and cyclamate/saccharin were the only sweeteners that had significantly different sweetness I_{\max} values compared with sucrose. Results from ANOVA and PCA showed that neotame, aspartame, and sucralose had the most similar temporal profiles when compared with sucrose in the espresso coffees. Ott et al. (1991) used TI to evaluate sweetness and bitterness profiles of aspartame, acesulfame-K, and sucrose in water. Aspartame was characterized by greater sweetness intensity than sucrose with a lingering sweet aftertaste. Acesulfame-K was characterized by an intense and lingering bitter taste not present in sucrose or the other non-nutritive sweeteners tested.

At equivalent sweetness concentrations, mixtures of alternative sweeteners had similar maximum sweetness intensities in fruit jams compared with sucrose, and overall had very similar time-intensity sweetness profiles (Souza et al., 2013). The sweeteners evaluated

included sucralose, sucralose/acesulfame-K (3:1), and sucralose/stevia (2:1). The alternative sweetener mixtures differed from sucrose for time-intensity bitterness curves, however. Samples sweetened with sucrose had significantly lower I_{\max} , T_{\max} , and curve area values for bitter taste compared with jam samples sweetened with sucralose/acesulfame-K/stevia. Palazzo et al. (2011) used TI to evaluate sucrose substitution with neotame, rebaudioside, and sucralose in diet chocolate. Sucralose had the most similar sweetness curve compared with sucrose. Samples sweetened with 0.075% sucralose were not statistically different from 43% (w/w) sucrose chocolate samples for I_{\max} , T_{\max} , T_{tot} , or sweetness curve area, thus making sucralose the best choice as a sweetener substitute in diet chocolate. Zamora et al. (1998) evaluated samples of fructose and sucrose in water at varying concentrations (5-45% w/v) using TI. Fructose had higher maximum sweetness intensity at concentrations of 25, 30, and 35% compared with sucrose.

Zorn et al. (2014) and Morais et al. (2014) used TDS to evaluate sweeteners in orange juice and chocolate dairy desserts, respectively. The attribute list for TDS evaluation of orange juices consisted of the terms sweet, sour, orange flavor, bitter, astringent, and off-flavor. Samples sweetened with sucrose, sucralose, and four types of stevia were evaluated. Sucrose samples were characterized by a dominant sweet taste with a dominant sour taste towards the end of the time window. Sucralose has similar TDS curves to sucrose, and was also dominated by sweet/sour tastes. Stevia-sweetened samples were different from sucrose TDS curves, due to the dominance of bitterness and off-flavor attributes. Sucrose substitutes in chocolate dairy desserts included sucralose and stevia. TDS curves for sucrose were initially dominated by sweet taste, followed by bittersweet chocolate taste. Sucralose-sweetened samples showed similar TDS profiles, characterized by dominance of sweet taste

followed by bittersweet chocolate taste. Samples sweetened with stevia were again characterized by a lasting, dominant bitter taste. Sweet taste and bittersweet chocolate flavor were also dominant, but for fractions of the length of bitter taste.

Temporal Check-All-That-Apply

Temporal Check-All-That-Apply (TCATA) is a novel temporal method based on and serving as an extension of the traditional Check-All-That-Apply (CATA) method. CATA questions list several response options, from which respondents may choose as many selections as they feel apply (Smyth et al., 2006). CATA questions don't limit consumer responses, thus allowing focus on multiple attributes at once. Attributes included as response options for CATA questions are often selected by a trained panel to provide an accurate representation of the products (Varela and Ares, 2012). Raw data is organized in matrices, with each response option in a separate matrix. Multiple Correspondence Analysis can be used on the matrices to determine the similarities and differences between samples (Ares et al., 2010). While CATA questions are often very useful to evaluate food products, they also have some limitations. CATA questions don't provide quantitative information about attribute intensity, and results can be largely skewed by response order. Consumers tend to select responses that occur near the top of the list, making it important to randomize response choices (Castura, 2009; Varela and Ares, 2012). Additionally, CATA is not the best choice to use for the discrimination of products, as the same terms will likely be selected for similar products.

TCATA differs from the previously described TI and TDS in that panelists are continually selecting or deselecting *all* attributes they perceive as applicable to a sample throughout the test duration. Multiple attributes can be selected at once, allowing for

panelists to show the evolution of various traits for a product (Castura et al. 2015). TCATA is presented using a computer or tablet, on which all previously determined attributes appear during the test.

Castura et al. (2015) used TCATA to evaluate flavored yogurts over 30-second sessions. TCATA curves were constructed using a procedure similar to the procedure used for TDS curve construction (Pineau et al., 2009) (Figure 10). Proportions for each attribute selection across time for all runs were calculated and plotted against time, then smoothed using the smoothing the smoothing spline function in R. The resulting plot displayed all attribute TCATA curves for each product on a separate plot. TCATA is also useful for non-food applications, such as cosmetic products. Boinbaser et al. (2015) used TCATA to evaluate the evolution of sensory characteristics in cosmetic creams. Evaluation consisted of nine attributes and lasted 60 seconds. Raw data was transformed into line plots based on the proportion of times each attribute was selected at each time during the evaluation.

It is suggested that variance in panelist thresholds for selecting vs. unselecting an attribute may affect results and needs to be researched further (Castura et al., 2015). There is also little known about the ideal number of terms to include on a TCATA ballot, although it is suggested it is similar to the number of terms used for a TDS ballot.

CONCLUSIONS AND OBJECTIVES

The objectives of this project are to compare the temporal effects of natural non-nutritive sweetener blends in neutral pH powdered whey protein beverages at two different protein loads (15 and 25 grams per serving) using the temporal methods TI, TDS, and TCATA and to determine what formulations are preferred by consumers.

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TABLES

Table 1.1 Composition ranges of dried whey products (adapted from Foegeding and Luck (2011)).

Ingredient	Protein (% dry wt.)	Moisture (%)	Lactose (%)	Fat (%)	Ash (%)
WPC 35 range	34.0-36.4	2.9-4.0	47.0-56.0	2.5-4.0	1.6-8.0
WPC 80 range	72.9-82.8	3.0-5.2	0.15-7.4	1.0-10.0	2.5-11.0
WPI range	90-95	4-6	0.2-2.0	0.2-1.5	0.3-4.5

FIGURES

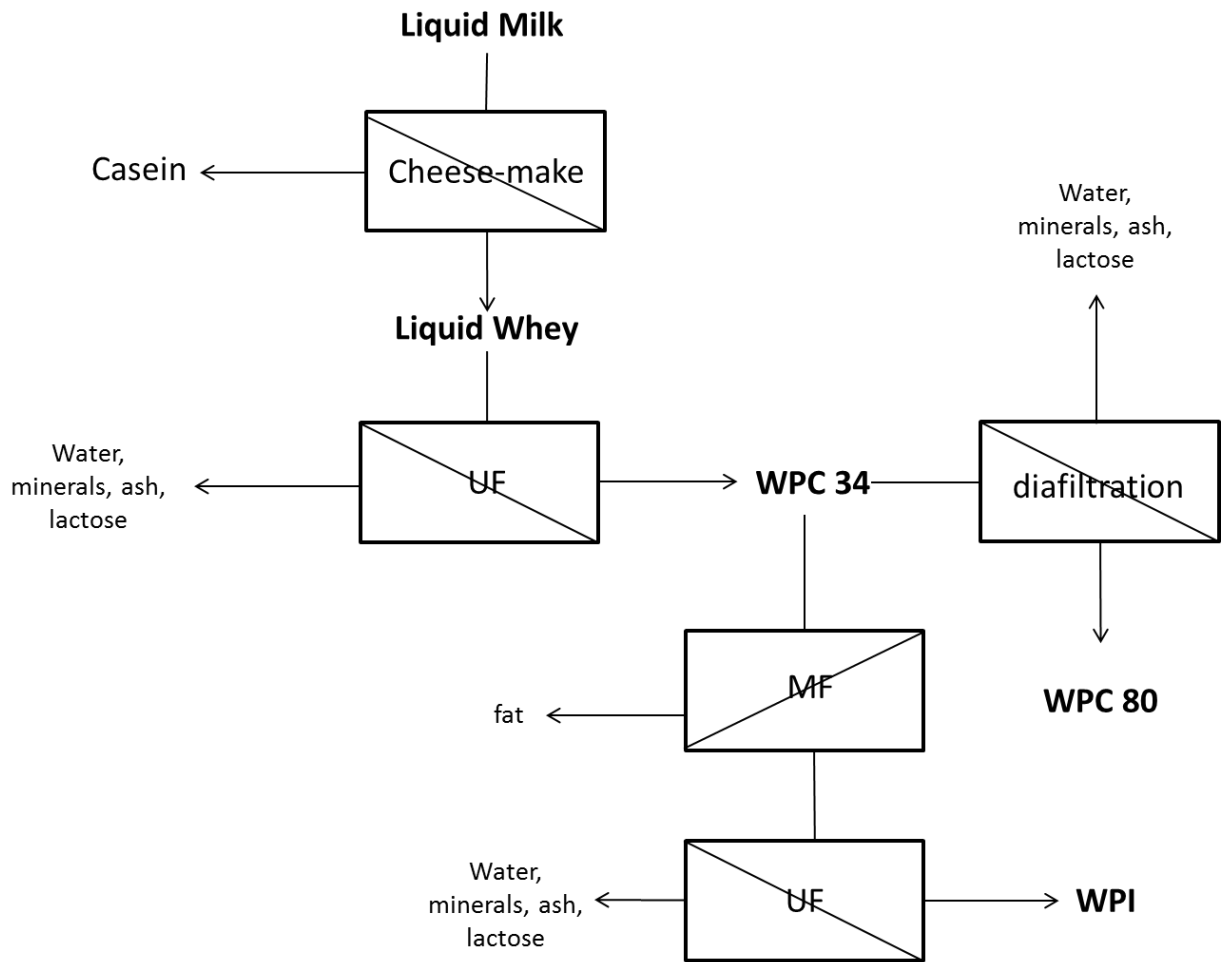


Figure 1.1 Summary of liquid whey processing (adapted from Bylund, 2003).

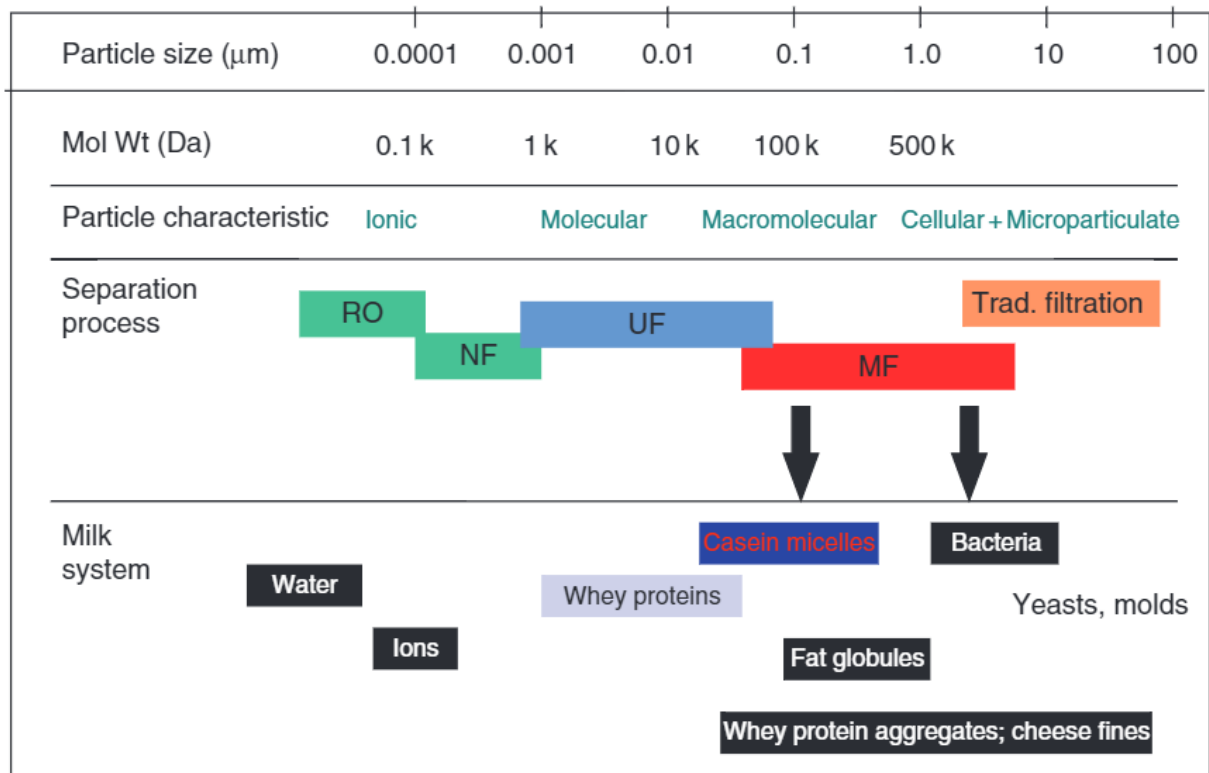


Figure 1.2 Membrane separation processes used in whey processing and corresponding molecular weight cutoffs (taken from Kelly, 2011).

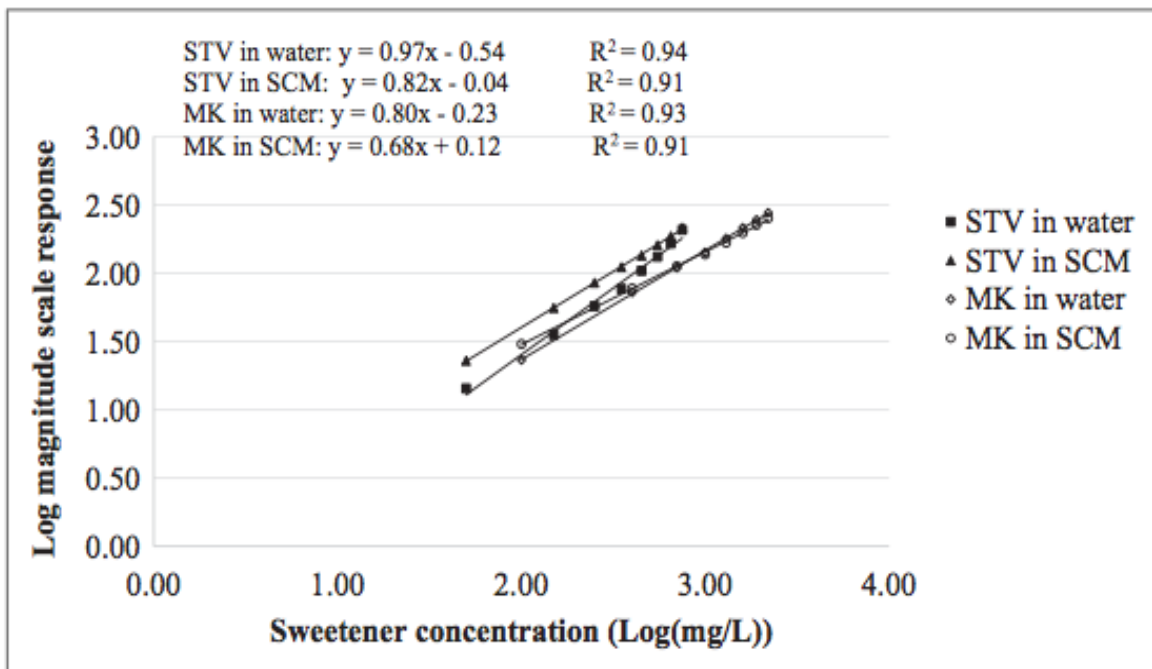


Figure 1.3 Plot of results from magnitude estimation scaling of monk fruit and stevia sweetness equivalence in water and skim chocolate milk compared with sucrose (taken from Li et al., 2015).

