

ABSTRACT

KEEFER, HEATHER RAE. The Role of Natural Sweeteners on Temporality and Bar Hardening of High Protein Low Carbohydrate Bars. (Under the direction of Dr. MaryAnne Drake).

Protein bars are one product that meet consumer demands for a low carbohydrate, high protein food. With such a large protein bar market, protein bar producers need to find the correct texture and sweetness levels to satisfy consumers while still delivering a high protein, low carbohydrate bar. In the bar industry, bar hardening is a major concern and currently the effect of non-nutritive sweeteners on bar hardening is unknown. Due to the negative implications of bar hardening, it is important to investigate the sweetener-protein relationship to bar hardening. The objective of this study was to characterize the impact of sweetener and protein source on flavor, texture, and shelf life of high protein, low carbohydrate bars. The iso-sweet concentration of sweeteners (sucralose, sucrose, monk fruit, stevia, and fructose) in pea protein (PP), milk protein (MP) and whey protein isolate (WPI) bars were established using Magnitude Estimation Scaling (MES) and 2- Alternative Forced Choice (2-AFC) Testing. Descriptive Analysis and Temporal Check All that Apply were then conducted to determine flavor and temporal differences between the different protein types and sweeteners in the protein bars. Finally, an accelerated shelf life study was completed to understand how sweetener and protein types affected the shelf life of protein bars. The fifteen protein bars formulated at iso-sweet concentration were all stored at 35 °C and 55% humidity for 35 days and measurements were taken every 7 days beginning at day 1 (1, 7, 14, 21, 28, 35). MP required significantly less sweetener compared to PP and WPI to reach equal sweetness ($p < 0.05$). Bars sweetened with stevia or monk fruit had distinct bitter and metallic tastes, and sucralose had a low metallic taste. WPI bars were the most cohesive, and PP and whey protein bars were more bitter and metallic compared to MP bars ($p < 0.05$). WPI bars made with fructose were initially the hardest protein bar but after day 14, scored at parity with

PP sucrose. There was no significant difference between bars in terms of hardness by day 21.

WPI bars were consistently more dense at all time points than bars made with PP or MP. Bars made with PP were the driest, least cohesive and had the fastest rate of breakdown in the study.

Non-nutritive sweeteners did not have a negative effect on bar hardness in low carbohydrate, high protein bars. Findings from this study can be applied to commercially produced protein bars for naturally sweetened bars with different protein types without negative effects on protein bar texture.

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The Role of Natural Sweetener on Temporality and Bar Hardening of High Protein Low
Carbohydrate Bars

By
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DEDICATION

This thesis is dedicated to my husband and my family. Without their constant support and encouragement, I would not have made it through this program. Thank you for all of your support, I love you.

BIOGRAPHY

Heather Rae McCain Keefer was born on May 13th, 1994. Heather grew up in Santa Clarita, California and attended Santa Clarita Christian School from 1st -12th grade. After high school, Heather went to college at Cal Poly San Luis Obispo, where she met her husband Bryce Keefer on a backpacking trip to Yosemite in 2015. Heather graduated in 2016 with a B.S. in Food Science and moved to Raleigh to pursue a M.S. degree at North Carolina State University under the direction of Dr. MaryAnne Drake. After a year of long distance, Heather and Bryce got married on December 30th, 2017. When she is not in the lab, Heather enjoys playing with her puppy Banksy, running marathons, and cooking.

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CHAPTER 1: Literature Review. Sugar Reduction in Dairy Products.

TITLE: Sugar Reduction in Dairy Products

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Abstract

Sugar overconsumption continues to increase worldwide and contributes to multiple health-related issues. Dairy foods are a large market grossing more than \$125-billion per year. Consumer demands for healthier products are leading to a large push for sugar reduction in dairy foods. Sugar plays an important role in dairy foods for not only flavor but also texture, color and viscosity. Replacing sugar can have negative effects, making substitution inherently difficult. Natural and artificial nonnutritive sweeteners exist for sugar reduction. Natural nonnutritive sweeteners are popular, particularly for label appeal, but many consumers still prefer the taste of artificial nonnutritive sweeteners. Sweet taste perception can also be effected by texture of the food matrix and the presence of fat. Other sugar reduction techniques include hydrolysis of lactose, ultrafiltration and direct reduction. This review will address the role of sugar, alternative sweeteners and sugar reduction in ice cream, yogurt and flavored milk.

Key Words: Sugar reduction, dairy foods, sweeteners

Introduction

Sugar consumption in the US

Humans are born with an innate preference for sweet taste (Behrens et al., 2011). Infant studies have shown that the sweeter a beverage is, the more babies will consume of it (Ganchrow et al., 1983; Yebra-Biurrun, 2005). Sugar increases the perceived pleasure of eating and if removing sugar causes a negative impact on product sales through lower consumption, companies will not have motivation to invest the money to decrease sugar content unless positive solutions are developed (Yebra-Biurrun, 2005). Furthermore, consumers who tend to consume more sugar sweetened beverages have a higher affinity for sweet taste which further encourages companies to add more sugar into products until it reaches consumers “bliss point” (Mahar and Duizer, 2007; Moss, 2013). Moss (2013) defines bliss point as the precise amount of sugar that will send consumers over the moon for the product, addicting them to the product, and causing them to overeat. However, overconsumption of sugar can contribute to a whole host of issues such as hypertension, type 2 diabetes, cardiovascular disease, and dental cavities (WHO, 2003; Grembecka, 2015). Daily sugar consumption is close to 500 calories per day worldwide, whereas the Recommended Daily Intake (RDI) of sugar for a normal weight individuals is about 25 grams or 96 calories per day (Lustig et al., 2012; Mann, 2017; WHO, 2017). The Centers for Disease Control and Prevention reports that an estimated 36% of adults over the age of 20 and 17% of adolescents ages 2-19 are obese (Jeong et al., 2014). The World Health Organization (2003) suggested that sugar should only compose 10% or less of total dietary energy consumption, but on average, sugar contributes much more. In the United States, a simple reduction in the consumption of added sugar is projected to potentially prevent 2.4 million cases of diabetes, 8,000 strokes, and 26,000 premature deaths per year (Wang et al., 2012; Mekonnen

et al., 2013). Although sugar is not the sole cause of the obesity epidemic, it is a major contributor to obesity (Pruthi, 2015).

The prevalence and increase of numerous diseases have continued to spark consumer interest in reducing sugar in dairy and other food products. Sugar-sweetened beverages (SSB), such as flavored milk, soda, sport drinks, sweetened tea, etc., are the highest contributors to added sugar in American diets and in 2009, 13.8 billion gallons of SSB were consumed (Wang et al., 2012). “Added sugar” refers to sugar added to food prior to consumption, during preparation, or during processing to enhance flavor and/or texture of foods, as opposed to sugar inherently present in a food (Anton et al., 2012; Yoo et al., 2017). There is no chemical difference between added sugar and sugar naturally present in food, but there is a large health concern about added sugar due to the addition of unnecessary calories (Edwards et al., 2016).

Over the past two decades, consumer focus on health has increased, leading to a significant push for healthier food choices (Thompson and Moughan, 2008; Chen, 2011; Küster and Vila, 2017). In general, consumers have a basic understanding of nutrition in regards to sugar but what differentiates a healthy consumer from an unhealthy consumer is how they use their nutritional knowledge to make food choices (Yeon and Lee, 2016). Many parents desire reduced-sugar products for their children, and current studies emphasize the importance of developing healthy eating habits at a young age (Chan et al., 2011; Byrd-Bredbenner et al., 2016). Sigman-Grant and Hsieh (2005) found that consumers that selected “reduced-sugar” versions or a combination of reduced-sugar and full sugar products in general had more favorable diets. All socioeconomic levels of consumers can achieve healthier dietary practices by simply switching from a full sugar food product to similarly priced reduced sugar product. Food and dairy companies should offer consumers healthy alternatives that could help reduce sugar consumption (Küster and Vila,

2017). This review will investigate sugar alternatives and sugar reduction techniques in dairy foods.

Why is the dairy industry interested in sugar reduction?

Health Benefits

Dairy foods are popular in consumer food choices and its sales gross more than \$125-billion per year (IDFA, 2017). With dairy product popularity comes new demands from consumers for healthier, low-calorie products that taste the same as higher calorie counterparts. Dairy products such as yogurt, chocolate milk, custards, shakes, and ice cream are potentially high in unwanted added sugar. Some of the standard processes for developing healthier food products such as fat, sugar, and salt reduction produce unacceptable flavor in dairy products (Biguzzi et al., 2014). Solutions to this dilemma of how to reduce sugar content without sacrificing flavor and negatively impacting product sales are challenging. Various methods exist for reducing sugar to improve the healthiness of dairy products without losing flavor, include direct reduction, sugar substitution, ultrafiltration, lactose hydrolysis, and many others, which will be addressed in this review.

Regulatory Perspective

In order to protect consumers from falsely advertised foods, sugar and sugar-free terms are regulated by the Food and Drug Administration (FDA) in CFR 21.101. When a food is labeled “sugar-free”, the claim represents that the food contains no sugars or sweeteners and the food should be low in calories or significantly reduced in calories. The term “no sugar added” is reserved for foods that have no amount of sugar added and do not contain any ingredient with sugar added, such as jam or fruit juice concentrate. In the Nutrition Facts and Supplement Facts label new rule, added sugar will be declared separately from sugar naturally present in the food

item (FDA, 2016a). For example, if the food has 12 g of sugar but 10 g are from added sugar, that will be declared under the new label regulations. The compliance date for this new rule is currently July 2018 for companies making \$10 million or more in sales and July 2019 for companies making less than \$10 million. Recently the FDA proposed a rule to extend the Nutrition Facts and Supplement Facts label final deadline to January 2020 for large companies and January 2021 for smaller companies, but the proposal is still pending at this time (FDA, 2016a). Goldfein and Slavin (2015) point out that this new labelling regulation will create additional cost for ingredient and food manufacturers that will eventually be passed onto consumers. Additionally, the group pointed out that the new labelling will make added sugar the enemy to consumers when it is not the sole cause of obesity.

“Reduced sugar” can be used to describe foods that contain at least 25 percent less sugar than the reference amount. In general, consumers prefer the “no sugar added” claim as opposed to the “reduced sugar” claim, most likely because they assume that ingredients are added in place of sugar (Patterson et al., 2012). “Reference amount” refers to the specific amount of that food customarily consumed per eating occasion (21 CFR 101.12). For example, when a chocolate milk is labeled reduced sugar, consumers can know that the milk contains at least 25% less sugar than the original product at the same reference amount (240 mL or 8 fl. oz.).

Sweeteners

Role of Sweeteners

Sugar has many roles in foods. Sugar not only makes foods more palatable but is also a bulking agent, adds viscosity, enhances flavor, provides texture, adds color, is a preservative, and inhibits protein coagulation (Davis, 1995; Silcock, 2017). Sugar acts as a bulking agent for many foods. When sugar is replaced or reduced, another bulking agent, such as insoluble fiber or polydextrose systems, often must take its place (Silcock, 2017). However when sucrose is

replaced with a bulking agent, the bulking agents, like maltodextrin (4 kcal/gram), also contribute calories to the product and may negate the original purpose of removing the sugar (Cardoso and Bolini, 2008). For example, maltodextrin is a common sugar replacer/bulking agent and it contributes the same amount of calories as sugar (4 kcal/gram). Sugar can also add viscosity to foods and when it is removed, viscosity is reduced (Muir et al., 1998; Nurgel and Pickering, 2005; Kappes et al., 2006; Saint-Eve et al., 2009, 2010; Cadena et al., 2012; Lekrisompong et al., 2012). Sugar reduces the water activity of foods and beverages, which makes water unavailable for bacteria and fungi growth. Thus, when sugar is removed, it has to be replaced with another preservative, which is often less appealing to consumers. Sugar acts as an anticoagulant agent in that sugar delays a liquid from changing into a solid or semi-solid state (Mizukoshi et al., 1979). For example, sugar delays the coagulation of proteins in many desserts, such as custards.

Another critical role that sugar plays is color through Maillard browning and caramelization. Maillard browning is a chemical reaction that occurs between an amino acid and a reducing sugar that requires the addition of heat (Damodaran, 2008; Varzakas, 2012). Maillard browning is responsible for the color and flavors in caramel, the browning of bread, maple syrup, condensed milk, cookies and many other foods (Harrison and Dake, 2005). Caramelization (browning) occurs when carbohydrates are heated without nitrogen containing compounds (protein) and creates sweet and nutty flavors and brown color (Harrison and Dake, 2005; Damodaran, 2008). Both caramelization and Maillard browning are types of non-enzymatic browning that play critical roles in flavor and appearance of many foods.

Terminology

Terminology of sweeteners include natural, nutritive, high-intensity, artificial, and non-nutritive sweeteners. These terms are currently not regulated by the FDA but definitions based on literature are displayed in Table 1.1 (Antenucci and Hayes, 2014).

Difference between Natural and Artificial Sweeteners

Sweeteners can be divided into two categories: natural and artificial (Servant et al., 2010; Lustig et al., 2012; Shankar et al., 2013). Currently there are many FDA approved natural nutritive sweeteners, two natural non-nutritive sweeteners and seven FDA approved artificial sweeteners with more being developed (FDA, 2015a). Natural nutritive sweeteners provide energy (calories) to the body in the form of carbohydrates (FDA, 2015a). Examples include fructose (found in honey and agave), lactose (found in milk) or sucrose (table sugar). Sugar alcohols are another type of natural nutritive sweetener that have similar sweetness intensity to sucrose but contribute fewer calories to the body due to slower and incomplete absorption in the intestine (Yebra-Biurrun, 2005; Grembecka, 2015). Sugar alcohols are additionally differentiated by a lower blood glucose response because they can be metabolized without insulin (Yebra-Biurrun, 2005; Grembecka, 2015). An example of a sugar alcohol that can be metabolized without insulin would be lactitol or iso-malt. Stevia and monk fruit are natural non-nutritive sweeteners found in the plants *Stevia rebaudiana* and Lo Han Guo (monk fruit) and can be extracted from the plants, dried, and used as sweeteners. The first natural non-nutritive sweetener was approved for use by the FDA in 2009 (purified steviol glycosides with rebaudioside A as the principal component).

Artificial sweeteners are not found in nature and are synthesized. The first artificial sweetener was approved by the FDA in 1958 (Saccharin) and the most recent artificial sweetener

to get approved by the FDA was Advantame in 2014. Artificial sweeteners can be synthesized from a natural source. For instance, sucralose is derived from sucrose but, since the sweetener is derived or synthesized from sucrose, the sweetener is no longer considered “natural”. Many consumers consider artificial sweeteners to be “unhealthy” perhaps due to studies dating back to the 1970’s linking artificial sweetener to cancer in animals (Saccharin) (Kroger et al., 2006; Larsen, 2012; Bearth et al., 2014; Chattopadhyay et al., 2014). These studies have been disproven, and are no longer relevant but nonetheless contribute to the image of artificial sweeteners. More recently, a 2005 study in Italy linked aspartame with various types of cancer in rats (Kroger et al., 2006). Again, the study was disproven but nonetheless continue to cast artificial sweeteners in a negative light enough so that Diet Pepsi claims to be “aspartame free” (Kroger et al., 2006). Wardy et al. (2017) found that even the color of the sweetener packet (yellow, green, pink, blue, and white) elicited an emotional and hedonic response from consumers caused by negative connotations of artificial sweeteners in specific color packets.

Consumers today desire an all-natural label and are willing to pay a premium for these foods as they are perceived as healthier (Rozin and Rozin, 2005; Anstine, 2007; Parasidis et al., 2015). Walters and Long (2012) found consumers with little nutrition knowledge were positively influenced by the “all natural” label no matter what the nutrition facts panel displayed. Ironically, McLean et al. (2017) determined that consumers preferred an all-natural label on bacon packages. However, this claim is not legally possible for bacon due to the addition of nitrates. Both Li et al. (2015a) and Oltman et al. (2015) determined that consumers preferred naturally sweetened labels on chocolate milk and protein beverages. Jervis et al. (2012a,b) also reported that “all natural” and “organic” claims were considered of equal importance to consumers and all

natural was considered a very important term for consumers in sour cream and latte-style coffee beverages.

Natural and artificial sweeteners differ greatly in their sweetness potency. Natural sweeteners range from 0.1-450 times sweeter than sucrose (lactose and monk fruit, respectively) whereas artificial nutritive and non-nutritive sweeteners can be up to 20,000 times as sweet as sucrose (Advantame) (Nofre and Tinti, 2000). When addressing sugar and calorie reduction, artificial sweeteners have more desirable taste profiles and when only flavor is considered (blind tasting), foods and beverages sweetened with artificial sweeteners generally scored better than natural non-nutritive sweeteners (Morais et al., 2014; Voorpostel et al., 2014; Zorn et al., 2014; Kubica et al., 2015; Rocha and Bolini, 2015). Consumers have shown interest in natural sweeteners but many refuse to compromise on taste as the food must have a desirable flavor (Gerdes, 2012; Cernivec, 2014; Li et al., 2014; Oltman et al., 2015). Contrary to this, many consumers still will choose a natural sweetened labelled product over an artificial labelled product (Li et al., 2015a; Oltman et al., 2015).

Additional consideration must be given to stability and cost before sugar can be substituted. There is a large cost difference between natural and artificial sweeteners, saccharin is currently the cheapest sweetener on the market at about \$3/pound which is even cheaper than sugar (\$0.27/pound) when considering sweetness potency. Natural non-nutritive sweeteners are currently much more expensive, monk fruit is about \$329/pound and stevia is about \$130/pound. Stability is another factor of sugar reduction. Many artificial sweeteners cannot be used in certain applications due to heat or pH instability (Chattopadhyay et al., 2014). Even with some instability, due to their sweetener potency and similar temporal profile, artificial sweeteners are currently more common in the dairy industry for sugar reduction than natural non-nutritive

sweeteners. However, artificial sweeteners are increasingly becoming less popular due to the artificial stigma and the changing landscape of food, the use of the term “natural” is currently under FDA review (Bearth et al., 2014; FDA, 2016b).

Non-Nutritive Sweeteners

Whether natural or artificial, sugar reduction in dairy foods has been most successful by replacing sugar with non-nutritive sweeteners. This can be attributed to the fact that nonnutritive sweeteners are able to give the sweet taste desired by consumers without added calories (Yebrabiurun, 2005). Non-nutritive sweeteners are at least 30 to 20,000 times sweeter than sugar and since non-nutritive sweeteners require such small amounts to reach equal sweetness compared to sucrose, manufacturers also can label these products as sugar free or reduced calorie (Mitchell, 2007; Zyglar et al., 2011). Non-nutritive sweeteners are currently consumed by at least 28% of the American population and are ubiquitous in many food products, including beverages, ice cream, chewing gum, chocolate, jams/jellies, yogurt, and salad dressings (Shankar et al., 2013; Antenucci and Hayes, 2014).

The ideal non-nutritive sweetener is a safe and stable molecule with high potency that gives the perception of sweet taste without compromising quality and palatability (Behrens et al., 2011). The goal of identical sweet taste is difficult because non-nutritive sweeteners have a sensory temporality different to that of sugar. Temporality is defined as how flavor or taste intensities/sensations of a food product change over time (Ott et al., 1991; Hanger et al., 1996; Fujimaru et al., 2012). Many different types of non-nutritive sweeteners exist but none of the non-nutritive sweeteners currently on the market are able to match the temporality of sucrose perfectly (Lawless and Heymann, 2010; Palazzo et al., 2011; Morais et al., 2014; Zorn et al.,

2014; Azevedo et al., 2015). Non-nutritive sweeteners can also contribute other sensory attributes including metallic mouthfeel and bitter taste.

Chemistry of Sweet Perception

Sweet Taste Receptors

Taste perception occurs in taste receptor cells (TRCs) which are scientifically classified as modified epithelial cells. Taste receptor cells are packed densely into taste buds located on the surface of the tongue and soft palate with up to 100 epithelial cells forming a single taste bud (Collings, 1974; Lindemann, 2001). In these taste buds, humans have the ability to detect sweet, salty, sour, bitter, or umami tastes, but the mechanism of reception for each of these tastes varies (Lindemann, 2001; Margolskee, 2002; Scott, 2005; Bachmanov and Beauchamp, 2007; Lawless and Heymann, 2010).

Sweet taste is believed to be perceived by G-Protein Coupled Receptors (GPCRs); specifically, sweet taste receptor subunits (Behrens et al., 2011). Each GPCR has a large amino-terminal ectodomain (ATD) with an area similar in appearance to a “Venus-Flytrap” domain (VFTD) searching for something to bind. The VFTD likely contains multiple binding sites for different sweet molecules called T1R2 and T1R3. The higher affinity a molecule has to the VFTD binding site, the more intense the sweetness is perceived (Servant et al., 2010; Behrens et al., 2011). Sweeteners not only bind with different affinities but bind on different spots on the T1R2 and T1R3 receptors and many are characterized by a lingering sweet aftertaste (DuBois and Lee, 1983; Meyers and Brewer, 2008). Both of these facts explain why non-nutritive or artificial sweeteners taste so sweet when compared to sucrose (Meyers and Brewer, 2008; Nie et al., 2008). For example, aspartame is known for a slight delay of onset of sweetness and with moderate lingering sweetness in the aftertaste whereas stevia is known for delayed onset on

sweetness and a long lingering sweet and bitter aftertaste (DuBois and Prakash, 2012; Morais et al., 2014; Zorn et al., 2014).

Some sweeteners additionally contribute other sensory attributes, such as bitter taste or metallic sensation. This sensation may be a taste caused by the sweetener's ability to interact with multiple binding sites within the taste receptor (Allen et al., 2013) while others have described it as a mouthfeel or trigeminal response – or more recently, an aromatic involvement was proposed (Waldrop and Ross, 2014; Skinner et al., 2017). Sucralose, stevia and other non-nutritive sweeteners are generally documented to elicit a metallic sensation. Bitterness is another common problem with nonnutritive sweeteners and food producers try to mask this by blending sweeteners (Pineiro and Oliveira, 2005).

