IMPACT OF EMULSIFIERS ON PHYSICAL, SENSORY, AND MICROSTRUCTURAL PROPERTIES IN FORMULATED DARK CHOCOLATE WITH AN INNOVATIVE EDUCATIONAL APPROACH

BY

MELISSA ANNE TISONCIK

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Food Science and Human Nutrition
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Adviser:

Associate Professor Nicki J Engeseth

Abstract

Dark chocolate has both a complex flavor profile and compositional matrix consisting of sugar and cocoa particles dispersed in a continuous phase of cocoa butter. The crystal structure of cocoa butter contributes to both the smooth mouthfeel and melting properties of chocolate that are favorable characteristics as perceived by consumers. Chocolate has a long shelf life of about a year; however, during storage structural changes occur, which may lead to development of fat bloom or sugar bloom, either of which compromises textural and visual quality. Due to unique interactions between structural lipid polymorphs in cocoa butter, quality parameters of chocolate such as texture and flavor release are impacted by the structural alterations during storage. Previous research in our laboratory involved characterization of physical, structural and microstructural properties of chocolate, dramatically affected by storage at different temperatures and with ranging relative humidity values. Temperature cycling of chocolate led to fat bloom formation and also had a dramatic impact on quality parameters. Some changes observed were speculated due to breakdown of emulsifier in the formulation. Thus, my research was focused on characterization of the impact of emulsifier type and concentration on fat bloom formation, physical, sensory, and microstructural properties in formulated dark chocolate. Three different emulsifiers were used in this study: soy lecithin, polyglycerol polyricinoleate (PGPR), and ammonium phosphatide. Chocolate was formulated with 0.2% or 0.5% emulsifier, conched/refined, tempered, and molded in the laboratory. Sample storage treatments included temperature cycling and long term storage. Instrumental analyses were utilized to evaluate lipid polymorphism, fat bloom formation, texture and sensory perceptual changes in chocolate. Texture and flavor of samples in long term storage were evaluated by a descriptive analysis panel. Temperature cycling significantly impacted appearance and texture in samples. Specifically, samples cycled at 34°C had increased whiteness index, and were harder than samples cycled at 37°C, which experienced dramatic changes of dimension and surface roughness. Samples stored at 37°C recrystallized into polymorph V and contained characteristics similar to untempered chocolate. Chocolate formulated with PGPR had the greatest impact on textural, melting, and physical properties undergoing long term storage and temperature cycling. Increased concentrations of all emulsifiers

had the greatest impact on textural, physical, and melting properties in samples. Chocolates stored at 34°C experienced polymorphic transition to form VI, experienced increased hardness and surface roughness, and were perceived as hard, grainy, crumbly, had longer melt time, left a dry mouthfeel, and had a bland, chalky flavor. Sensory study results indicated samples stored at ambient temperature were not visually or texturally compromised and were creamy, cohesive, melted quicker, left a fatty mouth coating, and had a chocolaty, roasted, bitter, and sweet flavor. Sensory results indicated the type of emulsifier used in formulation did not have a significant impact on texture or flavor attributes assessed by panelists.

An innovative educational effort to recruit bright, science-minded high school students to the field of food science complimented my research on lipid polymorphism and the role of emulsifiers in chocolate. The 2009 Chocolate Food Science Education Program taught students who may not have been exposed to food science about the many facets of this exciting field through the study of chocolate. Students learned about the concentrations of food science such as chemistry, microbiology, safety, processing, product development, sensory science, nanotechnology, and nutrition. Experiential learning sessions were taught through means of lectures, demonstrations, activities, field trips, research, and interactions with faculty, staff, graduate students, and industry professionals. Evaluation of the program documented that it was a beneficial program for participants and a powerful educational recruitment tool for future food scientists. Success of this experiential learning program will serve as a model for creative efforts of recruitment to fields that need more visibility at the high school level.

Acknowledgements

I would like to take this opportunity to thank all of the people who have made this project possible. First and foremost, I want to thank my wonderful advisor, Dr. Nicki Engeseth. She gave me the amazing opportunity to work with her and study a food I am very passionate about. I thank you for challenging me as scientist, writer, teacher and student, for all your encouragement, and for your exhaustive efforts trying to understand my rambling. I want to thank my thesis committee members, Dr. Keith Cadwallader and Dr. Shelly Schmidt for use of their laboratories, for their lessons, and their support on the defense of my project. Thank you to Dr. Faye Dong for introducing me to faculty and students during ExploreACES in 2007, when I was first looking at the Department for graduate school. That is where it all began. I want to recognize the amazing opportunities the University and specifically the Food Science Department have given me the past three years. I have had the opportunity to collaborate with many people and learn many instrumental techniques that were accessible to me as a student. Specifically, I want to thank Dr. Soo-Yeun Lee for use of the sensory laboratory and her advice during my sensory study. Thank you to Scott MacLaren and Mauro Sardela for teaching me how to use cutting edge microscopic technology while working at MRL CMM laboratories. I am privileged to have studied with many great minds and future pioneers of science. I thank you for all your knowledge and experiences you have shared with me.

I would like to thank those people who have been a great support throughout my three years at the University. Thank you to the two best research assistants I have ever had, Pieter Lim and Rustin Meister. Without their help I would not have been able to organize, prepare, and sometimes consume the thousands of chocolate samples I produced during my research. I would like to thank my lab members, Kelly Van Haren, Monica Garces, Yanhua He, and especially Crystal Goshorn. Crystal challenged me to always be better and helped me grow as a scientist. She is a respected fellow scientist and I am honored to call her my friend; let the ‘adventures’ continue. Thank you to my friends Veronica Jacome and Sharlene Denos for all their friendship and support during the tough moments. My closest friends know “this thesis is a piece of work that—like the molds Melissa used to make her chocolate—she has poured a fair bit of her soul into.” With those words, I especially want to thank Nick for his unfaltering support every step

of the way, the man who truly believed in me, loved me, and made me laugh every rainy day. I thank you with all my heart; I could not have done it without you.

Lastly, I would like to dedicate this research to those who mean the most to me, who have been my role models my whole life, to my family. I grew up in an environment that emphasized the importance of education. My parents have always taught me to go beyond, find what I am passionate about and to make it happen. They have supported me in every endeavor and been there for me everyday. To my mom, she is a true force of nature. She will forever inspire me with her love, perseverance, and endless support. To my dad, he has helped me overcome the many obstacles and challenges on my path to becoming a food scientist. He is the one person who has shown me how to keep my passion alive and never give up trying. He taught me how to be tough. I am proud to call myself their daughter. I am even more proud to call myself a sister. I dedicate this work to my only sister, Jenny. Growing up, I saw every challenge she overcame to get where she is today, and I strive to be like her everyday of my life. She has always paved the way for me and I am forever grateful. She will always be my idol and my choop. Thank you for your love.

Finally, I would like to thank my partner in crime, my subject matter, sweet concoction, dark chocolate. Without chocolate there would be no research. Not only am I eternally obsessed, but I now understand the words ‘a true chocolate lover finds ways to accommodate her passion and make it work with her lifestyle.’ Here’s to you and unraveling more of your mysteries! Smudge.

Table of Contents

List of Tables

List of Figures

Introduction

Dark chocolate has a complex flavor profile and compositional matrix consisting of sugar and cocoa particles and emulsifiers dispersed in a continuous phase of cocoa butter. This is due to the unique interactions of polymorphic lipid structures of cocoa butter. Specifically, the orientation of cocoa butter crystals in polymorph V contributes to the even melting properties associated with favorable mouthfeel (Hartel, 2001). During storage structural changes occur. Previous research indicates with improper storage these changes are magnified, causing increased particle size and dramatic changes in microstructural properties (Hartel, 2001; Andrae, 2006). The human tongue can detect particles up to 20-30 microns (Hoskin, 1994), thus, particle size must be controlled to ensure smooth mouthfeel and meet consumer standards (Morgan, 1994). As crystal size increases, appearance and flavor may also be compromised. Different storage conditions lead to the development of fat bloom or sugar bloom in chocolate, which is known to compromise textural and visual quality (Andrae, 2006). Bloom is the main cause of quality loss in the chocolate industry (National Confectioners Association, 2005). This has a dramatic impact on chocolate sales, which total nearly $7.08 billion in the US (Information Resources Inc., 2010). Exact market loss due to fat bloom is difficult to verify, since changes may arise many months after processing. Several studies have investigated the mechanism of fat bloom formation (Wille and Lutton, 1969; Bricknell and Hartel, 2006; Rousseau and Sonwai, 2008; James and Smith, 2010); however the literature is lacking reports on the impact emulsifiers have on cocoa butter stability, textural perceptions, and overall impact on fat bloom formation. Emulsifiers have been used in chocolate to improve rheology, or flow properties (Rector, 2000). Manufacturers use emulsifiers as an opportunity to optimize their process and formulations to minimize cost. Emulsifiers have also been used to influence structural properties impacting consumer perception of chocolate texture and flavor. They are also believed to impact properties of solidified chocolate, including susceptibility to fat bloom, stability against fat migration from fillings, and stability against oxidation in filled/nut laced chocolates (Schantz and Rohm, 2005). The overall goal of this study was to obtain a better understanding of the mechanistic features of how various emulsifiers influence fat bloom

formation in stored chocolate and to decipher the impact of emulsifier concentration in this role.

Different emulsifiers are used in chocolate, such as the most commonly noted soy lecithin, a mixture of natural phosphoglycerides; recently approved GRAS certified (2007) emulsifier ammonium phosphatide, and polyglycerol polyricinoleate (PGPR), obtained by polycondensation of castor oil and glycerol. Each emulsifier has different properties due to its structure and intermolecular interactions with both sugar particles and continuous fat phase in chocolate. Further investigations are needed to better understand the impact these specific emulsifiers have on physical, perceptual, and microstructual properties in dark chocolate, allowing for optimization of quality during storage. This will provide insight into emulsifier selection for chocolate manufacturing.

Chapter 1 is a review of literature involving the background, processing, and science of dark chocolate. Specifically, there are details on chocolate history, manufacturing, introduction to lipid polymorphism, fat bloom and sugar bloom formation, techniques on crystal characterization, as well as flavors associated with chocolate.

Chapter 2 describes the impact of emulsifier structure and concentration on physical, textural, and microstructual properties in formulated dark chocolate undergoing temperature fluctuations. I hypothesized that changes which occur during storage of chocolate would impact the stability of emulsifier-TAG complexes, which may be a precursor to phase separation of lipid and sugar in chocolate and ultimately lead to bloom formation. These changes would impact intermolecular mobility of the continuous fat phase which would be detrimental to the overall stability of the cocoa butter matrix. Specifically, the emulsifier-TAG complex stability is directly correlated to the type of emulsifier used and the concentration in chocolate formulation.

Quality analysis of chocolate was focused on melting behavior and initial lipid polymorph characterization using Differential Scanning Calorimetry (DSC), confirmation of polymorph transition by Powder X-Ray Diffraction (XRD) and topographical surface analysis using Atomic Force Microscopy (AFM), including roughness.

Chapter 3 describes long term storage of chocolate formulated with different emulsifiers at one concentration and their impact on sensory texture and flavor perception, as well as instrumental color, texture, and microstructural properties. Based on results from Chapter 2, I hypothesized that storage at high temperature, conductive to fat bloom formation, will cause detrimental changes in flavor, texture, and perceived quality in chocolate; the type of emulsifier used in formulation may impact these properties in all storage conditions. Sensory changes were analyzed using the technique of sensory descriptive analysis.

Chapter 4 demonstrates the creation/implementation of an innovative educational program designed to attract bright, science-minded high school students to the field of food science through the study of chocolate. An overview is provided for the 2009 Chocolate Food Science Education Program and details are given about the lessons taught, assessment tools used to evaluate the program, goals achieved, and overall impact the program had on participants. My hypothesis for Chapter 4 is that this program using apprentice based, hands-on style of teaching will foster an environment that encourages career aspirations, promotes understanding of scientific ideas and principles, instills confidence and self efficacy, and develops intellect and scientific skills for younger aspiring scientists. Success of this experiential learning program will serve as a model for creative efforts of recruitment to fields that need more visibility at the high school level.

Chapter 1. Review of Literature

1.1 Chocolate Background

Chocolate History

The delicious form of chocolate, as we know it today, comes from the cacao seed produced by *Theobroma cacao (*Linnaeus's *"food of the gods")* which is perfectly adapted to grow in the tropics, in a fairly narrow region, between 20° N and 20° S of the Equator (Presilla, 2001). It originated in Central and South America approximately 300 A.D. (Young, 1994). The cacao tree produces vine-like tree branches bearing pale flowers and oblong pods, which encase the cacao beans. The ancient Mayan civilization first cultivated these revered beans approximately 2,000 years ago (Rosenbum, 2005). Originally, the beans were used as currency (Young, 1994). In 1502, when Columbus, and years later when Cortez, presented this rich food to the Old World, cacao became recognized as a golden commodity (Guittard, 2009). Today, there are three cacao cultivars known as criollo, the northern South American strain, forastero, the Amazon Basin strain, and trinitario, the hybrid strain born in Trinidad. The criollo and forastero are pure species; while trinitario arose from conscious cross pollination of the two, engineered to provide more abundant crop yield (Scharffenberger and Steinberg, 2006). The forastero bean is known to have a stronger and flat flavor, but not as complex and mellow as the light color criollo bean. The rise of trinitario created a heartier distinct and different flavor cacao than its parent ancestors. Today, the origin and type of bean are driving factors of purchase for chocolate manufacturers around the world.

Cacao Consumption and Production World Wide

The hearty and bountiful forastero cacao, known as 'bulk beans', account for more than 95% of cacao used by the World's chocolate manufacturers today (Guittard, 2009). In comparison, the ancient fragile criollo cacao with its 'fine flavor' only accounts for 1% of the World crop (Presilla, 2001; Guittard, 2009). After numerous years and thousands of cross pollinations between cultivars, it is rare to find a pure cacao today. According to the International Cocoa Organization (ICCO), 3.4 million tonnes of cacao were produced in 2006/07; the majority being produced in West Africa (70.4%), specifically Ivory Coast (38%) and Ghana (18%) (ICCO, 2006/07). The amount of cacao

produced and corresponding areas of production are listed in **Table 1.1**. Cacao consumption by region was summarized by ICCO (**Table 1.2**) with Europe leading at 42.7%.

Table 1.1: Production of cacao beans (thousand tonnes)

Africa	**2,393**	**70.4%**
Ivory Coast	1,292	
Ghana	614	
Nigeria	190	
Cameroon	166	
Others	129	
America	**411**	**12.1%**
Brazil	126	
Ecuador	114	
Dominican Republic	47	
Others	124	
Asia & Oceania	**597**	**17.5%**
Indonesia	490	
Papua New Guinea	50	
Malaysia	31	
Others	25	
World (estimate)	**3,400**	

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol XXXIII, No. 4 2006/07.

Table 1.2: Consumption of cacao, measured by bean grindings (thousand tonnes)

Europe	**1,540**	**42.7%**
Germany	357	
Netherlands	465	
Others	719	
Africa	**514**	**14.3%**
Cote d'Ivoire	336	
Others	179	
America	**853**	**23.7%**
Brazil	224	
United States	418	
Others	212	
Asia & Oceania	**699**	**19.4%**
Indonesia	140	
Malaysia	270	
Others	289	
World Total	**3,608**	

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol XXXIII, No. 4 2006/07

Single Origin Chocolate

Information Resources Inc. (2010) projected US chocolate sales to be approximately $7.08 billion. Recently, there has been demand for premium chocolate, including organic, Fair Trade, single origin, reduced sugar, dark and high cocoa content chocolates across mature markets. The dark chocolate global market is now estimated to represent between 5-10% of the total market for chocolate bars (ICCO, 2006/07). With recent research findings on multiple health benefits of chocolate, paired with demand for 'functional foods', consumer interest in high quality products has increased. This phenomenon is manifested in increased sales of "bean to bar" quality dark chocolate products from independent chocolate manufacturers (Pollard, 2007). Introduction of single origin chocolate (meaning the origination of the cocoa beans) has exposed consumers to the charismatic flavor profiles within each bar (Guittard, 2009). As consumers are better educated on health aspects of chocolate, the ICCO initiates programs to increase cacao quality, and individual chocolatiers arise in the market; demand for high quality chocolate will ensue. The "bean to bar" single origin

methodology begins with individual selection of the cacao bean during harvest and is diligently overseen through the steps of chocolate processing to the finished molding of the chocolate product.

1.2 Chocolate Processing

Harvesting

When pods from the *Theobroma cacao* tree are ripe, they change from a green/red to yellow/orange color (Beckett, 1999). Pods are cut with machetes and manually harvested by local farmers. Harvest occurs every 2 to 4 weeks during a period of several months. Pods are opened to release the beans; on average there are 40 beans per pod.

Fermentation

The cacao bean is encased in sweet white pulpy mucilage inside large oblong pods. The pulp contains sugars necessary to feed yeast and bacteria during fermentation. Prior to fermentation, cacao beans are bitter and astringent. Fermentation is the first step in development of chocolate flavor. Pulp and beans are fermented 5-6 days, using a heap or box style method to generate heat. During the initial stage of this anaerobic process, yeasts and bacteria convert sugars into ethyl alcohol under conditions of reduced oxygen and decreased pH (<4) (Martelli and Dittmar, 1961). Specifically, lactic acid bacteria (LAB) convert sugars and organic acids to lactic acid while temperatures rise and acetic acid bacteria (AAB) convert alcohol to acetic acid. In addition to producing alcohol, the yeast hydrolyze pectin covering the seeds. Through this process the temperature of the mass rises to 50ºC, the embryo dies, and autolysis of the cotyledons is induced. Throughout fermentation, different distinct flavor notes associated with chocolate are produced. Fermentation leaves a slightly darkened bean. There is approximately 10% moisture loss in the bean during this process (Beckett, 1999). The pulp consists of mostly water with 10-15% sugar (Case, 2010). **Table 1.3** lists detailed pulp composition before and after fermentation. Proper fermentation and drying removes unpleasant flavors, such as the sour, bitter, and astringent compounds (organic acids, anthocyanins, tannins, catechins) and initiates chemical changes necessary to produce true cocoa and chocolate flavors that emerge after roasting (Minifie, 1989).

Table 1.3: Cacao pulp composition (adapted from Case, 2010)

	Before Fermentation	After Fermentation
Sucrose	12%	0%
Citric Acid	1-3%	0.5%
Pectin	1-1.5%	-
pH	3.7	6.5
Ethyl alcohol	-	0.5%
Acetic Acid	-	1.6%

Drying and Roasting

Once fermentation is complete, beans are spread on a concrete floor and dried in the sun for 5-6 days. During drying the beans develop their characteristic brown color. Many oxidation reactions occur with polyphenols, catalyzed by polyphenol oxidase (PPO). Chemical changes within the bean gradually slow down and stop when the moisture content drops below 8% (Beckett, 1999). It is important for the beans to have low moisture content to prevent molding, which would negatively alter bean flavor (Hansen and Keeney, 1970).

High temperatures (110-220°C) during roasting are important as they amplify the complex reactions for flavor development. Research demonstrates that roasting reduces acidity in bean flavor as indicated by a significant decrease in concentration of volatile acids (e.g., acetic acid) and non-volatile acids (e.g., oxalic, citric, tartaric, succinic, and lactic acids) (Jinap et al., 1995). An important process during roasting occurs through the Maillard reaction, non-enzymatic browning, where the cacao bean gains sweetness and floral/caramel notes.

Winnowing

Winnowing occurs once the bean has reached optimal moisture content and has loosened from the outer shell during drying. Shells are discarded and the beans are broken into 'nib' pieces which become ready for grinding (Beckett, 1999). After roasting, typical bean composition is 87.1% nib; 12% shell; 0.9% germ. A winnower machine is used to break beans and separate the shell and remaining germ by centrifugal force. After winnowing there is approximately 82% pure nib yield (Minifie, 1989).

Refining and Grinding

Refining and grinding of the cacao nib is necessary to produce chocolate liquor and reduce particle size. Nibs contain 53% fat, 14% protein, and have 1.5% maximum

moisture content; they are typically ground using roll or ball refiners to form a thick cocoa paste (Minifie, 1989). The paste is further refined to optimize particle size. The melangeur, first used in the 18th century, is one of many versatile machines used to refine cocoa paste, as it allows the mass to pass between two rotary stone slabs (Presilla, 2001).

Chocolate Liquor Pressing

After nibs are ground to "non alcoholic" chocolate liquor, the liquid can be directed one of two ways for further processing: 1) high pressure hydraulic press to yield cocoa powder and cocoa butter products or, 2) agitation and heating to prepare chocolate. Once cocoa powder is separated from cocoa butter after pressing, it may be alkalized to modify the color and flavor (Minifie, 1989). In the later case, the chocolate liquor can be processed into chocolate with sugar, emulsifiers, and cocoa butter during conching.

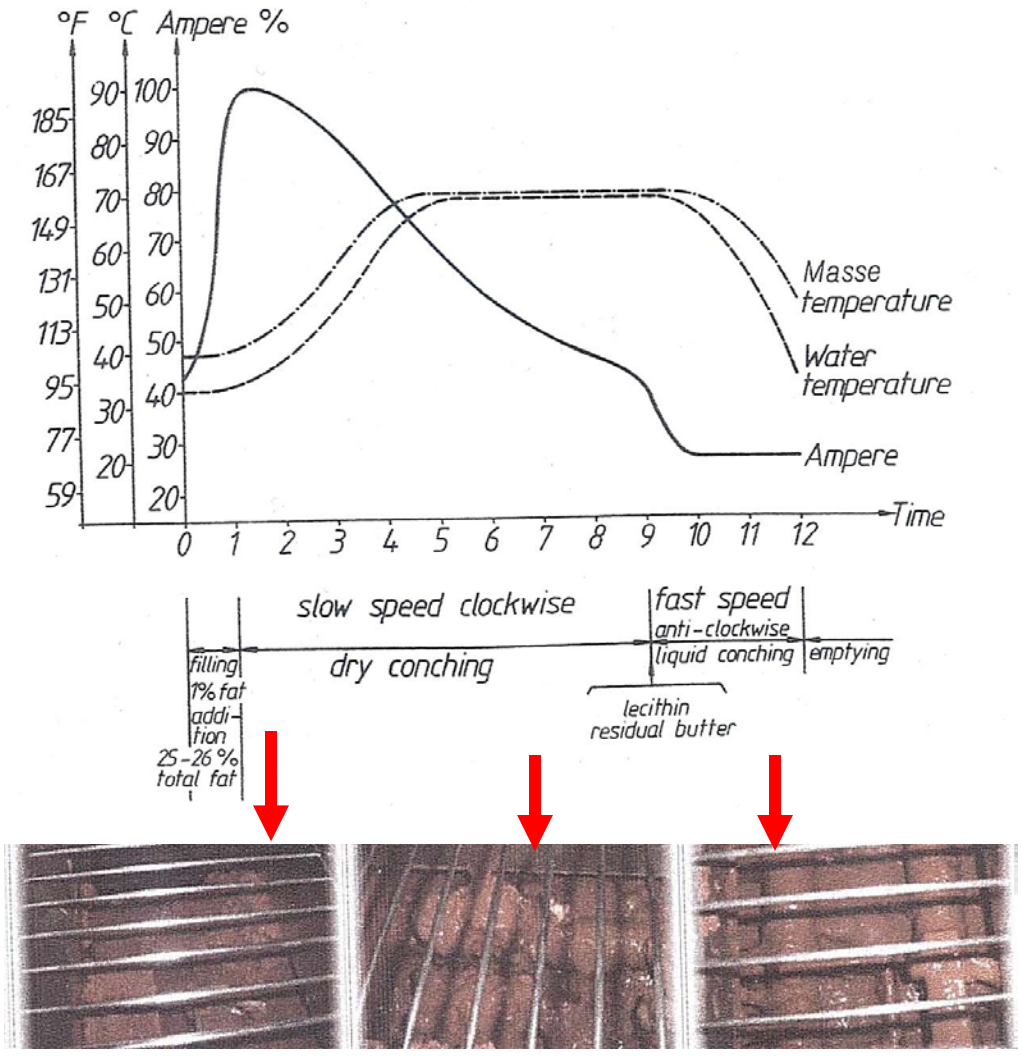
Conching

After Rodolphe Lindt developed the process known as conching in Switzerland in 1879, the graininess associated with chocolate disappeared, and chocolate had a new silky texture and even melting for consumer appeal. This process occurs when chocolate liquor is mixed, either with the addition of cocoa butter or without, in a sloshing-kneading apparatus known as a 'conche' (Precilla, 2001). Beckett (1999) describes the design of conching to optimize viscosity and flow properties by mechanically "smearing" the fat over the ground sugar and cacao surfaces to increase particle flow and further reduce particle size to <18 microns (Lucisano et al., 2006). During conching particles are ground, disassociated by friction and become rounded (Mentink and Serpelloni, 1994). Particle size is important for even melting, smooth mouthfeel, and volatile release in chocolate. The human tongue can detect particles a minimum size of 20-30 microns (Liang and Hartel, 2004); thus, it is important during refining to reduce particles below the human detection threshold to uphold favorable chocolate texture.

Conching is also essential for chocolate flavor development by removing the distasteful bitter, astringent, and sour flavors. These favorable textural and flavor attributes developed during conching are dependent upon duration of agitation, temperature during mixing, and addition of additives during the process. The developing chocolate mass is heated at approximately 50°C and agitated 4 to 72 hours (Cidell and Alberts, 2006). Since the 19th century chocolate producers have been optimizing production to minimize cost and increase productivity by shortening conching time at a

higher shear (Bolenz et al., 2005). Research indicates that short conching time results in problems with moisture removal. During refining moisture migrates from cocoa liquor to amorphous surfaces or ground sugar particles, forming a solvate layer which lets particles stick to each other (Franke and Tscheuschner, 1991). Beckett (1999) concluded it is necessary to remove moisture at the beginning of the conching cycle if a chocolate mass is to be conched for long periods of time (over 12 hours). However, there must be a balance because conching too long will result in dull, gummy chocolate and too little agitation results in grainy, acidic chocolate (Schumacher et al., 2009). There are three phases associated with conching that detail the process and the importance of speed and duration of conching (**Figure 1.1**) (Becket, 1999). During the first, dry phase, raw sugar and chocolate liquor (or cocoa mass) is agitated at a slow speed. The second, pasty phase, involves melting of natural fat. The third, liquid phase, involves rapid stirring to incorporate any additional fat or emulsifiers into the mixture.

Figure 1.1: Three phases of chocolate conching and associated phase images (Beckett, 1999; images adapted from K&K Chocolate-Confectionery Machinery)



1. 2. 3.

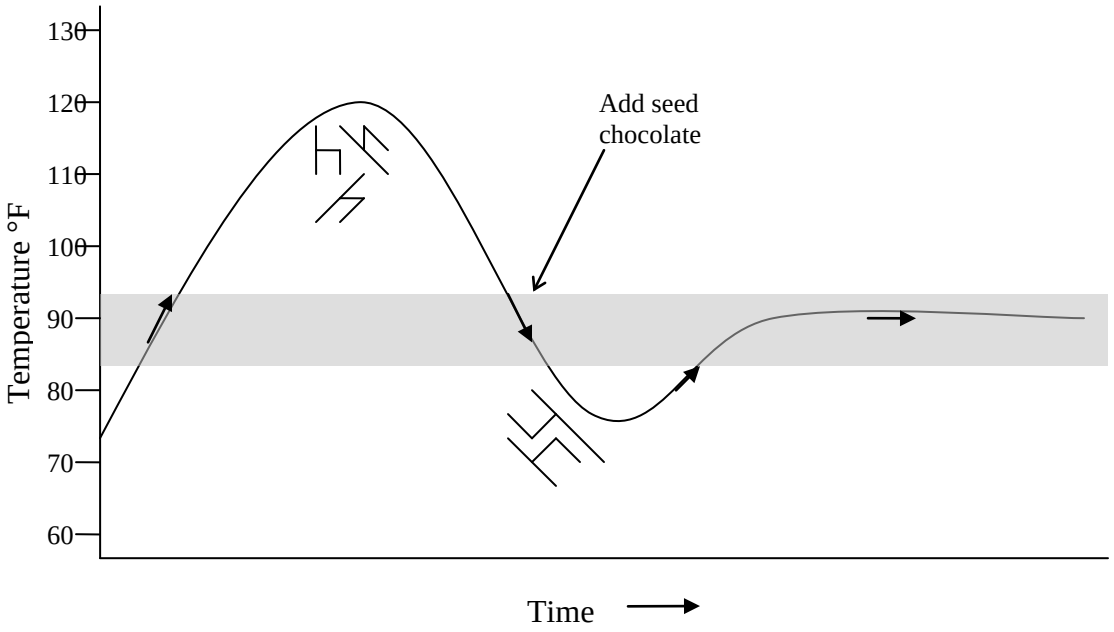
1. Dry phase- mass is crumbly and moisture is removed
2. Pasty phase- chocolate is thick paste
3. Liquid phase- high speed stirring to mix in final fat and emulsifier

Tempering

Once conching is complete, the flavorful chocolate product undergoes its final step of processing. Tempering involves controlled cooling of melted chocolate that will promote a stable crystalline structure for a finished product. Therefore, development of a sufficient number of seed crystals to provide this stable fat crystalline scaffolding is crucial. Recent research denotes that a break down in structure can occur when no seed crystals are formed, for example, when solidification occurs without tempering (Kinta

and Hartel, 2010). This can be compromising to visual, textural, and physical appeal for finished chocolate products. Stable cocoa butter crystals will provide desirable properties such as snap, gloss, proper texture, contraction for de-molding, and less permeability (Hartel, 2001). During tempering (**Figure 1.2**) chocolate is completely melted to 120ºF. Completely melted chocolate is cooled under the stable-crystal temperature (approximately 90ºF) to approximately 78.5ºF to induce crystallization and seed formation, followed by heating (to approximately 88.7ºF) to melt unstable crystals while retaining stable ones.

Figure 1.2: Tempering process in multiple steps (adapted from McGee, 1999)



Molding

Once chocolate is properly tempered, it is immediately transferred to desired molds to form the base of most chocolate confections. There are several types of molding techniques ranging from drop depositing, used in retail production; solid molding, resulting in chocolate bars and retail blocks; shell molding or 'enrobing', usually a three step operation with an encapsulated center confection; and hollow molding, involving a two piece figure spun mold (Patric, 2006).

1.3 Standard of Identity (SOI)

According to the Food and Drug Administration (FDA), the standards of identity for dark chocolate are as follows: must contain at least 35% chocolate liquor and a maximum of 12% milk solids (Code of Federal Regulations Title 21, 2003). Milk chocolate (based on dry matter basis) must contain no less than 25% cocoa solids and a minimum of specified milk solids between 12-14%, including a minimum of milk fat between 2.5-3.5%. Whereas, white chocolate must contain no less than 20% cocoa butter and no less than 14% milk solids, including a minimum milk fat between 2.5-3.5%.

1.4 Physical Properties of Chocolate

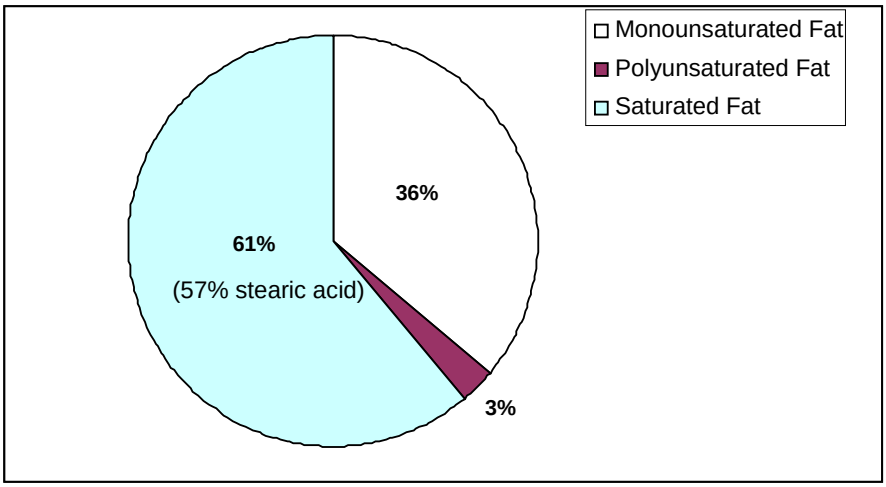
Chocolate is a suspension of sugar, cocoa, and/or milk solid particles in a continuous fat phase. It has solid particles in the liquid phase, but exhibits non-Newtonian flow properties (Beckett, 2000). One of the fascinating things about chocolate is that it has a long shelf life with virtually no microbial concerns. The smooth texture and mouthfeel of chocolate is due to unique interactions of polymorphic lipid structures of cocoa butter along with adequate volatile release associated with these interactions (Loisel et al., 1998). However, storage of chocolate results in structural changes consequently affecting the favorable texture and flavor attributes. With improper storage, these changes are magnified, causing an increase in particle size, and the development of either fat bloom or sugar bloom, which compromises mouthfeel, visual, and textural quality (Morgan, 1994). Bloom is the main cause of quality loss in the chocolate industry (National Confectioners Association, 2005); this is important because chocolate sales total nearly $7.08 billion in the US (Information Resources Inc, 2010). As bloom forms, particle size increases and microstructural and perceptual changes also occur (Andrae, 2006).

1.5 Cocoa Butter Polymorphism

Cocoa butter, the main structural material in chocolate consists of monounsaturated, polyunsaturated, and primarily saturated fats represented in **Figure 1.3**. Davis and Dimick (1989) reported that cocoa butter crystals formed during early crystallization contain high amounts of glycolipids (11.1%), phospholipids (6.6%) and

mainly triacylclycerols (67.7%). There are a mixture of about 40-50 different triglycerides (TAGs) in the cocoa butter matrix, dominated by three main "symmetrical" triglycerides: 1,3-dipalmitoyl-2-oleoyl-glyerol (POP), 1,3-distearoyl-2-oleoyl-glycerol (SOS), and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) at 17%, 27%, and 37%, respectively (Loisel et al., 1998; Lipp and Anklam, 1998; Lipp et al., 2001; Schenk and Peschar, 2004). Identification and characterization of these TAGs may be quantified using chromatographic techniques high pressure liquid chromatography (HPLC), gas chromatography-olfactory (GC-O).

