Lowering Sugars in Dark Chocolate through Alternative Sweeteners

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Abstract

With the recent new food labeling guidelines requiring that added sugars be listed on nutrition labels, both consumers and the food industry are concerned about sugar and added sugars in food. The literature review in this report evaluated studies that focused on a reduction of sugar in chocolate, a popular food that many people associate with containing sugar. The studies reviewed here included reduced sugar or sugar-free chocolates that used polyols, rare sugar, inulin, and high-potency sweeteners. Rare sugars are monosaccharides and their derivatives, which are rarely found in nature. One rare sugar that was included in the literature review was D-allulose. The review also looked at models of reducing added sugars in foods. From the review, a study was conducted to look at different sweeteners in dark chocolate. In this study, agave and fructose were compared to the control (sucrose); the reduction of sucrose in samples in this study was 30% from the control. The study evaluated how the sweeteners affect the physical attributes of dark chocolate to determine the best sweetener to use to reduce sucrose and further reduce added sugar. The study found that lowering sugar for taste is not the only aspect a product developer must consider when reducing sugar in a product; different sweeteners also affect physical parameters in chocolate. For example, the moisture and particle size distribution affect the physical properties of the chocolate. The moisture in the agave-sweetened chocolate bar was 54.54% higher than in the sucrose-sweetened chocolate bar; the agave-sweetened bar was 41.67% higher in moisture than the fructose-sweetened chocolate bar. The higher moisture of the agave-sweetened chocolate samples resulted in higher agglomeration; moisture created sticky patches that induced agglomeration and a higher reduction of particles. The hygroscopicity of agave affected the rheology of the chocolate because higher agglomeration of particles leads to higher yield values in the agave-sweetened chocolate. The

smaller particles have more surface area to get coated in fat, which affects rheology. The sucrose-sweetened chocolate treatment, which had larger particles and lower surface area, had a higher viscosity. However, the agave- and fructose-sweetened chocolates made in this study can be considered standard of identity while non-nutritive sweeteners would not be. When developing new chocolate formulations with reduced sugar, the scientist needs take the physical parameters of the non-sucrose sugars used into account.

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Chapter 1 - Introduction

In May 2016, the U.S. Food & Drug Administration (FDA) released information about the new nutrition facts label. The upcoming changes to the nutrition label, specifically for packaged foods, are intended to show scientific evidence linking diet with chronic diseases (such as obesity and heart disease) in order to help consumers make better decisions (FDA, 2018).

One of the changes will be the declaration of "added sugars" on the label, indented under the total sugars. Added sugars are sugars that contribute energy to the diet but that have little nutritional benefit (Yeung et al., 2017). As Mooradian et al. (2017) state, "the rapid increase in the prevalence of obesity worldwide has been partially attributed to the consumption of added sugar" (p. 1).

In 2015, the World Health Organization (WHO) provided recommendations on the reduction of free sugars to reduce non-communicable diseases in adults and children. They strongly recommended a reduction of free sugar to equal less than 10% of the total energy intake. They also recommended a further reduction of 5% for additional health benefits based on evidence regarding the relationship between free sugar, body weight, and dental caries (WHO, 2015). Per the WHO (2015), free sugars are the same as added sugars; "free sugars include monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates" (p. 12). With the nutrition facts label change and the link between an increased consumption of sugar with obesity, according to some of the authors in the literature review, manufacturers are incentivized to formulate products with less sugar.

Consumers are also becoming more concerned with the sugar content of food. According to a sweetener study by Innova Market Insights (2018) in 2015, 37% of United States consumers

were looking for natural sweeteners when selecting sweetened food and beverages. The Compounded Annual Growth Rate (CAGR) increased 29% for low-sugar products globally from 2011-2016 (Innova Market Insights, 2018). One popular product that could benefit from lower added sugars is chocolate.

Chocolate, initially invented by the Aztecs and Mayans in Central America around 600 A.D., originally took the form of a drink. It consisted of whole cocoa beans, sugar, and spices cooked together. In the 1600s, when the Spanish conquered Mexico, Don Cortez took the drink back to Spain (Beckett, 2008). From there, it spread to other parts of Europe and in that process was optimized for easier preparation. Van Houten developed the cocoa press in 1828 to mill the cocoa bean into cocoa powder. It wasn't until the 1840s, however, that chocolate was molded and used to cover other confectionary products (Minifie, 1989). Chocolate bars were developed to utilize the cocoa butter that was removed after pressing the cocoa bean into the cocoa powder.

According to the U.S. Food & Drug Administration in 21 CFR 163, the standard of identity for chocolate is the "solid or semiplastic food prepared by intimately mixing and grinding chocolate liquor with one or more optional nutritive carbohydrate sweeteners" (Sec. 163.123 (a)). To be more specific, according to Minifie (1989), the basic ingredients for chocolate manufacturing include cocoa nibs, cocoa liquor, sugar, other sweeteners, cocoa butter, butter fat (oil), milk powder, milk crumb, and emulsifiers. Sugar typically makes up about 50% of chocolate. Most of the sugar in chocolate comes from sucrose; however, some sugar comes from the lactose (from milk) in milk chocolate.

Sugar is not just a sweetener. Sugar, specifically sucrose, in dark chocolate, plays a critical role in both flavor and quality attributes of dark chocolate. Sugar is also a functional ingredient and provides bulk to the chocolate (Aidoo, 2013). Because sugar is a humectant, it

can help to control water activity and prevent microbial growth in products. Sugar affects particle size, rheology, texture, and mouthfeel. Some desirable attributes of chocolate include creaminess, sweetness, pleasant taste, smoothness, and melting (Saputro et al., 2016). Processing and ingredients (including sugar) affect these quality attributes.

According to Thamke et al. (2009), there is a trend toward increased consumption of dark chocolate. Chocolate consumption, sometimes considered an indulgence, can lead to positive emotional changes in the consumer, including reduced hunger and elevated mood. Additional nutritional benefits from chocolate consumption come from polyphenols, which can help prevent heart and vascular problems. With chocolate's high sugar content, however, the dietary benefits might be outweighed by the excess energy it contains.

With that in mind, some studies have looked at strategies for reducing sugar in foods, including chocolate. Yeung et al. (2017) looked at ways to reduce added sugar by modeling the diet of children and adolescents in Australia with data from a national nutrition survey. The study looked at the impact on the population's intake of energy, sugar, total fat, saturated fat, and fiber, and suggested product reformulation as a way to achieve the necessary added sugar reduction to positively impact health. According to Zhang et al. (2017), low-calorie rare sugars are getting more attention by researchers because of consumers' excessive intake of high sugar foods and the corresponding health impacts.

Although added sugars are dangerous to our health, chocolate, a historically popular treat worldwide, doesn't have to be given up in the growing consumer trend to reduce the intake of added sugar (especially considering that chocolate has both emotional and health benefits). Considering this, the following literature review looks specifically at the reduction of sucrose in chocolate and the use of nutritive and non-nutritive sweeteners in that process. With the

literature analyzed, a study was then performed on the reduction of added sugar in chocolate and the role of nutritive sweeteners. We've been eating chocolate for 2000 years; who wants to give it up now?

Chapter 2 - Literature Review

1: Articles that provided an overview of alternative sweeteners to sucrose

Both Mooradian et al. and Aidoo et al. conducted reviews of alternative sweeteners to sucrose. Mooradian et al. (2017) reviewed natural and artificial sweeteners and their role in reducing sugar consumption. This article focused on how the medical community looks at sugar reduction, compared to the Aidoo et al.'s article, which focused on the food science of sugar as an ingredient. This article also provided an overview of the different types of sweeteners. They stated that the consumption of added sugar contributes to obesity. They also noted that non-caloric sweeteners may have unfavorable effects on health because of glucose intolerances and failure to reduce weight. Finally, they stated that the consumption of caloric sweeteners has increased steadily over the past four decades (Mooradian et al., 2017).

Mooradian et al. (2017) reviewed many studies that evaluated the metabolic and clinical effects of sucrose, fructose, and artificial sweeteners. Some of the metabolic functions they evaluated included triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), fasting blood sugar, and systolic blood pressure. According to their evaluations, there is an increase in the association between added sugars and atherogenic lipid profile. They also reviewed studies that evaluated the association between obesity and caloric-sweetened beverages. These beverages typically contain high fructose corn syrup, which studies show has predicted greater cardiovascular risk and diabetes prevalence (Mooradian et al., 2017).

According to Mooradian et al. (2017), increased consumption of added sugar is due to the low-fat diet trend. The authors also believe that humans have natural cravings for sweets. Artificial sweeteners were developed in an attempt to lower the rate of obesity and to continue to satisfy cravings for sweets. This study references the WHO's sugar reduction guidelines, which

suggest that no more than 10% of daily caloric intake be from added sugar, and that lowering it to 5% or less be done for optimal health. In addition, Mooradian et al. (2017) describe the types of sugar substitutes, including nutritive and non-nutritive sweeteners, which are generally recognized as safe (GRAS). The non-nutritive sweeteners are regulated as food additives. The six FDA-approved non-caloric sweeteners are aspartame, acesulfame-K, neotame, saccharin, sucralose, and advantame. Mooradian et al. (2017) describe the different types of non-nutritive sweeteners in detail, including their relative sweetness to sucrose and their heat stability. Heat stability is important for a food scientist in determining the type of sweetener that can be used based on processing conditions.

Stevia and monk fruit (Luo Han Guo) are also discussed by Mooradian et al. (2017); both are considered natural because they come from natural sources (plants). High purity steviol glycosides include Rebaudioside A (Reb A), Stevioside, and Rebaudioside D and come from the stevia leaf. Some nutritive sweeteners include sugar alcohols, also called polyols, such as sorbitol, lactitol, xylitol, mannitol, erythritol, trehalose, and maltitol. Sugar alcohols' relative sweetness varies from 25-100% of the sweetness of sucrose. They have fewer calories per gram than sucrose and do not promote tooth decay, which is why they are used in many gum and breath mint products. One of the concerns with sugar alcohols that Mooradian et al. (2017) point out is that they may cause gastrointestinal discomfort when used in high quantities.

Mooradian et al. (2017) reviewed studies that evaluate artificial sweeteners (artificial sweeteners are non-nutritive). Per the review, several existing studies saw a correlation between artificial sweeteners and weight gain; the users of artificial sweeteners were more likely than non-users to gain weight by 0.5-1.5 lbs. According to Mooradian et al. (2017), "The weight changes were not explicable by differences in food consumption patterns" (p. 6). There are

theories that artificial sweeteners may increase cravings of sweets. Some of these were shown to be associated with the motivation to eat. Eating more, regardless of sugar content, could cause weight gain.

The authors suggest future work with long term trials to determine clinical benefits of reducing added sugars. Limiting consumption of any sweeteners may be the best advice for health, according to (Mooradian et al., 2017).

With consumers' increasing concern about sugar consumption, sugar-free products are becoming more popular. According to Aidoo et al. (2013), "A food product can assume a 'light' or 'sugar-free' claim if it provides less than 40 calories per serving or provides less than 0.5 g of sugars per serving, respectively" (p. 84). They stated that alternatives to sucrose do not have the same physical attributes as sucrose. Some sweeteners that provide bulk in chocolate are polyols. In addition, trehalose, tagatose, and isomaltulose function much like polyols; however, they are considered sugars.

Aidoo et al. (2013) provided an overview of various sweeteners, both nutritive and non-nutritive, that can be used in chocolate. Like Mooradian et al. (2017), Aidoo et al. (2013) discussed individual sweeteners; however, they looked at them from a food science point of view rather than a medical perspective. Aidoo et al. (2013) specifically described the types of sweeteners and their characteristics. They also discussed the functionality of sucrose in chocolate. They concluded that sugar is not just a sweetener, but it also provides function to chocolate. Other studies reviewed by Aidoo et al. (2013) concurred.

2: Formulating products by reducing sugar

Aidoo et al. (2013) stated that the development of sugar-free chocolate has proved challenging since all sugar needs to be replaced. Sugar provides bulk to a product and that needs

to be replaced with a bulking agent such as fiber or maltodextrin. Fibers, such as inulin, are important in the reduction of sugar. Sweeteners that Aidoo et al. (2013) discussed included rare sugars, polyols, and high-potency sweeteners. Additionally, they looked at low digestible carbohydrates, including fibers and maltodextrin.

Yeung et al. (2017) explored creating model formulas to reduce sugar because of the 2015 WHO guidelines, stating that, "Food reformulation has been suggested to be a potentially useful option to reduce the population added sugars intake, as it allows minor yet positive changes to be made to diets without consumers making major changes to their dietary patterns" (Yeung et al., 2017, p. 1). However, they stated, reducing sugar in processed foods can be challenging. Sugar is not just a sweetener; it also provides color, bulk, texture, flavor, and preservative qualities.

Yeung et al. (2017) looked at formulation strategies for replacing sugar through four models, including: simple removal of sugar with no replacement/replacement with non-nutritive sweeteners only; replacement with polyols and non-nutritive sweeteners; replacement with 50% fiber and non-nutritive sweeteners; and finally, replacement with 50% maltodextrin and non-nutritive sweeteners. They conducted tests examining changes in intake of energy, total sugar, added sugar, total fat, saturated fat, and fiber by evaluating Australian children and adolescents. One study looked at a 25% reformulation of sugar, which made the greatest change in total sugar intake in the population studied. Their study illustrated that the population's diet could be improved. The strategies they utilized were also seen in studies that looked at chocolate.

In theory, it is possible to reformulate, but difficulties and effectiveness need to be considered. When reformulating with sugar reduction, loss of sweetness and functionality need to be replaced to produce a food for consumer acceptability. Some functionality of sugar during

processing includes bulking and humectancy, so those factors would need to be considered in any product reformulation.

The strength of the Yeung et al. (2017) study was that it provided different strategies based on existing literature and industry practices. A limitation to the study is that it is theoretical. They make assumptions that consumers will consume the same amount of reformulated product and they did no sensory work. Yeung et al. (2017) suggests further work needs be performed. Each food has different properties and requires specific reformulation. Also, reformulation needs to be evaluated and analyzed for cost effectiveness.

Low-digestible carbohydrate polymers such as fibers can act as bulking agents in sugar-free chocolate. They provide viscosity and body because they have a molecular weight (Aidoo et al., 2013). Some examples include polydextrose, inulin, oligofructose, and maltodextrin. Polydextrose is hygroscopic and can control water activity. It provides bulk, texture, and a mouthfeel similar to sucrose. It can be combined with high-potency sweeteners in sugar-free chocolate. Polydextrose can lead to gastrointestinal issues if over-consumed. Inulin and oligofructose are fructans. They do not have off-flavors. They also do not add to viscosity to the product. Oligofructose poses similar functional qualities as sugar (Aidoo et al., 2013). Inulin has prebiotic properties. If over-consumed, oligofructose and inulin can also cause gastrointestinal issues. Some maltodextrins have a dextrose equivalence (DE) of less than 20. It is very soluble, but not hygroscopic, and is well-tolerated (Aidoo et al., 2013).

High-potency sweeteners are many times sweeter than sucrose, so they are used in small quantities. They are often referred to as non-nutritive sweeteners because they do not provide any calories. They can be either natural, such as stevia, or artificial, such as sucralose or acesulfame K. A challenge in using high-potency sweeteners is that they do not provide the

bulking properties that sucrose provides. Therefore, they require an ingredient that has bulking properties. Using a high-potency sweetener with a bulking agent is one of the strategies for reducing added sugar that Yeung et al. (2017) suggested.

3: Studies on polyols

Sugar-free chocolate has been around for years to allow those with diabetes to enjoy chocolate. Historically, sugar-free chocolate reduces sugar by using sugar alcohols; however, there are some challenges with intestinal distress with sugar alcohols (polyols). Sugar alcohols can act as a bulk sweetener, providing bulk to the product, but they vary in sweetness and physical characteristics. Also, some sugar alcohols are poor in mimicking the physical attributes of sucrose, such as body, mouthfeel, and texture (Aidoo et al., 2013). Maltitol is widely used in sugar-free chocolate because of the sweetness and because its technological properties are very close to sucrose (Aidoo et al., 2013; Coelho & de Jesus, 2016). Erythritol and xylitol have a cooling effect, which does not make for a good tasting chocolate. Isomalt is only 40% as sweet as sucrose, so it requires a high-potency sweetener in addition to it. However, there are regulatory limits to the maximum amount of polyols added to sugar-free chocolate. According to Coelho and de Jesus (2016), "Sugar alcohols […] are sugar derivatives, usually obtained by the reduction of aldehyde or ketone groups (of saccharides) to hydroxyl groups" (p. 2986). Because they are absorbed by the intestine, they have lower calories than monosaccharides (Coelho & de Jesus, 2016).