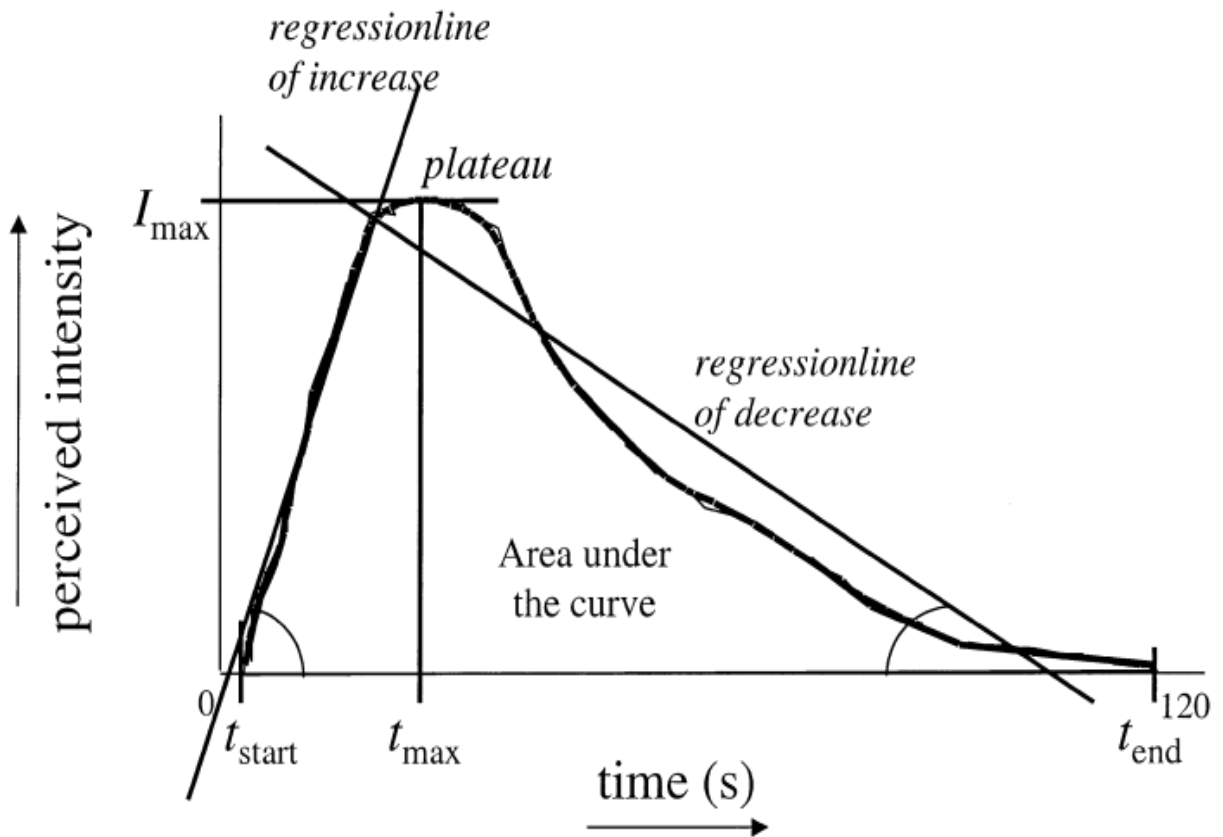


Figure 1.4 Time intensity curve with common parameters labeled (taken from Dijksterhuis and Piggot, 2000).

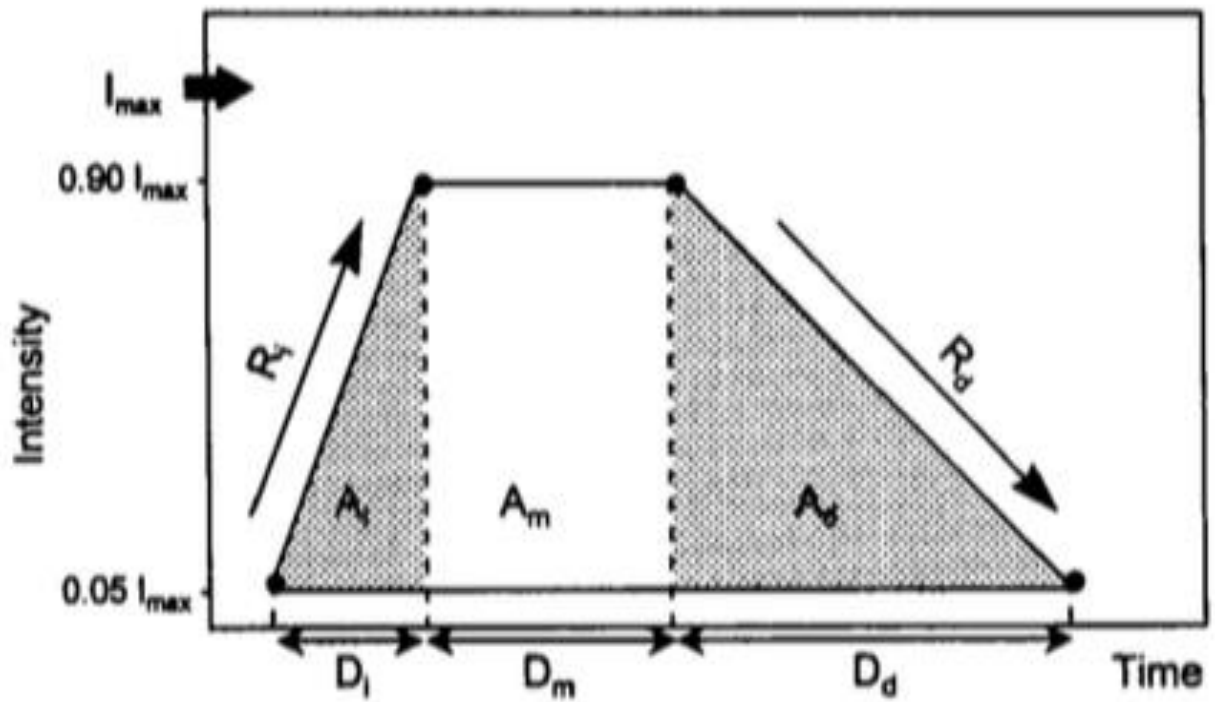


Figure 1.5 Trapezoid model used to display and analyze time-intensity data (taken from Lallemand et al., 1999).

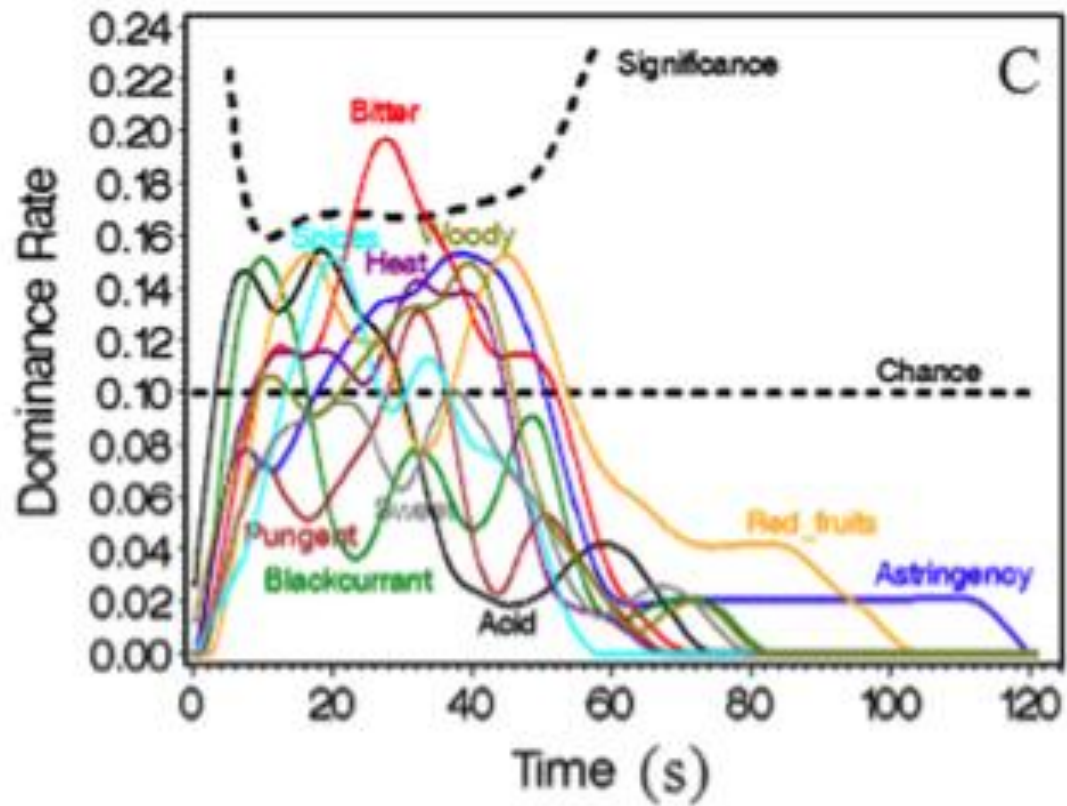


Figure 1.6 TDS curves from red wine in which several attributes have been superimposed on one graph (taken from Meillon et al., 2009).

$$P_s = P_0 + 1.645 \sqrt{\frac{P_0(1 - P_0)}{n}}$$

Figure 1.7 Equation for calculation of significance level for TDS results (Pineau et al., 2009).

P_s = Significance level

P_0 = Chance level

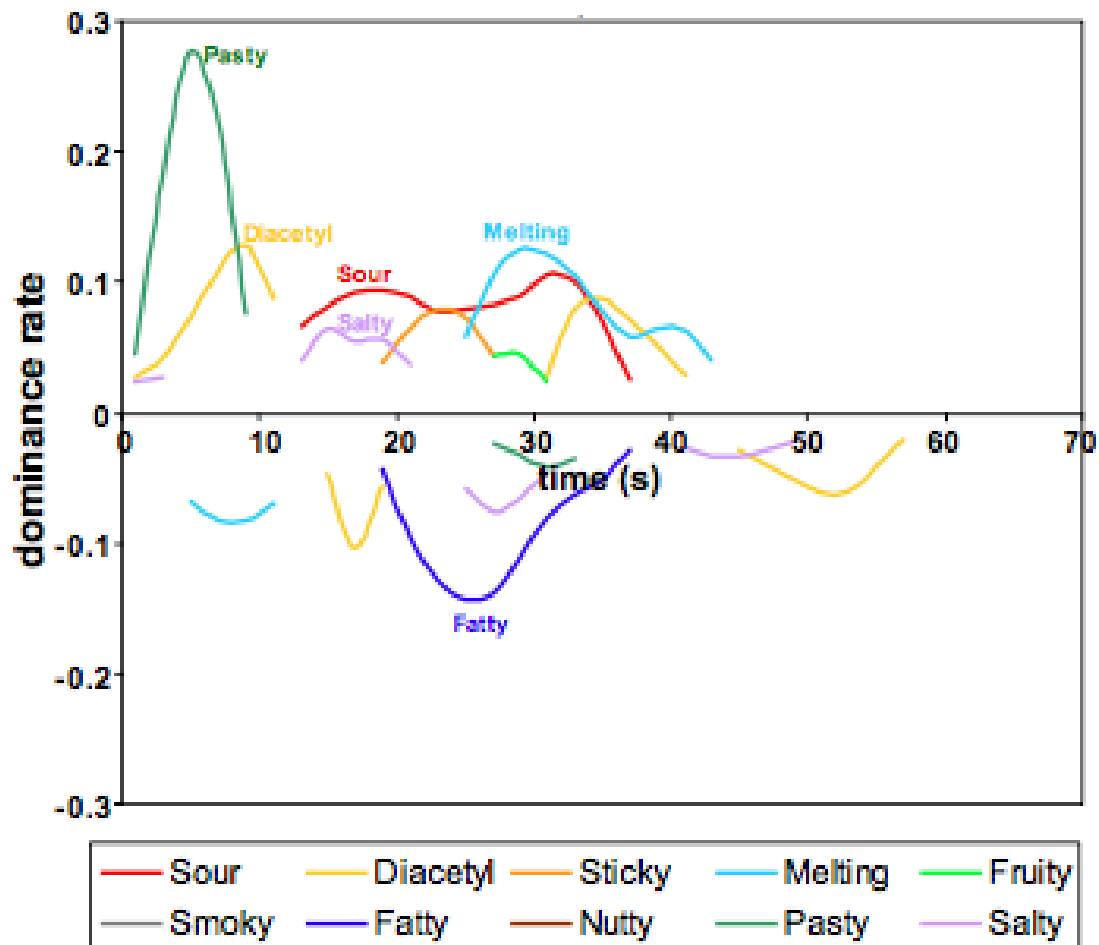


Figure 1.8 Temporal dominance of sensations difference curve illustrating the differences between two (unidentified) dairy products (taken from Pineau et al., 2009).

$$\text{SCORE} = \left(\sum_{\text{Scoring}} \text{Intensity} \times \text{Duration} \right) // \left(\sum_{\text{Scoring}} \text{Duration} \right)$$

Figure 1.9 Equation for calculation of a TDS score (taken from Labbe et al., 2009).

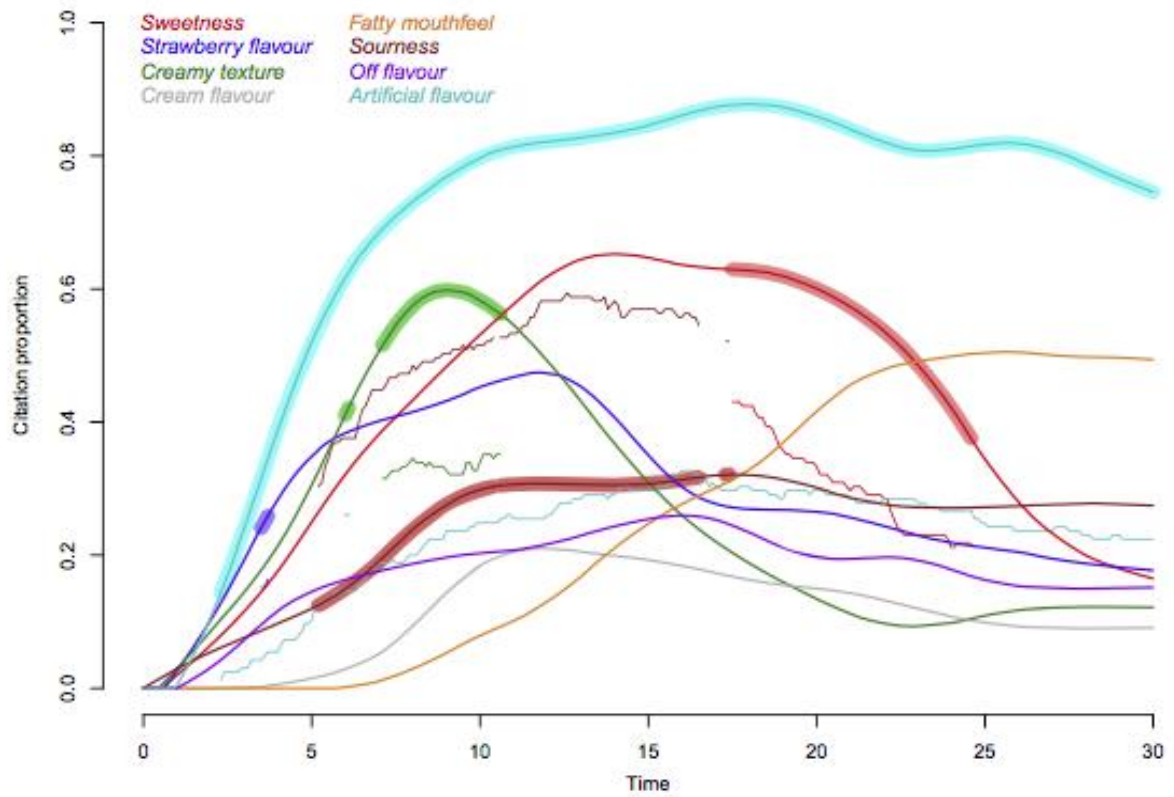


Figure 1.10 Temporal check-all-that-apply curve for one yogurt sample, constructed by plotting attribute proportion over time (taken from Castura et al., 2015).

CHAPTER 2:
COMPARISON OF NATURAL SWEETENERS IN PROTEIN BEVERAGES USING
TIME-INTENSITY, TEMPORAL DOMINANCE OF SENSATIONS,
AND TEMPORAL CHECK-ALL-THAT-APPLY.