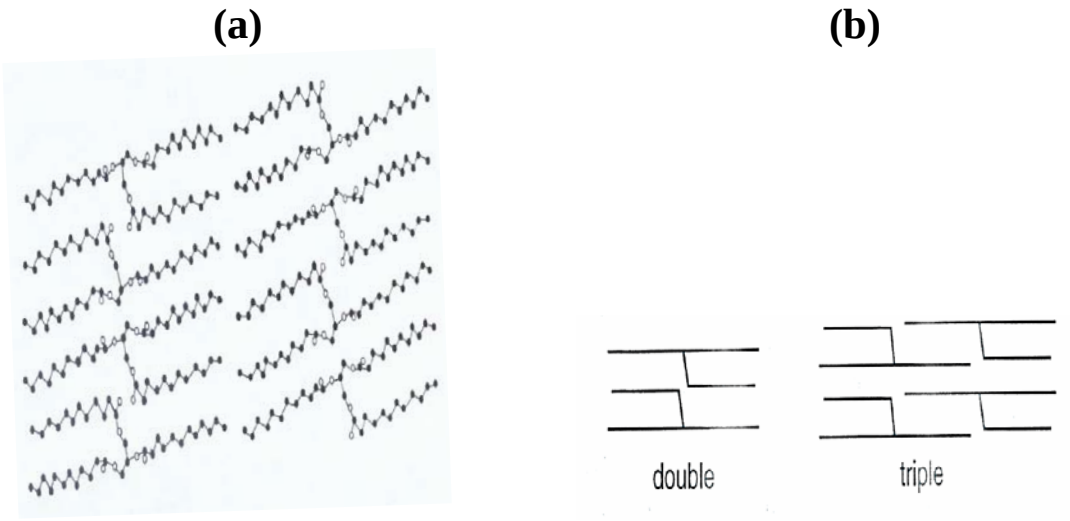
Figure 1.3: Lipid composition of cocoa butter (adapted from Davis and Dimick, 1989)



TAGs have several ways to crystallize into polymorphic forms (Sato and Koyano, 2001). Due to interactions between TAGs in the cocoa butter matrix, polymorphic crystallization is known to greatly affect the physical properties of a finished chocolate product, such as gloss, snap, and contraction after tempering (Loisel et al., 1998). The complex polymorphism of TAGs is influenced by many external factors such as heat, mass, and momentum transfer during crystallization, diversity of fatty acid composition, TAG structure, level of liquid oil in the sample, and temperature fluctuations during storage (DeMan and DeMan, 2001; Mazzanti et al., 2003). Specifically, the chain length of a TAG, its ability to pack, and its structure influence the stability of polymorphic forms. **Figure 1.4** represents an idealized packing arrangement of TAGs in a cocoa butter lattice. A double chain length TAG has three fatty acid moieties of a similar type, and

the triple chain length TAG is formed due to fatty acid moieties largely different in length or chemical structure (saturated, unsaturated, branched) (Sato, 1999).

Figure 1.4. (a) Ideal spatial arrangement of triglycerides in chair formation for packing; (b) chain length packing of double and triple triglycerides (Sato, 1999).



The three main cocoa butter crystal polymorphs, in order of increasing stability, are α (hexagonal subcell), several β' (orthorhombic subcell) and several β (triclinic subcell) forms (Hartel, 2001; Mazzanti et al., 2003; Min, 2003) (**Figure 1.5).** During initial stages of lipid crystallization, lower stability polymorphs form first (α, β', β, respectively) (Hartel, 2001). Polymorph β' signifies an intermediate polymorph (Wille and Lutton, 1969), and is seen to exist as a phase transition rather than as two separate phases (Schenk and Peschar, 2004). Research by the Schenk group indicates in β' polymorph no isothermal phase transitions have been observed and its stability can be estimated by temperature studies. A complete isothermal phase-transition scheme of cocoa butter polymorphs under static conditions is presented in **Figure 1.6**. Langevelde et al. (2001) describe that all phases except the stable β phase can be crystallized from melted cocoa butter, while only the β polymorph is crystallized after solidification. However, research suggests that direct crystallization of β polymorph from the melt is observed when cocoa butter is heated just slightly above the melting point of chocolate (33°C) before cooling. This effect is defined as the 'memory effect'. Van Malssen et al.

(1996) concluded memory effect crystallization is correlated with both the β phase melting point and the amount of SOS triglyceride and stearic acid in cocoa butter.

Figure 1.5: Basic structure of alpha, beta prime, and beta polymorph crystals (Min, 2003).

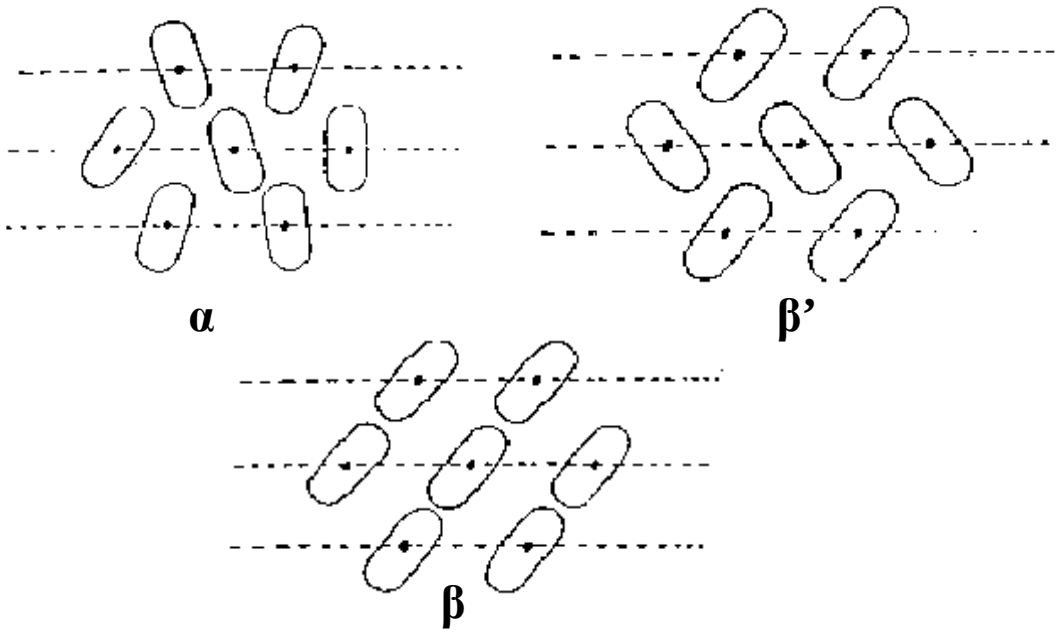
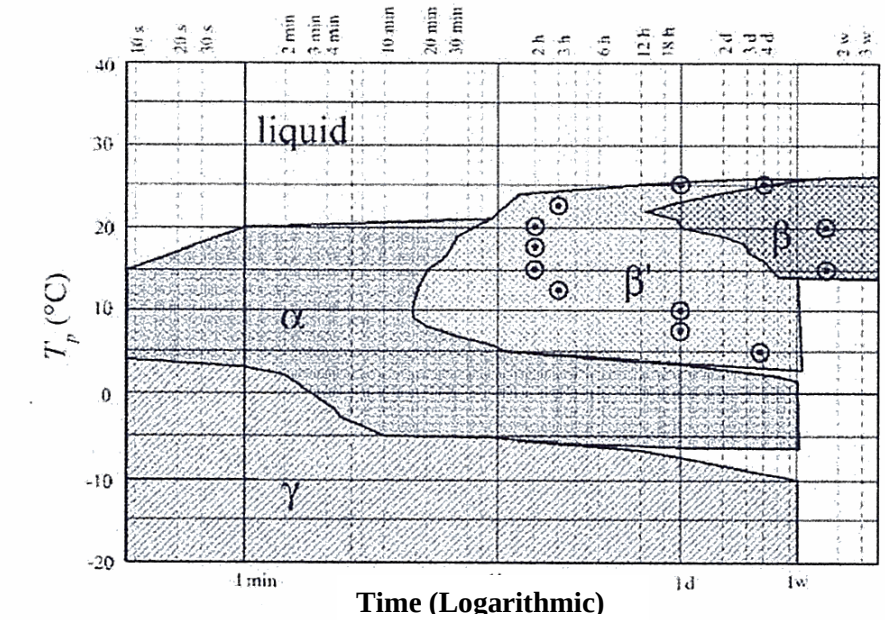


Figure 1.6: Qualitative isothermal phase-transition scheme of static cocoa butter (Schenk and Peschar, 2004).



There have been many research efforts to characterize cocoa butter polymorphs in chocolate. Between four and six polymorphs have been identified using a combination of thermal and spectrometric techniques (Fryer and Pinschower, 2000). Vaeck (1951; 1960) first used Greek letters $\gamma \rightarrow \alpha \rightarrow \beta$ to describe cocoa butter crystal polymorphs, increasing in melting point. Wille and Lutton (1969) later confirmed six polymorphs in cocoa butter, which was supported by multiple research groups as summarized in **Table 1.4**. These polymorphs are labeled with Roman numerals I-VI, increasing in stability. Differential scanning caloritmetry (DSC) results indicate that each cocoa butter phase has its own physical characteristics such as melting point and relative stability. There are many hypotheses surrounding formation of these polymorphs. Merken and Vaeck (1980) suggested Form III is a mixture of Form II and IV, later confirmed by Hartel (2001). Forms that are more dense and lower in energy, such as Form VI, are harder to melt with an average melting range of 32-37°C. The most unstable Form I, occurs under rapid cooling of molten chocolate; whereas polymorph V, the most desirable β polymorph, forms after proper tempering and is characterized to have a quick favorable melting range of 31-34°C (Schlichter-Aronhime et al., 1988). Direct solidification of β (V) polymorph from the melt has been reported by Schichter-Aronhime et al. (1988); however, Schenk and Peschar (2004) could not confirm direct crystallization of any β cocoa butter polymorph under static conditions. Form VI cannot be crystallized directly from the melt (Metin and Hartel, 2005); it occurs during storage of chocolate in polymorph V after a period of time (Wille and Lutton, 1969).

Cocoa butter polymorph V is the optimal form necessary to obtain a quality chocolate product. As stated before, polymorphic crystallization has great impact on the physical properties of a finished chocolate product, such as gloss, snap, and contraction after tempering (Loisel et al., 1998). Lonchampt and Hartel (2006) suggest as cocoa butter molecules in polymorph V rearrange into more compact crystals, the gaps between crystals are regions where cocoa solids and sugar crystals become entrapped; thus, the liquid fraction becomes less mobile and the transition from less stable to more stable polymorph forms is inhibited. Garti and Sato (1988) confirm that this non-liquid fat in cocoa butter is not completely solid, but it is plastic enough to allow slight reorganization into a more intricate and compact crystal pattern.

Table 1.4: Classification and melting temperature (°C) of cocoa butter polymorphs (adapted from Manning and Dimick, 1985)

Vaeck (1951)		Vaeck (1960)		Duck (1964)		Wille and Lutton (1966)		Lovengren et al. (1976)		Reverend (2010)	
γ	18.0	γ	17.0	γ	18.0	I	17.3	I	13.0	I	14.0
α	23.5	α	21-24	α	23.5	II	23.3	II	20.0	II	20.0
						III	25.5	III	23.0	III	22.0
β''	28.0	β'	28.0	β''	28.0	IV	27.5	IV	25.0	IV	24.0
β	34.5	β	34-45	β'	33.0	V	33.8	V	30.0	V	30.0
				β	34.4	VI	36.3	VI	33.5	VI	32.0

This cocoa butter structure is dependent upon previous organization of molecules; crystals that form parallel to the direction of cocoa butter liquid flow slow down particle rotation, while crystals that form perpendicular speed up particle rotation due to lipid-lipid interactions (Mazzanti et al., 2003) noted that the rate of cooling chocolate also contributes to the type of crystal orientation formed. Rapid growing crystals turn into clusters and begin to agglomerate (Chaisier and Dimick, 1995). Crystals that form fast lead to disorderly formations (α polymorph); crystals will not be properly packed and gaps are left in the lattice formation. Fats that crystallize slowly will form orderly lattices (β polymorphs) to further stabilize the cocoa butter matrix.

Chocolate has a unique mouthfeel since cocoa butter has a very narrow melting point, which is close to body temperature. The formation of the crystal cocoa butter lattice and crystal orientation in polymorph V contributes to this smooth mouthfeel and even melting properties (Hartel, 2001). Chocolate particle size is also extremely important to sample mouthfeel. The human tongue can detect particles up to 20-30 microns, anything at/above this range is considered 'gritty'; thus, particle size must be controlled to ensure a smooth mouthfeel, uniform melting, and proper volatile release in chocolate (Hoskin, 1994; Morgan, 1994). Extensive research has been conducted on the effects of various lipid polymorphs on texture perception (Tsheuscher and Markov, 1986a, 1986b, 1989; Franke et al., 1991; Full et al., 1996; DeMan and DeMan, 2001). Results expressed that formation of β crystals caused an increase in crystal size, also indicating an increase in hardness and decrease in spreadability (Tsheuscher and Markov,

1986a, 1986b, 1989). As crystal size increases, appearance and flavor may also be compromised. With improper storage, textural and mouthfeel changes are magnified, causing an increase in particle size, and the development of either fat bloom or sugar bloom, which compromises quality of chocolate (Morgan, 1994). The stability of cocoa butter matrix is not only essential to produce favorable mouthfeel, smooth melting in chocolate, and proper volatile release, but is also important to prevent fat bloom formation (Hartel, 2001).

1.6 Bloom Formation

Fat Bloom Formation

Fat bloom is a physical structural defect which appears during storage of chocolate and is characterized as a whitish coating on the outer surface shown in **Figure 1.7** (Loisel et al., 1997). This is a major quality concern for the confectionery industry, which has annual chocolate sales of $7.08 billion in the US (Information Resources Inc., 2010). Chocolate with fat bloom is not only visually unappealing, but theorized to affect flavor and textural qualities, which are important determinants of consumer preference. This phenomenon is not fully understood, but research suggests there are many factors which may contribute to fat bloom formation such as poor tempering (Kinta and Hartel, 2010), mixture of incompatible fats (Lonchampt and Hartel, 2006), disrupted cooling methods (Cohen et al., 2004), temperature fluctuations and storage conditions (Andrae-Nightingale et al., 2009), and abrasion or finger marking (Pastor et al., 2007).

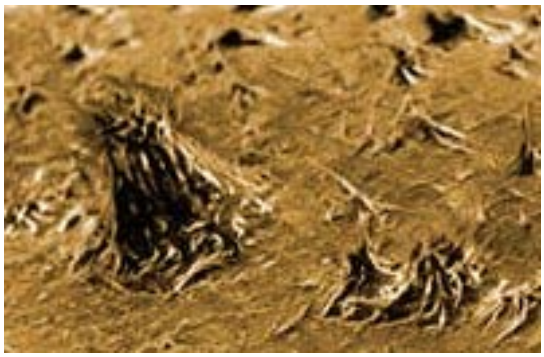
Figure 1.7: Fat bloom formation in dark chocolate (adapted from European Synchrotron Radiation Facility).

Numerous studies and resulting theories surround the mechanism of fat bloom formation. Beckett (2000) loosely describes fat bloom formation as a result of incorrect tempering of cocoa butter, as a transition from polymorph V to VI over a period of storage, and a melting, migration, and recrystallization of low melting point TAGs on the surface (Walter and Cornillon, 2001). Bricknell and Hartel (1998) further categorize fat bloom theories into two distinct groups: phase separation and polymorphic transition theories. The first involves separation of high and low melting point TAG fractions in cocoa butter (Beckett, 2000; Hartel, 2001). The higher melting TAG fails to completely melt; thus becoming a seed crystal left in the melt inducing unfavorable crystal packing. In polymorph VI, the high melting TAG becomes a phase separation of beta crystals (Bomba, 1993) while the lower melting liquid fat fraction migrates and crystallizes on the outer surface of the chocolate (Loisel et al., 1997). Aguilera et al., (2004) proposed capillary action to be a contributing factor to fat migration due to chocolate's porous microstructure. Hartel (2001) attributes liquid fat migration to a 'pumping action' mediated by temperature fluctuations. Wille and Lutton (1969) initially stated that fat bloom is not observed from pure polymorph V and has never been observed to exist in the absence of polymorph VI. It has been since noted that form VI is generally associated with fat bloom formation which is confirmed by later studies (Fryer and Pinschower, 2000).

The second theory, known as polymorphic transition, states that cocoa butter polymorphs transition from unstable to stable polymorphs (Bricknell and Hartel, 1998). Polymorph V is formed after proper tempering and slowly transforms into polymorph VI during prolonged storage with the appearance of fat bloom on the surface (Lipp et al., 1998; Aguilera et al., 2004; Afoakwa et al., 2007). The fact that polymorph VI is not obtained directly from the melt suggests that polymorph transformation from V to VI is not liquid mediated (Garti et al., 1986). Recent research confirms Wille and Lutton's (1969) hypothesis that polymorph VI is an indicator of fat bloom (though not a precursor). Quevedo et al. (2005) explain fat bloom as dispersion of light on small fat crystals (>5μm) that are formed on the surface. These fat crystals are characterized by extending 'needle and spike' formations that cause diffuse reflections of light, leading to dulling of chocolate (Bricknell and Hartel, 1998; Rousseau and Sonwai, 2008). **Figure**

1.8 is a visual representation of 'needle and spike' formations occurring during fat bloom formation. Over time, these crystals increase in number and size as bloom progresses (Adenier et al., 1993).

Figure 1.8: Visual depiction of needle and spike fat bloom formations on the surface of chocolate as observed by SEM (Rousseau and Sonwai, 2008).



Kinta and Hartel's (2010) study on untempered chocolate indicates areas with lower fat content become light colored and consist of mainly sugar and cocoa solids. This type of bloom is due to the porosity of the chocolate microstructure that is caused by a coarsened fat crystal network and liquid fat migration (Kinta and Hatta, 2007; James and Smith, 2009). It was hypothesized in previous research by Kinta and Hatta (2005) that this type of bloom is independent of the polymorphic state of cocoa butter and suggests a mechanism of bloom development involving separation associated with growth of xenomorphic fat crystals. This theory differs from the conventional visual fat bloom model suggesting the whitish layer on the surface of chocolate is recrystallized cocoa butter (Schlichter-Aronhime et al., 1988; Bricknell and Hartel, 1998; Sato, 2001). This new idea of fat bloom formation is being thoroughly investigated and confirms the complexity of multiple fat bloom mechanisms in chocolate.

Sugar Bloom Formation

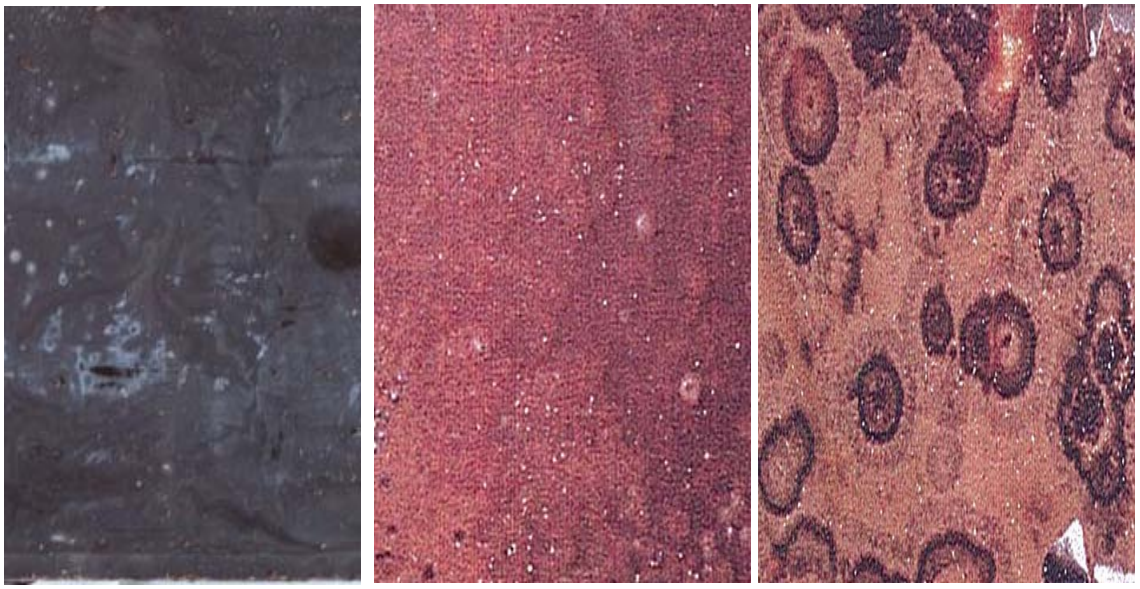
Sugar bloom is formed when water condensation dissolves sugar found on the surface of chocolate and then evaporates. Dissolved sugar particles separated from the chocolate matrix are allowed to recrystallize on the exterior, leaving a spotty white appearance. This process occurs when chocolate samples are exposed to warmer environments or conditions with high relative humidity. According to Andrae-

Nightingale et al. (2009), sugar bloom may also be seen as a function of temperature. Sugar bloom generally occurs during storage with relative humidity higher than 50-55% (Jensen, 1931). Chocolate stored at 94% relative humidity resulted in wetter, gritty, chewy chocolate, but did not form noticeable sugar bloom because the water was not allowed to evaporate; thus recrystallization of sugar did not occur (Andrae-Nightingale et al., 2009). Optimal storage for chocolate would be in a climate with relative humidity less than 50% and without temperature fluctuations.

Impact of tempering on bloom formation

Tempering involves controlled cooling of melted chocolate that will promote a stable crystalline structure for a finished product. Research suggests one type of fat bloom formation results from not tempering chocolate (Afowaka et al., 2008c). Fat bloom depends on the initial quantity of stable seed crystals formed during tempering (Garti and Sato, 1988). Therefore, development of a sufficient number of seed crystals to provide this stable fat crystalline scaffolding is crucial (Loisel et al., 1997). Lonchampt and Hartel (2006) noted that tempering induces crystallization of cocoa butter into a relatively stable polymorph that protects chocolate against bloom. Tempering first produces seed crystals of β polymorph in cocoa butter to form stable polymorph V (Cohen et al., 2004). If seed concentration is not high enough or in not the β(V) polymorph form, chocolate will crystallize into an unstable form (Kinta and Hatta, 2005). There are three types of finished chocolate masses that have been studied related to tempering that affect the overall quality: under-tempered chocolate, over-tempered chocolate, and untempered chocolate (Lonchampt and Hartel, 2006; James and Smith, 2009). **Figure 1.9** depicts quality defects on chocolates that have been improperly tempered. Untempered chocolate means that chocolate has not undergone the thermal melting process at all, under-tempering signifies the chocolate does not have sufficient β(V) seed content to completely crystallize the entire mass, and over-tempered means the mass has either too high concentration of β(V) seed after tempering or the seeds are too large. In all cases, chocolate forms bloom quickly.

Figure 1.9: Visual depiction of over-tempered (a), under-tempered (b), and untempered (c) dark chocolate (Lonchampt and Hartel, 2006; James and Smith, 2009).



(a) **(b)** **(c)**

Kinta and Hatta (2005) suggested the surface of over-tempered chocolate exhibited a dulling appearance on the surface as demonstrated in **Figure 1.9a (**Lonchampt and Hartel, 2006**)** and had a slight increase in sugar content, but the average composition had lipid concentrations similar to well-tempered chocolate. Under-tempered chocolate recrystallizes from the unstable β' seed crystals into large β form crystals which give a sandy structure **(Figure 1.9b)** (Lonchampt and Hartel, 2006). This theory was confirmed by Afoakwa et al. (2009b) stating the unstable polymorph formed in under-tempered chocolate was promoted by Ostwald ripening, the growth of larger crystals at the expense of smaller ones, and concluded that fat bloom development in under-tempered chocolate is dependent upon particle size distribution and storage time. Untempered chocolate results in unstable polymorphic cocoa butter crystal formation which stops the chocolate from contracting; thus, makes chocolate stick to the molds (Cohen et al., 2004). Recent results by Kinta and Hartel (2010) describe untempered chocolate to have black spots on a light brown surface area composed primarily of sugar and cocoa powder substances as demonstrated in **Figure 1.9c** (James and Smith, 2009). This theory supports a new mechanism for fat bloom formation that has been confirmed by both Differential Scanning Calorimetry (DSC) and Polarized Light Microscopy (PLM)

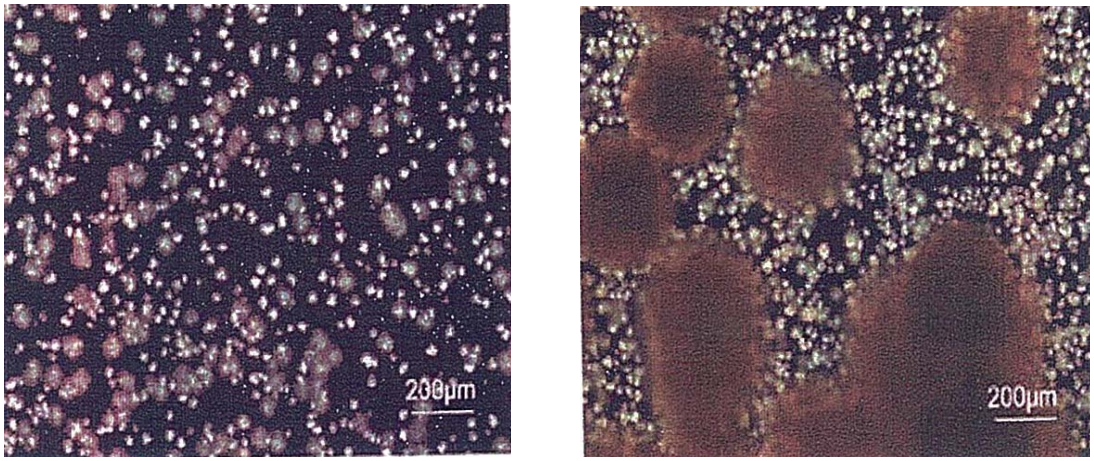
scanning techniques. This research and insight on the chocolate fat bloom mechanism is essential for understanding methods necessary to help inhibit formation and optimize quality.

1.7 Crystal characterization

Microscopic Analyses

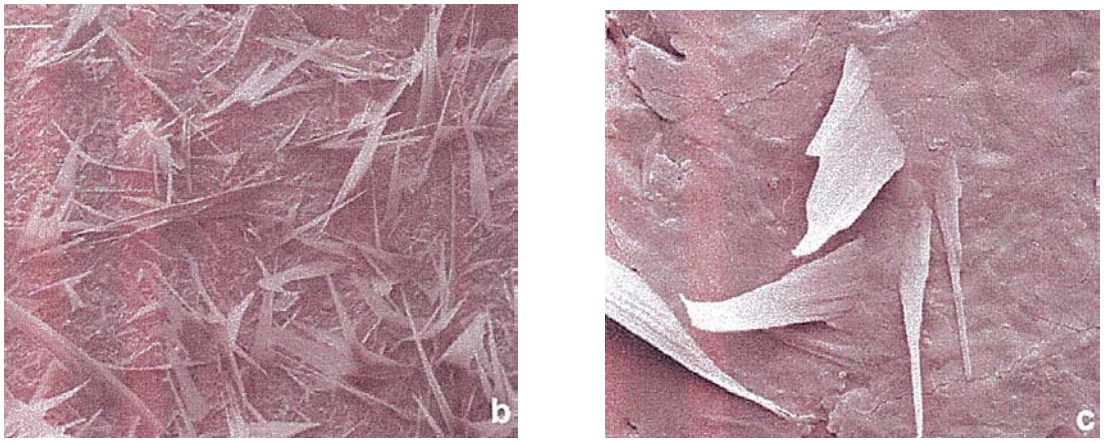
Microscopy is widely used to characterize crystals in many different media. Optical or light microscopy involves passing visible light through or reflecting off a sample through a lens to view a magnified image of the sample. PLM utilizes polarized light to form a detailed image of an object (Narine and Marangoni, 1999). This technique can be used to observe differences in cocoa butter crystallization in chocolate samples and determine crystal morphology (Kinta and Hartel, 2010). Specifically, PLM can be used to better understand microscopic structural details of the type of fat bloom crystals formed. For example, Kinta and Hartel (2010) used PLM to observe the rate and type of crystals formed from the addition of seed crystals. **Figure 10** demonstrates qualitative changes with time after addition of seed crystal and shows dramatic changes in sugar crystals (large brown agglomerations). Scanning electron microscopy (SEM) involves scanning a high-energy electron beam in a pattern over a sample to provide information on the surface topography, composition, and electrical conductivity. SEM is used to image the morphology of the surface of chocolate and produces images as small as 1 to 5 nm in size. Using SEM can help identify the structure of an individual crystal and give insight to the type of fat bloom crystals forming and their arrangement; specifically, whether the crystals are rounded or 'needle and spike' formations (**Figure 1.11)**. Environmental Scanning Electron Microscopy (ESEM) is a recent development in SEM, operating under sufficient pressure (up to 2.7kPa) and maintains an environment allowing liquid water to be present in the sample (James and Smith, 2009).

Figure 1.10: Cocoa butter crystallization with the addition of 5.5 ppm seed crystals after 30min (a), and 90min (b) as visualized using polarized light microscope (Kinta and Hartel, 2010)



(a) **(b)**

Figure 1.11: Fat bloom formation of freshly bloomed dark chocolate depicting "needle and spike formations" at the 50µm (a), and 5 µm (b) scale as visualized by Scanning Electron Microscopy (James and Smith, 2009).

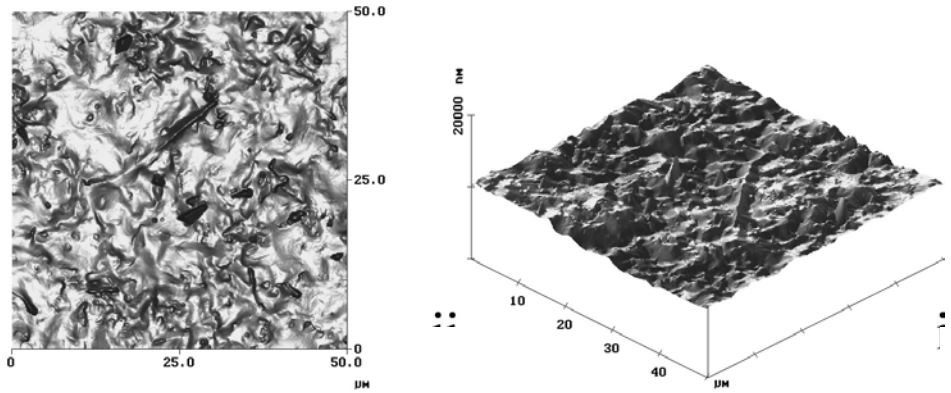


(a) **(b)**

Another microscopic technique used to characterize crystal structure is atomic force microscopy (AFM). AFM is a high-resolution scanning probe microscope that can image, measure, and manipulate matter at the nanoscale. A cantilever with a sharp tip probe is utilized to scan the surface of a sample in the *x* and *y* directions. The tip interacts with the sample surface while scanning and forces between the tip and the sample move the cantilever in the *z* direction. A 2-D or 3-D map of sample topography is created from these line scans. The AFM software can further measure surface roughness, grain

number, grain size, height, and phase difference. Hodge and Rousseau (2002) used AFM to analyze surface topography (**Figure 1.12**); as well as measure roughness in chocolate samples that had previously undergone temperature fluctuations.

Figure 1.12: Atomic force microscopy 2-D (a) and 3-D (b) scans of bloomed dark chocolate (Hodge and Rousseau, 2002).

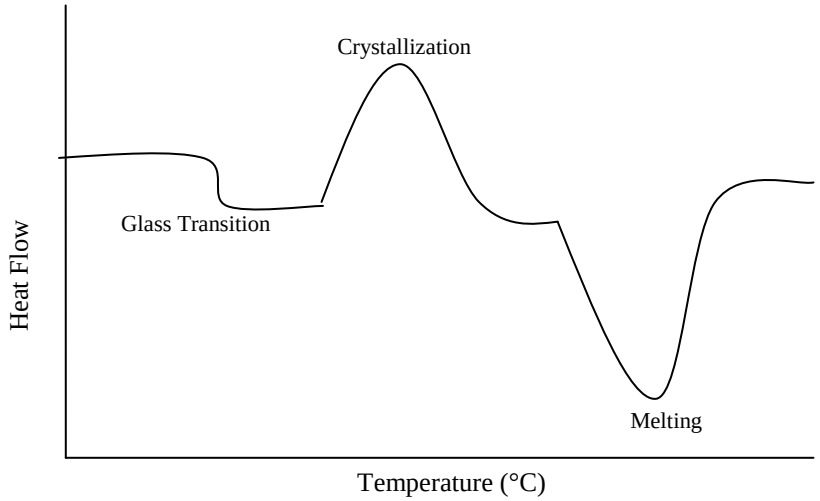


a **b**

Thermal Analysis

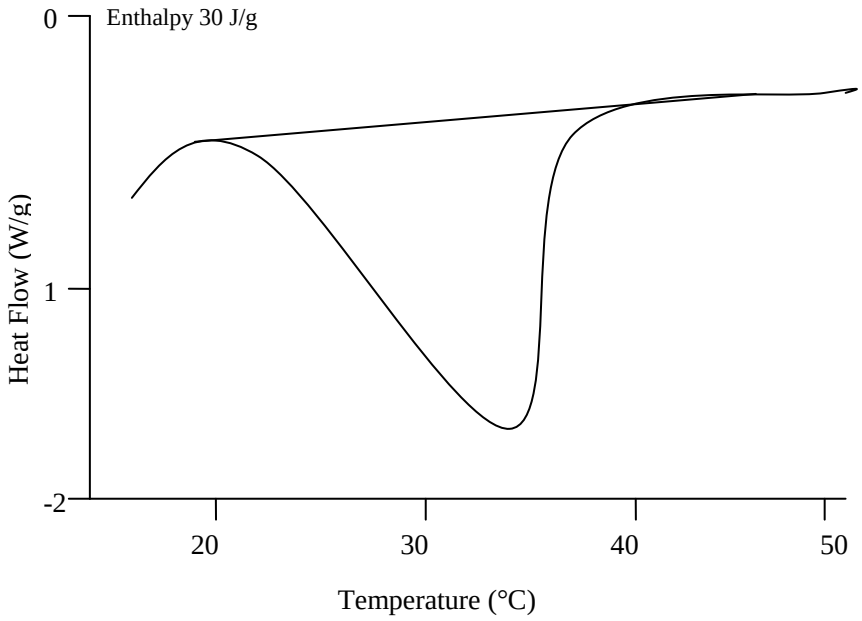
The most common thermal technique to determine crystallinity of foods is calorimetry; specifically differential scanning calorimetry (DSC). Differential scanning calorimetry is a thermo analytical technique used to measure the difference in heat flow between a sample and a reference measured as a function of temperature (Manning and Dimick, 1985). The main application is to study phase transitions, such as melting, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity. **Figure 1.13** depicts glass transition, crystallization, and melting in a DSC heat flow curve as a function of temperature. As temperature increases, the DSC instrument measures heat given off by the sample (crystallization) and heat absorbed (melting).