Coelho and de Jesus (2016) conducted a study using capillary electrophoresis to determine polyols in sugar-free chocolate. Previously, this method has been used for pharmaceutical formulas. Coelho and de Jesus (2016) stated that while polyols are increasingly being seen in products such as chocolate, analytical methods for testing them are not as

prominent. Because of the complexity of chocolate, they stated that polyols need to be extracted with water prior to capillary electrophoresis separation (Coelho & de Jesus, 2016).

If one develops a sugar-free chocolate, using capillary electrophoresis is important to test the level of polyols. Additionally, a developer can use capillary electrophoresis to evaluate competitive samples to see the amount of the polyols in the chocolate.

4: Articles on D-allulose and other rare sugars

Of the literature reviewed, alternative sweeteners to sucrose were included in order to better understand the sweeteners and their physical properties. As part of the review, the sweetener profiles were also studied. One of the sweeteners that was mentioned in the studies was D-allulose (D-ribo-2-hexulose or D-psicose). It is a new low-calorie functional rare sugar. The International Society of Rare Sugars states specifically that rare sugars are monosaccharides (and their derivatives) that exist in nature (Zhang et al., 2017). Only seven rare sugars are known to be common and abundantly occurring in nature: D-glucose, D-fructose, D-galactose, D-mannose, D-ribose, D-xylose, and L-arabinose (Zhang et al., 2017).

According to Zhang et al. (2016), D-allulose is an ultra-low-calorie sweetener and an ideal substitute for sucrose with 70% of the sweetness. It has distinct physiological functions as well as physical functions such as increased gelling, an increase in pleasantness of flavor, and reduced oxidation through Maillard reaction (Zhang et al., 2016). In 2014, it received GRAS status for food ingredients and dietary supplements.

The absorption rate of D-allulose is lower than other sweeteners, especially D-glucose. According to Zhang et al. (2016), "The suppressive uptake of D-glucose and D-fructose by D-allulose contributed to health benefits" (p. 128). One of the health benefits is insulin resistance, which could have some anti-diabetic effects. It can also demonstrate anti-obesity activity by

reducing adipose tissue weight in animals and humans. With its physiochemical properties, it has promising market potential in the food industry. Zhang et al. (2017) fully described the biotechnological production of D-allulose, including the evaluation of optimal temperature, pH, and metal ions.

Zhang et al.'s (2017) article on the enzymatic approaches to rare sugar production described enzymatic techniques to synthesize rare sugars such as D-allulose. Production of rare sugars such as D-allulose, D-tagatose, xylitol, mannitol, and erythritol can be done through biological methods. As Mooradian et al. (2017) mentions, naturally-occurring rare sugars have recently emerged as an alternative category of sweeteners.

Per Aidoo et al. (2013), D-tagatose is a rare sugar (and monosaccharide) with 92% of the relative sweetness of sucrose. According to Mooradian et al. (2017), D-tagatose is "structurally similar to D-fructose and has good palatability, good bulk properties, and [is] low in calories" (p. 6). Because it provides bulk, it can be a replacement for sucrose. It was GRAS-approved in 2001 and is a reducing sugar. Trehalose is a disaccharide formed by two α-glucose units. It has 50% of the sweetness of sucrose, so it would need to be used in combination with a bulk sweetener. It has the advantage of being chemically stable and received GRAS status in 2000 by the United States FDA; it was granted regulatory approval as a new food or food ingredient in Europe in 2001. Isomaltulose is a disaccharide that is 50% as sweet as sucrose and contains no aftertaste. It can be found naturally in honey and sugar cane extract (Aidoo et al., 2013). The physical properties of Isomaltulose are similar to sucrose. It has a low glycemic index.

5: Studies on Stevia

Another sweetener that studies have evaluated is stevia (*Stevia rebaudiana*). With the increase of reduced sugar products, Azevedo et al. (2016) studied bittersweet chocolate with

added insulin and stevia at different Rebaudioside (Reb A) contents. Reb A is considered to have superior sweetness quality and flavor (Azevedo et al., 2016). Azevedo et al. (2016) formulated chocolate with different sucrose concentrations to determine the ideal sucrose content using just-about-right scores so they could compare those samples to bittersweet chocolate with different levels of Reb A added.

Stevia sweeteners contain steviol glycosides such as steviosides and rebaudiosides (A, B, C, D or E), which are derived from *Stevia rebaudiana* Bertoni (Azevedo et al., 2016). They are 200-300 times sweeter than sucrose. Because of this, sensory studies are important to evaluate the sweetener to obtain desired sweetness. Fat content can also alter sweetness. When using a high intensity sweetener, viscosity and thickening properties are reduced. In the samples they created, Azevedo et al. added inulin (in addition to stevia) to their sugar-free chocolate to improve the texture and they also used it as a fat substitute because it provides mouthfeel and mouth-coating.

Time intensity analysis is a tool used to compare sweetness perception with time (Azevedo et al., 2016). It includes Imax (the maximum intensity recorded), TImax (the time at which the maximum intensity was recorded), Area (the area of time x the intensity curve), and Ttot (the total duration time at the stimulus) (Azevedo et al., 2016).

Azevedo et al.'s (2016) study established the ideal sucrose concentration and the equivalent concentration of stevia at different Reb A levels. First, they determined the ideal sucrose concentration to bittersweet chocolate. Next, they determined the equivalent concentration of sweetness. Then, time intensity curves of the sweeteners at different concentrations were compared to sucrose. The time was measured in seconds and the sweetness intensity was measured by a nine-point hedonic just-about-right scale (Azevedo et al., 2016).

By determining the ideal sweetness, they could discover the amount of sucrose to add to bittersweet chocolate. They noted that the sweetness intensity was the same for 97% and 60% Reb A due to synergies with polyols. The reduction of fat did not alter the perception of sweetness. According to Azevedo et al. (2016), "The bitterness in the bittersweet chocolate could have made the perception of sweet taste of the product by the tasters difficult" (p. S3012). Throughout the study of different stevia levels, they evaluated the following parameters: Tmax, Imax, TImax, Ttot, and area. They concluded that there was no significant difference in the concentrations tested at the respective Reb A values. They also concluded that the ideal sucrose concentration was 47% w/w. When formulating, one can optimize for sweetness using that percentage. Future work they recommend includes additional studies to determine product acceptability and bitterness intensity (Azevedo et al., 2016).

As Azevedo et al. (2016) studied time intensity of sweetness of stevia, Aidoo et al. (2013) described time intensity as a perception of sucrose compared to other sweeteners; they mentioned a study in which it was found that sucralose was best compared to sucrose. Aidoo et al. (2013) also noted that *Stevia rebaudiana* saw a decrease in sweetness with an increase in concentration.

Another evaluation of stevia and chocolate is Torri et al.'s (2017) study on steviosides and stevia "green" extract-sweetened chocolate. They looked at the feasibility of producing high quality chocolate sweetened with crude (or "green") extracts of stevia. Stevioside is the most abundant of the steviol glycosides. The challenge is that it has a bitter aftertaste. Steviol glycoside became the first high-potency sweetener of natural origin when it was authorized for use in 2008 in the United States and 2011 for the European Union (Torri et al., 2017). Torri et al. (2017) also state that "steviol glycosides present other bioactivities in humans, including

antihyperglycaemic, antihypertensive, and anticancer activity" (p. 2347). They also contain phenolic compounds and other antioxidants.

Crude stevia extracts have not been approved for use as a food or additive in the United States or European Union. Studies have been conducted using microwave extraction to extract dry plant material. They also compared sensory and antioxidant content using the green extract. There were seven samples created at different proportions of the extract. In the study, green extraction of *Stevia rebaudiana* Bertoni was performed using microwave extraction. They found that the extract is important. Green extract is an advantage because it makes the production faster, cheaper, and more sustainable (Torri et al., 2017).

Torri et al. (2017) determined the relative sweetness of samples by comparing them to 3% w/v sucrose. The sweetness was compared using an untrained panel and compared to 0.5% w/v crude stevia extract or 0.2% w/v commercial stevioside. They used an equation to determine the relative sweetness compared to sucrose. Crude stevia extract was 50. Commercial stevioside was 220.

From there, Torri et al. (2017) used a 70% dark chocolate formula and made seven different isosweet formulas. Sensory analysis was conducted using a nine-point hedonic scale for appearance, aroma, taste, flavor, texture, and overall liking. They also statistically evaluated flavanol concentration using t-tests. There was no significant difference between the samples for flavanol content. There was an increase in ORAC value, which suggests stevia extracts and steviosides contribute to antioxidant capacity (Torri et al., 2017).

Based on the sensory results, Torri et al. (2017) found that consumers based their preferences more on pleasantness than healthiness; the control with the sucrose formula had the highest overall consumer liking. That agrees with other studies that evaluated stevia-sweetened

chocolate compared to sucrose. The sample with a 50% substitution of sucrose by the steviol glycosides or green extracts had a liking of higher than five on the nine-point scale. Samples with 100% commercial stevioside or extract did not meet consumer acceptability. Two clusters of consumers were formed. After conducting cluster analysis, they found there was a significant difference between the two segments. The difference between the clusters was the samples with 50% sucrose substitution. The study showed higher discrimination in cluster 2 than cluster 1.

6: Studies on sweeteners and their effects on chocolate

6.1: Studies on chocolate and its interaction with flavanols and sugar

Mellor et al. (2018) reviewed how sugar and cocoa work together with flavanols. Sugar plays a key role in chocolate and cocoa products' flavor. Cocoa flavanols, such as epicatechins, catechins, and polyphenol's bioactivity and bioavailability, have been studied. They have been shown to improve health in ways that include reducing blood pressure and lowering the risk of chronic disease through nitric oxide. Mellor et al. (2018) did mention that sugar content could negate the impact of cocoa flavanols.

Sugar helps to mask the bitterness that is associated with flavanols. Mellor et al. (2018) wanted to understand the optimal formulation with sugar content and sweetness in respect to flavanols to maximize the bioavailability and bioactivity of the flavanols. When reformulating to reduce sugar in chocolate, the flavanols need to be considered.

According to Yuan et al. (2007), while the increase of chocolate consumption in moderation does have benefits, it is important to evaluate chocolate's association with the risk of coronary heart disease, stroke, and diabetes. Diet is a key step in preventing cardiometabolic diseases. Since chocolate has gained attention for its potential benefits with flavanols, Yuan et al. (2007) conducted a meta-analysis of studies to look at the link between cardiovascular

disease, stroke, and diabetes with chocolate consumption. They found 829 publications and included 14 of them in their analysis. From their findings, six studies reported risk of cardiovascular disease with chocolate consumption.

According to the meta-analysis, Yuan et al. (2007) found that the highest consumption of chocolate was associated with decreased risk of cardiovascular disease, stroke, and diabetes (there was a non-linear association). The flavanols in chocolate, including epicatechins, catechins, and procyanidins, are the reason for chocolate having a cardiometabolic benefit.

Both Mellor et al. (2018) and Yuan et al. (2007) illustrated the importance of cocoa flavanols in dark chocolate. With the addition of the consumption of chocolate in moderation to help prevent diseases, looking at reducing added sugar in chocolate is important. Chocolate has the potential to be considered a "health" food.

6.2: Studies on physical properties of chocolate

Three articles on the physical properties of chocolate that were reviewed for this report were by Saputro et al. (2016; 2017a; 2017b). All three articles examined replacing sucrose in chocolate with palm sugar. Saputro wanted to evaluate it compared to sucrose because palm sugar has different quality attributes in chocolate (Saputro et al., 2017a). Saputro et al. (2016) also wanted to evaluate palm sugar because it is a natural alternative to sucrose; the saccharides in palm sugar are glucose, fructose, and sucrose. They stated that palm sugar has a low glycemic index; "It contains proteins, reducing sugars, and relatively large amounts of moisture" (Saputro et al., 2016, p. 956). Because of the high moisture, glucose, vitamins, and minerals in palm sugar, they wanted to evaluate how they affect the physical attributes of chocolate.

One of Saputro et al.'s studies evaluated the feasibility of small scale production of palm sugar-sweetened chocolate. It also looked at the influence of palm sugar on particle size, rheology, and the aroma profile of dark chocolate. Another article focused on rheology, microstructural, and textural properties of palm sugar-sweetened chocolate. The third article discussed quality attributes of palm sugar-sweetened dark chocolate.

In Saputro et al.'s (2016) article, they discussed the conventional production of chocolate, which includes mixing, roll-refining, and conching. They found another study that looked at alternative methods of chocolate production. An alternative method includes using a ball mill, which mixes and refines the chocolate. This method also uses a Stephan mixer with a vacuum attachment that prevents moisture and acidity reduction and produces promising rheological properties.

Saputro et al. (2016) compared palm sugar and sucrose using the alternative process listed above and the conventional process. After processing, they took samples for particle size distribution, rheology, and moisture content. They tempered the chocolate and tested the bars for melting and aroma profile. They tested moisture of the sweeteners and the chocolate with the Karl Fischer method; they tested particle size distribution using a Malvern mastersizer and looked at the distribution at the 90%, 50%, and 10% percentiles. In addition, they used the differential scanning calorimeter (DSC) for both the chocolate and sweetener samples. They used the AR2000 rheometer (with the International Confectionary Association Method 46) to evaluate Casson yield, Casson viscosity, and thixotropy. Finally, they used HS-SPME-GC-MS to identify volatile compounds (Saputro et al., 2016).

Based on their test results, Saputro et al. (2016) found the higher particle size of palm sugar led to higher viscosity and higher particle volume fraction. They found palm sugar had a

higher degree of agglomeration. Additionally, the alternate process had a higher degree of agglomeration than the conventional process. The chocolate sample that contained palm sugar made using the alternate process had the highest degree of agglomeration. They stated that the glucose and fructose in palm sugar are hygroscopic, leading it to have a higher initial moisture content; per Saputro et al. (2016), "conventional processing removes moisture more effectively, creat[ing] chocolates with a lower particle agglomeration degree" (p. 959).

All samples (palm, sucrose, conventional, and alternate process) had a similar peak melting temperature of the fat. With regard to sugar melting, palm sugar had a lower melting peak temperature than those formulated with sucrose. Also, the alternative processing method resulted in less moisture evaporation than conventional processing. Saputro et al. (2016) stated that glucose, fructose, sucrose, and high moisture might develop amorphous sugar during refining, which would lead to a reduction of endothermic enthalpy of the sugar phrase.

Saputro et al. (2016) obtained their rheology by fitting the results in a Casson model for viscosity and yield stress. They found that the rheology was dependent upon the sweetness and the process. The particle size distribution, moisture, and final fat content affects chocolate flow behavior. They found that the Casson viscosity was largely influenced by moisture and particle density. Saputro et al. (2016) stated that chocolate with a high moisture content requires more fat to coat, which reduces "free" fat and causes high viscosity. Thixotropy is an indicator of the degree of particle agglomerates in suspension. If a chocolate is well-conched, it should not be thixotropic according to them.

Another attribute that was observed was the aroma profile. They found that palm sugar impacts the aroma regardless of the processing method. They also found that there were some volatiles present in chocolate that were found in alternate processing and not conventional

processing. In addition, they found that the chocolate with palm sugar created with the alternate process showed significantly higher ($p < 0.05$) aroma volatiles than did chocolate with sucrose created using the alternate process (Saputro et al., 2016).

Saputro et al. (2016) concluded that palm sugar chocolate had a higher viscosity due to higher agglomeration, higher moisture level, and lower particle density. They thought this could provide a more cost-effective way of producing chocolate in small chocolate industries. They recommended future work to improve the alternate process to remove moisture and acid volatiles to produce a high-quality palm sugar dark chocolate (Saputro et al., 2016).

In a subsequent study, Saputro et al. (2017a) examined palm-sap sugars in dark chocolate to look at color, hardness, melting profile, and flow behavior. Palm-sap sugars include coconut sugar and palm sugar. They have a similar sweetness to sucrose and contain fructose, glucose, and sucrose.

Saputro et al. (2017a) developed different kinds of coarse coconut and coarse palm sugars. As they had done in their 2016 study, they used the alternate process of a Stephan mixer and ball mill to produce the chocolate, and then they tempered and molded the chocolate into bars. They used the same analytical methods as in their 2016 study for moisture, particle size distribution, melting profile, rheology, and aroma profile. They also tested the chocolate for color with a colorimeter, evaluating the L* a* and b* parameters; they tested the chocolate hardness using an Instron texture analyzer. One thing they found was that "'[f]ree' moisture affects the flow behaviour of chocolate to a large extent by promoting particle agglomeration" (Saputro et al., 2017a, p. 180). By "creat[ing] sticky patches on the surface of the sugar particles, resulting in the sugar particles sticking together and an increase in flow parameter

values" (Saputro et al., 2017a, pp. 180-181). The moisture of both palm sap sugars was significantly higher than sucrose (p <0.05).

