STRATEGIES TO INFLUENCE TEMPERATURE STABILITY OF DARK CHOCOLATE IN TROPICAL REGIONS

BY

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THESIS

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Abstract

Chocolate is a complex matrix, for which the preparation involves several physical and chemical processes, requiring a diverse set of technological operations to obtain the desirable quality and sensory characteristics. Fat bloom formation and melting point are challenging to control in the chocolate industry, especially in tropical regions. Tempering, is the process where chocolate obtains the appropriate polymorphic form in cocoa butter (form V) which influences important physical and functional characteristics; it also is associated with avoiding fat bloom formation and, consuming significant time and energy, thus, conventional processing of chocolate has its disadvantages. The independent addition and fractionation of various lipids and ultrasonication technology have been explored to avoid bloom formation and increase melting point. However, the combination of these technologies has not been reported.

The use of palm oil in the chocolate industry has gained attention for its ability to imitate the cocoa butter Triacylglycerol (TAG), its β-carotene content (500-1500 ppm), and its low cost when compared to cocoa butter. Thus, it is viewed as a good cocoa butter replacer. High Intensity Ultrasound (HIU) application to dark chocolate formulation has been demonstrated to influence crystallization by promoting primary and secondary nucleation through the mechanisms of cavitation and acoustic streaming, creating the right polymorphic (form V).

The overall goal of this project was to develop optimal conditions for producing high quality chocolate in developing countries within tropical regions such as Honduras. The central hypothesis was that the combination of fractionated lipids (palm oil and cocoa butter) and ultrasonication will produce high-quality chocolate, reduce fat bloom formation and increase melting point. This thesis work was divided into two phases.

In Phase 1, the objective was to evaluate the addition of palm stearin (5%) in the chocolate formulation as a partial replacement for cocoa butter. Dark chocolate was formulated with and without 5% palm stearin, conched, and either tempered or sonicated. In phase 2, the objective was to compare chocolate that was made with palm stearin (5%) and cocoa butter stearin with chocolate that was made with only cocoa butter stearin. Dark chocolate was formulated with fractionated lipids (stearin fraction), conched, and either tempered or sonicated. In both phases, cycling experiments were conducted. Various parameters were investigated, including whiteness index (WI), physical dimensions, X-ray Diffraction (XRD), textural attributes, and melting point. Treatment differences were determined using a two-way analysis of variance (2-way ANOVA) and mean differences were calculated using the Tukey's honest significant different (HSD). Data were analyzed using the Statistical Software RStudio (RStudio, Boston, MA).

In Phase 1, temperature cycling resulted in decreased visual and textural quality. Tempered samples with 5% palm stearin presented less WI at 34°C cycle 3 as compared to tempered controls, indicating that chocolate with 5% palm stearin can resist some degrees of temperature changes. Tempered samples without palm stearin presented lower hardness than sonicated samples before cycling. Cycling at 34°C exposed greater hardness in tempered chocolate w/o palm stearin than in sonicated samples w/o palm stearin. Samples with 5% palm stearin, tempered or sonicated had less physical changes after cycling at 37°C for 3 cycles. Polymorphic form 5 was obtained with a melting point range of 32 to 35°C.

In Phase 2, temperature cycling resulted in decreased visual and textural quality. Sonication and fractionation of lipid were able to significantly reduce bloom formation after 3 cycles at 34°C, indicating that chocolate with cocoa butter stearin and 5% palm stearin can resist some temperature fluctuations. Sonication applications, w/o palm stearin after cycling experiment, did not present

significant changes in hardness value, indicating that sonication with fractionated fats can maintain the same value of hardness after cycling experiment. Chocolate with fractionated lipid did not spread dimensionally after cycling, while control samples spread dramatically. Polymorphic form 5, the optimal form in chocolate, was obtained with ultrasound and fractionation technologies with a melting point range of 31 to 34°C.

This research demonstrates the potential application of fractionation with ultrasonication technology for producing quality chocolate. Optimization of this combination of ultrasonication and fractionation may have great implications for the chocolate industry in tropical regions.

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CHAPTER 1: INTRODUCTION

Chocolate is a unique product that involves several physical and chemical processes that require diverse technological operations to obtain desirable quality and sensory characteristics. The final product is a complex emulsion which could be used to create a different range of products in the confectionary industry. The appropriate melting behavior is one of the main aspects of chocolate; solid at room temperature that melts on ingestion when it is dissolved in oral saliva, with the final assessment of texture. Chocolate characteristics are evaluated in terms of appearance, taste, mouthfeel, flavor and aftertaste. Chocolate quality can be affected by storage conditions, improper processing (e.g., tempering), and solid fat content; potential negative impacts include fat bloom or sugar bloom formation (Afoakwa, 2014).

The cacao industry is growing rapidly in tropical countries, i.e., Ecuador, Honduras, Guatemala, and Brazil. The feasibility of producing chocolate in developing countries is affected by costs, raw materials, and weather conditions. These aspects play an important role; yet they are often difficult to control. Optimizing production of high-quality chocolate should result in reduced time and production costs as well as reduction of post-production defects.

Palm oil is one of the most commonly produced vegetable oils worldwide. It is produced in tropical regions and contains larger amounts of carotenes and tocols than other vegetable oils (Nisha, 2015). Palm stearin has been used to replace cocoa butter because it is less expensive and has similar triacylglyceride (TAG) composition (Lipp & Anklam, 1998). Also, the characteristic red-orange color of palm oil can be hidden in the formulation due to the dark color of the chocolate.

Lipid crystallization is the formation of a solid from a liquid; during this process, different phases occur involving, achievement of a thermodynamic driving force for crystallization, nucleation, crystal growth, and recrystallization (Akoh, 2017). Recently, high intensity ultrasound

(HIU) has gained considerable attention for its ability to use acoustic waves at low frequencies (20 to 100 kHz) and high power (10 to 10,000 W cm^{-2}) to control nucleation and crystallization of lipid systems (Higaki, Ueno, Koyano, & Sato, 2003). It has been demonstrated that HIU application to dark chocolate formulation results in optimization of crystallization behavior of cocoa butter into polymorphic form V. According to Akoh (2017), the most remarkable effects of sonication are perceived in the physical properties such as hardness, elasticity, and melting behavior. Rosales *et al.* (2014) reported that HIU manipulated the crystallization behavior of dark chocolate obtained, and that polymorph V was achieved during the nucleation phase.

This research is focused on the formulation and processing of dark chocolate to enhance quality and temperature stability during storage. One of the goals was to create sustainable businesses with more stable chocolate in tropical regions. During summer 2017, a trip to Honduras allowed us to visit a small cacao foundation (DACHOJ) that produces cacao plants as well as chocolate. A collaboration to provide solutions to improve chocolate production was established and in turn this enriched our learning of the entire chocolate production chain and provided us with research areas centered on our theme of improving temperature stability of chocolate for production in tropical areas.

CHAPTER 2: LITERATURE REVIEW

2.1 Chocolate

Chocolate is a mixture of cocoa mass and sugar suspended in a cocoa butter matrix. The expectation of consumers is that they will experience the smooth texture and mouthfeel of the chocolate as it slowly melts in the mouth. This is due to the interaction between polymorphic lipid structures of cocoa butter (Loisel, Keller, Lecq, Launay, & Ollivon, 1997). According to Hosking (1994), the human tongue can detect particles small as 20-30 microns; emphasizing the importance of chocolate processing. The continuous lipid phase in chocolate influences mouthfeel and melting properties. Chocolate triglycerides are composed of primarily saturated stearic (~34%) and palmitic (~27%) acids and monounsaturated oleic acid (~34%). Chocolate is solid at room temperature (20-25°C) and melts at body temperature (37°C). Chocolates contain antioxidants such as polyphenols, and flavonoids that have potential to reduce the risk of cancer. Additionally, chocolate consumption is associated with reduced risk of cardiovascular disease, blood pressure lowering, and improvement of endothelial function, increase in insulin sensitivity, decreased platelet activation and function, and modulation of immune function and inflammation (Afoakwa, 2016).

2.2 Standard of identity in the United States

The Food and Drug Administration (FDA) is the organization that regulates the composition of cocoa products. Title 21 in the Code of Federal Regulations (2010) describes that chocolate and chocolate products must contain cocoa materials with sugar and may contain sweeteners, milk products, and flavoring substances. Dark chocolate must contain not less than 35% of total cocoa solids, of which not less than 18% must be cocoa butter and not less than 14% fat-free cocoa solids and up to 12% milk solids. The addition of vegetable fat, other than cocoa

butter, shall not exceed 5% of the finished formulation, after deduction of the total weight of any other added edible ingredients, without reducing the minimum content of cocoa materials and vegetable fats permitted for this purpose may be approved in applicable legislation.

2.3 Chocolate processing

2.3.1 The cacao bean

Theobroma Cacao grows in warm, moist places in areas approximately 20 degrees latitude north and south of the Equator. The cacao bean consists of an inner nib portion protected by an outer shell. After drying, the cacao bean is composed of different components: Shell (12 to 15%), nibs (fats 48-57%, theobromine 0.8 to 1.3%, caffeine 0.1 to 0.7%, nitrogen 2.2 to 2.5%, ash 2.6 to 4.2%, and water 2.3 to 3.2%) (Kealey, Snyder, Romanczyk, Geyer, Myers, Whitacre, & Schmitz, 2003).

2.3.2 Fermentation of the bean

Fermentation of cacao beans is important for the development of flavor. Cacao pods contain white pulp and cacao beans. During fermentation, the pulp and cacao beans are removed from the pods. Fermentation lasts approximately 5-8 days, during which time the sugar is fermented to alcohol by yeast (Ozturk, & Young, 2017). For the first 24-36 hours the fermentation is anaerobic where loss of fluids occurs due to "sweating" and an increase of pH that inhibits yeast proliferation. When the oxygen concentration increases, lactic bacteria begin to convert ethyl alcohol to acetic acid. The seeds become brown as the tannins polymerize. During fermentation, disruption of cellular membranes occurs resulting in release of packaged enzymes. During anaerobic fermentation, key flavor development occurs; some of these flavor notes include chemicals from the following: enzymatic reactions involving amino acid decarboxylase, amylase, lipase, pectin esterase, peroxidase, polygalacturonase, polyphenol oxidase, phosphatase, and

invertase. Polyphenols are converted to quinones by polyphenol oxidase, then further complexed with amino acids and proteins, decreasing astringency (Zak, 1998). Fermentation is followed by drying to reduce moisture content from approximately 60% to 7%. Fermented cocoa beans are processed into cocoa liquor or cocoa butter, while unfermented cocoa beans do not result in a desirable processed cocoa liquor and are processed only into cocoa butter (Kealey et al., 2003).

2.3.3 Roasting

Roasting is essential for flavor and aroma development and moisture content reduction of the bean (to less than 2%). According to Kealey et al., (2003), roasting is typically a combination of time/temperature, between 5 to 120 minutes and from 100 to 150°C.

2.3.4 Winnowing

Winnowing, or shell removal, is done to obtain the desired portion of the bean (nib). Standard winnowing machines use a combination of sieving and air aspiration. The shells are loosened during roasting; the beans are broken in rollers to shatter the shells and to facilitate their removal. Others processing techniques include a heat pre-treatment such as infrared heating or thermal shock to separate the shell from the nibs (Kealey et al., 2003).

2.3.5 Cocoa liquor formation

The formation of liquor is the next step after winnowing, this involves nib grinding. During grinding two steps are performed, an initial step to obtain a fluid paste and a second step to achieve the desired particle size. Cocoa liquor can be used to make cocoa butter and cocoa powder. Also, cocoa liquor can be mixed with sugar and cocoa butter to make chocolate (Kealey at al., 2003).

2.3.6 Principles of chocolate

Chocolate is defined by Codex Alimentarius as a product obtained by chemical transformation from a mixture of cocoa nibs, cocoa mass, cocoa press cake, cocoa powder including reduced fat powder, and permitted ingredients and/or flavoring agents (Roberts and others, 2005). Dark chocolate is produced by addition of sugar and cocoa butter to the liquor (Manning & Dimick, 1985). Chocolate has solids that are dispersed in the fat matrix. Many factors affect chocolate's mouthfeel, including particle size distribution of the solid, properties of the fat matrix, and how the chocolate is made.