Figure 1.13: Feature of a DSC heat flow curve as a function of temperature and associated phase transition states.



Many DSC studies on chocolate and cocoa butter involve heating rates of 0.2-0.5°C/min ranging from -20°C to 60°C which often allow annealing (formation of agglomerates) to occur (Bolliger et al., 1998; Walter and Cornillon, 2001; Afowaka et al., 2008b; TA Instruments, 2009; Reverend et al., 2010). Chapman et al. (1970) identified that at slow heating rates, the melting of one pure cocoa butter polymorph does not produce a sharp peak, but the solid melts over a range. Annealing can cause a shift in melting endotherms or decrease peak resolution; thus, a higher heat rate above 20°C/min is recommended to help correct for this effect (Manning and Dimick, 1983). However, faster heating rates may also mask crystallization and melting effects in the endotherms (Hodge and Rousseau, 2002). Sample size may also affect melting endotherms. A large sample size (>2 mg) masks smaller individual endotherms; while a sample size of 1-2 mg will give a more detailed account of melting peaks (Manning and Dimick, 1983). The maximum peak temperature in a DSC thermal scan indicates the sample has completely melted, giving off energy to its surroundings, and can be recorded as the official melting point (TA Instruments, 2009) (as depicted in **Figure 1.14)**. A limitation to DSC is that it cannot provide direct structural information. For this reason, melting points by DSC should not be used alone to identify polymorphs; the use of spectroscopic analyses can confirm DSC results (Chapman et al., 1970).

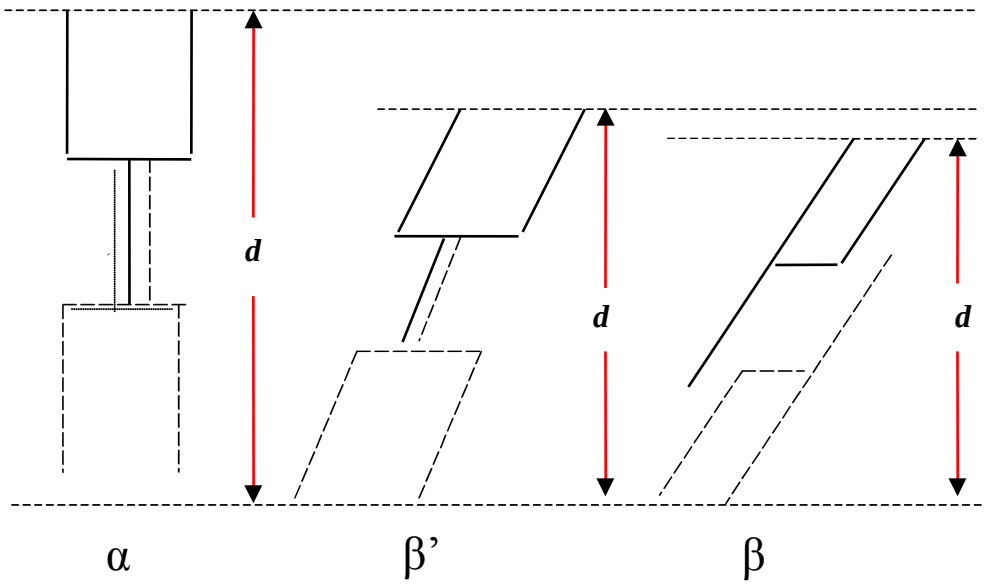
Figure 1.14: Typical DSC thermogram for chocolate melting point (adapted from Hodge and Rousseau, 2002)



Spectroscopic Analyses

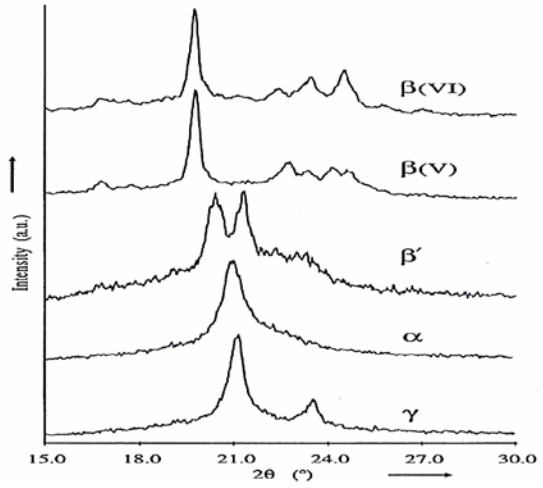
Spectroscopic techniques are used to characterize crystalline material and determine their structure. Powder X-ray diffraction (XRD), nuclear magnetic resonance (NMR), and infrared (IR) spectroscopy are the most widely used techniques for crystal analysis (Hartel, 2001). Powder XRD is often used on food products to determine molecular orientation of crystals in a crystalline lattice structure while the sample is presented in powder form, consisting of fine grains. Once the material has been identified, X-ray crystallography may be used to determine its structure such as how the atoms pack together in the crystalline state and their inter-atomic distance. As X-rays pass through the crystalline material, the wavelengths and the space between the crystal planes, *d*-spacings, are measured. The relationship between the two is specified by Bragg's law: $n\lambda = 2d\sin\theta$ (where n is a positive whole number, λ is the X-ray wavelength, *d* is the space between the crystal planes and θ is the angle of incidence). **Figure 1.15** illustrates *d*-spacings of triglyceride packing in α, β', β lipid polymorphs.

Figure 1.15: Changes in *d*-spacings for triglycerides in cocoa butter and their associated polymorphs (Min, 2003).



Cocoa butter polymorphs are identified by their short *d* spacings found in the range of 16-25°, known as cocoa butters' 'fingerprint region'. Short *d* spacings are independent of chain length and refer to the packing of hydrocarbon chains (DeMan and DeMan, 1992). An α polymorph is identified by a short spacing around 4.15Å, a β' polymorph contains two short spacings around 3.8Å and 4.2Å, and a β polymorph contains a strong spacing at 4.6 Å with weak spacings around 3.8Å and 5.4Å (Hartel, 2001). Diffraction patterns for polymorphs γ through β (polymorphs I-VI) are shown in **Figure 1.16.**

Figure 1.16: An example XRD scan of cocoa butter polymorphs I-VI (Langevelde et al., 2001).



NMR can be used to study chemical environments, physical domains, and molecular dynamics of a food product (Walter and Cornillon, 2001). NMR is the noninvasive technique used to exploit the magnetic properties of nuclei being excited. An applied electromagnetic (EM) pulse excites nuclei in higher spin states and causes them to absorb energy and radiate it back out. This radiated energy is measured at a specific resonance frequency which depends on the strength of the magnetic field and other factors. This technique has been used to measure solid fat content in foods and identify lipid polymorphs (Miquel et al., 2001). IR spectroscopy is used to identify molecules by analysis of their vibration frequencies in constituent bonds (Hartel, 2001).

Each crystal characterization analysis has its own unique feature that compliments other microscopic, thermal, and spectroscopic techniques. When characterizing cocoa butter, it is necessary to use more than one technique to confirm polymorph data. For example, it is necessary to confirm DSC melting point results with XRD polygraphs to identify the transition of polymorph V to VI and fat bloom formation (Chapman et al., 1970). Using AFM can confirm structural differences in crystal formation that relate results to PLM imagery of crystal formation (Rousseau and Sonwai, 2008). The disadvantage to using such sensitive analytical techniques is that external environmental forces can have a great impact on samples being analyzed. Relative humidity can have a great effect on melting of small (1-2mg) samples while using DSC and thus cause detrimental differences in melting points (Manning and Dimick, 1983). Also, sample scanning using AFM, SEM, or PLM must be conducted in a low moisture environment in order to prevent annealing, sticking, or melting of a sample to instrument probes. The sensitivity of such analyses requires precise conditions while running each instrument. Overall, all these techniques assist in characterization of crystal structure; microscopic techniques give visual insight, thermal techniques give insight to structural details, and spectroscopic techniques give insight to surface compositional and molecular differences.

1.8 Impact of emulsifiers in chocolate

Role of emulsifiers

An emulsion is a mixture of at least two immiscible liquids, (usually oil and water), where one liquid is dispersed as small droplets in the other (McClements, 2005).

Emulsions are made up of a dispersed and a continuous phase; the boundary between these phases is called the interface. An oil-in-water (O/W) emulsion is categorized as a system that has oil droplets dispersed in an aqueous phase (i.e., ice cream), whereas a water-in-oil (W/O) emulsion is water droplets dispersed in a fat phase (i.e., margarine) (Johansson and Bergenstahl, 1992a). The droplets are considered the internal phase and the surrounding liquid is the continuous or external phase. In most foods, droplet diameter ranges between 0.1-100μm (Surh et al., 2007). Emulsions are thermodynamically unstable mixtures because of interfacial tension and repulsive forces between oil and water (Beckett, 2008). Thus, something is needed to enhance the stability of an emulsion, such as an emulsifier. An emulsifier is a surface active substance (surfactant) that can help stabilize an emulsion by increasing its kinetic stability and decreasing interfacial tension to prevent the emulsion from changing significantly (Schneider, 1986).

Emulsifiers, as surfactants, absorb to the surface of particle droplets and form a protective membrane around the internal phase to prevent particles from aggregating (Beckett, 2008). By decreasing interfacial tension, emulsifiers help disperse particles throughout the continuous phase. Most emulsifiers are amphipilic molecules—they have polar and nonpolar regions on the same molecule (McClements, 2005). The most common emulsifiers used in the food industry are small molecule surfactants, phospholipids, proteins, and polysaccharides. Some examples of natural food emulsifiers are egg yolk (lecithin), honey, mustard, and proteins. The type of emulsifier selected is based on its interaction with other components in the food system. Thus, emulsifiers are classified according to their electric charge and their solubility in various polar and non polar solvents determined by the ratio of lipophilic and hydrophilic molecules (Surh et al., 2007). A standard was developed to evaluate the relative strength of an emulsifier in a system—known as the HLB value (hydrophilic/lipophilic balance) on a scale 1-20. Lipophilic emulsifiers have a low HLB (<10) and hydrophilic emulsifiers have a high HLB number (>10) based on emulsifier solubility in each phase. It is important to select a proper emulsifier based upon HLB values for a specific formulation to ensure optimal emulsion stability.

Emulsion stability refers to the ability of an emulsion to resist change in its properties over time (McClements, 2005). Instability of an emulsion is due to various internal physicochemical processes as well as external environmental conditions affecting the emulsion. Physical instability is caused from a spatial distribution or structural reorganization of the molecules, whereas chemical instability is due to an alteration in molecules present. Negative emulsion effects that can occur are creaming, flocculation, coalescence, phase inversion, and Ostwald ripening that result from physical instability (Dickinson and Golding, 1992; Walstra, 1996). Specifically, spatial distribution of molecules is governed principally by their noncovalent interactions such as electrostatic, van der Waals, and steric overlap (McClements, 2005). The strong repulsion that arises from steric hinderence has a strong influence on the packing of molecules in liquids and solids. Oxidation and hydrolysis are common examples of chemical instability and the rate at which an emulsion breaks depends on its composition, microstructure, and the external environmental conditions such as temperature fluctuations, mechanical agitation, and storage conditions (Fennema, 1996; McClements et al., 1993). Emulsifier investigations in various food systems have been crucial for optimizing processing to minimize emulsion instability (Beckett, 2008).

Emulsifiers in chocolate

Chocolate is considered a "dry" emulsion with hydrophilic sugar and lipophilic cocoa particles dispersed in the continuous fat cocoa butter phase (Nieuwenhuyzen and Szuhaj, 1998). Emulsifiers regulate rheological properties and/or crystallization of fats (Johansson and Bergenstahl, 1992a). In the chocolate matrix, emulsifiers coat the sugar particles to help facilitate flow in the continuous fat phase; this helps distribute particles evenly throughout the emulsion and prevent agglomeration. Emulsifiers have the capability of altering viscosity in specific foods (Walter and Cornillon, 2001; Schantz and Rohm, 2004; Afoakwa et al., 2009a). This becomes important in the chocolate production for example during enrobing, panning, molding, or depositing (Rector, 2000). The ability to reduce viscosity and control overall rheology of chocolate allows confectionary manufacturers to optimize their processes and help minimize production costs. Some of the research described here is directly in reference to chocolate coatings; however, it is also important to emulsifier selection and formulation in chocolate bars.

In 1930, Hanse-Mühle demonstrated that small amounts of suitable surface active lipids produce an immediate reduction in viscosity, thus, patenting the action of lecithin as a viscosity-reducing agent (as discussed by Chevalley, 1999). Using a viscometer, one can use the Casson equation to solve for two crucial flow parameters: yield value and plastic viscosity (Beckett, 2000). The Casson equation is used to describe the flow behavior of chocolate, where τ (yield stress); τ_{CA} (Casson yield stress); μ_{CA} (Casson plastic viscosity); and γ (shear rate):

$$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}}\sqrt{\gamma}$$

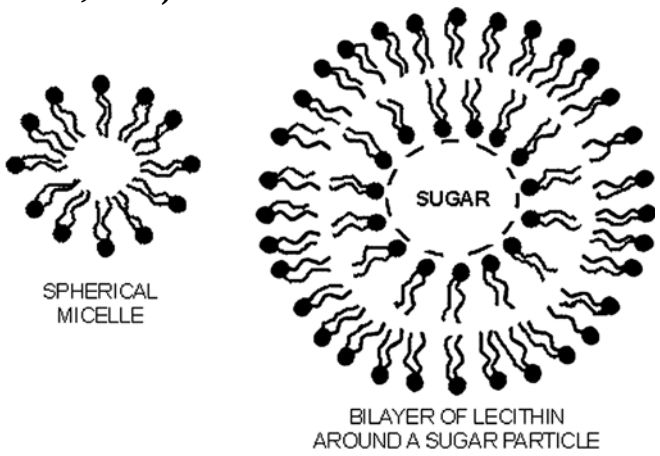
Casson plastic viscosity (1 Pa.s=10poise) is the energy required to keep fluid in motion. It relates to pumping characteristics, filling of rough surfaces and coating properties; whereas, yield value (1Pa= 10 dyn/cm^2) is the minimum force that must be applied to initiate flow. Yield value relates to shape retention, pattern holding, inclined surface coating and bubbles in processing (Seguine, 1988). In addition to altering flow properties, emulsifiers added at certain concentrations can help reduce overall fat content, and enhance functionality in chocolate (Walter and Cornillon, 2001). Specifically, in certain cases, emulsifiers have been used to substitute for cocoa butter. For example depositing chocolate (forming chocolate morsels) requires high yield values to maintain shape prior to chocolate setting, with the addition of an emulsifier, less cocoa butter is utilized while yield values are held constant (Rector, 2000). Chocolate manufacturers could potentially reduce the overall fat content from cocoa butter of the product and reduce cost by adding small amounts of emulsifier to control these yield values (Bamford et al., 1970). Emulsifiers have also been used to influence sensitivity to relative humidity, sensitivity to temperature, and tempering behavior (Afoakwa et al., 2007). Emulsifiers impact properties of solidified chocolate, including susceptibility to fat bloom and stability against fat migration and oxidation from fillings (Schantz and Rohm, 2005).

Different emulsifiers are used in chocolate, such as the most commonly noted soy lecithin (Schantz and Rohm, 2005), a mixture of natural phosphoglycerides. Polyglycerol polyricinoleate (PGPR), obtained from the condensation of polyglycerol and condensed castor oil fatty acids (Bamford et al., 1970; Wilson et al., 1998) and ammonium phosphatide, an extract from rapeseed oil are two additional emulsifiers used in chocolate. Additionally, citric acid esters of mono-, di-glycerides have also been studied

as potential emulsifiers for chocolate formulation. Previous studies demonstrated that, in contrast to lecithin, PGPR is very effective at binding water in chocolate (Afoakwa et al., 2007). Loisel et al. (1998) showed the Casson plastic viscosity was reduced and yield value was increased for lecithin; while the opposite was observed for PGPR chocolate blends.

Thickening of chocolate depends on particle size distribution, as smaller particles require more emulsifier to coat sugar surfaces (Beckett, 2000). In the chocolate matrix, plastic viscosity measurements are important in determining coating thickness on a confection. Yield value measurements relate to the initial movement of chocolate; thus, if yield value is high, the chocolate will tend to stand up (i.e., chocolate morsels). A low yield value results in a thin coating of chocolate over a biscuit (Fletcher, 2006). Increasing yield value corresponds to micelle formation in the fat phase possibly as multi-layers around sugar **(Figure 1.17)**, which hinders flow. Thus, Beckett (2008) recognized it is ideal to have reverse micelles form in the fat phase and interact with covered sugar particles to increase yield value. A study by Bartusch (1974) recognized that the efficiency of coating sugar by an emulsifier was found to be dependent upon the concentrations, approximately 50, 67, and 85% sugar coated for lecithin concentrations of 0.2, 0.3, and 0.5%, respectively. With increasing concentrations of lecithin, the interface between the particles and the fat reduces friction and facilitates “lubrication” (Nieuwenhuyzen and Szuhaj, 1998). Most importantly, the addition of emulsifiers is extremely effective in reducing chocolate viscosity, approximately 10 times greater than natural cocoa butter (Beckett, 1999). My research is designed to characterize behavior and impact of emulsifiers used in chocolate under different storage conditions. Specifically, three emulsifiers: soy lecithin, PGPR, and ammonium phosphatide were the subject of this study.

Figure 1.17: Schematic diagram of spherical micelle and bilayer of lecithin on a sugar molecule (Beckett, 2008).



Soy lecithin

The most common emulsifier used in chocolate manufacturing is soy lecithin (Schantz and Rohm, 2005). Soy lecithin is a by-product in soya oil preparation and results as a light to medium pasty brown product of a liquid to paste consistency (Surh et al., 2007). Lecithin has soya oil content of 33-35% and remainder polar lipids called phospholipids (Scholfield, 1980). Under Food and Drug Administration (FDA) regulations (Code of Federal Regulations Title 21, 2003), it can only be added up to 1% (w/w) of chocolate formulations. Soy lecithin has an HLB value ranging from 3-9. The ratio of phospholipids varies within standard lecithins resulting in ranging viscosities from chocolate batch to batch (Schmitt, 1995). The phospholipid head attaches to sugar and phosholipid tail flows in the fat phase to help maintain desirable flow (**Figures 1.18 & 1.19**). Vernier (1997) illustrates the coating of sugar particles by fluorescent lecithin using confocal laser scanning microscopy (**Figure 1.20**).

Figure 1.18: Structure of soy lecithin (Schnieder, 1986)

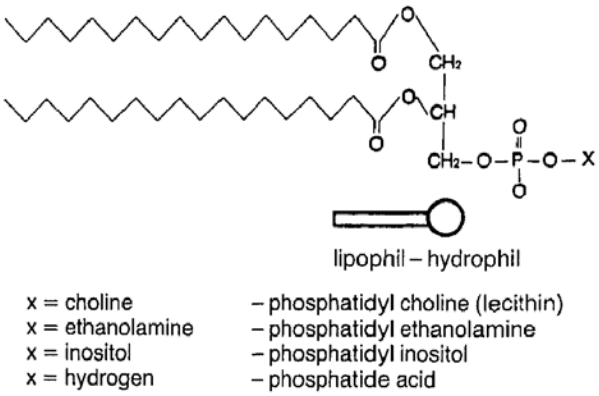


Figure 1.19: Schematic diagram of lecithin molecules surrounding a sugar particle in the continuous fat phase in chocolate (Beckett, 2008).

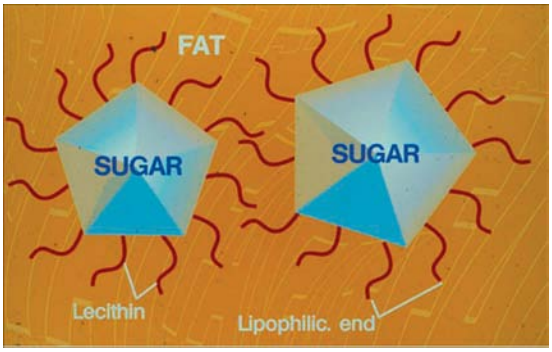
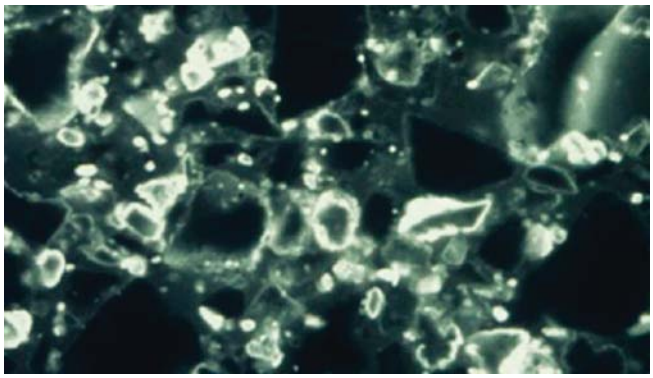


Figure 1.20: Confocal laser scanning microscope image of lecithin (fluorescing) surrounding solid particles within chocolate (Vernier, 1997; Beckett, 2008).



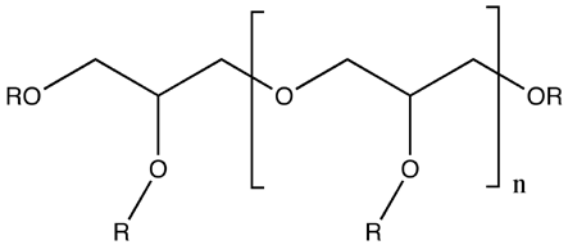
Dhonsi and Stapley (2006) demonstrated that lecithin retarded crystallization in cocoa butter and sugar mixtures, with no change in melting point as observed by DSC. Lecithin migrates to sugar/fat interfaces and coats sugar crystals, aids dispersion of sugar

crystals in cocoa butter, and influences the rheology of chocolate (Vernier, 1997). Lecithin may provide a rough surface for nucleation to occur since it covers the sugar; also lecithin might reduce shear rates by creating a greater 'gap' between the fat phase and sugar particles (Dhonsi and Stapley, 2006). Lecithin added at concentrations of at least 0.1-0.3%, reduces chocolate viscosity and enhances moisture level tolerance. At more than 0.5%, yield value increases while plastic viscosity continues to decrease (Chevalley, 1999; Schantz and Rohm, 2005). Currently, the main drawback of using soy lecithin is consumer concern that it may be a byproduct of genetically modified soybean plants; manufacturers have thus considered using different emulsifiers as new alternatives (Fletcher, 2007).

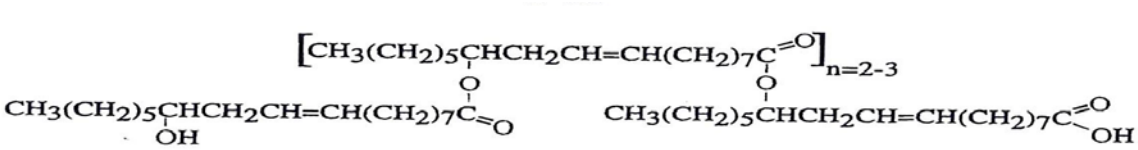
Polyglycerol Polyricinoleate (PGPR)

Polyglycerol polyricinoleate (PGPR) has the ability to greatly reduce or even eliminate the yield value of chocolate, essentially turning it into a Newtonian liquid to flow more readily, a valuable characteristic for molding and enrobing techniques (Schantz and Rohm, 2005). PGPR has an HLB value of approximately 1.5 (Rector, 2000). Polyglycerol polyricinoleate is produced from either esterification of polyglycerols with polymerized ricinoleic acid or polycondensation of castor oil and glycerol. It is a mixture with a polyglycerol backbone dominated by di, tri, and tetraglycerols (**Figure 1.21)** (Wilson et al., 1998). Extensive toxicology research has been conducted using PGPR and it is been GRAS certified (Wilson et al., 1998). Legally approved by the FDA, it can be added up to 0.5% (w/w) of the chocolate formulation and is considered a non-genetically modified product that is not an allergen (Code of Federal Regulations Title 21, 2010). Both lecithin and PGPR work synergistically with other emulsifiers, such as ammonium phosphatide and citric acid esters (Stier, 2009). PGPR is most efficient in enhancing the flow of chocolate into molds, helping reduce incorporation of air into chocolate, and improving coating abilities of chocolate (Fletcher, 2006).

Figure 1.21: Structure of Polyglycerol Polyricineoleate (adapted from Surh et al., 2007).



At least one **R** group is polyricinoleate (below) or fatty acid residues



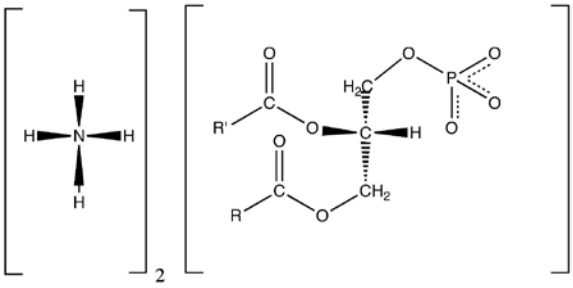
PGPR did not have dramatic effects on plastic viscosity; however it reduced yield value by 50% at 0.2% or eliminated it at 0.8% (Rector, 2000). PGPR effectively stabilizes formulations by extending its lipophilic tail into the lipid phase of chocolate and simultaneously, its hydrophilic head coats solid sugar particles (Dedinaite and Campbell, 2000). Schantz and Rohm (2005) noted that PGPR binds free water more effectively than lecithin in chocolate, thus preventing swelling of solid cacao particles. Walter and Cornillon (2001) suggested PGPR had less effect in inhibiting bloom formation, compared to other emulsifiers.

Ammonium Phosphatide

In 2007, ammonium phosphatide, was GRAS certified as an emulsifier in chocolate and vegetable fat coatings at a level of up to 0.7% (Agency Response Letter GRAS Notice No. GRN 000219). The FDA recognizes it for having a non-genetically modified status and for not being an allergen (Code of Federal Regulations Title 21, 2010). Ammonium phosphatide (**Figure 1.22**) is a flavor neutral product either manufactured synthetically from a mixture of ammonium salts of phosphorylated glycerides or from a mixture of glycerol and partially hardened rapeseed oil and has an HLB value range from 2-3 (Chevalley, 1999; Shur et al., 2007). Rapeseed oil is mainly used as it offers certain advantages in respect to taste, availability, and price (Schneider, 1986). Ammonium phosphatide is currently being investigated to determine its benefits

and optimal concentration for chocolate formulation. Preliminary studies suggest it lowers plastic viscosity without increasing yield value at a concentration of 0.5% (Fletcher, 2007).

Figure 1.22: Structure of ammonium phosphatide (adapted from Garti, 2001)



1.9 Chocolate Flavor

Effect of Processing on Flavor

Chocolate has over 600 volatile compounds associated with its flavor profile (Counet et al., 2002), contributing to consumer perception of taste and aroma. The flavor matrix is heavily dependent upon concentration of volatile components and is influenced by the rate of release from chocolate which is dependent on temperature, molecular interactions, and partition coefficients of the particular compounds (Kinsella, 1990). Besides cacao bean origin, there are four crucial external factors in chocolate processing that affect flavor development: fermentation, roasting, conching, and storage. Extensive studies have been conducted on the specific compounds responsible for the distinctive chocolate flavor (Schnermann and Schieberle, 1997; Deibler et al., 1999; Andrae, 2006). Key compounds that give cocoa and chocolate its distinct flavor are molecules such as pyrazines (roasted), aldehydes (cocoa aroma, nutty), esters (fruity), and phenolic compounds (astringence); many of these compounds are generated during the Maillard reaction (Schwan and Wheals, 2004).

Cambrai et al. (2010) concluded that aromatic quality of the cacao bean is linked to origin, variety, soil, climate, area of culture, and processing steps during chocolate production. Cacao beans, differing in origin and cultivars (crillo, forastero, and trinitario) are known to have distinct flavor differences that are often perceived by chocolate buyers

(Guittard, 2009). Specifically, compounds such as linalool or (E, E)-2,4-decadienal are noted to be characteristic of chocolate's different geographical origins. Cambrai et al. (2010) found that benzaldehyde (hazelnut/almond), linalool (floral smell), and phenylacetaldehyde (honey note) were characteristic of African beans; while 2-phenyl-2-butenal (roasting intensity) and 4-methyl-2-phenyl-2-pentenal (bitter) were compounds identified with the Madagascar cacao bean. After harvest, fermentation reduces bitter, sour, astringent, and acidic components (Bekett, 2000). Cacao beans can also be subjected to improper fermentation techniques such as over fermenting and under fermenting which negatively affect the flavor of the finished chocolate by giving the final product an astringent moldy or dry dark taste, respectively (Guittard, 2009). Research by Jinap et al. (1995) indicates that the acidic environment created during fermentation causes off-flavors due to production of volatile fatty acids. The variation of acidic characteristics of cacao beans can be correlated with certain fermentation techniques and duration of the process.

Maillard browning is nonenzymatic browning that occurs during roasting of cacao and produces two main classes of flavor compounds found in chocolate: pyrazines and aldehydes (Counet et al., 2002). Early stages of Maillard browning produce strong chocolate notes from Strecker aldehydes, 2-methylpropanoal, 3-methylbutanal, and 2-methybutanal while later stages produce characteristic pyrazines, pyrroles, and oxazoles that have been found in cocoa (Hoskin and Dimick, 1983). Urbanski (2001) noted that the type of beans and roasting conditions affect acidity and flavor profile of chocolate. Low roasts tend to maintain the volatility of the flavor profile; medium roasts produce less acid and contribute more balanced notes; while high roasting conditions are least acidic and least aromatic. Many flavor compounds are lost during this high heat process. Duration of roasting is also important; Misnawi et al. (2004) indicated longer roasting times resulted in higher pyrazine concentrations and significantly affected flavor.

After roasting, dried beans undergo another flavor development step known as conching. Conching is the process of continuous mixing of the chocolate liquor, either with or without the addition of cocoa butter to optimize flavor and further refine particle size. Conching involves volatilization of fatty acids and aldehydes and development of smooth texture (Prawira, 2009). Conching strips dark chocolate of its volatile acids and

aromatic complexes, reduces bitterness and develops typical chocolate flavor (Beckett, 1999). The favorable textual and flavor attributes developed during conching are dependent upon duration of agitation, temperature during mixing, and addition of additives during the process. Prawira (2009) noted that longer conching produced smoother, more mouthcoating chocolate. However, conching too long will result in gummy, bland chocolate (Beckett, 1999) and too little agitation results in grainy, acidic chocolate (Schumacher, 2009). Cambria (2010) noted that (E,E)-2-4-decadienal (fatty, waxy odor) is a compound generated at high temperatures applied during conching of chocolate. Chocolate can emit burnt, fatty, and astringent off-flavors if chocolate is subjected to conching at high temperatures for too long (Beckett, 1999).

Storage, another external factor affecting flavor development, causes structural changes in the cocoa butter liquid phase and may alter volatile release. Controlled storage is known to chocolate professionals as “aging” and is a technique used with dark chocolate for approximately 30-60 days (Urbanski, 2001). Famous chocolatier Art Pollard of Amano Chocolate relies on ‘aging’ his chocolate because he believes the potential full flavor does not develop for at least three weeks after the chocolate has been molded (Pollard, 2007). Research indicates that during aging, volatile acids and aromatics dissipate, leaving a balanced profile with fewer sour and pungent notes. This aging of chocolate is seen as beneficial; however, aging associated with improper storage negatively affects chocolate flavor and quality. Studies by Engeseth and Nightingale, (2007) indicate storage of chocolate at ambient, freezer, or high relative humidity conditions results in a significant increase in volatile loss. Improper storage of chocolate exhibiting fat bloom formation was seen to negatively impact flavor and volatile release as well.

Flavor and sensory analysis of cocoa products

Characterization of chocolate flavor is done using sensory and instrumental quantitative and qualitative analyses. Flavor analysis has been performed on many cocoa products, such as cocoa beans, cocoa mass, nibs, and chocolate. Flavor analysis involves a variety of potential techniques used to isolate volatiles such as, Static Headspace Sampling, Distillation-Solvent Extraction (DSE), Solvent-Assisted Flavor Evaporation (SAFE), and Solid Phase Microextraction (SPME) (Cadwallader, 2008). Following these

techniques, isolated volatiles are separated and identified using high pressure liquid chromatography (HPLC), gas chromatography-olfactory (GC-O), and gas chromatography-mass spectrometry (GC-MS). **Table 1.5** gives an example of 25 key volatile compounds identified in dark chocolate. Over 600 volatile compounds have been identified and associated with chocolate (Counet et al., 2002).