**Comparison of Natural Sweeteners in Protein Beverages using Time-Intensity,
Temporal Dominance of Sensations, and Temporal Check-All-That-Apply.**

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ABSTRACT

Protein beverage consumption by Americans has increased in recent years. Coupled with this increased consumption is an interest in natural sweeteners. Each sweetener has unique temporal properties that can influence beverage sensory properties and consumer acceptability. Iso-sweet concentrations of sweeteners (sucralose, sucrose, fructose, stevia, monk fruit) in ready-to-mix (RTM) vanilla whey protein beverages (25 g protein/12 oz. water) were established using magnitude estimation scaling and 2-alternative forced choice testing. Temporal sensory profiling was then conducted on each beverage by a trained panel using Time Intensity (TI), Temporal Dominance of Sensations (TDS), and Temporal Check-All-That-Apply (TCATA). These findings were used to formulate seven natural sweetener blends that more closely matched the temporality of sucrose sweetened RTM vanilla protein beverages. These blends were also subjected to temporal sensory profiling in RTM beverages. One sugar free blend (25% stevia/75% monk fruit) and one reduced sugar blend (25% stevia/25% monk fruit/25% fructose) were then selected for consumer testing, in addition to 3 control RTM beverages containing sucralose, stevia, or monk fruit. A two-day crossover design study (n=150 consumers) was conducted to investigate the impact of priming statements about sweetener as well as sweetener source on consumer acceptance of RTM protein beverages. Two distinct consumer clusters were identified. The 'label conscious' segment of consumers preferred beverages sweetened with natural blends when primed. The 'flavor driven' segment of consumers conceptually preferred naturally sweetened beverages, but preferred sucralose sweetened beverages when primed. An "all natural" label claim was most preferred across all consumers. Application of these findings to commercially produced RTM protein beverages aids in development of naturally sweetened

protein beverages with reduced calories and desirable sensory properties, and highlights the importance of label claims to consumers overall, but in particular to a ‘label conscious’ segment of consumers.

Key Words: protein beverages, natural sweeteners, consumer acceptance

INTRODUCTION

Protein beverages have increased in popularity among American consumers in recent years as more Americans aim to increase their dietary protein intake (Gerdes, 2012; Jacobson, 2015). ‘High protein’ is also a top beverage industry trend, and was selected as the #1 trend in a 2015 Beverage Industry Survey (Jacobson, 2015). One of the most common protein sources for protein beverages is whey protein; either in the form of whey protein concentrate 80 (WPC80) or whey protein isolate (WPI). Both products are produced from membrane filtration of liquid whey, a process that concentrates protein and removes lactose, fat, and minerals (Foegeding and Luck, 2011). Whey proteins are a complete source of essential amino acids and are high in branched-chain amino acids that may aid in muscle recovery after exercise, making it an ideal protein source in protein beverages (Blomstrand and Saltin, 2001; Hazen, 2006).

Sweetener type, flavor, amount of protein, low calorie, and protein source are of top importance to consumers when selecting protein beverages for purchase (Oltman et al., 2015; Mintel Group Ltd., 2016b). Previous research has also demonstrated the importance of naturally sweetened protein beverages with low carbohydrate content to consumers (Gerdes, 2012; Jacobson, 2015; Oltman et al., 2015). However, sweet taste is also a desirable attribute to many consumers, and direct sugar removal may negatively impact consumer liking. Previous literature demonstrates a threshold for direct sugar removal in various dairy products of 25-40% without negatively impacting consumer acceptance (Cadena et al., 2012; Chollet et al., 2013; Hoppert et al., 2013; Li et al., 2015; Oliveira et al., 2015). Beyond this threshold, non-nutritive alternative sweeteners can be used to replace sugar and maintain sweet taste.

Natural non-nutritive sweeteners have a more positive public perception than artificial non-nutritive sweeteners, especially in protein beverages (Bearth et al., 2014; Oltman et al., 2015). Natural non-nutritive sweeteners are derived from plants and are composed of natural compounds, typically sweet glycosides (Kim and Kinghorn, 2002). *Siratia grosvenorii* (monk fruit) is a fruit native to southern China that contains sweet glycosides mogroside IV, mogroside V, and mogroside VI (Kinghorn and Compadre, 2001; Pawar et al., 2013; Mintel Group Ltd., 2015). Stevia is another popular natural sweetener that is extracted from the leaves of *Stevia rebaudian*, a sweet herb native to northern Paraguay (Savita et al., 2004). Stevia is composed of sweet glycosides stevioside and rebaudioside A (Kinghorn et al., 2001). Crystalline fructose is a caloric sweetener option, but has a higher relative sweetness than sucrose (White and Osberger, 2001) and can be found in protein beverages for this reason.

In order to best formulate sucrose replacement with alternative sweeteners, the sweetness equivalence (iso-sweetness), or the amount of alternative sweetener that produces the same sweetness intensity must first be determined to maintain sensory properties of the product (Cardello et al., 1999). This is done by conducting magnitude estimation scaling, followed by descriptive analysis and 2-Alternative Forced Choice Testing (2-AFC) (Lawless and Heymann, 2010; Li et al., 2015). Magnitude estimation scaling (MES) has been used previously to determine iso-sweetness of various alternative sweeteners in dairy products and other food matrices (Cardoso and Bolini, 2007; Lisak et al., 2012; Reis et al., 2011; Souza et al., 2013; Azebedo et al., 2015; Li et al., 2015).

Following confirmation of iso-sweetness, temporal sensory evaluation methods are often useful to illustrate how the perceived sweet taste and/or other sensations and intensities

of alternative sweeteners evolve over time (Lawless and Heymann, 2010). These temporal methods include Time Intensity (TI), Temporal Dominance of Sensations (TDS) and Temporal Check-All-That-Apply (TCATA). TI involves the trained panel evaluation of a single attribute over time, with results yielding parameters of maximum intensity (I_{\max}), lag time (T_{lag}), time to maximum intensity (T_{\max}), total time (T_{end}), and area under the curve (Dijksterhuis and Piggott, 2000; Lawless and Heymann, 2010). TDS and TCATA do not involve scaling of individual attribute intensities; instead multiple attributes (previously determined by descriptive analysis) are presented to the panelists at once using a computer screen (Pineau et al., 2009). For both of these methods either trained panelists or consumers can be used. Panelists select the *single* attribute they perceive as dominant at any given time in TDS, and select *all* attributes they detect in TCATA. Results from TDS and TCATA are displayed as attribute curves or band plots, with chance and significance levels calculated using a binomial proportion (Pineau et al., 2009). Temporal sensory evaluation is especially useful for alternative sweeteners because although alternative sweeteners are useful to reduce sugar, carbohydrates, and calorie content, they may have a different sweetness temporality compared to sucrose at iso-sweetness and they also may contain undesirable off flavors such as bitter or metallic tastes (Kim and Kinghorn, 2002; Souza et al., 2013; Morais et al., 2014; Zorn et al., 2014).

Several previous studies have evaluated sweeteners in food and beverage products using temporal sensory methodologies (Palazzo et al., 2011; Azevedo et al., 2015; Souza et al., 2013; Morais et al., 2014; Zorn et al. 2014). These methods have also been used for other food products such as hot beverages, flavored gels, red wine, and dairy products (Le Reverend et al., 2008; Labbe et al., 2009; Meillon et al., 2009; Pineau et al., 2009; Castura et

al., 2015). While previous work has investigated the temporal evaluation of alternative sweeteners in food and beverage products, a large majority of this work has focused on artificial non-nutritive sweeteners. In comparison, relatively little work has been done to investigate and characterize the temporality of natural non-nutritive sweeteners, particularly monk fruit. In addition, there has yet to be a study that investigates how the addition of alternative sweeteners to whey protein beverages affects their sensory properties. The objective of this study was to profile the sensory properties of ready-to-mix (RTM) whey protein beverages sweetened with natural sweeteners stevia, monk fruit, and crystalline fructose, and blends of these sweeteners using three temporal methods: Time Intensity (TI), Temporal Dominance of Sensations (TDS), and Temporal-Check-All-That-Apply (TCATA). Through temporal sensory evaluation of these beverages, the ultimate goal was to formulate a natural sweetener blend that could be added to RTM protein beverages to provide sweetness without added sugar, calories, or carbohydrates, while still appealing in taste to consumers.

MATERIALS AND METHODS

Sample Preparation

Protein beverages were prepared according to a common industry formulation to contain either 15 or 25g protein per 360mL (12 oz.) in water (Table 2.1). Two sources of whey protein isolate (WPI) (Cheddar, microfiltration, northwest commercial supplier; Cheddar, anion exchange, midwest supplier) were used to make ready-to-mix (RTM) protein beverages, using deionized water, sweetener, and vanilla flavoring (Natural Vanilla 1032, Natural Cream 0151, Natural French Vanilla 1068; Flavor Artistry, Corona, CA). Both WPI sources were obtained as spray dried powders directly from the plant, within 30 days of

production and stored at -80° C throughout the duration of the study. Sucrose (Imperial Sugar Co., Sugar Land, TX), crystalline fructose (KRYSTAR® 300; Tate & Lyle, Decatur, IL), sucralose (Hard Eight Nutrition; Henderson, NV), monk fruit extract (PUREFRUIT™ Select Monk Fruit extract; Tate & Lyle, Decatur, IL) and stevia leaf extract (TASTEVA™ Stevia Sweetener; Tate & Lyle, Decatur, IL) were added as sweeteners to the RTM vanilla protein beverage base. All subsequent sensory testing was approved by the NCSU Institutional Review Board for human subjects.

Power Function Curves of Natural Non-Nutritive Sweeteners in Water, Protein Base, and Vanilla Flavored Protein Beverages

Power function curves were generated for stevia, monk fruit, fructose, and sucralose in water, WPI solution (15 or 25g protein/360 mL), and vanilla flavored WPI beverages (15 or 25 g protein/360 mL) by trained panelists (n=8, 5 females, 3 males, ages 22-30 y). Each panelist had a minimum of 40 h of previous descriptive analysis experience using the Spectrum™ method (Meilgaard et al., 2007) with an additional 6 h of training with magnitude estimation scaling.

Magnitude estimation scaling (MES) was used to build power function curves for each sweetener at both high and low protein levels for each WPI source. MES curves were first established for each sweetener in water only, as a training exercise. Next, curves were determined for each sweetener in a WPI solution at each protein level (15 or 25g protein/360 mL) and for each protein source (microfiltered or anion exchange) to investigate differences in iso-sweet values between protein levels and sources. Lastly, curves were determined for vanilla flavored WPI beverages at each protein level.

For MES exercises, a 7% sucrose solution was used as the reference sweet solution, and was assigned an arbitrary value of 100. The reference sweetness intensity was chosen based on a category screening of commercial vanilla-flavored RTM and ready-to-drink (RTD) protein beverages. Samples were served at 21 °C as 20-mL aliquots in lidded 60-mL soufflé cups labeled with random 3-digit codes. Serving order was randomized within each session. Panelists were instructed to taste each sample against the reference solution and assign a ratio score based on the sample's relative sweetness compared to the reference. For example, if a sample was half as sweet as the reference, the panelist would give it a score of 50. Each MES series contained 7 samples that covered a concentration range from detectable sweetness to well above the sucrose reference. Concentration ranges and factors were determined based on previous literature values (Cardello et al., 1999; Savita et al., 2004; Reis et al., 2011; Lisak et al., 2012; Morais et al., 2014; Zorn et al., 2014; Azevedo et al., 2015; Li et al., 2015). Each replicate of each series was evaluated in a separate session. Panelists expectorated solutions. A 5 min rest was enforced between samples, during which panelists rinsed with DI water. All series were evaluated in triplicate by each panelist using paper ballots.

Determination and Confirmation of Iso-sweetness Values in Vanilla Flavored Protein Beverages

Descriptive analysis was used to confirm and adjust iso-sweetness values from MES (Li et al., 2015). No significant differences in iso-sweetness values were documented between protein amounts (15 vs 25g/360 mL) or source (microfiltration vs anion exchange) in MES ($p>0.05$), so all beverage formulations were confirmed at one protein level (25 g/360 mL) and one WPI source (microfiltered) with and without vanilla. A trained panel evaluated

each sweetened protein beverage in duplicate compared to the 7% sucrose reference (described later). Iso-sweetness values for sweetened vanilla protein beverages were also confirmed with 2-Alternative Forced Choice Testing (2-AFC) (n=40, ASTM E2164; Li et al., 2015). Untrained consumers were recruited from the Sensory Service Center database, and were required to be >18 y. Food treats were provided as incentive after each day of testing. Up to three sample pairs were tested in each session.

For 2-AFC, samples of vanilla flavored protein beverage sweetened with stevia, monk fruit, sucralose, or fructose were presented against a reference vanilla protein beverage sweetened with 70g sucrose/L. All samples were served as 20-mL aliquots in lidded 60-mL soufflé cups labeled with random 3-digit codes. Samples were served at 21 °C. The presentation order was balanced within and between sample pairs (Meilgard et al. 2007). Stevia was always served in the last position due to lingering sweetness (Dubois and Prakash, 2012). Subjects were instructed to taste the samples from left to right and select the sample they thought was sweeter. A 5 min rest was enforced between each sample, during which panelists rinsed with DI water and unsalted crackers. All data was collected using Compusense Cloud software (Compusense, Guelph, Canada).

Formulation of Natural Sweetener Blends

Results from power function curves and iso-sweetness confirmation for individual sweeteners were used to formulate seven natural sweetener blends consisting of monk fruit, stevia, and/or fructose (Blend 1=25% stevia/75% fructose; Blend 2=25% monk fruit/75% fructose; Blend 3=50% stevia/50% monk fruit; Blend 4=25% stevia/25% monk fruit/50% fructose; Blend 5=50% stevia/50% fructose; Blend 6=50% monk fruit/50% fructose; Blend

7=25% stevia/75% monk fruit). Blends were divided into two categories: reduced sugar (Blend 1, Blend 2, Blend 4, Blend 5, Blend 6) and sugar free (Blend 4, Blend 7) Each blend was added to the vanilla flavored protein beverage base. Iso-sweetness of blends to a 7% sucrose solution was again confirmed using descriptive analysis and 2-AFC testing as previously described (n=40).

Descriptive Analysis

Descriptive analysis using the Spectrum™ method (Meilgard et al. 2007) was conducted to evaluate protein beverages sweetened with sucrose, fructose, sucralose, monk fruit, stevia, and the seven natural blends of fructose, monk fruit, and stevia. Evaluations were done for each sweetener or sweetener blend at one protein level (25 g/360 mL) and one protein source (microfiltered) to confirm iso-sweetness. Samples were evaluated with and without vanilla flavoring to determine the effect of the vanilla flavor on sweet taste.

All samples were evaluated in triplicate by a trained descriptive panel, consisting of 8 trained panelists (all female, ages 22-55y). Each panelist had a minimum of 40 h descriptive analysis training using the the Spectrum™ method (Meilgard et al. 2007) and at least 40 h of additional experience documenting the sensory properties of dairy products including protein beverages (Russell et al., 2006; Wright et al., 2006; Drake et al., 2009). Samples (45 mL) were served at 21 °C in lidded 60-mL soufflé cups labeled with random 3-digit codes. A 5 min rest was enforced between samples. Attributes scored were aroma intensity, vanilla flavor, cardboard flavor, bitter taste, sweet taste, metallic taste, astringency, and aftertaste intensity. Paper ballots were used.

Temporal Sensory Evaluation Methods

The temporal sensory properties of vanilla flavored RTM protein beverages sweetened with sucrose, fructose, stevia, monk fruit, sucralose, and natural sweetener blends at protein levels of 15g and 25g per 360 mL serving were investigated by trained panelists. All samples were served at 21 °C as 20-mL aliquots in lidded 60-mL soufflé cups labeled with 3-digit codes. Preparation occurred one day prior to testing, by mixing all dry ingredients, rehydrating with DI water, and pouring into sample cups. All samples were stored at 4 °C overnight, and evaluated at 21 °C. All data was collected on I-Pads using Compusense Cloud Software (Compusense, Guelph, Canada). All evaluations were done in triplicate.