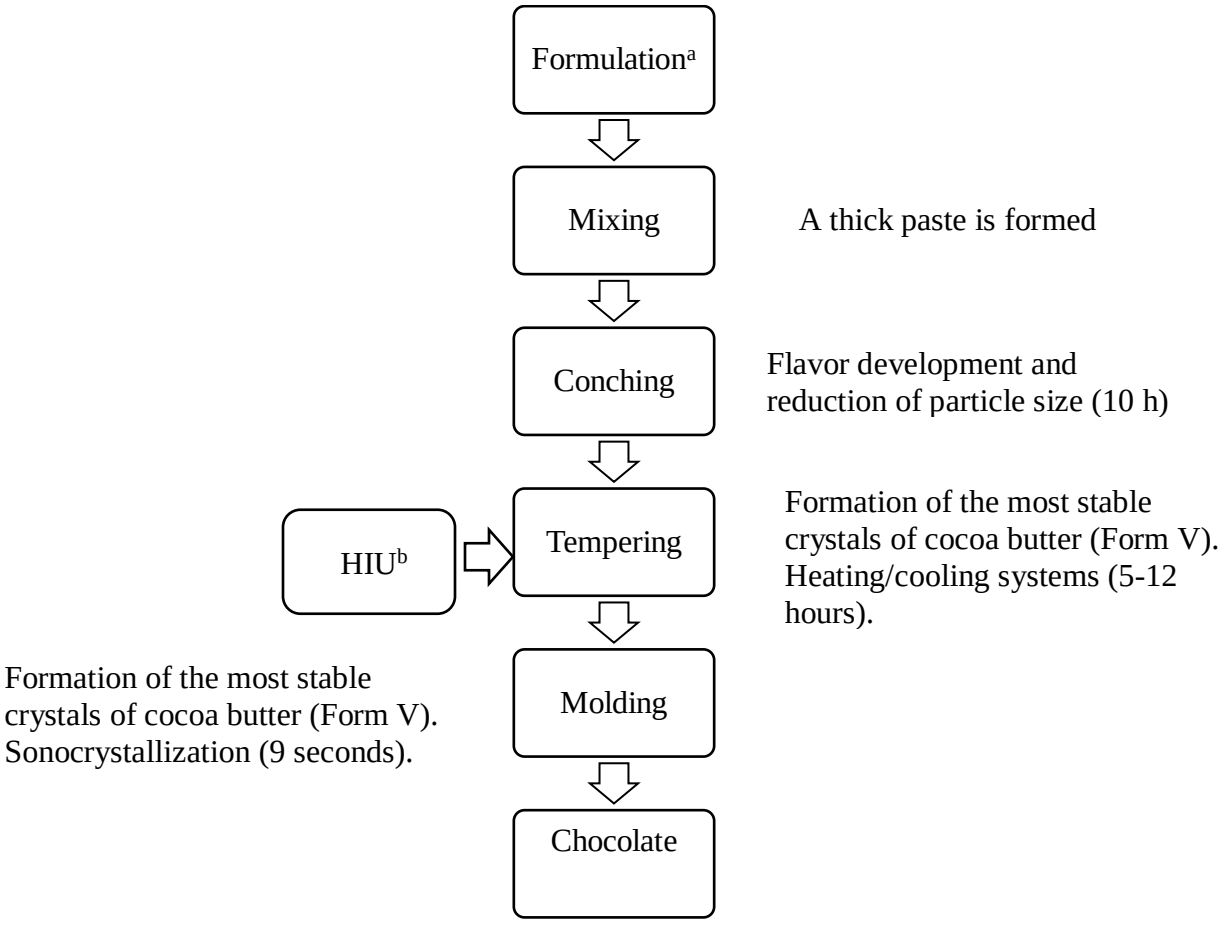

Figure 2.1. Basic processing steps to make chocolate
[a] Includes: cocoa liquor, sugar, palm oil, cocoa powder, and emulsifier
[b] HIU: High Intensity Ultrasound. This is where the ultrasound process was applied
(Adapted from Rosales, 2014)

2.3.7 Conching

Conching is the process of reducing particle size. It also results in the production of desirable flavor **(Figure 2.1.)**. It promotes a continuous fat phase that evenly coats the sugar and cocoa solids (Manning & Dimick, 1985). According to Hui (2005) conching is a process that can vary upon formulation and final product desired, the process can take from 10 to 12 hours up to several days. Conching temperatures range from 49 to 71°C. During this process, additional cocoa butter, flavors, and emulsifiers may be added.

2.3.8 Tempering

Tempering is the last step in chocolate processing and is the controlled cooling of melted chocolate with agitation (**Figure 2.2**). This process promotes the formation of small, stable fat crystals. Tempering includes: heating the chocolate to 43-46°C to melt fat crystals, cooling with agitation to 27-29°C to induce nucleation, and reheating to approximately 30-31°C to promote stable crystal formation (Hui, 2005). To obtain shelf-stable chocolate, tempering is important. Well-tempered chocolate has a glossy appearance, correct textural snap, and a stable lipid phase, which is important in preventing fat bloom (Afoakwa, 2008).

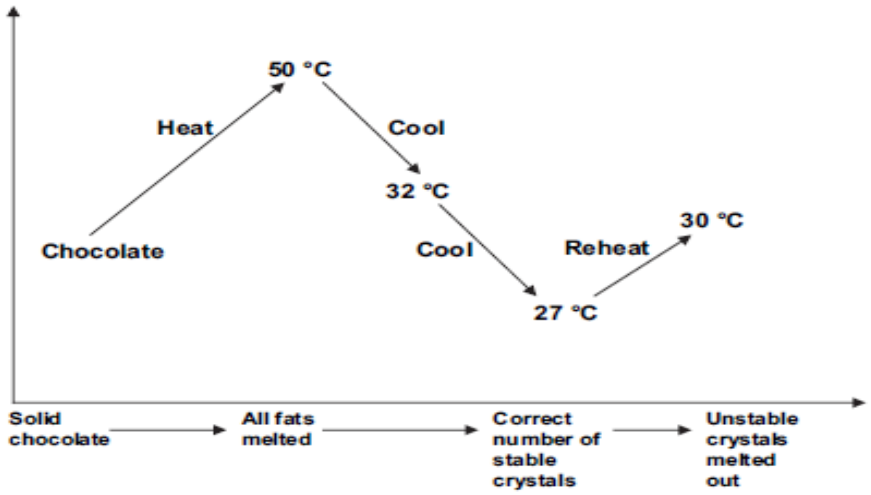

Figure 2.2. Tempering profile during lipid crystallization in dark chocolate (Beckett, 2001)**.**

2.4 Chocolate quality

Shelf life in food is defined as the specific time when a product does not have the expected quality for consumers. Shelf life, safety, and quality of food products are related, but the quality loss should not compromise safety (Side, 2008). Chocolate has a long shelf life without microbial growth (Loisel et al., 1997). However, quality in chocolate can be compromised, as improper storage conditions affect texture, flavor, and visual changes. Fat and sugar bloom are two of the main quality defects in chocolate. The development of either fat bloom or sugar bloom in chocolate is mainly due to improper storage (Morgan, 1994). Chocolate quality also depends on the particle sizes of the cocoa mass and sugar that are suspended in a cocoa butter matrix (Nightingale, Lee, & Engeseth, 2011).

2.4.1 Quality defects in chocolate

2.4.1.1 Fat bloom

Fat bloom is one of the principal problems in the chocolate industry, resulting in a large sales losses (Confectionery News, 2017). Fat bloom appears as a whitish coating on the chocolate surface (Briones and Aguilera, 2005). Fat bloom in chocolate can be a result of improper tempering, incompatible fats, and storage conditions (i.e., temperature fluctuations). Polymorphic transitions, incompatibility, fat dissolution, and lipid migration are some of the theories that have been proposed to explain fat bloom formation in chocolate during storage. For example, in temperature fluctuation three steps are proposed: dissolution of higher melting fat into the lower melting fat, migration of liquid fats to the surface, and recrystallization of higher melting fat on the surface to the most stable polymorph (Jin & Hartel, 2005). The polymorphic transformation theory suggests that cocoa butter crystals change from less stable to more stable polymorphic forms. According to Bui and Ross (2014), polymorphic forms have different melting points. Form I, the least stable

with lowest melting point (16–18°C); Form II and IV are soft in texture, melting at 24-26°C, and 26-28°C, respectively. Form III is a mixture of form II and IV, melting at 25°C. Form V, is most desirable because it melts below body temperature (32–34°C), gives a good snap, and imparts a glossy appearance to the chocolate. Finally form VI, is the transformation of form V and where blooming develops with a melting range of 34–36°C.

2.4.1.2 Sugar bloom

Sugar bloom is another problem in the chocolate industry, caused by water dissolving sugar on the chocolate surface; then evaporation of water occurs. Dissolved sugar recrystallizes on the surface of the chocolate causing whitish spots. Sugar bloom is less common than fat bloom but its appearance looks similar. Furthermore, chocolate with sugar bloom feels dry to the touch and does not melt, as compared to fat bloom. Warmer environments, high relative humidity without proper packaging, and time to gradually decrease the temperature are the causes of this defect (HUI, 2005). According to Nightingale, Lee, and Engeseth (2009), chocolate stored at 94% relative humidity did not present sugar bloom because water was not able to evaporate; thus recrystallization did not occur. However, chocolate quality was impacted as it was more wet, more cohesive, gritty, and chewy than properly stored chocolate. They also concluded that optimal storage for chocolate would be with relative humidity less than 50% and without temperature fluctuation.

2.4.2 Texture, mouthfeel, and flavor

Interaction of the polymorphic forms of cocoa butter, is responsible for the smooth texture and mouthfeel in chocolate (Loisel et al., 1997). According to Morgan (1994), chocolate is one of the most mystifying flavors known; there is not a single molecule that can describe its flavor. However, combinations of compounds have been identified. Improper storage conditions of

chocolate can result in structural changes, affecting texture, mouthfeel, and flavor. This is due to increases in particle size and development of either fat bloom or sugar bloom.

2.4.2.1 Role of lipids in quality

Lipids play an important role in the quality of foods by influencing texture, mouthfeel, and flavor. There are many physical and functional properties of interest to the food industry such as crystal size and morphology, hardness, viscoelasticity, melting behavior, solid content, and polymorphism (Wagh, Birkin, & Martini, 2016). The crystallization behavior of lipids has two important applications: (1) production of end products made of fat crystals such as chocolate, shortening, and whipping cream, and (2) separation of fat or lipids from natural resources (fractionation). In the crystallization of lipids, at least three types of polymorphism exist: alpha (α), beta-prime (β'), and beta (β). The type of polymorphism of TAGs has a significant impact on the functionality of the end lipid. The least organized crystal structure is the α-form, exhibits a hexagonal crystalline form and is characterized by low-density structure, resulting in low melting point. This form can be identify using X-ray diffraction short spacing with a single diffraction pick (d=0.415 nm) which corresponds to the hexagonal packing of the TAGs chains. The β'-form is more stable than the α-form, yet less stable than the β-form. **Figure 2.3**, is a depiction of the structure of the three forms. The β-form and β'-form are characterized by two diffraction peaks (d=0.420 nm and 0.380 nm) that are associated with the orthorhombic perpendicular configuration. The β-form is commonly associated with chocolate (Polymorphic form V), which gives its characteristic properties. Fractionation of lipids has been increasing over the last several years because of market demands, e.g., creation of trans-fat free products (Sato, 2001; Hashimoto, Nezu, Arakawa, Ito, & Maruzeni, 2001; Lorente, Hapońska, Clavero, Torras, & Salvadó, 2017).

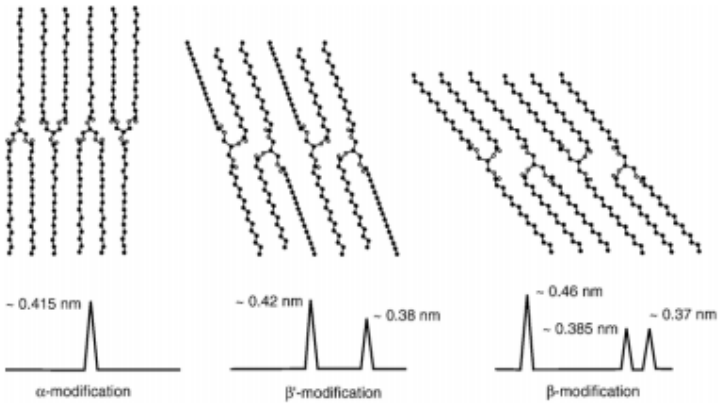

Figure 2.3 Molecular arrangement in the different triglyceride polymorphs and of the resulting XRD patterns (Akoh, 2017).

2.4.2.1.1 Cocoa butter

Cocoa butter (CB) is the main lipid in chocolate, which is solid at room temperature (21-24°C). Above these temperatures, the fat melts gradually until is completely melted at 36°C. This characteristic, melting at body temperature, of the cocoa butter provides the smooth, creamy mouthfeel of chocolate (Kealey at al., 2003). CB plays an important role in chocolate; it forms the continuum of the dispersion forming block aggregates dispersed in its solid phase. Melting CB can reverse the polymorphic structure, allowing it to return to its original structure. Cocoa butter provides a unique property to confectionary products due to its melting characteristics and links to flavor release (Ollivon, 2004).

2.4.2.1.2 Red palm oil

Red palm oil (RPO) comes from the fruit of the Oil Palm tree (*Elaeis guineensis jacq*). It has a peculiar red-orange color that can be used to create different products in the confectionary industry (**Figure. 2.5**). It contains compounds beneficial for human health, e.g., triacylglycerols, vitamin E, carotenoids, and phytosterols. Some of these phytochemicals, are recognized as antioxidants that act against reactive oxygen species, play a role in aging, boost the immune system,

anti-inflammatory, antiviral, antibacterial, and cancer prevention (Mayamol, Balachandran, Samuel, Sundaresan, & Arumughan, 2007; Chandrasekaram, 2009; Mancini, Imperlini, Nigro, Montagnese, Daniele, Orrù, & Buono, 2015). RPO is the richest natural resource of β-carotene (500-1500 ppm), which is a precursor of vitamin A or retinol. Vitamin A deficiency affects many children in developing countries. Vitamin A is essential for good vision, growth, and embryonic development (Mayamol at al., 2007).