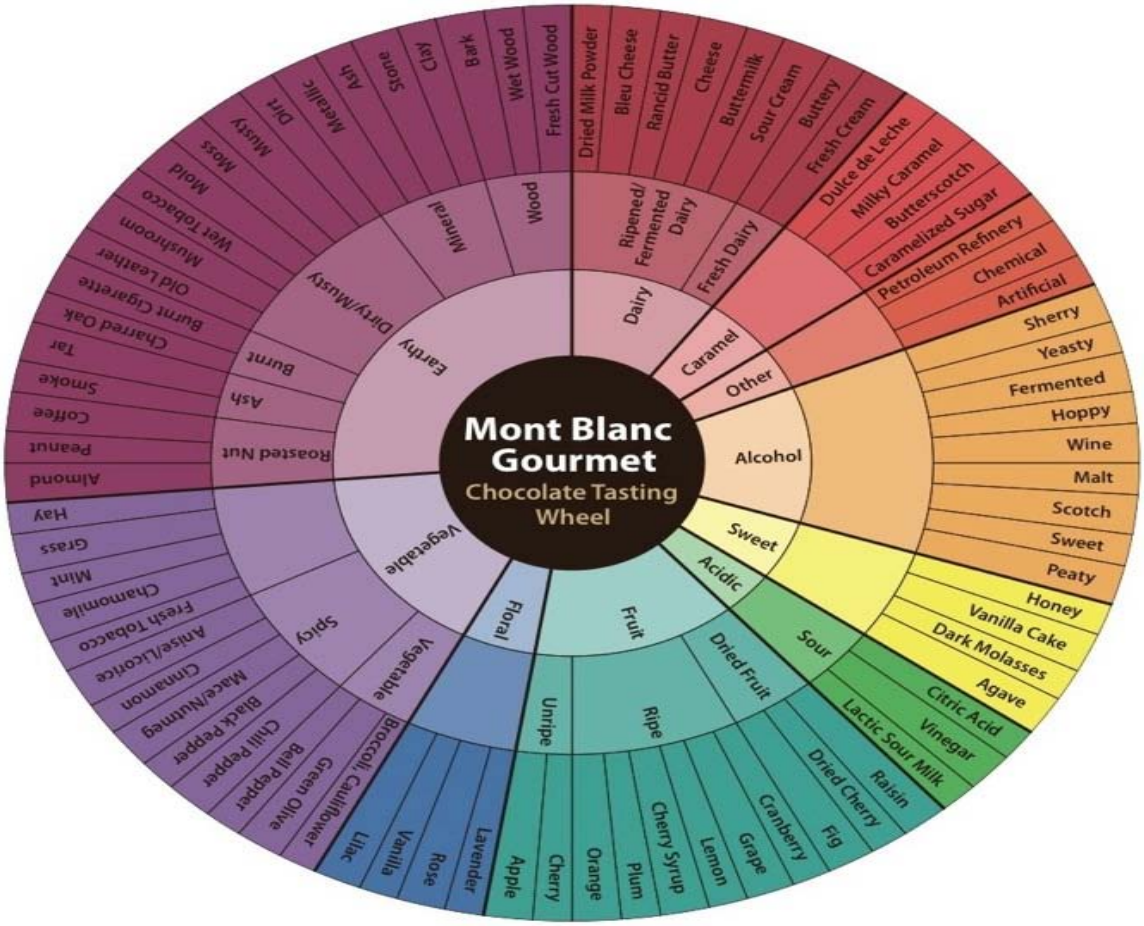
Sensory techniques have been used to characterize chocolate flavor and are useful to describe consumer preference. Consumers usually use broad general terms to describe chocolate flavor (Sune et al., 2002). Descriptions of the full sensory profile of chocolate can be grouped into three categories: texture, flavor, and melting properties. Chocolate must be evaluated at all points of tasting to fully describe the complete flavor profile of volatiles released prior to, during, and after mastication. Trained sensory panelists recognized that consumers evaluate chocolate at different points during consumption: initial bite or breaking (hardness) while chewing (melting, stickiness) and when rubbing melted chocolate between mouth and tongue (viscosity, smoothness, graininess), all properties essential to fully describe chocolate (Tscheuschner et al., 1986a). Thamke (2009) used this technique of evaluating chocolate at different points to describe chocolate bitterness, sweetness, acidity, cocoa flavor, and melting character. Other studies have evaluated bitterness associated with chocolate (Pelchat and Beauchamp, 1999), sensory properties of milk chocolate (Guinard and Mazzucchelli, 1999), and chocolate flavor alterations during storage over a range of conditions (Andrae-Nightingale et al., 200X). Surprisingly, there are limited reports in the literature focused on sensory properties of stored dark chocolate. Dark chocolate stored at high temperature with fluctuations resulted in decreased volatile concentrations, signifying that prolonged storage is detrimental to chocolate volatile loss (Andrae-Nightingale et al., 200X). This study confirms that storage of chocolate has negative effects on both physical and sensory properties associated with chocolate. Research thus far shows that sensory descriptors and volatiles identified with chocolate flavor are endless due to the complex flavor profile of chocolate as depicted in **Figure 1.23**.

Table 1.5: Flavor Compounds identified in dark chocolate samples used for quantification (Andrae, 2006).

Peak No.	Compound	Concentration[a]	RI[b]	m/z[c]
1	2-methylpropanal	0.197	806	72
2	2(or3)-methylbutanal	0.531	911	58
3	dimethyl disulfide	0.135	1057	94
4	hexanal	0.112	1068	44
5	myrcene	0.098	1147	93
6	limonene	0.171	1184	68
7	methyl pyrazine	0.406	1259	94
8	2,5(or6)-dimethylpyrazine	0.644	1316	108
9	2,3-dimethylpyrazine	0.149	1341	108
10	dimethyl trisulfide	0.105	1357	126
11	2-nonanone	0.127	1370	8
12	2-ethyl-5(or6)-methylpyrazine	0.144	1373	121
13	2-ethyl-3-methylpyrazine	0.162	1393	121
14	trimethylpyrazine	0.228	1395	122
15	1-octen-3-ol	0.174	1416	57
16	2(or3)-ethyl-2(or3),5-dimethylpyrazine	0.358	1440	135
17	2-furfural	0.133	1445	96
18	tetramethylpyrazine	0.099	1463	54
19	propanoic acid	0.366	1496	74
20	linalool	0.102	1514	71
21	2-methylpropanoic acid	1.241	1522	73
22	butyric acid	1.061	1595	60
23	3-methylbutyric acid	1.068	1626	60
24	2-methoxyphenol	0.324	1831	109
25	acetylpyrrole	0.100	1939	94

[a]Superstock concentration measured as g/mL. [b]RI = Retention Index. [c]Ion spectra used to identify and quantify respective volatiles.

Figure 1.23: A tasting wheel of terms used to describe chocolate flavor (adapted from Mont Blanc Gourmet Site).



Chapter 2: The impact of varying emulsifier concentrations on fat bloom formation in stored dark chocolate undergoing temperature fluctuations

2.1 Introduction

Chocolate is in essence cocoa mass and sugar suspended in a cocoa butter matrix. Cocoa butter, the main structural material in chocolate, is composed of three main "symmetrical" triglycerides (TAG's) (Lipp and Anklam, 1998; Lipp et al., 2001). These TAG's are responsible for crystallization and melting attributes of chocolate. The TAG's have several ways to crystallize, known as polymorphism (Sato, 1999). Structural lipid polymorphs are critical to quality parameters of chocolate such as texture and flavor release, and the smooth mouthfeel of chocolate is due to the unique interactions of polymorphic lipid structures of cocoa butter (Hartel, 2001). During storage structural changes occur. Bloom is the main cause of quality loss in the chocolate industry (National Confectioners Association, 2005). This has a dramatic impact on chocolate sales, which total nearly $7.08 billion in the US (Information Resources Inc., 2010). Exact market loss due to fat bloom is difficult to verify, since changes may arise many months after processing. As bloom forms, particle size increases and microstructural and perceptual changes also occur (Rousseau and Sonwai, 2008). Temperature cycling is often used to investigate bloom formation in chocolate. Previous research indicates temperature cycling regimens have varying and dramatic impacts on physical and chemical structural aspects of chocolate (Andrae, 2006). Increased temperature during cycling regimes increased intermolecular mobility of the lipid fraction and led to structural changes in the cocoa butter lattice which, in turn, resulted in fat bloom formation. Many studies have investigated the mechanism of fat bloom formation (Wille and Lutton, 1969; Bricknell and Hartel, 2006; Rousseau and Sonwai, 2008; James and Smith, 2010), but literature reports are lacking on the impact emulsifiers have on cocoa butter stability.

Emulsifiers have long been used to modify the texture of chocolate especially in commercial coatings (Walter and Cornillon, 2001). They influence several properties in chocolate, including sensitivity to relative humidity, sensitivity to temperature, and tempering behavior. Emulsifiers have also been used to influence structural properties impacting consumer perception of chocolate texture and flavor. They are also believed to

impact properties of solidified chocolate, including susceptibility to fat bloom, stability against fat migration from fillings, and stability against oxidation (Schantz and Rohm, 2005). Various emulsifiers are used in chocolate, such as the most commonly noted soy lecithin, a mixture of natural phosphoglycerides; recently approved GRAS certified (2007) emulsifier ammonium phosphatide, and polyglycerol polyricinoleate (PGPR), obtained by polycondensation of castor oil and glycerol. Previous research demonstrated that, in contrast to lecithin, PGPR is more effective at binding water in chocolate (Rector, 2000). The overall goal of this study was to obtain a better understanding of the mechanistic features of how various emulsifiers influence fat bloom formation in stored chocolate and to decipher the impact of emulsifier concentration in this role. I hypothesized that changes which occur during storage of chocolate would impact the stability of emulsifier-TAG complexes, which may be a precursor to phase separation of lipid and sugar in chocolate and ultimately lead to bloom formation. These changes would impact intermolecular mobility of the continuous fat phase which would be detrimental to the overall stability of the cocoa butter matrix. Specifically, the emulsifier-TAG complex stability is directly correlated to the type of emulsifier used and the concentration in chocolate formulation.

Characterizing instrumental and sensory changes associated with emulsifier formulations will assist in predicting appropriate storage conditions and optimal emulsifier composition/concentrations to minimize negative influences on flavor and textural qualities of chocolate. Quality analysis of chocolate was focused on melting behavior and initial lipid polymorph characterization using Differential Scanning Calorimetry (DSC), confirmation of polymorph transition by Powder X-Ray Diffraction (XRD) and topographical surface analysis using Atomic Force Microscopy (AFM), including roughness. Although temperature cycling is not typical for normal storage conditions, specific details in this study may lead to a better understanding of the impact emulsifier type and concentrations have on fat bloom formation during chocolate storage and how that impacts flavor, texture and human sensory perception in chocolate stored at different temperatures. This will provide insight into emulsifier selection for chocolate manufacturing applications.

2.2 Materials and Methods

2.2.1 Chocolate Materials

Dark chocolate was prepared using a SPECTRA 10 Stone Melangeur (SanthaUSA, Yoncalla, OR), and a Revolution 2 Chocolate Tempering System (Chocovision™, Poughkeepsie, NY). Chocolate liquor was obtained from Peters Chocolate (ILA, Springfield, IL) and granulated sugar was acquired from a local grocery store. Three different emulsifiers were used in formulation: soy lecithin LECIGRAN® 1000P (Cargill, Decatur, IL), GRINDSTED® PGPR 90 (Danisco, Cedar Rapids, IA), and ammonium phosphatide PALSGAARD® 448 (Palsgaard®, Morristown, NJ).

2.2.2 Chocolate Preparation

2.2.2.1 Formulation

Chocolate formulations were designed to ensure emulsifier concentrations were consistent and could be changed between chocolate batches for experimental design and storage studies. Formulation was based upon previous studies and Standards of Identity for dark chocolate. Chocolate was formulated from chocolate liquor (50% cocoa butter), granulated sugar, and an emulsifier. Batches (1.5 lb) were formulated for both 0.2% and 0.5% (w/w) emulsifier concentrations. Chocolate was formulated to have 32% total fat and 48% total sugar content

2.2.2.2 Refining and Conching

Dark chocolate liquor was subjected to continuous refining and conching in a SPECTRA 10 Stone Melangeur (SanthaUSA, Yoncalla, OR). Solid chocolate liquor was chopped and melted in a Revolution 2 Chocolate Tempering System (Chocovision™, Poughkeepsie, NY). Melted liquor and finely ground sugar were placed in the melangeur and continuously refined and conched for 10 hrs. At 30 min prior to completion of the process, emulsifier at 0.2% or 0.5% (w/w) was added to the dark chocolate mixture as the batch continued to mix. The mixture was then allowed to recrystalize in an ambient controlled temperature room overnight. Chocolate batches were made in duplicate.

2.2.2.3 Tempering and Molding

Dark chocolate was tempered to ensure proper cocoa butter crystallization. Tempering is essential in chocolate production as preliminary prevention against fat bloom formation (Becket, 2008). Tempering protocol was based upon McGee (2003) as shown in **Figure 2.1**. Cocoa butter crystals are heated, melted, and allowed to

recrystalize in a proper lattice formation. The 1.5 lb batch was chopped and allowed to melt in the temper following the Revolution 2 Manual Procedure (**Appendix A.1**). Freshly tempered chocolate was immediately molded in 25mm*25mm*10mm square polycarbonate molds (Kerekes, Brooklyn, NY). Molds were tapped to decrease air bubble formation and chocolate was allowed to recystallize in an ambient temperature controlled room overnight. Chocolates were de-molded and immediately placed in designated storage.

2.2.3 Chocolate Storage

Dark chocolate samples were subjected to rigorous temperature cycling regimes immediately after molding. Control samples were analyzed immediately after preparation and stored in an ambient temperature controlled (23°C) storage room. Fluctuating samples were stored in a digitally controlled incubator (±0.1°C), with temperature cycling as listed in **Table 2.1**. Chocolates were subjected to 2 hour cycling (2hr on and 2hr off) at 34°C or 37°C for both 3 and 5 cycles. Total cycling conditions consisted of: 3 cycles, 12 hours at 34°C; 5 cycles, 20 hours at 34°C; 3 cycles, 12 hours at 37°C; and 5 cycles, 20 hours at 37°C for each emulsifier. Samples were cycled at 3 and 5 cycles respectively based upon previous research, indicating the rate of polymorphic transition and fat bloom formation is dependent upon cycling temperature and duration (Andrae, 2006). Dark chocolate samples were allowed to completely recrystallize for 24 hours before analysis. Samples were analyzed in duplicate.

2.2.4 Instrumental Techniques

2.2.4.1 Texture Analysis

Physical properties such as hardness, cohesiveness, adhesiveness, and gumminess of dark chocolate were characterized (**Figure 2.2**). Sample texture was analyzed with a TA-XT2 Texture Analyser (Texture Technologies Corp; Scarsdale, NY) and Texture Expert Software v. 1.11. A 4mm cylinder stainless steel probe (P4 DIA) was used for the two-bite compression test (25% compression). Test settings, as described by Afoakwa et al. (2008a), were as follows: 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 seconds, and force of 20 g. Samples were clamped to a plate and held still during all textual measurements. Samples were analyzed in duplicate.

2.2.4.2 Color Evaluation

Color changes were analyzed after storage using a HunterLab LabScan II 0/45 (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were analyzed with HunterLab Universal Software™ Version 3.8. Color was evaluated in triplicate on full chocolate samples. Data recorded from the colorimeter resulted in an average lightness and darkness based on three distinct color measurements (L: 100=white, 0=black; a: 100=red, -100=green; b: 100=yellow, -100=blue). The lightening of chocolate can be measured by whiteness index (WI) which can be an indicator of fat bloom formation. Whiteness index was calculated based on the following equation (Briones and Aguilera, 2005):

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

2.2.4.3 Dimension Analysis

Physical dimension change during chocolate storage was analyzed using standard laboratory calipers. Full chocolate sample dimensions (l*w*h) were analyzed in triplicate. Control samples were analyzed immediately after preparation and stored samples were analyzed after storage experiment completion. Percent change in dimensions was calculated between 0, 3, and 5 cycles for all samples.

2.2.4.4 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermal analysis technique that determines the energy differential between a sample and a reference. Melting profiles of stored chocolate were conducted 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 hermetically sealed aluminum pan as a reference. Melting points were determined for chocolate samples (1-2mg), and sealed in Tzero hermetic aluminum pans (TA Instruments; New Castle, DE). The sample chamber was cooled to an initial temperature of -20°C and samples were manually placed in the chamber. Pans were heated at a rate of 10°C/min from a range of -20°C to 80°C in a dry N_2 gas stream. Melting points were reported at the temperature (Tpeak) where the maximum energy was absorbed by the sample. Samples were analyzed in duplicate.

2.2.4.5 Atomic Force Microscopy

Atomic Force Microscopy (AFM) was used to illustrate nanoscale surface topography. Images are visualized by measuring the force between the tip and sample

surface as detected by deflection of the cantilever. Chocolate samples (25mm*25mm*5mm) were fixed onto a glass slide by gently heating one side of the sample. A Dimension 3100 AFM with Nanoscope IIIa controller (Digital Instruments; Santa Barbara, CA) was used to generate images of a 15 x 15 μm area of prepared chocolate. Height and phase differences were analyzed in duplicate using tapping mode. Sample roughness, and average number of grains were determined using Nanoscope software. Sample roughness was based on root means square (RMS) of height deviations from the sample.

2.2.4.6 Powder X-ray Diffraction

Sample Preparation

Chocolate polymorphs were identified using powder X-ray diffraction (XRD). Chocolate samples were prepared as described by Cebula and Ziegleder (1993) to eliminate diffraction interference by extracting the sugar. Samples were prepared as follows: 5 g chocolate was finely chopped, mixed with 500 mL cold deionized water water, and allowed to sit for 4-6 hrs to extract the sugar. The mixture was then filtered under vacuum using Whatman #1 filter paper and allowed to dry overnight. After drying, the chocolate powder was stored in small vials until analysis.

XRD Analysis

Crystal polymorph transitions were confirmed by the short *d* spacings of an X-ray diffraction pattern. X-ray diffraction patterns were measured at room temperature (23ºC) 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 1º divergence slit was used. The powder sample was pressed into a polycarbonate cell and mounted in the machine. A 2θ scan from 18º to 26º, step of 0.008, and a scan rate of 0.2 degrees/min was utilized to analyze samples in duplicate.

2.2.4.7 Statistical Analysis

Data was analyzed using Statistical Analysis Software (SAS) 9.1 (SAS Institute Inc; Cary, NC) to determine the analysis of variance (ANOVA) and Fischer's least significant difference (LSD) using PROC GLM code for all results.

2.3. Results

Temperature cycling of prepared dark chocolate using varying emulsifiers at 0.2% and 0.5% (w/w) of chocolate formulation, at 34ºC and 37ºC led to distinct changes in physical dimensions, textural attributes, polymorphic transitions, microstructural characteristics, melting points, and fat bloom formation. Impact of temperature cycling on dark chocolate appearance (formulated with soy lecithin, PGPR or ammonium phosphatide as emulsifiers) is displayed in **Figures 2.3-2.5**. Chocolate stored at 34ºC showed the greatest change in WI for all emulsifiers. Chocolate stored at 37ºC, experienced dramatic changes in physical dimensions and were the least visually appealing, most notable with the formulation of 0.5% emulsifier concentration.

2.3.1 Cycling at 34ºC

2.3.1.1 Physical Appearance

Temperature cycling at 34ºC did not dramatically impact the physical dimensions of chocolate samples after 3 and 5 cycles (**Figure 2.3 & Table 2.2).** All emulsifier formulations did not experience a significant change in dimensions (width) and held their physical structure when cycled just above the melting point of dark chocolate (33ºC); whitening of chocolate occurred primarily on the exterior of these samples (**Figures 2.3)**. **Figure 2.5** depicts an example cross sectional interior image for all chocolate samples. The interior of control chocolate samples was dark brown throughout; mottling occurred in small whitish spots on the interior of the samples cycled at 34ºC. Fat bloom was observed around the porous bubbles on the interior of the chocolate. WI on the exterior increased in samples cycled at 34°C for all emulsifiers. Increasing concentrations of PGPR and ammonium phosphatide resulted in an increase in WI at 5 cycles; while increasing concentrations of soy lecithin formulations experienced a decrease in WI at both 3 and 5 cycles **(Figure 2.6**). Lecithin samples at 0.2% experienced the greatest percent change in WI at 5 cycles compared to all emulsifiers. PGPR samples exhibited an increase in WI for samples at 0.5%, but resulted in a repeatable decrease in WI from 3 to 5 cycles at 0.2%. After 3 cycles, ammonium phosphatide samples show the least percent increase in WI for all emulsifiers at both 0.2% and 0.5%.

2.3.1.2 Textural Analysis

Tables 2.3 & 2.4 present detailed textural data for all samples and emulsifiers at 23ºC and 34ºC for both 0.2% and 0.5% concentrations. During temperature cycling at 34ºC, samples formulated at 0.2% PGPR were the hardest, most cohesive, and gummiest at 3 and 5 cycles. At 0.5% formulation, ammonium phosphatide samples were the softest, least adhesive for 3 and 5 cycles. Ammonium phosphatide was the only emulsifier to decrease gumminess between 3 and 5 cycles for all concentrations. With increasing concentrations of emulsifier, lecithin and PGPR samples experienced decreased hardness; while ammonium phosphatide samples increased in hardness but were the softest overall. The most cohesive samples were PGPR and ammonium phosphatide at 0.5%, while lecithin samples had the least cohesive values at 0.2% for all cycles respectively. All samples decreased in gumminess as concentrations increased for both 3 and 5 cycles.

2.3.1.3 Differential Scanning Calorimetry (DSC)

Confirmed structural changes were reflected by DSC for all emulsifiers. **Table 2.5** shows melting point averages by DSC for emulsifier samples. Control samples (23ºC) had the lowest melting points for 0.2% and 0.5% emulsifier concentrations. During cycling at 34ºC, melting points increased for all samples between 0 and 3 cycles at 0.2% and 0.5% (**Figure 2.7**). At 0.2%, lecithin samples had the highest melting points for control samples, as well as at 3 and 5 cycles. PGPR samples had the statistically highest melting points for samples at/between 3 and 5 cycles at 0.5%; while ammonium phosphatide samples had the lowest melting points. Overall, at 23ºC as emulsifier concentration increased in ammonium phosphatide and PGPR samples, the melting points increased; however, lecithin melting point decreased. At 34ºC cycling as emulsifier concentration increased, PGPR and ammonium phosphatide melting points increased; while melting points decreased for lecithin samples.

2.3.1.4 Powder X-Ray Diffraction (XRD)

A change in polymorphic structure in cocoa butter can be identified from observation of *d*-spacings using X-ray Diffraction. The diffraction pattern of polymorph V has four distinct peaks between 22º-25º; the pattern for polymorph VI contains only 3 peaks **(Figure 2.8).** During transition from polymorph V to VI, the peaks merge and begin to increase in magnitude; the first peak becomes less pronounced. **Tables 2.6 & 2.7** give detailed *d*-spacings and polymorph data for each emulsifier formulation cycled

at 34ºC. Control samples (23ºC; not cycled) for each emulsifier had four distinct peaks decreasing in size, indicating a polymorph V stable pattern (**Figure 2.9**). Diffraction pattern analysis indicated that chocolate samples stored at 34ºC transitioned to varying degrees to polymorph VI, with each emulsifier formulation (**Figure 2.10**). For all emulsifiers at 0.2% and 0.5% concentrations, chocolates transitioned to polymorph VI; confirmed by three diffraction peaks, increasing in size. With increasing concentration after 5 cycles, all emulsifier diffraction patterns had a more distinct merging of peaks (**Figure 2.11**).

2.3.1.5 Atomic Force Micrscopy (AFM)

AFM images of surface topography of dark chocolate formulated with 0.5% emulsifier are depicted in **Figure 2.12**. Storage in all conditions increased surface roughness for chocolates formulated with each emulsifier (**Figure 2.13**). Surface roughness measurements correlated with AFM imagery. Control samples (23°C) are significantly different in roughness; ammonium phosphatide formulated samples are the smoothest. There was no significant difference in surface roughness due to formulation for chocolate stored at 34°C for both 3 and 5 cycles. Previous research noted temperature fluctuations at 34°C and above lead to growth of smooth rounded crystals on the surface of chocolate while storage at 34ºC without temperature fluctuations lead to the formation of 'needle and spike' crystal formation on the surface which had direct impact on surface roughness as observed by AFM (Bricknell and Hartel, 1998; Hodge and Rousseau, 2002; Andrae, 2006).

2.3.2 Cycling at 37ºC

2.3.2.1 Physical Appearance

Temperature cycling at 37ºC resulted in a drastic change in shape for all emulsifier formulations (**Figure 2.4)**. Samples were unable to retain their square molded shape for both 3 and 5 cycles. Formulations containing 0.5% emulsifier experienced the greatest change in physical dimensions (width) during cycling (**Table 2.8 & Figure 2.14)**; PGPR showed the greatest change followed by lecithin and ammonium phosphatide samples**.** As emulsifier concentration increased, PGPR and ammonium phosphatide samples experienced greater changes in dimension at 3 and 5 cycles, respectively. Whitening of chocolate samples, both exterior and interior, can be

visualized in **Figures 2.4 & 2.5** for all emulsifiers cycled at 37ºC. On the exterior of the samples, there was a significant increase in WI from 0 to 3 cycles for all concentrations of emulsifiers **(Figure 2.15).** At 5 cycles, the most significant change in WI was seen from samples with ammonium phosphatide at both 0.2% and 0.5% concentrations from 3 to 5 cycles. Between 3 and 5 cycles, lecithin samples decreased in WI for both concentrations; however, there was no immediate impact from increasing emulsifier concentration. **Figure 2.4** demonstrates that lightening of chocolate stored at 37ºC for 5 cycles was more visible on the chocolate interior for all samples. A strategy needs to be developed to quantitatively asses color changes in the interior.

2.3.2.2 Textural Analysis

Detailed textural data of dark chocolate stored at 37ºC is presented in **Table 2.9.** Samples formulated with PGPR (both 0.2 and 0.5%) were hardest during temperature cycling at 37C. Ammonium phosphatide samples were softest and least gummy at 0.5% concentration. Cohesiveness values increased with increasing PGPR and lecithin concentrations. At 0.2%, formulations prepared with lecithin were lowest for cohesiveness and gumminess. Samples formulated with lecithin and PGPR increased in gumminess with increasing emulsifier concentrations; while those formulated with ammonium phosphatide decreased in gumminess.

2.3.2.3 Differential Scanning Calorimetry (DSC)

Table 2.10 shows melting point averages by DSC for emulsifier samples. At 37ºC, melting points for lecithin and PGPR increased, while ammonium phosphatide melting points decreased between 3 and 5 cycles at 0.2%. At 0.5%, ammonium phosphatide had the lowest melting point at 23ºC; PGPR samples consistently had the highest melting points for the duration of 37ºC cycling (**Figure 2.16**). At 37ºC as emulsifier concentration increased, all PGPR and ammonium phosophatide samples experienced increased melting points; while melting points decreased for lecithin samples for both 3 and 5 cycles, respectively.

2.3.2.4 Powder X-Ray Diffraction (XRD)

Figure 2.8 shows a typical XRD pattern in the range of 22º-25º for dark chocolate. All samples stored at 23ºC (control) had four distinct peaks illustrating a polymorph V stable pattern (**Figure 2.9**). **Tables 2.6 & 2.7** give detailed *d*-spacings and polymorph data for each emulsifier cycled at 37ºC. Diffraction pattern analysis indicated

that none of the chocolate samples stored at 37ºC transitioned to polymorph VI **(Figures 2.17 & 2.18).** All emulsifiers cycled at 37ºC for both 3 and 5 cycles, at 0.2% and 0.5% concentration had four distinct peaks similar to the control samples. Based on *d*-spacings, ammonium phosophatide samples at 0.5% (3 and 5 cycles respectively), and lecithin samples at 0.2% (5 cycles only) are the only emulsifiers to have a slight merging of peaks 3 and 4 which may signify transition to polymorph VI.

2.3.2.5 Atomic Force Microscopy (AFM)

AFM images of surface topography of dark chocolate are depicted in **Figure 2.19**. Chocolate undergoing temperature fluctuations at high temperatures lead to smooth yet uneven surfaces due to extreme melting during storage. With higher temperature cycling, there occurs more alteration of structural and compositional elements on the chocolate surface and interior (**Figures 2.4 & 2.5**). Storage in all conditions increased surface roughness for chocolates formulated with each emulsifier (**Figure 2.20**). Control samples (23°C) are significantly different in roughness; ammonium phosphatide formulated samples are the smoothest. At 37°C, samples formulated with soy lecithin had the highest degree of roughness when comparing all three emulsifiers. Surface roughness was notably different between emulsifiers 0.5% (w/w) at 37°C as cycles increased from 0, to 3, and 5 cycles, respectively. Ammonium phosphatide samples were the smoothest at 3 and 5 cycles for all concentrations.

2.4 Discussion

Temperature fluctuations and cycling had a great impact on appearance, texture, and exacerbated microstructural changes in dark chocolate as expected. Polymorphic transition from V to VI must occur before fat bloom is formed (Bricknell and Hartel, 2006). The structural rearrangements of TAG molecules are the driving force behind polymorphic transitions. This transition between polymorph V and VI may be melt mediated when chocolate is fluctuated slightly above its melting point (Van Malssen et al., 1996). The role emulsifiers take on fat bloom formation is still unclear, but emulsifiers have potential as specific inhibitors of this phenomenon. Emulsifiers are known to influence the rheology of fat and sugar dispersions in oils (Johansson and Bergenstahl, 1992b). Specifically, the direction and degree of chocolate flow varies with

the type of emulsifier and with concentration. The magnitude of these changes is affected by both sugar and fat dispersions. The stability and properties of emulsifiers in chocolate are dependent upon the interactions between the hydrophilic head and the sugar particles as well as the lipophilic tail flowing in the continuous cocoa butter phase. Reports on TAG-emulsifer interactions are limited in literature. Emulsifier interactions with TAGs in the lipid phase may also contribute to structural stability in chocolate. It is possible both these interactions between emulsifier and sugar particles and TAGs could influence intermolecular mobility in the continuous fat phase which would influence overall structural TAG arrangement and crystal formation.

In this study, chocolate formulated with three distinct emulsifiers exhibited different, significant changes in structural, visual, and textural properties associated with fat bloom formation. Temperature fluctuations exacerbated theses changes in chocolate samples. Chocolate cycled at both 34°C resulted in a melt mediated polymorphic transition to form VI. The rate of polymorphic transition was similar for both cycling temperatures; both conditions are expected to have a large percentage of melted chocolate, causing an increase in molecular mobility, lipid migration, and melt mediated transition. Chocolate cycled at 34°C is just above the normal melting point of chocolate (~33ºC), and thus may be exhibiting a 'memory effect' recrystallization as seen in previous studies (Van Malssen et al., 1999; Schenk and Peschar, 2004). Chocolate cycled at 37°C transitioned to polymorph V as early as 3 cycles into the experiment; therefore, completely melting the sample caused disorder in the chocolate matrix and forced recrystallization into polymorph V. Van Malssen et al. (1999) determined that chocolate heated sufficiently above the melting point of chocolate will melt the lipid crystals completely and recrystalize first into a less stable polymorphic form I-IV before transitioning into more stable polymorph V. In this study, the type and concentration of emulsifier did not have a direct affect on polymorphic transitions in chocolate cycled at high temperature fluctuations.

There is extensive research on emulsifier concentration and influence on rheological properties associated with chocolate such as a change in viscosity, shear factor, and yield stress (Rector, 2000; Water and Cornillon, 2001; Schantz and Rohm, 2005; Baker et al., 2006; Afowaka et al., 2007). These properties are influenced by the

structure of the emulsifier which helps reduce interfacial tension between the sugar and lipid particles (Schneider, 1986). Emulsifiers adsorb more strongly to sugar and form tightly packed monolayers with hydrocarbon chains directed into the lipid phase and crystals are then stabilized sterically; this indicates the adhesion between them is weaker and the particles are more compact. Cocoa butter crystalline instability is seen as a precursor to fat bloom formation. It is recognized that an enhancement of crystallinity is associated with thermodynamic stability and leads to an increase in endothermic values (Garti et al., 1986). Chocolate cycled at 34°C transitioned to stable polymorph VI and DSC melting point results indicate that all samples had an increase in melting point (**Figure 2.7**). This supports the Garti group theory that crystalline structure in polymorph VI is more stable. On the other hand, samples cycled at 37°C did not have a significant increase in melting point for all samples with each emulsifier. This indicates that the crystalline structure of these samples had greater degrees of disorder, leading to decreased thermodynamic stability; this result is confirmed by a polymorphic transition back to polymorph V as observed by XRD analysis.

Cycling at 34°C and 37°C lead to a significant increase in melting point by DSC specifically for PGPR emulsifier samples at 0.5% as compared to 0.2%. Increasing emulsifier concentrations above 0.6% (w/w) causes thickening of the chocolate mass (Nebesny and Zyzelewicz, 2005). This indicates that more emulsifier causes hindrance between the long polyricinoleic acid chains of the PGPR molecule. These chains are meant to provide maximum wetting of the fatty acid chains in the lipid phase, but steric hindrance created between molecules in the cocoa butter phase may lead to agglomeration of individual solid particles (Rector, 2000). Agglomeration of particles could affect cocoa butter melting properties in chocolate. Our results indicate that increasing PGPR concentrations resulted in increased melting points, up to 0.5%. According to Nebesny and Zyzelewicz (2005), at concentrations lower than 0.6%, there may be increased molecular mobility and thus a decrease in melting point. This is not what we observed. Due to particle agglomeration, samples formulated with increased concentrations of PGPR may not be experiencing increased molecular mobility. More studies on TAG composition and structural interactions between emulsifiers and TAG need to be characterized further to make a clear distinction between varying reports. It

was noted that addition of high concentrations (0.5-0.8% (w/w)) of PGPR causes 'clumping' of chocolate which will cause a negative effect on textural qualities of chocolate such as increased gumminess, hardness, and adhesiveness (Nebesny and Zyzelewicz, 2005). These textural changes were seen with increasing concentrations of PGPR in chocolate cycled at 37°C. PGPR dramatically impacts yield values in chocolate, which is confirmed from changes in width during temperature cycling. PGPR samples with 0.5% emulsifier experienced the largest change in shape (width) at 37°C cycling due to reduced yield value and immediate loss in structure with increased temperature storage (**Table 2.9; Figure 2.14**). Samples lost their initial molded shape after 3 cycles, but then maintained this newly deformed shape after continued cycling occurred. Expansion in width of chocolate samples during cycling was seen in ammonium phosphatide formulated samples as well.