Processing affects not only the composition and roughness of the chocolate's surface, but also the color. A rougher surface on the chocolate will scatter light differently and have a low L* value, causing a lighter colored chocolate. With a lower particle density sugar, the chocolate will also be lighter in color because it is more dense and better able to scatter light. Finer particle size chocolate also appears lighter. Saputro et al. (2017a) found that palm-sap sugar was lighter than sucrose due to lower particle density of the sugars.

According to Saputro et al. (2017a), hardness of chocolate is affected by fat content, particle volume fraction, particle size distribution, and tempering. They found sucrose chocolate hardness was significantly lower (p <0.05) than palm-sap sugar chocolate. They believed it was due to particle density, particle size distribution, moisture content, and sugar composition. The increase in particle fractions increased particle-to-particle interaction. Impurities such as proteins, minerals, and reducing sugars can also complicate the particle-to-particle interaction (Saputro et al., 2017a).

The melting profiles, per Saputro et al. (2017a), were similar to their 2016 study's results. The palm sugar melting was at a slightly lower onset temperature compared to the coconut sugar- and sucrose-sweetened chocolate (Saputro et al., 2017a). The reduction of the melting point can be due to the impurities in the palm-sap sugars.

Saputro et al. (2017a) also agreed with their 2016 study that higher agglomeration caused higher moisture and a higher reduction in particle size. In chocolate, the palm-sap sugars had more agglomeration than sucrose. Sugars with a higher specific surface area (because of a smaller particle size) have a higher yield. Agglomeration also indicated lower reducing sugars

and moisture content in the chocolate. The Casson viscosity was influenced by the particle density and moisture content; the sample that had the lowest moisture content and highest particle density had the lowest Casson viscosity. Thixotropy was higher in the palm-sap sugar chocolate versus sucrose-sweetened chocolate, which is supported by the level of agglomeration. It also matches the results in Saputro et al.'s 2016 study.

Saputro et al. (2017a) found that sucrose does not influence the aroma of chocolate. However, palm-sap sugars do affect the aroma profiles. A Maillard reaction from the palm-sap sugars and the heat from chocolate production affects the aroma profile in the chocolate. They concluded that the aroma profile is different in palm-sap sugar-sweetened chocolate than in sucrose-sweetened chocolate.

They concluded that the high moisture content results affect color, hardness, and viscosity of chocolate. The color was lighter in the palm-sap sweetened chocolate. It was also harder and had a higher viscosity. They suggested further improvements in processing techniques of palm-sap sweetened chocolate (Saputro et al., 2017a).

Saputro et al. studied palm sugar in another study in 2017(b). Saputro et al. (2017b) studied the rheology of palm sugar blends compared to sucrose-sweetened chocolate. Their results agree with their previous studies; the viscosity and thixotropy in chocolate was a result of the moisture content, glucose, and fructose. The palm sugar also influenced the hardness of the chocolate.

As they had previously, they employed the alternate chocolate making process of using the Stephan mixer and ball mill, and then they tempered and molded the bars for analysis. They used the same analytical methods as in their other two studies for rheology, moisture content, particle size distribution, hardness, and particle size distribution. Additionally, they studied the

microstructure using a microscope to observe sugar crystals and agglomerates. They also looked at the surface morphology of the sugar and chocolate using a scanning electron microscope (Saputro et al., 2017b).

They found that the yield declined as the palm sugar increased (Saputro et al., 2017b). They did not see a trend exhibited in the Casson viscosity of palm sugar (Casson viscosity is the stress needed to maintain the chocolate flow during shearing). They found the flow behavior and hardness was influenced by combination of moisture, sugar composition, and particle size distribution. Hardness increased as the palm sugar increased up to 50% and leveled off as the palm sugar amount increased over 50%.

Saputro et al., (2017b) found the yield stress of 100% palm sugar was significantly lower ($p < 0.05$) than the other samples. They believe that is due to the larger particle size of palm sugar. They also found that the 100% palm sugar product had the highest agglomeration, which agrees with their other studies. The sucrose-sweetened chocolate had no agglomeration. Finally, the 100% palm sugar chocolate had the highest Casson viscosity because the 100% palm sugar product had the lowest surface area and highest moisture (Saputro et al., 2017b). The thixotropy, due to the agglomerates, agreed with their previous studies discussed above.

The melting profile of palm sugar chocolate also agrees with their previous studies. According to Saputro et al. (2017a; 2017b), the impurities in palm sugar lowered the melting point and enthalpy of the sugar phase in the chocolate. The hardness was influenced by the proportion of palm sugar. Chocolate with a smaller particle size should result in higher hardness, thus, the 0% palm sugar sample should have been the hardest. However, the 0% palm sugar sample had the lowest hardness; the moisture content effected the hardness more than the particle size (Saputro et al., 2017b).

Saputro et al. (2017b) found that all the chocolates had similar surface morphology due to the fat content in chocolate. The agglomerates could be seen using light microscopy. Polarized light microscopy was used to look at the sugar crystals and particle-to-particle interactions. Amorphous sugar was observed because of the glucose and fructose. More amorphous sugar was formed, creating a stronger sugar network (Saputro et al., 2017b).

They concluded that the agglomeration in palm sugar has an effect on the physical properties of chocolate. This agrees with their other studies on sugar-sweetened dark chocolate. The amorphous parts of palm sugar-sweetened chocolate were observed by microstructure to "visualize the impact of moisture and/or amorphous sugar as well as chemical 'impurities' on the sugar and chocolate morphology, the agglomerates formation and the sugar network of molten chocolates" (Saputro et al., 2017b, p. 1737).

As previously discussed, sugar is not just a sweetener in chocolate, it also provides functional properties in chocolate and impacts such things as rheology. In Belščak-Cvitanović et al.'s (2014) study, they evaluated chocolates made with sucrose alternatives for their physical and sensory properties. They studied particle size distribution and texture; additionally, they evaluated the samples for antioxidant capacity. The samples they used had a lower than 20% caloric value compared to control chocolate.

In the introduction of the study, Belščak-Cvitanović et al. (2014) discussed the different types of sweeteners such as polyols and high-potency sweeteners and the impact they have on products (their statements agree with what Aidoo et al. (2013) stated about polyols and high-potency sweeteners). They wanted to perform the study because they wanted to evaluate natural sweeteners' effects on the physical parameters of chocolate. Previously, much work had been done on high intensity sweeteners and polyols-sweetened chocolate. They discussed natural

alternatives including agave, honey, brown rice syrup, and fruit or vegetable sugars; per their research, the natural alternatives tend to have a low glycemic index.

Belščak-Cvitanović et al. (2014) studied sucrose-free semisweet chocolate (50% cacao) samples that used naturally-derived sweeteners. There were a number of sweeteners, including polyols, fibers, syrups and natural sweeteners. Their control was chocolate made with sucrose.

After processing the chocolate with a refiner and conche, it was tempered and molded prior to analyzing the physical properties of the chocolate. This included testing particle size with a mastersizer, which was the same method as Saputro et al. (2016; 2017a; 2017b). They used a TA.HDPlus texture analyzer to measure the maximum force needed to penetrate the chocolate. The type of sugar in the ingredients and chocolate was determined by HPLC. Polyphenol and antioxidant capacity were also measured in the sweeteners and chocolates (Belščak-Cvitanović et al., 2014).

Sensory evaluation of the chocolate was conducted using quantitative descriptive analysis according to ISO (International Standards Organization) standards. The panelists underwent extensive sensory training. They evaluated the chocolate for the following attributes: color, gloss, surface breakage, structure, melting, odor, taste, mouthfeel, aftertaste, sweetness, astringency, bitterness, herbal, and overall acceptability (Belščak-Cvitanović et al., 2014).

Belščak-Cvitanović et al. (2014) found that the median particle size (d(0.05)) was directly related to the hardness of the chocolate samples. In addition, they found that all the variants were harder and had higher elasticity then the control; the variants also had a larger particle size than the control. They found no direct relationship between hardness, particle size distribution, and elasticity. When they compared the samples, they found 25% less sugar in the variants

compared to the control. Based on regulatory claims, the samples could be called "reduced sugar" or a "less sugar" product.

According to the sensory results in the study, Belščak-Cvitanović et al. (2014) found that the control scored the highest in textural properties. They found that the sample that contained fructose, isomalt, stevia leaves, oligofructose, lucuma, agave syrup, and peppermint scored the highest for overall acceptability for flavor. The sample that contained fructose, maltitol, stevia leaves, yacon, and rice syrup scored the lowest in overall acceptability. The highest acceptability sample was closest to the control for the attributes of mouthfeel and aftertaste; however, the control scored the highest in mouthfeel and aftertaste. The samples with the highest acceptability also had the highest sweetness intensity. The stevia samples had the highest bitterness and astringency attributes. Belščak-Cvitanović et al. (2014) also found that these samples had a different perceived sweetness due to the other ingredients in the formula.

They concluded that the samples with stevia and peppermint were closest sensorially to the control. They also noted that the hardness increased and the elasticity decreased in the variants compared to the control. The hardest samples had a large particle size (Belščak-Cvitanović et al., 2014).

Similarly, Aidoo et al. (2014) evaluated the physical properties of sugar-free dark chocolate that contained polydextrose, inulin, stevia, and thaumatin (an intensely sweet-tasting protein). Just as Saputro et al. (2016; 2017a; 2017b) evaluated their samples, Aidoo et al. tested their samples for rheology, hardness, color, and melting. They used similar analytical methods to Saputro et al. (2016; 2017a; 2017b) and Belščak-Cvitanović et al. (2014). Aidoo et al. (2014) found the Casson viscosity was significantly higher in the alternative sugar samples ($p < 0.05$) than in the control. Also, the Casson yield was significantly ($p < 0.05$) greater than the control.

The moisture content of the sugar-free chocolate was within the limits of the moisture control of standard chocolate; they found the control was harder. Based on a* and b* color values, sweeteners also effected the color. From the melting properties, the enthalpy of melt was higher for the control (Aidoo et al., 2014). They suggested future work to include sensory evaluation. Understanding the oral melting properties in chocolate is important to developing alternative sweeteners in chocolate, from a standpoint of the "components of flavor release and also epithelial sensation" (Aidoo et al., 2014, p. 596).

The five articles that were previously mentioned (Aidoo et al., 2014; Belščak-Cvitanović et al., 2014; Saputro et al., 2016; 2017a; 2017b) were similar because they studied the physical properties of alternative sweeteners in chocolate. The five articles used the same methods for testing the rheology, particle size distribution, hardness, color, and melting profile of chocolate.

Aidoo et al. (2013) went into great detail on how alternative sweeteners affect processing of chocolate. One of the effects on processing is that polyols need to be conched at different temperatures depending on the type used. Aidoo et al. (2013) discussed rheology of chocolate and how different sweeteners affect chocolate. They also discussed how the particle size distribution of the chocolate influences rheology. This concurs with Saputro et al. (2016; 2017a; 2017b) and Belščak-Cvitanović et al. (2015). Aidoo et al. (2013) evaluated isomalt, maltitol, and xylitol samples at different particle intervals. The isomalt chocolate had the highest plastic viscosity; it was observed at lower particle sizes (Aidoo et al., 2013).

In a study, Aidoo et al. (2013) evaluated the replacement of sucrose with inulin, polydextrose, and maltodextrin. The hardest sample evaluated had a ratio of 50:25:25 inulin: polydextrose: maltodextrin. The melting rate of samples increased with increasing polydextrose and inulin. That correlated with mouth coating sensory acceptability overall. According to

Aidoo et al. (2013), "higher inulin and polydextrose and lower proportions of maltodextrin greatly improved sensory attributes of the milk chocolates" (p. 93).

Another study that Aidoo et al. (2013) discussed "replaced sucrose with inulin (HP, HPX and GR) with different degrees of polymerization and polydextrose as bulking agents together with the intense sweetener stevia in the development of sugar-free chocolates" (p. 93). They found that the chocolate was darker in color with the sucrose-replaced ingredients. The melting point temperature was higher in the control and in one of the inulin types. One reason they believe the increase occurred was due to the effect of inulin and its average degree of polymerization (Aidoo et al., 2013). The study also evaluated hardness, viscosity, and flow behavior. They observed that the "Replacement of sucrose with stevia as a sweetening agent and inulin and polydextrose as bulking agents had no major impact on elastic behavior of chocolate mixes during the initial stages of tempering" (Aidoo et al., 2013, p. 93). Sensorially, inulin had significantly lower smoothness acceptability and mouthfeel (Aidoo et al., 2013). They also stated that flavor acceptability decreased with a decrease in inulin. Inulin does not affect particle size, melting point, and composition in sucrose-free chocolate.

The studies in the literature review included reduced sugar or sugar-free chocolates that used polyols, rare sugar, inulin, and high potency sweeteners. In addition, the review also looked at modeling products to reduce added sugars. Finally, the review examined studies that looked at alternative sweeteners in chocolate and their effect on physical properties of the chocolate.

Chapter 3 - Experiment on the physical properties of dark chocolate using alternative sweeteners

Based on the literature review, a study was conducted to evaluate how sweeteners affect the physical attributes of dark chocolate to determine the best sweetener to use to reduce sugar. The alternative sweeteners used in the study were fructose and agave and the reduction of sucrose was 30% from control.

Fructose and agave were the two sweeteners evaluated in the study (compared to sucrose) for many reasons. According to Beckett (2008), fructose has a low glycemic index. Fructose's relative sweetness compared to sucrose is 1.1, so one can formulate a product with less fructose to get the same sweetness impact as sucrose. Because fructose is hygroscopic, it requires special processing conditions for temperature and humidity (Beckett 2008). According to White (2014), fructose is better at moisture binding and controlling water activity than glucose and sucrose because of its hygroscopicity and humectancy.

Agave was evaluated because it can be considered a humectant, which would help with the water activity in chocolate. The relative sweetness of agave is 1.4 times as sweet as sucrose. One can use less agave to get the same sweetness as sucrose. Per the Innovadatabase.com, in 2017 there were 135 new products that launched using agave.

According to the U.S. Food & Drug Administration in 21 CFR 163, the standard of identity for chocolate is the "solid or semiplastic food prepared by intimately mixing and grinding chocolate liquor with one or more optional nutritive carbohydrate sweeteners" (Sec. 163.123 (a)). In this study, it was decided to maintain the standard of identity for chocolate; therefore, sweeteners were chosen that were nutritive carbohydrates. D-allulose or stevia were not used in this study because they are not considered to be nutritive carbohydrate sweeteners. If

D-allulose was used, it could be considered to be a modified standard of identity for chocolate according to 21 CFR 130.10. To be have a modified standard of identity for chocolate, the manufacturer is required to label for the nutrient claim. For example, it would need to state, "reduced sugar milk chocolate." The characteristics of the modified chocolate need to behave similarly to those of the standard of identity for chocolate. It would be the product developer's responsibility to understand their company's and brand's desire on the standard of identity for chocolate.

This study focused on the physical properties of dark chocolate specifically. Analysis was conducted similar to Saputro et al.'s three studies (2016; 2017a; 2017b), Aidoo et al.'s (2014) study, and Belščak-Cvitanović et al.'s (2015) study. The rheology, particle size distribution, moisture content, melting point, and hardness were tested.

1: Materials and Methods:

1.1 Materials

Ingredients (Table 3.1) and equipment used in this study were from The Hershey Company (Hershey, PA).

Table 3.1: Ingredients and suppliers used in study to see the physical attributes of chocolate

Ingredient	Supplier
Low roast West African Chocolate Liquor	Cargill Cocoa and Chocolate (Elizabethtown, PA)
Medium Granulated Sugar	Domino Sugar (Baltimore, MD)
Crystalline Fructose	Tate and Lyle (London, United Kingdom)
Cocoa Butter	Blommer Chocolate (East Greenville, PA)
Alkalized Cocoa Powder	Olam International Limited (Singapore)
Anhydrous Milk Fat	Grassland Dairy Products (Greenwood, WI)
Vanillin	Solvay S.A. (Neder-Over-Heembeeck, Belgium)
Powdered Organic Blue Agave	Health Gardens of New York Inc. (Spring Valley, NY).
Soy Lecithin	Cargill Texturizing Solutions (Wayzata, MN).

The lot numbers for each ingredient was identical for all treatments. The sweeteners, sucrose, fructose, and agave, were tested for moisture, particle size distribution, and saccharide profile.