The role of matrix on sweet taste

Texture and fat in the food matrix play an important role in sugar reduction studies as both highly influence sweet taste perception. Several studies have been conducted to identify the relationship between texture and flavor, very few have been able to accurately define the relationship, primarily because so many relationships exist (Baines and Morris, 1987; Wilson and Brown, 1997; Malone et al., 2003; Di Monaco et al., 2014). Baines and Morris (1987) reported that the perceived intensity of flavor and sweetness was independent of guar gum concentrations up to c^* . Food polymers are proteins, polysaccharides, or peptides and c^* is defined as the critical concentration which marks the transition from a dilute solution of random coil polymers to an entangled network of overlapping hydrodynamic volumes at high polymer concentration. At guar gum concentrations above c^* , perceived flavor and sweetness decreased steeply. The authors hypothesized that this was due to inefficient mixing that inhibited transport of tastant molecules, causing a sweetness and flavor reduction once the critical

overlap concentration was surpassed. Practically speaking the effect of viscosity on taste/flavor is unclear, but clear suppression occurs at $c > c^*$. Particles weren't entrapped until c^* was exceeded. In summary, at higher viscosities it is harder to perceive sweet taste, and thicker foods require higher concentrations of sugar to obtain equivalent sweetness (Baines and Morris, 1987). Wilson and Brown (1997) found that the higher the concentration of gelatin, the more panelists had to chew the samples. This prolonged exposure resulted in lower intensity of flavor but in addition gave a prolonged perception of the flavor. This model suggests that the longer a consumer has to chew a sample, the longer the flavor lasts, but at much lower intensities. Malone et al. (2003) reported that knowledge of in-mouth behavior of food microstructures and flavors could be used to design food product microstructures in order to control flavor release and perception. For example, as more components get added to a food or as complexity of food increases, the perception of acidity decreased (Malone et al., 2003). Based on the Malone et al. (2003) hypothesis, reduced sugar dairy foods can be designed to mimic full sugar products because a reduced sugar dairy foods can be designed to control flavor release and sweetness perception to taste like a full sugar product.

All these models can be applied to understanding dairy foods in terms of sugar reduction. For example, chocolate milk is a complex, viscous food matrix and based on the models above, adjusting viscosity might enable some sugar reduction (Arabie and Moskowitz, 1971). Iso-sweetness of monk fruit in water versus that of skim chocolate milk was determined by magnitude estimation scaling and the reported exponent for monk fruit in water was 0.80, and in skim chocolate milk was 0.68 (Li et al., 2015a). The higher the exponent, the more sweetening power a solution has. Sweeteners in water have a higher sweetening power than sweeteners in chocolate milk which is consistent with the previous studies (Drake et al., 2011; Li et al., 2015a;

b). Additionally, although thicker foods are perceived as less sweet, there is a prolonged flavor exposure time throughout oral processing (Wilson and Brown, 1997; Di Monaco et al., 2014). Staying with the chocolate milk example, chocolate flavor will be perceived longer in a thicker food. All of these studies reveal that maximum sweetness intensity depends upon not only temporal properties of the sweeteners, but additionally textural properties as well (Bayarri et al., 2007).

Sweet taste can be further hindered by other components of the food matrix, besides viscosity, such as a higher fat content (Malone et al., 2003). Fat plays a critical role in the perception of sweet taste and in general, solutions are perceived to be less sweet in emulsions with higher fat content (Bayarri et al., 2006; Zahn et al., 2013). Bayarri et al. (2006) suggested that the lower perception of sweet taste in an emulsion may be triggered by some trigeminal sensation that leads to a decrease in taste perception. Hoppert et al. (2012) found that trained panelists had higher sensitivity to sweetness differences at higher fat content. Therefore, chocolate milks with higher fat content require higher sugar contents to be perceived as iso-sweet, and if the chocolate milk is a high fat food, regular consumers of the product will be highly sensitive to a reduction in sugar. The same principle could be applied to ice cream, yogurt, or any dairy product, suggesting that sugar reduction in higher fat foods may be more challenging.

Milk and sugar

Lactose

Lactose, a disaccharide naturally present in milk, is an inherent part of mammalian milks and can be found in goat, cow, human milk, as well as in all other animal milks. Lactose is exclusively found in milk and not found naturally in any plants or plant products (Schaafsma,

2008). Lactose is 9.4 times less sweet than standard sucrose but when lactose is hydrolyzed into glucose and galactose the combination is only 1.5 times less sweet when compared to sucrose (Pangborn, 1963). Flavored milks, as such, have sugar added to increase the flavor and overall sweetness, and the acceptability.

Lactose accounts for about 30% of the caloric value of whole milk but provides little sweetness (American Society for Clinical Nutrition, 1988). Lactose is formed when one glucose molecule is converted to UDP-galactose and is then combined with an unmodified glucose that contains a β -1-4 glycosidic linkage in the mammary glands or udder of the mother cow (Schaafsma, 2008). Lactose is unique in that it only exists in its free form in milk where it is unattached to any other molecule (Wiley, 2002). Lactose is the single most abundant milk solid particle and is an important starting material for probiotic bacteria (Mazza, 1998). Many manufactures add lactose to baby formula so that formula-fed infants still get probiotic growth in their gut (Mazza, 1998). As consumers age, lactose will continue to be feed for the bacteria in their gut but many consumers develop hypolactasia or lose the ability to digest lactose as they grow older (Keusch et al., 1969; Sahi et al., 1983; Tadesse et al., 1992; Rasinpera, 2004; Troelsen, 2005). Lactose-intolerant individuals have a deficiency of lactase, the enzyme needed to break down lactose, which is believed to be caused by polymorphisms across the 50 kb *LCT* gene (lactase nonpersistence) (Bhatnagar and Aggarwal, 2007; Ingram et al., 2009). Without lactase, the lactose becomes food for gas-producing bacteria in the gastrointestinal (GI) tract, leading to gastrointestinal distress including bloating, abdomen pain, and diarrhea (Zadow, 1986; Wiley, 2002). In America: 6-25% of Caucasians and 45-81% of African Americans, Asians, and Hispanics suffer from lactose intolerance (Baadkar et al., 2014; Abbasi and Saeedabadian, 2015). Lactose intolerance is defined as experiencing the symptoms defined above 30 minutes to a few

hours after consuming dairy products. Discomfort can vary widely across individuals and there is currently no set limits for what defines lactose intolerant from not lactose intolerant (Cook and Dahlqvist, 1968; Suarez et al., 1995; Ingram et al., 2009).

It is important to note that lactose intolerance is not a milk allergy. Lactose intolerance is the inability to breakdown lactose which causes (GI) discomfort. A milk allergy is an immune system response to milk protein that occurs after a person consumes milk (FARE, 2013; FDA, 2015b). Allergies can only occur in the presence of proteins. In the case of milk, a person can be allergic to casein protein, whey protein, or in some cases both. Milk allergy is the most common allergy among children affecting approximately 2% of children under the age of 4 but milk allergy is uncommon among adults with only 0.1-0.3% with confirmed cases in adults (Woods et al., 2001; Rona et al., 2007). Sugar reduction in milk and lactose intolerance does not affect consumer allergies or sensitivities to milk protein.

Status of lactose in different dairy products and dairy ingredients

The lactose content in dairy products varies greatly. Bovine milk including whole, skim, low fat, buttermilk and flavored milk ranges from 4.2-5% lactose (Varnam and Sutherland, 2001). Spray dried milk powders contain higher amounts of lactose on a weight to weight basis compared to fluid whole or skim milk (43-59.6% lactose) due to water removal (Nijdam and Langrish, 2006). Other dairy products contain lower percentages of lactose. Butter is a high-fat dairy product that contains only trace amounts of lactose (Portnoi and MacDonald, 2011). Cheeses can have a large range of lactose, ranging from as low as being non-detectible to as high as 14.2% in some process cheeses (Kapoor et al., 2007; Davila-Vazquez et al., 2008). Hard cheeses have low levels of lactose because much of the lactose is strained out with the liquid whey during the cheese making process (Ramchandran and Vasiljevic, 2012). Since soft cheese

do not undergo the same process as hard cheese, they have a higher lactose content. Yogurt mix contains about 6-8% lactose after pasteurization, but lactose concentrations decrease during fermentation to 6.4% 45 minutes after inoculation. The yogurt culture ingests the lactose and continues to break it down to 1-5% at the end of fermentation (Wolf et al., 2015).

Whey Protein Isolate (WPI) contains less lactose than Whey Protein Concentrate (WPC) 34 (0.1-2.6% and 54.8% respectively)(Silvestre et al., 2012; Norwood et al., 2017). In contrast, whey permeate is a high-lactose dairy ingredient that is produced as a by- or co-product of whey protein manufacture and contains mostly lactose (65-85%) and minerals (Adamczak et al., 2009; Frankowski et al., 2014; Smith et al., 2016).

General Method of Sugar Reduction

Lactose hydrolysis

Lactose hydrolysis is a method for sugar reduction in dairy foods. Currently, enzymatic lactose hydrolysis is used to make lactose-free milk (Fox and McSweeney, 2003; Harju et al., 2012). The FDA has not yet set a definition for the terms "lactose-free" or "lactose-reduced," in dairy products (FDA, 2015b). However, food manufacturers must label products truthfully. A lactose-free product should not contain lactose, and lactose-reduced product must have a meaningful reduction (FDA, 2015b). Although the importance of lactose hydrolysis in dairy products has been known for decades, it has only been within the past few years that industrial production of β -galactosidase became feasible (Abbasi and Saeedabadian, 2015).

Lactose hydrolysis can be done many ways. Single-use hydrolysis is performed 2 ways: the first method consists of adding β -galactosidases to pasteurized milk and holding the mixture at 35-45 °C for a set amount of time or by holding it overnight at refrigeration temperatures (Zadow, 1986). The lactase enzyme is then deactivated by an additional heat treatment. The

second method for lactose hydrolysis can be achieved by adding a sterile lactase to Ultra High Temperature (UHT) milk prior to packaging. In this method, lactose is broken down during the first few days of packaging. Both single use enzyme methods are costly (Panesar et al., 2010). More cost effective lactose hydrolysis methods include membrane recovery of soluble enzyme or immobilized systems (Zadow, 1986). In the membrane recovery system, the enzyme is added to milk permeate after ultrafiltration and the enzyme can then be recovered by filtration and reused. The hydrolyzed lactose permeate is then reintroduced into the milk (Zadow, 1986).

Lactose hydrolysis has been investigated as a sugar reduction alternative because hydrolysis of 70% of the lactose in milk increases the sweetness of milk or yogurt to the same degree as adding 2% sugar would (Zadow, 1986; Mahoney, 1998; Adhikari et al., 2010). Lactose free milk is sweeter than regular milk (Jelen and Tossavainen, 2003; Adhikari et al., 2010; Skryplonek et al., 2017). Li et al. (2015b) investigated the impact of using lactose hydrolysis to sweeten chocolate milk naturally. The sweetness achieved from hydrolysis of lactose naturally present in the milk was not sufficient to sweeten chocolate milk to a palatable level. The authors suggested that this method might be more successful in a simpler flavored milk other than chocolate milk since cocoa is inherently bitter and requires more sugar than vanilla or strawberry milk (Li et al., 2015b). Li et al. (2015b) additionally investigated the impact of adding lactose directly through the addition of a permeate followed by hydrolysis. The addition of the permeate powder created an overwhelming salty taste due to the minerals in permeate which made this an unsuitable approach to sweeten chocolate milk (Li et al., 2015b). Lactose hydrolysis of natural lactose in milk has also been investigated as a means of sugar reduction in yogurt (Engel, 1973; Tamime and Deeth, 1980). Hydrolysis of lactose in yogurt leads to a milder, sweeter yogurt without added sugar (Engel, 1973). Whalen et al. (1988) reported that consumers could not

detect a difference between yogurt sweetened with sugar (4 g sucrose/ 100 g yogurt) and lactose-hydrolyzed yogurt with less sugar added (2-3 g sucrose/100 g yogurt) (n=25, p<0.01). This approach (lactose hydrolysis) is applied for modest sugar reduction in yogurt today.

Lactose hydrolysis has also been used to reduce sugar content in ice cream through either hydrolyzing lactose in ice cream mix or using lactose-free skim milk powders (Abbasi and Saeedabadian, 2015). El-Neshawy et al. (1988) found that lactose-reduced reconstituted skim milk caused a decrease in the formation of an undesirable sandy texture in reduced sugar ice cream and improved the textural properties. Sandy texture in ice cream is an undesirable characteristic that is caused by large lactose crystals (Arbuckle, 1972). Large lactose crystals form in ice cream due to the low solubility of lactose. When the available free water freezes in the ice cream mix, high concentration of lactose are left behind to form large crystals (Skryplonek et al., 2017). El-Neshawy et al. (1988) recommended hydrolysis of 75% of the lactose in skim milk powder to prevent formation of the sandy texture and reduce sugar content by 12.5-25%. Abbasi and Saeedabadian (2015) implemented this finding and found that lactose hydrolysis at the suggested 75% usage increased the apparent viscosity of the ice cream mix, lowered the freezing point, increased the sweetness, lowered sandiness, and improved overall acceptability of ice cream. In this study, lactose hydrolysis of milk led to a 25% reduction in sugar content of ice cream.

What happens to label declaration when lactose is hydrolyzed?

There is currently a debate over whether lactase is considered a processing aid or if lactase must be declared as an ingredient on food labels (FDA, 2004, 2015c). Processing aids are substances approved by both the FDA and the U.S. Department of Agriculture (USDA) which are not present in significant amounts in the finished product and do not affect the appearance or

taste of the final product (USDA, 2008). As stated earlier, it is impossible to hydrolyze all of the lactose in the product and therefore in lactose free milk such as Lactaid™ and Fairlife™, additional lactase is added into the packaged product to breakdown the remainder of the lactose present in the milk prior to consumption (Jelen and Tossavainen, 2003). These products declare “lactase enzyme” as an ingredient because some lactase is never deactivated by heat and this type of lactose-free milk must declare lactase on the label. The FDA considers this an added ingredient and it must then be declared on the label (USDA, 2008). However, if lactase is naturally produced by the body, does it need to be declared on the label? This is the dilemma that is the subject of much debate. It is impractical as stated in 21 CFR 101.100 (a) (3) to declare “incidental additives” (processing aids) in nonfunctional trace amounts; however, when it is present in active and functional amounts in the final product, it should be declared on the label. Additionally, consumer education is of vital concern. If some milks need to declare lactase as an added ingredient per the FDA definition and some are not required to declare lactase on the label, consumers then become confused. This phenomenon creates large obstacles for the dairy industry when lactase is used as a method of sugar reduction in dairy foods. Education and consistency are important for consumers to understand how sugar --or lactose— reduction is being achieved.

Ultrafiltration

Ultrafiltration (UF) is a well-established method for decreasing lactose in milk (Jelen and Tossavainen, 2003). UF is a pressure-driven process that separates compounds in milk by molecular weight. UF is widely used in the dairy industry for lactose removal and in turn can be used for sugar reduction. Heavier molecular weight compounds (protein, fat) are retained by the UF whereas the lower molecular weight compounds (lactose, water, minerals, and vitamins) pass

through the membrane. Water can then be added back into the suspended solids to create lactose-free milk that is not sweet like lactose hydrolyzed milk.

The final sweetness of UF produced lactose free milk can be improved through milk chromatography or through the addition of non-nutritive sweeteners (Jelen and Tossavainen, 2003; Harju et al., 2012). Milk chromatography, the process of separating lactose from milk without removing the salts, splits milk into two streams: a main stream and a secondary steam. In the main stream, where a majority of the milk goes, lactose is removed from the milk through ultrafiltration. In the secondary stream, lactose is hydrolyzed with enzymes. The two streams are then brought back together so that the sweetness of the final product matches that of regular milk (Jelen and Tossavainen, 2003; Harju et al., 2012). UF milk can also be improved through the addition of non-nutritive sweeteners following ultrafiltration. This method of lactose removal has been investigated as a means of sugar reduction in yogurt and cheese (Kosikowski, 1979; Özer and Robinson, 1999; Magenis et al., 2006; Karam et al., 2013). The lactose is removed from the milk before further processing into yogurt or cheese.

Direct Reduction

Direct reduction of sugar is a method for a gradual reduction of sugar consumption (MacGregor and Hashem, 2014). The sugar content of dairy products is slowly and progressively reduced so that consumers will gradually get used to the lower sugar concentrations without noticing a difference (MacGregor and Hashem, 2014). This reduction can be achieved through threshold testing to determine what change in sugar concentration causes a perceivable change in sweetness intensity by 50% of consumers (Boring, 1946). This is also called a “Just Noticeable Difference Threshold (JND). The calculated JND can then be used to determine gradual sequential sugar reductions of up to 30% that can be implemented without consumer awareness

(Li et al., 2015b). This gradual reduction has been achieved in the United Kingdom in regards to salt reduction (MacGregor and Hashem, 2014). As a further example of the application of JND values to reduction of target ingredients, Drake et al. (2011) evaluated JND salt concentrations in different dairy matrices (cottage cheese, cheese sauce, and milk-based soup). They found that consumers were sensitive to salt reduction in dairy foods and noticed reductions at less than 20% in all the products tested. The JND values were different among the three different dairy foods tested. However, if consumers were informed about the reduced sodium and its health benefits, overall liking scores were not significantly different between the regular and reduced sodium options (Drake et al., 2011). JND have not been investigated thoroughly in terms of sugar reduction in dairy foods but could be a valuable option that should be explored. In Harwood et al. (2013) recommended using rejection thresholds, or the point at which a consumer preference occurs for a sample not containing a substance, to see at what point a consumer will no longer accept a product for quality control and product optimization (Lawless and Heymann, 2010). In terms of sugar reduction studies, the rejection threshold is important to determine when a consumer will no longer purchase a product. JND has been used for sugar reduction in dairy-based emulsions and chocolate milk (Hoppert et al., 2012; Oliveira et al., 2016). Hoppert et al. (2012) reported a matrix specific sugar reduction; the higher the concentration of fat the more sensitive a panelist is to a sugar reduction (lower the JND value). Based on these studies on model foods, a modest sugar reduction of 5-20% may be possible by direct reduction (Hoppert et al., 2012; Oliveira et al., 2016). Even if consumers notice a difference in sweetness, the product in its sugar reduced form may still be liked. Li et al. (2015b) reported that direct sugar reduction under 30% did not change consumer acceptance.

Sugar Substitutions

Since consumers desire sweet taste, sugar substitution is a preferred method among sugar reduction techniques to reduce calories while preserving sweet taste. Currently, there are many different types of sweeteners available for sugar substitution (Table 1.2), but as stated earlier many issues arise with sugar substitution with the most troublesome issue being that sugar substitution is very product specific. The relationship between the matrix and the concentration of sweetener to create an iso-sweet relationship cannot be consistently defined by any single model. Sugar substitution has been successfully done, however, in many products such as frozen yogurt, yogurt, and chocolate milk. Isik et al. (2011) found that isomalt could be substituted for sugar in frozen yogurt for a similar sweetness and no added sugar. Narayanan et al. (2014) reported that stevia, at 0.7- 5.5% wt./wt. substitution concurrently with mildly sweet bulk fillers or some sucrose, could be used in naturally flavored vanilla low-fat yogurt. Guggisberg et al. (2011) investigated the effects of a total substitution of 8% sugar in yogurt with Actilight (a fructooligosaccharide) combined with stevia and Palatinose and found that 6% Actilight combined with stevia lead to a temporality most similar to the sucrose control. Li et al. (2015a) also reported that chocolate milk could be partially sweetened with monk fruit and stevia to successfully reduce sugar and maintain sweet taste and a similar temporal sweetness profile.

Formulation challenges for reduced sugar dairy products

A reduction in sugar is easier to perceive than a reduction in fat or salt content, making formulation of reduced sugar dairy products a challenge (Biguzzi et al., 2014). One critical challenge that makes these studies so inherently difficult is that the perceived intensity of the sweetener is entirely dependent upon the food matrix. Wilson and Brown (1997) and Li et al. (2015a, 2015b) reported that iso-sweet concentrations of sugar compared to different sweeteners

differed when the viscosity of the matrix was increased. Biguzzi (2014) found that a reduction in sugar content caused little change in perception of fat in biscuits, whereas a reduction in fat content sometimes induced a reduction in sweetness perception. This means that the relationship between the matrix and the concentration of sweetener to create an iso-sweet relationship cannot currently be consistently defined by any model. Therefore, there cannot be an overarching study for all non-nutritive sweetener substitutions, instead, each substitution is product specific. This item makes sugar reduction in dairy foods challenging.

Sugar reduction studies in dairy foods

Ice Cream

Ice cream is one of the most heavily consumed dairy products in the world (Sun-Waterhouse et al., 2013). Ice cream has a highly complex food matrix that can be defined as an aerated suspension of fat and water in a concentrated sugar solution (Frøst et al., 2005; Erkaya et al., 2012). To achieve the sweet taste desired by consumers, a substantial amount of sugar needs to be added ranging from 10-14% (Goff, 2015). Therefore, reduced sugar and reduced fat products, such as ice cream, in general, tend to show a higher propensity for a bitter aftertaste and a lower intensity of creaminess (Cadena et al., 2012). Even with higher bitter tastes due to replacement by alternative sweetener, a 25% reduction in sugar was not linked to decreased consumer acceptance of vanilla ice cream (Cadena et al., 2012). Furthermore, Cadena and Bolini (2011) found that calorie-reduced ice creams, with a sugar reduction of 3.4%, sweetened with sorbitol and sucralose were most accepted compared to other “light” vanilla ice creams, or ice cream with a minimum reduction of 25% of the total energy, sugar, or lipid. Sugar substitutes such as sugar alcohols have also been investigated to reduce the sugar content of ice cream. Erythritol and lactitol are sugar alcohols that have both been used to create low-calorie ice cream

(Grembecka, 2015). Erythritol is more commonly used for sugar reduction in ice cream because it provides volume and texture and is only a fraction of sucrose calories (0.2 kcal/g).

Another hurdle in sugar reduction of ice cream is flavor. Chocolate flavored ice creams are typically formulated with higher sugar content to decrease the bitterness associated with cocoa (Harwood et al., 2013). Guinard et al. (1996) found that the optimum level for sugar in vanilla ice cream was around 13.5% but other research has shown that chocolate ice creams had to have much higher sugar levels to compensate for the bitterness of cocoa (Prindiville et al., 1999). Marshall et al. (2003) suggested this higher level of sugar to be a 1:1 ratio of sugar to cocoa powder. Chocolate ice cream sugar reduction is thus difficult because of cocoa bitterness and the decreased flavor perception that comes with sugar reduction (Guinard et al., 1996; Koeferli et al., 1996; Roland et al., 1999). When sugar is reduced in chocolate ice cream, not only does it taste more bitter, but the ice cream tastes less chocolatey (Prindiville et al., 1999). Harwood et al. (2013) proposed a solution to this problem by marketing sugar reduced chocolate ice cream to dark chocolate lovers who already desire and tolerate substantially higher levels of bitterness.