Soy lecithin and ammonium phosphatide samples cycled at 34°C exhibited different melting points and physical characteristics associated with lipid polymorphism. With increasing concentrations of emulsifier to 0.5% (w/w), these samples transitioned to polymorph VI and decreased in melting point (soy lecithin) or stayed the same (ammonium phosphatide). This can be attributed to the increased molecular mobility of liquid fat in the cocoa butter and confirms the idea that enhanced molecular mobility means melting points are decreased (Nebesny and Zyzelewicz, 2005). One role of an emulsifier is to soften the fat while promoting a more liquid component in the mixture (Cebula and Ziegleder, 1993). The continued presence of emulsifier molecules in the lattice acts as a block to propagation of the transition from one crystal formation to the next, thus retarding form V to form VI transition. However, this theory of polymorphic retardation was only seen in ammonium phosphatide and soy lecithin samples fluctuated at 37°C. Addition of lecithin at 0.2% appears to retard crystallization of cocoa butter, but does not change the melting point of the product formed (Dhonsi and Stapley, 2006). The way lecithin or ammonium phosphatide covers the surface of the sugar particles may provide a less favorable surface for heterogenous nucleation to occur or these emulsifiers may enable greater gaps between the fat phase and sugar particles reducing shear rates. Overall, lecithin is found to be more interactive with coarse sugar particles due to their smaller specific surface area (Lee et al., 2002).

Johansson and Bergenstahl (1992b) recognized that increased concentrations will increase packing densities of molecules. Essentially, molecules pack more efficiently to provide space for new molecules and create a tighter packing which contributes to the adsorption energy in the system. The outermost layer of emulsifier is always directed with the hydrocarbon chains towards the oil, and a strong steric stabilization is created. Specifically, lecithin reduces adhesion between sugar crystals, resulting in much denser structures. A decrease in particle volume with increasing emulsifier concentration indicates weaker attraction and/or stronger repulsion between the particles due to the presence of adsorbed layers. In the present study, emulsifier type or concentration did not significantly impact surface roughness in chocolate samples. The impact emulsifiers have on microstructural changes is not thoroughly reported in literature. However, storage cycling temperature both at 34°C and 37°C, did impact surface roughness as seen in previous studies (Andrae, 2006; Rousseau and Sonwai, 2008).

Polymorphic transition to polymorph VI was consistent with significant increase in surface roughness (>600nm) for all samples cycled at 37°C. The increased roughness due to fat bloom formation on these samples was also visually apparent, indicating large particle size formation; the matrix was visibly less homogenous compared to freshly prepared samples (**Figure 2.5**). This visual difference indicates phase separation of liquid and solid particulate matter in the chocolate matrix. All samples cycled at 37°C crumbled during textural analysis and were softer, indicating a less compact structure. These samples experienced bloom similar to what Kinta and Hartel (2010) saw in untempered chocolate. Light colored areas on the surface consisted of sugar/cocoa particles and the lipid fractions migrated to the center of the chocolate. Samples fluctuated at temperatures near the melting point of chocolate, 34°C, experienced less of an increase in roughness. This can be attributed to samples forming more smooth agglomerates overlapping and packing on top of each other rather forming ‘needle and spike’ formations on the surface. The type of fat bloom on the surface of both these samples (34°C and 37°C) needs to be examined and characterized to reveal compositional details of such areas.

2.5 Conclusions

Cycling temperature and type of emulsifier significantly impacted fat bloom formation, microstructural characteristics, and textural attributes of formulated chocolates. Fresh, unstored samples were smooth, with favorable melting properties. Cycling at 34°C increased roughness, melting point, textural hardness, and whiteness index for all samples. These samples transitioned to polymorph VI as indicated by XRD. Cycling at 37°C increased roughness and whiteness for all samples. These samples were in polymorph V as indicated by XRD. Specifically, PGPR had the greatest impact on hardness and had the highest melting points. Soy lecithin and ammonium phosphatide samples experienced the greatest textural changes such as decreased gumminess, adhesiveness, and hardness with increasing concentrations of emulsifier (0.5%). Increased concentrations of emulsifier lead to increased dimensions (width) for all samples cycled at 37°C. These samples exhibited a transition back to polymorph V due to the increased molecular disorder under high temperatures which severally impacted the cocoa butter lattice stability. Samples formulated at 0.5% with PGPR may have experienced steric hindrance and agglomeration of intermolecular particles; while samples formulated with soy lecithin and ammonium phosphatide experienced intermolecular mobility. Bloom was apparent in all samples cycled at 34°C and 37°C, although the composition of bloom formation was undetermined. Future studies should focus on the compositional characteristics of these samples after temperature fluctuations. This future data should be complimented with rhelogical analysis; both particle size and viscosity need to be measured and results compared between chocolates formulated with different emulsifiers.

2.6 Figures

Figure 2.1: Tempering protocol (adapted from McGee, 1999).

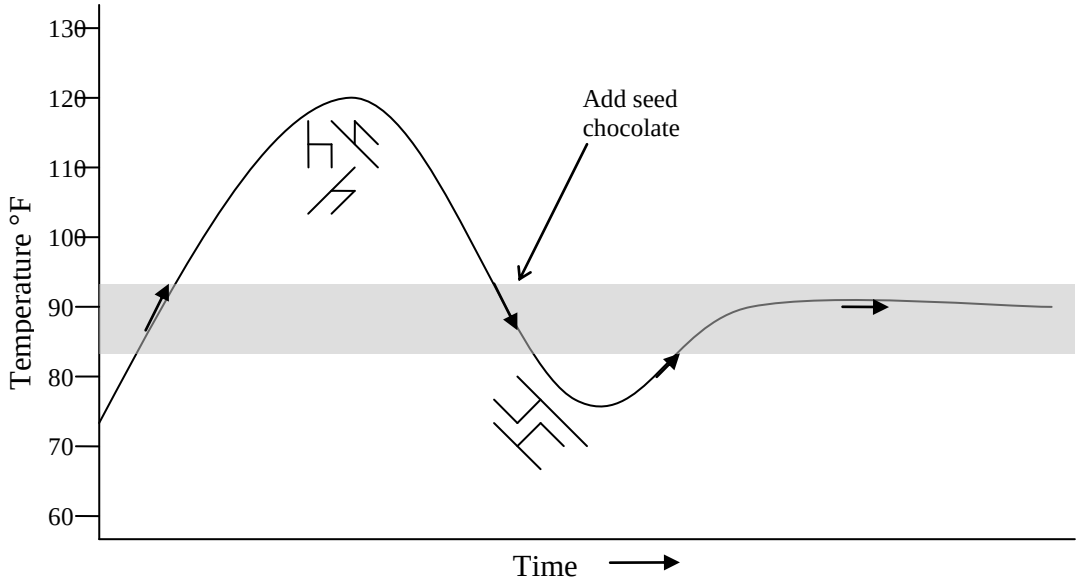
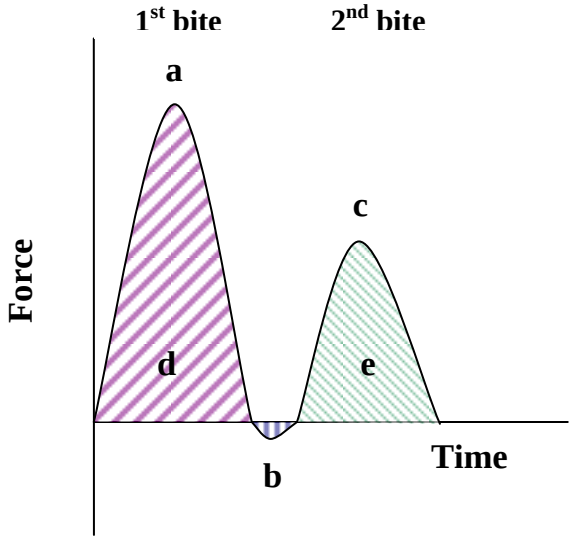


Figure 2.2: A common texture profile analysis curve for a two bite compression test (adapted from Rosenthal, 1999)



Measurement	Definition
Hardness	**a:** peak force during the 1st bite
Coheisveness	**d/e**: area of 2nd bite divided by the area of the 1st bite
Adhesiveness	**b:** negative force area for the 1st bite
Gumminess	product of hardness and cohesiveness

Figure 2.3: Image of dark chocolate formulated with soy lecithin, polyglycerol polyricinelorate, and ammonium phosphatide at 0.2% (w/w) (a), (c), (e), and 0.5% (w/w) (b), (d), (f) stored at 34°C, for 0, 3, 5 cycles, respectively.

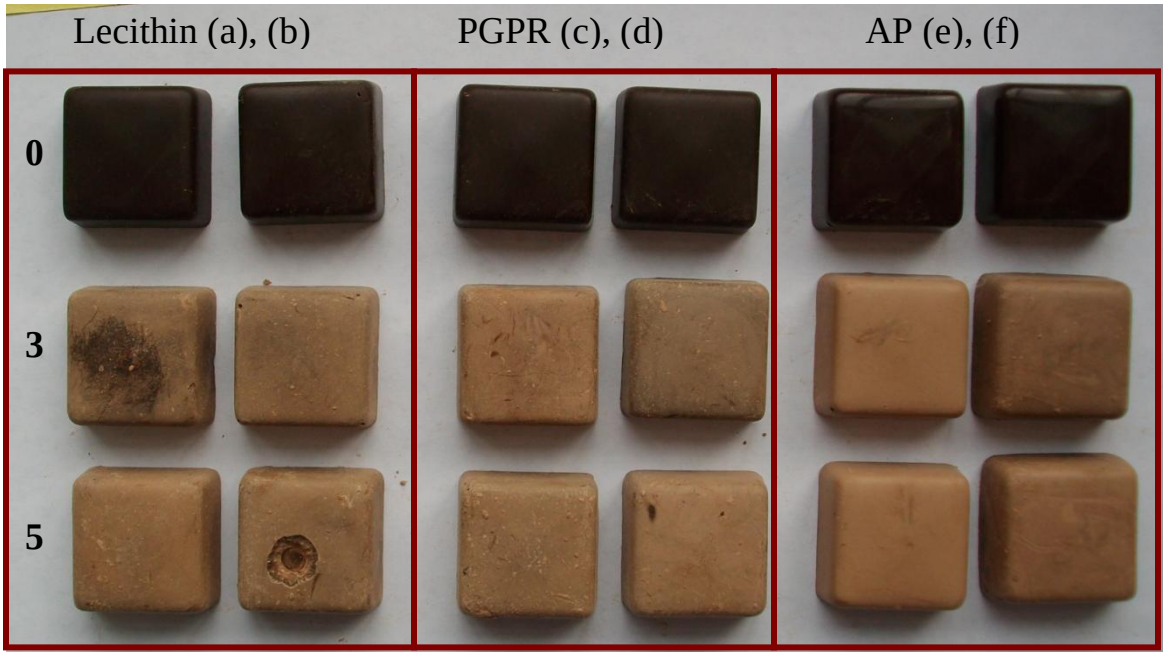


Figure 2.4: Image of dark chocolate formulated with soy lecithin, polyglycerol polyricinelorate, and ammonium phosphatide at 0.2% (w/w) (a), (c), (e), and 0.5% (w/w) (b), (d), (f) stored at 37°C for 0, 3, 5 cycles, respectively.

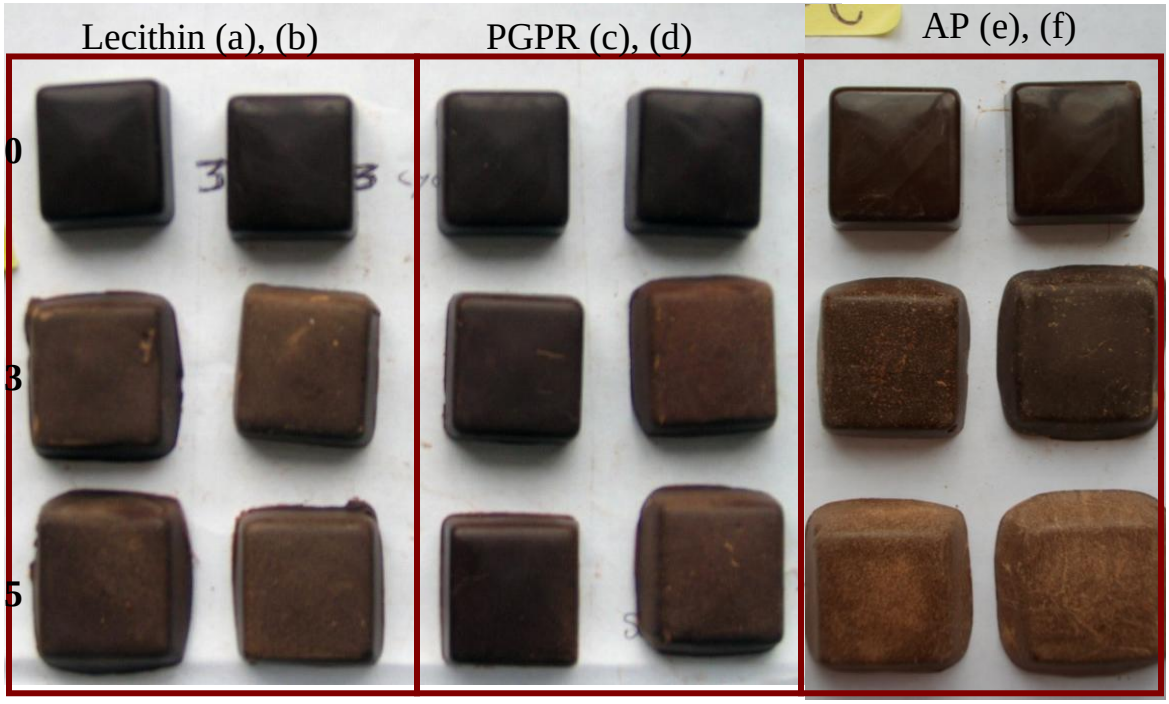
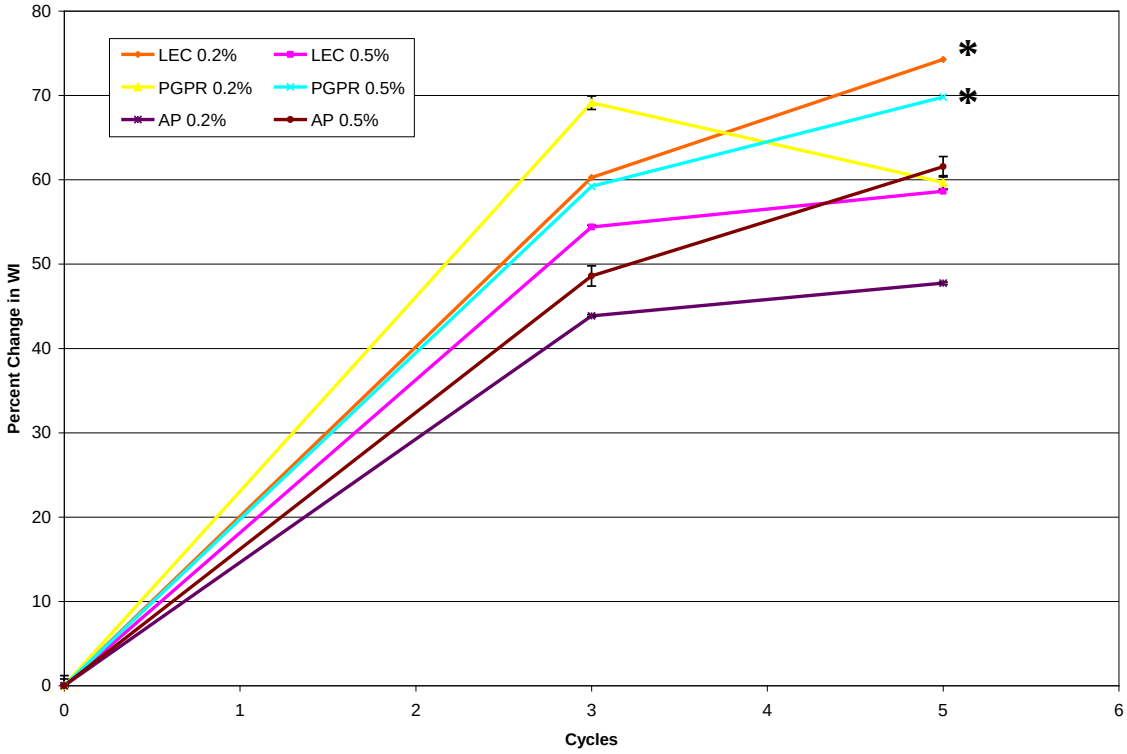


Figure 2.5: Cross sectional image of dark chocolate with ammonium phosphatide emulsifier 0.2% (w/w) for unstored control, 0 cycles (a), stored at 34°C (b), and 37°C (c) for 5 cycles.

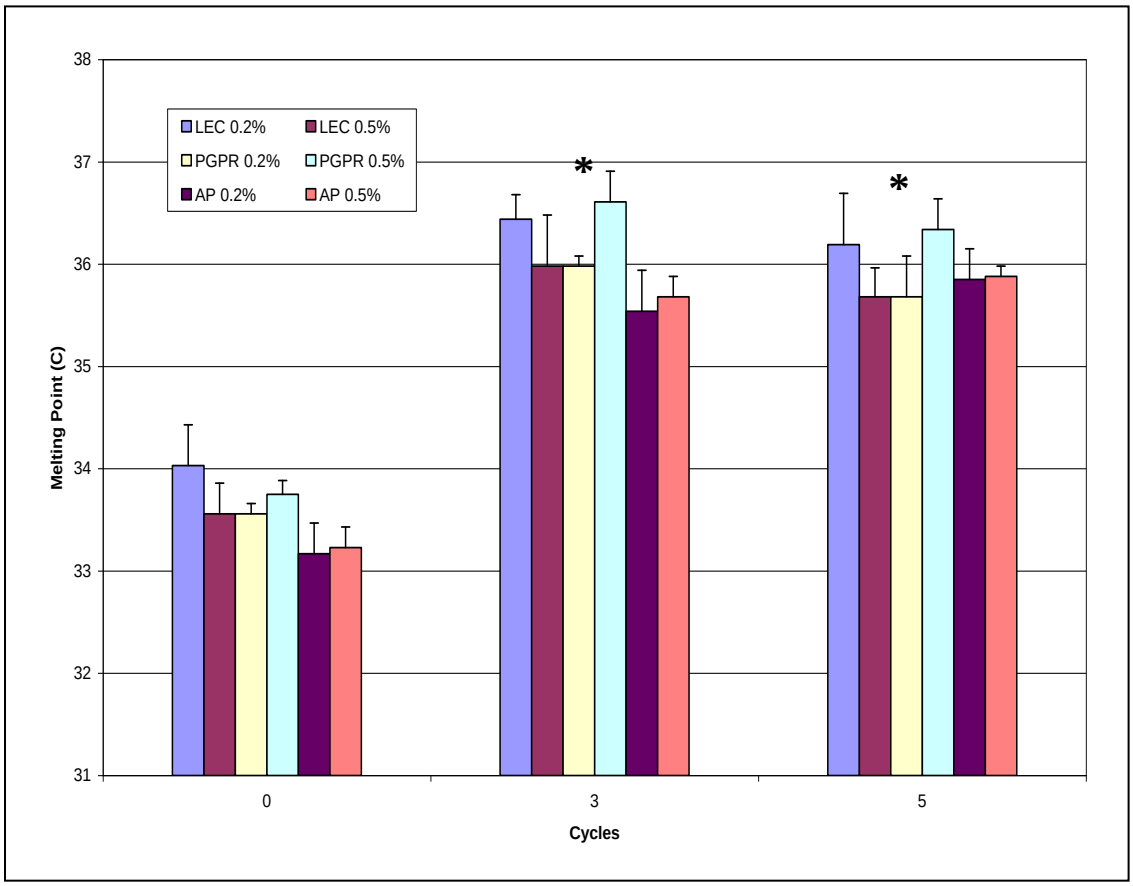
(a) **(b)** **(c)**

Figure 2.6: Impact of temperature fluctuations on whiteness index (WI) of dark chocolate stored at 34°C for 0, 3, and 5 cycles, respectively.[a]



[a]Percent change in WI was significantly different after 3 cycles for all emulsifers ($p<0.05$).
***Percent change in WI was significantly different at $p<0.05$.**

Figure 2.7: Impact of increasing emulsifier concentrations on melting points by DSC for samples cycled at 34°C for 0, 3, 5 cycles, respectively.[a]



[a]Melting points were significantly different from control sample after 3 cycles for all emulsifers ($p<0.05$). *Emulsifier concentration was significantly different at $p<0.05$.

Figure 2.8: Example of XRD patterns for polymorph V and VI in dark chocolate with "fingerprint" region between 22-25° (Talbot, 1995).

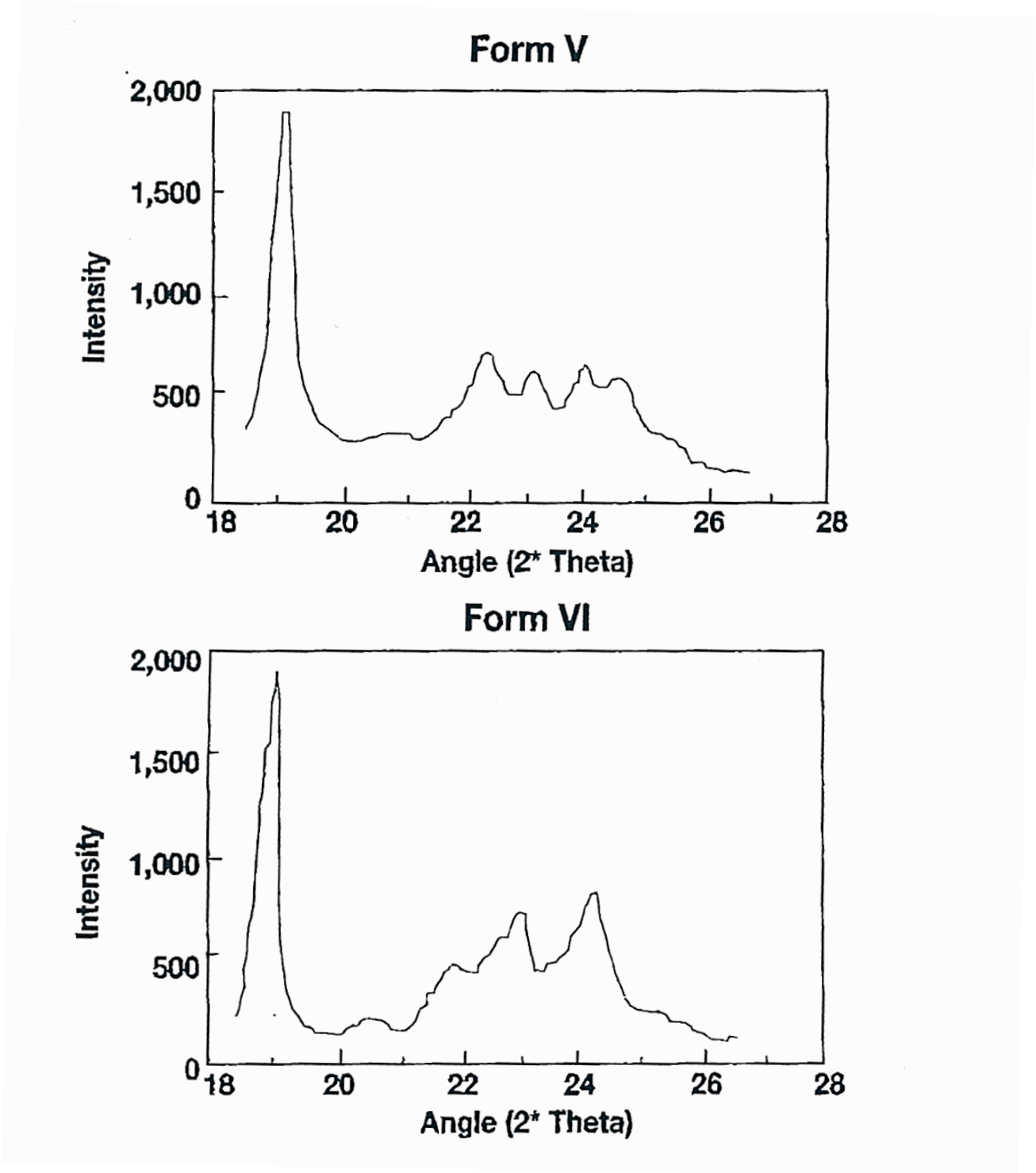


Figure 2.9: X-ray diffraction patterns for control samples (23°C) for all emulsifiers.

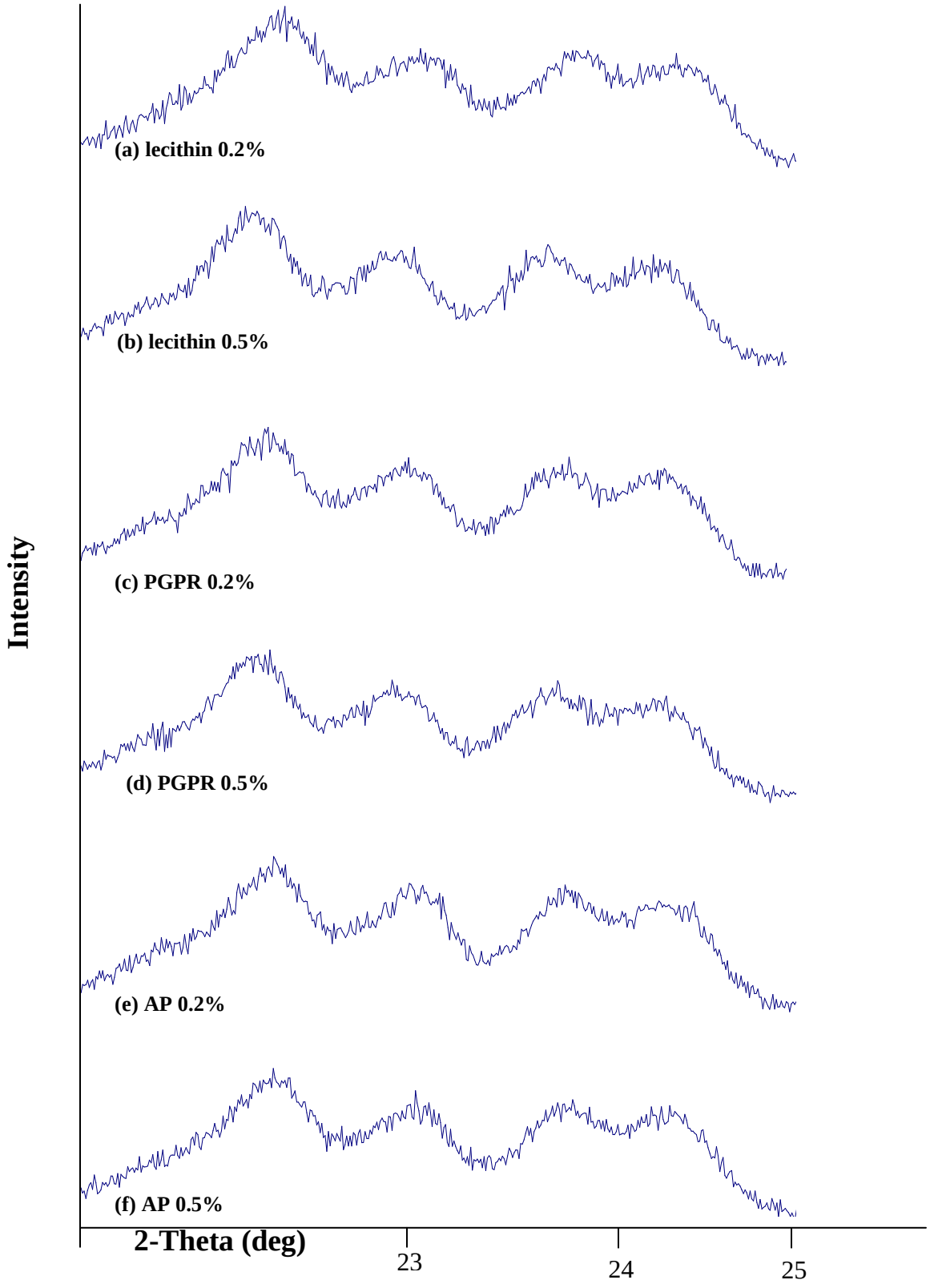


Figure 2.10: X-ray diffraction patterns for samples cycled at 34°C formulated with 0.2% emulsifier for 3 & 5 cycles, respectively.

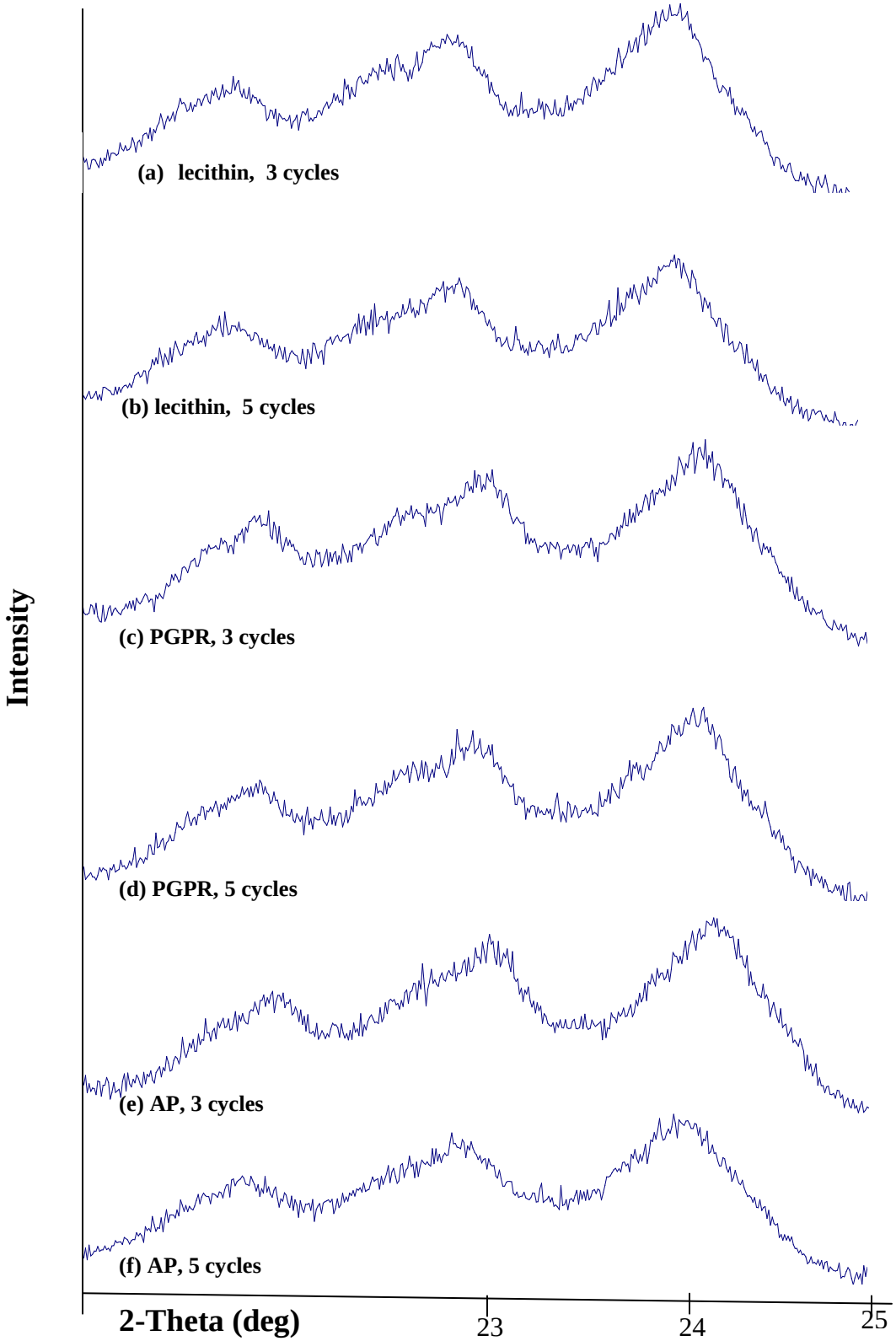


Figure 2.11: X-ray diffraction patterns for samples cycled at 34°C formulated with 0.5% emulsifier for 3 & 5 cycles, respectively.