Table 3.2: Sucrose-, fructose-, and agave-sweetened dark chocolate formulas for study that looks at the physical attributes of dark chocolate

Treatment	**Sucrose %**	**Fructose %**	**Agave %**
Sugar	49.2	14.2	14.2
Fructose		35.0	
Agave			35.0
Chocolate Liquor	33.0	33.0	33.0
Alkalized Cocoa Powder	3.10	3.10	3.10
Deodorized Cocoa Butter	11.1	11.1	11.1
Anhydrous Milk Fat	3.30	3.30	3.30
Lecithin	0.30	0.30	0.30
Vanillin	0.02	0.02	0.02
Total	100	100	100

1.2 Preparation and Processing Methods

Chocolate was produced based on the formulas in Table 3.2 using a three-roll refiner from Buehler (Plymouth, MN) with a target of 25 microns using a hand-held micrometer. To achieve this, product was refined using a double pass process. The first pass resulted in particles of 70 microns. The rolls were adjusted to a particle size target of 25 microns. To test the micron size using a hand-held micrometer, a 50:50 blend of the refined product and mineral oil were mixed together and a drop was placed on the micrometer. The product that was refined had a fat level of 25%. This was kept consistent for all treatments. According to Minifie (1989), “the

main purpose of a roll refiner is to grind the paste fed to it" (p. 143). It also rubs out agglomerates and wets the particulate with the cocoa butter (Minifie, 1989). Refining reduces the particle size of the chocolate to below 30 microns. It also allows for not too many small particles. Particles that are too small make the chocolate thicker (Beckett, 2008).

After the chocolate was refined, it was conched for 2 h. More anhydrous milk fat and cocoa butter were added to the refined chocolate until it had a conching fat of 28%. This was kept consistent for all treatments. Product was mixed in an 11.36 L Hobart Bowl (Hobart Corporation, Troy, OH) with a heat lamp. Throughout the 2 h, the temperature was taken. The refining and conching conditions are listed in Table 3.3.

Table 3.3: Refining and conching attributes of sucrose-, fructose-, and agave-sweetened dark chocolate treatments

Treatment Type	**Sucrose**	**Fructose**	**Agave**
Microns[1]	23	24	26
Conching Time (h)	2	2	2
Final Conching Temperature (°C)	59.4	47.5	43.7

[1]Using hand-held micrometer technique.

After the product was conched, the chocolate was standardized. Vanillin, cocoa butter, and soy lecithin were added to the conched chocolate mixture. The standardizing fat was 32.85%. This was kept consistent for all three treatments. The product was mixed without heat for 30 min using an 11.36 L Hobart mixer (Hobart Corporation, Troy, OH). Half of the lecithin was added at the beginning and then the other half was added halfway through the mixing. After 30 min of mixing, the chocolate was stored in an air-tight container until the chocolate was ready to be molded. A sample of the chocolate paste for all treatments was collected for rheology testing, particle size distribution, and moisture content.

Chocolate was tempered in a Chocovision Revolation 3210 tempering unit (Poughkeepsie, NY). The temper was set to dark chocolate on the unit. Product was placed in the temper unit and the temperature was increased to 42°C. The temperature then went down to 32°C. At 31.7°C, seed chocolate (dark chocolate) was added and mixed for five min. After five min, the temper was checked using a Chocolate Temper Meter 530 from Tricor Systems Inc. (Elgin, IL). The Chocolate Temper Units (CTU) and slope were recorded for all samples. When the temper reached a slope of +/- 2, chocolate was ready to mold. The CTU is a relative number that is an indicator of the quality of the chocolate temper. The smaller the number, the less the temper will be. The larger the number, the greater the temper will be. The temper was calculated based on the rate of cooling and the rate of crystallization. The slope is an interpretation of the amount of heat of crystallization produced during cooling of the sample. Table 3.4 shows the product temper for all three treatments.

Table 3.4: Product temper of sucrose-, fructose-, and agave-sweetened dark chocolates in study that evaluated sweeteners' effect on physical attributes of chocolate

Treatment type	**CTU***	**Slope**
Sucrose-sweetened chocolate	4.8	+1.28
Fructose-sweetened chocolate	2.7	+1.47
Agave-sweetened chocolate	3.7	+2.00

*CTU – Chocolate Temper Units

Chocolate was molded using a bar mold of 13.8 cm x 5.5 cm x 0.6 cm. There were 10 bars in a mold. The molds were placed on a vibrating table (FMC Syntron J-50 Jogger; FMC Technologies Inc., Saltillo, MS) and set to one to remove air bubbles from bars. Molds were placed in a refrigerator for 15 min. The temperature was around 3.7°C. After 15 min, the bars were de-molded and placed on a tray. The bars were wrapped in aluminum foil (Reynolds Consumer Products, Lake Forest, IL) and stored for one week at a temperature of 21°C until analytical testing took place. About 30 bars of each treatment were made. Table 3.5 shows the average weight of five bars. Figures 3.1-3.4 illustrate pictures of the three treatments.

Table 3.5: Sucrose-, fructose-, and agave-sweetened dark chocolate bar weights

Treatment Type	**Sucrose**	**Fructose**	**Agave**
Average Weight (g)	70.98	71.54	68.18

Figure 3.1: Chocolate treatment made with sucrose

Figure 3.2: Chocolate treatment made with fructose

Figure 3.3: Chocolate treatment made with agave

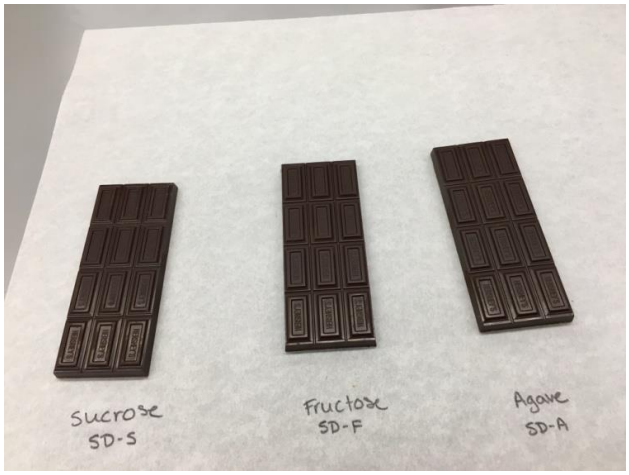


Figure 3.4: Three chocolate treatments: sucrose, fructose, and agave, which were used in study that looked at the effect of sweeteners on the physical properties of dark chocolate.

2: Analytical Methods

2.1 Fat

The fat of the chocolate was analyzed using the ANKOM XT15 Extraction System (Ankom Technology, Macedon, NY) using the AOCS official procedure Am 5-04. It determined the crude fat by extracting the sample with petroleum ether under elevated temperature and pressure.

After a labeled filter bag was zeroed on a balance, 1.5 g of melted chocolate (50°C) was added to the bag and recorded (W1). The filter bag was heat-sealed within 4 mm of the top. The sample bag was placed in a tared weigh pan during oven drying. The samples were placed in the drying oven for 3 h in a 100°C air oven. The dried sample was then cooled in a box desiccator for 20 min and weighed (W2). The sample was extracted for 60 min at pressure between 45 and 55 psi at 90°C. After extraction, the sample was placed in the 100°C oven for 30 min. The filter bag was placed in the desiccator for 20 min and then weighed (W3). Three samples per treatment were evaluated.

Calculation

$$\% \text{ Crude Fat} = \frac{100\ (W2-W3)}{W1}$$

W1: original weight of sample

W2: Weight of pre-dried sample with the filter bag

W3: Weight of dried sample and filter bag after extraction

2.2 Saccharide Profile

The saccharide profile was analyzed with High Performance Liquid Chromatography (HPLC) using refractive index detection. The sample was first defatted with petroleum ether and then the saccharides were extracted with hot water. The particulates were removed by centrifugation and filtration. The filtered extract was assayed for the saccharides, then the sample was injected in the HPLC. The sweeteners were analyzed as well as the chocolate.

The chocolate was heated at 50°C until melted. Three samples per treatment were tested. In a 2 ml HPLC vial, 1 ml of acetonitrile and 1 ml of sample extract were mixed. The sugar was extracted from the matrix. In a 50 ml volumetric flask, 25 ml of 2 V filtered extract was

transferred. Acetonitrile was added within 1 ml of the volumetric fill level. It was then mixed and sat until warmed to 25°C. The acetonitrile was added to the fill mark and mixed. The diluted sample was ultracentrifuged. The supernatant was filled through a 0.45 μm filter into a HPLC vial to ready for injection. Three samples per treatment were evaluated.

2.3 Moisture

The moisture of the chocolate treatments was derived by Karl Fischer titration method. The sample was prepared by weighing 1 g of chocolate. A ratio of 20:10:10 of Formamide: Methanol: Chloroform solvents were dispensed into a 50 ml graduated cylinder. In the vessel, the solvents were pre-titrated to bring the solvents to dryness. In a test tube, 1 g of the chocolate was added. The combined weight was recorded. A sample of the chocolate treatment was added to the reaction vessel. Titration was initiated. The empty tube was reweighed and recorded. That weight was subtracted from the combined weight to obtain the sample weight. After the titration was completed, the total mass and tare mass were entered and recorded. Three samples per treatment were evaluated.

The moisture of the sweetener types was derived by the AOAC official method 925.45 vacuum oven drying method. In a pan, 2 g of the sweetener was weighed, covered with a lid, and placed in the vacuum oven. The oven was bled by a current of inlet air passing through a desiccator filled with fresh desiccant before entering the oven to remove water vapor. After the treatment was in the oven for 2 h at <70 °C and the pressure was set to 6.7 kPa of mercury, the weight of the pan, lid, and treatment were measured. The sweetener was re-dried for 1 hr and the process was repeated until changes in weight between successive dryings at 1 hr intervals were

<2 mg. The difference in weights from before the oven and after the oven was measured and considered moisture loss. Three samples per sweetener were tested.

Calculation

Sweetener weight (g) = (wt pan + wt lid + wt sample) – (wt pan + wt lid)

Percent moisture = [(initial wt-final wt)/sample wt (g)] x 100

Initial wt = weight of pan, lid, and sample before heating (g)

Final wt = weight of pan, lid, and sample after heating (g)

2.4 Particle size distributions

The particle size distribution of the chocolate paste and the chocolate bar samples were analyzed using Sympatec HELOS Laser Diffraction (Sympatec GmbH, Clausthal-Zellerfield, Germany). Dispersed samples were passed through the path of a low power helium-neon laser. Angular deflected and diffracted light intensity were measured and calculated to result in the volume percent distribution and parameters of the particles over the range of the lens. The R4 lens (range 0.5/1.8- 350 μm) was used. Data were then analyzed with the Quixel wet dispersion unit.

Prior to starting the measurement, the wet dispersion unit was cleaning with 2-propanol. The Quixel tank was filled with the 2-propanol to the bottom of the overflow opening. The pump speed was set to 60%, and the ultrasound was turned on. After 45 s, the ultrasound was turned off and drained.

The chocolate was placed into a 50°C oven to melt. After the sample was mixed thoroughly, 0.5 g of chocolate was diluted in 10 ml of isoproponal, and it was drawn into a 10-cc syringe. The syringe was placed in a heater block. A reference measurement was taken by clicking the reference button. The program re-aligned the laser and performed a background

measurement. After the background measurement, the sample was measured. The measurement button was clicked, and the ultrasound was turned on. The sample was injected into the tank and the measurement took place, displaying the results on the computer. The results displayed on the report were D(v,0.9), D(v,0.5), D (v,0.1), volume mean, Sauter diameter, and surface area.

2.5 Rheology

Rheology of the samples were evaluated using two methods – Brookfield viscometer (Ametek Brookfield, Middleborough, MA) and Anton Paar rheometer (Anton Paar GmbH, Graz, Austria) to determine chocolate flow parameters.

For Anton Paar, two bars of each treatment were melted in a 52°C hot box. The rheometer was prepared by warming up the cup and bob. The rheometer used the International Confectionery Association (ICA) official program method. Samples were added to the cup to the line inside (approximately 20 g). Samples were pre-sheared at 5 s^{-1} at 40°C for 15 min prior to measurement. The shear stress was determined by measuring the shear rate from 2 to 50 s^{-1} (ramp up). It was held at 50 s^{-1} for 60 s and the shear rate was then decreased from 50 to 2 s^{-1}. The data was fitted to the Casson model to determine Casson viscosity and yield stress. Thixotropy was determined by the difference between the ramp up and ramp down at 5 s^{-1}. This was a similar method to Saputro et al.'s 2017 study on palm sugar.

Brookfield viscosity measures the torque needed to rotate the spindle through various rates, which are converted into shear rates. The torque was converted to shear stress. A Brookfield HA was used. The sample was heated to 50°C to melt the chocolate, and then cooled to 40°C before being placed in the chamber. In the sample chamber, 11 ml of sample were added. A number 27 spindle was placed in the sample. The sample went through five speeds (5

rpm, 10 rpm, 20 rpm, 50 rpm, and 100 rpm) and the torque was measured. The NCA viscosity program was selected to calculate the yield stress and plastic viscosity. The shear rate (1 s^{-1}) was plotted against the shear stress to calculate the Casson viscosity using the confidence of fit of the model.

A Brookfield viscometer is typically used in the chocolate industry at the manufacturing facility; however, it is used more often for liquids. The Anton Paar viscometer was used in the studies from the literature review. It is considered to be a research tool rather than a tool to use at a manufacturing facility; it is also more functional for semi-solids.

2.6 Color

The color of the chocolate was measured by an X-RITE Color i5 Spectrophotometer (Xrite Inc., Grand Rapids, MI). It uses pulsed xenon illumination to measure the color. The color is expressed in the CIELab color space where L* is lightness (100 – white and 0 – black), a* is the red/green attribute (positive value is redness and negative value is greenness), and b* value is the yellow/blue attribute (positive value is yellowness and negative value is blueness). A 20 g sample of the chocolate bar was placed upside down into a 50 x 9 mm petri dish base that was free from scratches and covered with the lid, making sure the entire bottom of the dish was covered. The sample was immediately analyzed. Prior to analysis, the bottom of the petri dish was wiped with KimWipe (Kimberly-Clark, Irving, TX). The sample was placed over the viewport. The e-Job for chocolate was selected on the computer. The measure trial icon was selected, and the chocolate was read for the L*a*b measurements. The top, middle, and bottom of the bar were measured. The 0 SCE LAV 25 mm aperture was used on the spectrophotometer.

2.7 Peak Hardness

The peak hardness of bars was evaluated using a 30 kg load cell with a TA.XT2 (Texture Technologies, Slurry, England) texture analyzer at 20°C. The chocolate bar was compressed using a 2 mm diameter blunt cylinder at 0.1 mm/s to 50% strain. The area under the curve from 1-3 s was integrated to provide work in g*mm and converted into Newtons as the maximum force during sample penetration. This method was similar to Saputro et al. (2017a).

2.8 Melting Profile

The melting profile was tested using a Discovery 2500 differential scanning calorimeter (DSC) with a refrigerated cooling system (TA Instruments, Dover, DE). Approximately 15 mg of the chocolate was sealed in hermitically-sealed cups. The samples were equilibrated at 20°C for 1 min followed by heating to 200°C at a rate of 10°C/min. This was similar to Saputro et al.'s (2017a) study. The data was interpreted using Trios (TA Instruments, Dover, DE).

2.9 Aroma Profile

The aroma profiles were determined by qualitative analysis using gas chromatography/mass spectrometry (GC-MS) (Agilent Technologies, Santa Clara, CA). The components present in the sample were separated with capillary gas chromatography and then identified with mass spectrometry. The column was an Agilent HP-5ms UI - 30 m x 0.25 mm with a film thickness of 0.25 µm.

The SPME fiber was placed in the GC injection port, which was heated to >200°C to allow the fiber to bake out for at least 5 minutes. A blank run was performed by injecting the SPME fiber to ensure that the fiber and the chromatographic system was free of contaminants.

The chocolate was frozen using liquid nitrogen and then ground. After being ground, 2 g of each treatment was placed into a 20 ml headspace vial and cap using a vial crimper.

To analyze, the head space vial was placed into a heat block that was set to 85°C for 5 min. The SPME fiber was placed into the heated GC injection port (>200°C) for 5 min. The SPME unit was removed from the heated GC injection port and inserted into the device into the vial through the septum. The fiber was exposed into the headspace of the sample. The SPME fiber in the vial was left undisturbed for 20 min. After the 20 min, the fiber was withdrawn back into the sheath, removed the vial, and inserted into the GC injection port where it was exposed into the heated GC injection port. The GC started and ran for 2 min. After the 2 min, the fiber was withdrawn into the sheath and removed from the SPME device from the GC injection port. The chromatograms were overlaid onto the instrument software to look for differences. The mass spectral differences between the samples were evaluated using library matching.