Fat reduction is another way the dairy industry is trying to decrease energy density of ice cream. Fat reduction impacts not only the mouthfeel of ice cream but can lead to a change flavor release and possibly sweetness perception (Karaca et al., 2009). Prindiville et al. (1999) found that decreasing the fat content of chocolate ice cream increased the perceived sweetness. The study found that ice cream with fat at 0.5% was significantly sweeter than other ice creams at 4.0, 6.0 and 9.0% because the fat was not there to impede sweetener exposure to tongue (Malone et al., 2003). Conflicting research from Roland et al. (1999) reported that there was an increase in sweetness perception in vanilla ice cream when fat was used above 7%. In turn, Rolon et al.

(2017) found that, although there was a physical change in texture properties of vanilla ice cream when substituting maltodextrin for fat (fat 6-14% with respectively maltodextrin 8-0%), there was no change in sweetness perception of ice cream when using an untrained consumer panel. However, the trained panel did detect a sweetness difference among the different fat reduction treatments (Rolon et al., 2017).

Frozen yogurt is often viewed as the healthy alternative to ice cream due to its lower fat content and the presence of lactic acid bacteria even when frozen (Lopez et al., 1998). The typical sugar content of frozen yogurt is around 10-14 g/100 g which is about the same amount of sugar as ice cream (14 g/100 g) (Marshall et al., 2003). Skryplonek et al. (2017) investigated lactose hydrolysis in frozen yogurt to increase solubility of lactose, increase sweetness of the yogurt and expand frozen yogurt available to lactose intolerant individuals. When a trained panel compared the lactose hydrolyzed frozen yogurt to the control, the trained panel documented that the lactose hydrolyzed yogurt was creamier and sweeter. Isik et al. (2011) reported that substituting inulin and iso-malt for sugar and fat in frozen yogurt led to a similar sweetness and a reduction in fat with no added sugar.

Yogurt

Yogurt is a popular product in the United States helping the dairy industry by grossing more than \$125-billion a year (IDFA, 2017). Yogurt is generally recognized as a healthy food due to its nutritional content but is generally sweetened with sugar to increase palatability (Guggisberg et al., 2011). Yogurt is made when lactic acid producing bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, are added to cream, milk, or skim milk when lactose is present (FDA, 21 CFR 131.200). The bacteria culture ferments the lactose present in the milk to lactic acid causing the milk to thicken and also gives yogurt its characteristic sour taste

(Tamime and Deeth, 1980; Harper et al., 1991). The first step to making yogurt is mixing all the ingredient such as milk, flavoring, and sugar to make a milk base. Sugar, up to 5%, is added to the milk base before homogenization, heat treatment and before incubation/fermentation (Tamime and Deeth, 1980; Sodini et al., 2017). The sugar is added to yogurt to lessen the impact of sour taste due to the production of acids and acetaldehyde in yogurt by the bacteria (Harper et al., 1991). The bacteria ferment about 40-50% of the lactose in the yogurt mix (Alm, 1982).

Several studies reported that yogurt liking was influenced by texture, aroma, and taste (Kälviäinen et al., 2003; Bayarri et al., 2011; Routray and Mishra, 2011; Desai et al., 2013; Grygorczyk et al., 2013). Sweetness is a major driver of liking for yogurt and yogurt-like products (Bayarri et al., 2007; Thompson et al., 2007). Consumers find yogurt without any added sugar to be too sour (Harper et al., 1991). Allgeyer et al. (2010) found that consumers preferred drinkable yogurts with a medium level of sweetness and high viscosity. Hoppert et al. (2013) reported that sugar content (112 g/kg verse 160 g/kg) had a significant effect on the overall liking of the yogurt when investigating different types of dietary fibers in the yogurt. The group found that higher sugar content lead to higher overall liking. However, Hoppert et al. (2013) did find that many consumers rated the regular sugar products as too sweet and flavor as too low and that increasing the flavor of sugar reduced yogurt could lead to better hedonic scores. Yoo et al. (2017) additionally suggest that sugar reduction is positively perceived by children and adolescents as both had an increase in expected overall liking scores when primed with a sugar reduction claim. Chollet et al. (2013) found that although consumers preferred yogurts with 10% sugar they found yogurt containing 7% sugar acceptable.

Non-nutritive sweeteners were additionally investigated as a means of sugar reduction in yogurt. Pinheiro and Oliveira (2005) reported that it was possible to produce a probiotic yogurt

successfully using sweeteners (sucrose, aspartame, aspartame + saccharin, and sucralose) without affecting the viability of the probiotic microorganisms. The addition of nonnutritive sweeteners did not have a negative impact on the yogurt making process as the sweeteners did not breakdown over time (Pinheiro and Oliveira, 2005). Sweetener blends of non-nutritive sweeteners have been very successful in reducing sugar content of yogurt (Gelardi, 1987; Birch, 1996; Hanger et al., 1996; Pinheiro and Oliveira, 2005; Waldrop and Ross, 2014; Narayanan et al., 2014; Guggisberg et al., 2011).

Flavored Milk

Flavored milk continues to gain popularity among children and adults, due to its special taste and its ability to meet the dietary requirements for dairy foods in the United States (IDFA, 2010). Chocolate milk is the most popular flavored milk for both children and adults in the United States (Thompson et al., 2004, 2007). In order to be considered chocolate milk, the chocolate flavoring must meet the standard of identify of cacao products as defined by 21 CFR 163. Studies have shown that flavored milk increases milk consumption, therefore chocolate milk plays an important role in getting Americans to consume the recommended 3 cups of milk per day (Murphy et al., 2008; Li et al., 2015b; USDA-HHS, 2015). Yon et al. (2012) found that on average students in grades 3-5th consumed about 5.5 oz. of flavored milk per day and elementary students were just as likely to drink lower calorie flavored milk as regular milk. Li and Drake (2015) pointed out that the Yon et al. (2012) study did not have a huge calorie reduction so the flavor differences were likely small and most likely not detected by children. In May 2017, US Agriculture Secretary Sonny Perdue signed an interim rule to bring 1% chocolate milk back to the National School Lunch Program (NSLP). Previously, only unflavored low fat

milk or flavored/unflavored skim milk was allowed in elementary or secondary schools as part of the NSLP (8 oz. and 12 oz. respectively) (American Heart Association, 2017).

Despite having similar nutritional benefits as regular fluid milk, chocolate milk is known for a higher sugar content and thus chocolate milk is a large target for sugar reduction techniques (Johnson et al., 2002; Murphy et al., 2008). From 2006 to 2010 there was a decrease in the calories of chocolate milk 165.9 to 154 (Van Horn et al., 2010). However sugar reduction of chocolate milk is quite costly and many school directors choose the higher sugar alternative to reduce cost or choose to eliminate chocolate milk entirely (Van Horn et al., 2010; Li and Drake, 2015). Quann and Adams (2013) found that eliminating chocolate milk from schools resulted in a 37.4% decrease in milk consumption. Furthermore, when chocolate milk is removed from a child's diet, in order to replace the nutrients given from milk, 3 or 4 additional foods needed to be added into the diet adding additional calories (16 to 141 kcal) and cost (\$2200 to \$4600 annually per 100 students)(Quann and Adams, 2013). Therefore, sugar reduced product should be considered the cheaper alternative.

Chocolate milk has many factors that influence consumer choice. Kim et al. (2013) reported that fat content and sugar content (or intrinsic factors) were the biggest drivers of choice for adult consumers of chocolate milk selection and had a significant effect on consumer purchase habits. This study found that consumers were more likely to purchase chocolate milk based on intrinsic factors (fat content and sugar content) as opposed to extrinsic factors (brand name and packaging labels) especially when the consumers were able to taste the milk. Additionally an organic label had a positive influence on consumer satisfaction of chocolate milk even if it did not directly correlate to higher purchase intent (Kim et al., 2013). Thompson et al. (2007) reported that having a "natural" chocolate flavor was the most important attribute for

Hispanic and Caucasian adult consumers when choosing chocolate milk. Thompson et al. (2004, 2007) also found that sweet taste was a driver of liking but many adult consumers reported commercial chocolate milk to be too sweet. Li et al. (2014) reported that parents preferred natural non-nutritive sweeteners over nutritive sweeteners as the sweetener source in chocolate milk. However, adults preferred both nutritive and natural non-nutritive sweeteners over artificial non-nutritive sweeteners in chocolate milk. Kim et al. (2013) found that although consumers were aware of lower fat product and lower sugar products, most still preferred a higher calorie product due to taste. Childs and Drake (2009) reported a similar phenomenon with fat replacement; even if a consumer knows an alternative food was better for them, some consumers do not want to sacrifice flavor while seeking lower fat alternatives. Li and Drake (2015) predicted that innovation of calorie reduced chocolate milk with natural sweeteners should help increase milk consumption while increasing sugar reduction. Because there are known off-tastes associated with natural non-nutritive sweeteners, Li et al. (2015a) investigated adult and child acceptance of chocolate milk using blends of sucrose and non-nutritive sweeteners. Young adults and children found chocolate milks with 25% monk fruit with sucrose and 25% stevia with sucrose (9.39 g/serving sucrose and 10.8 mg/L monk fruit or 7.09 mg/L stevia) to be acceptable (Li et al., 2015b). However, greater substitutions of stevia or monk fruit were not as well liked. Li et al. (2015b) reported that sugar could be directly reduced in chocolate milk (16.8 g sugar/serving (benchmark control) to 13.3 g (sugar/serving) and still be accepted by children and adults as long as it does not exceed 30%.

Oliveira et al. (2016) investigated direct sugar reduction strategy regarding chocolate milk using JND values in Uruguay. The group reported that consumers could detect a sugar reduction of approximately 6.00% (24.41 g sucrose/240 mL). However, Li et al. (2015b)

reported that even though consumers could detect small differences in sweetness in chocolate milk, and milks that did not exceed a 30% reduction in sugar were still liked. Consumers reported a noticeable difference in the sensory attributes of sweetness, bitterness, and chocolate flavor in chocolate milk (Oliveira et al., 2016). Interestingly, Harwood et al. (2012) found that consumers who self-reported that they preferred dark chocolate had a higher bitterness rejection threshold (2.3 times) than consumers who preferred milk chocolate. However, there was no difference in bitterness detection threshold between consumers that preferred dark chocolate and those that preferred milk chocolate milk suggesting that chocolate preference was not due to bitterness sensitivity. This may be a contributing factor to Oliveira et al. (2016) finding that even though consumers could detect a reduction in sugar at 6%, a sugar reduction of 28.9% did not result in significant differences in overall liking of chocolate milk. This results is also consistent with Oliveira et al. (2015) who reported a 20% reduction in added sugar did not cause significant differences in liking in chocolate milk. Li et al. (2015b) also reported that sucrose reduction in chocolate milk for both children and young adults was possible as long as it did not exceed a 30% reduction (from 70.17 g/240 mL). Oliveira et al. (2016) suggested that sugar could gradually be reduced over time without a significant change in consumer liking (MacGregor and Hashem, 2014). Furthermore, this approach can be implemented for all commercially available dairy products to make more healthful processed products to meet current nutritional recommendations (Oliveira et al 2016).

Practical Application: Understanding current sugar reduction techniques, research, and consumer response to sugar reduction in dairy products is important for dairy manufacturers to design and produce sugar reduced products. Sugar reduction is an inherently difficult task due to the many functions of sugar in food products, but progress is being made to make consumer

accepted products. Currently, substitution of sugar using non-nutritive sweeteners has been the most successful approach for sugar reduction, but direct reduction of sugar and lactose hydrolysis methods show promise. Manufacturers can use different sugar reduction techniques to produce high quality products to help reverse negative health effects with high sugar consumption.

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Table 1.1. Terminology used to describe sweeteners in dairy foods

Term	Definition
Nutritive	provide energy in the form of carbohydrates while additionally contributing calories (FDA, 2015a)
Non-nutritive	very low in calories (0.08 cal/g) or contain no calories at all and are often sweeter than sugar on a weight by weight basis (Antenucci and Hayes, 2014; USDA, 2015)
High-intensity*	are many times sweeter than sugar on a weight to weight basis but contribute only a few to no calories when added to food (Shankar et al., 2013), for example: aspartame contains two percent of the calories of an equivalent amount of sugar (sugar 4 cal/g and aspartame 0.08 cal/g).
Natural	refers to sweeteners already present in a food substance or occurring commonly in nature (Anton et al., 2012). Examples: sucrose, fructose, stevia, Monk Fruit, lactose, etc.)
Artificial/synthetic	are synthetic sugar substitutes such as sucralose or aspartame that are non-caloric sweeteners (Sirshendu et al., 2013).
Non-Caloric	contribute no calories (Prakash et al., 2008; Sehar et al., 2008)

*Note: All approved non-nutritive sweeteners are considered high intensity sweeteners (Shankar et al., 2013).

Table 1.2. Sweeteners used in Dairy Foods

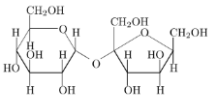
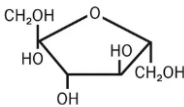
Category	Sweetener (Source)	Characteristics?	Pros	Cons	Sucrose Equivalence
Nutritive Sweeteners	Sucrose	 <p>simulates sweet proteins receptor in the taste cells</p>	Add color, flavor, lowers water activity, preservative, bulking agent, antimicrobial	Adds unnecessary calories into diets	1
Nutritive Sweeteners	Fructose	 <p>Ketohexose, known as D-fructose or levulose, and is considered the sweetest sugar found in nature</p>	Considered the sweetest carbohydrate found in nature, natural	Does not affect satiety in the same way as glucose, Eat more total calories (Page et al., 2013)	1.2-1.8 x

Table 1.2. (continued) Sweeteners used in Dairy Foods

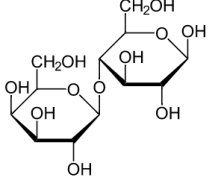
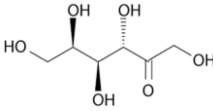
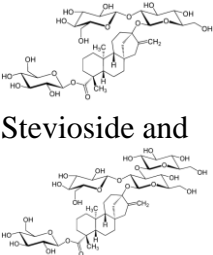
<p>Nutritive Sweeteners</p>	<p>Lactose</p>	 <p>dissaccharide naturally present in milk</p>	<p>Natural, single most abundant milk solid particle and is an important starting material for probiotic bacteria</p>	<p>Accounts for 30% of the caloric value of whole milk but provides little sweetness</p>	<p>0.11-0.125 x (Pangborn, 1963)</p>
<p>Nutritive sweetener</p>	<p>Tagatose</p>	 <p>Rare natural hexoketose found in dairy products, is an isomer of d-galactose</p>	<p>Lower glycemic index and virtually zero calories (1.5 calories per gram) Successfully produced by lactic acid bacteria</p>	<p>Rare in nature and has to be produced artificially using a calcium catalyst</p>	<p>0.92 x (Oh, 2007; Patra et al., 2009; Fujimaru et al., 2012; Shankar et al., 2013)</p>
<p>Natural non-nutritive sweetener</p>	<p>Stevia rebaudiana (Sweet herb native to South America) (Truvia® PureVia® Enliten®)</p>	 <p>Stevioside and rebaudioside A</p>	<p>Natural, Stable at high temperatures, GRAS</p>	<p>Off flavors reported, Differences between ingredient suppliers, very different temporality</p>	<p>210 x (Kim and Kinghorn, 2002; Pawar et al., 2013; Sirshendu et al., 2013; FDA, 2014, 2015a; Narayanan et al., 2014)</p>

Table 1.2. (continued) Sweeteners used in Dairy Foods

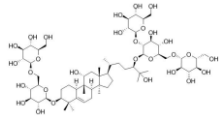
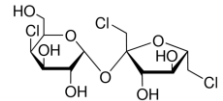
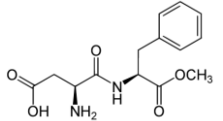
<p>Natural non-nutritive sweetener</p>	<p><i>Siraitia grosvenorii</i>, monk fruit or “Lo Han Guo” (Nectresse® Monk Fruit in the Raw® PureLo®)</p>	 <p>Mogroside V</p>	<p>Natural, Mogroside V is the most abundant mogroside in the plant when ripe and recently has been engineered into crystalline form. GRAS, GRN No. 359</p>	<p>Bitter and Metallic tastes associated</p>	<p>250-425 x (Kim and Kinghorn, 2002; Pawar et al., 2013; Cho, 2014)</p>
<p>Artificial non-nutritive sweetener</p>	<p>Sucralose (Splenda®)</p>	 <p>Replacing three hydrogen-oxygen groups on the sucrose molecule with three chlorine atoms</p>	<p>Temporality most similar to sucrose-easiest non-substitute for sugar (Zorn et al., 2014)</p>	<p>Metallic aftertaste, Artificial</p>	<p>750 x (Nofre and Tinti, 2000; Palazzo et al., 2011; Pawar et al., 2013)</p>
<p>Artificial non-nutritive sweetener</p>	<p>Aspartame (Nutrasweet®, Equal®, Sugar Twin®)</p>	 <p>L-aspartyl-L-phenylalanine methyl ester Dipeptide composed primarily of two amino acids, phenylalanine and aspartic acid</p>	<p>More pleasant taste when compared with stevia, FDA approved</p>	<p>Artificial Not heat stable so can not be used in baking applications, not pH stable</p>	<p>200 x (FDA, 1981, 1983; Bell and Labuza, 1991; Nofre and Tinti, 2000; Anton et al., 2012; Pandurangan et al., 2014; Kumari et al., 2016; Toniolo and Temussi, 2016)</p>

Table 1.2. (continued) Sweeteners used in Dairy Foods

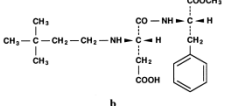
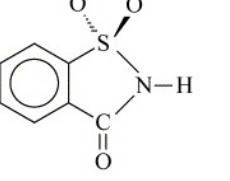
<p>Artificial non-nutritive sweetener</p>	<p>Neotame (Newtame®)</p>	 <p>Modified Acesulfame K</p> <p>substituent was the 3,3-dimethylbutyl group binds to both hydrophobic binding pockets of the human sweetness receptor</p>	<p>very clean sweet taste, close to sucrose, with no undesirable bitter or metallic off taste FDA approved</p>	<p>Artificial</p>	<p>11,000 x (Nofre and Tinti, 2000)</p>
<p>Artificial non-nutritive sweetener</p>	<p>Saccharin (Sweet and Low®, Sweet Twin®, Sweet'N Low®, Necta Sweet®)</p>	 <p>Shape and hydrogen are important to sweet taste</p>	<p>FDA approved</p>	<p>Artificial, it exhibits an unpleasant bitter and metallic aftertaste</p> <p>Caused cancer in rats when fed high doses but studies proven to be irrelevant to humans and no longer needs to have warning label associated with it</p>	<p>400 x (Nofre and Tinti, 2000; Baran and Yilmaz, 2006; Acar et al., 2017)</p>

Table 1.2. (continued) Sweeteners used in Dairy Foods

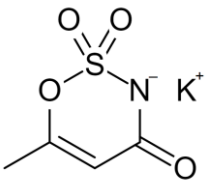
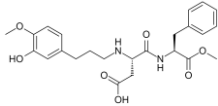
<p>Artificial non-nutritive sweetener</p>	<p>Acesulfame potassium (Sweet One®, Sunett®)</p>	 <p>interacts with the human sweetness receptor through one hydrophobic interaction shared between its phenyl ring and a hydrophobic binding pocket of the receptor</p>	<p>very clean sweet taste, close to sucrose, with no undesirable metallic off-taste, heat stable, synergistic with aspartame, FDA approved</p>	<p>Artificial, slight bitter aftertaste when used as sole sweetener</p>	<p>250 x (Chattopadhyay et al., 2014)</p>
<p>Artificial non-nutritive sweetener</p>	<p>Advantame</p>	 <p>N-substituted (aspartic acid portion) derivative of aspartame like neotame</p>	<p>Heat stable, can be used at much lower levels than sugar because of the sweetness potency, FDA approved</p>	<p>Artificial, more bulking agents needed for substitution, associated with weight gain due to sweetness and reduced calorie count (people eat more)</p>	<p>20,000 x (Ellison, 2014)</p>

Table 1.2. (continued) Sweeteners used in Dairy Foods

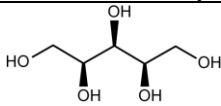
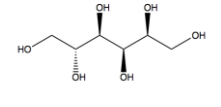
<p>Sugar Alcohol</p>	<p>Xylitol</p>	 <p>5-carbon polyol which is produced from d-xylose</p>	<p>one-third calories of sucrose (1.32 cal/g)</p> <p>similar sweetness intensity as sucrose but fewer calories, meaning they can be substituted on a weight-by-weight basis for sucrose while still decreasing calorie content</p>	<p>Potent laxative effect and other gastrointestinal symptoms such as flatulence, bloating, and abdominal discomfort when more than 50 grams are ingested, cooling trigeminal effect</p>	<p>1 (Equal sweetness)</p>
<p>Sugar Alcohol</p>	<p>Sorbitol</p>	 <p>Obtained by substituting and aldehyde group with a hydroxyl one on aldose sugars</p>	<p>Naturally present in apples, pears, peaches, apricots, nectarines and some vegetables</p> <p>is a bulking agent, humectant, sequestrant, stabilizer, sweetener. and thickener Successfully produced by lactic acid bacteria</p>	<p>Similar to Xylitol</p>	<p>0.5-0.7 x (Patra et al., 2009; Grembecka, 2015)</p>

Table 1.2. (continued) Sweeteners used in Dairy Foods

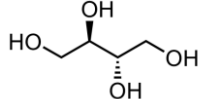
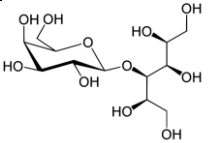
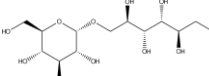
Sugar Alcohol	Erythritol	 <p>1,2,3,4-Butanetetrol, produced using osmophilic yeast</p>	Highly stable, low-calorie(0.2 kcal/g) , tooth-friendly, and a bulk sweetener that provides volume, texture, and microbiological stability	gastrointestinal symptoms observed with more than 1000 mg/kg of body weight	0.7 x (Grembecka, 2015)
Sugar Alcohol	Lactitol	 <p>Disaccharide polyol composed of sorbitol and galactose, produced from lactose</p>	low-calorie(1.9 kcal/g), low-fat and/or sugar-free food for diabetics	Similar to Xylitol	0.3-0.4 x (Grembecka, 2015)
Sugar Alcohol	Isomalt	 <p>Disaccharide polyol composed of glucome and mannitol</p>	low-calorie (2 kcal/g), tooth-friendly, resistance to loss of sweetness from heating	Similar to Xylitol	0.45-0.65 x (Yebra-Biurrun, 2005; Grembecka, 2015)