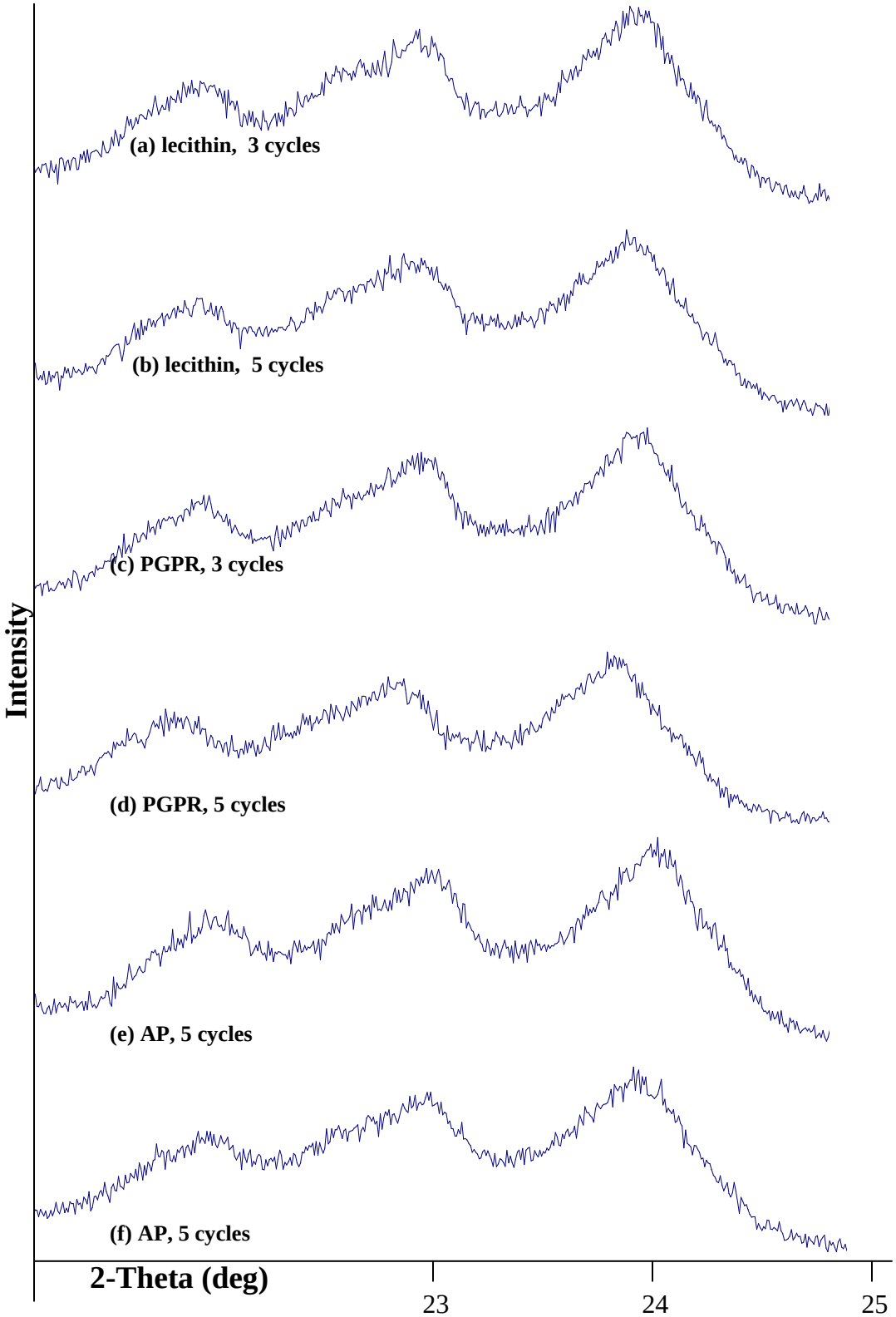
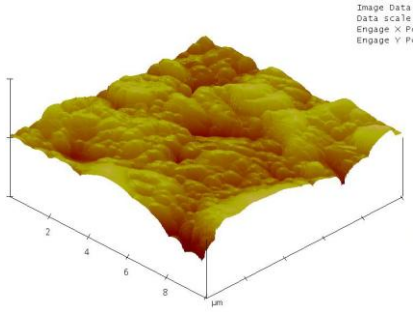
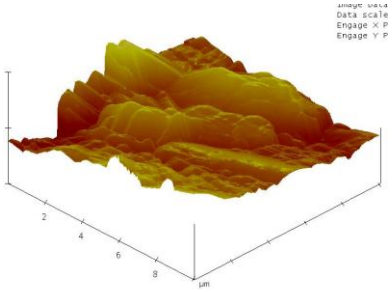


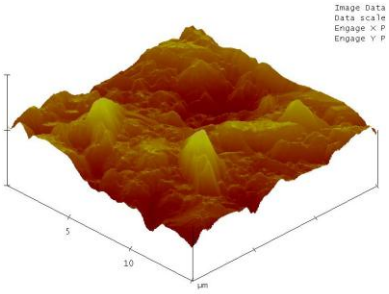
Figure 2.12. AFM images of chocolate samples formulated with soy lecithin 0.5% (w/w) stored at 23°C, 0 cycles (a) 34°C, 5 cycles (b), with PGPR 0.5% (w/w) stored at 23°C, 0 cycles (c) 34°C, 5 cycles (d), and with ammonium phosphatide 0.5% (w/w) stored at 23°C, 0 cycles (e) 34°C, 5 cycles (f) (z=15µm).



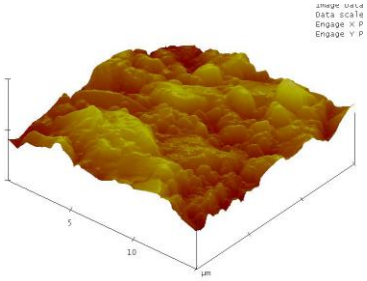
(a) Soy lecithin (0.5%), 23°C, 0 cycles



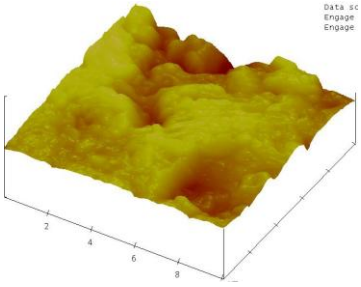
(b) Soy lecithin (0.5%), 34°C, 5 cycles



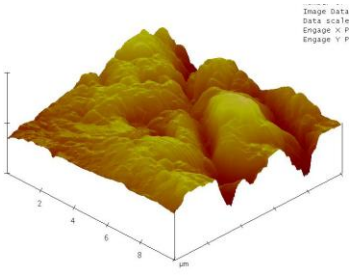
(c) PGPR (0.5%), 23°C, 0 cycles



(d) PGPR (0.5%), 34°C, 5 cycles

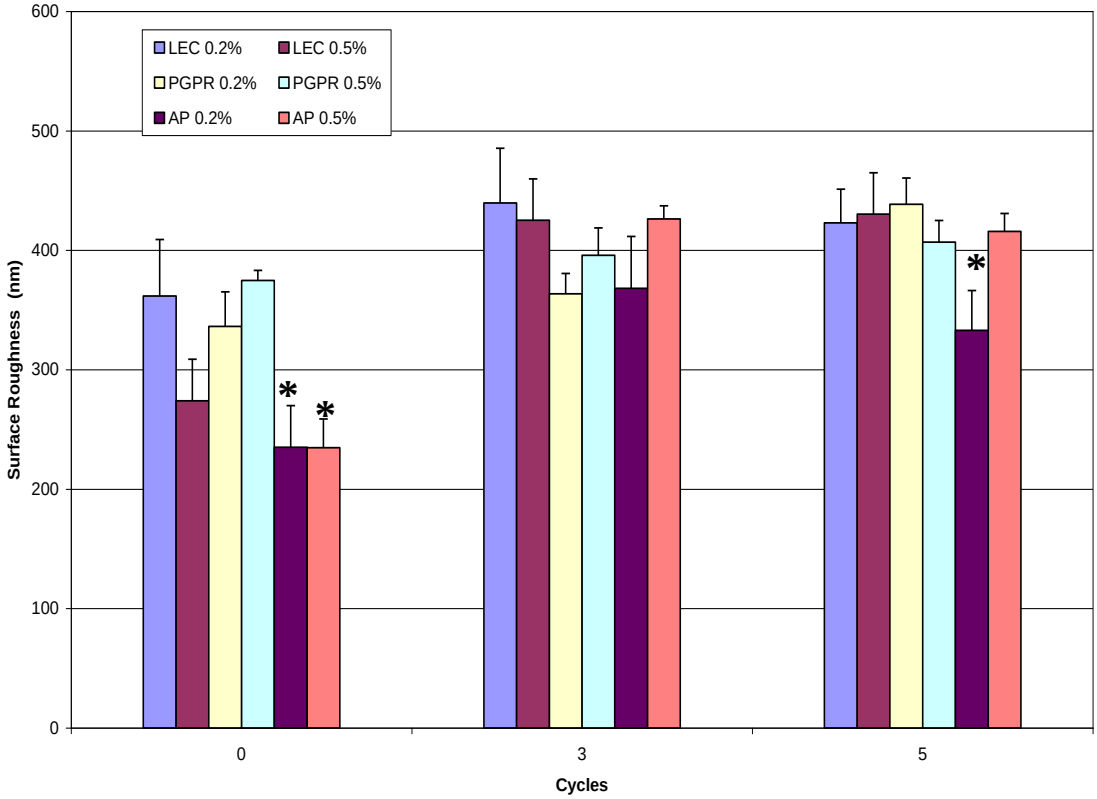


(e) AP (0.5%), 23°C, 0 cycles



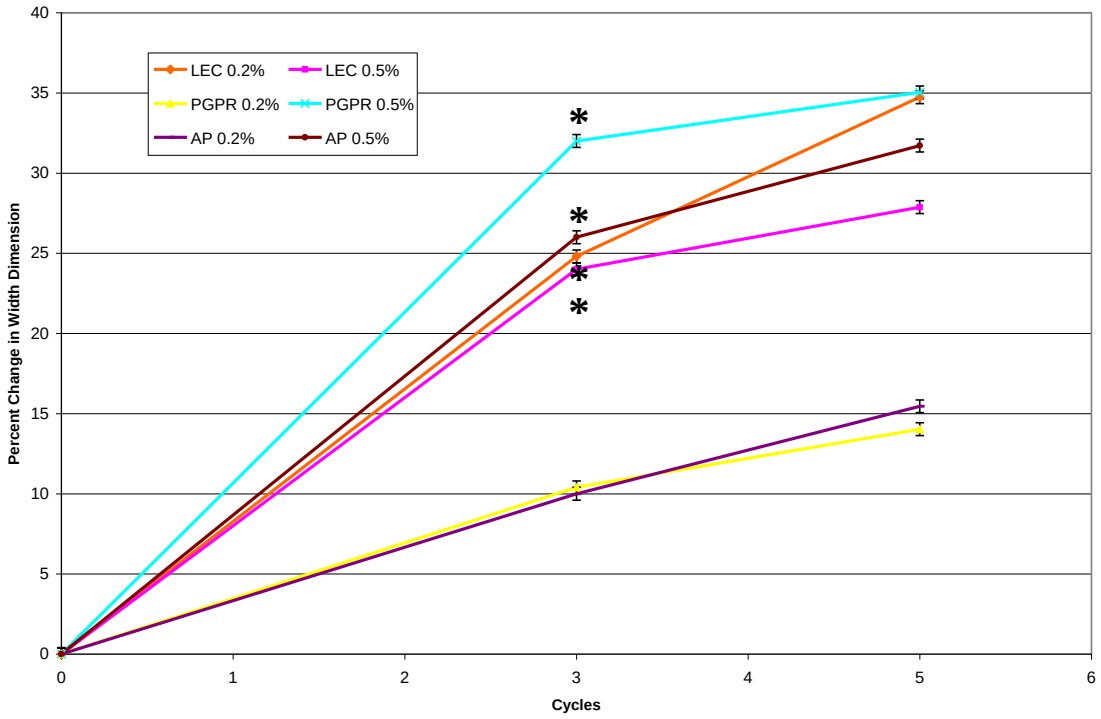
(f) AP (0.5%), 34°C, 5 cycles

Figure 2.13: Impact of temperature fluctuations on surface roughness of dark chocolate stored at 34°C with emulsifiers for 0, 3, 5 cycles, respectively.[a]



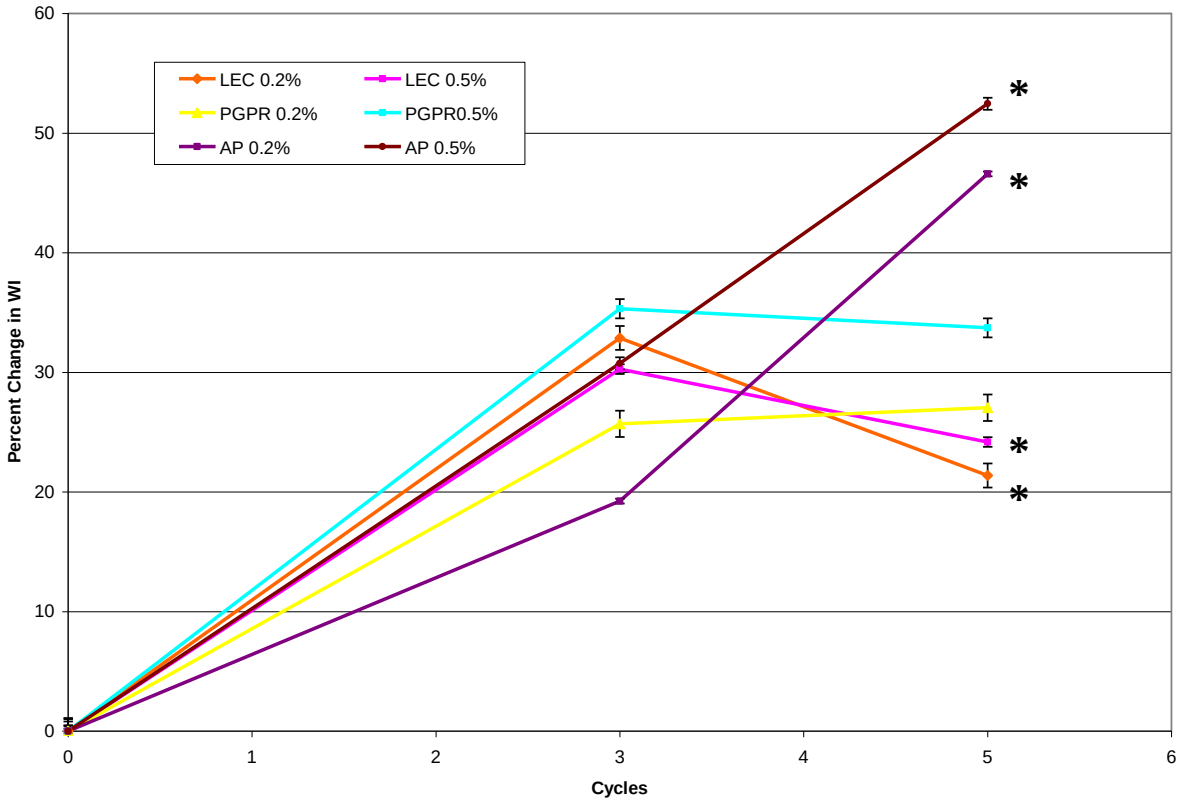
[a]Storage conditions significantly impacted surface roughness for all emulsifier type and concentration at $p<0.05$. *Emulsifier significantly impacted surface roughness at $p<0.05$.

Figure 2.14: Impact of temperature fluctuations on physical dimensions (width) for dark chocolate stored at 37°C for 0, 3, 5 cycles, respectively.



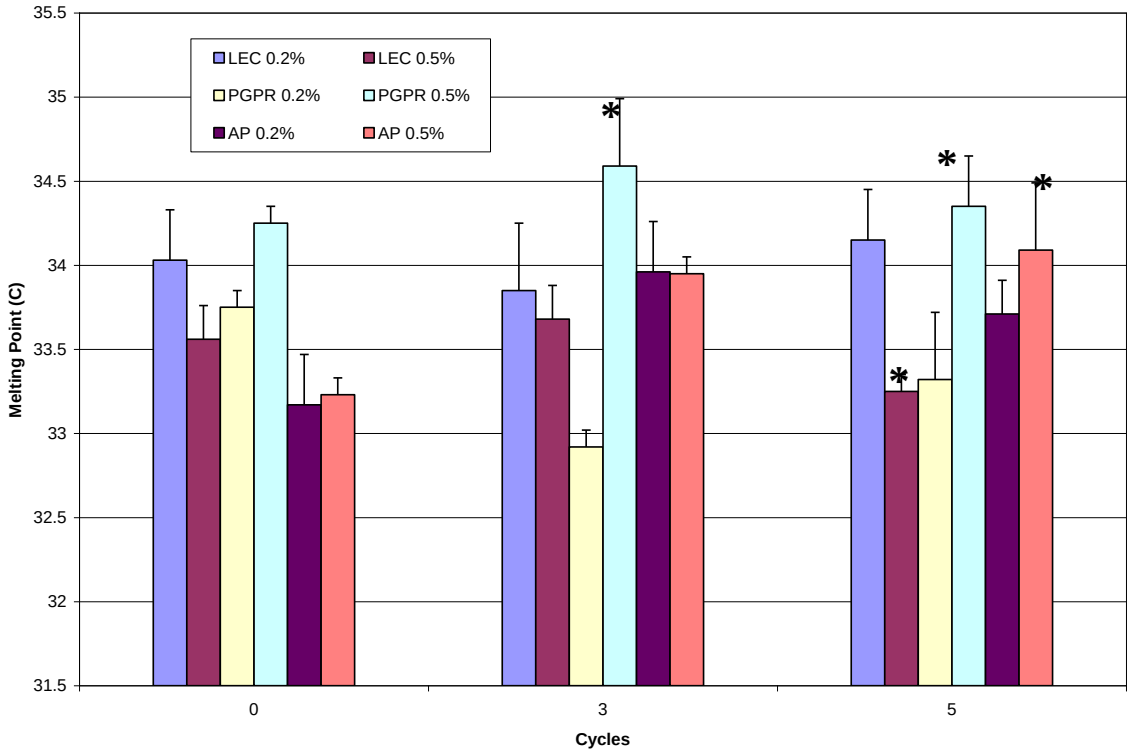
***Percent change in dimension (width) was significantly different after 3 cycles ($p<0.05$).**

Figure 2.15: Impact of temperature fluctuations on whiteness index (WI) of dark chocolate stored at 37°C for 0, 3, and 5 cycles, respectively.



***Percent change in whiteness index was significantly different after 5 cycles ($p<0.05$).**

Figure 2.16: Impact of increasing emulsifier concentrations on melting points by DSC for samples cycled at 37°C for 0, 3, 5 cycles, respectively.



***Emulsifier concentrations (0.5%) were significantly different at p<0.05.**

Figure 2.17: XRD patterns for samples cycled at 37°C formulated with 0.2% emulsifier for 3 & 5 cycles, respectively.

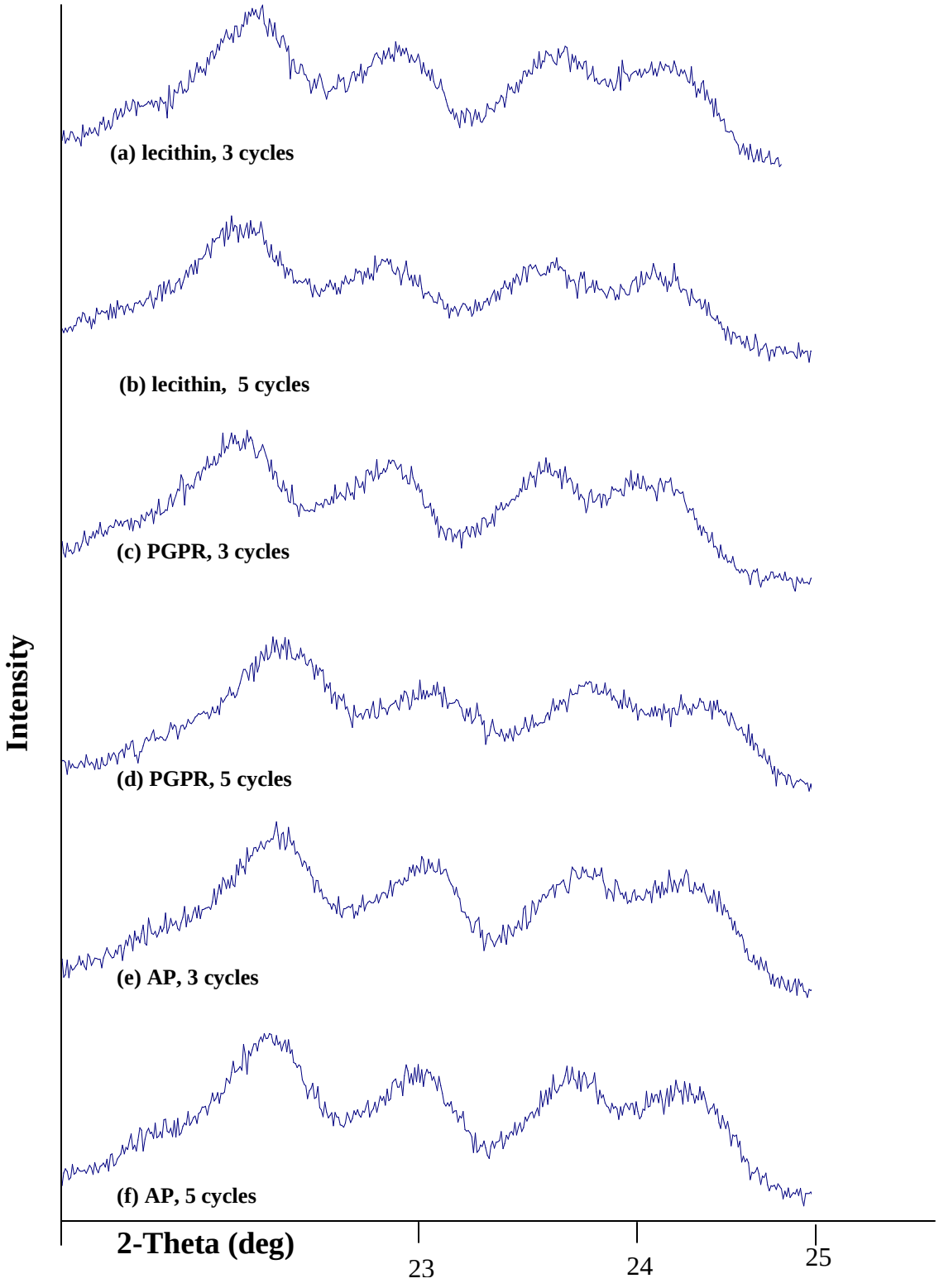


Figure 2.18: XRD patterns for samples cycled at 37°C formulated with 0.5% emulsifier for 3 & 5 cycles, respectively.

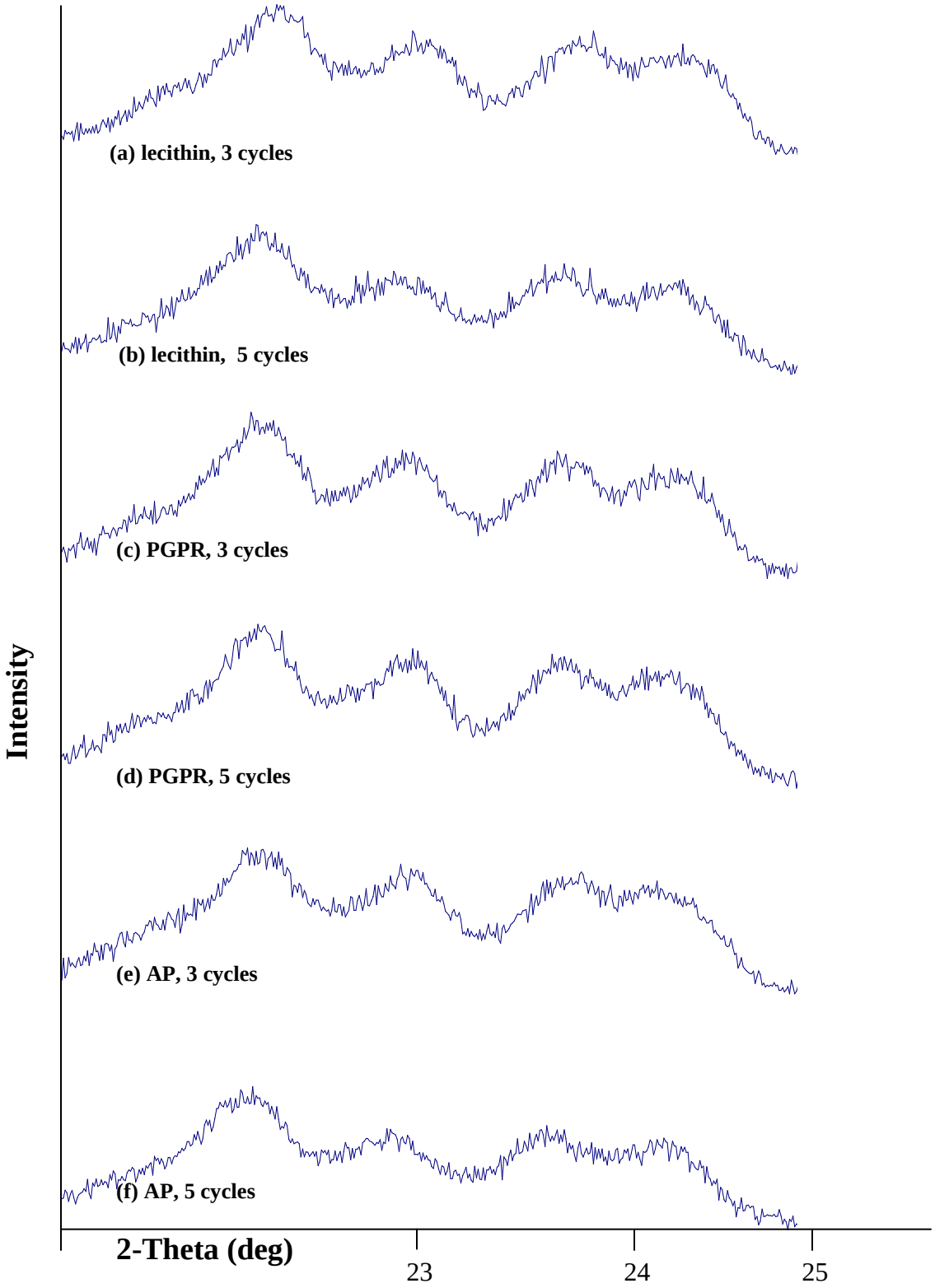
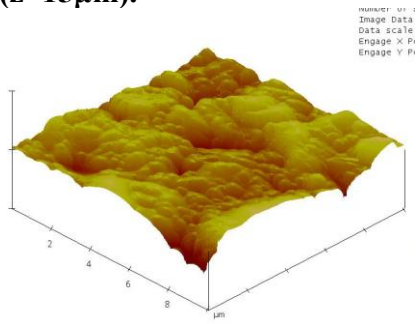
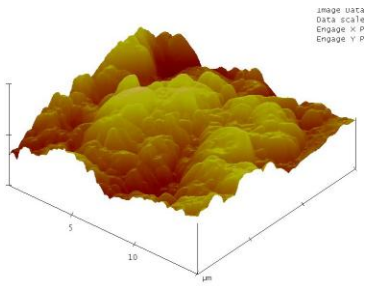


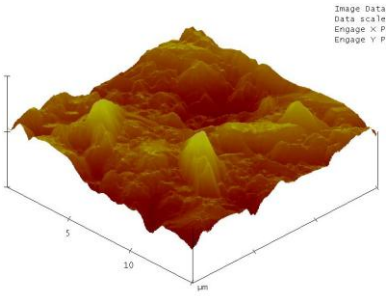
Figure 2.19: AFM images of chocolate samples with lecithin 0.5% (w/w) stored at 23°C, 0 cycles (a) 37°C, 5 cycles (b), with PGPR 0.5% (w/w) stored at 23°C, 0 cycles (c), PGPR (0.5%), 37°C, 5 cycles (d), ammonium phosphatide 0.5% (w/w) stored at 23°C, 0 cycles (e), and with ammonium phosphatide 0.5%, 37°C, 5 cycles (f) (z=15µm).



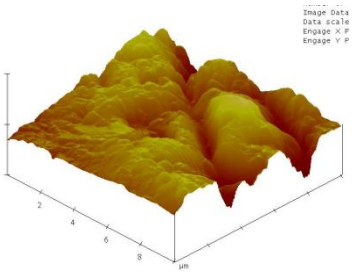
(a) Soy Lecithin (0.5%), 23°C, 0 cycles



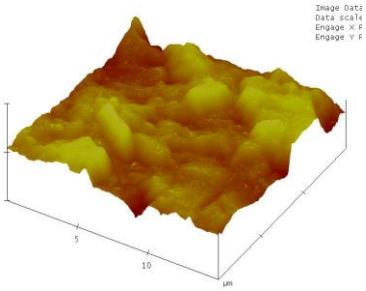
(b) Soy Lecithin (0.5%), 37°C, 5 cycles



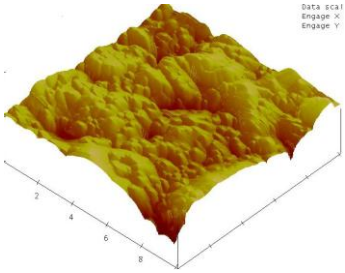
(c) PGPR (0.5%), 23°C , 0cycles



(d) PGPR (0.5%), 37°C, 5 cycles

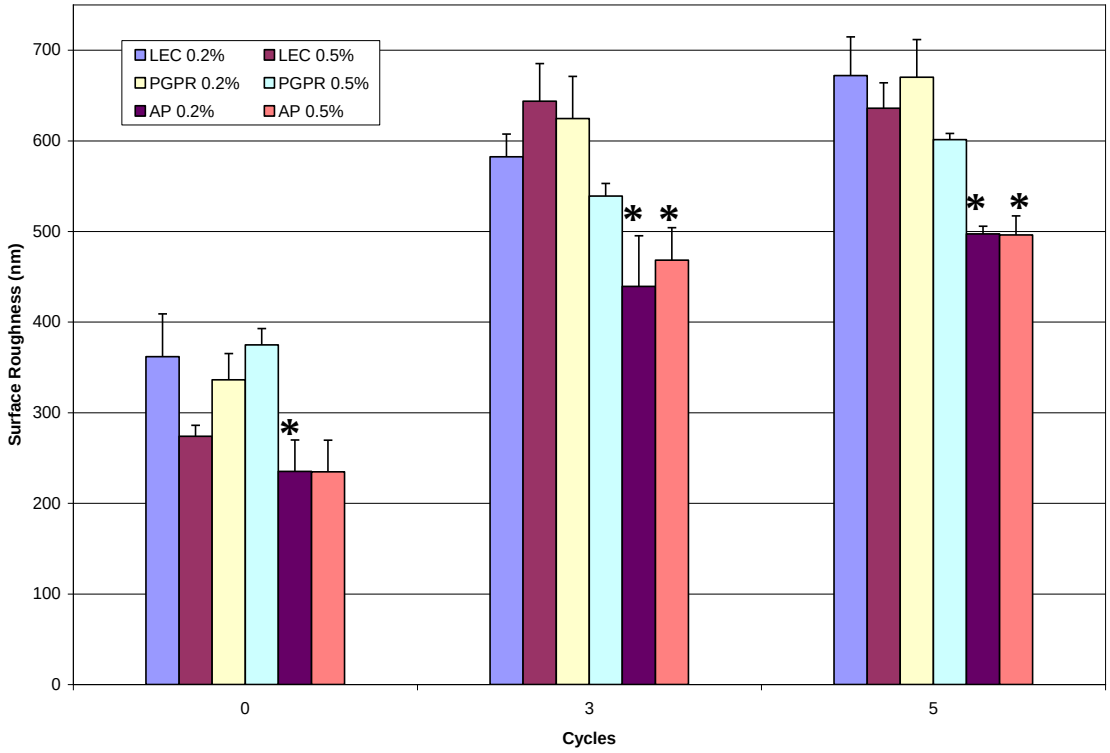


(d) AP (0.5%), 23°C , 0 cycles



(f) AP (0.5%), 37°C, 5 cycles

Figure 2.20: Impact of temperature fluctuations on surface roughness of dark chocolate stored at 37°C with emulsifiers for 0, 3, 5 cycles, respectively.[a]



[a]Storage conditions significantly impacted surface roughness for all emulsifier type and concentration at $p<0.05$. *Emulsifier significantly impacted surface roughness at $p<0.05$.

2.7 Tables

Table 2.1: Storage conditions for dark chocolate samples

Emulsifier (Concentrations w/w)	Storage Temperature (°C)	Cycles Analyzed
Soy Lecithin	23.0	0
(0.2%, 0.5%)	34.0	3, 5
	37.0	3, 5
Polyglycerol Polyricinelorate	23.0	0
(0.2%, 0.5%)	34.0	3, 5
	37.0	3, 5
Ammonium Phosphatide	23.0	0
(0.2%, 0.5%)	34.0	3, 5
	37.0	3, 5

Table 2.2: Sample dimensions (l*w*h) for samples stored at 34°C for 0, 3, 5 cycles, respectively. [a,b]

Sample	length (mm)	width (mm)	height (mm)
Soy Lecithin			
0.2%, 0 cycles	25.0	25.0	12.0
0.2%, 3 cycles	25.0	25.0	11.5
0.2%, 5 cycles	25.0	25.0	12.0
0.5%, 0 cycles	25.1	25.1	11.9
0.5%, 3 cycles	25.1	25.1	11.9
0.5%, 5 cycles	25.1	25.1	12.0
Polyglycerol Polyricinelorate			
0.2%, 0 cycles	25.0	25.0	12.0
0.2%, 3 cycles	25.0	25.0	12.1
0.2%, 5 cycles	24.8	25.0	12.0
0.5%, 0 cycles	25.0	25.0	12.0
0.5%, 3 cycles	24.9	25.0	12.0
0.5%, 5 cycles	25.0	25.0	12.0
Ammonium Phosphatide			
0.2%, 0 cycles	25.0	25.0	12.0
0.2%, 3 cycles	25.0	25.0	12.0
0.2%, 5 cycles	24.9	25.0	12.0
0.5%, 0 cycles	25.0	25.0	12.0
0.5%, 3 cycles	25.0	24.9	12.1
0.5%, 5 cycles	25.2	25.2	12.1

[a]Samples measured with calipers (±0.1 mm). [b]Samples were not significantly different ($p<0.05$).