2.10 Statistics

Statistical analysis was performed using Microsoft Excel 2007 software (Microsoft Corporation, Redmond, Washington). Mean values of three replications of each treatment were calculated.

3: Results and Discussion

3.1 Initial Observations

The fructose- and agave-sweetened treatments had lower temperatures after 2 h of conching. The conching time was kept as a constant for all three treatments. The agave-sweetened sample appeared thicker before molding when pouring the chocolate paste in the mold than the fructose-sweetened sample.

3.2 Fat

Because the cocoa butter and anhydrous milk fat were kept constant, the fat percentage of all three treatments should have been similar. According to Table 3.6, the mean percentage of fat in the sucrose- and fructose-sweetened chocolate bar treatments appeared similar. The agave-sweetened chocolate bar mean was 3.32% lower than the sucrose-sweetened chocolate bar treatment and 3.22% lower than the fructose-sweetened chocolate bar treatment.

Table 3.6: Percent fat of sucrose-, fructose-, and agave-sweetened chocolate bars

Treatment	**Fat (%)**
Sucrose-Sweetened Chocolate	31.13
Fructose-Sweetened Chocolate	31.10
Agave-Sweetened Chocolate	30.13

Mean analysis of samples within the same treatment tested in triplicate.

3.3 Saccharide Profile

The saccharide profile of the three sweeteners was tested to see what sugars were in each of the sweeteners. Table 3.7 shows the breakdown of saccharides in the different sweeteners. The granulated sugar sample is made up primarily of sucrose, which was to be expected; the rest of the saccharides were non-detectable. The crystalline fructose sample was primarily made of fructose; the rest of the saccharides were non-detectable. The detection limit for glucose and maltose was <0.20%. The detection limit for lactose was <0.70%. The powdered agave sweetener contained sucrose, fructose, and glucose. Fructose made up the majority (mean 83.67%) of the agave. The total sugar percentage in the agave was 6.21% lower than the sucrose

and 6.53% lower than fructose. The total sugar percentage in fructose was 0.31% higher than the sucrose.

Table 3.7: Saccharide profile of granulated sucrose, crystalline fructose, and powdered agave

Sweetener Type	**Sucrose (%)**	**Fructose (%)**	**Glucose (%)**	**Maltose (%)**	**Lactose (%)**	**Total Sugar (%)**
Granulated Sugar	99.26	N.D.	N.D.	N.D.	N.D.	99.26
Fructose, Crystalline	N.D.	99.57	N.D.	N.D.	N.D.	99.57
Agave, Powdered	2.20	83.67	8.13	N.D.	N.D.	93.47

Mean analysis of samples within the same treatment tested in triplicate.
N.D. = not detected. The detection limit for glucose and maltose was <0.20%. The detection limit for lactose was <0.70%.

Table 3.8 shows the saccharide profile of the three chocolate samples. It shows that the sucrose sample's sucrose percentage was higher than the percentage listed in the proposed formula. The sucrose in the agave treatment also showed that there was a higher amount than the proposed formula. Statistics were not performed comparing the formula percentage and the saccharide profile to see significant difference.

Table 3.8: Saccharide profile of the sucrose-, fructose-, and agave-sweetened chocolate bars

Treatment	**Sucrose (%)**	**Fructose (%)**	**Glucose (%)**	**Maltose (%)**	**Lactose (%)**	**Total Sugars (%)**
Sucrose-Sweetened Chocolate	51.33	N.D.	N.D.	N.D.	N.D.	51.80
Fructose-Sweetened Chocolate	14.47	33.8	N.D.	N.D.	N.D.	48.27
Agave-Sweetened Chocolate	15.20	28.23	2.70	N.D.	N.D.	46.13

Mean of samples within the same treatment tested in triplicate.
N.D. = not detected. The detection limit for glucose and maltose was <0.20%. The detection limit for lactose was <0.70%.

3.4 Moisture

Based on the saccharide profile of the sweeteners (Table 3.7), agave contains glucose and fructose, which makes it hygroscopic. Similar to the palm sugar that Saputro et al. (2016; 2017a; 2017b) studied, the hygroscopicity of agave caused it to have a higher moisture content than the fructose and sucrose (Table 3.9). The agave was 2800% higher than the sucrose and 1600% higher than fructose.

Table 3.9: Moisture of granulated sucrose, crystalline fructose, and powdered agave

Sweetener Type	**Moisture %**
Sucrose	0.040
Fructose	0.043
Agave	1.160

Means of samples within the same treatment tested in triplicate.

Moisture of the sweeteners (Table 3.9) was evaluated using the vacuum oven method. The moisture in the agave-sweetened chocolate bar (Table 3.10) was 54.54% higher than in the sucrose-sweetened chocolate bar. Moister in the agave-sweetened chocolate bar (Table 3.10) was 41.67% higher than in the fructose-sweetened chocolate bar. It can be inferred that the higher moisture of the agave chocolate samples resulted in higher agglomeration.

Table 3.10: Moisture of sucrose-, fructose-, and agave-sweetened chocolate bars

Treatment	**Moisture (%)**
Sucrose-sweetened	0.44
Fructose-sweetened	0.48
Agave-sweetened	0.68

Means of samples within the same treatment tested in triplicate.

3.5 Particle Size Distribution

The particle size of the chocolate is very important, and it affects rheology of the samples. Particle size distribution at D(v,0.5) and D(v,0.1) can predict viscosity. A lower D(v,0.1) can lead to a higher viscosity. Based on Saputro et al.'s (2016) study, that would

suggest that the agave has strong particle-particle interaction. Because the agave has strong particle-particle interaction, it can result in a higher rheological yield stress value.

Table 3.11: Particle size distribution of sucrose-, fructose-, and agave-sweetened dark chocolate bars

Treatment	Distribution percentiles			Derived Diameter[1]		Specific Surface Area
	D(v,0.9) μm	D(v,0.5) μm	D(v,0.1) μm	D (4,3)[1] μm	D (3,2) μm	(m^2/m^3)
Sucrose-Sweetened Chocolate	32.62	8.95	1.78	13.56	4.69	1.28
Fructose-Sweetened Chocolate	19.72	6.21	1.56	8.75	3.85	1.56
Agave-Sweetened Chocolate	17.59	5.60	1.42	7.82	3.54	1.7

Means of samples within the same treatment tested in triplicate.
[1] Derived Diameter determined by laser diffraction.

The sucrose-sweetened chocolate bar treatment had a larger particle size distribution at D(v,0.9); D v(0.5), and D(v,0.1). The sucrose-sweetened chocolate bar treatment was also the highest Sauter mean (3,2). The derived Sauter mean is the surface mean. The derived diameter D(4,3) is the volume mean. Surface area of the treatments was directly proportional to the distribution percentiles D(v,0.5); D(v,0.1) and Sauter mean (3,2) (Table 3.11). The agave-sweetened chocolate sample had the highest surface area and smallest particles. The agave-sweetened treatment particles were 46.08% smaller than sucrose-sweetened treatment at D(v,0.9). The fructose-sweetened chocolate particles were 39.55% smaller than sucrose-

sweetened treatment at D(v,0.9). The agave-sweetened chocolate treatment's surface area was 32.81% larger than sucrose-sweetened chocolate. The agave-sweetened treatment's surface area was 7.59% larger than the fructose-sweetened. Products that have a higher surface area and smaller particle size get coated more with fat, which affects the rheology of the product.

Figure 3.5 shows an overlay of the particle size distribution curves for the sucrose, fructose, and agave samples plotting cumulative and density distributions versus particle size. The sucrose sample had a bimodal distribution. The fructose and agave samples appear similar; this could be because agave is made up of a mean of 83.67% fructose (Table 3.7).

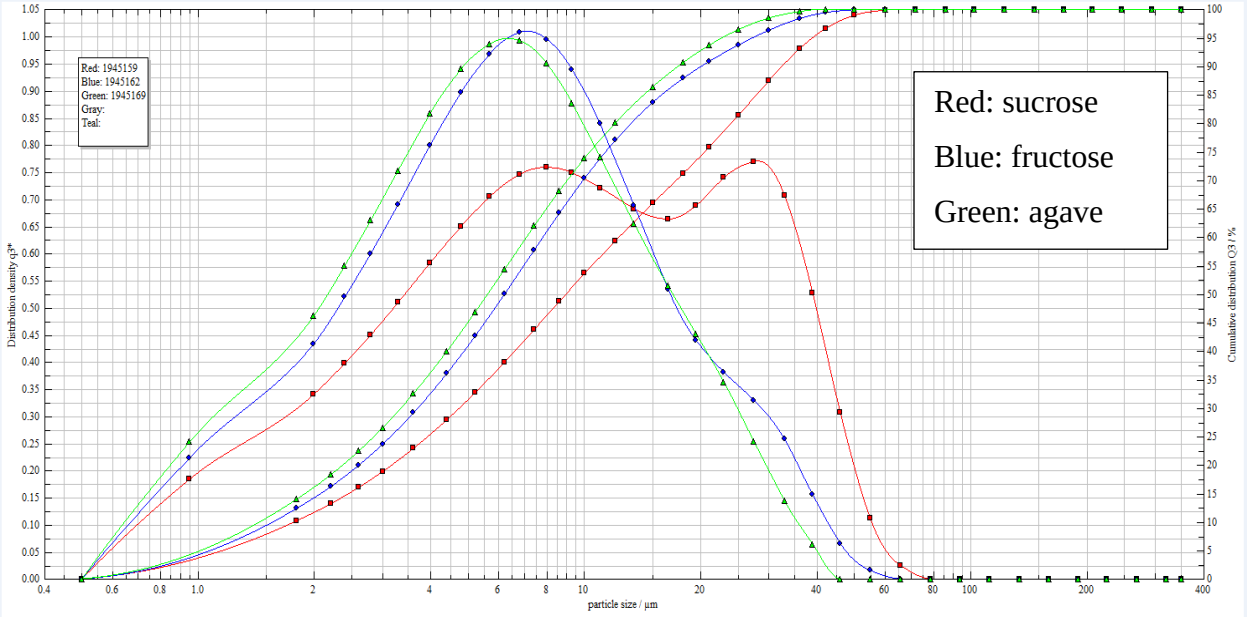
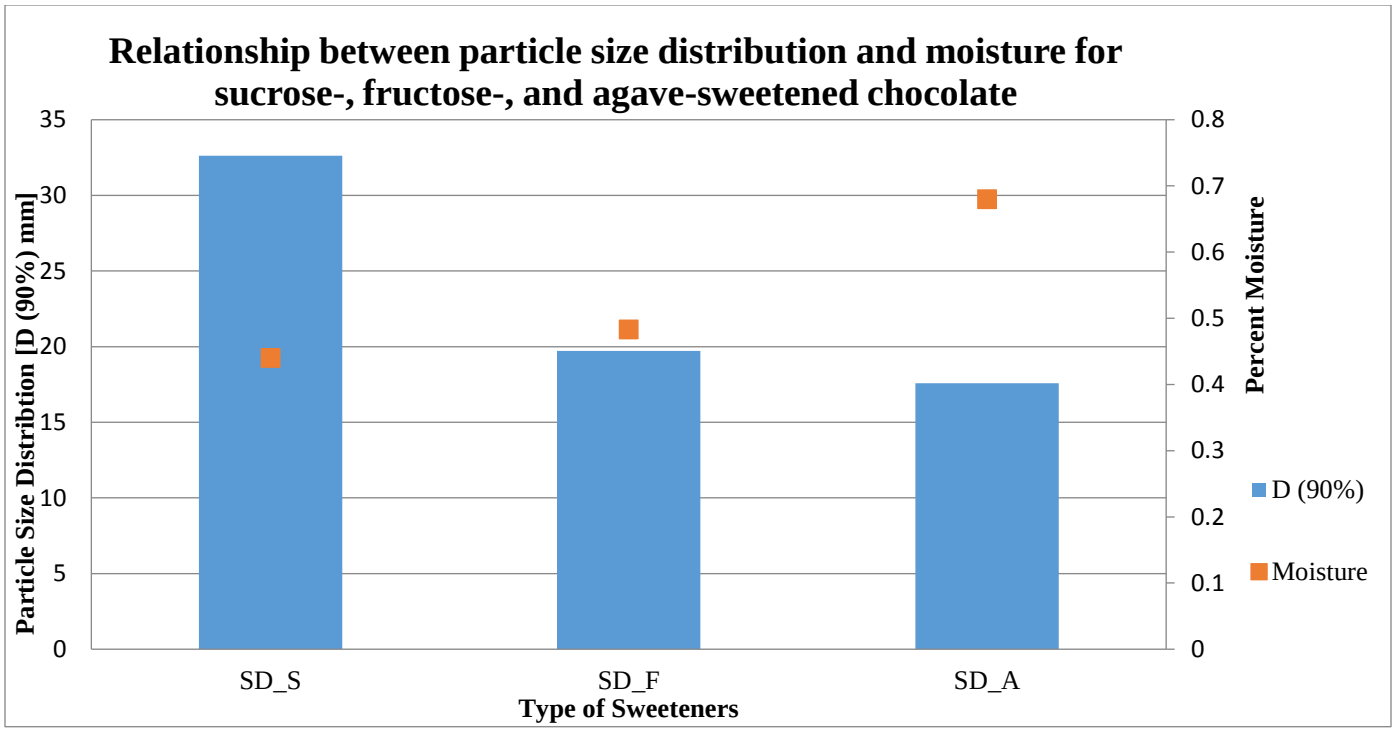


Figure 3.5: Particle size distribution curve of sucrose-, fructose-, and agave-sweetened chocolate samples

Particle size is important in the sensory of chocolate. According to Saputro et al. (2017a), a chocolate with larger particle size (>30 microns) is considered to be "gritty" while particles of 20 µm create creamier chocolate. This study targeted a micron size of 25 µm when refining.

Moisture plays a role in the particle size distribution of chocolate. According to Saputro et al. (2016), "the moisture of chocolate creates sticky patches on the surface of the sugar particles inducing agglomeration" (p. 959). In Figure 3.6, the particle size distribution at 90% of the particles was compared to the percentage of moisture in the chocolate bars. The agave-sweetened chocolate, which had the highest percent moisture, had the lowest particle size at a mean of 17.59 μm. Higher size reduction of particles is related to a higher degree of agglomeration.



Data used from Tables 3.10 and 3.11

Figure 3.6: Relationship between particle size distribution and moisture in sucrose-, fructose-, and agave-sweetened chocolate

3.6 Rheology

Rheology measures the viscosity and yield stress of a product. According to Saputro et al. (2016), molten chocolate exhibits a non-ideal plastic behavior, so they suggested using the Casson model. Chocolate is considered non-Newtonian, which means that its "viscosity is

affected by the presence of solids in suspension as well as the temperature" (Minifie, 1989, p. 124). The Casson viscosity is the stress needed to maintain chocolate flow during shearing (Saputro et al., 2016). According to them, Casson viscosity is influenced by moisture, particle density of the sweetener, and particle size. As they stated, if the chocolate contains the same lecithin and fat levels, flow includes the particle size distribution, particle volume fraction, and moisture content. In this experiment, lecithin and cocoa butter levels were constant in all the formulas. The Casson yield value is more shear thinning.

Based on the Anton Paar rheology method, sucrose had the highest viscosity. It was followed by agave and then fructose. The higher viscosity for the sucrose sample was due to the particle size. At D(v,0.9), the sucrose-sweetened chocolate treatment had the highest particle size and the lowest surface area (Table 3.12). This could be why it had the highest viscosity. The sucrose sample was bimodal (Figure 3.6), which can also influence the Casson viscosity by agglomerates reducing the "free" fat availability in decreasing the viscosity. According to Aidoo et al. (2014), "Casson viscosity relates to pumping characteristics, filling of rough surfaces, coating properties, and sensory character of body" of chocolate (p. 594).