RPO can be fractionated into a liquid fraction, palm olein, that is used to produce cooking oil, and a solid fraction, palm stearin, that is sold as an inexpensive byproduct (Chandrasekaram, 2009) (**Figure 2.4**). Palm stearin has a high melting point (47-54°C) favoring the β crystal structure, which is desirable in chocolate.

Figure. 2.4 Palm oil fractionation, crystallization process (left) and filtration (right).

Figure. 2.5 Process of red palm oil fractionation. Red palm tree (top left), red palm nut (top center), commercial red palm oil (top right), Separation of different fats (bottom right), palm stearin (bottom center), and palm olein (bottom left; left) and palm stearin (bottom left; right). (Adapted from Garrow, 2015)

2.4.3 Processing impact on chocolate lipids

2.4.3.1 Tempering

Tempering is a process requiring controlled temperatures, involving mixing, heating, and cooling the chocolate liquid. This process gives the chocolate the most desirable crystal form, Form V (Hui, 2005). Polymorphic form V provides the chocolate characteristics that are desired by consumers (Ollivon, 2004). According to Afoakwa (2008), tempered chocolate should have a good shape, color, gloss, stability, be more heat resistant, and have a longer shelf life. Highly sophisticated machines have been developed to create well-tempered chocolate. Control of tempering is important to obtain quality chocolate because, under tempered, over tempered, and non-tempered chocolate can produce fat bloom enabling the formation of unwanted crystals. Polymorphic form VI is normally associated with fat bloom formation, which is formed as a result of poor storage conditions or poor manufacturing of chocolate.

2.4.3.2 Ultrasonic processing of lipids

High intensity ultrasound (HIU) has been demonstrated to change the crystallization behavior of lipids (Rosales, 2014; Suzuki, Lee, Padilla, & Martini, 2010; Ye & Martini, 2014). According to Wagh at al., (2016), sonication can induce crystallization, increase crystallization rate, and create a harder and more elastic crystalline network with smaller crystals and a sharper melting profile. HIU is an invasive technique that utilizes acoustic waves (20-100 kHz) and high power (10-10000 W Cm^{-2}); this promotes primary and secondary nucleation of fats through cavitation and streaming (Ye & Martini, 2014). HIU promotes the formation of stable polymorphic forms. Previous research demonstrated the use of this technology in chocolate (Rosales, 2014), where 20 KHz with 100 % amplitude for 9 seconds, induced polymorph V in chocolate; tempering was compared to sonication, demonstrating that ultrasonication can be used as an alternative to tempering in chocolate. Also, HIU has been utilized to induce crystallization in palm oil (Suzuki et al., 2010; Ye & Martini, 2014); 20 kHz operation frequency, 75 W of power at 35°C crystallization temperature was utilized to generate a crystalline network with higher solid fat content, higher elasticity, and sharper melting profile than a non HIU control.

CHAPTER 3: STRATEGIES TO INFLUENCE TEMPERATURE STABILITY OF STORAGE DARK CHOCOLATE

3.1 Introduction

Chocolate is a complex matrix of cocoa butter, cocoa and sugar. When eating chocolate consumers expect smooth texture and mouthfeel as it slowly melts in the mouth. These characteristics are due to the interaction between polymorphic lipid structures of cocoa butter. Tempering, is the process where chocolate obtains the appropriate polymorphic form in cocoa butter (form V) which influences important physical and functional characteristics in the chocolate (Hachiya, Koyano, & Sato, 1989). This is a cooling/heating system with a long time requirement, thus is costly. Uncontrolled storage due to variations in temperature may lead to the development of specific polymorphic crystals associated with fat bloom or sugar bloom, which compromises chocolate's visual and textural quality and flavor release.

Susceptibility to melting and fat bloom formation are the main causes of quality loss in the chocolate industry, especially in tropical areas. Many factors are responsible for this including tempering, storage conditions, and solid fat content; these have been identified as critical to fat bloom developmetn in chocolate (Jin & Hartel,, 2015). High Intensity Ultrasound (HIU) of lipids has been demonstrated to influence crystallization by promoting primary and secondary nucleation of lipids through the mechanisms of cavitation and acoustic streaming (Ruecroft et al., 2005). It has been demonstrated that ultrasonication technologies at low frequencies (20 KHz) for 6-9 seconds can be utilized to yield similar characteristics to traditional tempered chocolate (Rosales et al, 2014).

Multiple theories exist about the complexity of bloom formation. Some of the critical factors include storage condition and solid fat content. It has been demonstrated that increase of

solid fat content in chocolate could result in less bloom formation and higher melting point. Chocolate with low solid fat content increases the amount of high-melting fats in the liquid phase, thus leading to more fats mobilized and recrystallized on the surface. Also, chocolate with lower solid fat content would increase the amount of liquid fats to migrate in the system resulting in greater bloom formation (Jin & Hartel., 2015).

Recently, the use of palm oil in the chocolate industry has gained attention for its ability to imitate chocolate triacyglycerides (TAGs), its content of pro-vitamin A β-carotene (500-1500 ppm), and its lower cost as compared to cocoa butter. Several groups have shown its ability to replace cocoa butter in great efficiency (Hashimoto et al., 2001; Lipp & Anklam 1998; Mayamol at al., 2007).

The objective of this study was to evaluate the combination of treatments, i.e., formula modification and HIU, in order to increase the quality of dark chocolate, especially in the tropics. Cocoa butter in the initial formula was replaced with 5% plam stearin and combined with HIU as a treatment. The secondary objective was to evaluate the combination of lipid fractionation, obtained from either palm oil or cocoa butter, and HIU as treatments to increase melting point and reduce bloom formation.

The central hypothesis was that the combination of fractionated fats from raw palm oil and cocoa butter and HIU will result in high-quality chocolate with reduced fat bloom formation and increased melting point.

3.2 Materials and Methods

Chocolate liquor was purchased from Peters Chocolate (ILA, Springfield, IL); granulated sugar (C&H, Dominos Foods Inc., ASR group) was acquired from a local grocery store; cocoa butter (Mary Tylor Naturals, LLC, Corinth, NY); cocoa powder (The Hershey's Company, PA Red palm oil (Spicy World, Houston, TX) and liquid soy lecithin (Fast Easy Bread; http://fasteasybread.com/) were acquired from Amazon. This research was divided into two phases. Phase 1; Dark chocolate was created with palm stearin (5%, replacement of cocoa butter). It was conched and either tempered or sonicated. Phase 2; Dark chocolate was formulated with fractionated lipids (stearin fraction) and cocoa powder, conched, and either tempered or sonicated. Since, the described two phases have similar materials and methods, the results are presented here in one section.

3.2.1 Chocolate samples

Dark chocolate was formulated according to Rosales (2014) using a Premier Chocolate Refiner (Sivanesan Company, Chennai, India) and a Revolution 2 Chocolate Tempering System (Chocovision^{TM}, Poughkeepsie, NY). Samples were prepared in triplicate 1.5 lb batches. The original formulation was composed of chocolate liquor (353.80 g), sugar (326.59 g), and emulsifier (soy lecithin) at 0.5% (w/w); 3.40 g. This Chocolate liquor contained ~50% lipid and ~50% solids. Based on this 5% palm stearin was added based on a calculation of the lipid component of the liquor (8.85 g). In order to have a balance in the formulation, 8.85 g cocoa solids was also added. The final formulation for phase 1 was 336.1 g chocolate liquor, 8.85 g cocoa solids, 8.85 g palm stearin, 326.59 g sugar, and 3.40 g lecithin. In phase 2, chocolate was made with the stearin part of each lipid source, resulting in 168.05 g cocoa butter stearin, 8.85 g palm stearin, 176.90 g cocoa powder, 326.59 g sugar, and 3.40 g lecithin. For both phases, controls were the same formulation

without the addition of palm stearin. After addition of emulsifier, samples were divided into four groups and either a) tempered and molded, containing palm oil, and b) tempered and molded, without palm oil, or c) sonicated and molded, containing palm oil, and d) sonicated and molded, without palm oil (**Table 3.1, 3.4**).

3.2.2 Palm oil fractionation

Red palm oil (RPO) was fractionated to yield a high olein fraction (P-O; liquid at room temperature) and stearin fraction (P-S; solid at room temperature). According to Deffense (1985), dry fractionation is economical, easy, and no harsh chemicals are involved. RPO was heated to 60-80°C to remove all crystal memory. The temperature was decreased to 20°C to crystallize the palm stearin fraction and left overnight. The crystallized lipid mixture was filtered in a Büchner funnel lined with Whatman #1 qualitative filter paper, under vacuum. The solid mass was melted at 80°C, then a second filtration, to remove impurities of the solid mass, was performed. The collection flask was heated (to 60°C) to minimize solidification. The filtered mass was transferred to an amber jar fitted with a screw-cap plastic lid and stored in the dark at ~23°C.

3.2.3 Cocoa butter fractionation

Cocoa butter stearin (CB-S) was fractionated from cocoa butter and used as high melting fat. Dry fractionation was performed (According to Jin & Hartel, 2015). CB was heated to 60°C for 1 hr to destroy any crystal structure. The melted mass was cooled and left undisturbed for 24 hrs at 26°C. The crystallized coca butter was filtered in a Büchner funnel lined with Whatman #1 qualitative filter paper, under vacuum. The filtered mass was transferred to a dark jar fitted with a screw-cap plastic lid and stored in the dark at ~23°C.

3.2.4 Tempered samples, with and without palm oil

Dark chocolate samples formulated with and without palm oil was tempered using Revolution 2 Chocolate Tempering System (ChocovisionTM, Poughkeepsie, NY). Tempering protocol was established based upon protocols of Tisoncik (2010) and Rosales (2014). Chocolate (300 g) was transferred into the stainless-steel bowl and stirred by a U-shaped stainless-steel mixer with a scraper blade. The continuous movement encouraged the formation of crystal nuclei and influenced growth and agglomeration of crystals. Constant mixing also removed the crystallized mass from the cool chamber of the temperer, thus permitting uniform heat transfer throughout the chocolate. Tempered chocolate was directly molded in 25mm x 25mm x 10mm square polycarbonate molds (Kerekes, Brooklyn, NY). Molds were tapped for two min to decrease air bubble formation and chocolate was allowed to recrystallize at room temperature overnight (23°C). Chocolates were de-molded and placed at room temperature (23°C). The tempering profile used for chocolate was as follows: melt at 44°C and maintain movement for approximately 25 min to melt fat crystals. This was followed by cooling to 28°C at a rate of ~ 2°C/min, holding 8 min to induce crystallization and finally increasing to 29°C and holding 6 min to melt lower melting polymorphs, leaving the mixture with the high melting polymorphic form V.

3.2.5 Sonicated samples, with and without palm oil

Ultrasonication of sample formulations was conducted using a laboratory scale batch-mode utilizing a 750 W ultrasonic processor (Sonics and Materials, Inc., Newtown, CT). All samples were processed at 20 kHz with 100% amplitude. Sonication protocol was based upon that of Rosales (2014). A water-jacketed beaker (100 mL) was utilized (at 33°C) to assure temperature control, containing 100 g liquid chocolate (33°C). The temperature of the chocolate was monitored with Digital Temperature Controller ETC-111000-000 (Ranco North America, Plain City, OH),

and the target temperature to begin sonication was 33°C. The ultrasound probe (25 mm dia., 750 W generator rating power was submerged in the liquid chocolate to a depth of 15 mm from the bottom of the jacked beaker. Samples were exposed to ultrasound for 9 s. Ultrasound-treated samples were molded at 29°C.

3.2.6 Chocolate storage: cycling experiments

To induce fat bloom formation in chocolate, cycling experiments are usually conducted (Lan & Ross, 2014). After molding and recrystallization, samples were exposed to different temperature cycles: 2 h cycling (2 h on and 2 h off) at 34°C or 37°C for 3 cycles. Samples were cycled in a digitally controlled incubator (± 0.1°C; BRINKMANN Incubator 1000; 1085 Vista Sorrento Parkway Suite 200 San Diego, CA) and removed after 2 h and left 2 h at room temperature. All samples were cycled a day after preparation and stored at ambient temperature (23°C). Characteristics of quality in chocolates samples were evaluated to assess the impact of ultrasound and palm oil before and after cycling.

3.2.7 Texture profile analysis (TPA)

Chocolate samples were examined with a TA-XT2 Texture Analyser (Texture Technologies Corp; Scarsdale, NY) and Texture Expert Software v. 1.11. Texture profile analysis was executed using a 4 mm cylinder stainless steel probe (P4 DIA) for the two-bite compression test (25% compression). Settings of the test were established as defined by Afoakwa and others (2008a): pretest speed of 2 mm/s, test speed of 5 mm/s, post-test speed of 5 mm/s, 25% deformation, relaxation time of 5 s, and force of 20 g. As defined by Texture Technologies (2003); hardness, gumminess, and adhesiveness were calculated (**Figure 3.1**).