Table 2.3: Textural data for all control samples stored at 23°C.[a]

Sample	Concentration	Hardness ($x10^3$)	Cohesiveness ($x10^{-1}$)	Adhesiveness	Gumminess ($x10^3$)
Soy Lecithin	0.2%	15.14±0.6[b,d]	1.64±0.1[b]	9.58±0.2[b]	2.48±0.5[b]
Soy Lecithin	0.5%	16.56±3.1[b]	1.59±0.8[b]	10.01±0.2[b]	2.63±0.4[b]
PGPR	0.2%	19.76±0.7[c]	0.76±0.1[c]	-	1.49±0.4[c]
PGPR	0.5%	20.61±0.6[c]	2.28±0.2[d]	17.96±0.4[c]	4.71±0.9[d,f]
Ammonium Phosphatide	0.2%	14.61±0.8[d]	2.05±0.4[d]	14.22±0.3[d]	2.99±0.1[b]
Ammonium Phosphatide	0.5%	10.76±0.2[e,f]	1.77±0.1[b]	25.63±3.2[e,f]	1.90±0.1[e]

[a]Results stated as mean±SD (n=2). [b-e]Numbers with the same letter in columns were not significantly different for emulsifier type at $p<0.05$. [f]Emulsifier concentrations were significantly different at $p<0.05$.

Table 2.4: Textural data for samples cycled at 34°C for 3 & 5 cycles, respectively. [a]

Sample	Hardness ($x10^3$)	Cohesiveness ($x10^{-1}$)	Adhesiveness	Gumminess ($x10^3$)
Soy Lecithin				
0.2%, 3 cycles	$24.14\pm0.6^{b,c}$	0.16 ± 0.1^{b}	8.56 ± 0.3^{b}	0.38 ± 0.4^{b}
0.2%, 5 cycles	21.44 ± 0.7^{g}	$0.33\pm0.2^{g,h}$	6.73 ± 0.2^{g}	0.83 ± 0.3^{g}
0.5%, 3 cycles	22.97 ± 0.9^{b}	$0.07\pm0.0^{b,c,f}$	10.61 ± 0.5^{b}	0.16 ± 0.1^{b}
0.5%, 5 cycles	24.18 ± 0.4^{g}	$0.17\pm0.1^{g,i}$	5.91 ± 0.4^{g}	0.41 ± 0.2^{g}
Polyglycerol Polyricinelorate				
0.2%, 3 cycles	$24.24\pm0.4^{c,d}$	0.27 ± 0.3^{b}	32.22 ± 1.1^{c}	0.65 ± 0.2^{b}
0.2%, 5 cycles	22.95 ± 0.5^{g}	0.73 ± 0.4^{h}	$0.92\pm0.1^{g,h}$	1.68 ± 0.3^{h}
0.5%, 3 cycles	$24.34\pm0.5^{b,c}$	$0.06\pm0.0^{b,c}$	$17.85\pm0.8^{c,f}$	$0.15\pm0.1^{b,c,f}$
0.5%, 5 cycles	21.17 ± 0.7^{g}	$0.37\pm0.2^{f,j}$	$3.78\pm0.5^{f,h}$	$0.79\pm0.3^{f,i,j}$
Ammonium Phosphatide				
0.2%, 3 cycles	$21.58\pm1.1^{d,e}$	0.20 ± 0.1^{b}	13.56 ± 0.8^{b}	0.43 ± 0.1^{b}
0.2%, 5 cycles	20.12 ± 0.9^{g}	0.52 ± 0.2^{i}	42.01 ± 1.5^{g}	1.05 ± 0.1^{i}
0.5%, 3 cycles	$22.11\pm0.4^{d,e}$	$0.17\pm0.1^{c,f}$	$0.82\pm0.1^{c,f}$	$0.37\pm0.1^{c,f}$
0.5%, 5 cycles	$20.55\pm0.5^{f,g}$	$0.14\pm0.1^{f,j}$	$2.55\pm0.4^{f,g,h}$	$0.28\pm0.0^{f,j}$

[a]Results stated as mean±SD (n=2). [b-e]Numbers with the same letter in columns were not significantly different ($p<0.05$) for emulsifier type cycled for 3 cycles. [f]Emulsifier concentrations were significantly different at $p<0.05$. [g-j]Numbers with the same letter in columns were not significantly ($p<0.05$) different for emulsifier type cycled for 5 cycles.

Table 2.5: Melting points by DSC for samples formulated with 0.2% (w/w) and 0.5% emulsifier cycled at 34°C for 0, 3, 5 cycles, respectively.[a,b]

Sample	melting point (°C)
Soy Lecithin	
0.2%, 0 cycles	34.03±0.4
0.2%, 3 cycles	36.44±0.2
0.2%, 5 cycles	36.19±0.5
0.5%, 0 cycles	33.56±0.4
0.5%, 3 cycles	35.98±0.2
0.5%, 5 cycles	35.68±0.6
Polyglycerol Polyricinelorate	
0.2%, 0 cycles	33.75±0.1
0.2%, 3 cycles	36.01±0.1
0.2%, 5 cycles	35.72±0.4
0.5%, 0 cycles	34.25±0.1
0.5%, 3 cycles	36.86±0.3
0.5%, 5 cycles	36.75±0.5
Ammonium Phosphatide	
0.2%, 0 cycles	33.17±0.3
0.2%, 3 cycles	35.54±0.4
0.2%, 5 cycles	35.85±0.3
0.5%, 0 cycles	33.23±0.3
0.5%, 3 cycles	35.68±0.5
0.5%, 5 cycles	35.88±0.4

[a]Results stated as mean±SD. [b]Melting points were not significantly different ($p<0.05$) for samples with different formulations.

Table 2.6: **Differences in *d*-spacings and polymorphs by XRD for samples stored at 34°C &37°C with 0.2% (w/w) emulsifier.**

Sample	2-theta	*d*- spacing (Å)	Polymorph
Soy Lecithin			
23°C, control	22.35, 23.05, 23.73, 24.28	3.97, 3.86, 3.75, 3.66	V
34°C, 3 cycles	21.96, 22.93, 23.98	4.04, 3.88, 3.71	VI
34°C, 5 cycles	21.00, 22.87, 23.87	4.06, 3.89, 3.72	VI
37°C, 3 cycles	22.3, 22.92, 23.7, 24.18	3.98, 3.88, 3.75, 3.68	V
37°C, 5 cycles	22.16, 22.88, 23.55, 24.12	4.01, 3.88, 3.78, 3.69	V
Polyglycerol Polyricinelorate			
23°C, control	22, 27, 22.94, 23.68, 24.18	3.99, 3.87, 3.75, 3.68	V
34°C, 3 cycles	22.03, 23.05, 24.02	4.03, 3.86, 3.70	VI
34°C, 5 cycles	21.99, 22.99, 24.01	4.04, 3.86, 3.70	VI
37°C, 3 cycles	22.23, 22.9, 23.62, 24.04	3.99, 3.87, 3.76, 3.69	V
37°C, 5 cycles	22.35, 23.03, 23.82, 24.41	3.97, 3.85, 3.73, 3.64	V
Ammonium phosphatide			
23°C, control	22.26, 22.89, 23.69, 24.30	3.99, 3.88, 3.74, 3.66	V
34°C, 3 cycles	21.97, 22.97, 24.01	4.04, 3.87, 3.70	VI
34°C, 5 cycles	21.83, 22.89, 23.86	4.07, 3.88, 3.73	VI
37°C, 3 cycles	22.36, 23.01, 23.68, 24.24	3.97, 3.86, 3.75, 3.67	V
37°C, 5 cycles	22.29, 23.00, 23.66, 24.23	3.99, 3.86, 3.76, 3.67	V

Table 2.7: Differences in *d*-spacings and polymorphs by XRD for samples stored at 34°C & 37°C formulated with 0.5% (w/w) emulsifier.

Sample	2-theta	*d*- spacing (Å)	Polymorph
Soy Lecithin			
23°C, control	22.31, 23.00, 23.70, 24.24	3.98, 3.86, 3.75, 3.66	V
34°C, 3 cycles	21.95, 22.96, 23.93	4.05, 3.87, 3.72	VI
34°C, 5 cycles	21.94, 22.97, 23.95	4.05, 3.87, 3.71	VI
37°C, 3 cycles	22.38, 23.09, 23.78, 24.32	3.97, 3.85, 3.74, 3.66	V
37°C, 5 cycles	22.28, 22.92, 23.71, 24.26	3.99, 3.88, 3.75, 3.67	V
Polyglycerol Polyricinelorate			
23°C, control	22.22, 22,89, 23.58, 24.27	3.99, 3.88, 3.77, 3.67	V
34°C, 3 cycles	21.97, 22.97, 24.01	4.04, 3.87, 3.70	VI
34°C, 5 cycles	21.83, 22.89, 23.86	4.07, 3.88, 3.73	VI
37°C, 3 cycles	22.36, 23.01, 23.68, 24.24	3.97, 3.86, 3.75, 3.67	V
37°C, 5 cycles	22.29, 23.00, 23.66, 24.23	3.99, 3.86, 3.76, 3.67	V
Ammonium phosphatide			
23°C, control	22.30, 23.04, 23.66, 24.19	3.98, 3.86, 3.76, 3.68	V
34°C, 3 cycles	22.09, 23.05, 24.09	4.02, 3.86, 3.69	VI
34°C, 5 cycles	21.95, 23.02, 23.98	4.05, 3.86, 3.71	VI
37°C, 3 cycles	22.33, 22.93, 23.79, 24.16	3.98, 3.86, 3.72, 3.68	V
37°C, 5 cycles	22.26, 22.89, 23.63, 24.22	3.99, 3.88, 3.76, 3.67	V

Table 2.8: Sample dimensions (l*w*h) and percent change in width between cycles for samples fluctuated at 37°C for 0, 3, 5 cycles, respectively.[a]

Sample	length (mm)	width (mm)	height (mm)	Percent change in width
Soy Lecithin				
0.2%, 0 cycles	25.0	25.0	12.0	0.0
0.2%, 3 cycles	31.1	31.2	9.1	24.8[c]
0.2%, 5 cycles	34.5	34.3	8.9	34.7[i,j]
0.5%, 0 cycles	25.0	25.0	12.0	0.0
0.5%, 3 cycles	31.0	31.0	9.5	24.0[c]
0.5%, 5 cycles	32.2	32.2	9.0	27.9[g]
Polyglycerol Polyricinelorate				
0.2%, 0 cycles	25.0	25.0	12.0	0.0
0.2%, 3 cycles	27.9	27.6	12.0	10.4[e]
0.2%, 5 cycles	28.7	28.6	12.0	14.0[f]
0.5%, 0 cycles	25.0	25.0	12.0	0.0
0.5%, 3 cycles	33.0	33.0[b]	9.6	32.0[d]
0.5%, 5 cycles	34.0	34.0[b]	10.0	35.0[j]
Ammonium Phosphatide				
0.2%, 0 cycles	25.0	25.0	12.0	0.0
0.2%, 3 cycles	27.5	27.5	10.5	10.0[e]
0.2%, 5 cycles	29.1	29.0	10.9	15.5[f]
0.5%, 0 cycles	25.0	25.0	12.0	0.0
0.5%, 3 cycles	31.5	31.5[b]	9.9	26.0[c]
0.5%, 5 cycles	33.5	33.3[b]	9.0	31.7[g,i]

[a]Samples measured with calipers (±0.1 mm). [b]Emulsifier concentration was significantly different for width of samples at ($p<0.05$). [c-e]Numbers with the same letter were not significantly different ($p<0.05$) for samples at 3 cycles. [f-e]Numbers with the same letter were not significantly different ($p<0.05$) for samples at 5 cycles.

Table 2.9: Textural data for samples cycled at 37°C for 3 & 5 cycles, respectively.[a]

Sample	Hardness ($x10^3$)	Cohesiveness ($x10^{-1}$)	Adhesiveness	Gumminess ($x10^3$)
Soy Lecithin				
0.2%, 3 cycles	16.65 ± 0.4^{b}	0.22 ± 0.1^{b}	$13.18\pm0.4^{b,c}$	0.37 ± 0.1^{b}
0.2%, 5 cycles	15.86 ± 1.4^{g}	0.22 ± 0.1^{g}	$8.82\pm0.6^{g,h}$	0.35 ± 0.1^{g}
0.5%, 3 cycles	16.36 ± 0.8^{b}	0.25 ± 0.0^{b}	13.14 ± 0.5^{c}	0.40 ± 0.1^{b}
0.5%, 5 cycles	15.96 ± 1.4^{g}	$0.32\pm0.1^{f,i}$	$15.14\pm0.6^{f,h,i}$	$0.51\pm0.0^{f,h,i}$
Polyglycerol Polyricinelorate				
0.2%, 3 cycles	17.48 ± 0.5^{b}	0.45 ± 0.2^{c}	6.97 ± 1.3^{d}	0.78 ± 0.1^{d}
0.2%, 5 cycles	17.72 ± 0.5^{g}	$0.43\pm0.2^{g,h}$	7.16 ± 1.1^{g}	$0.77\pm0.1^{g,h}$
0.5%, 3 cycles	14.10 ± 0.3^{b}	0.74 ± 0.1^{c}	$16.23\pm0.9^{b,f}$	1.05 ± 0.2^{d}
0.5%, 5 cycles	16.36 ± 0.5^{g}	$0.52\pm0.0^{g,h,i}$	3.43 ± 1.4^{g}	$0.85\pm0.2^{h,i}$
Ammonium Phosphatide				
0.2%, 3 cycles	16.08 ± 0.5^{b}	0.39 ± 0.1^{b}	33.16 ± 1.2^{e}	$0.53\pm0.3^{b,c}$
0.2%, 5 cycles	16.17 ± 0.6^{g}	$0.52\pm0.1^{h,i}$	$9.01\pm1.8^{g,h}$	$0.85\pm0.3^{f,i}$
0.5%, 3 cycles	13.21 ± 0.4^{b}	0.29 ± 0.0^{b}	$12.48\pm0.9^{c,f}$	$0.69\pm0.0^{c,d}$
0.5%, 5 cycles	$13.62\pm0.3^{f,h}$	$0.44\pm0.1^{f,g,h}$	$16.9\pm0.9^{f,i}$	0.50 ± 0.4^{i}

[a]Results stated as mean±SD (n=2). [b-e]Numbers with the same letter in columns were not significantly different ($p<0.05$) for emulsifier type at 3 cycles. [f]Emulsifier concentrations were significantly different at $p<0.05$. [g-j]Numbers with the same letter in columns were not significantly ($p<0.05$) different for emulsifier type at 5 cycles.

Table 2.10: Melting points by DSC for samples formulated with 0.2% (w/w) and 0.5% emulsifier cycled at 37°C for 0, 3, 5 cycles, respectively.[a]

Sample	Melting Point (°C)
Soy Lecithin	
0.2%, 0 cycles	34.01±0.3
0.2%, 3 cycles	33.85±0.4
0.2%, 5 cycles	34.15±0.3[b]
0.5%, 0 cycles	33.51±0.2
0.5%, 3 cycles	33.68±0.2
0.5%, 5 cycles	33.25±0.1[c,d,e]
Polyglycerol Polyricinelorate	
0.2%, 0 cycles	32.85±0.1
0.2%, 3 cycles	32.92±0.1
0.2%, 5 cycles	33.32±0.4[b,d]
0.5%, 0 cycles	34.33±0.1
0.5%, 3 cycles	34.59±0.4[e]
0.5%, 5 cycles	34.35±0.3[b,e]
Ammonium Phosphatide	
0.2%, 0 cycles	33.10±0.3
0.2%, 3 cycles	33.96±0.3
0.2%, 5 cycles	33.71±0.2[b,d]
0.5%, 0 cycles	33.34±0.1
0.5%, 3 cycles	33.95±0.1
0.5%, 5 cycles	34.09±0.4[b,d,e]

[a]Results stated as mean±SD. [b-d]Numbers with the same letter in column were not significantly different ($p<0.05$) for emulsifier. [e]Emulsifier concentrations were significantly different at $p<0.05$.

Chapter 3: Long term storage effects on fat bloom formation, textural properties, and sensory attributes in dark chocolate samples with different emulsifiers.

3.1 Introduction

Smooth texture and mouthfeel of chocolate is due to unique interactions of polymorphic structures of cocoa butter with adequate volatile release associated with these interactions. Specifically, the orientation of cocoa butter crystals in polymorph V contributes to the even melting properties associated with favorable mouthfeel. Previous research indicates with improper storage these changes are magnified, causing increased particle size and development of fat bloom (Hartel, 2001; Andrae, 2006). The human tongue can detect particles up to 20-30 microns, anything at/above this range is considered 'gritty'; thus, particle size must be controlled to meet consumer standards (Hoskin, 1994; Morgan, 1994). As crystal size increases, appearance and flavor may also be compromised. Extensive research has been conducted on the effects of various lipid polymorphs on texture perception (Tsheuscher and Markov, 1986a, 1986b, 1989). However, literature is lacking reports on the effect emulsifiers have on cocoa butter stability, textural perceptions, and overall effect on fat bloom formation. Emulsifiers have long been used in chocolate to improve the rheology, or flow properties (Rector, 2000). Manufacturers use emulsifiers as an opportunity to optimize their process and formulations to minimize cost.

Stability of the cocoa butter matrix is affected by polymorphic transition in chocolate during storage at high temperatures. In this study, the effect of different emulsifiers on fat bloom formation, sensory attributes, melting points, and surface properties of dark chocolate during long term storage is evaluated. It is hypothesized that during storage due to the instability of emulsifier, the TAG-emulsifier complexes will be impacted, leading to a break in emulsion between the liquid and solid phases in the cocoa butter matrix. Each emulsifier has different properties due to its structure and intermolecular interactions that will affect these TAG-emulsifier complexes. Emulsifiers examined include soy lecithin, polyglycerol polyricinoleate (PGPR), and ammonium phosphatide at 0.2% (w/w). All three emulsifiers have been investigated and are currently being studied for their optimal use in chocolate formulation. Chocolate stored 8 weeks at 23°C and 34°C was evaluated by a trained descriptive analysis sensory panel focused on

identification of differences in texture and flavor between stored samples. Specific details from sensory and instrumental analyses will lead to a better understanding of the impact different emulsifiers have on physical, chemical, and structural properties in dark chocolate, allowing for optimization of quality during storage. This will provide insight into emulsifier selection for chocolate manufacturing.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Sample Preparation

Sensory evaluation samples were prepared using a SPECTRA 10 Stone Melangeur (SanthaUSA, Yoncalla, OR), and a Revolution 2 Chocolate Tempering System (Chocovision™, Poughkeepsie, NY). Samples were prepared in duplicate 1.5 lb batches. Emulsifiers were added at 0.2% (w/w). Three emulsifiers were used in formulation: soy lecithin LECIGRAN® 1000P (Cargill, Decatur, IL), GRINDSTED® polyglycerol polyricinelorate (PGPR) 90 (Danisco, Cedar Rapids, IA), and ammonium phosphatide PALSGAARD® 448 (Palsgaard®, Morristown, NJ). Samples were stored 8 weeks at 23°C and 34°C; control samples (unstored) were prepared and analyzed immediately. **Table 3.1** explains sample conditions and experimental definitions used during this study. Sample preparation was staggered to coordinate with panel schedule. Training samples were prepared for the first four weeks of the panel; while testing samples were prepared for the fifth week of the panel. Samples were stored in sealed containers inside 34°C water baths and an incubator at 34°C (±0.1°C). Once storage was complete, samples were allowed to equilibrate at room temperature 24 hrs before consumption.

3.2.2 Instrumental Techniques

3.2.2.1 Texture Analysis

Physical properties such as hardness, cohesiveness, adhesiveness, and gumminess of dark chocolate were characterized (**Figure 3.1**). Sample texture was analyzed with a TA-XT2 Texture Analyser (Texture Technologies Corp; Scarsdale, NY) and Texture Expert Software v. 1.11. A 4mm cylinder stainless steel probe (P4 DIA) was used for the two-bite compression test (25% compression). Test settings, as described by Afoakwa et

al. (2008a), were as follows: 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 sec, and force of 20 g. Samples were clamped to the plate and held still during all textual measurements. Samples were analyzed in duplicate.

3.2.2.2 Color Evaluation

Color changes were analyzed after storage using a HunterLab LabScan II 0/45 (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were analyzed with HunterLab Universal Software^{TM} Version 3.8. Color was evaluated in triplicate on full chocolate samples. Data recorded from the colorimeter resulted in average lightness and darkness based on three distinct color measurements (L: 100=white, 0=black; a: 100=red, -100=green; b: 100=yellow, -100=blue). Whiteness index (WI) was measured as an indicator of fat bloom formation. Whiteness index was calculated based on the following equation (Briones and Aguilera, 2005):

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

3.2.2.3 Dimension Analysis

Physical dimension change during chocolate storage was analyzed using standard laboratory calipers. Full chocolate sample dimensions (l*w*h) were analyzed in triplicate. Control samples were analyzed immediately after preparation and stored samples were analyzed at end of indicated storage period.

3.2.2.4 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermal analysis technique that determines the energy differential between a sample and a reference. Melting profiles of stored chocolate were conducted 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 hermetically sealed aluminum pan as a reference. Melting points were determined for chocolate samples (1-2 mg), and sealed in Tzero hermetic aluminum pans (TA Instruments; New Castle, DE). Sample chamber was initially cooled to -20°C prior to manually loading the samples in the chamber. Pans were heated at a rate of 10°C/min from a range of -20°C to 80°C in a N_2 gas stream. Melting points were reported at the temperature (Tpeak) where the maximum energy was absorbed by the sample. Samples were analyzed in duplicate.

3.2.2.5 Atomic Force Microscopy

Atomic Force Microscopy (AFM) was used to illustrate nanoscale surface topography. Images are visualized by measuring the force between the tip and sample surface as detected by deflection of the cantilever. Chocolate samples (25mm*25mm*5mm) were fixed onto a glass slide by gently heating one side of the sample. A Dimension 3100 AFM with Nanoscope IIIa controller (Digital Instruments; Santa Barbara, CA) was used to generate images of a 15 x 15 μm area of prepared chocolate. Height and phase differences were analyzed using tapping mode in duplicate. Sample roughness was determined using the Nanoscope software. Sample roughness was based on the root means square (RMS) of height deviations from the sample.

3.2.2.6 Powder X-ray Diffraction

Sample Preparation

Chocolate polymorphs were identified using powder X-ray diffraction (XRD). Chocolate sample preparation was adapted from the method described by Cebula and Ziegleder (1993) to eliminate diffraction interference by extracting the sugar. Samples were prepared as follows: 5 g of chocolate was finely chopped, mixed with 500 mL cold deionized water, and allowed to sit for 4-6 hrs to extract sugar. The mixture was filtered under vacuum using Whatman #1 filter paper and allowed to dry overnight. Chocolate powder was stored in small vials until analysis.

XRD Analysis

Crystal polymorph transitions were confirmed by the short *d*-spacings of an X-ray diffraction pattern. X-ray diffraction patterns were measured at room temperature (23ºC) 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 30mA and a 1º divergence slit was used. The powder sample was pressed into a polycarbonate cell and mounted in the machine. A 2θ scan from 18º to 26º, step of 0.008, and a scan rate of 0.2 degrees/min was utilized to analyze samples in duplicate.

3.2.3 Sensory Evaluation

Twelve panelists (11 female, one male, aged 20-35) were recruited via email and trained in the method of descriptive analysis over a four week period. Prior to testing,

panelists were asked to sign consent forms approved by the Institutional Review Board of the University of Illinois (**Appendix B.1**), and to complete a questionnaire containing allergen information, chocolate preference, gender, and age questions and contact information (**Appendix B.2**). Most panelists indicated preference for dark chocolate over milk chocolate.

Panelists developed a lexicon of descriptive terms classifying flavor, taste and texture characteristics for all samples prepared. Panelists were given 9 samples/test session and asked to taste and expectorate the samples. They were instructed to rate samples for intensity of each of the generated characteristics using a 15 point scale (0=no signal, 15=extreme signal). A sample ballot is located in **Appendix B.3**. Panelists also rated melting characteristics of samples using a time-intensity melting technique. Descriptive analysis training included sessions on understanding sample tasting, for example learning how to rinse between samples and taste samples properly by identifying chewing, melting, and mastication techniques. Once panelists agreed upon the tasting methodology, they refined term generation and reference identification skills followed by proper methods to scale (or rate) attributes associated with each sample. Dove Dark Chocolate Promises^{TM} and laboratory prepared chocolates were used as training samples. The final rinsing protocol, unanimously agreed upon, consisted of three water rinses; first rinsing with warm water, carbonated water, and followed by cold water to completely remove all traces of chocolate between samples.

During term generation, prepared samples were used to initiate generating appropriate terms to describe chocolate texture and flavor. References for each chocolate attribute described were refined over a two week period. Sample texture, taste, and flavor attributes and corresponding references chosen are listed in **Tables 3.2 & 3.3**. Hardness, (defined as the initial force required to bite through the sample with central incisors) was measured at first bite of sample. Crumbliness (defined as the feeling of pieces breaking apart from one another during initial mastication) was evaluated after chewing five times. Tooth packing (the amount of sample left in teeth after chewing a few times) and cohesiveness (how the sample adhered to itself during mastication) were evaluated with crumbliness. Graininess and creaminess were evaluated during mastication. Fatty mouth coating and dry mouthfeel were evaluated after expectoration of the sample. Flavor

attributes analyzed during mastication included: chocolaty, chalky, bitterness, sweetness, and blandness. Aftertastes, bitterness and artificial sweetness, were analyzed 5 sec after expectorating the sample. All samples were rated using a 15 point scale. Panelists established a time intensity melting technique to measure the approximate time it took for a measured sample to completely melt, by placing sample on top of the tongue and pressing to the roof of the mouth until completely melted over a 2 min period; these times were recorded. Example time test scorecards are shown in **Appendix B.4**.

Individual computerized booth analyses were completed during the testing week. Panelists were randomly presented all samples in duplicate, testing 9 samples/session. Samples were distributed in 2 oz clear plastic cups with lids, labeled with random 3-digit codes for identification purposes. Samples were evaluated under red light at room temperature in individual booths to remove any sample appearance bias and/or group influence. All 9 samples were put into sets (3 rows of 3 samples, in random order) and given together. One minute breaks were timed between each set of samples for panelists to rest. Panelists analyzed one sample at a time, starting first with texture, then mouthfeel, followed by flavor. Separate 2 oz. clear plastic cups with previously measured samples (0.25-0.3 g) were given to panelists for time-intensity melting tests, following the textural and flavor rating for each coordinating sample. Compusense® Five 5.0 software was used for data collection and analysis.

3.2.4 Statistical Analysis

Data was analyzed using Statistical Analysis Software (SAS) v. 9.1 (SAS Institute Inc: Cary, IN) to determine analysis of variance (ANOVA) and Fischer's least significant difference (LSD) for each rated attribute using PROC GLM procedure. Mean ratings of significant attributes ($p<0.05$) were further analyzed using correlation matrices for principal component analysis (PCA) in SAS. Multivariate statistical analysis was performed by linear partial least squares regression analysis (PLS) using the SAS Software. PLS2 was used to correlate all instrumental and sensory attributes, while relationships between instrumental data and a single sensory attribute were evaluated by PSL1.

Regression analysis (PROC REG) was used to relate mean ratings of each sensory and instrumental attribute to storage temperature, type emulsifier used, their square

terms and interactions The full model consists of second-order polynomial regression model with two linear terms: storage temperature in degrees Celsius (x_1), type emulsifier used (x_2) and their squared terms and all interactions as shown:

$$\mathbf{Y=\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon}$$

Y is the attribue rating; β_0 is the intercept; $\beta\eta$ are parameter estimates for their respective terms; and ε is a normal random variable with mean 0 and constant standard deviation (Choi et al., 2005). For each attribute, models with adjusted $R^2 > 0.60$ and with equal number of terms were examined for their highest adjusted R^2 and lowest root mean square error (RMSE) were chosen as optimum. These models were tested against the full models using F-statistics to show no significant differences.

3.3 Results and Discussion

Dark chocolate has both a complex flavor profile and compositional matrix consisting of sugar and cocoa particles dispersed in a continuous phase of cocoa butter. The crystal structure of cocoa butter contributes to both the smooth mouthfeel and favorable melting properties of chocolate. The ideal polymorphic phase of cocoa butter is stable polymorph V consisting of triple chain packed β crystals (Sato, 2001). Storage of chocolate can dramatically affect the stability of this crystalline matrix. Specifically, storage at high temperatures can intensify the transition of polymorph V to more stable polymorph VI. Although, there are many studies on this phenomenon, the mechanism of fat bloom formation and its relationship with lipid polymorphism is still uncertain. Fat bloom is a major quality concern for the confectionery industry, which has annual chocolate sales of US $7.08 billion (Information Resources Inc., 2010). Chocolate with fat bloom is not only visually unappealing, but theorized to affect flavor and textural qualities, which are important determinants of consumer preference.

In this study, dark chocolate samples stored 8 weeks at 23°C and 34°C were both visually compromised (**Figure 3.2**) and experienced decreased textual and physical quality. Chocolate was stored 8 weeks (long term storage) to ensure ample exposure time to conditions to promote fat bloom formation. Sensory descriptive analysis indicated samples stored at 34°C were compromised in texture and flavor characteristics compared

to unstored samples (controls) or those stored at 23°C. All samples formulated with each different emulsifier stored at 34°C exhibited both a visual confirmation of fat bloom formation, shown from increased WI and a structural change, confirmed by polymorphic transition from polymorph V to VI using XRD analysis. This study focused on the impact emulsifiers had on fat bloom formation and on flavor and textural qualities associated with stored chocolate samples.

Scientists have proposed certain ideas/methods, such as the addition of emulsifiers, to inhibit fat bloom formation. Each emulsifier has a different structure which directly contributes to its effect on viscosity, yield value, and flow of formulated chocolate (Rector, 2000; Schantz and Rohm, 2005, Afoawka et al., 2007); however, the role of emulsifiers in fat bloom formation is still unclear. In the chocolate matrix, cocoa particles are highly hydrophobic and interact strictly with cocoa butter phase. Emulsifier stability and properties in chocolate are dependent upon the interactions with both sugar particles and cocoa butter phase; in detail, the hydrophilic head of an emulsifier attaches to the sugar particle while its lipophilic tail flows in the continuous phase. These interactions involve formation of emulsifier-sugar complexes as well as emulsifier-TAG complexes; however, the impact and significance these interactions have on cocoa butter crystal stability is still unclear. Walter and Cornillon (2001) suggested sucrose does not influence the stability of lipid crystals and migration of lipids to the surface will still occur. While Nebesny and Zyzelewicz (2005) stated the shape and interaction of the sugar-emulsifier complex are responsible for the liquid fraction flow properties. Both studies focus on intermolecular mobility of the continuous fat phase, which is hypothesized to impact crystal packing and fat bloom formation (Andrae, 2006). I speculated the stability of both emulsifier-TAG and emulsifier-sugar complexes are impacted by storage and have a direct affect on intermolecular mobility of the fat phase, which may be a precursor to phase separation of lipid and sugar in chocolate.

3.3.1 Instrumental Analysis

3.3.1.1 Physical Appearance

There was no significant change in dimension for all chocolates stored at 23°C and 34°C (**Figure 3.2**). Samples stored at 34°C exhibited fat bloom on the surface. Average colorimeter data are presented with whiteness index (WI) in **Table 3.4**.

Chocolate samples experienced significant increases in WI during storage at 34°C. There was no change in WI for control samples (unstored) and samples stored at 23°C for all emulsifiers, which confirms there was no visual formation of fat bloom on the surface of these samples. The greatest percentage increase in WI was seen in ammonium phosophatide samples; followed by PGPR and lecithin samples.

3.3.1.2 Textural Attributes

Storage of chocolate formulated with each emulsifier resulted in significant changes in certain textural attributes, namely hardness, cohesiveness, and adhesiveness (**Table 3.5**). Textural attributes were also affected by emulsifier type. Replicates of samples were not significant for each textural attribute measured. Although gumminess was not significant for all storage treatments, there was a noticeable trend that gumminess decreased for all emulsifiers with increasing storage temperature to 34°C. PGPR samples were the hardest in all storage conditions. As storage temperature increased from 23°C to 34°C, textural hardness measurements significantly increased for samples formulated with PGPR. This may be due to rearrangement of TAGs packing into tight crystalline structures and/or the decrease in the lipid fraction mobility. PGPR is known to significantly decrease yield values (minimum force required to initiate flow) in chocolate, but have no affect on viscosity (energy required to keep fluid in motion) (Rector, 2000). The structure of PGPR has a long lipophilic tail stretching into the lipid fraction. The nature of its chemical structure may interrupt emulsifier-TAG interactions and crystal packing by causing steric hindrance between intermolecular particles and thus create agglomeration of individual particles (Nebesny and Zyzelewicz, 2005). These agglomerates might affect TAG packing and the overall stability of the cocoa butter matrix. Lecithin samples were least hard for samples stored at 23°C. As storage temperature increased to 34°C, lecithin samples increased in hardness, and decreased in cohesiveness and gumminess. This trend seen in lecithin may be because it forms smaller aggregates of reversed micelles in cocoa butter which could positively affect the elastic properties of the particle network, and/or change of the particle network structure itself which would increase the overall textural appeal for chocolate (Johansson and Bergenstahl, 1992b).

Ammonium phosphatide samples were the least cohesive and least gummy for all storage conditions. Ammonium phosphatide samples were least hard for control samples. Similarly, ammonium phosphatide samples significantly increased in hardness, and decreased in cohesiveness, and gumminess as storage temperature increased. However, these changes in textural properties formulated with ammonium phosphatide samples stored dark chocolate at 34°C were not significant. I speculated this may be due to unchanged solid and liquid ratios found in these samples; however they were not determined in this study. Hardness is associated with solid fat content (SFC) in chocolate and crystal microstructure (Full et al., 1996). Solidification is dependent on the small amounts of low melting fatty components in the liquid phase, and the proportion of liquid to solid fat content (SFC) is a function of chocolate temperature (Tscheuschner and Markov, 1986a). Future studies will need to characterize the TAG composition and solid fat content (SFC) in samples formulated with each emulsifier especially if structural rearrangements of TAG molecules have an impact on polymorphic transitions. The texture analyzer was only able to measure four parameters; yet sensory results will show trained panelists were able to distinguish differences between nine textural attributes.