Because Casson viscosity is also largely influenced by moisture content, the agglomeration in the agave with the fructose and glucose could have caused the Anton Paar viscosity of the agave sample to be higher than the fructose sample. According to Saputro et al. (2017b), the higher degree of agglomeration can be fragmented during shearing, which creates "wet" surfaces and higher viscosity if the shear is higher than the product is thin. The Anton Paar method goes from low speed to high speed. The Brookfield method goes from high speed to low speed. Table 3.12 and Figures 3.7 and 3.8 compared the two rheology methods

Table 3.12: Brookfield and Anton Paar rheology of sucrose-, fructose-, and agave-sweetened dark chocolate

	Brookfield Casson Viscosity Centipoise(cP)	Confidence of Fit (%)	Anton Paar Casson Viscosity Centipoise(cP)	Brookfield Casson Yield Value (dynes/cm^2)	Anton Paar Casson Yield Value (dynes/cm^2)
Sucrose-Sweetened Chocolate	4352	99.35	4016	81	106
Fructose-Sweetened Chocolate	2533	98.7	1941	136	160
Agave-Sweetened Chocolate	6727	99.3	2860	232	280

Singular analysis of treatments

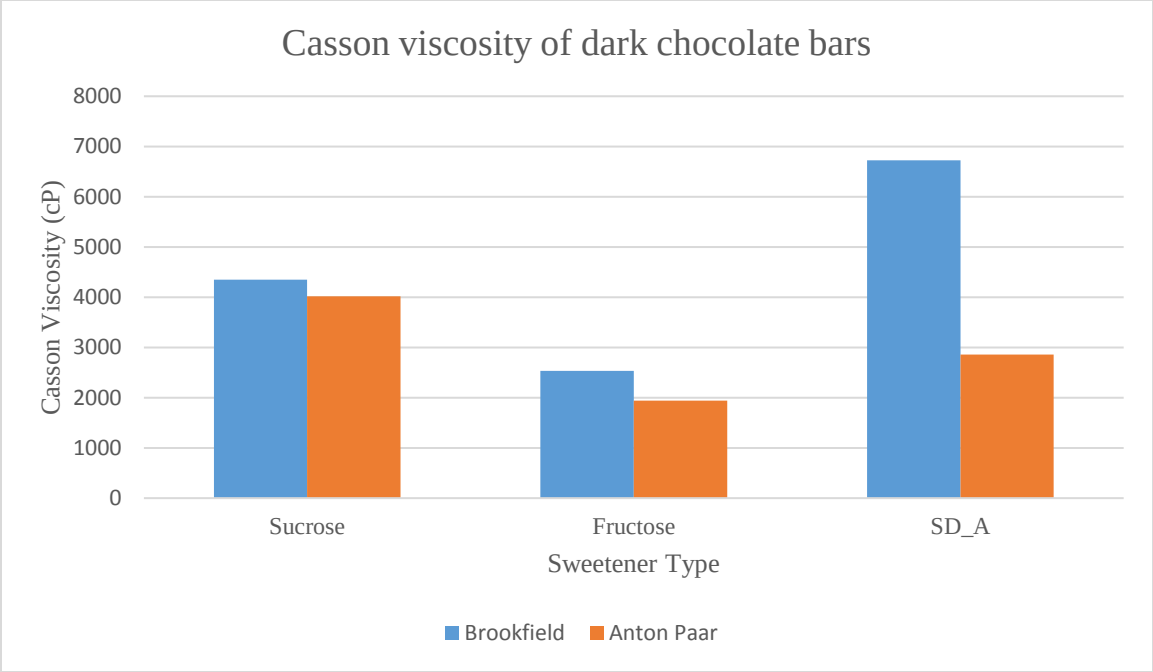


Figure 3.7: Casson viscosity of sucrose-, fructose-, and agave-sweetened chocolate bars comparing Brookfield and Anton Paar methods

The agglomeration in agave affects the rheology of the chocolate. The yield stress values for both Brookfield and Anton Paar methods were consistent with each method. Both methods had shown the yield stress for the agave-sweetened chocolate to be higher than the fructose- and sucrose-sweetened chocolate treatments. Both methods showed that the sucrose-sweetened chocolate treatment had the lowest yield stress value (Figure 3.8). The yield value of the agave-sweetened chocolate treatment was 75.00% higher than the fructose-sweetened chocolate treatment for the Anton Paar method and 70.59% higher based on the Brookfield method. The agave-sweetened chocolate treatment was 164.15% higher than the sucrose-sweetened chocolate treatment using the Anton Paar method. The agave-sweetened chocolate sample was 186.42% higher than the sucrose-sweetened chocolate treatment using the Brookfield method. The fructose-sweetened treatment was 50.94% higher yield stress using Anton Paar method and 67.90% higher for the Brookfield method (Figure 3.8 and Table 3.12); this is likely due to the

agglomeration of the particles. Based on Saputro et al.'s (2016) study, they suggested that the agave has stronger particle-particle interaction. This resulted in higher yield values, which is what was indicated in the rheology of the samples (Table 3.12).

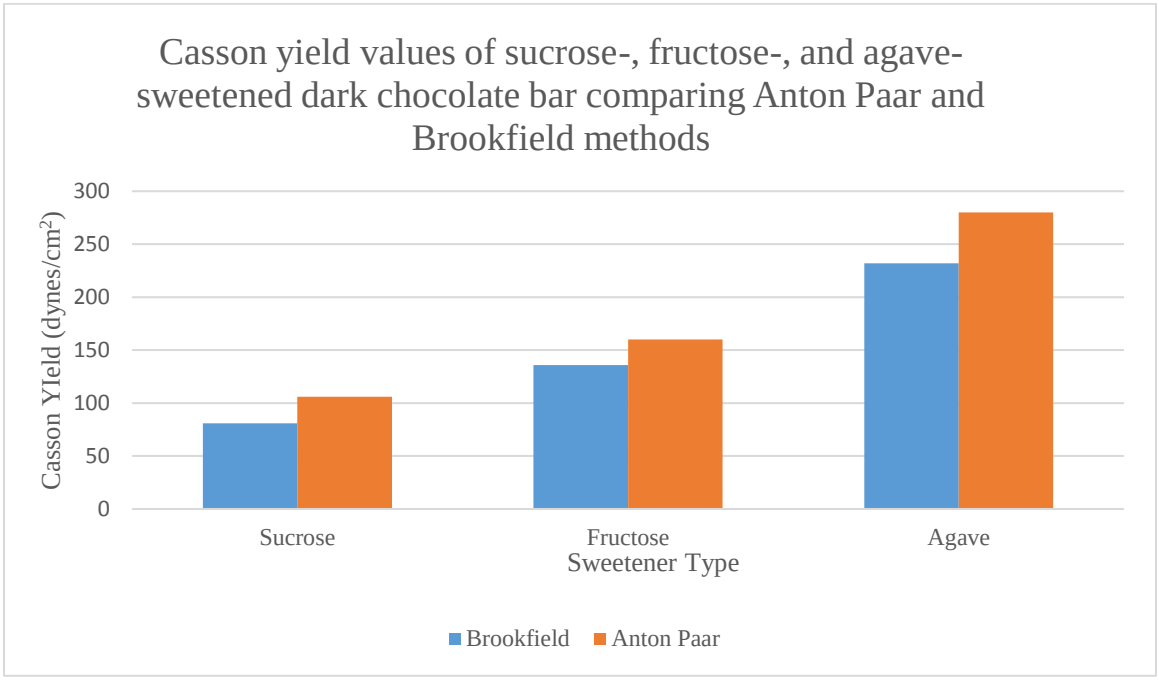


Figure 3.8: Casson yield values of sucrose-, fructose-, and agave-sweetened dark chocolate bar comparing Anton Paar and Brookfield methods

Thixotropy is the difference between the ramp up and ramp down of shear stress at 5 s^{-1} in centipoise (Figure 3.9). The agave-sweetened chocolate treatment was 2646% higher thixotropy than the sucrose-sweetened chocolate treatment. It was 369.50% (significantly higher) than the fructose-sweetened treatment. The fructose-sweetened treatment had a 484.84% higher thixotropy than the sucrose-sweetened treatment (Figure 3.9). This suggests it was not conched as well as the sucrose sample. That agrees with the lower product temperature of the conched agave and fructose samples. The conching temperature for the fructose-sweetened chocolate treatment was 25.05% lower than the sucrose-sweetened treatment. The conching temperature for the agave-sweetened chocolate sample was 35.92% lower than the sucrose-

sweetened treatment (Table 3.3). If a product is thixotropic, it is shear thinning. Agave was the most shear thinning.

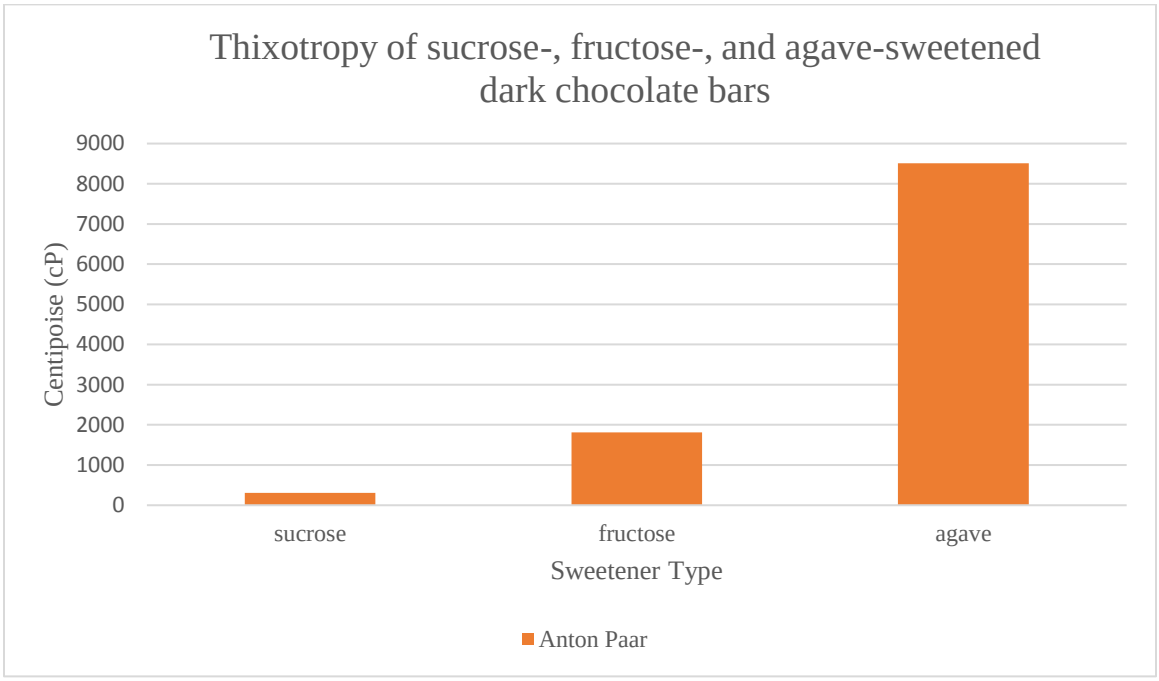


Figure 3.9: Thixotropy of sucrose-, fructose-, and agave-sweetened dark chocolate bars

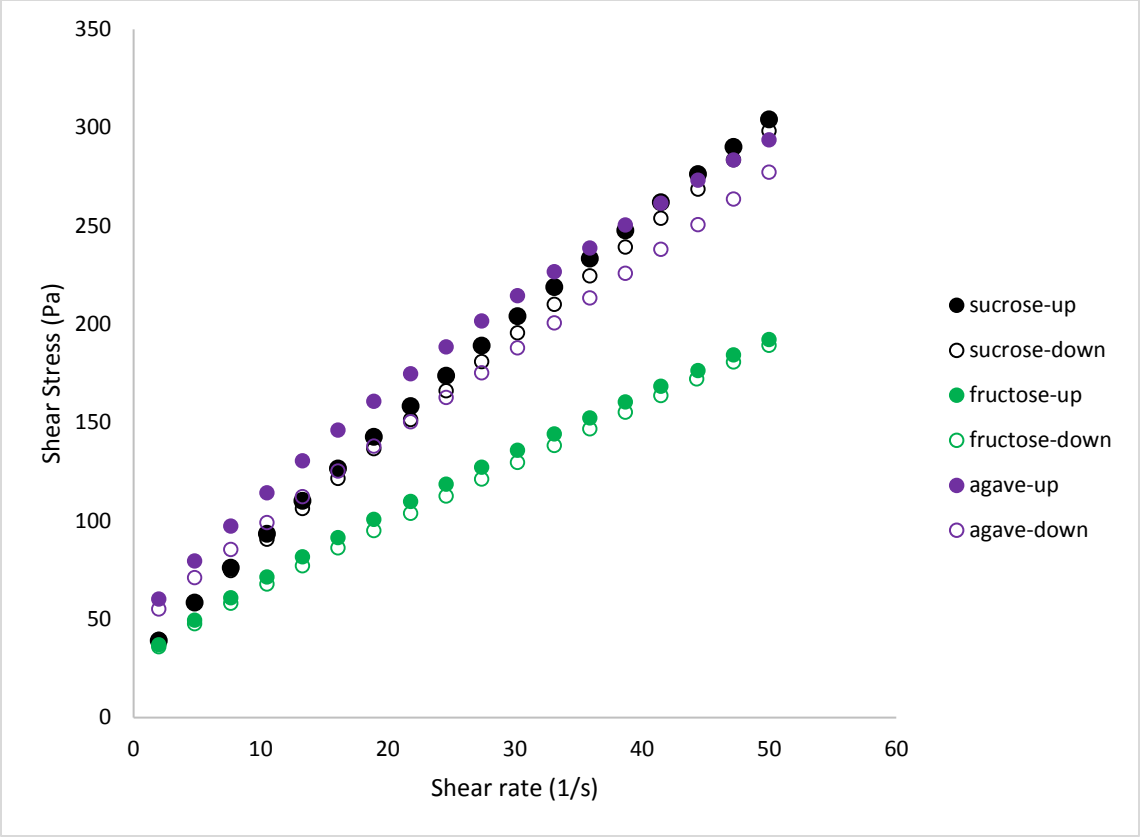


Figure 3.10: Sheer rate versus shear stress of sucrose-, fructose-, and agave-sweetened chocolate bars using Anton Paar rheometer

Rheology is important in chocolate. Some products are affected by rheology more than others. Rheology is more important for depositing or decorating confections. Rheology is also important for enrobing to make sure that the piece of candy is completely coated. Therefore, a lower viscosity chocolate is needed. For shell-molding, a higher yield stress value is needed. A higher yield chocolate is important for decorating chocolate and baking chips. If one uses bulk sweeteners such as polyols, they affect viscosity with their finer particles (Aidoo et al., 2013). Quality attributes of chocolate also can be indicated by rheology. If the viscosity and yield are too low for shell molding, it results in thin shells. If the viscosity and yield are too high, de-lamination of shells occurs.

3.7 Color

The color of the chocolate bars was observed. The L*value of agave-sweetened chocolate treatment was 4.77% higher than the sucrose-sweetened chocolate treatment and 1.40% higher than the fructose-sweetened chocolate treatment. The a* value for the agave-sweetened treatment was 0.22% higher than the sucrose-sweetened treatment. The fructose-sweetened chocolate treatment was 0.57% higher than the agave-sweetened treatment and 0.79% higher than the sucrose-sweetened treatment. The b* value of the fructose-sweetened treatment was 2.81% higher than the b* value of the sucrose-sweetened chocolate treatment and 0.21% higher than the b* value of the agave-sweetened chocolate.

The agave's lighter color resulted in the highest L* value. This could be due to the finer particles having more particle-particle interaction (Saputro et al., 2017a). Additionally, more particles result in a higher reflective surface. The differences between the treatments was not seen by the naked eye. The lightness might not have an effect visually for dark chocolate samples using agave with consumers, but it could affect milk or white chocolate products. When developing formulas using agave for milk chocolate and white chocolate, one should consider the L* value and its effect on consumer perception of the product.

Table 3.13 Color of sucrose-, fructose-, and agave-sweetened dark chocolate

Treatment	L*	a*	b*
Sucrose-sweetened chocolate	20.73	13.91	18.52
Fructose-sweetened chocolate	21.42	14.02	19.04
Agave-sweetened chocolate	21.72	13.94	19.00

Means of samples within the same treatment tested in triplicate.

3.8 Hardness of Chocolate

The hardness of chocolate is a measure of the strength of fat crystals. Peak hardness of all the chocolate samples was measured by Newton's force (Table 3.14). The sucrose-sweetened treatment measured 37.74% higher than the fructose-sweetened treatment and 16.48% higher than agave-sweetened chocolate treatment sample. According to Saputro et al. (2017a), hardness of chocolate is affected by fat content, particle volume fraction, particle size distribution, and tempering. Hardness is a good indicator of good tempering or degree to which a fat crystal network has been formed (Aidoo et al., 2013). Since the fat content and temper were kept constant for all the treatments, the particle fraction and particle size distribution affected the chocolate samples. Higher surface area and finer particles attribute to increased particle-particle interaction. That would suggest the agave-sweetened treatment would be harder. However, the sucrose-sweetened treatment was 16.48% higher than the agave-sweetened. Finer particles have more particle-particle interaction, resulting in harder chocolate (Saputro et al., 2017a).