Two separate panels were used: one for TI evaluation and one for TDS and TCATA evaluations. TI panelists (n=8; 1 male, 7 females; ages 24-55y) had at least 80 h of prior descriptive analysis experience and at least 30 h of previous experience with TI methodology and evaluation. TDS and TCATA panelists (n=8; 4 males, 4 females; ages 22-35 y) also had prior descriptive analysis experience (>80 h each) and participated in two 30 min instruction and practice sections for each temporal method prior to beginning data collection.

Time Intensity

Panelists were instructed to press the start button, immediately place the entire sample in their mouths, and begin evaluation. Instructions to expectorate appeared at 12s, and evaluation took place through 90 s. Throughout evaluation, panelists were instructed to move a cursor to the appropriate intensity level for the attribute using a 0 to 15-point Spectrum™ scale (Meilgard et al. 2007). A rest period of 5 min was enforced between each sample,

during which panelists were instructed to rinse with DI water and unsalted crackers. Attributes of sweet, bitter, and metallic were evaluated for each sample on separate days. Before beginning evaluation, panelists warmed up with appropriate reference solutions (5, 7, and 9% w/w sucrose solutions (sweet taste); 0.5% w/w caffeine solution (bitter taste); or 0.4% w/w copper sulfate solution (metallic)).

Temporal Dominance of Sensations

Panelists were instructed to place the entire sample in their mouths, press the start button, and begin evaluation. Instructions to expectorate were shown at 12 s, and evaluation took place through 192 s. A rest period of 5 min was enforced between samples. Panelists were instructed to select the single attribute perceived as dominant at any point during the evaluation period (Pineau et al., 2009). Unlimited selections as well re-selections were allowed. The attributes sweet, bitter, metallic, sweet aromatic, cardboard, and none were displayed simultaneously on the I-pad interface. After the point at which no sensations were perceived, panelists were instructed to select none.

Temporal Check-All-That-Apply

Temporal-Check-All-That-Apply (TCATA) followed the same evaluation procedure as Temporal Dominance of Sensations, except that selection of multiple attributes *at one time* was allowed (Castura et al., 2015). The attributes displayed simultaneously were sweet, bitter, metallic, sweet aromatic, cardboard, and none. Unlimited selections and re-selections were allowed for all applicable attributes, and panelists were instructed to select ‘none’ when sensations were no longer perceived. Attributes were not scaled for intensity.

Consumer Acceptance Testing

Subsequently, a central location test (CLT) was conducted at North Carolina State University to investigate consumer liking and acceptance for vanilla flavored RTM whey protein beverages sweetened with various natural sweeteners and natural sweetener blends. Beverages were sweetened with sucralose, monk fruit, stevia, a stevia/monk fruit blend ((25:25:50); Natural Blend 1), and a monk fruit/stevia/fructose blend ((25:25:50); Natural Blend 2). Sucralose, monk fruit, and stevia sweetened beverages served as controls. Sucralose was selected due to its prevalence in commercial food and beverage products. Monk fruit and stevia were selected as reference points to compare against the natural blends. All natural blends were divided into two categories (reduced sugar and sugar free). The blend from each group that most closely matched the temporal sensory properties of the sucralose control was also selected. Blend 7 (25% stevia/75% monk fruit) was selected as the sugar free blend and Blend 4 (25% stevia/25% monk fruit/50% fructose) was selected as the reduced sugar blend.

Consumers (n=150) evaluated five protein beverages with and without priming statements in a 2-day crossover design test (Li et al., 2015; Hubbard et al., 2016). Test days were separated by 4 weeks to prevent memory effects. Half of consumers read priming statements prior to tasting each sample for each testing day. The other half of consumers evaluated the same beverages and answered the same questions without seeing priming statements. The order of test days (primed vs. unprimed) was randomly assigned.

Consumers were recruited from the database of >8000 respondents maintained by the Sensory Service Center (NCSU, Raleigh, NC). In order to qualify, consumers were required

to be 18-54 y, self-reported exercisers (once a week or more), self-reported RTM protein powder consumers (at least 2 times per month), and vanilla flavor acceptors. Additionally, respondents were required to have an awareness of non-sucrose sweeteners in products they consumed (report consumption of non-sucrose sweeteners at least once in past 3 months). Participants were compensated with a gift card after completion of the second testing day.

Sample beverages contained 25g protein/360 mL serving size, as this is similar to protein concentrations in rehydrated commercial RTM protein powders. Dry ingredients were weighed, mixed, and stored in airtight containers 12 h prior to tests. Dry mixes were rehydrated in 500 mL of DI water (kept at 4 °C) 15 min prior to serving using shaker bottles with metal blender balls (Blender Bottle, Lehi, UT). Sixty mL (2 oz.) aliquots were served in lidded 120 mL soufflé cups labeled with random 3-digit codes. A 5 min rest was enforced between samples, during which panelists were instructed to cleanse their palates with DI water and unsalted crackers.

For each day of testing, consumers evaluated all samples in a complete balanced, randomized design and data was collected using Compusense Cloud (Guelph, Canada). Consumers tasted all five RTM protein beverage samples each session, and answered questions about overall liking, sweetness, vanilla flavor, thickness, quality, and purchase intent. Liking was scored on a 9-pt hedonic scale with 1=dislike extremely and 9=like extremely. Quality and purchase intent were scored on 5-pt scales with 1=extremely low quality/definitely would not purchase and 5=extremely high quality/definitely would purchase. Aftertaste liking was scored only when aftertaste was indicated.

Priming statements about the sweetener used in each beverage were displayed prior to tasting in primed sessions. For the sucralose control: “The protein beverage you are about to taste is sweetened with sucralose and is sugar/carbohydrate free.” For the monk fruit control: “The protein beverage you are about to taste is naturally sweetened with monk fruit extract and is sugar/carbohydrate free.” For the stevia control: “The protein beverage you are about to taste is naturally sweetened with stevia leaf extract and is sugar/carbohydrate free.” For Natural Blend 1: “The protein beverage you are about to taste is naturally sweetened with monk fruit and stevia leaf extracts and is sugar/carbohydrate free.” For Natural Blend 2: “The protein beverage you are about to taste is naturally sweetened with extracts from stevia leaf and monk fruit, and natural fructose crystals and has >50% less sugar than a regular sweetened protein beverage with sucrose.”

Statistical Analyses

Power curves were constructed using the geometric mean of the average response at each sweetness concentration level (ASTM E1697-05). The logarithm of this number was plotted against the logarithm of the sample sweetener concentration to create the power curve. Following power curve generation, the iso-sweet concentrations were confirmed using the minimum number of ‘correct’ judgments for significance through 2-AFC testing ($\alpha=0.05$; Meilgaard et al., 2007; ASTM E2164).

Linear regression models were generated to compare power function curves using SAS Version 9.4 (SAS Institute Inc., Cary, NC). Iso-sweet values between protein source and protein level for each individual sweetener (sucralose, fructose, monk fruit, stevia) were calculated and compared using the NLMIXED procedure in order to fit the nonlinear

magnitude estimation data. Descriptive analysis data was analyzed using a one-way ANOVA with means separation in XLSTAT (Fishers LSD, $\alpha=0.05$). The impact of protein level, protein source, and vanilla flavoring on descriptive analysis sweetness scores was analyzed separately using two-way ANOVA with means separation in XLSTAT (Tukeys HSD, $\alpha=0.05$).

Time intensity data was analyzed using an add-on macro provided by Compusense Cloud (Compusense, Guelph, Canada) in XLSTAT Version 2014.4.09 (Addinsoft, New York, NY). Parameters were extracted from the raw data and analyzed using a one-way ANOVA with means separation in XLSTAT (Fishers LSD, $\alpha=0.05$). SensoMineR (version 3.2.1) was used to create composite TDS and TCATA curves and difference curves. A binomial proportion was used to calculate chance and significance levels, which were displayed on the graphs ($\alpha=0.05$). TDS and TCATA data was also graphed using XLSTAT, and results were displayed as 'band plots,' with detected attributes shown as horizontal bands.

Consumer acceptance data was also analyzed in XLSTAT. Liking scores were analyzed using a one-way ANOVA with means separation (Fishers LSD, $\alpha=0.05$). 5-pt non-JAR questions were analyzed using the Kruskal-Wallis Test with Dunn's multiple comparisons test ($\alpha=0.05$). A two-way ANOVA (priming x beverage type) with means separation was used to determine the impact of priming statements on liking means for each beverage (Fishers LSD, $\alpha=0.05$). Consumers were grouped into clusters using k-means on differences between primed and unprimed overall liking scores across all panelists (Li et al., 2015). Partial least squares regression was conducted in XLSTAT to see how samples were characterized and how this related to liking for each cluster.

RESULTS AND DISCUSSION

Power Function Curves and Iso-sweetness Values

Results from magnitude estimation scaling and power function curve generation confirmed sucralose was the most potent sweetener, followed by monk fruit, stevia, and fructose. Iso-sweet values for each sweetener were 130.7 mg/L for sucralose, 642.6 mg/L for monk fruit, 786.5 mg/L for stevia, and 63.60 g/L for fructose. These results are consistent with previous literature, which reported sucralose potency in the range of 500-600 times that of sucrose, stevia potency is 97-240 times that of sucrose, mogrosides IV and V are 233-450 times sweeter than sucrose, and fructose is up to 1.8 times sweeter than sucrose (Kim and Kinghorn, 2002; Kinghorn and Compadre, 2001; White and Osberger, 2001; Cardello et al., 1999). Minor adjustments to sweetener concentrations were required following descriptive analysis and prior to 2-AFC tests (Table 2).

Impact of Protein Level and Protein Source on Iso-Sweetness Values

Protein level (15g vs. 25g per 360 mL serving size) and WPI type (anion exchange vs. microfiltered) had no impact ($p > 0.05$) on iso-sweetness concentration for any of the four sweeteners investigated in this study (fructose, sucralose, monk fruit, stevia) (results not shown). These interactions were important to investigate due to increasing consumer interest in greater dietary protein consumption (notably in the form of protein beverages) and greater protein concentration in beverages (Gerdes 2012; Jacobson 2015; Oltman et al., 2015). The lack of impact of WPI source (MF WPI contains GMP, anion exchange WPI does not) on sweetness perception suggests that glycomacropeptide (GMP) has no impact on sweet taste perception, which is also important to note since GMP may improve immune health, help

regulate gut function, and prevent dental caries (Malkoski et al., 2001; Manso and López-Fandino, 2004).

Descriptive Analysis

Descriptive analysis confirmed protein source and protein level had no effects on beverage sweetness ($p>0.05$) (results not shown). Additionally, vanilla flavoring did not significantly impact sweet taste perception for any of the beverages ($p>0.05$) (results not shown). Subsequent descriptive analysis results refer to beverages *with* vanilla flavorings using microfiltered WPI with 25g protein/360 mL.

No significant differences were seen between any of the sweeteners or sweetener blends for intensities of aroma, vanilla, cardboard, sweet taste, or astringency ($p>0.05$; Table 3). Cardboard flavor was detected above threshold level in all beverages, as this is a flavor that has been widely characterized in whey proteins (Drake et al., 2003). Samples were differentiated by differences in metallic taste, bitter taste, and aftertaste intensity (Table 3). Bitter and metallic tastes were not detected in beverages sweetened with sucrose, fructose, or natural blends with 50% or less monk fruit or 25% stevia (Blend 1, Blend 2, Blend 4, Blend 6). Metallic taste alone was not detected in Blend 5 (50% stevia/50% fructose) although bitter taste was present above threshold level ($p<0.05$). Beverages sweetened with sucralose, stevia, monk fruit, Blend 3 (50% stevia/50% monk fruit), and Blend 7 (25% stevia/75% monk fruit) displayed both bitter and metallic tastes above threshold level, with the highest intensities of bitter taste documented in stevia and Blend 3 beverages, and the highest intensities of metallic taste documented in beverages sweetened with sucralose, stevia, Blend 4, and Blend 7 ($p<0.05$).

Bitter and metallic flavors have been widely identified and characterized in sucralose, stevia and monk fruit (Hanger et al., 1996; Goldsmith and Merkel, 2001; Kim and Kinghorn, 2002; Pawar et al., 2013; Philippe et al., 2014). Previous studies also reported bitter and metallic off-flavors in dairy products formulated with non-nutritive sweeteners. Li et al. (2015) reported bitter taste increased in skim chocolate milks as stevia and monk fruit concentrations increased. Guggisberg et al. (2011) reported bitter and metallic off-flavors in whole milk yogurt sweetened with stevia. Morais et al. (2014) reported chocolate dairy desserts sweetened with stevia had higher bitter taste intensity than sucralose sweetened samples. Narayanan et al. (2014) reported stevia sweetened vanilla yogurts were less liked than sucrose sweetened yogurts due to bitter taste from stevia. These results demonstrate the utility of blending alternative sweeteners to minimize off flavors, as blends containing 25% stevia or monk fruit did not elicit bitter or metallic tastes above threshold, and blends with stevia and monk fruit at levels of 50% did not elicit bitter taste above threshold level. Aftertaste intensity was also significantly decreased in all natural blends ($p < 0.05$) with the exception of Blend 3 (50% stevia/50% monk fruit).

Time Intensity

Time intensity parameters and curves further characterized differences in the sensory properties of the beverages. Differing sweet TI curve shapes illustrated differences in sweetness temporality (Figure 1a). No differences were seen for sweet taste parameters ‘time to maximum intensity,’ ‘maximum intensity,’ ‘initial delay,’ or ‘increasing angle’ between any of the beverages ($p > 0.05$). Beverages sweetened with stevia displayed the longest lingering sweetness, shown by the greatest ‘stop time,’ ‘plateau length,’ and ‘area under the curve’ values ($p < 0.05$). Monk fruit beverages also displayed lingering sweet taste. Beverages

sweetened with fructose, Blend 1, Blend 2, and Blend 6 had the shortest lasting sweet taste. Similar results have been previously reported. Palazzo et al. (2011) reported that in chocolates, stevia and sucralose both had greater sweet taste curve area than sucrose sweetened samples. Additionally, these authors showed that stevia had longer lingering sweetness than sucralose or sucrose, and that sucralose sweet taste curves displayed the most similar temporality to sucrose sweet taste curves.

Bitter taste was not detected above threshold in beverages sweetened with sucrose, fructose, Blend 1, Blend 2, Blend 4, or Blend 6. Stevia sweetened beverages had the highest ‘maximum intensity’ ($\bar{x}=3.3$) and longest lingering bitter taste ($p<0.05$, Figure 1b). Monk fruit/Blend 3/Blend 7 sweetened beverages had the second highest intensities of bitter taste ($\bar{x}=1.9$) ($p<0.05$), followed by sucralose beverages ($\bar{x}=1.0$). All bitter TI curves displayed similar shapes, with maximum bitter intensity peaking in the first 30 seconds of evaluation, followed by a steady intensity decline (Figure 1b). Souza et al. (2013) also reported that alternative sweeteners (including stevia and sucralose) had bitter TI curves that were similar to each other. Morais et al. (2014) reported that in chocolate dairy desserts, stevia and sucralose sweetened samples both had longer lingering sweet taste than sucrose control samples. Stevia samples had the highest intensity and longest lasting bitter taste. Rocha and Bolini (2015) reported higher bitter taste intensity and sweet/bitter aftertaste intensities in stevia sweetened passion fruit juice than sucrose or sucralose sweetened juices.

Stevia sweetened beverages also had the highest metallic taste intensities ($\bar{x}=1.9$) ($p<0.05$), followed by monk fruit/Blend 3/Blend 7 ($\bar{x}=1.1$), and then sucralose ($\bar{x}=0.60$) (Figure 1c). Metallic TI curves illustrated differences in metallic taste temporality between samples. Beverages sweetened with Blend 3 and Blend 7 peaked at maximum metallic taste

intensity within the first 20 seconds of evaluation, while stevia, monk fruit, and sucralose sweetened samples did not peak at maximum intensity until 40-50 seconds. Stevia sweetened beverages again displayed the longest lingering metallic taste. Rocha and Bolini (2015) also reported higher metallic taste intensity in stevia sweetened passion fruit juice than in samples with sucrose or sucralose.