3.2.8 Color evaluation

Changes in color were examined using a HunterLab LabScan II 0/45 Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were investigated with HunterLab Universal SoftwareTM Version 3.8. Color was evaluated in triplicate on complete chocolate samples. Based on three color parameters (L: 100=white, 0=black; a: 100=red, -100=green; b: 100=yellow, -100=blue). Data were recorded based on those parameters, resulting in an average lightness and darkness. The whiteness index (WI), which is an indicator of fat bloom formation in chocolate was measured by the lightening of the chocolate. WI was calculated using the following equation as reported by Briones and Aguilera (2005).

$WI = 100 - [(100-L)^2 + a^2 + b^2]^{1/2}$

3.2.9 Dimensional analysis

Dimensional changes of chocolate samples before and after cycling were calculated using standard laboratory dial calipers (Scienceware, Pequannock, NJ). Samples were analyzed (l*w*h*) after cycling experiments.

3.2.10 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a technique commonly used in thermal analysis that determines the energy differential between a sample and a reference. When the temperature increases, crystals that are present in the lipid absorb energy from the system in order to melt (Manning and Dimick 1985). The melting point of chocolate samples was determined using a Q2000 Thermal Analysis DSC System (TA Instruments; New Castle, DE). The instrument was calibrated with indium (m.p. 156.59°C) at a scan rate of 5°C/min using an empty aluminum pan as a reference. Melting points were determined for different chocolate samples (1-3 mg) sealed in Tzero hermetic aluminum pans (TA Instruments; New Castle, DE). The DSC temperature in the

post test setting or unloaded temperature, was adjusted (20 to 25°C). The sample chamber was cooled to an initial temperature of -20°C (equilibrium temperature), and samples were automatically placed in the chamber. Pans were heated at a rate of 10°C/min from -20°C to 80°C in a N_2 stream (ramp temperature). Melting points were reported at the temperature (temperature peak) where the major quantity of energy was absorbed by the chocolate sample. Samples were analyzed in triplicate.

3.2.11 Powder X-ray Diffraction

3.2.11.1 Sample preparation

Powder X-ray diffraction (XRD) was utilized to identify the chocolate polymorphs. In order to avoid diffraction interference, sugar was extracted as described by Cebula and Zielgleder (1993). Chocolate samples were prepared as follows: 5 g chocolate was chopped and the powder was vigorously shaken with 500 ml of room temperature deionized water. The mixture was left to stand for approximately 4 h to dissolve the sugar. After 4 h the mixture was filtered under vacuum using Whatman #1 filter paper for approximately 1 h and allowed to dry overnight. After drying, samples were stored in disposable glass scintillation vials until analysis.

3.2.11.2 XRD analysis

Crystal polymorphic transitions were established by the short d spacings of an X-ray diffraction pattern. Patterns of X-ray diffraction were analyzed at room temperature (~25°C) on Siemens Bruker D5000 theta/theta Powder X-ray Diffractor (Siemens-Bruker Instruments, Billerica, MA). Copper radiation (CuKα) with an average wavelength of 1.5418 Å set at 40 kV and 30 mA and a 1° divergence slit was used. Chocolate powder was added to a polycarbonate cell and placed in the instrument. A 2θ scan from 18° to 26°, step of 0.008, and a scan rate of 0.2 degrees/min was utilized. X-ray diffraction is measured by Bragg's law:

$$n\lambda = 2d \sin\theta$$

Where n is a positive whole number, λ is the X-ray wavelength, d = space between crystal planes, and θ is the angle of incidence. Short d spacings are used in the 2θ range of 16-30° to identify polymorphs. Polymorph V will have four distinct d spacings and polymorph VI will have only three; this can be used to demonstrate a shift in crystal lattice orientation.

3.2.12 Statistical analysis

Data were analyzed using the Statistical Software RStudio (RStudio, Boston, MA). Treatment differences were evaluated using analysis of variance (2-ANOVA) with interactions. If factors effects were significant, mean differences were evaluated using Tukey's honest significant difference (HSD). A linear model was used to determine differences in each combination of factors. The level of significance was chosen as $p \leq 0.05$.

3.3 Results and Discussion

PHASE 1: Effect of the addition of palm stearin on dark chocolate properties

In phase 1, 5% of palm stearin was added to the chocolate formulation to evaluate the impact on melting point, polymorphic form, texture, color, and dimensions after cycling. Analysis of the four group of samples were performed a) tempered and molded, containing palm oil, and b) tempered and molded, without palm oil, or c) sonicated and molded, containing palm oil, and d) sonicated and molded, without palm oil (**Table 3.1**).

3.3.1 Effect of treatment on melting point

Addition of 5% palm stearin to the chocolate formulation, either tempered or sonicated, after cycling did not present significant changes in melting point. The combination of 5% palm stearin with sonication, achieved the desired crystal structure, polymorphic form V (**Table 3.2**); this indicates that sonication with 5% palm stearin addition to the chocolate formulation, results in

chocolate of similar melting properties to traditional tempered chocolate. These results are similar to the findings of Jin and Hartel (2015), who reported that chocolate with addition of different types of fats achieved polymorphic form V. Control samples (tempered or sonicated, without palm stearin addition) also achieved polymorphic form V. Rosales (2014) reported similar results where sonication (for 6 and 9 seconds) presented the desired crystal form, polymorph V.

Temperature fluctuations during cycling, did not affect melting point (**Table 3.3**). There are a range of possible outcomes predicted from the literature with regards to temperature cycling of chocolate. Jin and Hartel (2015) reported changes in melting points as a result of chocolate temperature cycling. However, different temperatures with longer time exposures were used than those used in this study (one cycle = 7 hours at 20 °C, and 7 hours at 30 °C. For 99 cycles).

Tempering is a traditional process in chocolate manufacturing because it induces crystallization of cocoa butter into the desired polymorphic form V. This polymorphic form is desired because is less susceptible to fat bloom. Tempering requires a series of energy intensive heating/cooling steps; while sonication only requires a brief burst of energy.

3.3.2 Effect of treatment on Whiteness Index (WI)

Addition of 5% palm stearin in the chocolate formulation, either tempered or sonicated, after cycling presented significant changes in WI (**Figure 3.2, 3.3**). Whiteness index increased after cycling as expected. Sonicated samples, presented less WI at 34°C after cycling as compared to tempered samples. Tempered samples with 5% palm stearin presented less WI at 34°C cycle 3 as compared to tempered controls ($P<0.05$). Thus, addition of 5% palm stearin and sonication has great promise in chocolate manufacturing.

The instrumental technique to measure WI is not as sensitive as visual determination (Lonchampt et al., 2006). Despite this limitation, WI data showed a general tendency to increase

after cycling (**Figure 3.2, 3.3**). At 37°C, samples showed an increase after cycle 1 but no difference between cycle 1 and cycle 3 was noted (**Figure 3.3**). These results are similar to those of Rosales (2014), where WI of chocolate cycled at 37°C was less dramatic than that of chocolate cycled at 34°C.

Cocoa butter has many implications in the food, pharmaceutical, and cosmetic industry, due to its unique triglyceride composition and melt characteristics. Low production and high demand make cocoa butter expensive as compared to other fats and oils (Jahurul et al., 2014). Tempering, the process to obtain the desired crystal form, polymorph V, is costly and time-consuming. The goal of this research was to use sonication as an alternative to tempering and palm stearin as replacement for cocoa butter. Thus, sonicated samples with 5% palm stearin might experience reduced production costs and less fat bloom formation after extreme temperature exposure.

Conching is another traditional process essential for quality optimization in chocolate. During our trip to Honduras, our chocolate samples were exposed to different temperatures, producing internal bloom. Longer conching time (24 hours) was suggested by members of the foundation (DACHOJ); they use this long conching time to produce their chocolate. Previously, the chocolate was prepared with 10 hours of conching. Experiments with 24 hours of conching were conducted, followed by chocolate samples being exposed to cycling. It was determined that longer conching time did not enhance chocolate quality characteristics. Thus, 24 hours of conching will increase production costs without having a positive impact on the final product.

3.3.3 Effect of treatment on chocolate hardness

Quality chocolate is defined as chocolate with good snap at room temperature, shiny surface, and melt at body temperature to obtain the pleasant mouth-feel sensation. (Afoakwa,

2016). The hardness of dark chocolate samples is the maximum force (N) in the instrumental penetration test; this has been related to simulate sensory analysis to determine texture of dark chocolate (Andrade, 2006).

The combination of 5% palm stearin in the chocolate formulation with sonication, after cycling experiments presented significant changes in hardness. Tempered samples without palm stearin were harder than sonicated samples without palm stearin (**Figure 3.4, 3.5**). Tempered samples without palm stearin were not different than those with palm stearin, indicating that is possible to achieve similar hardness with the addition of 5% palm stearin with tempering.

Cycling of chocolate samples to create temperature fluctuations at 34°C and 37°C influenced hardness (**Figure 3.4, 3.5**). Cycling at 34°C resulted in greater hardness in tempered samples w/o palm stearin than sonicated samples w/o palm stearin. No differences between cycle 1 and cycle 3 either at 34°or 37°C were found, presenting a disagreement with Rosales (2014), where all samples showed a decrease in hardness after cycling. Samples cycled at 37°C with 5% palm stearin, either tempered or sonicated did not present changes in hardness after cycling. Biswas et al. (2017), presented similar results, where addition of 15 grams of palm as cocoa butter replacement, had lower values of hardness.

3.3.4 Effect of treatment on physical dimensions after cycling

Cycling of chocolate samples with temperature fluctuation at 34°C (for 3 cycles) did not influence physical expansion, while cycling at 37°C dramatically impacted the physical dimensions (**Figure 3.6, 3.7**). Similar results were presented by other studies, temperature fluctuation at 37°C suffered significant changes in physical dimensions, appearance, and texture characteristics decreasing chocolate quality (Tisoncik, 2010; Rosales, 2014). Samples with 5% palm stearin, tempered or sonicated had less physical changes after cycling at 37°C for 3 cycles

(**Figure 3.7**). Jin and Hartel (2015), demonstrated that chocolate with the addition of different types of fats, and different solid fat content had better characteristics after cycling.

PHASE 2: Impact of lipid fractionation on dark chocolate properties

In phase 2, chocolate samples were formulated using palm stearin (5%), cocoa butter stearin, and cocoa powder to evaluate the impact on melting point, polymorphic form, texture, color, and dimensions after cycling. Analysis of the four groups of samples included: a) tempered and molded, containing palm oil, and b) tempered and molded, without palm oil, or c) sonicated and molded, containing palm oil, and d) sonicated and molded, without palm oil (**Table 3.4**).

3.3.5 Impact of fractionated lipid on melting point

The melting point of dark chocolate is an indicator of the polymorphic form and the resultant glassy appearance, good snap, and sensory mouthfeel. In tropical regions, the melting point of dark chocolate can be difficult to control because of the exposure to different temperatures during storage. Because of this, bloom formation is one of the main problems in those countries. In Honduras, for example, bloom formation and melting point are the main concerns. The results of this research can be applied in small businesses, e.g., the Honduras association (DACHOJ) to ensure chocolate quality. This foundation, was enthusiastic about the use of lipid fractionation to produce more stable chocolate upon exposure to temperature changes. However, they suggested to use coconut oil instead of palm oil because of the environmental issues that surround palm oil production.

Dry fractionation was used to create chocolate with high melting fats. According to Sato (2001), dry fractionation of lipids has been used to obtain high melting fats without creating trans-fats, to create better confectionery end products, and to obtain better functionality vegetable oils.

Fractionation and combination of lipids (cocoa butter and 5% palm oil) in the chocolate formulation, either tempered or sonicated, after cycling presented changes in melting point (**Figure 3.10**). Similar results were reported by Tisoncik (2010) and Rosales (2014), where cycling resulted in an increase in the melting point of dark chocolate. The combination of 5% palm stearin and cocoa butter stearin with sonication, achieved desired crystal structure, polymorph V (**Table 3.5**).

The melting point of chocolate with added fractionated lipid did not increase as expected (**Table 3.5**). These findings are similar to those of Biswas et al. (2017), where the chocolate with the addition of high melting fats showed a sharp melting peak, but no increase in melting temperature was observed. However, their study did not include sonication and fractionation of cocoa butter. According to Buscato et al. (2017), cocoa butter is composed of the mixture of several triacylglycerol (TAG) types, and those can vary depending on the variety, origin, and season. The saturated-unsaturated-saturated (SUS) symmetrical TAGs represent approximately 80 to 90% of cocoa butter composition, exhibiting a unique packing characteristic that affects crystallization and melting behaviors. In our study, dry fractionation of cocoa butter was used to obtain cocoa butter stearin to create high melting fat chocolate. A more stable matrix was created (**Figure 3.11, 3.12**), but no increase in melting point was found, an explanation of this could be the polymorph form of the chocolate after tempering or sonication. All samples showed the polymorphic form V, thus melting point approximately 32 to 34°C. Sato (2001), explained the impact of polymorphism and molecular interaction on the crystallization and melting properties of lipids.