Table 3.14: Peak hardness of sucrose-, fructose-, and agave-sweetened dark chocolate bars

Treatment	**Peak Hardness (N)**
Sucrose-sweetened chocolate	21.06
Fructose-sweetened chocolate	15.29
Agave-sweetened chocolate	18.08

Means of samples within the same treatment tested in triplicate.

More amorphous sugar could lead to a "stronger" sugar network of chocolate (Saputro et al., 2017a). The hardness of the agave-sweetened treatment was 18.25% stronger than the fructose-sweetened treatment, which could be because of the moisture of agave. Saputro et al. (2017a) suggested the moisture content could lead to amorphous sugar.

Hardness of chocolate is a quality parameter. The hardness of the chocolate could affect the consumer's eating experience if the chocolate is too hard. When evaluating for sensory characteristics, hardness should be included using just-about-right scoring. Since temper affects hardness, it might be more challenging for the production facility to achieve proper temper.

3.9 Melting Profile

The enthalpy and onset of the melt are critical parameters when evaluating the profile of chocolate. The onset is the steepest rate of energy. The melt peak occurs when sugar stops melting. The onset sugar melt in agave was lower than fructose and had a slightly wider peak and half height (Figures 3.12 and 3.13). These results suggest that agave contains both glucose and fructose. Agave is a less pure source of fructose. The sucrose sample had a higher onset and peak maximum sugar melt, which was the same as in Saputro et al.'s (2017a) study where the control sucrose had the highest onset and peak maximum as the variants. The reducing sugar (fructose and glucose) in agave could contribute to lower onset and peak temperature (Table 3.15). The same is true for fructose; the reducing sugars are considered "impurities" (Saputro et al., 2017a).

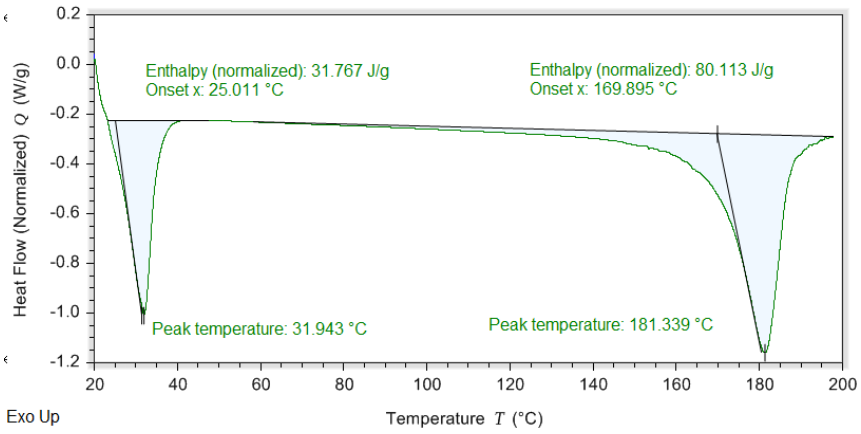


Figure 3.11: Fat and sugar melting profile of sucrose-sweetened dark chocolate bar

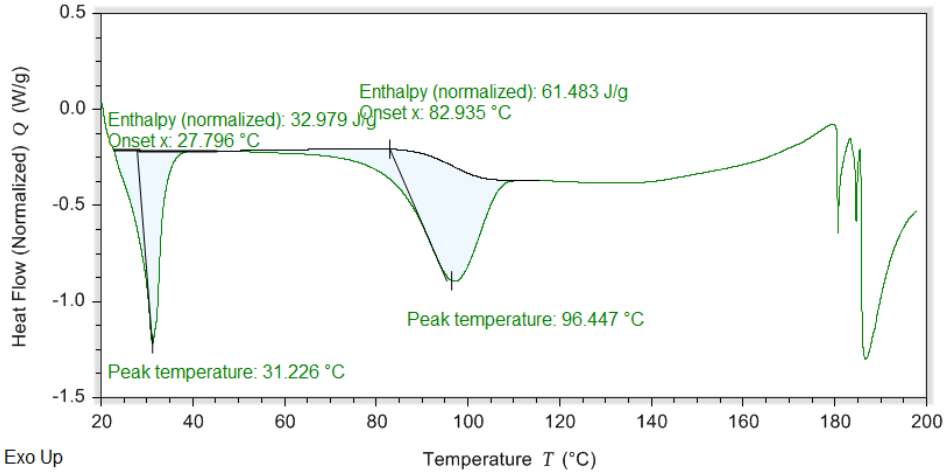


Figure 3.12: Fat and sugar melting profile of fructose-sweetened dark chocolate bar

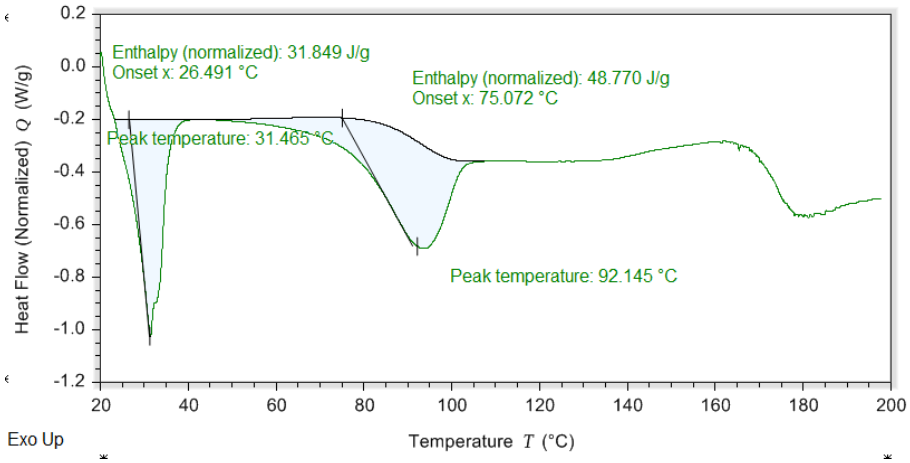


Figure 3.13: Fat and sugar melting profile of agave-sweetened dark chocolate bar

Table 3.15: Melting profile of sucrose-, fructose-, and agave-sweetened dark chocolate bars

	Fat Melt Energy (J/g)	Fat Melt Onset (C)	Fat Melt Peak (C)	Sugar Melt Energy (J/g)	Sugar Melt Onset (C)	Sugar Melt Peak (C)	Width Peak Half Height (C)
Sucrose-sweetened Chocolate	32.05	25.06	31.65	76.48	169.81	180.92	9.4
Fructose-sweetened Chocolate	32.85	26.78	31.38	61.48	89.69	89.73	13.6
Agave-sweetened Chocolate	32.12	26.72	31.61	48.99	73.12	92.88	14.6

Means of samples within the same treatment tested in duplicate.

3.10 Aroma Profile

Many of the volatiles in the aroma profile of chocolate come from chocolate liquor. In the samples, 108 peaks were identified. All the samples tested on the GC-MS had very similar peaks to each other; however, some of the peaks were higher depending on the sweetener. Table 3.16 identifies the peaks. In the chocolate sample sweetened by agave, it showed a presence of ethanol where the sucrose and fructose did not. It also showed a peak of ethyl lacetate. Benzaldehyde was shown to have a higher peak in the agave sample. One explanation in the

different peaks and peaks heights is that the reducing sugars in agave could lead to an interaction with amino acids and cause a Maillard Reaction. The peaks from 15-35 minutes looked the most similar of the three samples (Figures 3.14-3.18). A next step for evaluating the aroma profile is performing quantitative testing by testing the concentration (ng/g) as Saputro et al. (2016) performed.

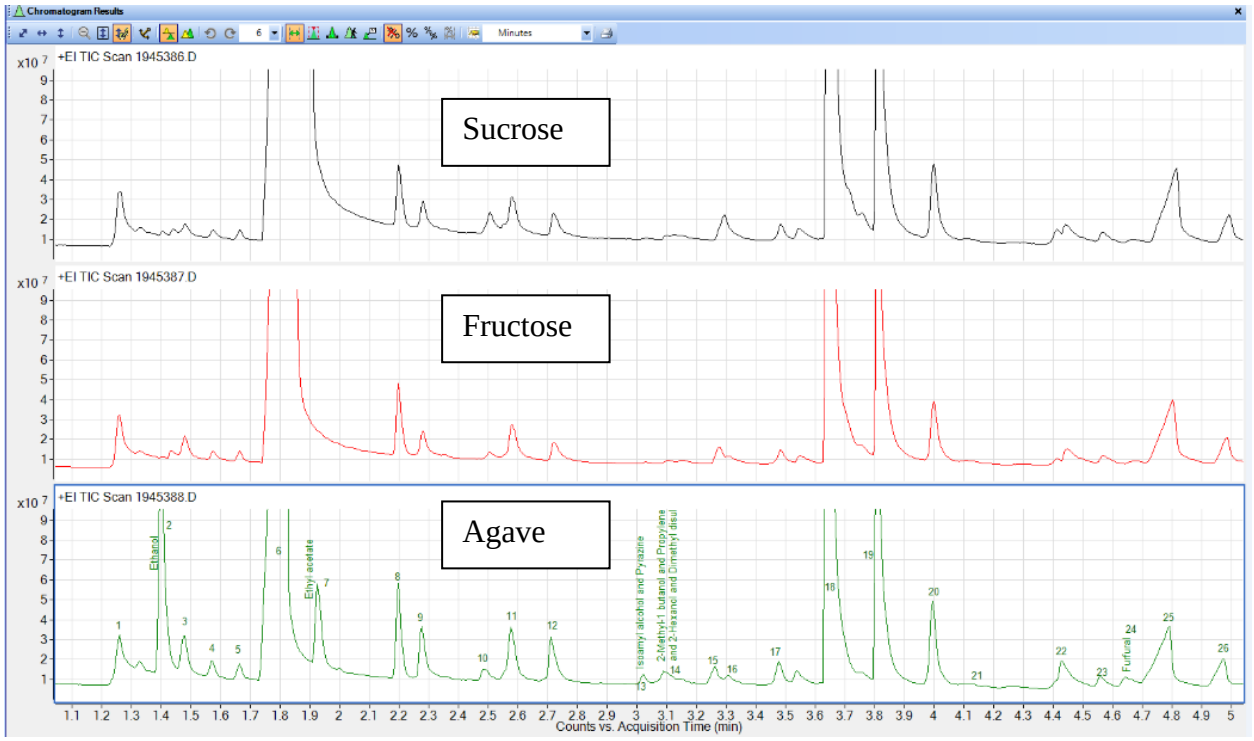


Figure 3.14: GC chromatogram 0-5 minutes for sucrose-, fructose-, and agave-sweetened dark chocolate

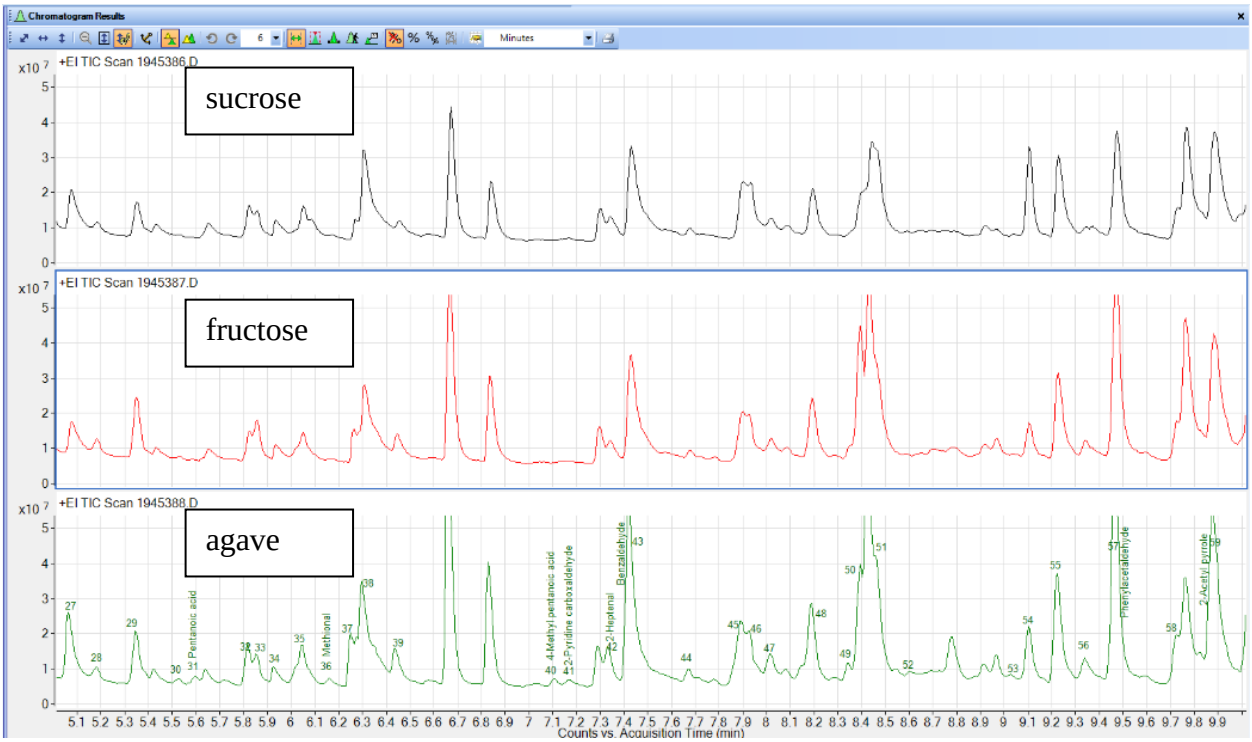


Figure 3.15: GC chromatogram 5-10 minutes for peaks of sucrose-, fructose-, and agave-sweetened dark chocolate

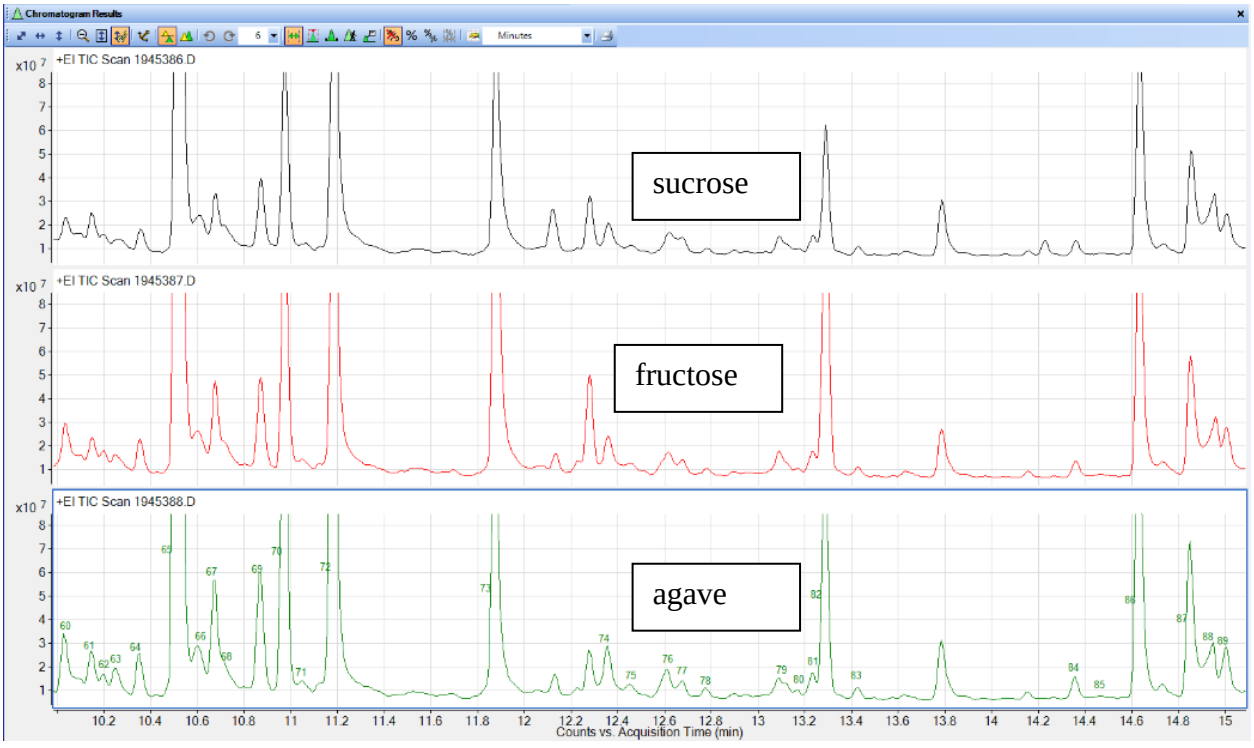


Figure 3.16: GC chromatogram 10-15 minutes for peaks of sucrose-, fructose-, and agave-sweetened dark chocolate