Temporal Dominance of Sensations

TDS band plots for sucrose, fructose, and sucralose sweetened beverages were similar (Figure 2a) They were characterized by dominant sweet taste, sweet aromatic, and cardboard flavor. Protein beverages sweetened with stevia or monk fruit were distinct in dominant temporal sensory attributes. Monk fruit beverages were characterized by initial dominant sweet taste followed by dominant, lingering metallic taste. Stevia beverages had the longest lingering dominant bitter taste followed by dominant and lingering metallic taste. All natural sweetener blends displayed similar TDS curves, and were characterized by dominant sweet taste only. Blends formulated with 50% or greater monk fruit and/or stevia displayed longer sweetness dominance than blends with 25% monk fruit or stevia. Of the beverages selected for consumer testing (sweetened with sucralose, stevia, monk fruit, Blend 4 (25% stevia/25% monk fruit/50% fructose), and Blend 7 (25% stevia/75% monk fruit)), dominant bitter taste was only characterized in stevia beverages (Figure 2a). These results were consistent with descriptive analysis and TI results, and provide additional information about the temporal differences in the sensory attributes of the beverages.

Previous studies reported similar TDS results for food and beverages formulated with non-nutritive sweeteners. Zorn et al. (2014) used TDS to evaluate sensory characteristics of orange juices prepared with different sweeteners and reported that sucralose sweetened juices

had the most similar TDS curves to sucrose and both samples were characterized by dominant sweet taste. Stevia sweetened juices had dominant sweet taste followed by dominant bitter taste and dominant off-flavor. Morais et al. (2014) used TDS to evaluate chocolates formulated with different sweeteners. Sucrose and sucralose sweetened samples were characterized by dominant sweet taste and stevia sweetened samples were characterized by dominant sweet taste followed by dominant bitter taste.

Temporal Check-All-That-Apply

The application of TCATA allowed further clarification of temporally dominant attributes in the beverages since multiple attributes are selected by panelists. Bitter taste was documented in TCATA band plots for monk fruit, sucralose, and stevia sweetened beverages, as well as for two of the natural blends (Blend 3 (50% stevia/50% monk fruit) and Blend 7 (25% stevia/75% monk fruit)) (Figure 2b). The temporality of bitter taste differed among beverages. It was the longest lasting in stevia beverages, followed by monk fruit (Figure 2b). Metallic taste was detected in sucralose, monk fruit, and stevia sweetened beverages as well as in the 2 blends previously mentioned, and was again longest lasting in stevia beverages, followed by monk fruit beverages. Attributes of cardboard, sweet taste, and sweet aromatic were also characterized in all beverages. Onset and duration of these attributes was similar for all beverages. Sweet taste was detected first and lasted the entire evaluation time, followed by onset of sweet aromatic and cardboard flavors which both had shorter durations. TCATA plots contained attributes not shown in TDS plots (addition of bitter and metallic in sucralose beverages, addition of bitter and sweet aromatic in monk fruit beverages, addition of cardboard and sweet aromatic in stevia beverages and Blend 4 and addition of bitter, metallic, sweet aromatic and cardboard in Blend 7, indicating these attributes were not

dominant but still detected). These attributes were also documented in these beverages by descriptive analysis, so the TCATA results are consistent with descriptive analysis but also provide insights on temporal perception of these attributes. Ares et al. (2015) also reported similar differences between TDS and TCATA results for several product categories. These authors also reported that TCATA delivered more detail about the sensory attributes present during evaluation and had higher discriminating power than TDS. These conclusions were supported by results of this study as well.

Consumer Acceptance Testing

A total of 150 self-reported ready-to-mix (RTM) protein powder consumers participated in the two-day consumer acceptance test. Males made up 60.7% of the consumers, and females 39.3%. All consumers were between the ages of 18-54 y, with the majority between 18-34 y (64.0%). All consumers self-reported exercising at least once a week on average, with most reporting they exercised 2-3 times per week (31.3%) or 4-6 times per week (46.0%). The majority of consumers (66.0%) reported that a label claim of ‘naturally sweetened’ was important to their RTM purchase decision, 48.7% reported reduced sugar was important, 42.7% reported sugar free as important, and 26.7% placed importance on a calorie-free sweetener in RTM purchase decision.

Cluster analysis of consumer scores for overall liking of sweetened protein beverages with and without priming statements identified two segments of consumers (Table 4). The interaction between sweetener, priming, and consumer segment was significant for overall liking as well as flavor liking, sweetness liking, vanilla flavor liking, and purchase intent ($p < 0.05$) (Table 4). The interaction between sweetener, priming, and consumer segment was not significant for thickness liking or quality rating ($p > 0.05$) (results not shown). The only

difference in demographic information and attitudinal responses was the types of alternative sweeteners typically consumed. Segment 1 consumers (n=75) reported a less frequent consumption of both sucralose and acesulfame-K compared to segment 2 consumers (n=75) ($p<0.05$). Segment 1 consumers scored primed sucralose beverages directionally lower for overall liking than unprimed beverages ($p>0.05$), while segment 2 consumers scored primed sucralose samples higher than unprimed ($p<0.05$) (Table 4). Segment 1 consumers reacted more positively to priming statements about both natural sweetener blends with increased overall liking scores ($p<0.05$), while segment 2 consumers scored primed and unprimed natural blends at parity ($p>0.05$) (Figure 3). These results suggest that segment 1 consumers were more ‘label conscious’ while segment 2 consumers were primarily ‘flavor driven’.

When asked concept liking based on priming statements (before tasting the beverages) both segments of consumers rated sucralose lowest in concept liking ($p<0.05$) (Table 4) Once beverages were tasted, however, segment 2 consumers scored sucralose beverages highest in flavor liking, sweetness liking, and vanilla flavor liking. Segment 1 consumers, in contrast, scored sucralose beverages highest in flavor liking when unprimed, but scored both sweetener blend beverages highest in flavor liking, sweetness liking, and vanilla flavor liking when primed. Additionally, segment 1 consumers scored monk fruit concept liking, overall liking, flavor liking, and sweetness liking lower than that for other natural sweeteners or blends when primed, but scored monk fruit overall liking, flavor liking, and sweetness liking at parity with stevia and Blend 4 beverages when unprimed. This may have been due to a negative reaction from the ‘discriminative’, ‘label conscious’ segment 1 consumers when shown an unfamiliar sweetener name. A 2015 survey reported that monk fruit awareness and recognition was much lower than that of stevia or sucralose (Mintel

Group Ltd., 2015). Only 10% of survey respondents reported they had used monk fruit as a sugar substitute, compared with 25% for both stevia and sucralose. These results for concept and actual tasting collectively suggest that segment 1 consumers were more positively influenced by priming statements about familiar natural sweeteners, while segment 2 consumers were most strongly influenced by beverage flavor.

Oltman et al. (2015) investigated the effect of priming statements ('great tasting' and protein content information) on overall liking of acidified whey protein beverages. Priming statements increased beverage concept liking ($p < 0.05$) but had no impact on overall liking ($p > 0.05$). Li et al. (2015) investigated parents' overall liking of reduced sugar chocolate milks formulated with natural sweetener blends with and without priming statements that identified reduced sugar and naturally sweetened milks. A 'label conscious' segment of consumers preferred sucrose sweetened milks when tasted blind and conversely preferred natural sweetener blends when primed prior to tasting ($p < 0.05$), similar to results observed in the current study.

Overall liking and purchase intent of protein beverages with sweetener label claims were also evaluated independently of tasting. 'Naturally sweetened' received the highest average overall liking score ($p < 0.05$), followed by 'reduced sugar' and 'sugar free' (at parity), and 'calorie free sweetener'. Purchase intent was also significantly higher for the 'naturally sweetened' label claim' ($p < 0.05$). All other label claims scored at parity ($p > 0.05$). No significant differences were seen for sweetener label claim liking or purchase intent between consumer segments ($p < 0.05$). These results are consistent with findings from Oltman et al. (2015) which reported that sweetener type was the most important protein beverage attribute, and within sweetener type, 'naturally sweetened' was the most appealing

attribute. Gerdes et al. (2012) also reported that results of a survey conducted by the US Dairy Export Council indicated increased consumer interest in sugar free, naturally sweetened protein beverages. Increased consumer interest in natural sweeteners in other beverage types has also been reported. A recent Mintel survey (2016a) reported that naturally sweetened soft drink consumption is increasing with Millennials, due in part to desire to live healthier lifestyle. Li et al. (2014) conducted a conjoint study to investigate factors driving parents' purchase of chocolate milk. The authors reported sweetener type was the most important purchase factor for parents. Natural, noncaloric sweeteners were most preferred by a 'health conscious' segment of parents, but flavor was ultimately more important to some consumers.

CONCLUSIONS

Two sugar free and five reduced sugar natural blends were formulated for application in RTM vanilla protein beverages. The natural blends had reduced bitter and metallic off-flavors initially characterized in beverages sweetened with stevia or monk fruit alone. Of the five reduced sugar natural blends formulated, Blend 4 (25% stevia/25% monk fruit/50% fructose) most closely matched the temporal sensory properties of sucrose control beverages and offered a >50% sugar reduction. Of the two sugar free natural blends formulated, Blend 7 (25% stevia/75% monk fruit) most closely matched the temporal sensory properties of sucrose control beverages. Consumer results supported these findings. Both natural blends scored at parity with sucralose beverages ($p>0.05$) for overall liking and flavor liking across all consumers in unprimed evaluations.

Two distinct consumer segments for RTM protein beverages were characterized. These results suggested that segment 1 consumers were more discriminative when reading

priming statements and were more interested in natural sweetener blends than segment 2 consumers. Segment 2 consumers were ultimately influenced by beverage taste, despite an interest in naturally sweetened beverages conceptually. Based on these results, naturally sweetened protein beverages should be marketed with label claims but must also taste comparable to artificially sweetened or sucrose sweetened products. The findings from this study about the temporal sensory properties of natural, non-nutritive sweeteners and formulation of natural sweetener blends may be useful to beverage formulators in accomplishing this objective.

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TABLES

Protein per 360 mL serving (g)	WPI Powder (g)	Natural Vanilla Flavor (g)	Natural Cream Flavor (g)	Natural French Vanilla Flavor (g)
15	16.8	0.60	0.45	0.15
25	28.0	0.60	0.45	0.15

Table 2.1 General formulation of vanilla ready-to-mix (RTM) whey protein beverages at high and low protein levels per 360 mL (12 oz) serving size.

Table 2.2 Formulations of all sweeteners and natural blends in vanilla ready-to-mix whey protein beverages at 25g protein/360 mL (12 oz).

	Sucrose (g)	Fructose (g)	Sucralose (mg)	Monk fruit (mg)	Stevia (mg)	Protein (g)	Vanilla Flavoring (g)	Cream Flavoring (g)	French Vanilla Flavoring (g)
Sucrose	25.20	-	-	-	-	28.02	0.60	0.45	0.15
Fructose	-	22.90	-	-	-	28.02	0.60	0.45	0.15
Stevia	-	-	-	-	283.1	28.02	0.60	0.45	0.15
Sucralose	-	-	47.06	-	-	28.02	0.60	0.45	0.15
Monk Fruit	-	-	-	231.4	-	28.02	0.60	0.45	0.15
Blend 1(25% stevia/75% fructose)	-	16.41	-	-	38.74	28.00	0.60	0.45	0.15
Blend 2 (25% monk fruit/75% fructose)	-	18.14	-	43.01	-	28.00	0.60	0.45	0.15
Blend 3 (50% stevia/50% monk fruit)	-	-	-	132.2	149.5	28.00	0.60	0.45	0.15
Blend 4 (25% stevia/25% monk fruit/50% fructose)	-	11.71	-	43.58	43.38	28.00	0.60	0.45	0.15
Blend 5(50% stevia/50% fructose)	-	9.95	-	-	100.4	28.00	0.60	0.45	0.15
Blend 6 (50% monk fruit/50% fructose)	-	11.43	-	102.0	-	28.00	0.60	0.45	0.15
Blend 7 (25% stevia/75% monk fruit)	-	-	-	205.1	51.06	28.00	0.60	0.45	0.15

Table 2.3 Trained panel profiles of RTM vanilla whey protein beverages at 25g protein/360 mL with different sweeteners and sweetener blends at iso-sweet taste intensities.

	Sucrose	Fructose	Sucralose	Stevia	Monk Fruit	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
Aroma Intensity¹	2.9	2.8	2.8	1.9	2.3	2.3	2.0	2.1	2.0	2.3	2.4	2.6
Vanilla Flavor¹	1.5	1.6	1.4	1.1	1.1	1.0	0.8	0.8	1.0	1.3	1.3	1.3
Cardboard Flavor¹	1.2	1.5	1.6	1.3	1.2	1.0	1.2	1.4	1.0	1.6	1.6	1.6
Bitter Taste	ND	ND	0.8b	1.3a	1.0b	ND	ND	1.3a	ND	0.8b	ND	1.0b
Sweet Taste¹	7.0	6.9	7.0	7.0	6.9	7.2	7.0	7.2	7.2	7.0	7.1	7.1
Metallic	ND	ND	1.6a	1.7a	0.8b	ND	ND	1.4a	ND	ND	ND	1.6a
Astringency¹	1.8	1.8	2.1	2.1	2.1	2.0	2.1	2.2	2.0	2.2	2.0	2.0
Aftertaste Intensity	1.1b	1.3b	1.9a	1.7ab	1.9a	1.3b	1.1b	1.8a	1.1b	1.0b	1.3b	1.3b

Attributes were scored on a 0 to 15 point universal intensity scale (Meilgaard et al., 2007). Letters in rows following means signify significant differences (p<0.05).

¹ No significant differences were documented for this attribute across samples (p>0.05).

²Blend 1(25% stevia/75% fructose)

Blend 2 (25% monk fruit/75% fructose)

Blend 3 (50% stevia/50% monk fruit)

Blend 4 (25% stevia/25% monk fruit/50% fructose)

Blend 5(50% stevia/50% fructose)

Blend 6 (50% monk fruit/50% fructose)

Blend 7 (25% stevia/75% monk fruit)

Table 2.4 Segmented overall liking scores for vanilla protein beverages with different sweeteners (n=150).

	Primed Overall Liking	Unprimed Overall Liking	Concept Liking (Primed Only)	Primed Flavor Liking	Unprimed Flavor Liking	Primed Sweetness Liking	Unprimed Sweetness Liking	Primed Vanilla Flavor Liking	Unprimed Vanilla Flavor Liking	Primed Purchase Intent	Unprimed Purchase Intent
S1 x Sucralose	5.7cdA	6.0aA	5.8c	5.8bcB	6.2aA	5.7bcA	6.1aA	5.7bA	6.0aA	2.9abA	3.0abA
S1 x Stevia	5.7cdA	5.6abcA	6.8a	5.6cA	5.7abcA	5.5cdA	5.6abcA	5.6bcA	5.7abA	2.8abA	2.6abcA
S1 x Monk Fruit	5.0eB	6.2aA	6.5ab	5.0dB	6.0abA	5.0dB	5.6abcA	5.0cB	6.0aA	2.4bA	2.9abA
S1 x Blend 4	6.2abcA	5.4abB	6.8a	6.2abcA	5.7abcB	6.2abA	5.8abB	5.9abA	5.3bB	3.2aA	2.5bcB
S1 x Blend 7	6.1abcdA	5.1cB	6.7a	6.0abcA	5.3cB	5.9bcA	5.2cdB	5.9abA	5.4bB	3.1aA	2.5abcB
S2 x Sucralose	6.5aA	5.2bcB	6.0bc	6.4aA	5.6abcB	6.5aA	5.2cdB	6.4aA	5.8abB	3.3aA	2.8abcA
S2 x Stevia	5.8bcdA	5.2bcB	6.7a	5.8bcA	5.3cA	5.7bcA	5.2cdB	5.9abA	5.6abA	2.9abA	2.6abcA
S2 x Monk Fruit	6.3abA	4.4dB	6.7a	6.3abA	5.7dB	5.9abcA	4.8dB	6.3aA	4.8cB	3.3aA	2.3cB
S2 x Blend 4	5.8bcdAA	5.4bcA	6.7a	5.7bcA	5.5bcA	6.0abcA	5.8bcA	5.8abA	5.3bcA	3.0aA	3.1aA
S2 x Blend 7	5.6deA	5.6abcA	6.7a	5.7bcA	5.7abcA	5.4cdA	5.5bcA	5.7bA	6.1aA	2.9abA	2.9abA

Liking was scored on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely.