3.3.6 Impact of fractionated lipid on Whiteness Index (WI)

Formulation of dark chocolate with cocoa butter stearin and 5% palm stearin, either tempered or sonicated, presented significant changes in WI after cycling. WI increased after cycling (**Figure 3.8, 3.9**). Samples cycled at 34°C showed lower WI than samples cycled at 37°C

(**Figure 3.11, 3.12**). Cycling at 34°C or 37°C resulted in more dramatic whitening at 3 cycles than at cycle 1. The WI of sonicated samples was similar to that of tempered samples before cycling. Chocolate samples containing cocoa butter stearin and 5% palm stearin did not experience significance differences in WI at cycle 1 at 34°C from the control (not cycled) samples (**Figure 3.8**), indicating that chocolate with these fractionated lipids can resist some levels of temperature fluctuations. These results are similar with Jin and Hartel (2015) where fractionated cocoa butter with the addition of another fat reduced fat bloom formation at some point of cycling experiment.

The temperature at which dark chocolate is stored can impact chocolate characteristics. It has been demonstrated that fluctuation in temperatures can be one of the main causes of fat bloom formation (Bui & Coad, 2015; Jin & Hartel, 2015; Nightingle et al., 2009). According to Jin and Hartel (2015), fat bloom formation, starts with temperature fluctuation where dissolution of higher melting into the lower melting fats, migration of the lipids to the surface, and recrystallization of the higher melting fat on the surface occur. This can explain why the chocolate with high melting fats with the longer fluctuation of temperatures (cycle 3) presented extremely changes on the surface when compared with no cycle or less intensive cycles (**Figure 3.12**).

3.3.7 Impact of fractionated lipid on hardness

The formulation of dark chocolate with cocoa butter stearin and 5% palm stearin, with tempering, presented significant changes in hardness after cycling. Sonication, without palm stearin, did not present significant changes in hardness after cycling, indicating that sonication with fractionated fats can maintain the same value of hardness after cycling (**Table 3.6, 3.7**). Samples cycled at 37°C did not present significant changes in hardness with exception of tempered samples with 5% palm stearin that showed decreased hardness. These findings are similar to Tisoncik (2010) where lower values of dark chocolate hardness were observed after cycling.

It was previously established that hardness (N) of dark chocolate, is directly correlated to the hardness determined by sensory analysis (Andrade, 2006). It is important to mention that during Texture Profile Analysis (TPA), most of the chocolate samples "broke" after the first bit or during the instrumental penetration. According to Narine (1999), the rheological properties of fats, including hardness, are related to the macroscopic properties that are influenced by the levels of the structure created during the formation of the final network, the TGA composition, and the polymorphic nature of the network. In this study, samples were formulated with stearin fractions, creating a stronger macrostructure network. This can explain why the samples "broke" during TPA analysis.

3.3.8 Impact of fractionated lipid on physical dimensions

Formulation of dark chocolate with cocoa butter stearin and 5% palm stearin, either tempered or sonicated, did not result in significant changes in dimensions after cycling. Previous studies demonstrated that cycling, especially at 37°C, can impact physical dimensions of chocolate (Rosales, 2014; Tisoncik, 2010). In this study, chocolate maintained its physical dimensions after cycling. An explanation of this could be the creation of a stronger network with fractionated fats (Narine, 1999). However, the visual appearance was affected at cycle 3 (**Figure 3.12**), where 37°C was more affected as mentioned in the WI section.

3.4 Conclusion

Palm stearin addition (5%) resulted in significant reduction of bloom formation in dark chocolate. Tempering is costly and time consuming, thus difficult to implement in small companies. As a result, some companies are forced to either eliminate or reduce timing of this process, resulting in lesser quality chocolate. Lipid sonocrystallization has great promises for reducing quality defects in the chocolate industry. It can be used to obtain high quality chocolate that is stable during storage. Cocoa butter is expensive when compared to palm stearin. One can adapt sonication technology with the addition of 5% palm stearin to decrease the need for tempering and reduce the cost of production, creating quality chocolate at a reasonable price.

Dry fractionation of lipids had significant impact on chocolate stability during temperature cycling. It is utilized to obtain better functionality in lipids; it is inexpensive and is a relatively safe and easy to implement process in small companies. Temperature fluctuation can create fat bloom formation and physical changes in chocolate. One of the main problems of DACHOJ was the stability of chocolate because of the high temperatures encountered in Honduras. This research demonstrated the powerful impact of combining sonication with a formulation containing fractionated lipids to achieve quality chocolate characteristics. The combination of sonication with lipid fractionation could be potentially be utilized in tropical regions to achieve more stable chocolate that will resist temperature fluctuation, thus creating a better quality chocolate.

3.5 Figures & Tables

Figures: PHASE 1. Effect of the addition of palm stearin on dark chocolate properties

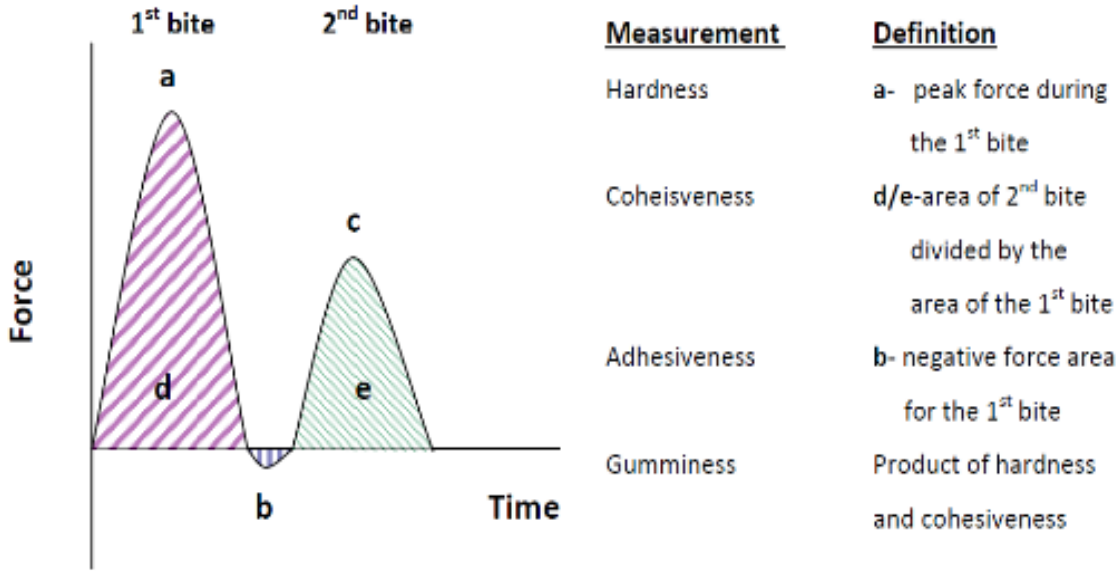

Figure 3.1 Curve for two bite compression test in texture analysis
(Adapted from Andrae, 2006)

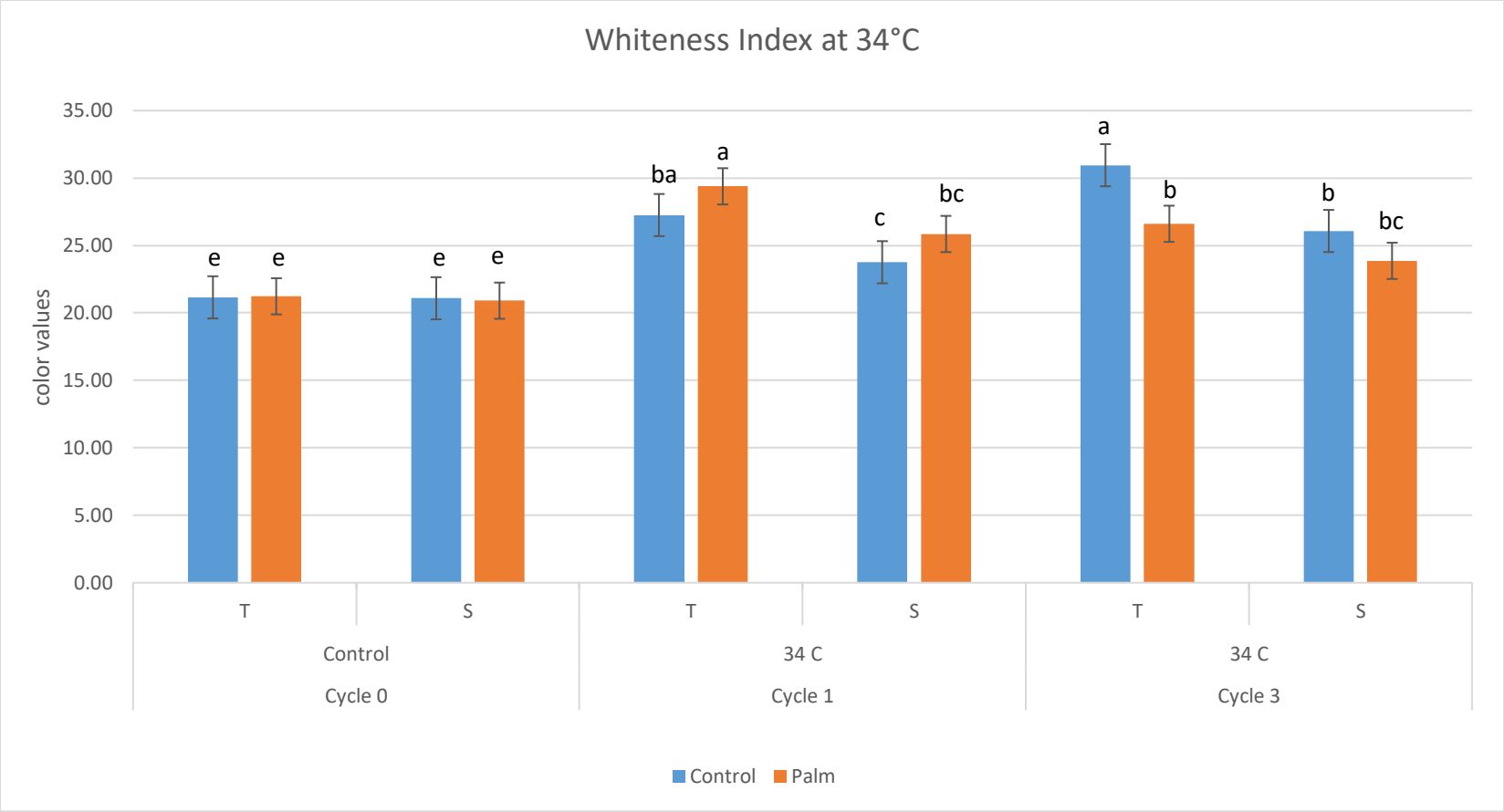

Figure 3.2 Effect of temperature cycling on whiteness index of dark chocolate samples

Bars represent means +SD

T: Tempered chocolate; w/o palm oil; S: Sonicated chocolate w/o palm oil;

a-b Bars with different letters were significantly different ($P<0.05$)

*Means of at least 3 replicates

*Letters were established with the interaction between treatment (tempered vs sonicated), cycle, and oil (palm vs control)

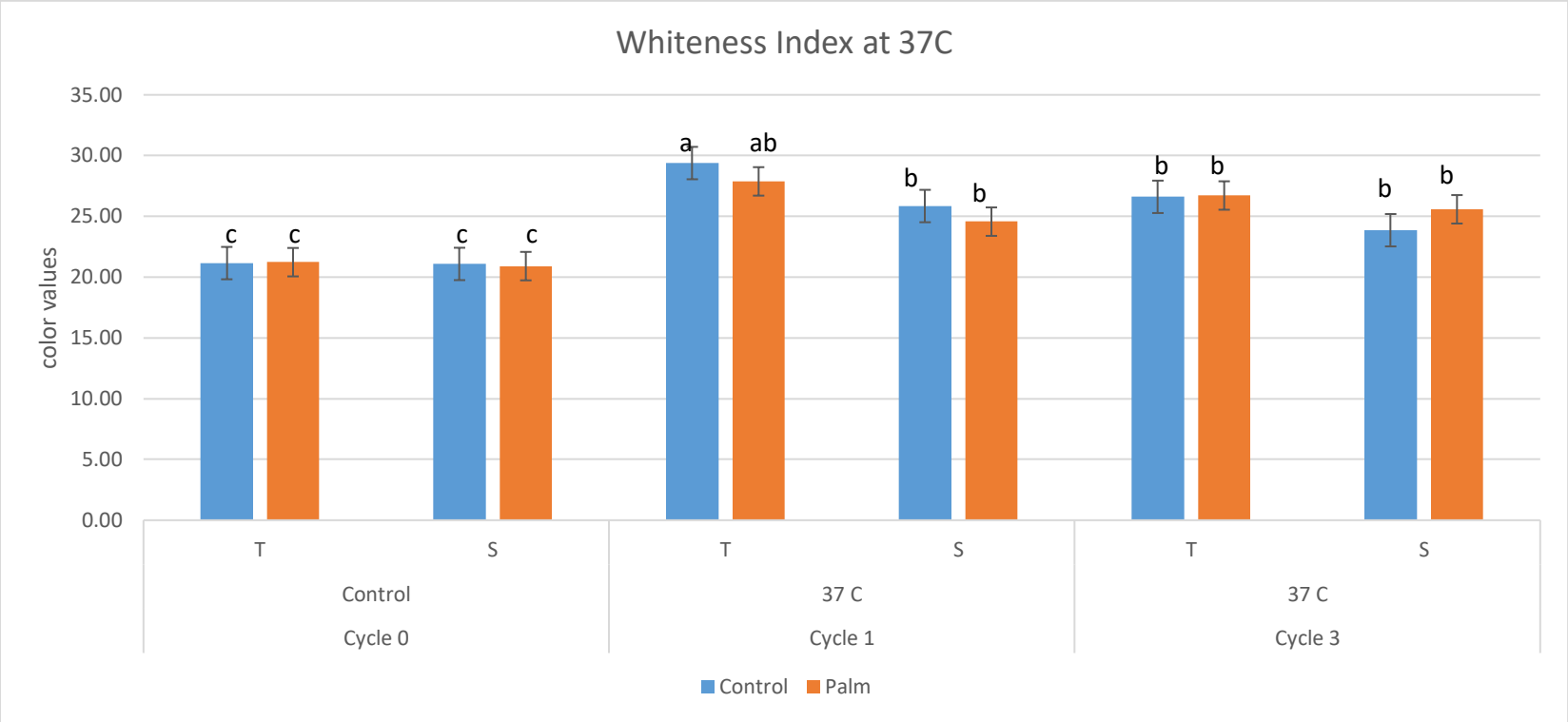

Figure 3.3 Effect of temperature cycling on Whiteness index of dark chocolate samples