Table 1.2. (continued) Sweeteners used in Dairy Foods

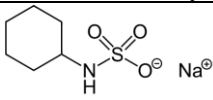
Cyclamates	Sodium Cyclamate	 <p>salt of cyclohexylsulfamic acid</p>	Non-nutritive sweetener and used in low sodium diets	Banned in the United States, Canada and UK in 1970, But currently available in over 90 countries including several European countries and China	30 x (Pinheiro and Oliveira, 2005)
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Table 1.3. Lactose content of various dairy products and ingredients

Dairy Product/Ingredient	Lactose amount	Source
Dried, Condense, and Evaporate Milk	43-59.6%	(Nijdam and Langrish, 2006)
Butter	ND	(Portnoi and MacDonald, 2011)
Whey Protein Isolate (WPI)	0.1-2.6 w/w%	(Fox and McSweeney, 2003; Majeed et al., 2017; Norwood et al., 2017)
Milk (whole, skim, low fat, buttermilk)	4-5%	(Varnam and Sutherland, 2001)
Whey Protein Concentrate (WPC 34 and WPC 80)	16-54.8 %	(Fox and McSweeney, 2003; Silvestre et al., 2012; Pérez et al., 2013)
Cheeses (Hard, soft and processed)	ND- 14.2%	(Kapoor et al., 2007; Davila-Vazquez et al., 2008)
Whey Permeate	65-85 %	(Adamczak et al., 2009; Smith et al., 2016)
Yogurt	1- 13%	(Adamczak et al., 2009)

ND= not detected

**CHAPTER 2: The Role of Natural Sweeteners on Temporality and Bar Hardening of High
Protein Low Carbohydrate Bars**

TITLE: The Role of Natural Sweeteners on Temporality and Bar Hardening of High Protein
Low Carbohydrate Bars

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Abstract

Protein bars are one product that meet consumer demands for a low carbohydrate, high protein food. With such a large protein bar market, protein bar producers need to find the correct texture and sweetness levels to satisfy consumers while still delivering a high protein, low carbohydrate bar. In the bar industry, bar hardening is a major concern and currently the effect of non-nutritive sweeteners on bar hardening is unknown. Due to the negative implications of bar hardening, it is important to investigate the sweetener-protein relationship to bar hardening. The objective of this study was to characterize the impact of sweetener and protein source on flavor, texture, and shelf life of high protein, low carbohydrate bars. The iso-sweet concentration of sweeteners (sucralose, sucrose, monk fruit, stevia, and fructose) in pea protein (PP), milk protein (MP) and whey protein isolate (WPI) bars were established using Magnitude Estimation Scaling (MES) and 2- Alternative Forced Choice (2-AFC) Testing. Descriptive Analysis and Temporal Check All that Apply were then conducted to determine flavor and temporal differences between the different protein types and sweeteners in the protein bars. Finally, an accelerated shelf life study was completed to understand how sweetener and protein types affected the shelf life of protein bars. The fifteen protein bars formulated at iso-sweet concentration were all stored at 35 °C and 55% humidity for 35 days and measurements were taken every 7 days beginning at day 1 (1, 7, 14, 21, 28, 35). MP required significantly less sweetener compared to PP and WPI to reach equal sweetness ($p < 0.05$). Bars sweetened with stevia or monk fruit had distinct bitter and metallic tastes, and sucralose had a low metallic taste. WPI bars were the most cohesive, and PP and whey protein bars were more bitter and metallic compared to MP bars ($p < 0.05$). WPI bars made with fructose were initially the hardest protein bar but after day 14, scored at parity with PP sucrose. There was no significant difference between bars in terms of hardness by day 21.

WPI bars were consistently more dense at all time points than bars made with PP or MP. Bars made with PP were the driest, least cohesive and had the fastest rate of breakdown in the study. Non-nutritive sweeteners did not have a negative effect on bar hardness in low carbohydrate, high protein bars. Findings from this study can be applied to commercially produced protein bars for naturally sweetened bars with different protein types without negative effects on protein bar texture.

Introduction

Currently, consumers desire low carbohydrate, high protein foods and protein bars are one food product that meet this demand. In 2016, the protein bar industry was valued at over \$837 million with several companies competing for a share of the market (Mordor Intelligence, 2018). With high competition and so many options for consumers, companies have to find the correct texture and sweetness to satisfy consumers while still delivering a high protein, low carbohydrate bar (Guinard and Mazzucchelli, 1996; Imtiaz et al., 2012). In the bar industry, there are many sources of plant and animal proteins available, and the type of protein used can have a significant effect on bar texture (Imtiaz et al., 2012). The most common bar protein sources are soy and whey protein (Mordor Intelligence, 2018). Other proteins are available and it is important to investigate implications of using different protein sources on the texture and sweetness of protein bars (Imtiaz et al., 2012).

In order to make a low carbohydrate bar while preserving sweetness, non-nutritive sweeteners can be substituted for sugar to reduce calories and to satisfy consumers (Yebra-Biurrun, 2005; Behrens et al., 2011). Past studies with other foods have shown that sugar can be reduced by 25% to 30% while maintaining consumer acceptance (Cadena et al., 2012; Li et al., 2015; McCain et al., 2018). Sugar contributes 4 calories per gram, whereas non-nutritive sweeteners can be 30 to 20,000 times sweeter than sugar which allows for less to be added for a similar sweetness, with fewer calories (Mitchell, 2007; Zygler et al., 2011). Although non-nutritive sweeteners are great alternatives to sugar, several recent studies have reported that sugar substitution is an inherently difficult task (Parker et al., 2018; Wagoner et al., 2018). Food structure, specifically texture, has a significant effect on consumer enjoyment of a food, and sugar substitution can be difficult because sucrose is a key ingredient in providing texture

(Wilkinson et al., 2001; Cardoso and Bolini, 2008; Silcock, 2017). Specifically, substituting non-nutritive sweeteners to reduce calories in protein bars has major textural implications. In the protein bar industry, where bar hardening is a major concern, the effect of non-nutritive sweeteners on bar hardening is unknown. Bar hardening occurs when the protein matrix hardens due to age and this problem is only intensified as protein content increases (Wilkinson et al., 2001). Once protein bars harden, the resulting texture can lead to consumer rejection or avoidance of the protein bars (Wilkinson et al., 2001; McMahon et al., 2009). Bar hardening negatively effects profit margins of companies and is a major issue for the bar industry.

Sugar substitution using non-nutritive sweeteners is also difficult because none of the non-nutritive sweeteners on the market are able to perfectly match the temporality of sucrose (Lawless and Heymann, 2010; Palazzo et al., 2011; Morais et al., 2014; Zorn et al., 2014; Azevedo et al., 2015; McCain et al., 2018; Parker et al., 2018). Due to the negative implications of bar hardening, it is important to investigate the sweetener-protein relationship to bar hardening. In order to compare the effects of bar hardening and the flavor implications each sweetener has on a protein bar, the iso-sweet concentration or sweetness equivalence must first be determined. This is the amount of non-nutritive sweetener or sugar substitute needed to reach sweetness equivalence with a sucrose target. Magnitude estimation scaling (MES) followed by descriptive analysis and 2-alternative forced-choice (2-AFC) testing is one set of methods to confirm sweet taste equivalency (Cardello et al., 1999; Lawless and Heymann, 2010; Li et al., 2015; Parker et al., 2018). Temporal methodologies can then be used to characterize differences between products over time (Palazzo et al., 2011; Morais et al., 2014; Zorn et al., 2014; Azevedo et al., 2015). Additionally, natural sweeteners have different flavor profiles from sucrose and temporally documenting the presence of other basic tastes and temporality of sweet taste is

important for the success of these natural non-nutritive sweeteners in foods (Parker et al., 2018). Previous research reported that protein product label claims of “naturally sweetened” and “low carbohydrate” content are important to consumers and therefore important to investigate in regards to protein bars (Gerdes, 2012; Oltman et al., 2015; Parker et al., 2018; Harwood and Drake, 2019). Several studies have investigated bar hardening, but to our knowledge, there has been no study that investigates how the addition of alternative sweeteners to protein bars affects the sensory and textural properties of protein bars. The objective of this study was to characterize the impact of sweetener and protein source on flavor, texture and shelf life of high protein low carbohydrate bars.

Material and Methods

Bar preparation

A model protein bar system consisting of a protein source, shortening, fiber, and sweetener was used. Three different protein sources, currently used in bar formulations, were used: whey protein isolate (BarFlex 191; Glanbia Nutritionals, Twin Falls, ID) (85.7% protein), milk protein isolate (BarPro 585, Glanbia Nutritionals) (81.7% protein), and pea protein (BarHarvest 801, Glanbia Nutritionals) (84.8% protein). All three protein powders were obtained as 22 kg lots and stored at -80 °C throughout the duration of the study. The sweeteners used were sucrose (India Tree Caster sugar, Seattle, WA), liquid sucrose (Liquid Sugar, Kelly's Delight; Woodway, TX), fructose (Krystar 300; Tate & Lyle, Decatur, IL), sucralose (Bulk Ingredients; Henderson, NV), monk fruit extract (Purefruit Select monk fruit extract; Tate & Lyle), and stevia leaf extract (Tasteva stevia sweetener; Tate & Lyle). Vegetable shortening was purchased from a local grocery store (Crisco, The J.M. Smuckers Company, Orrville, OH). Fiber was purchased from BioNeutra (VitaFiber, Edmonton, AB, Canada). Bars were prepared in 500 g batches for MES and temporal studies and 2,600 g batches for the bar hardening study. Bars contained 15 g of protein per 62 g serving.

All ingredients were combined into a stainless steel bowl and hand mixed for approximately 3-4 min until a soft dough was formed (Table 2.1) (McMahon et al., 2009). Fiber was adjusted in order to account for difference in mass when using non-nutritive sweeteners (Table 2.1). Bars then were rolled out to approximately 10 mm thickness and cut into 80 mm long by 40 mm wide squares. Each 500 g batch yielded about 9 bars at these dimensions. All sensory testing was approved as exempt by the North Carolina State University Institutional Review Board for human subjects. A pool of 20 descriptive analysis panelists with a minimum of

40 h of training and a range of descriptive analysis experience are maintained at North Carolina State University. Different groups of these panelists were utilized for TCATA, descriptive analysis of flavor or sweet taste and descriptive analysis of texture.

Selection of iso-sweet taste intensity from protein bars

A preliminary sensory assessment of 12 commercial protein bars with various protein sources, protein loads and sweeteners was conducted to determine a target sweet taste intensity for model bars. A target sweetness intensity of a 10% (w/v) sucrose solution was selected. Power functions for sweeteners were generated using magnitude estimation scaling (MES) in whey protein isolate (WPI), pea protein (PP), and milk protein (MP) bars (Lawless and Heymann, 2010). The experimental design consisted of five sweeteners (fructose, sucrose, monk fruit, sucralose, and stevia) and three protein sources (pea, milk, and whey protein) for a total of fifteen distinct protein bars. Panelists (n=9, 5 females and 4 males, ages 22 to 28 y) participated in 10 h of MES training until they were able to demonstrate replicable ratio scaling with a range of sweetened solutions and WPI model bars prior to data collection (Meilgaard et al., 2016; Parker et al., 2018). The MES scaling method was used to create power function curves using 10% (w/v) sucrose solution as a reference intensity of 100, and scale anchors of 5 and 20% (w/v) sucrose solutions representing intensities of 50 and 200, respectively. Panelists were instructed to refer to these references as they tasted each bar. Samples were served at 21°C in 120 mL lidded soufflé cups with a random 3-digit code. Each MES series contained 7 bars ranging from just noticeable sweetness to up to twice as sweet as the reference solution, and a step factor of 1.4 for sweetener concentration was used. Samples were randomized in each session and only one MES series was evaluated per session. A five min rest period was enforced between samples and

panelists were instructed to rinse with deionized water. Paper ballots were used and the results were collected in triplicate.

The iso-sweet point for each protein type and each sweetener determined by MES was confirmed using descriptive analysis (described below) and a directional paired comparison test (2-Alternative Forced Choice, 2-AFC) (ASTM, 2008; Parker et al., 2018). Bars were presented in lidded 120 mL soufflé cups with 3-digit codes using a balanced presentation order. Consumers for the 2-AFC were recruited from the university community. Consumers (n= 40) were instructed to taste each protein bar against the sucrose control protein bar in the order presented. Panelists selected the sample they believed was sweeter. A three-min rest period was enforced between each set of samples, during which panelists were instructed to rinse their mouth with spring water and take a bite of an unsalted cracker. No more than two 2-AFC tests were conducted in one session. Data for 2-AFC testing were collected on iPads using Compusense Cloud.

Descriptive analysis of Iso-Sweet taste and descriptive analysis of flavor following MES and prior to 2-AFC tests

To confirm the iso-sweet point in protein bars for each sweetener, six trained panelists (n=6; 4 females and 2 males; age 24-56 y) were utilized for descriptive analysis (Meilgaard et al., 2016). Each panelist had more than 100 h of training using the 0 to 15-point Spectrum™ universal intensity scale and participated in an additional 20 h of training on protein bars with different sweeteners prior to the evaluation. Descriptive analysis of sweet taste was conducted on all protein bars sweetened with sucrose, fructose, sucralose, monk fruit, and stevia in the three different protein types (MP, WPI, PP). Bars were served at 21 °C in lidded 120 mL soufflé cups labeled with random 3-digit codes. A 5-min rest was enforced between samples. Each sample

was evaluated by each panelist in duplicate and no more than five bars were evaluated in a session. Paper ballots were used.

Descriptive analysis of bar flavor attributes was conducted following confirmation of iso-sweet taste intensity (Table 2.2). Bars were evaluated in triplicate by a trained descriptive panel (6 females and 2 males, age 22–55 y). Each panelist had more than 100 h of training using the 0 to 15-point Spectrum™ universal intensity scale and participated in an additional 20 h of training on protein bars with different sweeteners prior to the evaluation (Meilgaard et al., 2016). Bars were served in the same fashion as for descriptive analysis for sweet taste. A 5-min rest was enforced between samples.

Temporal Check All That Apply

Protein bars formulated to iso-sweetness were evaluated by Temporal Check All That Apply (TCATA). TCATA was used to document how the flavor and taste intensities of the bars changed with the different protein sources and sweeteners (Ott et al., 1991; Hanger et al., 1996; Fujimaru et al., 2012). Bars were cut into 10 g aliquots and presented in lidded 120 mL soufflé cups labeled with 3-digit codes. The protein bars were evaluated at 21 °C. TCATA panelists (n=9; 6 females and 3 males; age 23-30 y) had prior descriptive analysis experience (>40 h each) and participated in two 30-min instruction and practice sections for TCATA before beginning data collection. Attributes scored were sweet aromatic and cardboard flavors, sweet taste, bitter taste, metallic taste, cohesive, adhesive, and astringency.

Data was collected in quadruplicate over eight sessions on iPads using the temporal check-all-that-apply method in Compusense Cloud (Compusense, Guelph, Canada). In each session, each panelist evaluated a random presentation of five protein bars. TCATA evaluations of each protein bar were performed over the course of 192 s and panelists were allowed

unlimited selection and deselection of all attributes perceived at any point over the course of evaluation (Ares et al., 2015; Castura et al., 2016). Panelists were instructed to place the entire sample in-mouth and press start for an initial profiling of flavor attributes, mouthfeel, and basic tastes from 0-20 s. The panelists were then instructed to expectorate the sample at 20 s followed by an aftertaste evaluation of flavor attributes and basic tastes from 20-192 s. Panelists underwent an enforced five min rest time between each sample and were instructed to rinse their mouths with deionized water before beginning the next sample.

Bar Hardening and Color

Bars were placed in labeled moisture barrier pouches (Mylar®, Sorbent Systems, Los Angeles, CA) and heat sealed. Bars were approximately 80 mm long by 40 mm wide and 10 mm thick and one bar was placed in each pouch. Bars were stored at 35 °C and 55% humidity for 35 d for accelerated shelf life testing in a Metro C 5 Series 3 Cabinet (Metro, Alton, IL). Temperature and humidity were confirmed using an Extech RH300 humidity meter. Accelerated shelf life testing of 42 d corresponds to approximately 12 mo storage at 21 °C so 35 day corresponds to approximately 10 mo storage (Li et al., 2008). Measurements were taken every seven days beginning at day 1 (1, 7, 14, 21, 28, 35).

Hardness was measured as the maximum load recorded during 7.5-mm penetration of a knife blade (Instron 5542 (UTM) (Instron, Norwood, MA) at a speed of 1 mm/s into the bar (Li et al., 2008; McMahon et al., 2009). Bars were laid on the platform and a hardness test was performed at 3 locations along each bar. Four replications were done at each time point. An average hardness value was calculated by using the peak force (g) of the force versus time graph (Li et al., 2008).

To measure color differences between the bars, Hunter L, and a, and Commission Internationale de l'Éclairage (CIE) b* values were measured on the bars at each time point (1, 7, 14, 21, 28, 35) using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). Luminosity (L) measures the degree of lightness from dark (0) to light (100), a measures the degree of redness defined by a positive number or greenness defined by a negative number, and b* measures the degree of yellowness defined by a positive number or blueness defined by a negative number (Quinones et al., 1997; Cheng et al., 2018). Hunter values and CIE values were computed from the reflectance data in the range of 360-750 nm at 5 nm intervals, Illuminant A with a 10 degree observer angle.

Descriptive Analysis of Bar Texture

Protein bars were evaluated by a trained sensory texture panel (8 females, ages 40 to 55 y). Each panelist had greater than 200 h of texture profiling experience using a 0 to 15-point product specific texture scale. Five- 2 h training sessions were conducted to assess and calibrate on the texture attributes of commercial and experimental protein bars. During the training sessions, panelists generated and practiced with the identified attributes (Table 2.2). Moisture barrier pouches labeled with a random 3-digit code were presented to each panelist at 21 °C. For each session, panelist were presented with one protein bar and one bar texture reference with attribute intensities previously defined by the panel in the training session as a warm up. Each panelist evaluated each sample in quadruplicate in a randomized balanced block design. A five min rest was enforced between each sample. Paper ballots were used and no more than 5 bars were evaluated in a session.

Statistical Analysis

Power curves for sweet taste intensity were created using XLSTAT version 19.5.2018 and constructed by plotting the logarithm of the geometric mean of the average response at each sweetness concentration level against the logarithm of the sample sweetener concentration (ASTM, 2012; Parker et al., 2018). The iso-sweet concentrations determined from the power curves were then confirmed using the minimum number of “correct” judgments for significance through 2-AFC testing ($\alpha = 0.05$) (ASTM, 2008; Meilgaard et al., 2016). A two-way ANOVA (protein type x sweetener concentration) was conducted to determine these effects on the slopes of the power functions iso-sweet ($\alpha = 0.05$). Descriptive analysis data was evaluated with analysis of variance (ANOVA) with Fisher’s least significant difference test to determine means separation ($\alpha=0.05$) (Addinsoft, New York, NY).

TCATA data was analyzed using the smoothing and statistical analysis of TCATA data conducted in tempR package in R version 3.4.0 (Castura et al., 2016). Comparisons between protein bar types were made at each second of evaluation using 2-sided Fisher’s Exact Test. Citation proportions and differences were graphed using XLSTAT version 19.5.2018 (Addinsoft, Paris, France). For bar hardening, due to a three way interaction between protein, sweetener, and time point, separate analyses were performed for each day. Linear mixed model analyses were conducted, with sugar source, protein source, and their interaction as fixed effects. Random effects included batch, interactions between batch and sweetener and batch and protein, as well as the three way interaction between batch, sugar source, and protein source. The mixed procedure in SAS version 9.4 (SAS Institute, Cary, NC) was used with differences in least squares means investigated. All statistical analyses were performed at 95% confidence level.

Results

Magnitude Estimation Scaling

Consistent with previous studies, MES results and power function curves established that sucralose was the most potent sweetener followed by monk fruit, stevia, and fructose (Cardello et al., 1999; Kim and Kinghorn, 2002; McCain et al., 2018; Parker et al., 2018; Wagoner et al., 2018) (Figure 2.1). As expected, non-nutritive sweeteners required smaller amounts than fructose to reach equivalency to 10% (W/V) sucrose based on the power function slope curves (Table 2.3, Figure 2.1). More importantly, different protein types required different sweetener amounts to reach iso-sweet concentration (Table 2.1). More sweetener was needed for bars with PP or WPI than MP (Table 2.3, Figure 2.1).

Descriptive Analysis

Descriptive analysis documented predominant flavor differences that were due to protein type rather than sweetener differences (Figure 2.2). MP bars were associated with milky, sweet aromatic, and tortilla flavors and sweet taste. PP bars were associated with yellow pea/beany, pyrazine, fecal, and cardboard flavor and low but distinct umami taste which was not documented in MP or WPI bars. WPI bars were characterized by cardboard flavor (along with PP bars) as well as sulfurous flavor and low bitter taste intensity. These protein specific and protein-type flavors have been previously documented in protein based products (Russell et al., 2006; Wright et al., 2009; Oltman et al., 2015; Smith et al., 2016).

Temporal Check All That Apply

Temporal effects of sweetener type and protein were investigated from the TCATA results. When averaged across the 3 protein types, distinct temporal differences among the sweeteners were documented ($p < 0.05$), similar to results reported by Parker et al. (2018) for

ready-to-mix (RTM) protein beverage (results not shown). Bitter and metallic taste was documented in bars made with monk fruit, sucralose, and stevia. Sweet aromatic, cardboard, and sweet taste were present in all protein bars with similar time onsets. Distinct temporal differences were also documented among the different protein types (Figures 2.1a-c). WPI bars were highly cohesive, had the highest metallic citation proportion, and was the only protein type that sweet taste did not go to 100% citation proportion (Figure 2.3a). PP and WPI bars had higher bitter citation proportions compared to MP (Figures 2.3a-c). MP had the highest sweet aromatic frequency (Figure 2.3c). All 3 protein bar types had a lingering astringency (Figure 2.3a-c).

Significant temporal differences between the proteins are shown in Figures 2.2a-c. In-mouth and just after expectoration, WPI bars were higher than PP bars in sweet aromatic, cohesiveness, metallic taste, sweet taste, and adhesiveness (Figure 2.4a)($p < 0.05$). Ninety seconds post expectoration, WPI bars was more bitter than PP bars (Figure 2.4a)($p < 0.05$). In-mouth and post expectoration, WPI bars were more cohesive, metallic, and bitter than MP bars (Figure 2.4b). MP bars were higher in sweet taste than WPI bars (Figure 2.4b)($p < 0.05$). Comparing MP bars to PP bars, MP bars were higher in sweet aromatic and cardboard flavors, whereas, PP bars were more cohesive, bitter, and metallic (Figure 2.4c). Post expectoration, PP bars were higher in bitter taste, metallic, and sweet taste compared to MP bars (Figure 2.4c) ($p < 0.05$).