Purchase intent was scored on a 5-point scale where 1=definitely would not purchase and 5=definitely would purchase.

Different lowercase letters in columns following means indicate significant differences (p<0.05).

Different uppercase letters in rows following means of each attribute indicate significant differences (p<0.05).

S1, segment 1 (n=75); S2, segment 2 (n=75).

Blend 4, 25% stevia/25% monk fruit/50% fructose; Blend 7, 25% stevia/75% monk fruit.

FIGURES

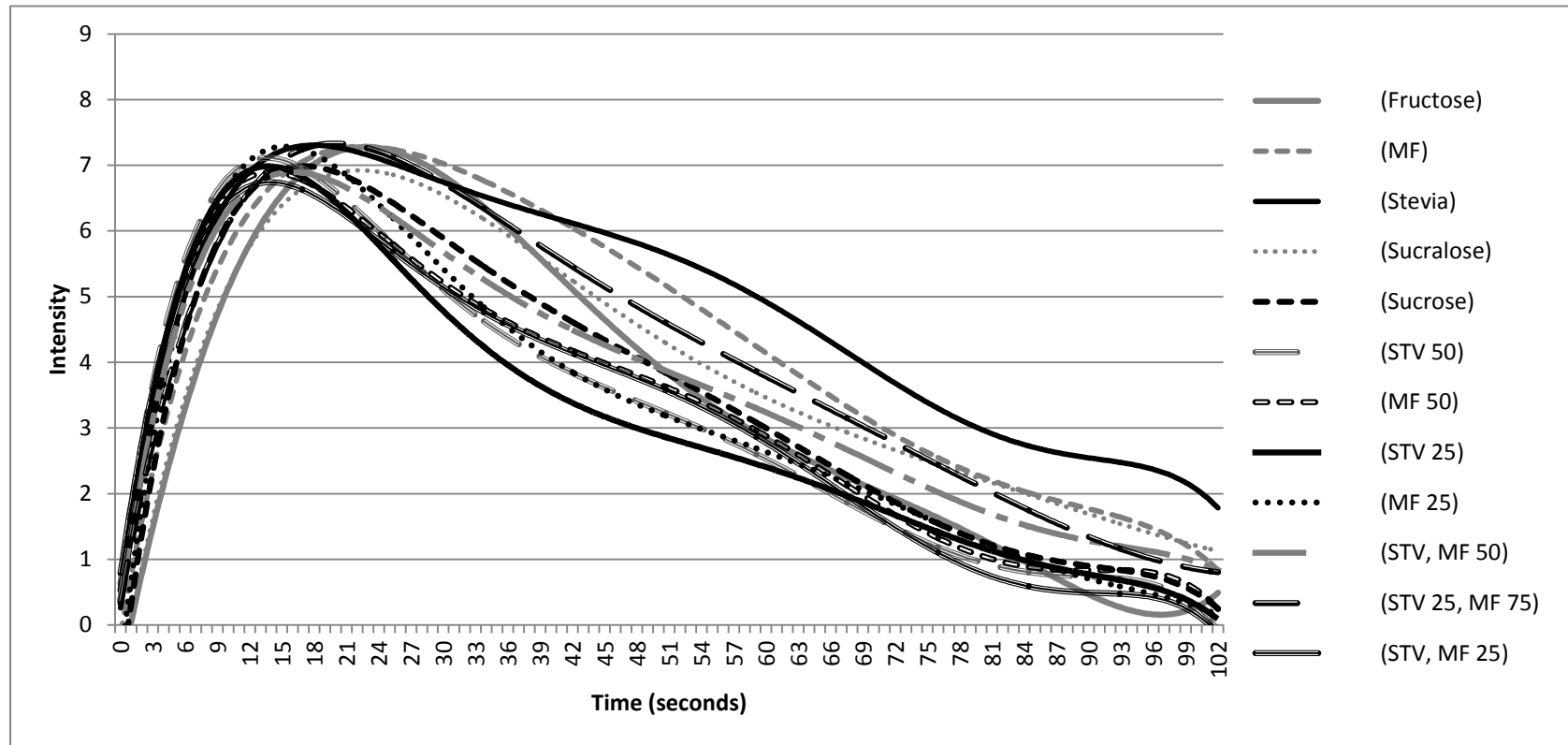


Figure 2.1a Sweet taste Time Intensity Curves for sweetened vanilla ready-to-mix protein beverages. The reference beverage (7% sucrose) is shown with a dotted line. Sweet taste intensity was scored on a 0-15 point scale (Meilegard et al. 2007).

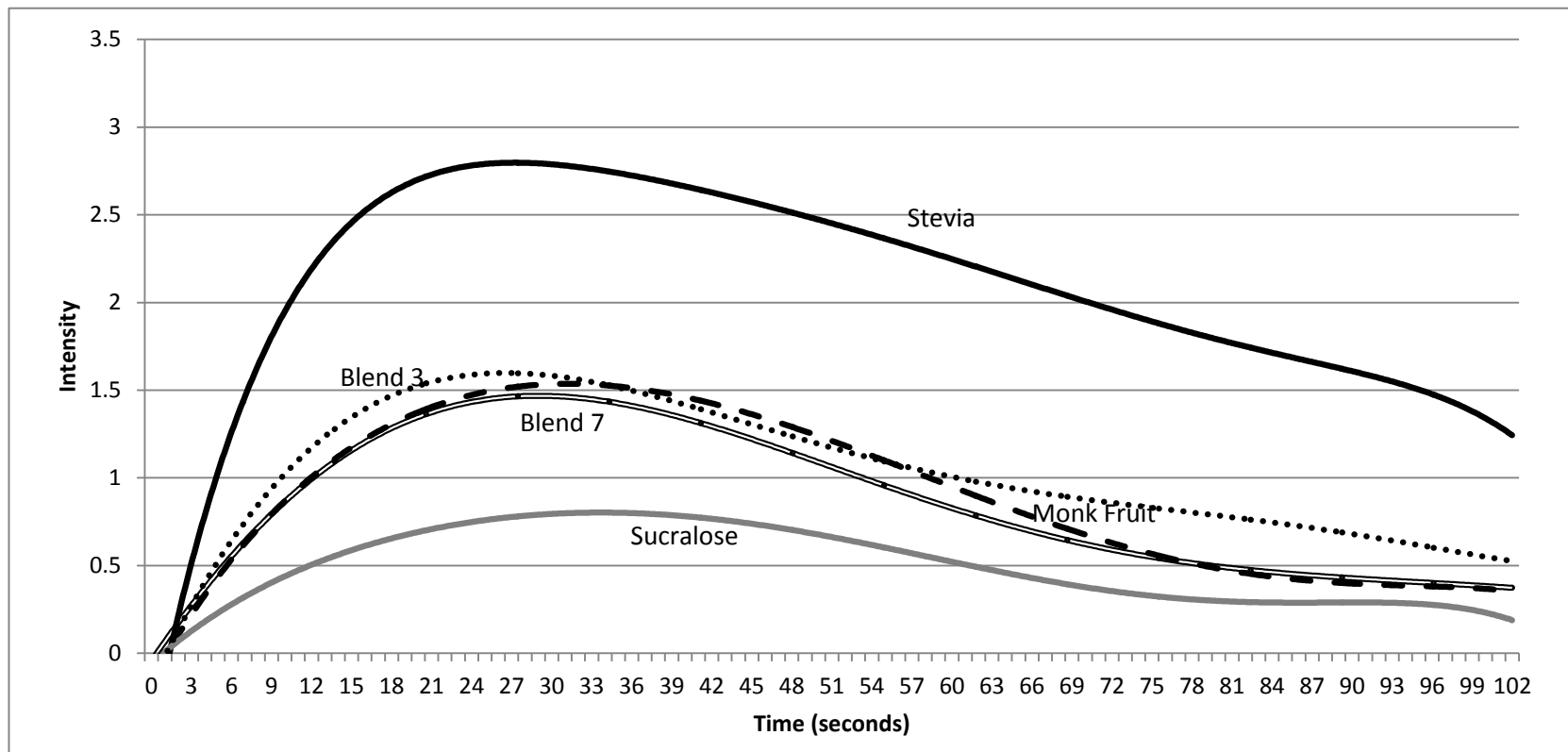


Figure 2.1b Bitter taste Time Intensity Curves for sweetened vanilla ready-to-mix protein beverages. Bitter taste intensity was scored on a 0 to 15 point scale (Meilegard et al. 2007).

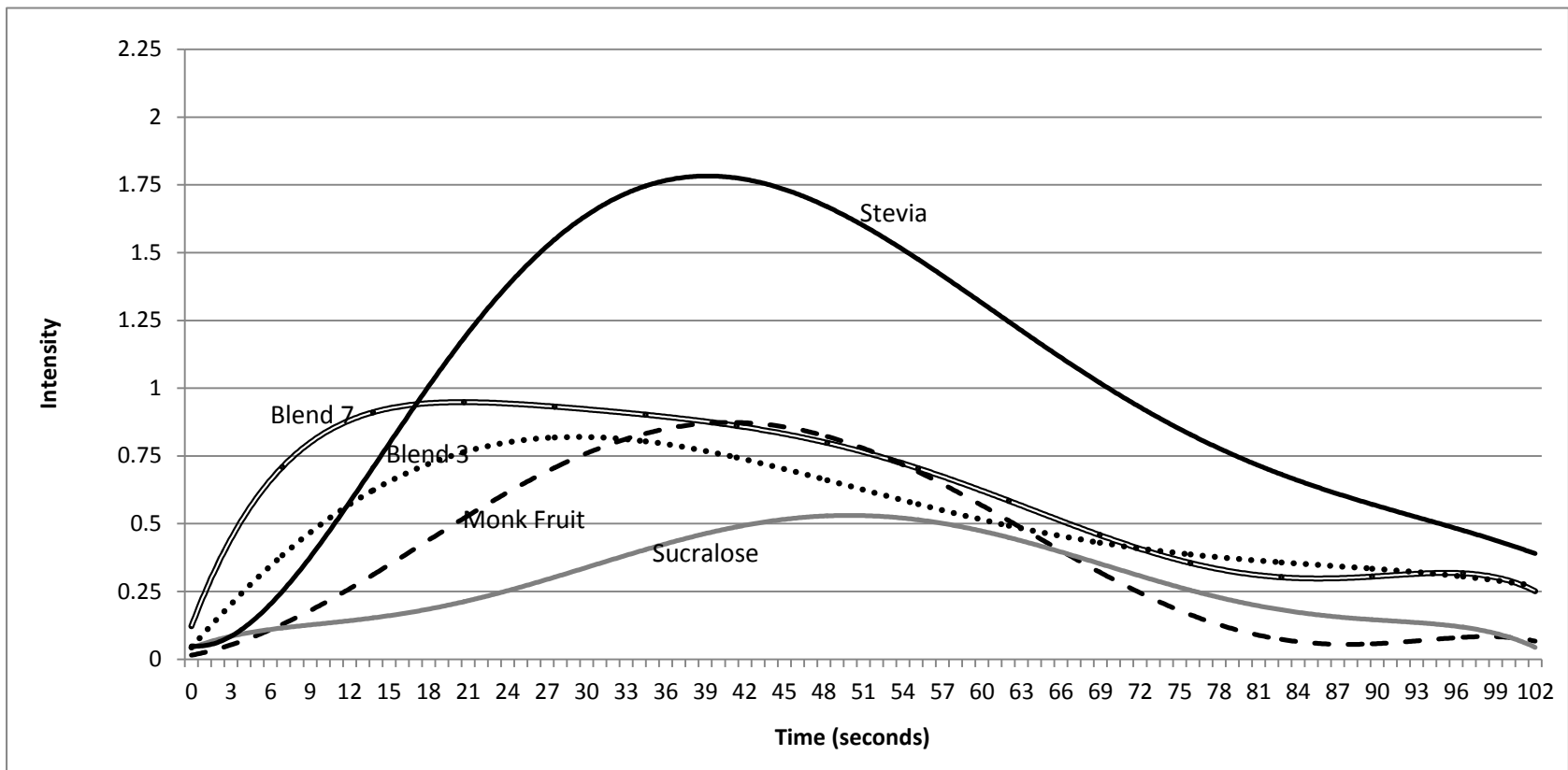


Figure 2.1c Metallic Time Intensity Curves for all sweetened vanilla ready-to-mix protein beverages. Metallic intensity was scored on a 0 to 15 pt scale (Meilegard et al. 2007).

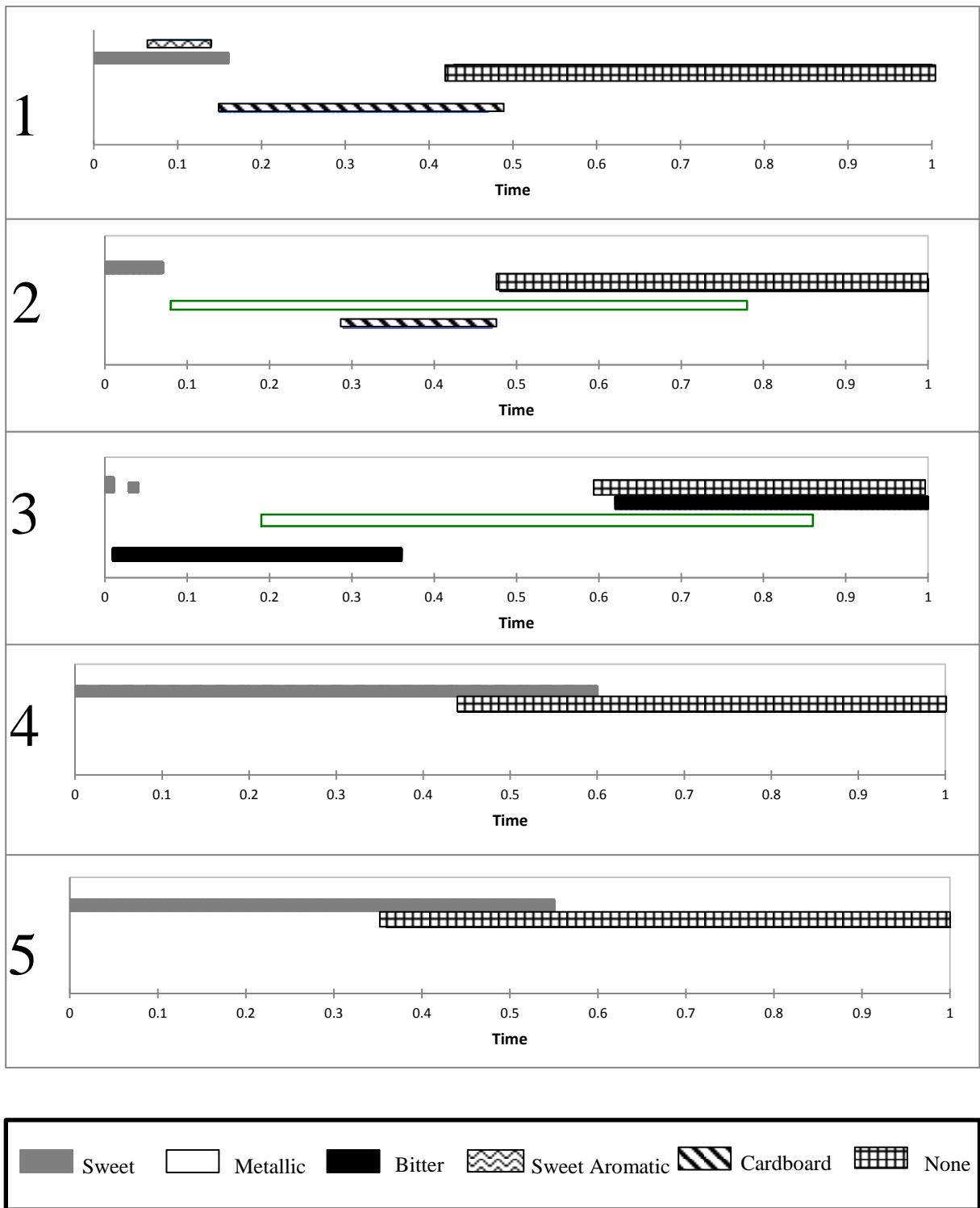


Figure 2.2a Temporal Dominance of Sensations band plots for the five beverages selected for consumer testing. Bands represent dominant attributes ($\alpha=0.05$). 1=sucralose control, 2=monk fruit control, 3=stevia control, 4=Blend 7 (25% stevia, 75% monk fruit), 5=Blend 4 (25% stevia, 25% monk fruit, 50% fructose).