T: Tempered chocolate; w/o palm oil; S: Sonicated chocolate w/o palm oil;

Bars represent means +SD

a-b Bars with different letters were significantly different ($P<0.05$)

*Means of at least 3 replicates

*Letters were established with the interaction between treatment (tempered vs sonicated), cycle, and oil (palm vs. control)

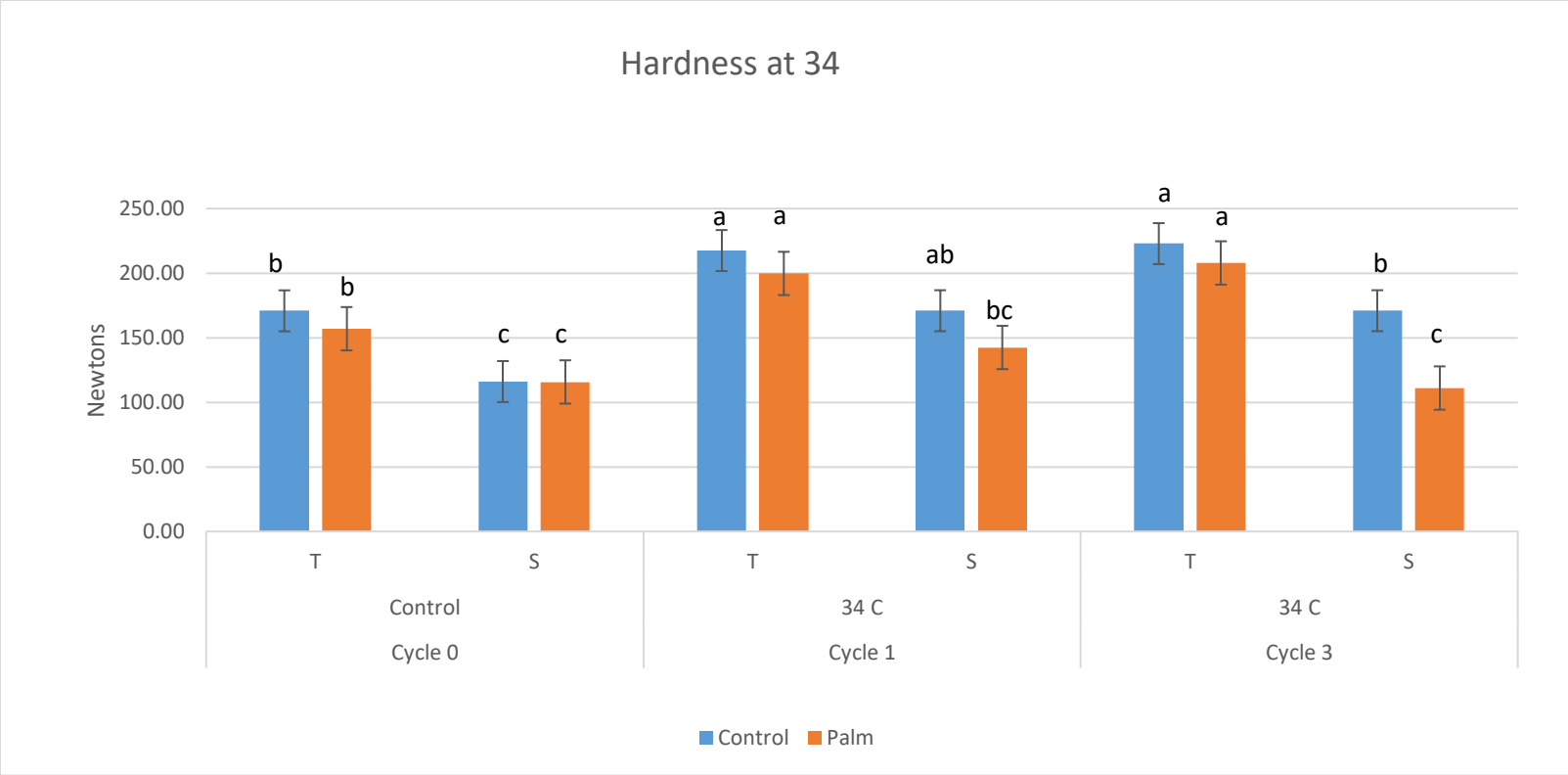

Figure 3.4 Effect of temperature cycling on Hardness of dark chocolate samples
T: Tempered chocolate; w/o palm oil; S: Sonicated chocolate; w/o palm oil
Bars represent means +SD
a-c Bars with different letters were significantly different ($P<0.05$)
*Means of at least 3 replicates
* Letters were established with the interaction between treatment (tempered vs. sonicated), cycle, and oil (palm vs. control)

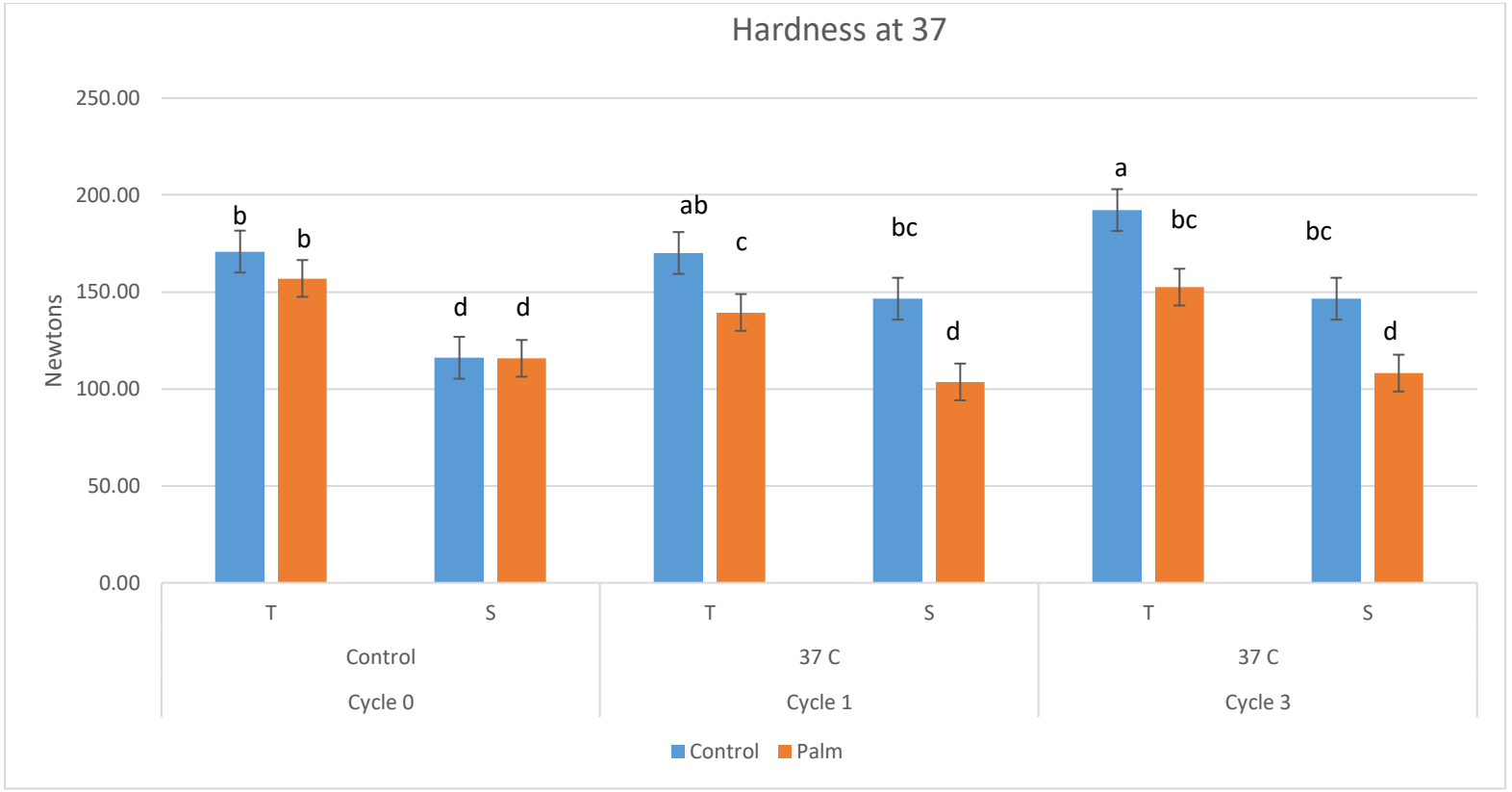

Figure 3.5 Effect of temperature cycling on Hardness of dark chocolate samples
T: Tempered chocolate; w/o palm oil; S: Sonicated chocolate; w/o palm oil
Bars represent means +SD
a-c Bars with different letters were significantly different ($P<0.05$)
*Means of at least 3 replicates
* Letters were established with the interaction between treatment (tempered vs. sonicated), cycle, and oil (palm vs. control)

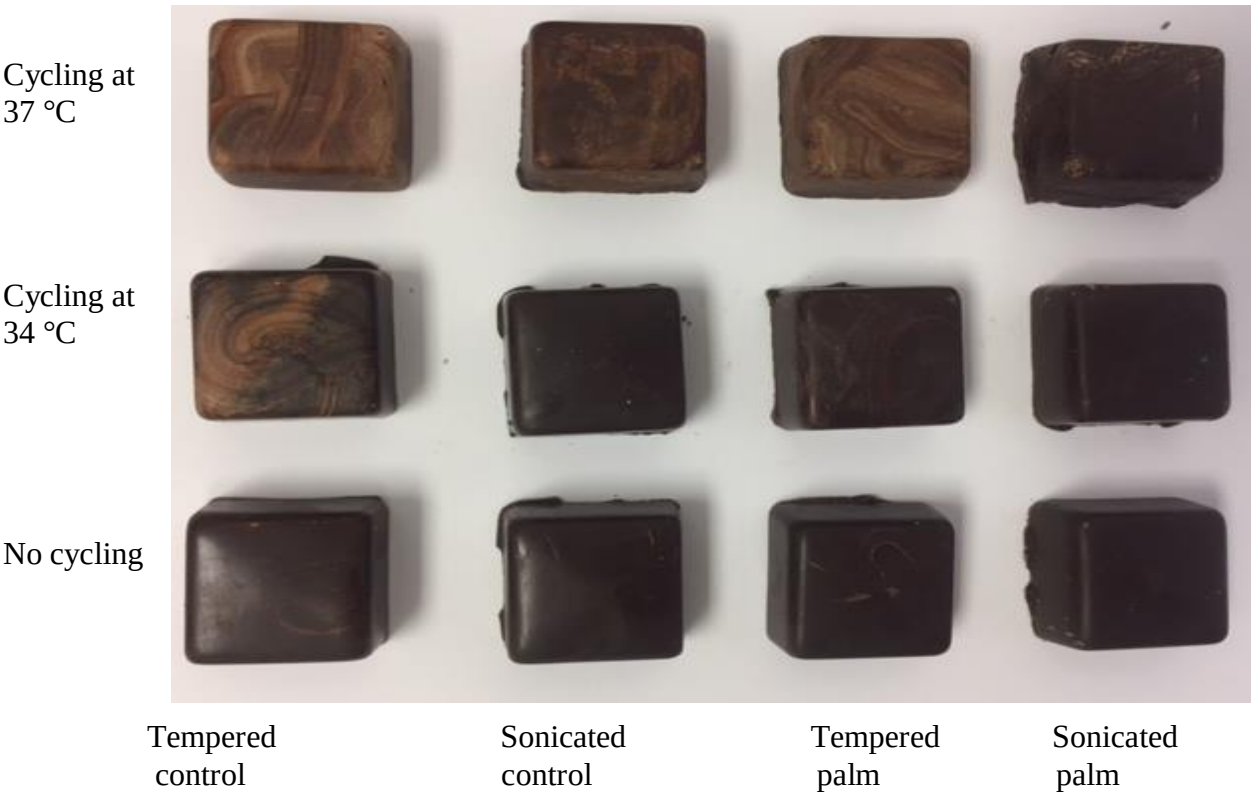

Figure 3.6 Effect of temperature (cycle 1) on visual characteristics of dark chocolate