Bar Hardening

Protein source and sweetener had a significant interaction or significant main effects for instrumental and sensory visual and texture properties across all attributes ($p < 0.05$) (Table 2.4). Bar hardening was seen in all bars regardless of protein type and sweetener source (Figure 2.5). Bar hardening is proposed to be caused by protein aggregation from disulfide linkage formation in protein bars, non-covalent interactions, and the maillard reaction with reducing sugars

(McMahon et al., 2009; Liu et al., 2011; Zhou et al., 2013). In this study, WPI bars made with fructose were consistently the hardest bar (Figure 2.5). Other bars, such as PP made with sucrose, had an upward trend of instrumental bar hardening to a greater degree than WPI bars at day 7 and day 14 (Figure 2.5). At day 21, there were no differences in instrumental bar hardening ($p>0.05$) (results not shown).

Sensory first bite hardness confirmed that WPI bars made with fructose were the hardest for the first 3 time points, consistent with instrumental hardness (Figure 2.5). At day 1 and day 7, all protein bars made with fructose were harder and denser than bars with other sweeteners (Figures 2.6 and 2.8b). By day 21-35, there was a protein main effect and WPI bars required more first bite force than PP or MP bars. Additionally, WPI bars were more dense than PP and MP bars at all time points (Figure 2.8a). In terms of cohesiveness, there was a significant interaction between sweetener and protein type at all time points ($p<0.05$). WPI bars were consistently the most cohesive, and PP bars were the least cohesive (Figure 2.10). All sweeteners had a significant impact on cohesiveness at all time points. PP bars were the driest whereas WPI bars had the highest surface moisture at all time points and appeared moist throughout the study (Figure 2.11a and b). Finally, PP and MP bars had a faster rate of breakdown than WPI bars at all time points with the exception of day 1 (Figure 2.12).

Instrumental and sensory color differences were also documented. WPI bars had the greatest instrumental change in color compared to PP or MP but all protein bar formulas visibly darkened and increased in yellow/red color over time (Figure 2.9a-c) ($p<0.05$). In terms of sensory visual effects, there was an interaction between sweetener and protein type (Table 2.4). All bars had visible color changes by sensory and instrumental measurements but bars with fructose displayed the largest change in color across storage ($p<0.05$).

A principal component biplot (PCA) of the protein bars across storage time provides a visual assessment of differences among the different bars (Figure 2.13). Principle component 1 (PC1) (54%) was positively correlated with sensory first bite hardness, sensory denseness, sensory cohesiveness, sensory adhesiveness, sensory tooth pack, sensory number of chews and negatively correlated with sensory rate of breakdown. WPI protein bars loaded positively with PC1 whereas MP and PP bars were negatively correlated with PC1. WPI bars were initially the hardest but hardened at a slower rate compared to MP and PP bars. WPI bars were hard, cohesive, dense bars, with high tooth packing whereas MP and PP bars were lower in these attributes and had a faster rate of breakdown. Principal component 2 (PC2) (26%) was positively correlated with sensory surface moisture and Hunter L, and negatively correlated with sensory color intensity, Hunter a, and CIE b*. Day 1 time points for MP and WPI bars loaded positively with PC2 whereas PP bars loaded negatively with PC2 at all time points as PP bars started and remained more brown throughout the study compared to other protein type bars.

Principal component analysis biplots of each protein type across sweetener and time point were also generated to visualize differences within each protein type (Figures 2.14a-c). For MP bars, PC1 (52%) was positively associated with sensory surface moisture, sensory adhesiveness, Hunter L, sensory tooth packing, and negatively associated with sensory denseness, sensory color intensity, sensory number of chews, instrumental texture, Hunter a, and CIE b*. PC 2 (14%) was positively correlated with sensory first bite hardness, and sensory cohesiveness. At day 1, bars loaded positively with PC1 whereas bars at day 7 and day 14, the bars loaded positively with PC2. MP bars at day 1 were light in color, moist and not very cohesive. As bars aged from day 1 to day 14, MP bars became harder, drier, darker, and more cohesive.

For PP bars, PC1 (48%) was positively associated with color intensity, instrumental texture, CIE b*, and Hunter a, and negatively associated with surface moisture, sensory denseness, sensory cohesiveness, sensory adhesiveness, and sensory tooth pack. PC2 (17%) was positively correlated with sensory first bite hardness and sensory # of chews, and negatively correlated with sensory rate of breakdown. Bars at day 21-35 loaded positively associated with PC1 and as the bars aged, PP bars became harder (as taken by instrument), less dense, and became darker in color.

For WPI bars, PC1 (57%) was positively correlated with sensory color intensity, sensory denseness, sensory cohesiveness, and Hunter L and negatively correlated with sensory surface moisture, sensory rate of breakdown, sensory adhesiveness, sensory tooth pack, sensory number of chews, Hunter a, and CIE b*(Figure 2.14c). Day 21-35 WPI bars loaded positively for PC1 whereas day 1 WPI bars loaded negatively on PC1. After day 1, there was no consistent trend with time suggesting that another factor besides sweetener source and time affected WPI bar hardening after day 1. PC 2 (19%) was positively correlated with sensory first bite hardness, and instrumental texture. PC 2 loaded most positively with the fructose WPI bars and these bars were consistently the hardest WPI bars.

Discussion

WPI and PP bars required significantly more sweetener than MP bars for an iso-sweet taste intensity within the same sweetener. Most likely, the bitter taste of the WPI and PP are the reason that these bars required additional sweetener to reach iso-sweetness (Liu et al., 2014; Zeeb et al., 2018). It is important to note for sugar reduction efforts, within a single specific formulation, it cannot be assumed that switching protein types does not change the iso-sweet taste. Additionally, results from this study suggest that, alone, the specific stevia used in this study is not a suitable sugar substitute in protein bars made with WPI or PP due to increased bitterness from the stevia in addition to that from the proteins, which would most likely result in consumer rejection. However, monk fruit or sucralose could be used for sugar reduction or replacement in WPI or PP protein bars without a huge increase in bitterness. Previous work has determined that consumers desire “naturally sweetened” and “low carbohydrate” product claims but many consumers are flavor driven and will choose taste over product claims (Li et al., 2015; Oltman et al., 2015; Parker et al., 2018; Harwood and Drake, 2019). Future work should evaluate different stevia sources with reported lower bitter levels, investigate sweetener blends to decrease bitterness, and determine consumer acceptance of the protein bars (Parker et al., 2018).

The results from TCATA additionally suggest that there was a temporal effect of protein and sweetener types that affected bitter, sweet and metallic tastes across all bars. This effect could have been caused by two possible factors. The first could be due to possible interactions between adhesiveness/tooth packing and the lingering tastes. Bar hardening data demonstrated a significant effect of all protein sources and sweetener sources at day 1 for sensory tooth packing and could have led to temporal differences in bitter, sweet and metallic tastes across all bars. WPI bars had significantly higher tooth packing than PP bars and MP bars at day 1 which could have led to temporal differences seen in TCATA frequencies (Table 2.4). The second possibility

could be due to an interaction between the protein and sweetener types. The interaction could have led to different binding affinities or delays in sweetener binding due to structural interactions causing different temporalities (Acevedo et al., 2018). Both possibilities described would result in temporal differences in bitter, metallic, and sweet taste seen in this study. Parker et al. (2018) evaluated beverages and there was no effect of texture so these differences were not observed.

All bars experienced differences in bar hardening across the study which would lead to differences in shelf life. For protein bars, the minimum shelf life is six months but producers often desire a shelf life of more than 12 months (Banach et al., 2014). Imtiaz et al. (2012) previously reported that the type of protein used in formulating a bar can influence bar firmness and a high protein bar typically contains around 20-50% protein (12.4 – 31 g of protein in 62 g bar). Bars with WPI, although initially harder than all other protein, equalized over time but were consistently more dense at all time points than PP bars or MP bars (Figure 2.3 and 2.8a). Imtiaz et al. (2012) reported high protein bars made with MP developed a crumbly texture over time and reported that the bars lacked cohesion. Consistent with previous studies, the crumbliness nature of the bars was increased in bars made with MP and PP (Li et al., 2008; Imtiaz et al., 2012). The PCA biplot (Figure 2.13) suggested that MP and PP bars were more closely related in how they behaved in accelerated shelf life testing. In this study, bars made with PP were the driest (Figure 2.11 a and b), least cohesive (Figure 2.10), and had the fastest rate of breakdown across shelf life (Figure 2.12) ($p < 0.05$) and bars made with MP had a lower density which was noted to cause a crumbly texture (Figure 2.8a) (Beck et al., 2017).

For effect of sweeteners, WPI bars made with fructose were harder than other bars until day 21 (Figure 2.3). Fructose is extremely hygroscopic and it is recommended to store fructose at

a relative humidity of below 50% (Davis, 1995; Hobbs, 2019). At the storage conditions in this study (55% humidity), fructose in the bar can slowly absorb the water in the air and by day 14 of this study, the fructose had absorbed enough water to form a visible fructose syrup. This syrup could have caused softening after day 14 and by day 21, the difference between the WPI bars made with fructose and all other bars may have been minimized due to the hygroscopic nature of fructose. Ultimately, the hygroscopic nature of fructose may have led to no significant interactions between sweetener and protein source in terms of texture and first bite hardness after day 14 (Table 2.4). The non-nutritive sweetener bars, with the slightly higher fiber content to maintain equivalence protein content, were less hard than their nutritive sweetener counterparts (fructose and sucrose) until the 6-month shelf life equivalent. This study demonstrates that non-nutritive sweeteners do not have a negative effect on bar hardness in low carbohydrate, high protein bars and would not negatively impact the shelf life of the bars.

All protein bars experienced color changes throughout the study but WPI bars experienced the most notable change beginning at day 7. This change in color occurred possibly due to increased maillard browning compounds caused by the accelerated shelf life conditions (McMahon et al., 2009). In general, the protein sweetener combinations evaluated resulted in protein bars that darkened in color over time (Figure 2.9a-c). Future work should address consumer testing with protein bars to confirm acceptable sensory properties and the use of other protein or protein blends with non-nutritive sweeteners (Loveday et al., 2010; Liu et al., 2011; Hogan et al., 2012; Zhou et al., 2013; Banach et al., 2014).

Conclusions

The results from this study demonstrated that the type of sweetener(s) and the type of protein chosen for a protein bar impact bar flavor and texture characteristics and should be chosen carefully. This is important to understand and predict how sugar reduction and/or sweetener replacement will affect the sensory and textural properties of protein bars within different protein types. PP and WPI bars both required more sweetener to reach iso-sweetness than MP bars. Flavor difference in protein bars were predominately due to protein type chosen rather than sweeteners. TCATA data additionally suggested that there was a temporal effect of protein and sweetener types that affected bitter, sweet and metallic tastes across all bars. Bar hardening (instrumental and sensory first bite) differences caused by sweetener and protein type were evident until day 21 at 35 °C 55% relative humidity. Bars made with WPI were consistently more dense at all time points than PP bars or MP bars and although initially harder than other protein bars, WPI bars were not different in hardness after day 14. Bars made with PP became dry and less cohesive over time and would benefit from another protein type incorporated into the bar to increase shelf life. The findings from this study may be useful to protein bar manufacturers.

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Tables and Figures

Table 2.1. Formulation of protein bars for sweeteners and protein types (500 gram batch) at iso-sweet taste intensity.

Milk Protein (g)	Whey Protein (g)	Pea Protein (g)	VitaFiber DP3 (g)	Vegetable Shortening (g)	Fructose (g)	Monk Fruit (g)	Stevia (g)	Sucralose (g)	Sucrose (g)	Liquid Sucrose (g)
145.0	--	--	230.6	80.0	44.434	--	--	--	--	--
--	145.0	--	201.7	80.0	73.256	--	--	--	--	--
--	--	145.0	202.6	80.0	72.432	--	--	--	--	--
145.0	--	--	274.4	80.0	--	0.575	--	--	--	--
--	145.0	--	273.8	80.0	--	1.235	--	--	--	--
--	--	145.0	273.0	80.0	--	2.002	--	--	--	--
145.0	--	--	274.4	80.0	--	--	0.576	--	--	--
--	145.0	--	273.2	80.0	--	--	1.754	--	--	--
--	--	145.0	273.0	80.0	--	--	2.016	--	--	--
145.0	--	--	274.8	80.0	--	--	--	0.182	--	--
--	145.0	--	274.3	80.0	--	--	--	0.692	--	--
--	--	145.0	274.3	80.0	--	--	--	0.661	--	--
145.0	--	--	191.7	80.0	--	--	--	--	41.662	41.662
--	145.0	--	120.6	80.0	--	--	--	--	77.189	77.189
--	--	145.0	110.7	80.0	--	--	--	--	82.135	82.135

Bars were formulated at 15 g protein/62 g serving

Bars were formulated to sweet taste equivalency to a 10% w/v sucrose solution

Table 2.2: Flavor and texture lexicon for protein bars

Attribute	Definition
Flavor Lexicon	
Sweet Aromatic	Sweet aromatic associated with grains such as oatmeal. Quaker oatmeal, 50 g soaked in 500 mL water
Cardboard	Aromatics associated with wet cardboard and brown paper. 2 cm x 2 cm piece of brown paper bag boiled in water for 30 min
Milky	Aromatics associated with milk. 1% fat high temperature short time pasteurized milk
Sulfurous	Aromatics associated with sulfurous compounds. Boiled mashed egg. Hydrogen Sulfide
Yellow Pea/Beany	Aromatics associated with dried beans and split peas. Canned pinto beans or dried yellow peas rehydrated in DI water
Green Pepper/Pyrazine	Aromatics associated with freshly cut green bell peppers. Methoxy pyrazines (5 µg kg ⁻¹); freshly cut bell pepper
Fecal	Aromatic associated with complex protein decomposition. Indole, skatole (20 mg kg ⁻¹)
Tortilla	The aromatic associated with cornmeal. Fresh white corn tortillas, Tostitos brand tortilla chips
Sweet Taste	Basic taste elicited by sweeteners.
Bitter Taste	Basic taste elicited by various compounds including caffeine and quinine.
Umami Taste	Basic taste elicited by certain peptides and nucleotides.
Metallic	Chemical feeling factor elicited by metallic objects in the mouth.
Texture Lexicon	
Color Intensity	Hue (light to dark). Light(L)=1, L/Medium(M)=4, M=7.5, M/Dark(D)=11, D=14
Surface Moisture	Degree to which the uncut upper surface appears moist. Moist=high number, dry=low number.
First Bite Hardness	Force required to bite completely through the sample with the front teeth. cream cheese=1; Velveeta=2; carrot slice=11
Denseness In Mouth (1 To 2 Compressions)	Compactness of the cross-section after 1-2 compressions. Cool Whip=0.5, Oscar Meyer brand hot dog=9, fruit jellies=13
Cohesiveness	Degree to which the sample is in a bolus @ 3-4 chews. Carrots=2, processed cheese=9
Rate Of Breakdown	How slow or fast the sample breaks down (slow=low #, fast=high #)
Adhesiveness	Degree to which sample sticks to any of the mouth surfaces throughout mastication
Tooth Pack	Degree to which the sample packs in and around the teeth. Fruit jellies =13
# Chews	Count the number of chews required to get a 3/4-inch square masticated to a state ready to swallow (varies for each panelist)

Table 2.3. Iso-Sweet Sweetener Slopes from MES scaling with each sweetener in milk protein, pea protein, and whey protein bars (500 gram batch)

	Pea Protein	Whey Protein	Milk Protein
Fructose	0.551 de	0.652 a	0.592 abcd
Monk Fruit	0.565 cde	0.646 a	0.545 de
Stevia	0.627 abc	0.638 ab	0.577 bcde
Sucralose	0.518 e	0.627 abc	0.536 de
Sucrose	0.546 de	0.656 a	0.534 de

Bars were formulated at 15 g protein/62 g serving.

Mean slopes without a common letter are different ($P < 0.05$).

Bars were formulated to sweet taste equivalency to a 10% w/v sucrose solution.

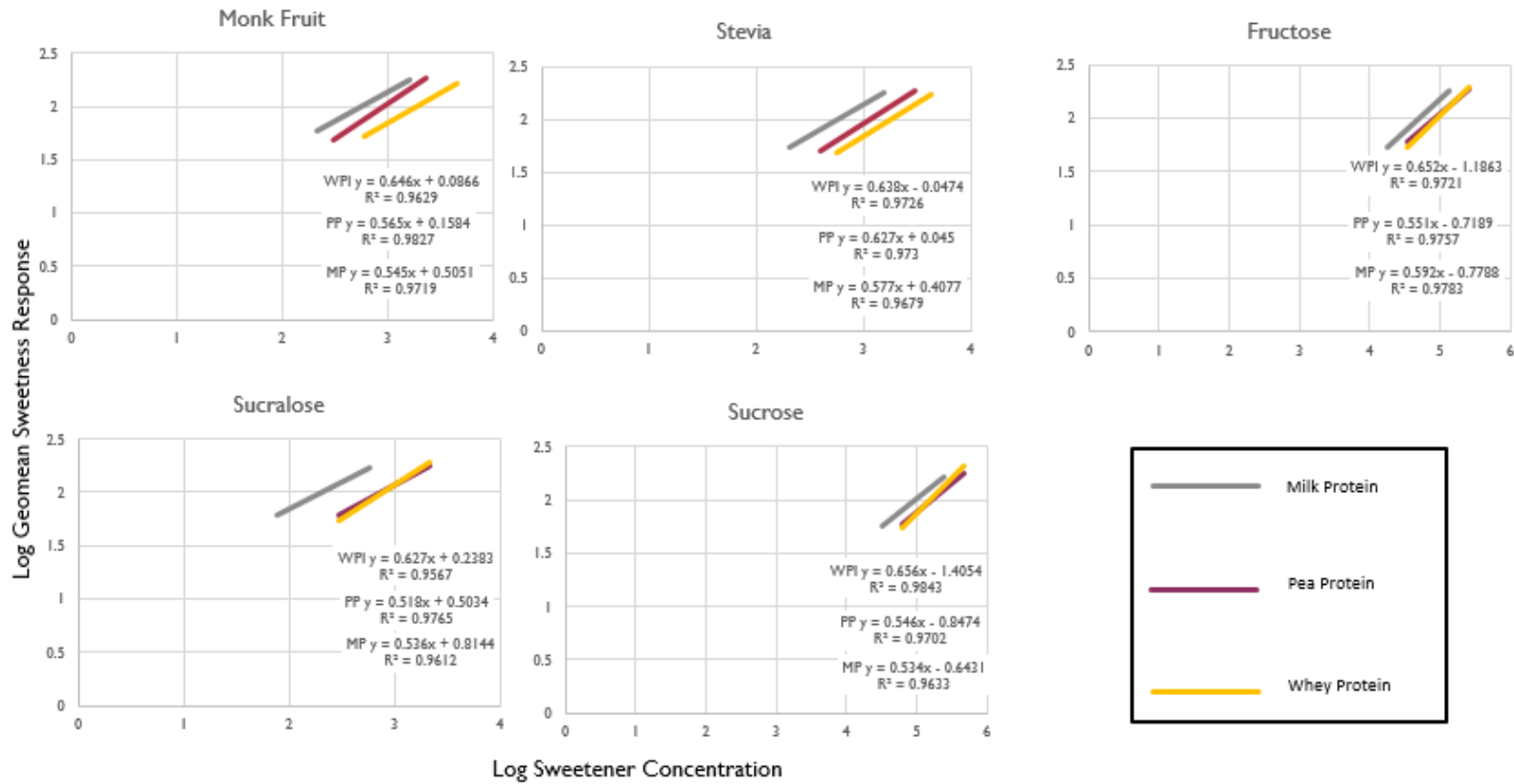


Figure 2.1. Magnitude estimation curves of iso-sweet amounts in milk protein, pea protein, and whey protein bars (500 gram batch)

Bars were formulated at 15 g protein/62 g serving

PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined

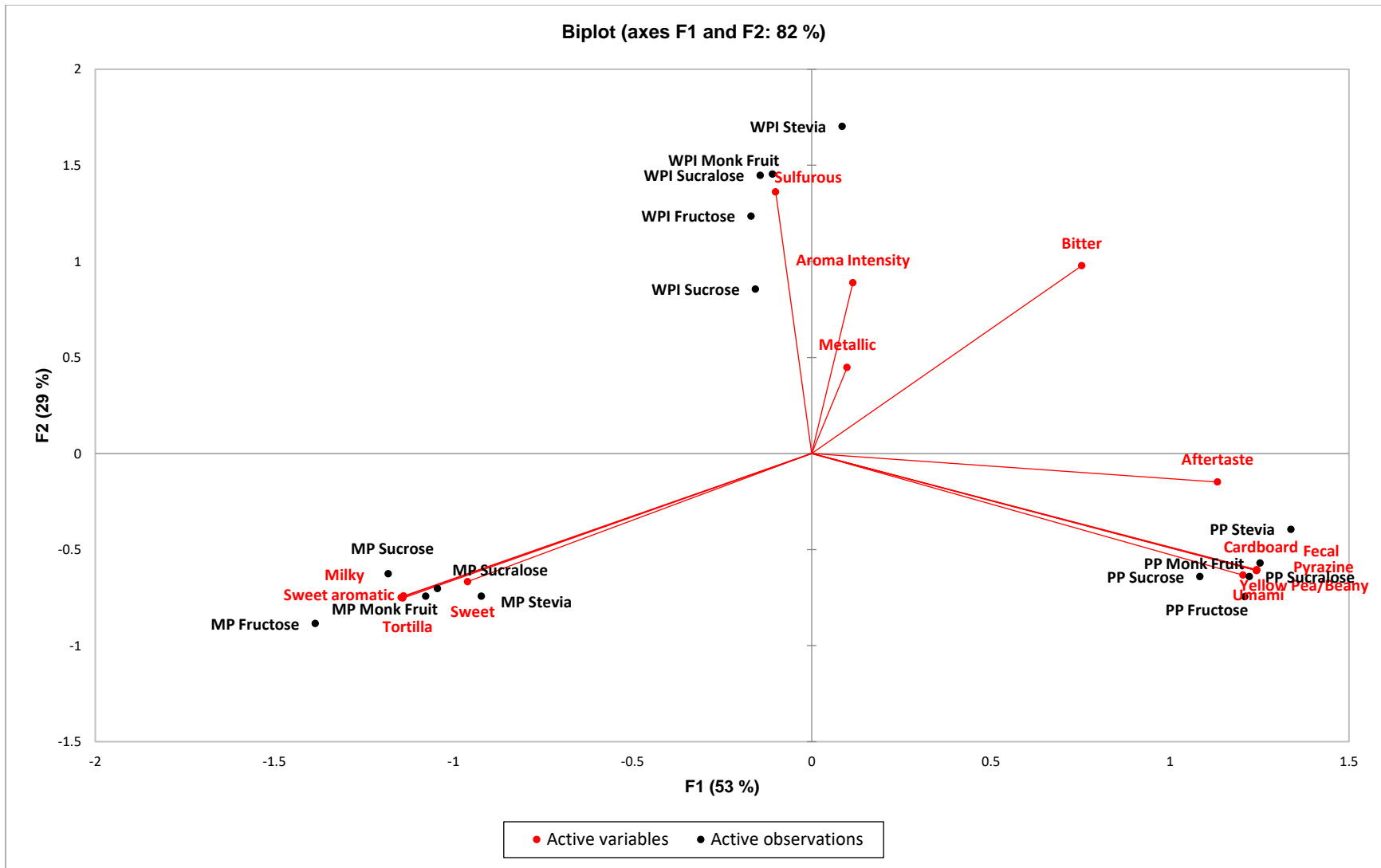


Figure 2.2. Principal component biplot of trained panel flavor profile of protein bars at iso-sweet taste

Bars were formulated at 15 g protein/62 g serving

PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined.