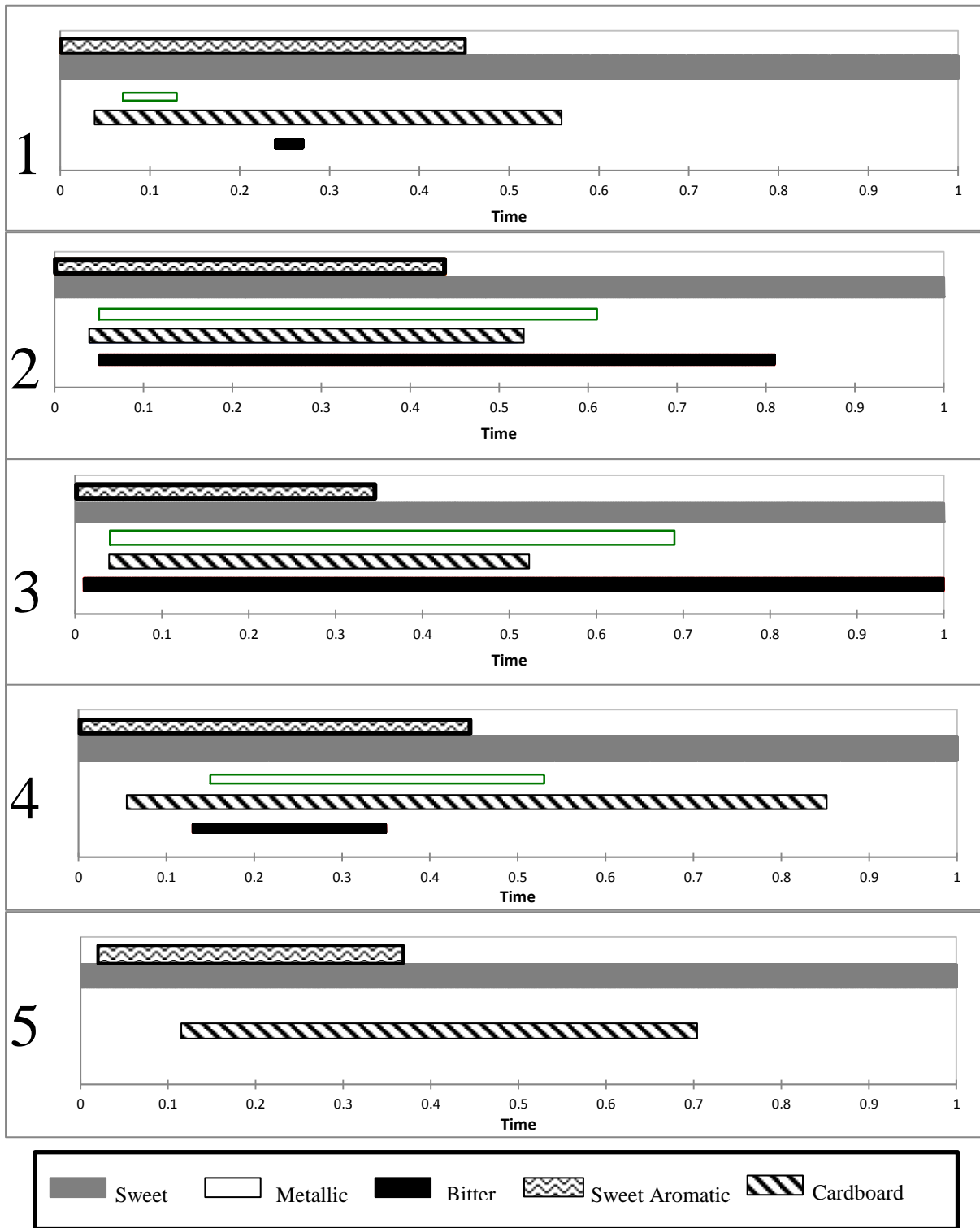


Figure 2.2b Temporal Check-All-That-Apply band plots for the five beverages selected for consumer testing. Bands represent **all detected** attributes ($\alpha=0.05$). 1=sucralose control, 2=monk fruit control, 3=stevia control, 4=Blend 7 (25% stevia, 75% monk fruit), 5=Blend 4 (25% stevia, 25% monk fruit, 50% fructose).

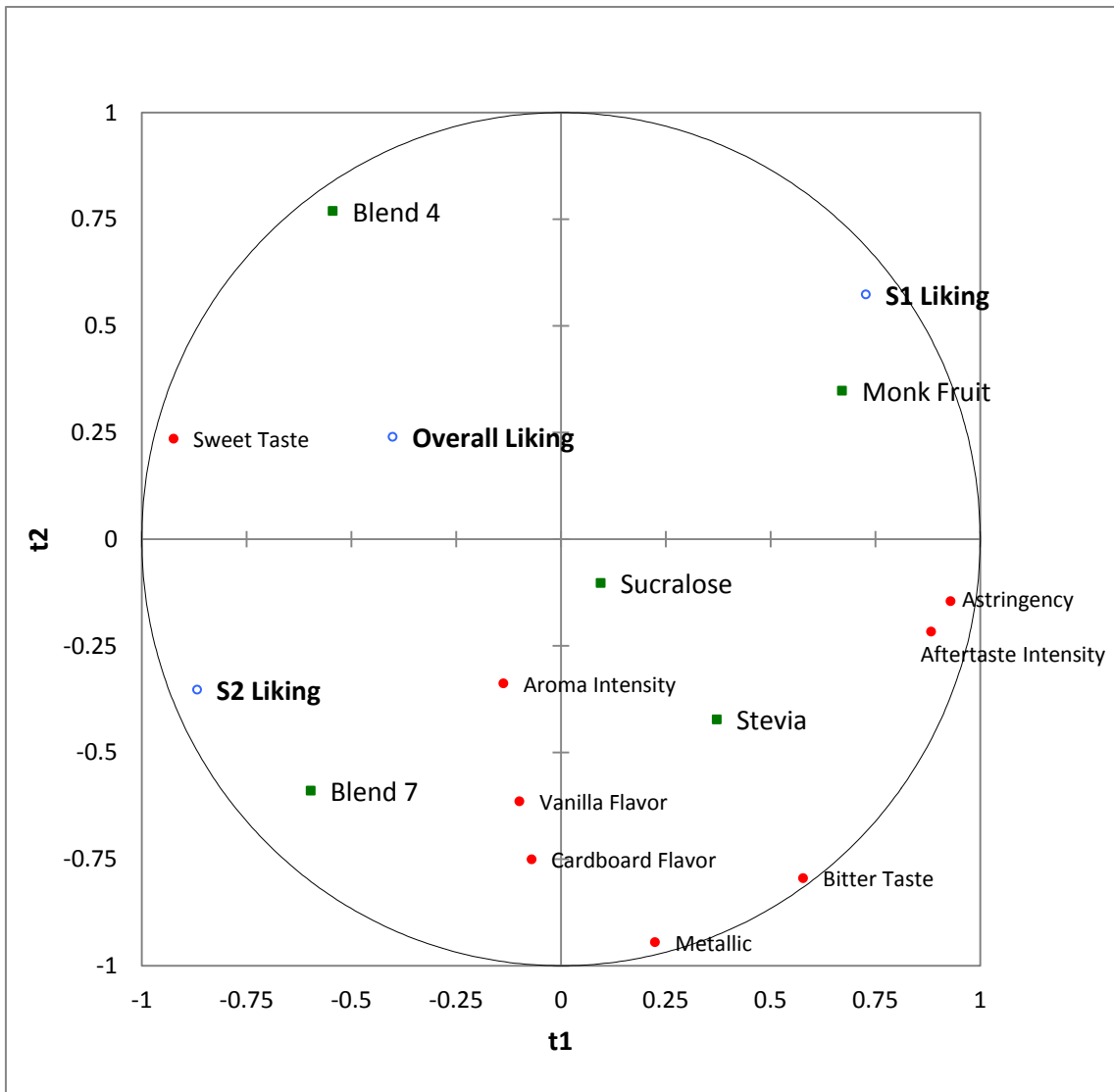


Figure 2.3a Partial least squares (PLS) regression biplot of unprimed overall liking scores of consumer segments for vanilla protein beverages with different sweeteners. Sensory attributes include aromatics and basic tastes. Variance explained as follows: $t_1=48.1\%$, $t_2=17.0\%$, and $t_3=16.8\%$. Blend 4 (25% stevia/25% monk fruit/50% fructose); Blend 7 (25% stevia/75% monk fruit). S1, segment 1($n=75$); S2, segment 2($n=75$).

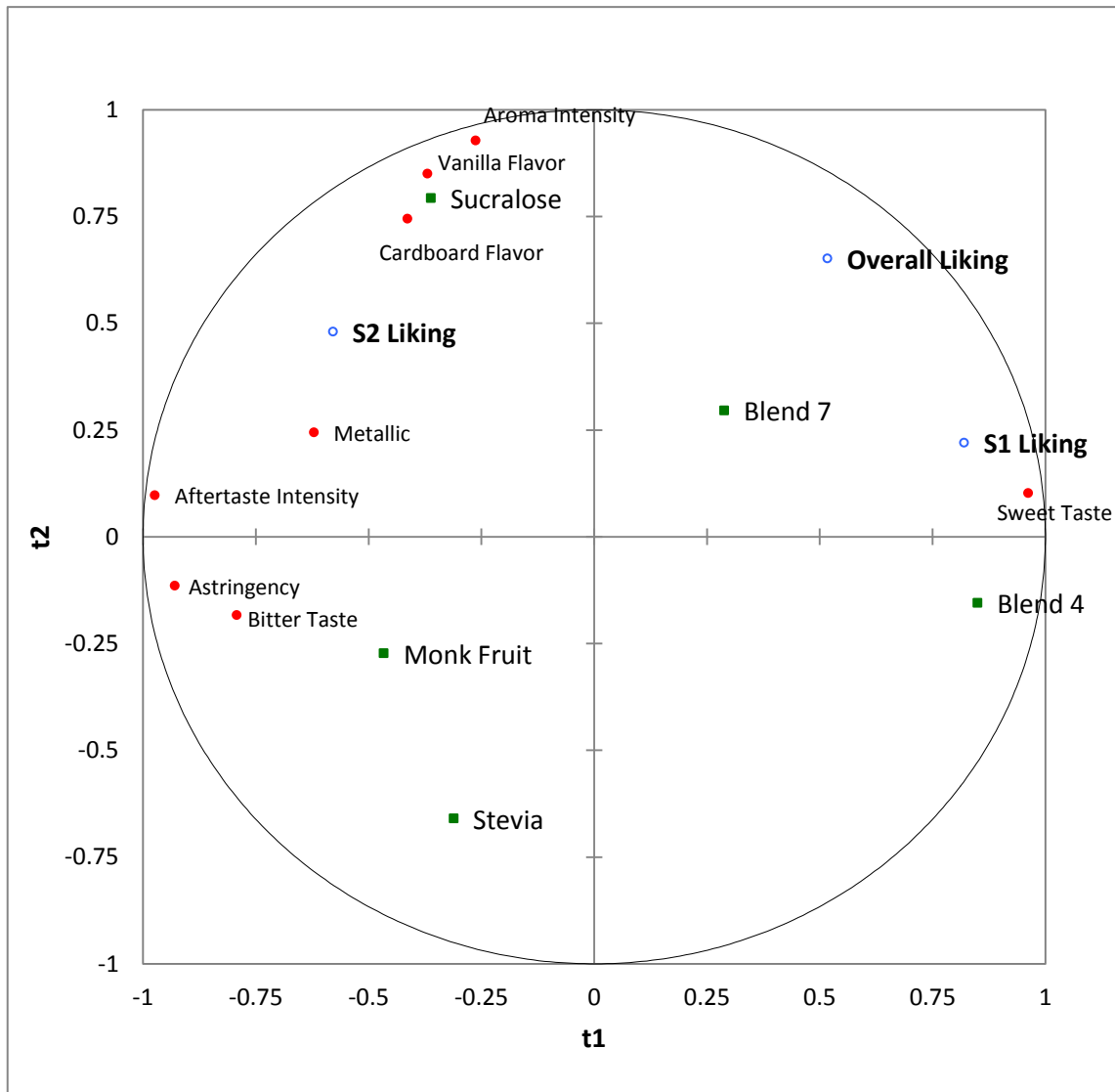
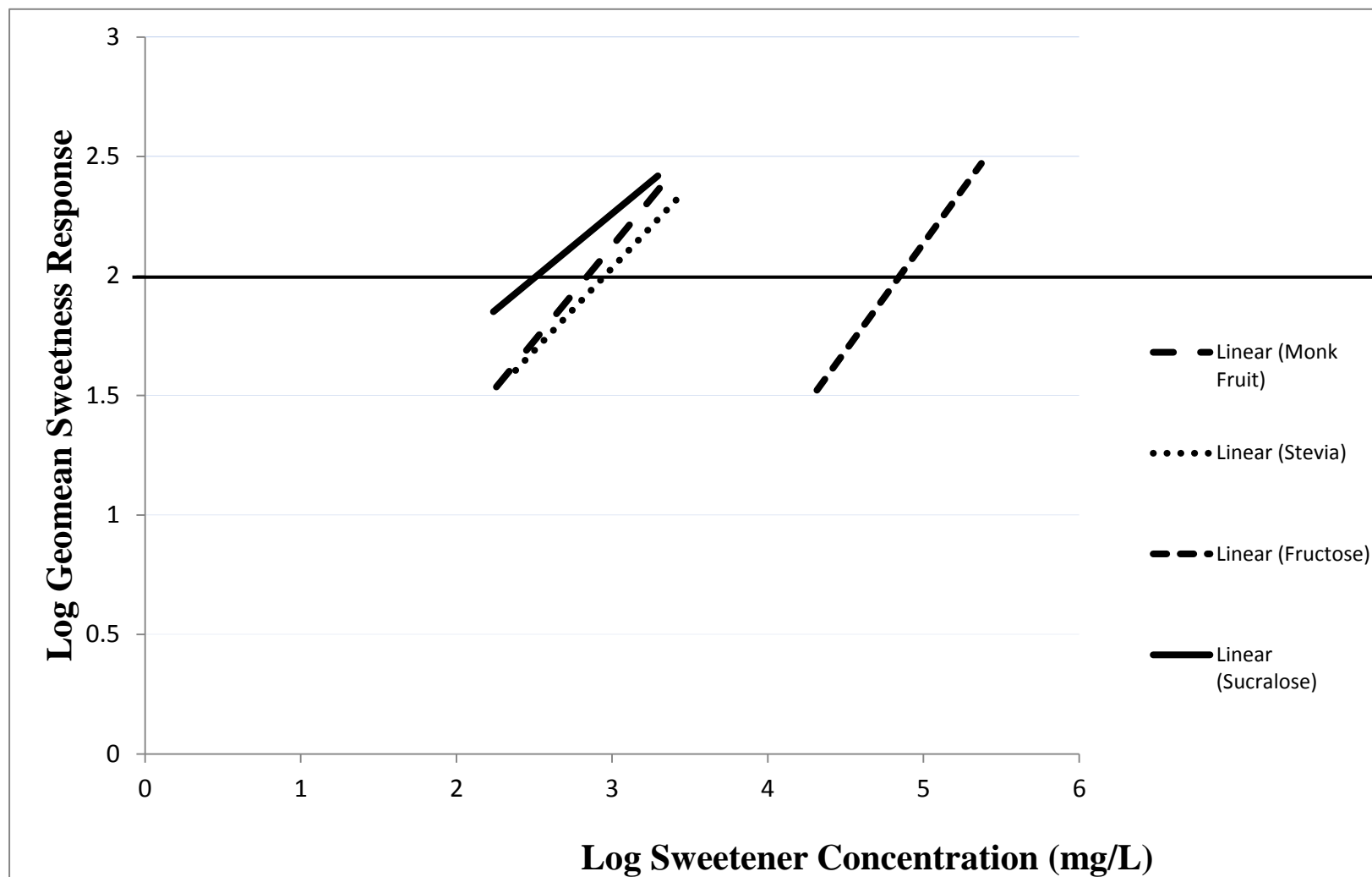


Figure 2.3b Partial least squares (PLS) regression biplot of primed overall liking scores of consumer segments for vanilla protein beverages with different sweeteners. Sensory attributes include aromatics and basic tastes. Variance explained as follows: $t_1=42.6\%$, $t_2=23.4\%$, and $t_3=24.1\%$. Blend 4 (25% stevia/25% monk fruit/50% fructose); Blend 7 (25% stevia/75% monk fruit). S1, segment 1($n=75$); S2, segment 2($n=75$).