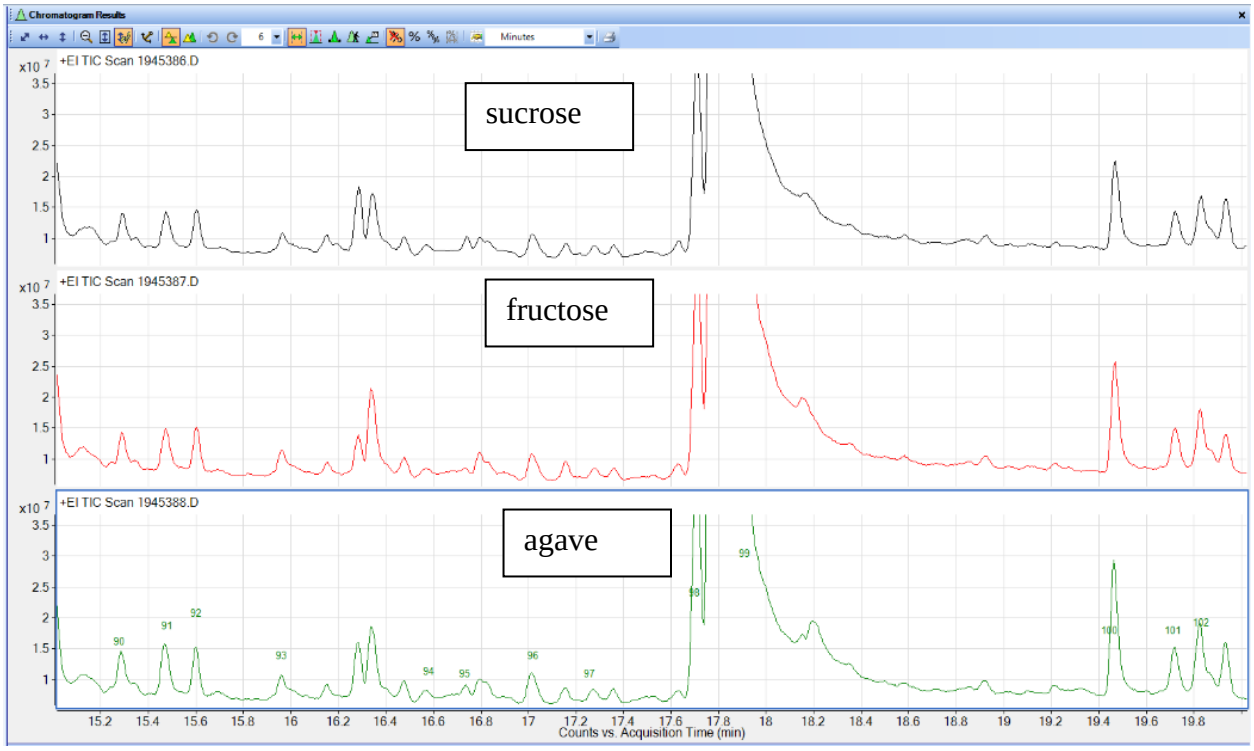


Figure 3.17: GC chromatogram 15-20 minutes for peaks of sucrose-, fructose-, and agave-sweetened dark chocolate

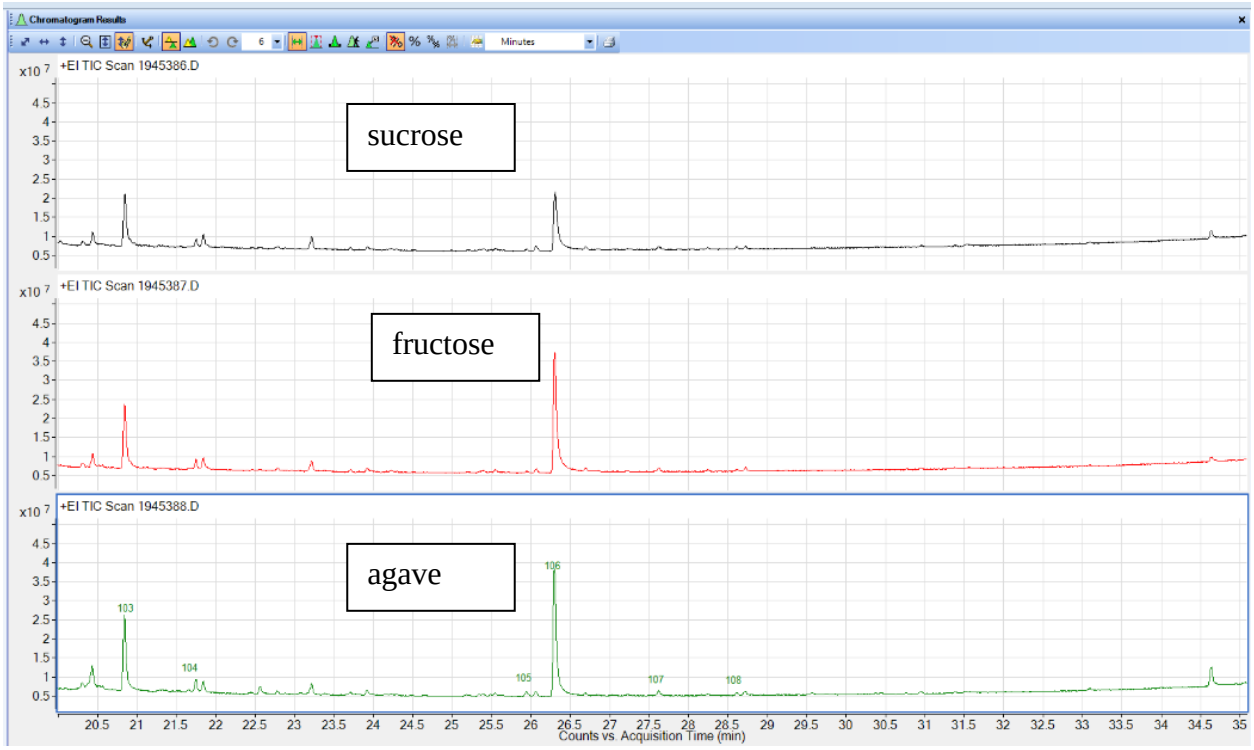


Figure 3.18: GC Chromatogram 20-35 minutes for peaks of sucrose-, fructose-, and agave-sweetened dark chocolate

Table 3.16 GC Peaks of chocolate sweetened with agave

Peak No.	FEMA No.	Compound(s)	Odor Descriptors from (http://www.thegoodscentscompany.com/)
1		Carbon dioxide	alcoholic, ethereal, medicinal
2	2419	Ethanol	
3		Acetone	
4	2676	Methyl acetate	ethereal, sweet, fruity, solvent, estery, winey, cognac, rum, green, whiskey
5		Isobutyraldehyde (2-Methyl propanal)	
6		Acetic acid	
7		Ethyl acetate	
8		Isovaleraldehyde (3-Methyl butanal)	
9		2-Methyl butanal	
10		Propanoic acid	
11		2,3-Pentanedione and Pentanal	
12		Acetoin	
13	2057 4015	Isoamyl alcohol Pyrazine	fusel, alcoholic, whiskey, fruity, banana, pungent, etheral, cognac, molasses, fermented pungent, sweet, corn, roasted, hazelnut, barley
14	3998 2940 not GRAS 3536	2-Methyl-1-butanol Propylene glycol 2-Hexanol Dimethyl disulfide	roasted, winey, onion, fruity, fusel, alcoholic, whiskey odorless, very slight alcoholic chemical, winey, fruity, fatty, terpenic, cauliflower sulfurous, vegetable, cabbage, onion
15		Isobutyric acid	
16		1H-Pyrrole	

17		1-Pentanol Toluene	
18		Butanediol Butyric acid	
19		Butanediol	
20		Hexanal	
21		Dihydro-2-methyl-3[2H]-furanone	
22		Methyl pyrazine	
23		Tetrahydrofurfuryl acetate	
24	2489	Furfural	sweet, woody, almond, baked bread, brown, caramelic, phenolic, nutty, burnt, astringent
25		Isovaleric acid (3-Methyl butyric acid)	
26		2-Methyl butyric acid	
27		Furfuryl alcohol	
28		a Dimethyl benzene or Ethyl benzene	
29		a Dimethyl benzene Acetol acetate	
30		Isoamyl acetate	
31	3101	Pentanoic acid	acidic, sweaty, rancid, sharp, cheesy, sour, milky, tobacco, fruity, dairy
32		2-Heptanone	
33		a Dimethyl benzene	
34		2,4-Pentanediol	
35		2-Heptanol Heptanal	

36	2747	Methional	musty, potato, tomato, earthy, vegetable, creamy, oily, yeasty, bready, cheesy, brothy
37		2,5-Dimethyl pyrazine	
38		.gamma.-Butyrolactone 2,6-Dimethyl pyrazine Ethyl pyrazine	
39		2,3-Dimethyl pyrazine	
40	3463	4-Methyl pentanoic acid	pungent, cheesy
41	not GRAS	2-Pyridine carboxaldehyde	caramellic, fatty
42	3165	2-Heptenal	green, fatty
43	2127	Benzaldehyde	sharp, sweet, bitter, almond, cherry, fruity, powdery, nutty, oily, nutty, woody
44		1-Heptanol	
45		1-Octen-3-ol	
46		Hexanoic acid and Phenol	
47		Benzonitrile 2,3-Octanedione	
48		2-Pentyl furan	
49		2-Ethyl-5-methyl pyrazine	
50		2-Ethyl-6-methyl pyrazine	

51		Trimethyl pyrazine 2-Ethyl-3-methyl pyrazine	
52		1H-Pyrrole-2-carboxaldehyde	
53		Methyl cyclopentenolone	
54		Limonene	
55		Pantolactone Benzyl alcohol	
56		trans-.beta.-Ocimene	
57	2874	Phenylacetaldehyde	green, sweet, floral, hyacinth, clover, honey, cocoa, rose, powdery, chocolate, earthy
58		Ethyl isobutyrate Furaneol	
59	3202	2-Acetyl pyrrole	musty, nutty, coumarinic, licorice, walnut, bready, sweet, tea
60		Acetophenone	
61		1-Octanol 3,5-Octadien2-one	
62		cis or trans Linalool oxide (furanoic)	
63		Butanediyl acetate	
64		3-Ethyl-2,5-dimethyl pyrazine or 2-Ethyl-3,5-dimethyl pyrazine	
65		Tetramethyl pyrazine	

66		cis or trans Linalool oxide (furanoic) 2-Methoxy phenol	
67		2-Nonanone	
68		.delta.-Hexalactone 2-Methoxy phenol	
69		Linalool	
70		Nonanal	
71		2-Isopropyl-5-methyl-2-hexenal	
72		Phenethyl alcohol	
73		2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	
74		a Diethyl methyl pyrazine or a Trimethyl ethyl pyrazine	
75		Benzyl acetate	
76		Ethyl benzoate	
77		cis or trans linalool oxide (pyranoic)	
78		Methyl phenyl acetate	
79		2-Decanone	
80		alpha-Phenethyl acetate	
81		Ethyl octanoate	
82		Dodecane	
83		Decanal	

84		Ethyl phenylacetate	
85		an Isoamyl methyl pyrazine	
86		Phenethyl acetate	
87		Massoilactone	
88		Nonanoic acid	
89		2-Phenyl-2-butenal	
90		.delta.-Octalactone	
91		2-Undecanone	
92		Tridecane	
93		a Dimethyl isoamyl pyrazine	
94		Methyl anthranilate	
95		Triacetin	
96		.gamma.-Undecalactone ? Nonalactone	
97		4-Methyl-2-phenyl-2-pentenal	
98		Isoamyl benzoate	
99		Vanillin	
100		Massoia lactone	
101		5-Methyl-2-phenyl-2-hexenal	
102		.delta.-Decalactone	
103		Mellein	
104		Ethyl dodecanoate	
105		Isopropyl myristate	
106		Caffeine	
107		Methyl palmitate	

108		Ethyl palmitate	

4. Conclusion

When reducing sugar in a product, taste is not the only aspect a product developer must consider. Physical parameters of chocolates using alternative sweeteners are very important. Knowing how the chocolates behave with alternative sweeteners is important for a product developer when developing a chocolate with specific physical needs depending on the application. The flow parameters of the chocolate affect the usage of chocolate. The higher yield value of the agave sample might cause issues molding or enrobing chocolate, but it would work well for a baking chip that requires it to stand up.

Additionally, the physical parameters are important in a manufacturing facility. Two of the key parameters that shape the product are the moisture content of the chocolate and the particle size distribution. Both parameters affected the rest of the parameters in this study, including the rheology, color, and melting profile. The moisture and particle size distribution affect the refining of chocolate. The peak hardness affects the temper of chocolate.

This study showed that different sweeteners affect physical parameters in chocolate. For example, the moisture and particle size distribution affect the physical properties of the chocolate. The hygroscopicity of agave affected the rheology of chocolate. Higher agglomeration of particles also leads to higher yield values. Finally, it is important to remember that the agave and fructose chocolates made in this study can be considered standard of identity for chocolate, while non-nutritive sweeteners would not be.

5. Future Work

Next steps include performing a sensory evaluation of the samples. Based on a study by Thamke et al. (2009), the Free Choice Profiling technique can be utilized on the chocolate samples tested. From that technique, sensory characteristics and key features of the chocolate samples would be identified. In addition to Free Choice Profiling, trained panelists would evaluate the samples using a nine-point hedonic scale for the attributes. Some attributes that should be included are overall acceptability, sweetness, mouthfeel, astringency, and bitterness. As Belščak-Cvitanović et al. (2015) evaluated the attributes, they used a five-point scale and created spider charts of the attributes. Individual spider charts for each of the chocolate samples can be created. From there, consumers' feedback would be incorporated. In Torri et al.'s (2017) study where they evaluated stevia and stevia green extract and chocolate, they tested the samples using 95 consumers. The attributes they tested with consumers were overall acceptability, appearance, odor, taste, flavor, and texture; they used a nine-point hedonic scale. Similar methods could be utilized for this study.

Saputro et al. (2017b) studied the microstructure of the chocolates to evaluate the morphology of the chocolate and the particle-to-particle interactions in the chocolate. Based on that, future considerations involve evaluating the micro structure of chocolate from this study.

Because the samples in this study had a different temperature after conching the chocolate for two hours, a recommendation would be to increase the conching time of the chocolate to have all samples reach the same temperature instead of the two hours conching time. Studies would be performed to see how the product temperature of the conched chocolate affects the physical and sensory properties of the chocolate samples. Additionally, it is important to see the change in moisture before and after conching affects the properties of the chocolate.

Conching is “crucial since the moisture reduction and fine-tuning of a desirable flavour profile occur in this step” (Saputro et al., 2016, p. 956). Conching chocolate develops the final texture and flavor of chocolate (Minifie, 1989). Rudi Lindt invented the chocolate conche to make chocolate smoother and modify the taste (Beckett, 2008).

Studying other sweeteners such as D-allulose would be included in future work with reducing sucrose in chocolate. Because it is 0.2 kcal/gram, it is considered to be a non-nutritive sweetener. One can use it in conjunction with other sweeteners including sucrose. The product would have a reduction of sucrose, but it would not be a full replacement of sucrose. Because D-allulose is a non-nutritive sweetener, the chocolate would not be standard of identity. The chocolate company would need to decide if that is acceptable for their business.

Economics of sweeteners need to be considered for developing chocolates with alternative sweeteners. One would need to see how the alternative sweeteners affect the profit margins of the formula. Additionally, availability of the sweeteners need to be considered. While Saputro et al. (2016; 2017a; 2017b) studied palm-sap sweeteners, commercial availability of them is a challenge. D-allulose is new, so that also might have commercial availability issues. This study used agave as a powder. Seeing if a commercially available source of granulated agave exists would be important to study. A future study would also include granulated agave and agave syrup to see how that affects the physical parameters of chocolate.

Since Yeung et al.’s (2017) article studied model formulas for reducing adding sugar, taking their strategies into effect for reducing sugar in chocolate should be evaluated. Using the fructose and agave or other alternative sweeteners with inulin would replace sucrose. Using the alternative sweeteners with maltodextrin as a replacement to sucrose would be another study to see how that affects the physical and sensory properties of chocolate.

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