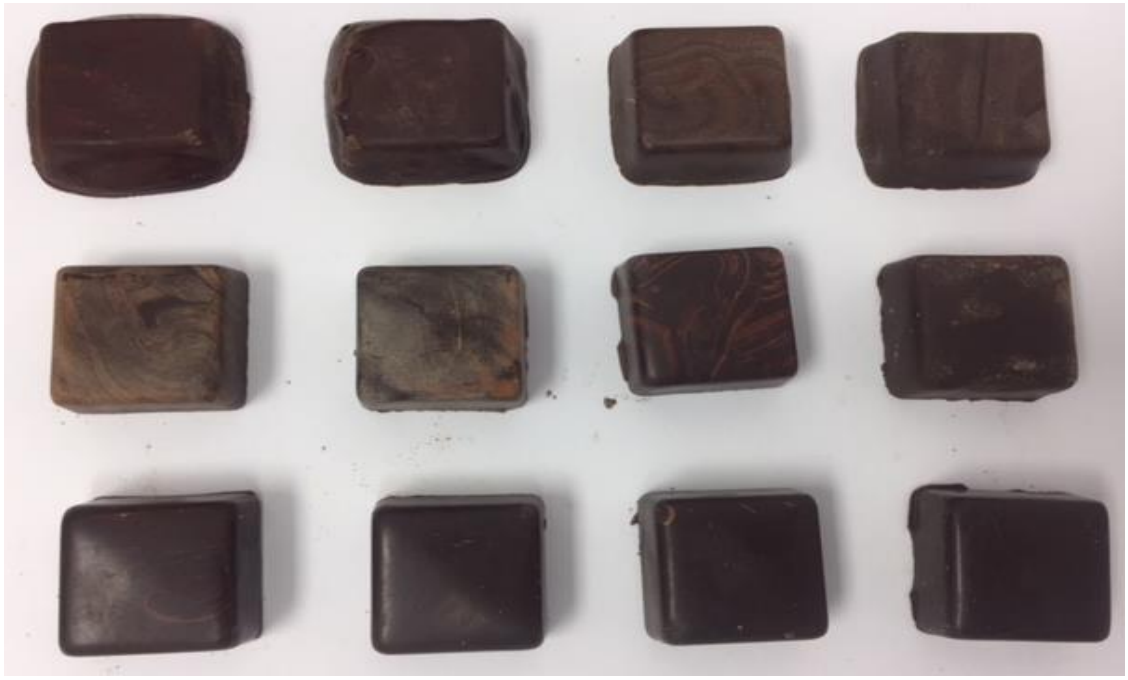

Figure 3.7 Effect of temperature (cycle 3) on visual characteristics of dark chocolate

Figures: PHASE 2. Impact of lipid fractionation on dark chocolate properties

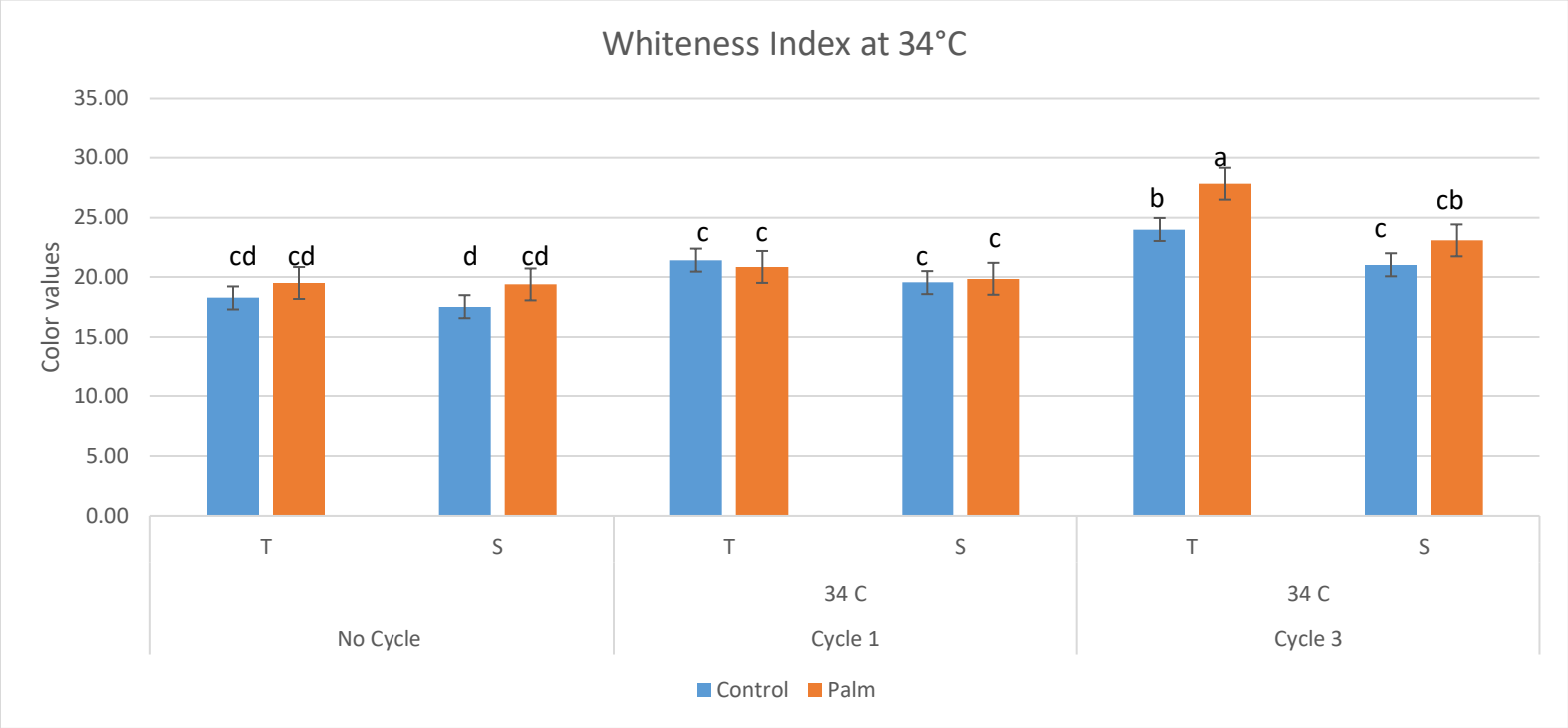

Figure 3.8 Effect of temperature cycling on Whiteness index of dark chocolate samples with fractionated fats

T: Tempered chocolate; S: Sonicated chocolate

Bars represent means +SD

a-b Bars with different letters were significantly different ($P<0.05$)

*Means of at least 3 replicates

*Letters were established with the interaction between treatment (tempered vs. sonicated), cycle, and oil (palm vs. control)

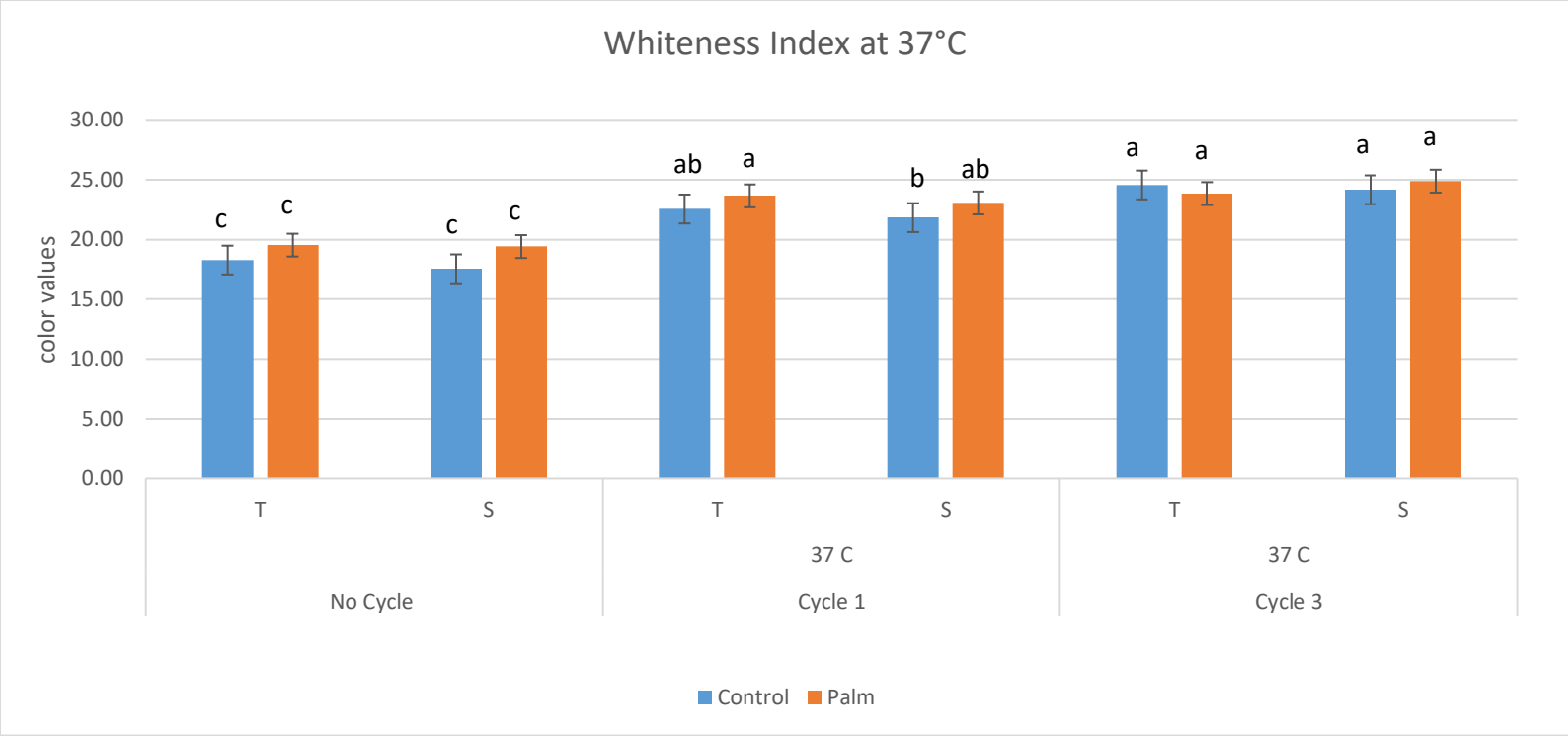

Figure 3.9 Effect of temperature cycling on Whiteness index of dark chocolate samples with fractionated fats

T: Tempered chocolate; S: Sonicated chocolate

Bars represent means +SD

a-c Bars with different letters were significantly different ($P<0.05$)

*Means of at least 3 replicates

*Letters were established with the interaction between treatment (tempered vs. sonicated), cycle, and oil (palm vs. control)

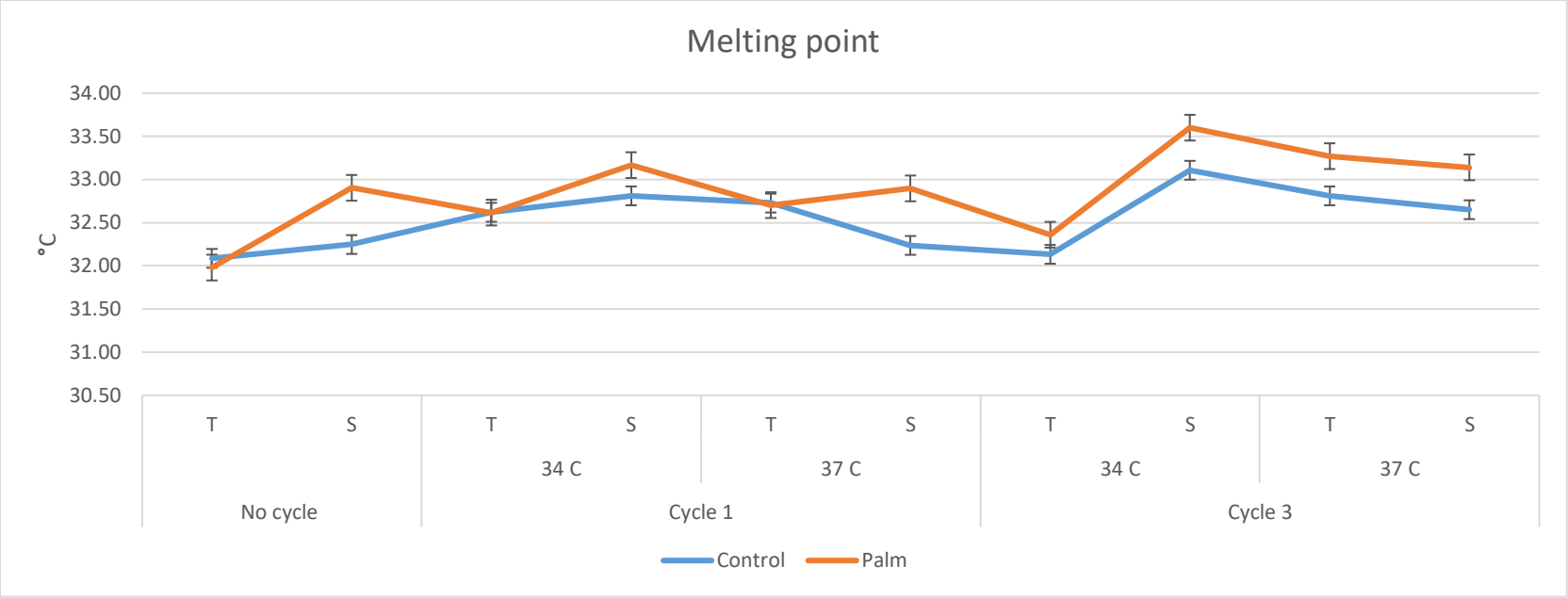

Figure 3.10 Effect of temperature cycling on the melting point of dark chocolate samples with fractionated fats
T: Tempered chocolate; S: Sonicated chocolate
*Means of at least 3 replicates