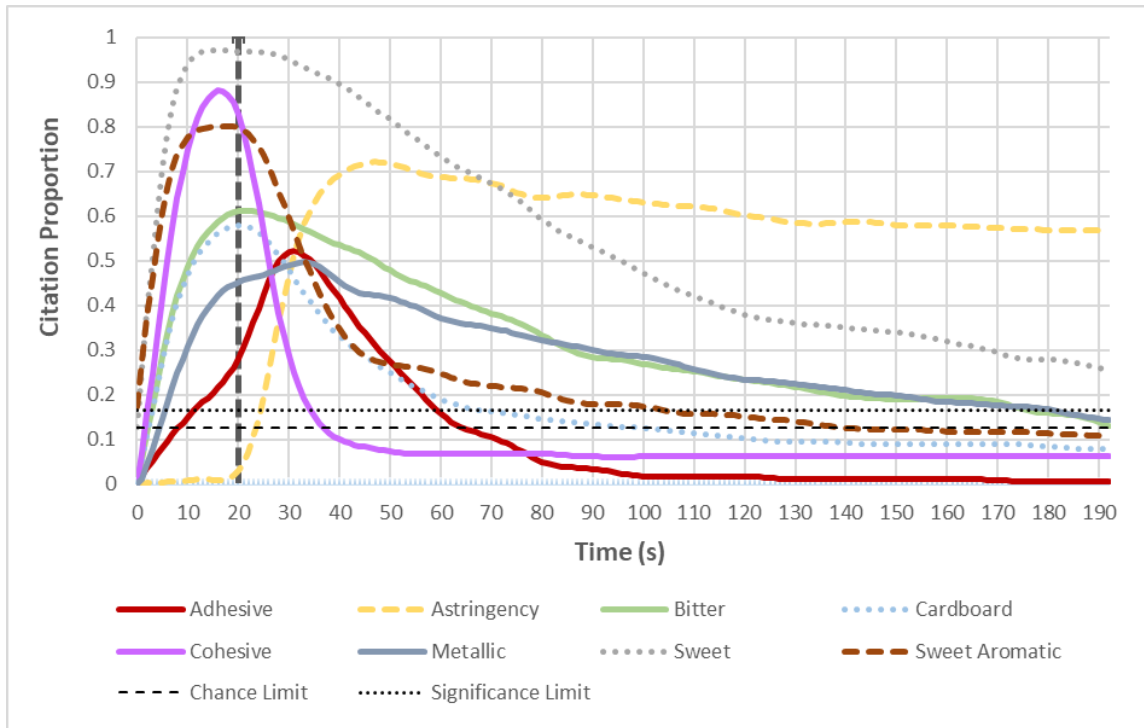


Figure 2.3a. Temporal Check All That Apply (TCATA) curves for protein bars made with WPI
 *Vertical lines denotes expectation of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)
 Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within all WPI bars

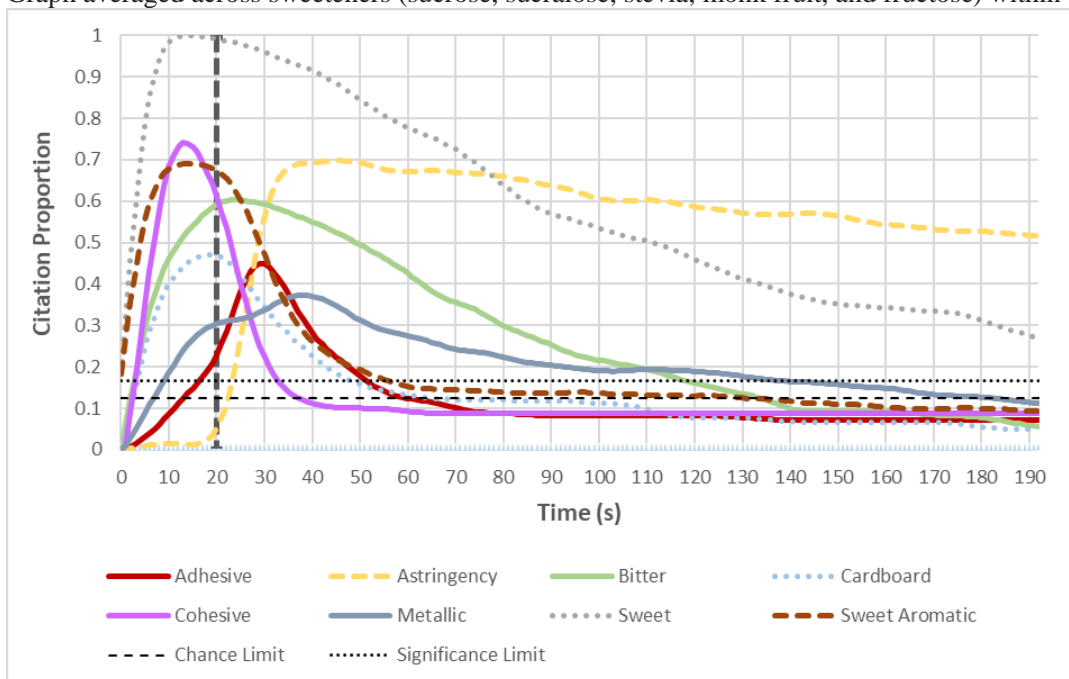


Figure 2.3b. Temporal Check All That Apply (TCATA) curves for protein bars made with PP
 *Vertical lines denotes expectation of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)
 Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within all PP bars

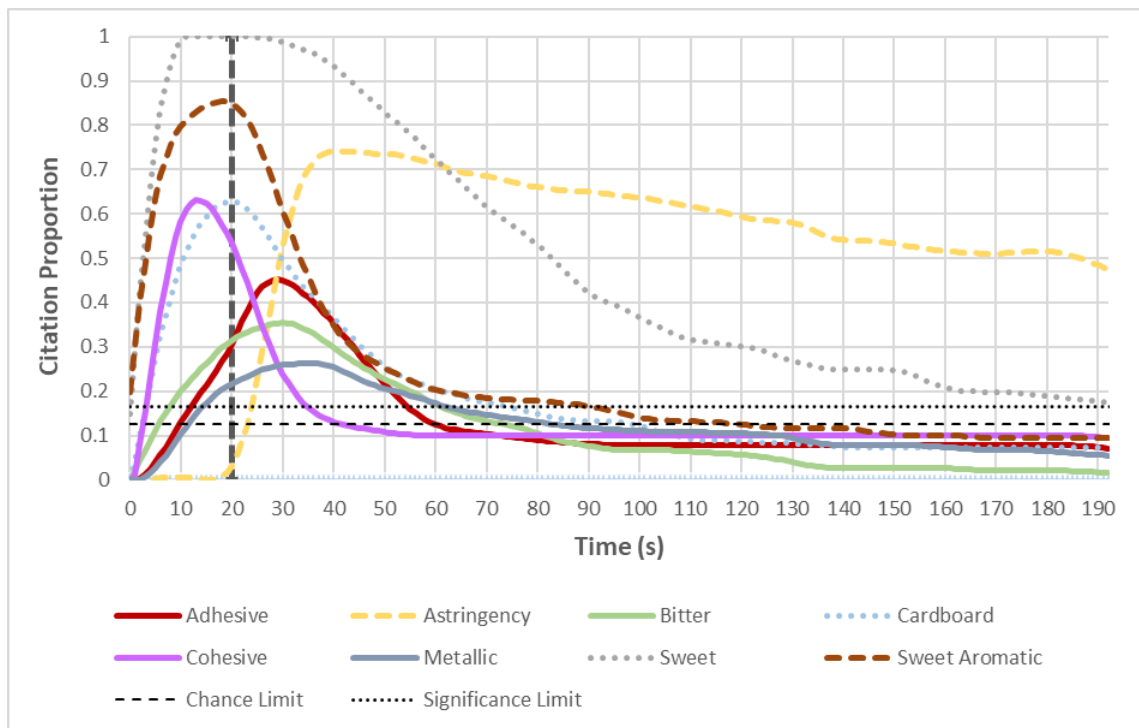


Figure 2.3c. Temporal Check All That Apply (TCATA) curves for protein bars made with MP
 *Vertical lines denotes expectation of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)
 Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within all MP bars

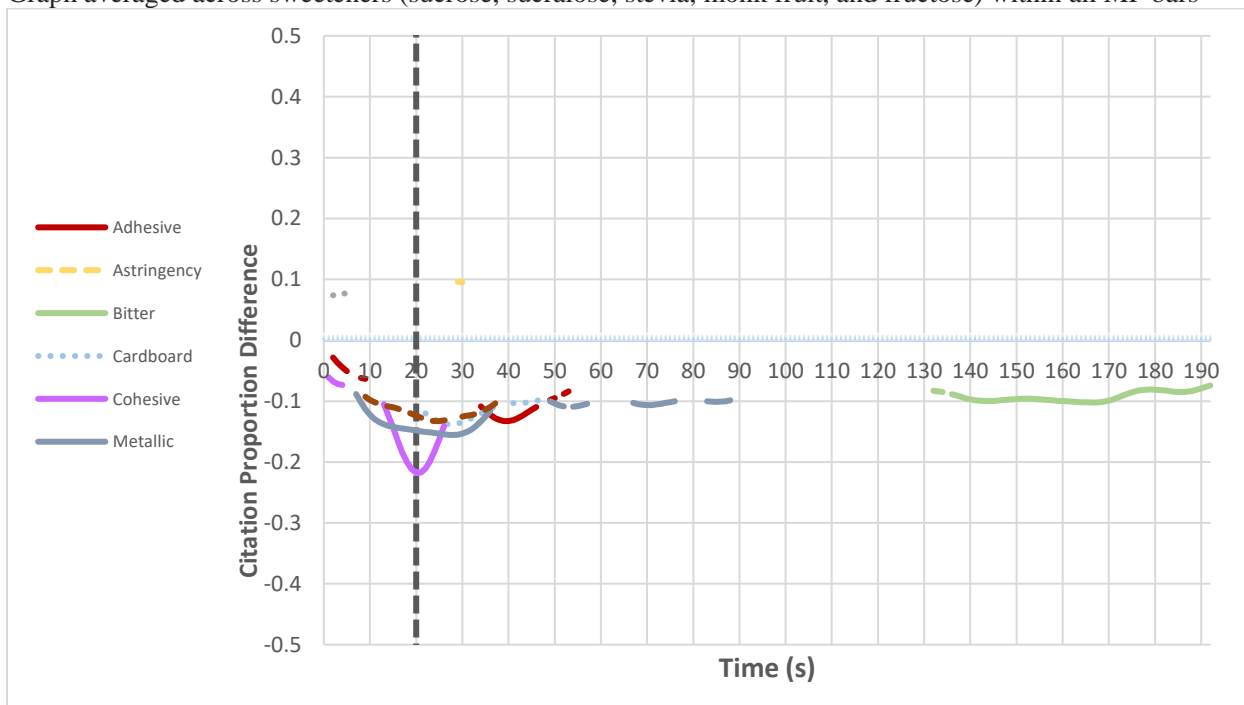


Figure 2.4a. Temporal Check All That Apply (TCATA) difference curves for protein bars made with PP or WPI ($p < 0.05$)
 *Vertical lines denotes expectation of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)
 Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within all WPI bars

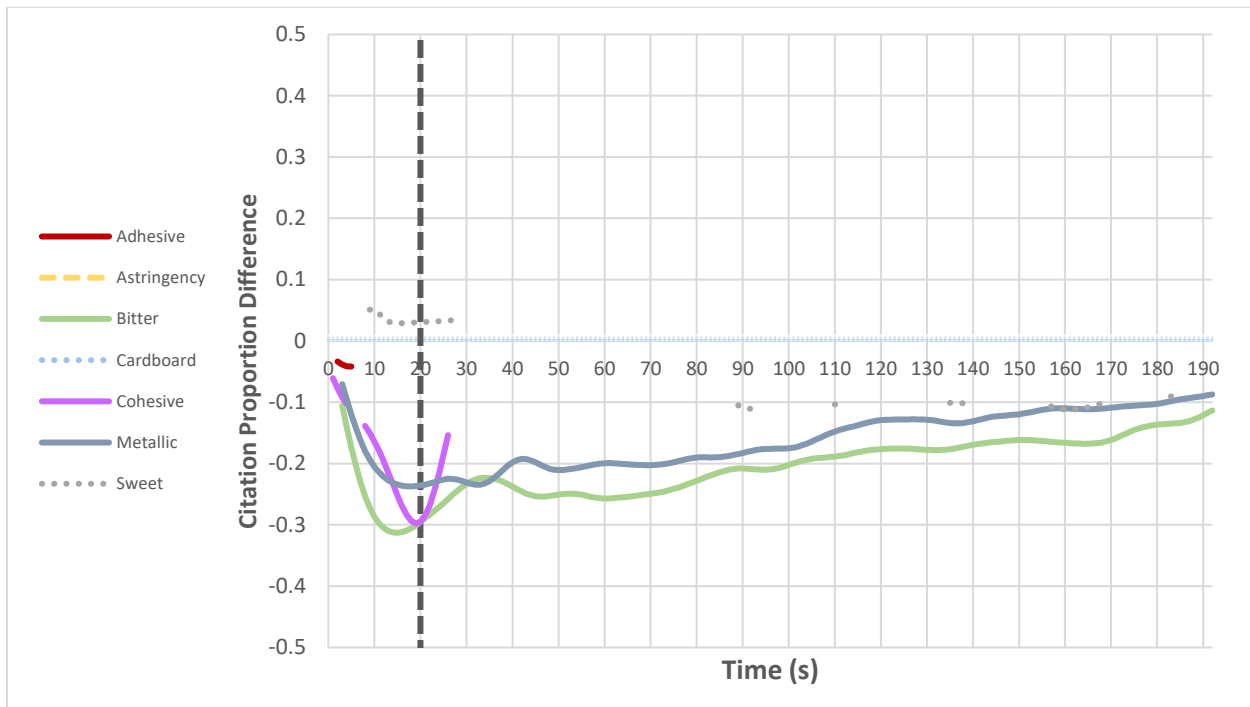


Figure 2.4b. Temporal Check All That Apply (TCATA) difference curves for protein bars made with MP or WPI ($p < 0.05$)

*Vertical lines denotes expectoration of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)

Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within protein type

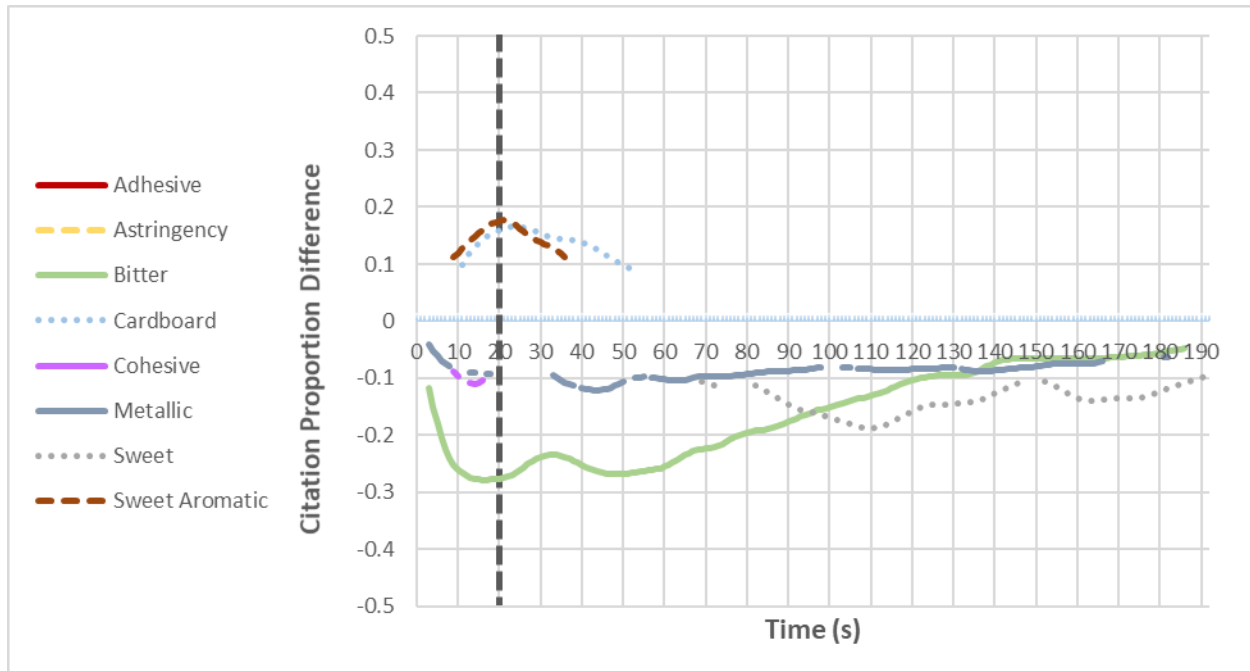


Figure 2.4c. Temporal Check All That Apply (TCATA) difference curves for protein bars made with MP or PP ($p < 0.05$)

*Vertical lines denotes expectoration of sample during evaluation (In-mouth: 0-20s, Aftertaste: 20-192s)

Graph averaged across sweeteners (sucrose, sucralose, stevia, monk fruit, and fructose) within protein type

Table 2.4. P-values for the main effects and interaction of protein and sweetener when analyzed within time for protein bar instrumental color and trained panel texture attributes

Attribute	Effect	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Instrumental p-values							
Texture	Sugar	0.0196	0.0122	0.0062	0.4421	0.4199	0.6089
	protein	0.0379	0.019	0.3035	0.2319	0.1676	0.3646
	Sugar*protein	0.0067	<.0001	0.0007	0.1357	0.4558	0.4699
L	Sugar	0.0055	0.0168	0.1477	0.2038	0.016	0.1604
	protein	<.0001	0.0004	0.0039	0.0324	0.0005	0.076
	Sugar*protein	<.0001	0.2741	0.0387	0.7531	0.0298	0.6592
a	Sugar	0.001	0.3649	0.028	0.3166	0.3159	0.9287
	protein	<.0001	0.022	0.0068	0.0154	0.0532	0.4393
	Sugar*protein	<.0001	0.0031	0.0123	0.1143	0.1045	0.4366
b*	Sugar	0.0006	0.3695	0.0513	0.5878	0.4952	0.9819
	protein	<.0001	0.0106	0.0427	0.0381	0.1063	0.3575
	Sugar*protein	<.0001	0.0141	0.0479	0.5365	0.0726	0.5079
Sensory Texture Descriptive Analysis p-values							
Color intensity	Sugar	0.287	0.0098	0.0491	0.0189	0.0044	0.0173
	protein	0.0001	0.0036	0.0024	0.0029	0.0013	0.0014
	Sugar*protein	0.0701	0.0004	0.0072	<.0001	0.0025	0.0237
Surface moisture	Sugar	0.2509	0.7579	0.5357	0.2435	0.1256	0.4236
	protein	0.2659	0.0168	0.0342	0.0251	0.0089	0.0094
	Sugar*protein	0.0224	0.5971	0.3947	0.1833	0.0446	0.1847
First bite hardness	Sugar	0.001	0.0087	0.0092	0.4136	0.5777	0.421
	protein	0.001	0.0022	0.0112	0.0064	0.0025	0.0032
	Sugar*protein	0.0036	0.0203	0.0012	0.3603	0.6884	0.7702
Denseness	Sugar	0.0193	0.0372	0.0743	0.0759	0.2303	0.2965
	protein	0.0047	0.0162	0.021	0.036	0.0254	0.0169
	Sugar*protein	0.1821	0.0583	0.1216	0.1301	0.2574	0.0854
Cohesive	Sugar	0.0859	0.0254	0.0071	0.0397	0.0368	0.0907
	protein	0.0125	0.0015	0.0031	0.0106	0.0139	0.0157
	Sugar*protein	0.0111	0.0003	0.0001	0.0001	0.0042	0.0132
Rate of breakdown	Sugar	0.2933	0.2865	0.5316	0.718	0.7127	0.492
	protein	0.0082	0.0191	0.0078	0.008	0.0053	0.0018
	Sugar*protein	0.0447	0.1827	0.1972	0.7961	0.4849	0.7787
Adhesiveness	Sugar	0.2229	0.3056	0.1018	0.1089	0.0294	0.0183
	protein	0.0048	0.0117	0.0031	0.0038	0.0015	0.002
	Sugar*protein	0.1018	0.1403	0.1095	0.1061	0.0062	0.0034

Table 2.4. (continued) P-values for the main effects and interaction of protein and sweetener when analyzed within time for protein bar instrumental color and trained panel texture attributes

Tooth pack	Sugar	0.0508	0.0763	0.1333	0.0113	0.0773	0.0569
	protein	0.0086	0.0305	0.0129	0.0033	0.0017	0.0014
	Sugar*protein	0.1665	0.0987	0.0148	0.0137	0.0306	0.0411
# of chews	Sugar	0.1378	0.0869	0.1575	0.0449	0.4952	0.6245
	protein	0.0056	0.0065	0.0019	0.0016	0.0016	0.0051
	Sugar*protein	0.5681	0.1418	0.0823	0.0763	0.4968	0.8096

Highlighted cells indicate significant effects and interactions ($P < 0.05$).