APPENDICES

APPENDIX A: COMPOSITE MAGNITUDE ESTIMATION SCALING CURVES



APPENDIX B: SWEETENER CONCENTRATIONS USED FOR MAGNITUDE ESTIMATE SCALING IN BOTH WATER AND RTM VANILLA PROTEIN BEVERAGES

	Stevia in Water	Monk Fruit in Water	Fructose in Water	Sucralose in Water	Stevia in Protein Beverage	Monk Fruit in Protein Beverage	Fructose in Protein Beverage	Sucralose in Protein Beverage
Concentration 1	178 mg/L	103 mg/L	7.5 g/L	63.8 mg/L	239.6 mg/L	180.6 mg/L	20.7 g/L	172.5 mg/L
Concentration 2	267 mg/L	155 mg/L	13.6 g/L	89.3 mg/L	359.4 mg/L	271.0 mg/L	31.0 g/L	258.9 mg/L
Concentration 3	400 mg/L	233 mg/L	24.4 g/L	125 mg/L	539.1 mg/L	406.4 mg/L	46.5 g/L	388.3 mg/L
Concentration 4	600 mg/L	400 mg/L	44 g/L	175 mg/L	808.6 mg/L	609.7 mg/L	69.7 g/L	582.4 mg/L
Concentration 5	900 mg/L	640 mg/L	79.2 g/L	245 mg/L	1212.9 mg/L	914.5 mg/L	104.6 g/L	873.6 mg/L
Concentration 6	1350 mg/L	960 mg/L	142.6 g/L	343 mg/L	1819.4 mg/L	1371.7 mg/L	156.9 g/L	1310.4 mg/L
Concentration 7	2025 mg/L	1440 mg/L	256.6 g/L	480.2 mg/L	2729.1 mg/L	2057.6 mg/L	235.3 g/L	1965.6 mg/L

APPENDIX C: ANALYSIS OF THE IMPACT OF PROTEIN LEVEL AND PROTEIN SOURCE ON ISO-SWEETNESS VALUES

	Low Protein Level (15g/360 mL)^a	High Protein Level 25g/360 mL)
Fructose (g)	77.5±18.8a ^b	73.2±18.4a
Sucralose (mg)	238.3±7.4a	234.3±7.0a
Monk Fruit (mg)	814.1±27.5a	860.3±31.3a
Stevia (mg)	895.4±32.1a	916.0±31.3a
	Anion Exchange WPI^c	Microfiltered WPI
Fructose (g)	71.8±18.2a	73.2±18.4a
Sucralose (mg)	228.4±7.1a	234.3±7.0a
Monk Fruit (mg)	856.0±31.0a	860.3±31.3a
Stevia (mg)	995.5±35.4a	916.0±31.3a

^a Assessed with no vanilla flavoring and microfiltered WPI.

^b Iso-sweet values reported as composite means, with means separation done at $\alpha=0.05$ level.

^c Assessed without vanilla flavoring at high protein level.

APPENDIX D: AVERAGE TIME INTENSITY PARAMETERS

Average sweet Time Intensity parameters for vanilla ready-to-mix protein beverages.

	Sucrose	Fructose	Sucralose	Monk fruit	Stevia	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
Time to Max¹	15	18	14	14	12	14	16	10	11	13	15	15
Intensity at Max¹	7.2	7.1	7.1	7.13	7.1	6.8	7.1	7.3	6.8	6.9	6.8	7.0
Initial Delay¹	2.6	3.8	3.4	2.7	1.7	1.8	2.3	1.9	1.8	1.9	2.2	3.0
Duration	74ab	66b	82ab	86ab	95a	65b	68b	83ab	67b	71ab	69b	82ab
Stop Time	77ab	70.b	86ab	88ab	96a	67b	70.b	85ab	69b	73ab	71b	85ab
Plateau Length	15b	16b	18ab	23ab	29a	15b	14b	19ab	17b	19ab	15b	19ab
Increasing Angle¹	21	18	25	28	36	27	30.	28	34	33	28	28
Decreasing Angle	9.3ab	12ab	6.9ab	6.6ab	5.6b	16ab	16ab	10.ab	12ab	19a	16ab	11ab
Area Under Curve	330bcd	290cd	360abcd	402b	450a	270d	272d	384abc	280cd	280cd	270d	350abcd

¹ No significant differences were seen between any of the sample means ($p>0.05$).

Average bitter Time Intensity parameters for vanilla ready-to-mix protein beverages.

	Sucrose	Fructose	Sucralose	Monk fruit	Stevia	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
Time to Max	ND	ND	16a	16a	16a	ND	ND	14a	ND	15a	ND	19a
Intensity at Max	ND	ND	0.96c	1.9b	3.3a	ND	ND	1.9b	ND	0.75c	ND	1.8b
Initial Delay	ND	ND	6.4a	9.9a	6.1a	ND	ND	6.2a	ND	4.4a	ND	5.6a
Duration	ND	ND	19cd	40.bc	79a	ND	ND	61ab	ND	23cd	ND	59ab
Stop Time	ND	ND	26c	50.bc	85a	ND	ND	68ab	ND	28c	ND	65ab
Plateau Length	ND	ND	66a	50.ab	36b	ND	ND	40.ab	ND	56ab	ND	32b
Increasing Angle	ND	ND	0.22d	5.3abcd	8.8a	ND	ND	6.8ab	ND	1.3bcd	ND	6.2abc
Decreasing Angle	ND	ND	1.1a	2.3a	1.9a	ND	ND	2.1a	ND	0.99a	ND	1.4a
Area Under Curve	ND	ND	37cde	86bcd	220a	ND	ND	105b	ND	28e	ND	88bc

ND signifies not detected above sensory threshold level.

Average metallic Time Intensity parameters for vanilla ready-to-mix protein beverages.

	Sucrose	Fructose	Sucralose	Monk fruit	Stevia	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
Time to Max	ND	ND	17a	24a	24a	ND	ND	26a	ND	ND	ND	31a
Intensity at Max	ND	ND	0.60c	1.1b	1.9a	ND	ND	1.2b	ND	ND	ND	1.1b
Initial Delay	ND	ND	4.8b	9.3ab	12ab	ND	ND	15ab	ND	ND	ND	17a
Duration	ND	ND	16b	19b	44a	ND	ND	42a	ND	ND	ND	43a
Stop Time	ND	ND	21c	29bc	56ab	ND	ND	57a	ND	ND	ND	60.a
Plateau Length	ND	ND	39a	53a	44a	ND	ND	41a	ND	ND	ND	39a
Increasing Angle	ND	ND	0.27b	0.33b	2.3b	ND	ND	9.2ab	ND	ND	ND	13a
Decreasing Angle	ND	ND	0.52a	0.56a	0.81a	ND	ND	1.5a	ND	ND	ND	3.9a
Area Under Curve	ND	ND	32b	39b	95a	ND	ND	55ab	ND	ND	ND	64ab

Analyses were done on beverages with 25g protein/12 oz. serving.
 Letters in rows following means signify significant differences (p<0.05).
 ND signifies not detected above sensory threshold level.

APPENDIX E: TRAINED PANEL SENSORY ATTRIBUTES FOR VANILLA WPI BEVERAGES

Term	Definition	Reference
Aroma Intensity ²	The overall orthonasal aroma impact, evaluated as the lid is removed from the cupped sample	
Vanilla Aromatics	Sweet aromatics associated with vanilla, vanillin, or marshmallows	Vanillin
Cardboard ³	Aromatics associated with wet cardboard and brown paper	Cardboard paper, soaked in skim milk overnight
Cabbage ⁵	Sulfurous aromatic associated with cooked cruciferous vegetables	Dimethyl trisulfide, boiled fresh cut cabbage
Soapy ⁴	Aromatics associated with medium chain fatty acids and soaps	White unscented soap bar, 50 g soaked in 500 mL water
Bitter Taste ¹	Fundamental taste sensation elicited by caffeine, quinine	Caffeine (0.5% in water)
Sweet Taste ¹	Fundamental taste sensation elicited by sugars	Sucrose (5% in water)
Metallic Taste ²	Taste elicited by metallic objects in the mouth	Ferrous sulfate solution (0.4% in water)
Astringency ¹	Chemical feeling factor on the tongue or oral cavity described as puckering or dry	Alum (1% in water)
Aftertaste Intensity ²	Evaluated after expectoration and up to 1 min after	

¹ Terms adapted from Civelle and Lyons (1996)

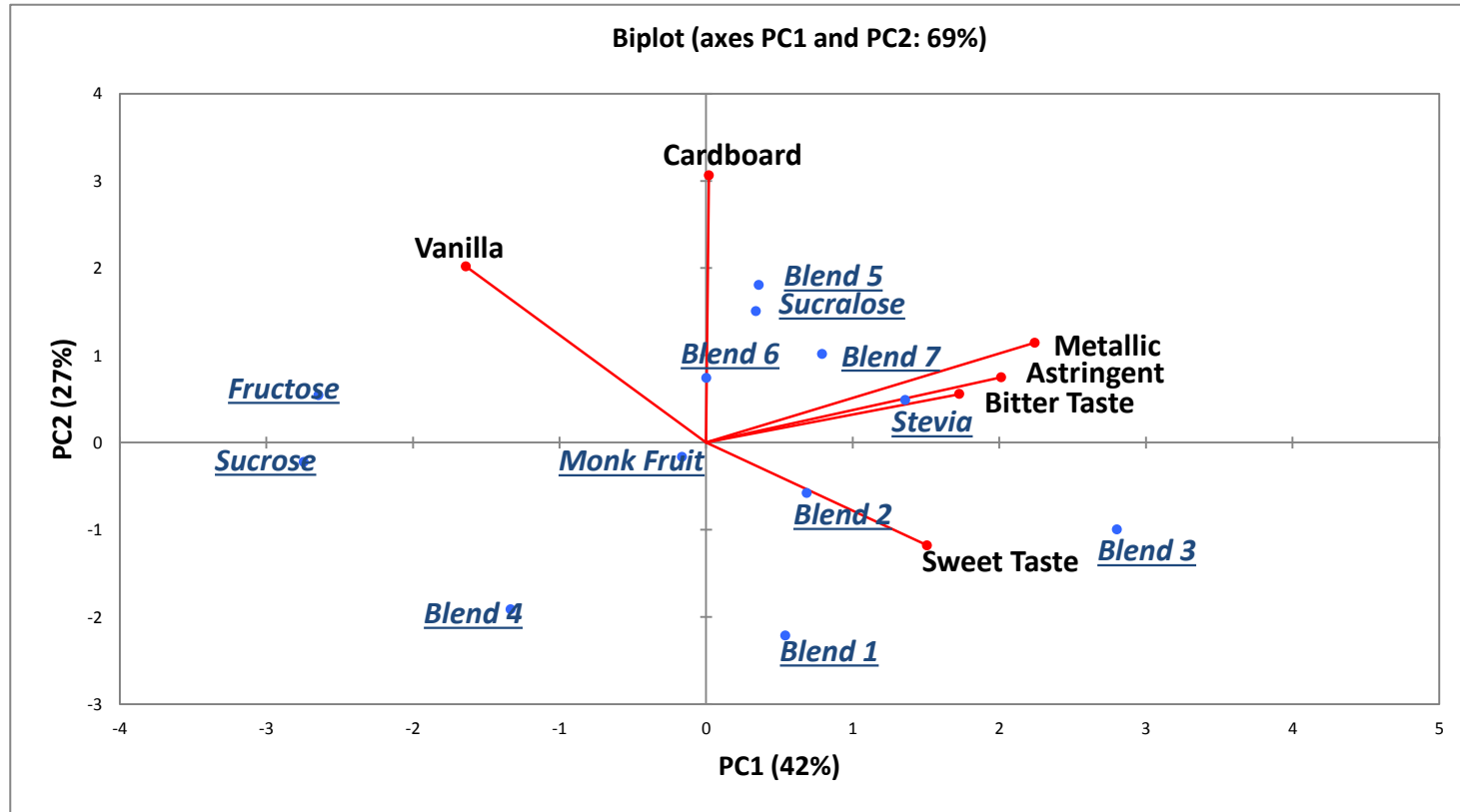
² Terms adapted from Leksrisonpong et al. (2012)

³ Terms adapted from Drake et al. (2003)

⁴ Terms adapted from Russell et al. (2006)

⁵ Terms adapted from Wright et al. (2006)

**APPENDIX F: PRINCIPAL COMPONENT BIPLLOT FOR VANILLA PROTEIN BEVERAGE SAMPLES
SWEETENED WITH EACH SWEETENER AND NATURAL BLEND**



PC-principal component

- Blend 1 (25% stevia/75% fructose)
- Blend 2 (25% monk fruit/75% fructose)
- Blend 3 (50% stevia/50% monk fruit)
- Blend 4 (25% stevia/25% monk fruit/50% fructose)
- Blend 5 (50% stevia/50% fructose)
- Blend 6 (50% monk fruit/50% fructose)
- Blend 7 (25% stevia/75% monk fruit)

**APPENDIX G: SELF-REPORTED ALTERNATIVE (NON-NUTRITIVE)
SWEETENERS TYPICALLY CONSUMED BY PARTICIPANTS IN THE
CONSUMER TEST**

	Segment 1 (n=75)	Segment 2 (n=75)	Total Population (n=150)
Sucralose	33.3% b	46.7% a	40.0%
Stevia	60.0% a	68.0% a	64.0%
Acesulfame-K	4.0% b	13.3% a	8.7%
Aspartame	33.3% a	37.3% a	35.3%
Monk Fruit	21.3% a	20.0% a	20.7%
Sorbitol	10.7% a	14.7% a	12.7%
Xylitol	13.3% a	12.0% a	12.7%
Other (please specify)	5.3% a	2.7% a	4.0%
I know I have consumed alternative sweeteners, but I don't know which ones	16.0% a	13.3% a	14.7%

Different letters in rows following means of each attribute indicate significant differences ($p < 0.05$).

APPENDIX H: OVERALL LIKING AND PURCHASE INTENT SCORES FOR SWEETENER LABEL CLAIMS BY CONSUMERS

Overall liking for sweetener label claims by consumers (n=150).

	Segment 1 (n=75)	Segment 2 (n=75)
Calorie Free Sweetener	5.9b	6.0c
Naturally Sweetened	7.0a	7.3a
Reduced Sugar	6.5b	6.6b
Sugar Free	6.6b	6.4bc

Liking was scored on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely. Different lowercase letters in columns following means indicate significant differences ($p < 0.05$). No significant differences were seen between segments ($p > 0.05$).

Purchase intent for sweetener label claims by consumers (n=150).

	Segment 1 (n=75)	Segment 2 (n=75)
Calorie Free Sweetener	3.3b	3.3b
Naturally Sweetened	3.8a	4.0a
Reduced Sugar	3.6ab	3.7ab
Sugar Free	3.5b	3.6b

Purchase Intent was scored on a 5-point scale where 1=definitely would not purchase and 5=definitely would purchase. Different lowercase letters in columns following means indicate significant differences ($p < 0.05$). No significant differences were seen between segments ($p > 0.05$).