Figure 3.11 Effect of temperature (cycle 1) on visual characteristics of dark chocolate with fractionated fats

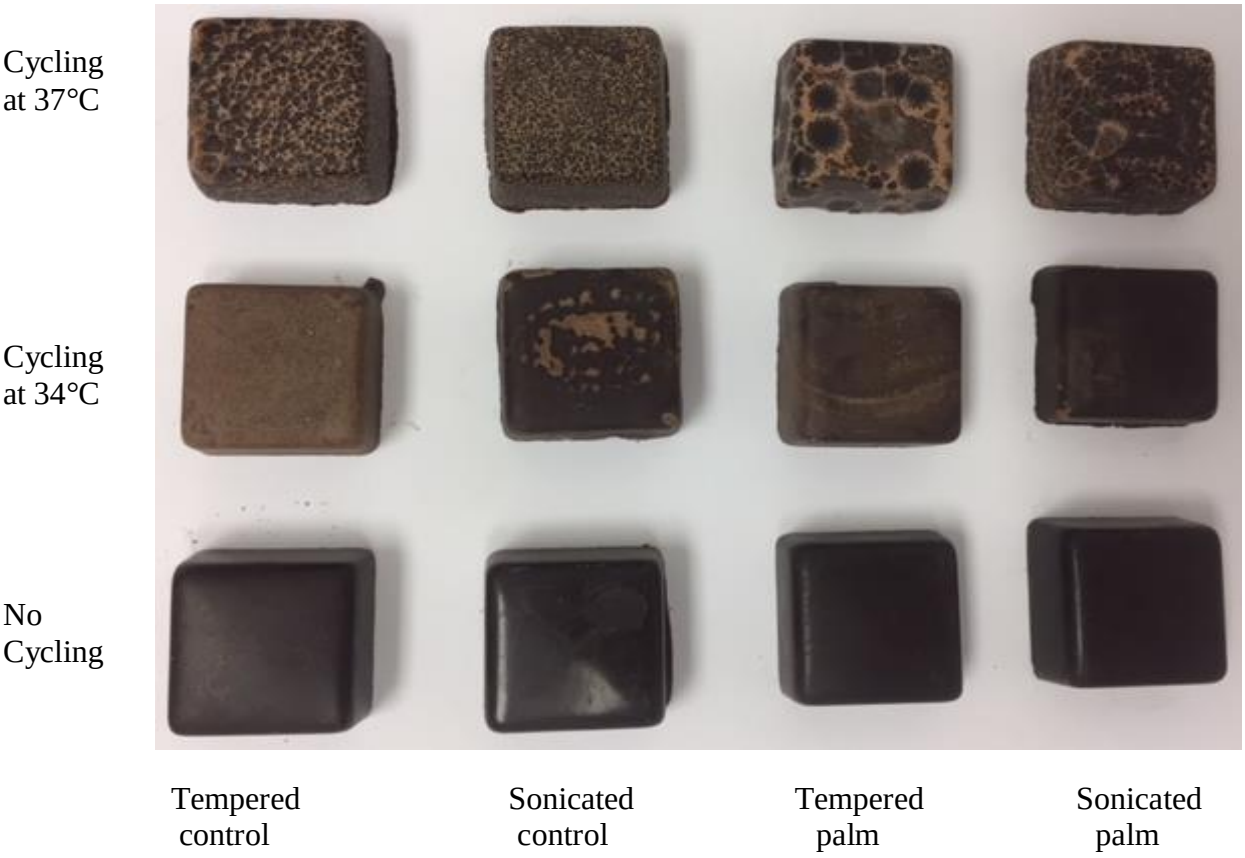

Figure 3.12 Effect of temperature (cycle 3) on visual characteristics of dark chocolate with fractionated fats

Tables: PHASE 1. Effect of the addition of palm stearin on dark chocolate properties

Table 3.1 Treatments and names of samples during preparation

Sample	Oil addition	Conching	Tempering	Sonication (9 s)	Molded	Cycles (34/37 C)
Control 1[a]	None	X	X		X	3
Control 2[a]	None	X		X	X	3
Palm1[ba]	Palm stearin	X	X		X	3
Palm 2[ba]	Palm stearin	X		X	X	3

[a]After conching, chocolate was allowed to cool and molded next day (either tempered or sonicated)
[b]Chocolate with addition of (5%) palm stearin

Table 3.2 Polymorph form and melting point of dark chocolate controls

[a-d] Numbers with different letters were significantly different (P<0.05).

Sample	Melting point (°C) (Mean ± SD)	Polymorph
Tempered control	33.38[a] ± 0.54	V
US Control	33.69[a] ± 1.66	V
Tempered palm	32.89[a] ± 0.41	V
US palm	33.76[a] ± 1.12	V

*Means of at least 3 replicates.

Table 3.3 Melting point of dark chocolate samples*

Sample	Melting point (°C) (Mean ± SD)
Tempered control	
0 cycle	33.38 ± 0.54
1 cycle, 34	32.70 ± 0.42
1 cycle, 37	32.54 ± 0.52
Tempered palm	
0 cycle	32.89 ± 0.41
1 cycle, 34	32.42 ± 0.62
1 cycle, 37	33.21 ± 0.89
Us control	
0 cycle	33.69 ± 1.66
1 cycle, 34	33.85 ± 0.93
1 cycle, 37	32.13 ± 0.38
US palm	
0 cycle	33.76 ± 1.12
1 cycle, 34	32.76 ± 0.19
1 cycle, 37	32.96 ± 0.91
Tempered control	
0 cycle	33.38 ± 0.54
3 cycle, 34	32.08 ± 0.62
3 cycle, 37	32.49 ± 0.26
Tempered palm	
0 cycle	32.89 ± 0.41
3 cycle, 34	32.39 ± 0.48
3 cycle, 37	32.49 ± 0.59
Us control	
0 cycle	33.69 ± 1.66
3 cycle, 34	33.05 ± 0.88
3 cycle, 37	33.2 ± 0.574
US palm	
0 cycle	33.76 ± 1.12
3 cycle, 34	33.48 ± 2.61
3 cycle, 37	32.66 ± 0.48

*Means of at least 3 replicates
No significantly differences ($P<0.05$) were found.

Tables: PHASE 2. Impact of lipid fractionation on dark chocolate properties

Table 3.4 Treatments and names of samples during preparation

Sample	Fraction	Fat	Conching	Temper	HIU	Mold	*Cycle
Control1[a]	X	C-stearin[b]	X	X		X	3
Control2[a]	X	C-stearin[b]	X		X	X	3
Palm1[a]	X	PCStearin[c]	X	X		X	3
Palm 2[a]	X	PCStearin[c]	X		X	X	3

[a]After conching, chocolate was allowed to cool and molded next day (either tempered or sonicated)
[b]Chocolate was formulated with fractionated cocoa butter (stearin)
[c]Chocolate was formulated with 5% palm stearin and cocoa stearin
*Chocolate was cycled either at 34 or 37C°

Table 3.5 Polymorph form and melting point of dark chocolate with fractionated fats*

Sample	Melting point (°C) (Mean ± SD)	Polymorph
Tempered control	$32.09^{a} \pm 1.05$	V
US Control	$32.25^{a} \pm 0.33$	V
Tempered palm	$32.08^{a} \pm 0.27$	V
US palm	$32.90^{a} \pm 0.85$	V

$^{a-d}$ Numbers with different letters were significantly different ($P<0.05$).
*Means of at least 3 replicates

Table 3.6 Hardness of dark chocolate with fractionated fats at 34°C*

	Hardness (N) Mean ± SD			
Cycle	**Tempered control**	**Tempered palm**	**US control**	**US palm**
0 cycle	130.57 ± 6.6[b]	121.89 ± 10.5[b]	104.17 ± 42.5[a]	105.12 ± 26.97[a]
1 cycle	166.03 ± 19.0[a]	152.77 ± 5.2[a]	144.98 ± 41.8[a]	108.42 ± 40.56[a]
3 cycle	176.83 ± 8.6[a]	148.38 ± 16.1[ab]	130.32 ± 2.8[a]	117.87 ± 57.63[a]

[a-c] Numbers with different letters were significantly different ($P<0.05$).
*Means of at least 3 replicates

Table 3.7 Hardness of dark chocolate with fractionated fats at 37°C*

	Hardness (N) Mean ± SD			
Cycle	**Tempered control**	**Tempered palm**	**US control**	**US palm**
0 cycle	130.57 ± 6.57[a]	121.89 ± 16.11[a]	104.17 ± 42.45[a]	105.12 ± 57.63[a]
1 cycle	127.63 ± 7.63[a]	117.65 ± 9.93[ab]	125.06 ± 12.70[a]	102.60 ± 40.76[a]
3 cycle	134.67 ± 27.15[a]	90.62 ± 5.59[b]	114.89 ± 3.13[a]	81.25 ± 0.98[a]

[a-c] Numbers with different letters were significantly different ($P<0.05$).
*Means of at least 3 replicates

CHAPTER 4: SUMMARY AND FUTURE STUDIES

The studies presented in this thesis show the important role that formulation and processing play to enhance quality and temperature stability of chocolate during storage. The goal was to create modifications to create more stable chocolate for high temperature conditions experienced in the tropics where the raw ingredient cacao is grown. A graduate student travel grant through ACES International Programs was gained and thus, was able to extend my thesis project to Honduras, where an effort of research/training was established with a Women's Association to help the cacao farmers. This work was divided into two phases.

Phase 1, was designed to illustrate the effect of the combination of 5% palm stearin and ultrasonication on melting point, physical dimensions, and bloom formation after cycling. Addition of 5% palm stearin to the chocolate formulation, either tempered or sonicated, did not present significant changes in melting point after cycling. The combination of 5% palm stearin with sonication, achieved the desired crystal structure, polymorphic form V. Sonicated samples, presented less WI at 34°C after cycling as compared to tempered samples. Tempered samples with 5% palm stearin presented less WI at 34°C cycle 3 as compared to tempered controls. At 37°C, samples showed an increase in WI after cycle 1 but not differences between cycle 1 and cycle 3 were found. Cycling of chocolate samples with temperature fluctuation at 34°C (for 3 cycles) did not influence physical expansion. While cycling at 37°C dramatically impacted physical dimensions. Samples with 5% palm stearin, tempered or sonicated, had less physical changes after cycling at 37°C for 3 cycles than samples without palm stearin cycling at 37°C for 3 cycles.

Phase 2, demonstrated the use of lipid fractionation in the chocolate formulation. The melting point did not increase after fractionation as expected. All samples showed polymorph V, thus had a melting point approximately 32 to 34°C. Cycling samples 3 times either at 34°C or

37°C increased their WI more than cycling them once. Sonicated and tempered samples presented similar WI before temperature cycling. Chocolate samples did not present significant differences in WI after one 34°C cycle 1 as compared with no cycling, indicating that this new chocolate formulation can resist some levels of temperature fluctuations. Overall, despite the treatments and cycling, the new dark chocolate formulation did not present changes in its dimensions.

Future studies should focus on evaluating the stability of palm stearin carotenoids after fractionation and cycling. This could potentially be commercialized in tropical regions as quality chocolate with nutritional value. The fractionation and use of other oils (e.g., coconut oil) should be examined; this was a suggestion of DACHOJ, because there are some environmental issues surrounding palm oil production. Furthermore, sensory analysis of chocolate with 5% palm stearin and chocolate with fractionated lipid should be performed to determine consumer preferences. Also, studies should focus on the economical and practical alternatives to implement dry fractionation with ultrasonication in small businesses. The combination of these technologies can be very useful for the minimal processing of chocolate, because of the "instantaneous" transfer of acoustic energy, resulting in a reduction of the total manufacturing time, and more stable chocolate with higher throughput and lower cost production.

CHAPTER 5: REFERENCES

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