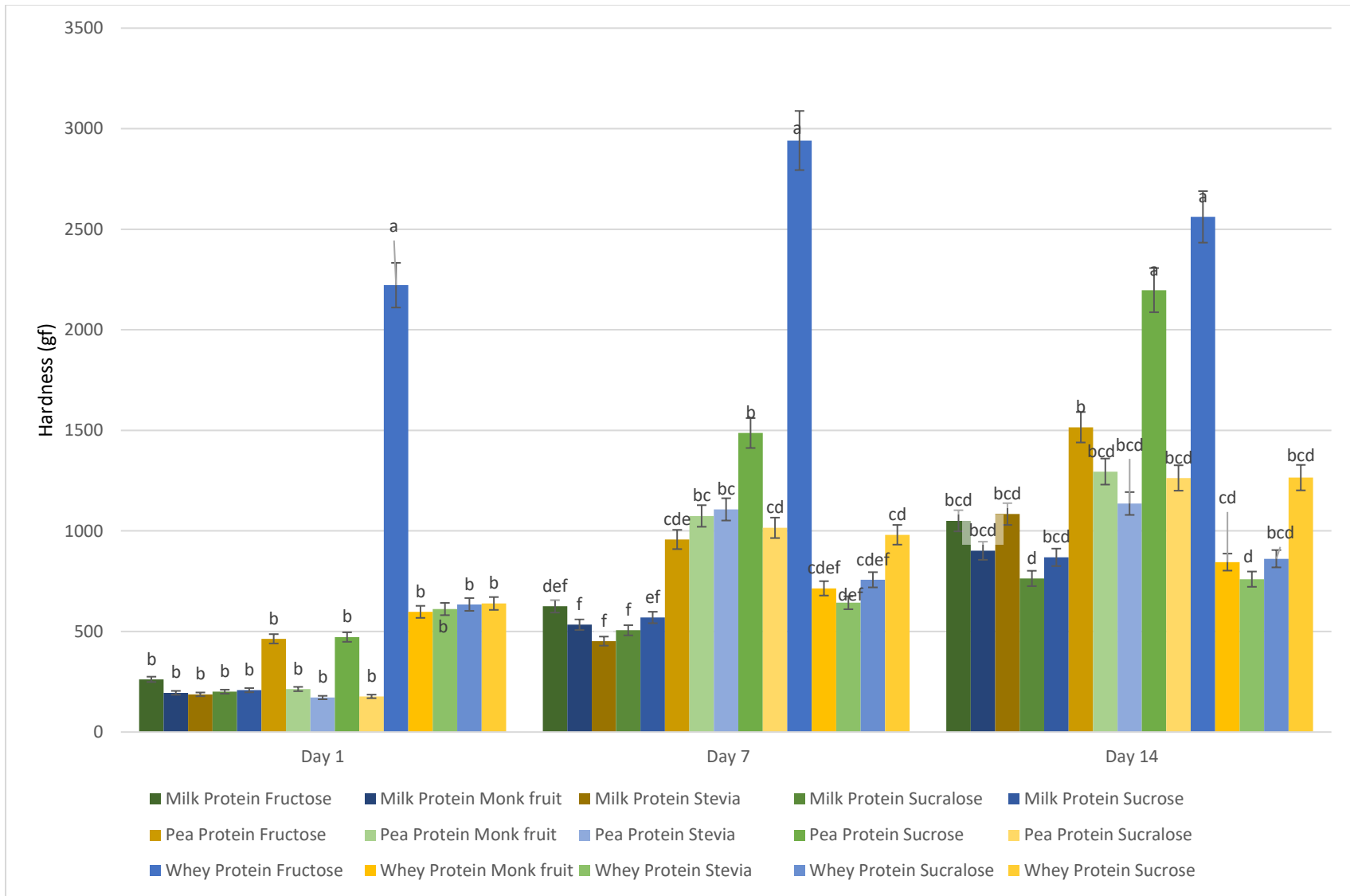


Figure 2.5. Instrumental hardness for protein bars separated by protein and sweetener within each storage time point. Protein bars without a common letter within the same time point are significantly different ($P < 0.05$). Standard error bars are included. Bars were stored at 35 °C

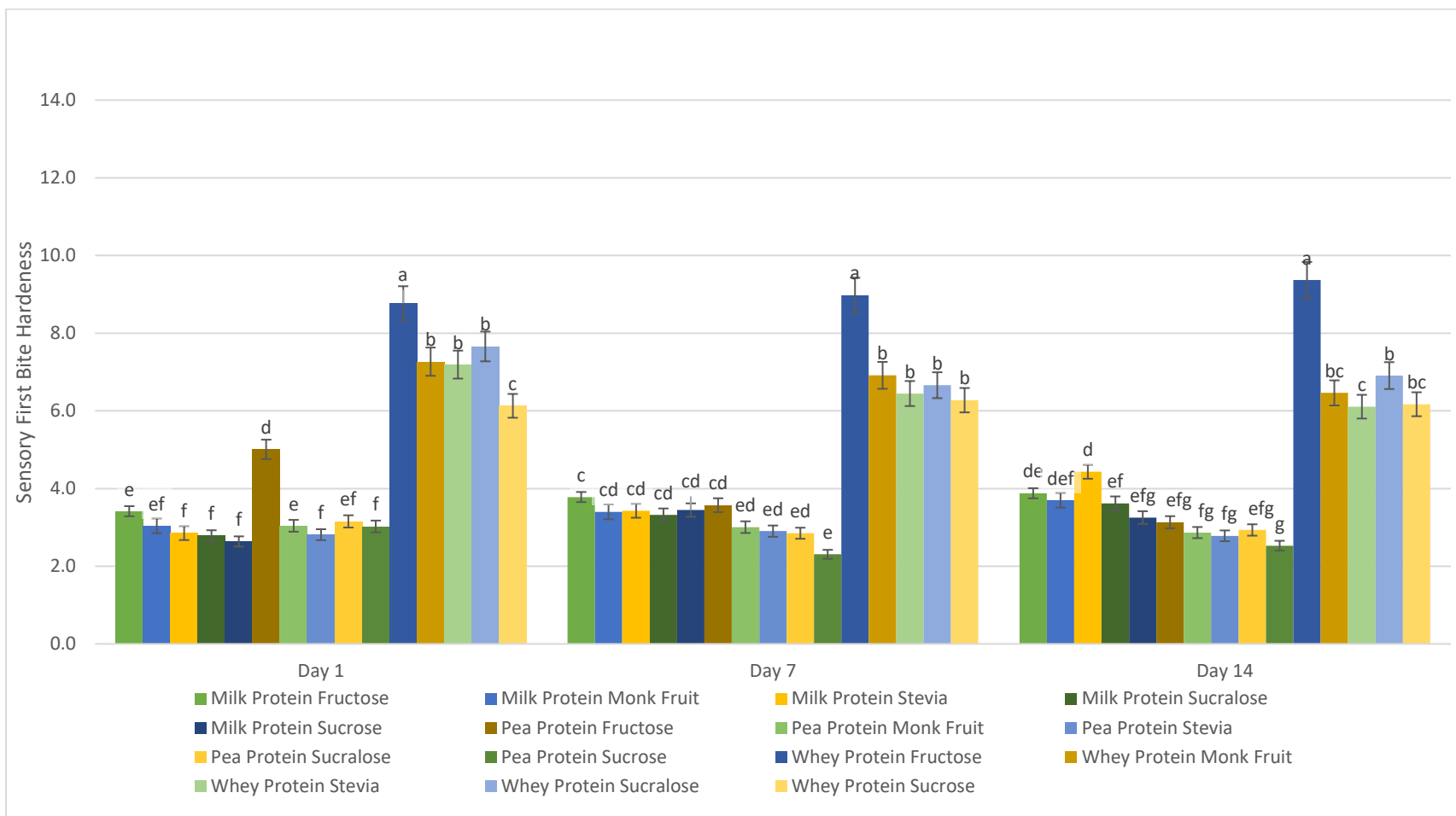


Figure 2.6. Sensory first bite hardness for protein bars separated by protein and sweetener within each time point

Sensory first bite hardness was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included.

Bars were stored at 35 °C



Figure 2.7. Protein type main effects for sensory first bite for protein bars

First bite hardness was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included.

Bars were stored at 35 °C

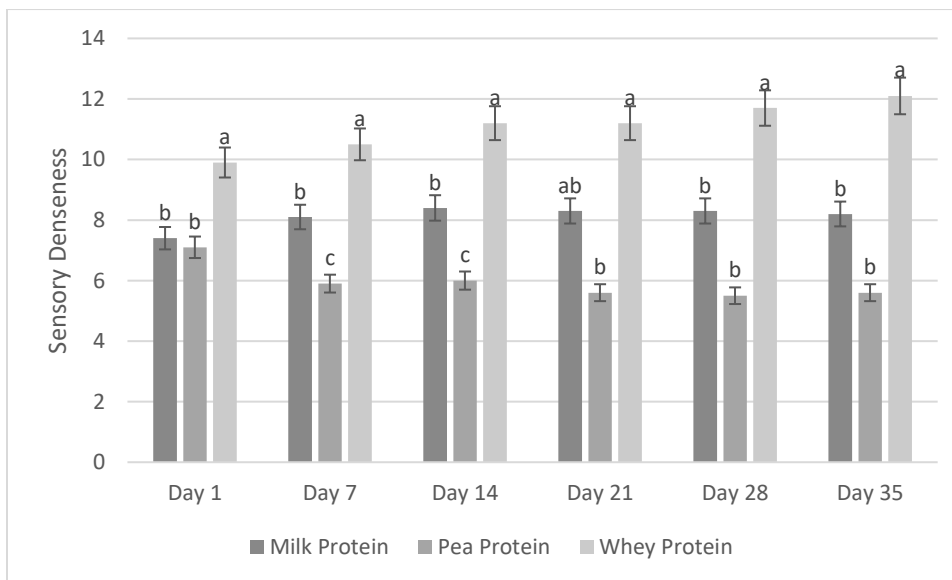


Figure 2.8a. Protein type main effects for sensory denseness for protein bars

Sensory Denseness was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included.

Bars were stored at 35 °C

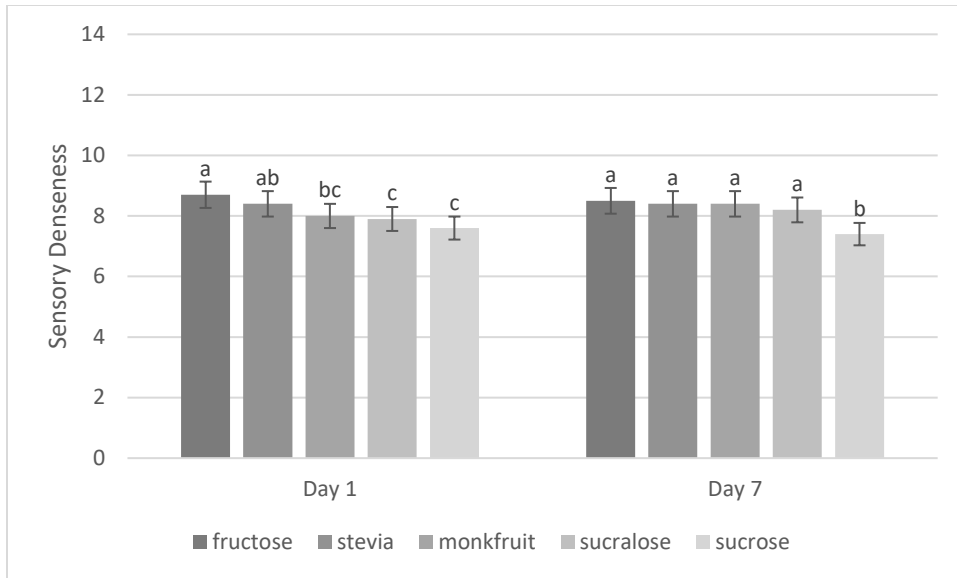


Figure 2.8b. Sugar main effects for sensory denseness for protein bars

Sensory Denseness was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included

Bars were stored at 35 °C

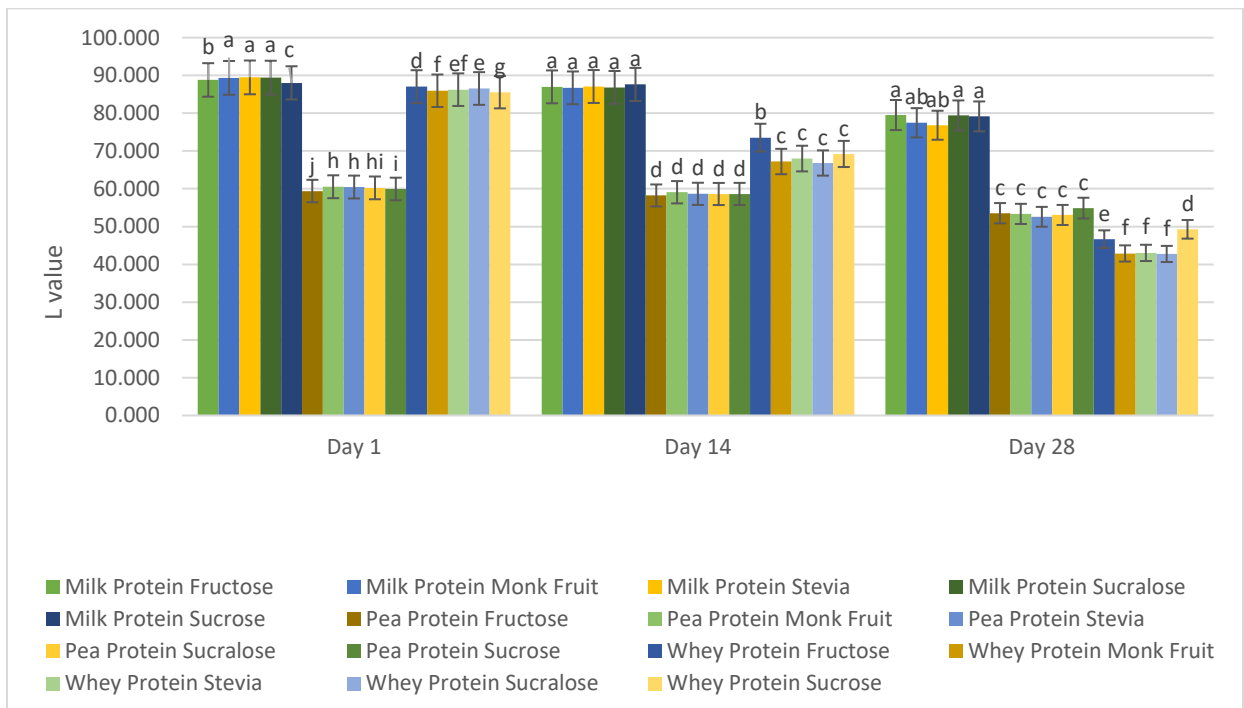


Figure 2.9a. Instrumental Hunter Lab L values color measurements for protein bars at a viewer angle of 10° separated by protein type and sweetener

Hunter Luminosity (L) measures the degree of lightness from dark (0) to light (100).

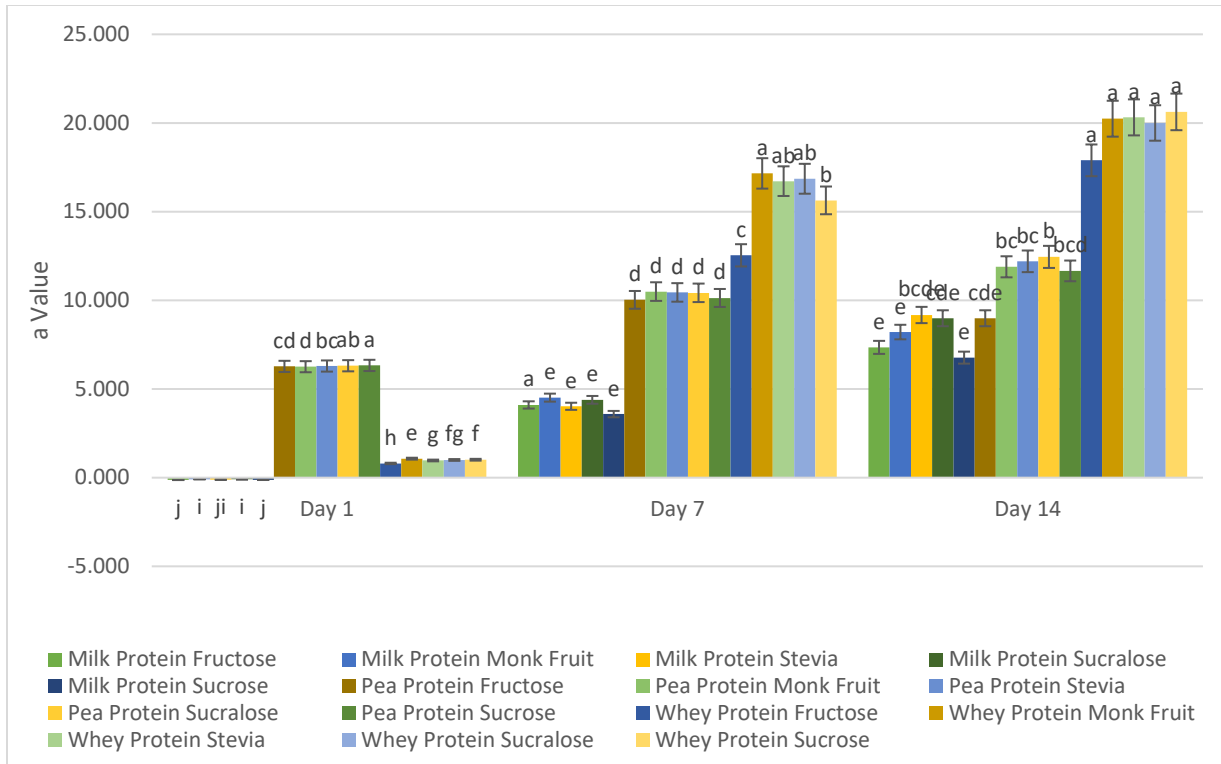


Figure 2.9b. Instrumental Hunter Lab a values color measurements for protein bars at a viewer angle of 10° separated by protein type and sweetener

Hunter a measures the degree of redness (+) or greenness (-).

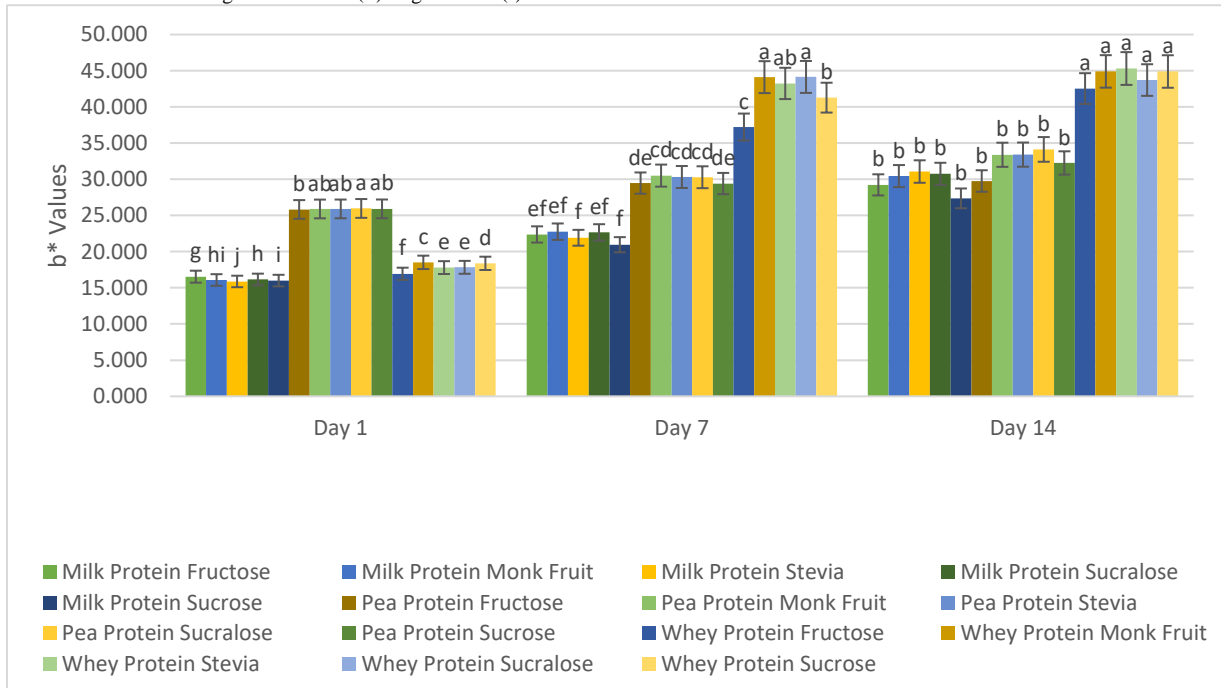


Figure 2.9c. Instrumental CIE b* values color measurements for protein bars at a viewer angle of 10° separated by protein type and sweetener

CIE b* measures the degree of yellowness (+) or blueness (-).

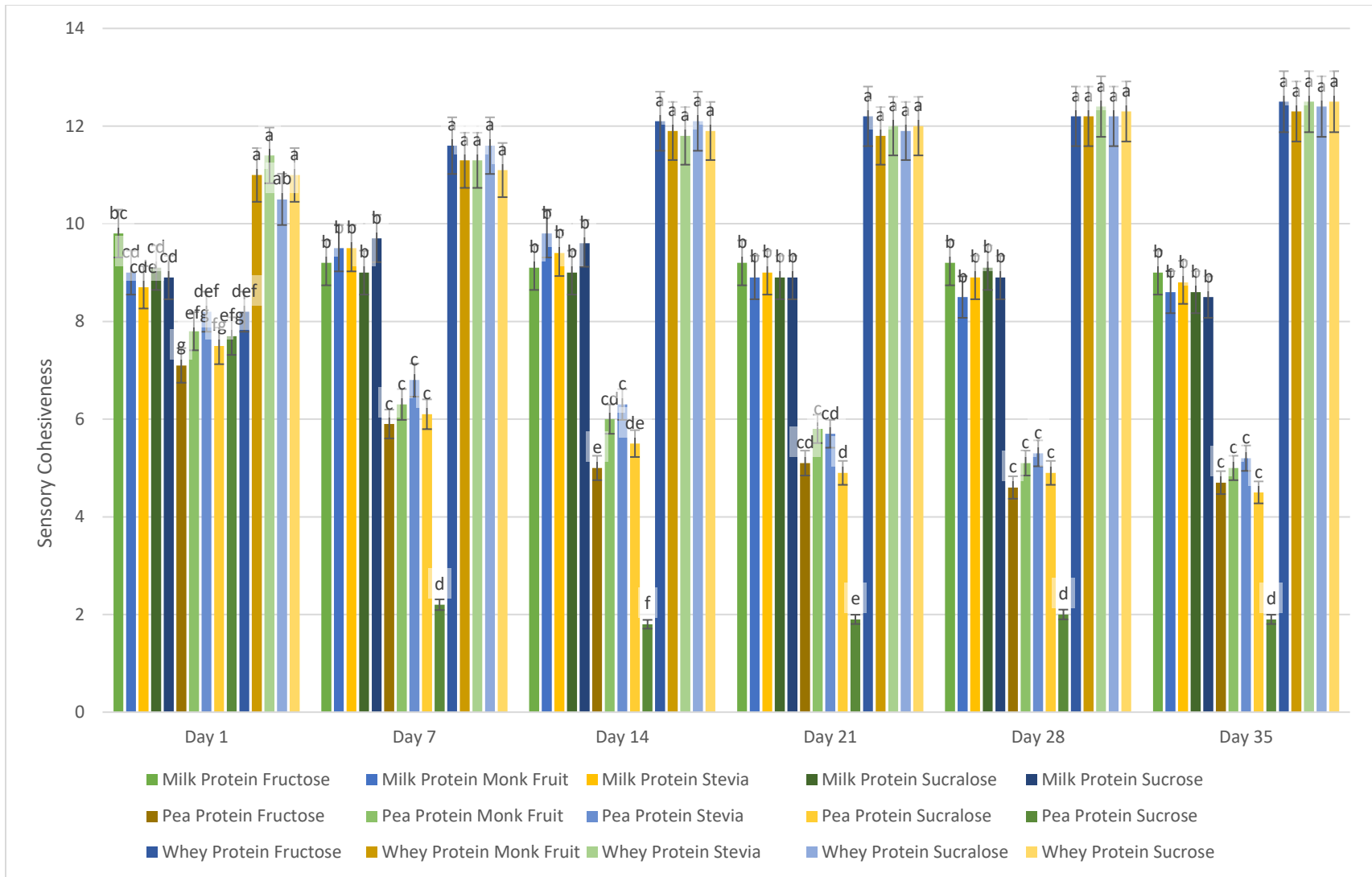


Figure 2.10. Trained panel cohesiveness for protein bars separated by protein type and sweetener

Sensory Cohesiveness was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included.

Bars were stored at 35 °C

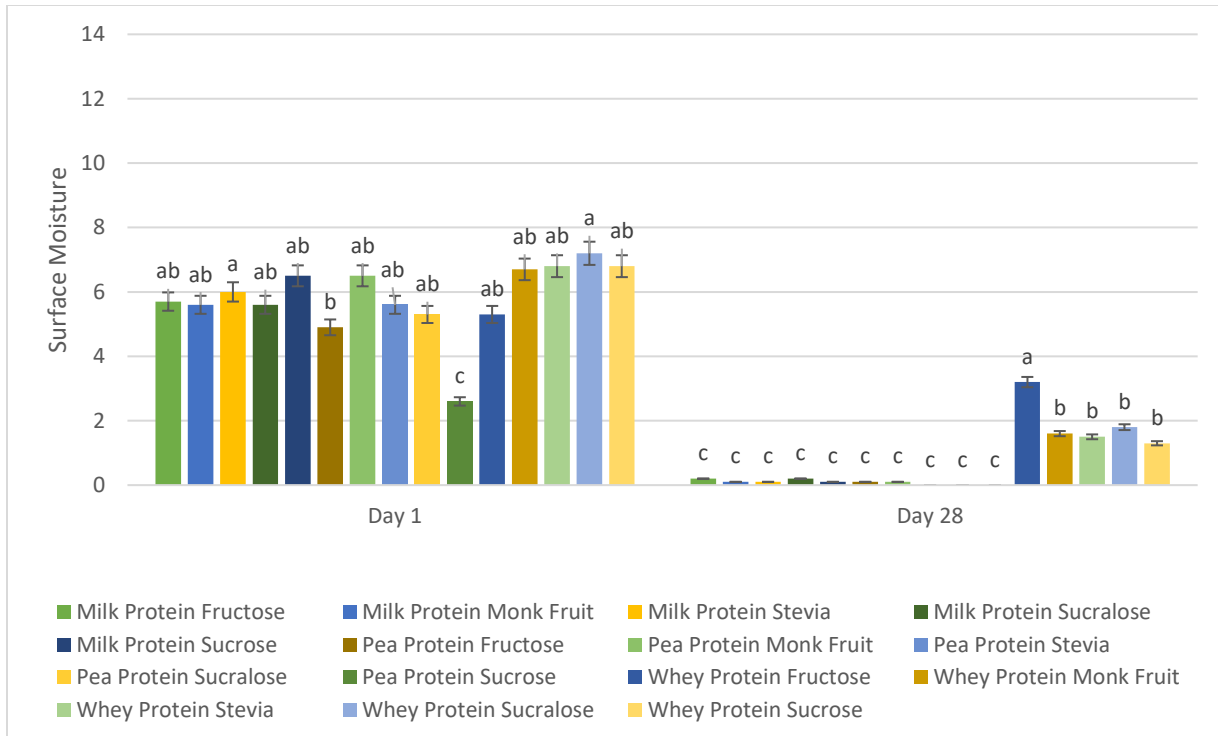


Figure 2.11a. Trained panel surface moisture for protein bars separated by protein type and sweetener

Sensory surface moisture was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein bars without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included.

Bars were stored at 35 °C



Figure 2.11b. Trained panel surface moisture protein type main effects

Sensory surface moisture was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low intensity and 15= very high intensity.

Protein types without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included

Bars were stored at 35 °C



Figure 2.12. Trained panel rate of breakdown intensities for protein bars separated by protein main effects

Sensory rate of breakdown was scored on a 0- to 15-point product specific intensity scale (Meilgaard et al., 2007) where 1= very low rate of breakdown and 15= very high rate of breakdown.

Protein types without a common letter within the same time point are significantly different ($P < 0.05$).

Standard error bars are included

Bars were stored at 35 °C

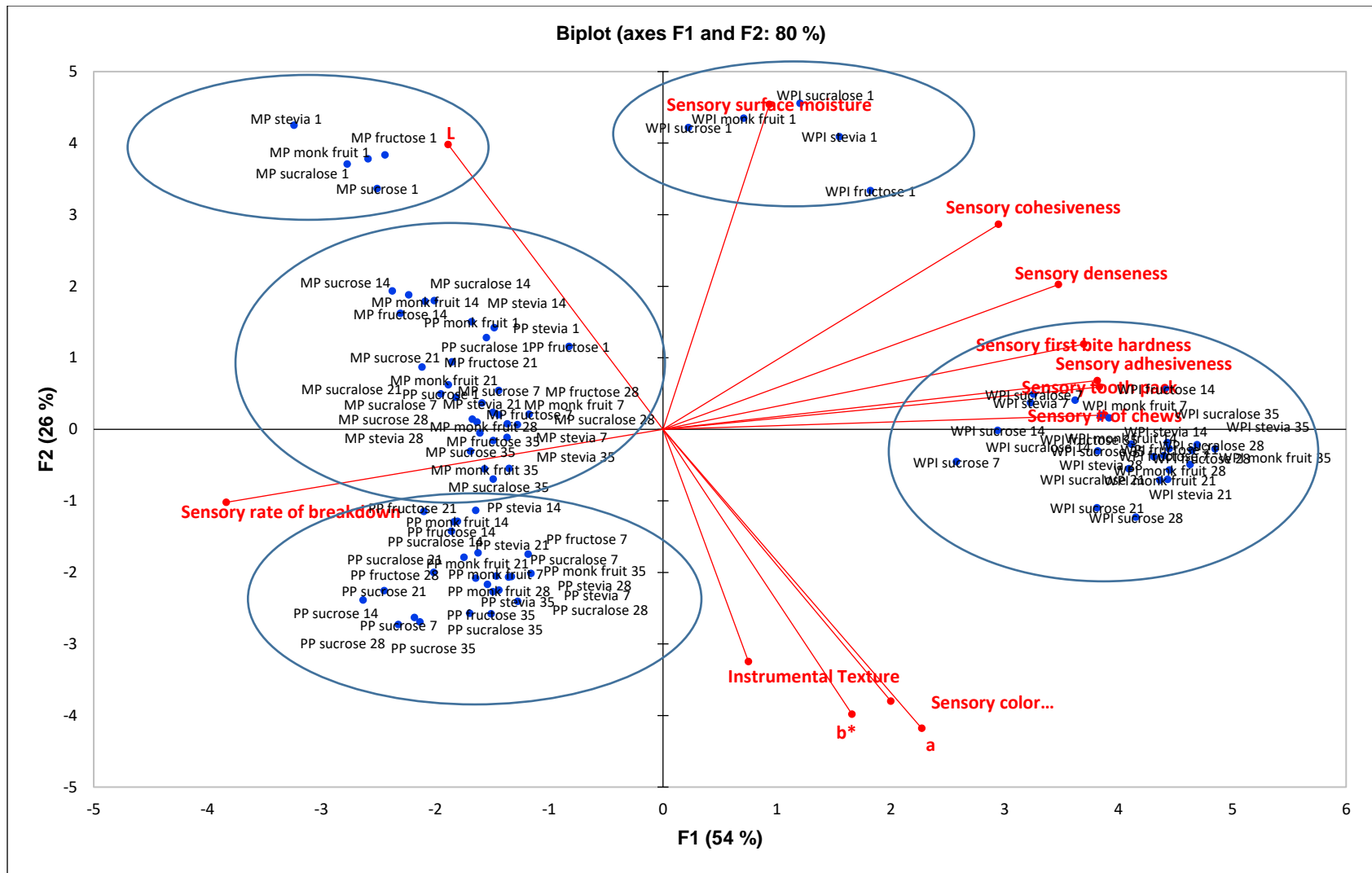


Figure 2.13. Principal component biplot of trained panel and instrumental measurements of protein bars across all time points

All protein bars within a circle are the same protein type.

Bars were stored at 35 °C

PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined. Number indicates days of storage 1, 7, 14, 21, 28, or 35

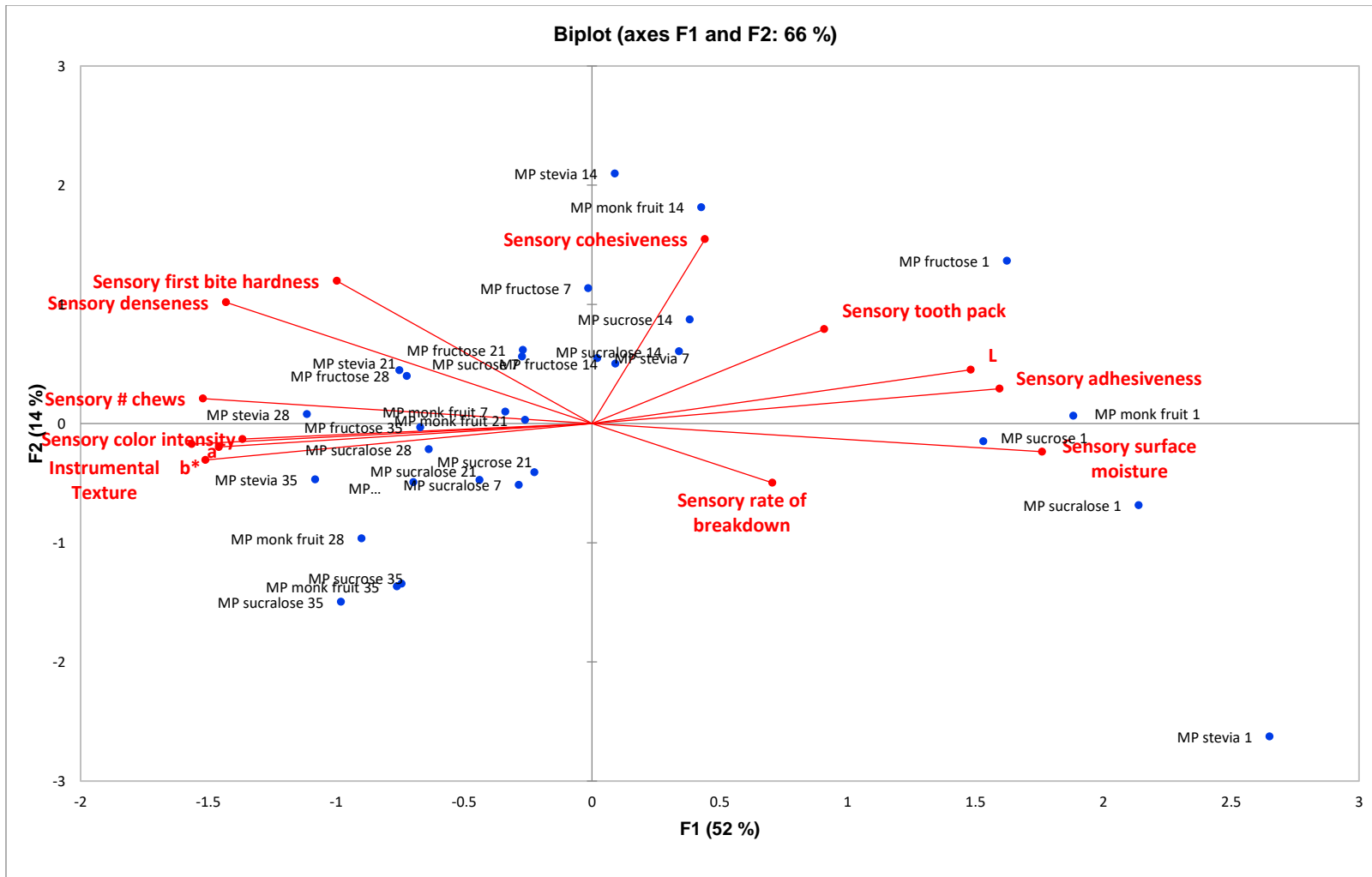


Figure 2.14a. Principal component biplot of trained panel and instrumental measurements of MP protein bars across all time points

Bars were stored at 35 °C

PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined. Number indicates days of storage 1, 7, 14, 21, 28, or 35

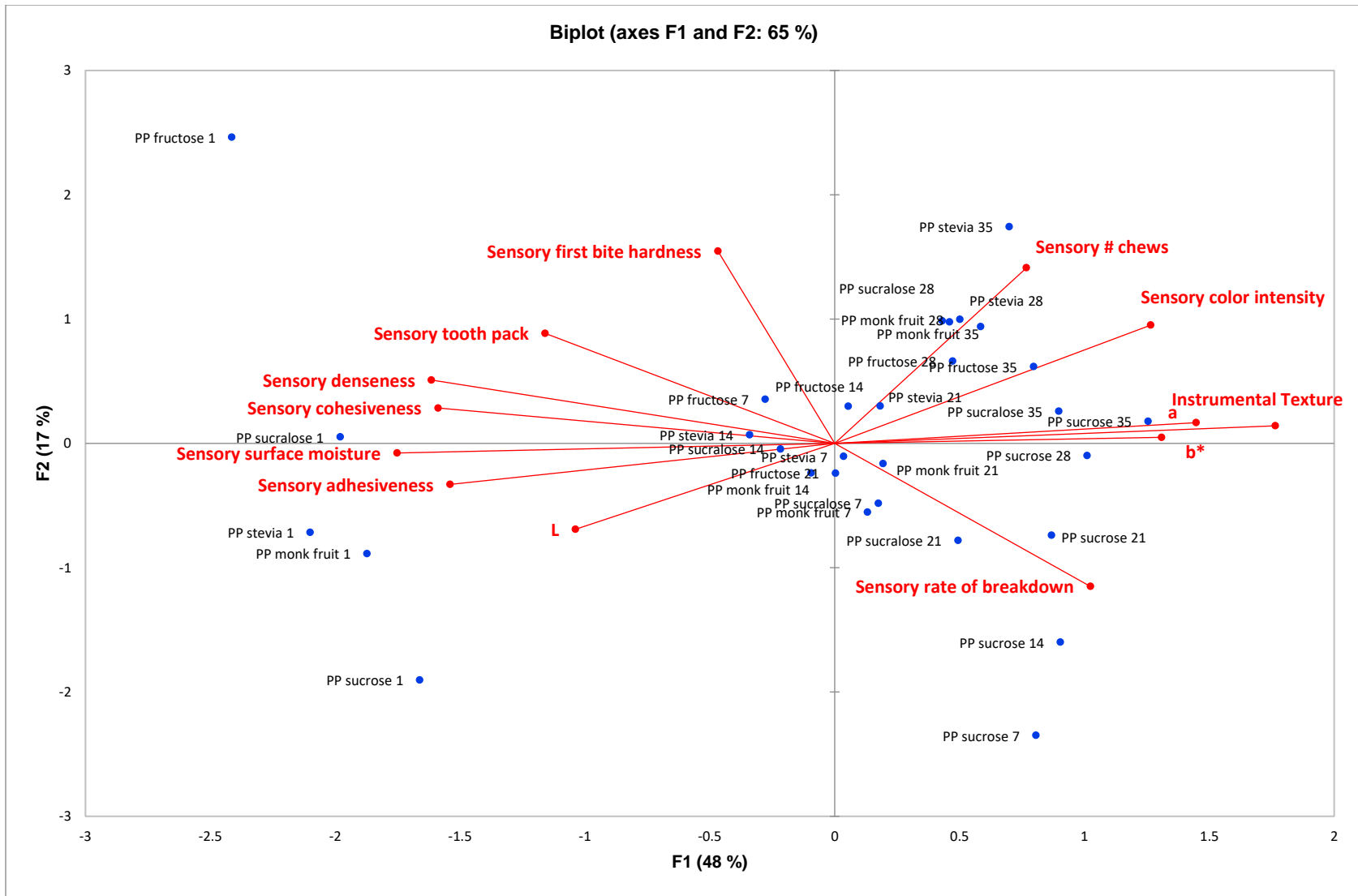


Figure 2.14b. Principal component biplot of trained panel and instrumental measurements of PP protein bars across all time points. Bars were stored at 35 °C. PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined. Number indicates days of storage 1, 7, 14, 21, 28, or 35.

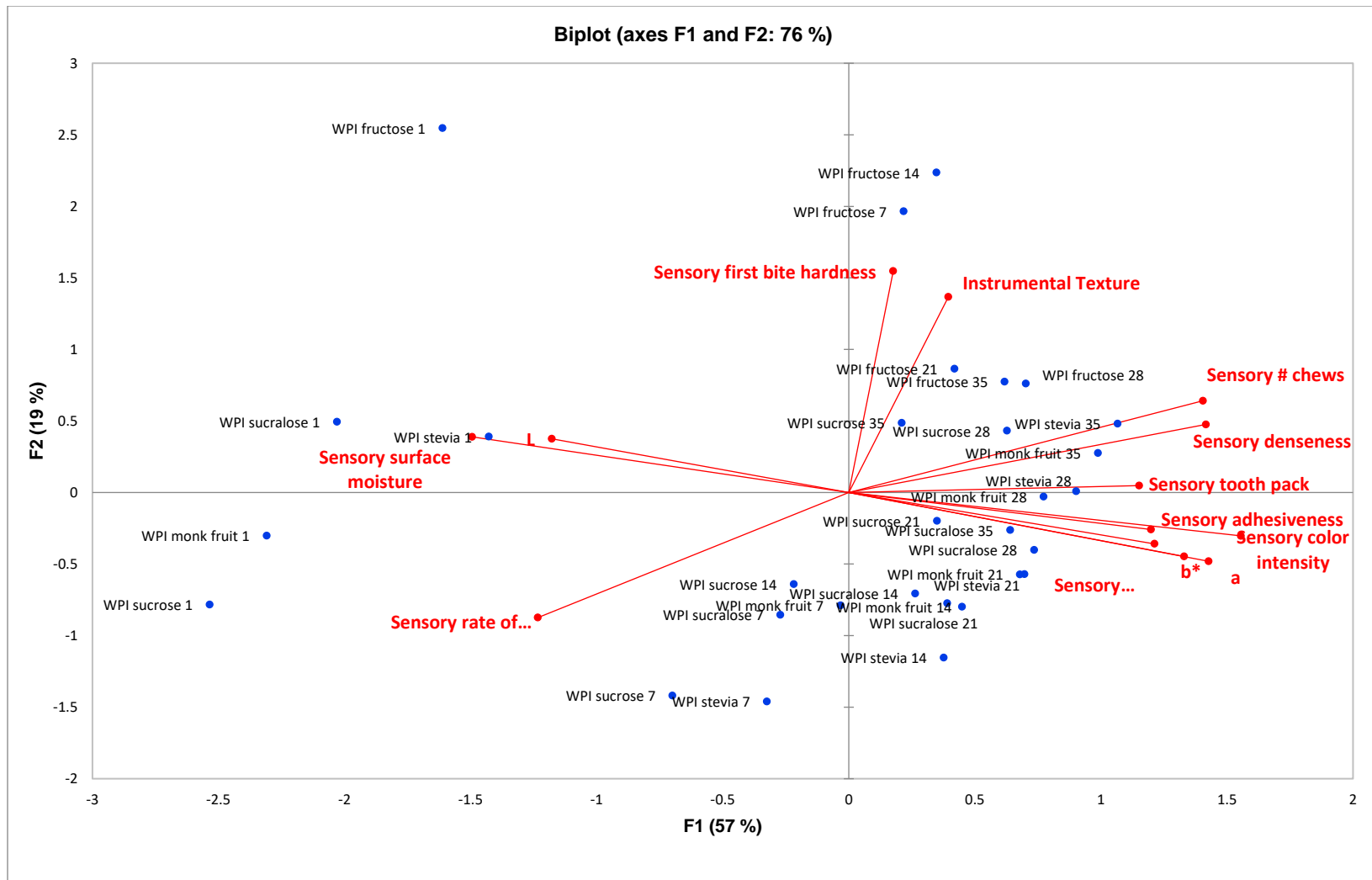


Figure 2.14c. Principle Component Biplot of trained panel and instrumental measurements of WPI protein bars across all time points. Bars were stored at 35 °C. PP- Pea protein, MP- Milk Protein, WPI- Whey Protein Isolate. Sweetener is defined. Number indicates days of storage 1, 7, 14, 21, 28, or 35.