3.3.1.3 Melting characteristics

Confirmed structural changes were reflected by DSC for all emulsifier samples either not stored (control) or stored at 23°C and 34°C. **Figure 3.3** shows changes in melting points assessed by DSC. As storage temperature increased to 34°C, melting points significantly increased for all emulsifier formulations. Maximum melting temperatures for all emulsifier formulations are listed in **Table 3.6**. Freshly prepared unstored (control) samples had an average melting point of 33.4°C. Lecithin significantly impacted melting characteristics of samples stored at 23°C and 34°C. Lecithin samples had the highest melting point for samples stored at 34°C; while PGPR samples had the lowest melting points for unstored samples and samples stored at 34°C. Samples stored at 34°C for 8 weeks had an average melting point of 36.14°C; lecithin had the greatest increase and highest melting point. Thermodynamic stability is associated with an increase in endothermic values and thus an enhancement of crystallinity; which contributes to polymorph VI being considered the most stable polymorph (Garti et al., 1986). Samples stored at 34°C, had the highest melting points which also indicates a

decrease in molecular mobility (Walter and Cornillon, 2001). TAGs can either be tightly packed, producing a more rigid structure, or TAGs can lack rigidity, which will permit undulation of carbon chains and movement of fat molecules. Samples formulated with soy lecithin had the highest melting point (37°C) after 34°C storage, indicating these samples had significant thermodynamic stability affecting the cocoa butter structure. Crystal XRD pattern analysis agreed with DSC melting points for all samples.

3.3.1.4 Crystal polymorphism

Changes in polymorphic structures were confirmed by analyzing *d*-spacings in XRD polygraphs. Typical polymorph V patterns contain four distinct peaks from 22-25° scan range. While polymorphs transition from V to VI, the peaks merge and begin to increase in magnitude; the first peak becomes less pronounced. Detailed *d*-spacings and polymorph data for each emulsifier during storage are presented in **Table 3.7**. XRD patterns for non-stored (control) samples exhibit 4 distinct peaks, indicating a polymorph V stable pattern (**Figure 3.4**). Patterns for samples stored 8 weeks at 23°C are presented in **Figure 3.5**. The last peak in the diffraction pattern for chocolate stored 8 weeks at 34ºC was more pronounced compared to the XRD diffraction patterns for samples stored at 23°C **(Figure 3.6)**. This occurred from a complete merging of peaks and shows samples stored at 34°C transitioned to polymorph VI, (Cebula and Ziegleder, 1993). Fat bloom formation is usually seen after a transition of polymorph V to VI (Wille and Lutton, 1969). Results from XRD, DSC, and color analysis confirm samples stored at 34°C exhibited fat bloom formation; while freshly made unstored samples and samples store at 23°C did not exhibit fat bloom formation.

3.3.1.5 Surface characteristics

AFM images of surface topography of dark chocolate formulated with 0.2% emulsifier stored at 23°C, 34°C, and freshly made samples confirm differences in microstructural characteristics on all samples stored 8 weeks at 34°C. Emulsifier type had no significant impact on surface roughness during long term storage. Storage at 34°C increased surface roughness for chocolates formulated with each emulsifier (**Figure 3.7**). As noted in Chapter Two, temperature fluctuations at 34°C and above lead to growth of overlapping smooth rounded crystals on the surface of chocolate. These samples were harder because of tight packing of TAGs in the cocoa butter matrix (Bricknell and Hartel, 1998; Hodge and Rousseau, 2002; Andrae, 2006). On the other hand, in this study

storage at 34°C without temperature fluctuations lead to formation of 'needle and spike' crystal formation on the surface which had direct impact on surface roughness as observed by AFM.

Sample storage at 23°C had no significant impact on surface roughness. Freshly made chocolate samples had no significant impact on surface roughness. Sample roughness after storage at 34°C was higher than that of samples stored at room temperature. This can be explained by the coarse and crystalline cluster formations noted by AFM. When chocolate is severely bloomed, crystal size increases and roughness of the sample increases, forming clusters of fat particles on the surface and interior of chocolate (Andrae, 2006). Difficulty associated with scanning these samples was more pronounced due to such dramatic formations on the surface.

3.3.2 Sensory Analysis

Sensory results in this study confirm important key descriptors associated with dark chocolate, but also give a more detailed lexicon of chocolate impacted by storage and affected by fat bloom. Definitions for sensory attributes determined by panelists are listed in **Tables 3.2 & 3.3**. Mean panelist scores for all attributes evaluated are listed in **Tables 3.8 & 3.9**. Of the 17 attributes assessed, 14 were significantly altered during chocolate storage. ANOVA results for sensory attributes are listed in **Table 3.10**. Chocolate storage condition (treatment) caused a significant source of variation in all attributes, except bitter aftertaste, artificial sweet aftertaste, and tooth packing. Judges were a significant source of variation in all 17 attributes. This variation is common and results from panelists using different rating methods and not utilizing the whole scale (Andrae, 2006). Replications were significant for dry mouthfeel, grainy, chalky, creamy, and bland indicating the panel was not reproducible over replications for these attributes. Judge by treatment interactions were significant for 16 attributes (with exception of crumbly) suggesting that panelists ranked intensity level differently for these attributes across chocolate samples. Interactions of judge by replication were significant for chocolate, bitter aftertaste, and time intensity melting point signifying panelists did not rate samples in the same order upon replications for theses attributes. Treatment by replication interactions were only significant for dry mouthfeel, hardness, and artificial

sweet aftertaste indicating that storage conditions caused uniform changes in chocolate samples, yielding reproducible results across most other attributes.

Cluster analysis results for sensory data indicate four distinct groupings exist (**Figure 3.8**). Samples stored at 34°C were separated from the other samples in one group. Samples stored at 23°C were grouped in a separate cluster; and the control samples were split into two smaller groups based upon emulsifier formulations. Principal component (PC) loading factors indicate relationships between attributes in a set and aid in determining where variation lies (Lee et al., 2002). Loading factors were considered high when the factor was greater than ± 0.5 for one factor, while less than ± 0.5 for the remaining factor. **Table 3.11** contains PC loading factors for all sensory attributes assessed; PC1 describes all attributes except tooth packing (described by PC2). Artificial sweet aftertaste was loaded relatively high for both factors. **Figure 3.9** illustrates relationships between instrumental data; PC1 explains 83.5% total variance and PC2 explains 8.4% total variance. Statistical analysis shows 95% of the total variance is explained in 3 total factors.

PCA data indicates samples were separated into two distinct groups, specifically by storage condition (**Figure 3.9**). Chocolate flavor was bland and chalky after storage at 34°C. These samples were also harder, more grainy, crumbly, had a dry mouthfeel, and melted slower compared to other samples. Previous research indicates grain size affects physical perception; crystal sizes larger than 20 μm can be detected by the human tongue and thus impact consumer appeal (Hodge and Rousseau, 2002). Chocolates stored at 34°C exhibited fat bloom formation and were perceived as grainy which may have resulted from separation of lipid and sugar in the chocolate matrix, caused from the break in emulsifier-TAG stability after being subjected to high temperatures. Research also indicates chocolate stored at 8 weeks at 34°C caused a decrease in flavor volatile concentrations which can be detrimental to quality chocolate flavor (Andrae, 2006). This can be due to these samples having a lower solid fat content (SFC) which can facilitate volatile loss throughout storage. These fat bloom attributes were less appealing to panelists as compared to samples stored at 23°C. Samples stored at 23°C were creamier, more cohesive, and had a fatty mouth coating; flavor associated with these samples were bitter, chocolate, sweet, and roasted. Unstored control samples were loosely scattered in

the PCA plot and did not form a distinct group, confirmed by cluster analysis results. Control samples provided the greatest variation during sensory testing; these samples were not defined by a set group of attributes. Artificial sweet, bitter aftertaste, and tooth packing did not correlate with any sample formulations or storage conditions. Bitter aftertaste was statistically proven to have no impact on chocolate flavor, even though the PCA plot (**Figure 3.9)** shows it clustered near bitter taste. Ammonium phosphatide and PGPR samples stored at both 34°C and 23°C were closely clustered together around similar sensory attributes.

As fat bloom occurred on samples stored at 34°C, the decrease in sweet and creamy flavor could be attributed to volatile release of the chocolate (Guinard and Mazzucchelli, 1999). Panelists rated samples stored at 23°C with higher intensities for these flavors which supports the method of controlled storage or proper 'aging' of chocolate. A 2007 interview with master chocolatier Art Pollard of Amano Chocolate disclosed that chocolates do not fully develop their flavor until at least 30 days after preparation: this is known to the experts as "aging" chocolate. Pollard noted that each chocolate ages differently and at different speeds, thus impacting final flavor notes. Sensory results confirm that flavor differences between freshly made control samples and samples stored at 23°C follow this trend; chocolates stored 8 weeks at 23°C are more clustered around rich flavor notes such as chocolate, creamy, sweet, and roasted. Unstored samples are not clustered together or tightly associated when it comes to flavor attributes as mentioned before. Detailed flavor analysis needs to be performed on these samples to quantify emulsifier interactions with important volatiles in chocolate.

Sensory results revealed that all attributes, for both texture and flavor, were rated similarly (**Tables 3.8 & 3.9**) and no attributes were dominant factors for each chocolate sample seen from PCA data results (**Table 3.11**). This may be due to inadequate panelist training, minimal volatile release, or a combination of both. Markov and Tscheuschner (1989) noted long sensory tests lead to fatigue and are disadvantageous because memory of intensity values deteriorates. Overall, sensory results indicate emulsifier type did not differentially impact chocolate flavor and texture. This confirms manufacturer claims that their emulsifier products added up to 0.5% (w/w) have no affect on chocolate flavor in formulated samples (Palsgaard, 2009). However, previous research confirmed soy

lecithin added at high concentrations (0.7%) negatively affects the taste and aroma of chocolate; sensory panelists rated samples “unpleasant” due to lecithin addition at that concentration (Sondergaard, 1987). At low concentrations, as used in this study, emulsifiers do not impact flavor of chocolate; therefore, manufacturers can rely on the consistency of flavor and focus on the role emulsifiers have on structural changes in the cocoa butter matrix or emulsifiers’ role in inhibition of fat bloom.

3.3.3 Instrumental and Sensory Correlation

Overall, 17 sensory attributes and 9 instrumental variables were influenced by storage condition for chocolate samples. Correlations between sensory and instrumental results were determined by cluster analysis, principal component analysis (PCA), and partial least squares linear regression (PLS). Three distinct groups are indicated by cluster analysis (**Figure 3.10**) of sensory attributes versus instrumental results such as melting point (DSC); hardness, gumminess, adhesiveness, cohesiveness textural variables; roughness; L, a, b, and WI color measurements. The first group consists of unstored control lecithin and PGPR formulations stored at 23°C. The second group contains the remainder of unstored control samples and those stored at 23°C, and the third group is composed of all samples stored at 34°C. PCA biplot indicated instrumental hardness and adhesiveness did not correlate with any storage condition or emulsifier type (**Figure 3.11**); PC1 explains 79.1% total variance and PC2 explains 19.4% total variance. Instrumental measurements reflecting gumminess and cohesiveness were highly correlated with samples stored at 23°C and unstored control samples, particularly lecithin and PGPR formulations. Color measurements, DSC, and roughness data were negatively correlated with unstored control samples and samples stored at 23°C (**Figure 3.12**); PC1 explains 88.7% total variance and PC2 explains 9.1% total variance. Chocolates stored at high temperature positively correlated with color and DSC melting data. These samples had higher WI, were harder, melted slower by sensory assessment and DSC measurements; these samples also had rougher surfaces as indicated by AFM.

PLS2 data decribes percent variance of sensory attributes and instrumental attriubtes. The PLS2 biplot of sensory and instrumental results indicates two highly correlated clusters **(Figure 3.13).** Textural gumminess and cohesiveness were highly correlated with creamy, sweet, cohesive, creamy, chocolate, fatty mouth coating, roasted

and bitter flavor sensory attributes. While textural adhesiveness and hardness, WI, melting point, and color were highly correlated with time intensity melting points, and sensory attributes of chalky, crumbly, bland, grainy, hardness, and dry mouthfeel. Toothpacking, artificial sweet aftertaste, bitter aftertaste, and instrumental adhesiveness did not correlate with any other sensory attribute or instrumental result. It was discovered later that toothpacking was misunderstood by panelists during testing; panelists were confused about the toothpacking definition and did not rate toothpacking attribute at the same consistent point during sample masitcation. **Table 3.12** demonstrates relationships between sensory and instrumental results in a correlation matrix. Textural hardness was positively correlated with dry mouthfeel ($r=.944$, $p<0.001$). Textural cohesiveness and gumminess were also positively correlated ($r=0.979$, $p<0.001$). All samples stored at 34°C were described as crumbly, grainy, chalky, had a higher T-I melting point, were also hard and adhesive. These samples also exhibited fat bloom formation on the surface. The attributes such as crumbly, grainy, and chalky have been recorded in chocolate with fat bloom, which is known to have impact the crystal cocoa butter lattice, smooth mouthfeel, and even melting properties in chocolate (Hartel, 2001; Andrae, 2006).

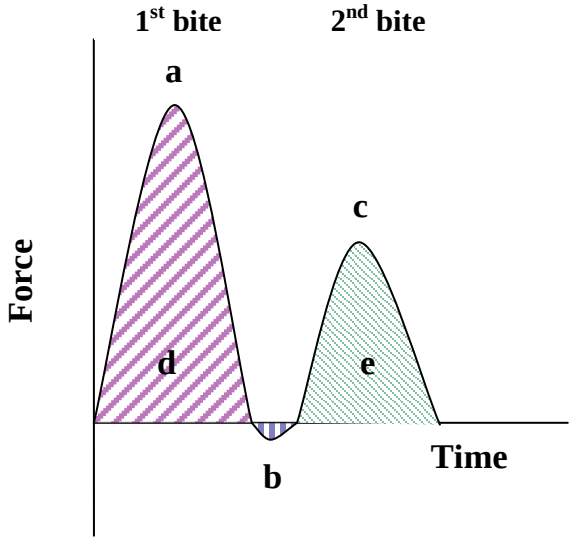
Correlations between sensory attributes and color are presented in **Figure 3.14 and Table 3.12**. Instrumental color was described by sensory attributes of dry mouthfeel, chalky, hardness, grainy, crumbly, bland, and T-I melting point. **Figure 3.15** illustrates the PLS biplot of sensory and DSC (melting point) data. DSC melting point was positively correlated with bland flavor ($r=0.934$, $p<0.01$), and crumbly texture ($r=0.939$, $p<0.001$), while negatively correlated with chocolate ($r=-0.934$, $p<0.01$) flavor and creamy ($r=-0.922$, $p<0.001$) attributes Instrumental textural data versus sensory attributes is split into two distint groups as demonstrated by PLS (**Figure 3.16**). Textural hardness and adhesiveness correlate with sensory attributes of dry mouthfeel, chalky, hard, grainy, crumbly, bland, and T-I melting; whereas instrumental gumminess and cohesiveness are correlated with sensory attributes of creamy, fatty mouth coating, and cohesive. Significant regression equations based on mean sensory and instrumental data with adjusted R^2 >0.60 are shown in **Table 3.13**. The lower values are likely due to a weaker relationship rather than scatter in the experimental data (Choi et al., 2005).

3.4 Conclusions

Long term storage of dark chocolate at different temperatures had impact on texture and flavor of dark chocolate formulated with different emulsifiers at 0.2% (w/w). Textural properties and melting properties were impacted by emulsifier addition. PGPR had the greatest impact on texture hardness, cohesiveness, and gumminess, as well as increased melting point. Analysis of dark chocolate stored at 34°C experienced a transition from polymorph V to VI and increased roughness as expressed by XRD and AFM. Lightening of chocolate on the surface indicated fat bloom formation; AFM results revealed that 'spike and needle' crystal formations occurred on lighter surface area of the chocolate samples. Chocolate samples freshly prepared and those stored at 23°C for 8 weeks had similar chocolate microstructural, lipid polymorphic and physical characteristics. However, samples stored at 23°C exhibited more intense flavor notes as described by panelists during descriptive analysis. Descriptive analysis revealed temperature conditions significantly impacted flavor and texture of dark chocolate stored for 8 weeks. Chocolate experiencing fat bloom (storage 34°C) was harder, grainy, crumbly, left a dry mouthfeel, and had a bland, chalky flavor. While chocolate stored at 23°C was described as cohesive, creamy, fatty mouth coating, and had a roasted, chocolate, bitter, and sweet flavor. The addition of different emulsifiers did not have any affect on flavor and texture attributes of chocolate. Overall, chocolate storage temperature had greater impact on sensory appeal and textural analysis than emulsifier addition. Future studies are necessary to confirm compositional and structural rearrangement of TAGs in dark chocolate formulated with soy lecithin, PGPR and ammonium phosphatide. There should be a focus on confirming the type of structural interactions between TAG and emulsifiers at the molecular level.

3.5 Figures

Figure 3.1: A common texture profile analysis curve for a two bite compression test (adapted from Rosenthal, 1999)



Measurement	Definition
Hardness	**a:** peak force during the 1st bite
Coheisveness	**d/e**: area of 2nd bite divided by the area of the 1st bite
Adhesiveness	**b:** negative force area for the 1st bite
Gumminess	product of hardness and cohesiveness

Figure 3.2 Image of dark chocolate formulated with either soy lecithin, polyglycerol polyricinoleate (PGPR), or ammonium phosphatide (AP) at 0.2% (w/w) for unstored samples (a), samples stored at 23°C (b), and samples stored at 34°C (c).

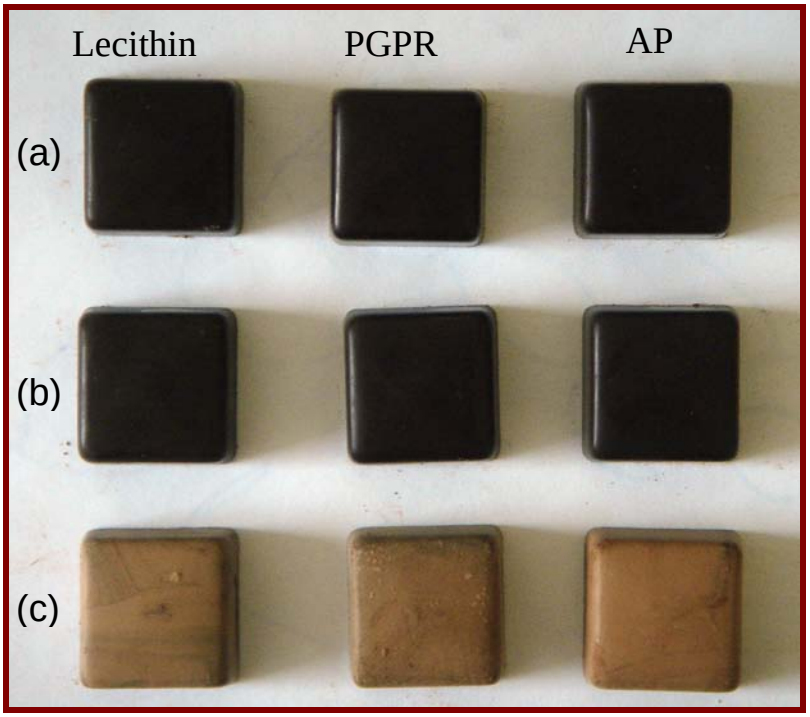
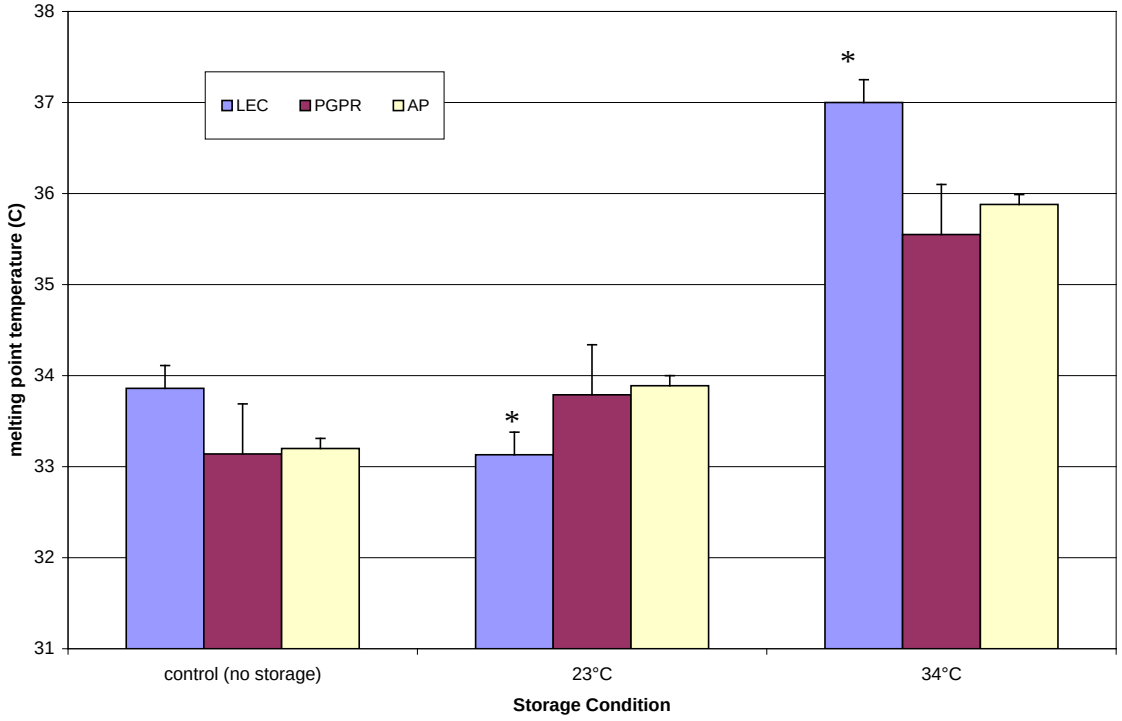


Figure 3.3 Melting point averages, by Differential Scanning Calorimetry for all samples with 0.2% (w/w) emulsifier.



***Emulsifiers at specified temperature are statistically different, p<0.05.**

Figure 3.4 X-ray diffraction patterns for control samples (unstored) for each emulsifier.

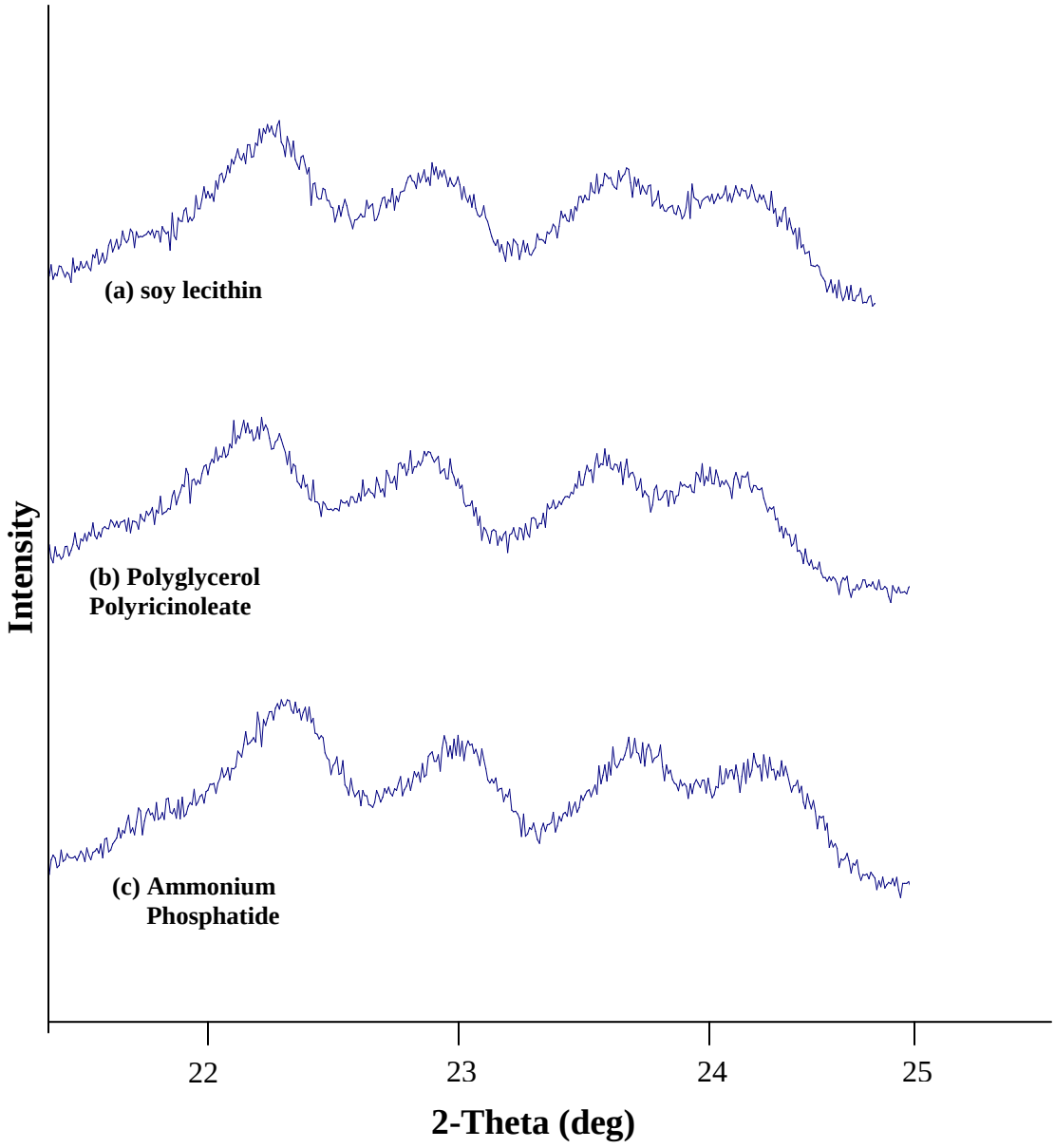


Figure 3.5 X-ray diffraction patterns for sensory samples stored for 8 weeks at 23°C for all emulsifiers.

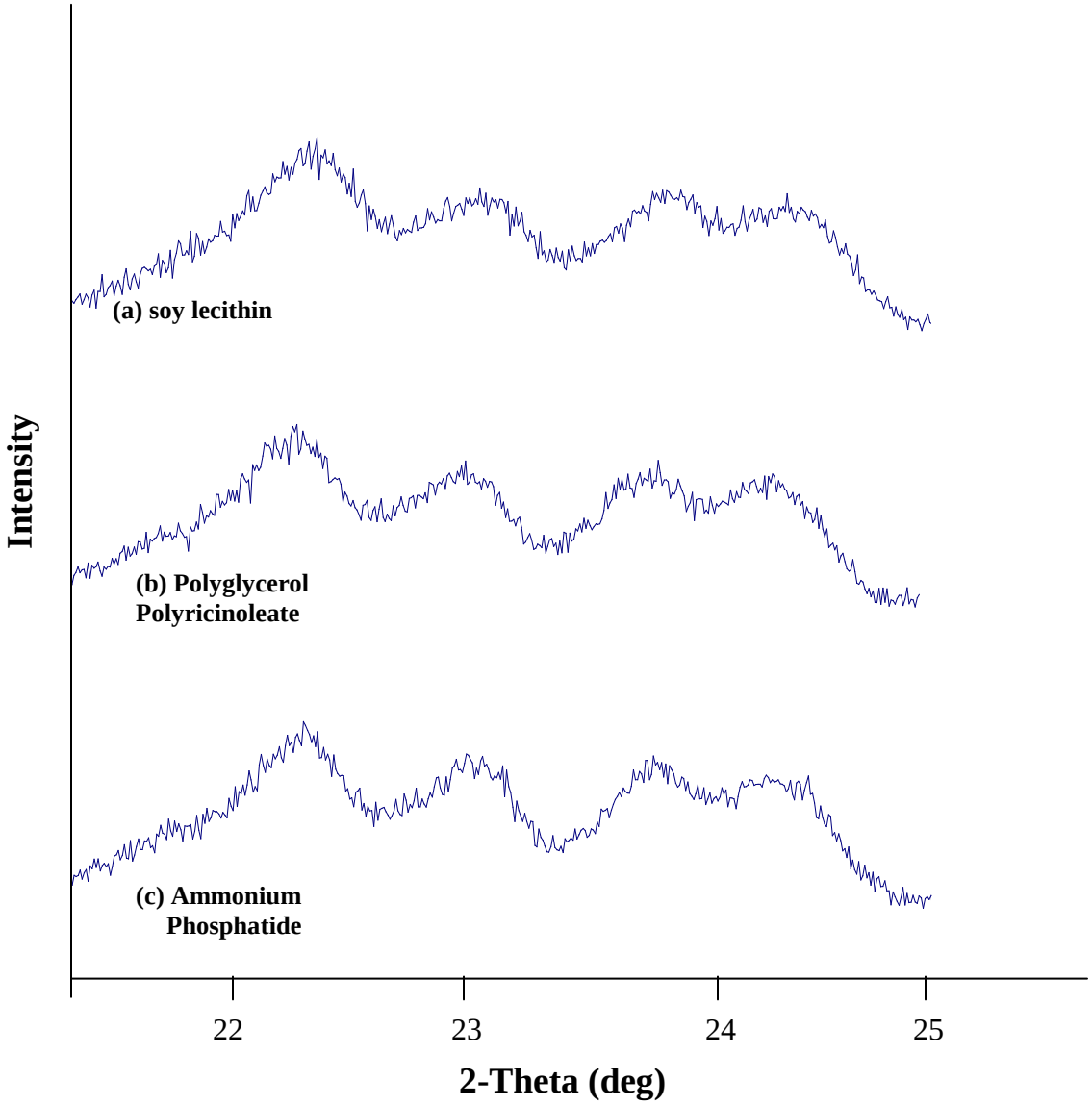


Figure 3.6 X-ray diffraction patterns for samples stored at 34°C for 8 weeks for all emulsifiers.

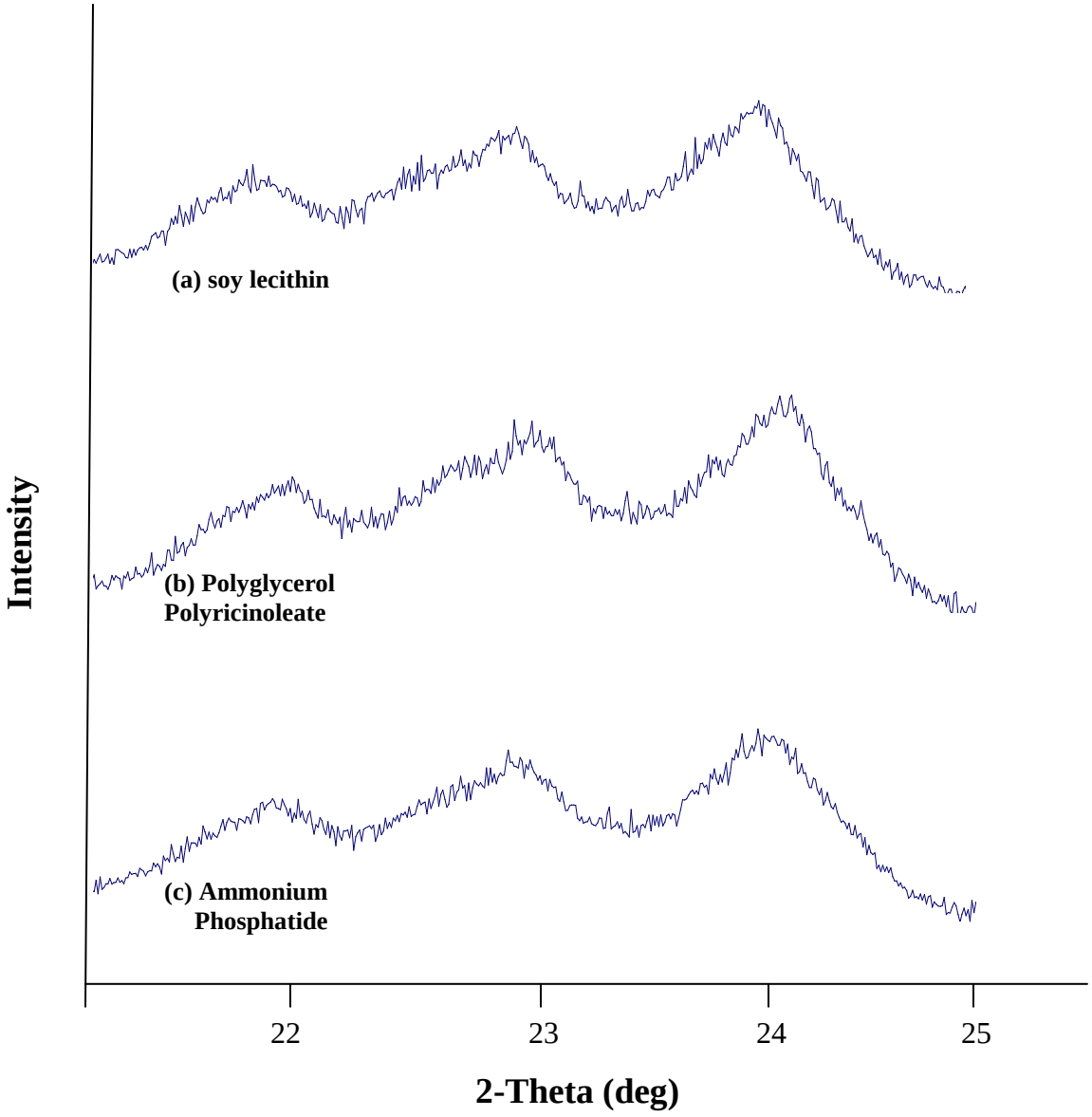
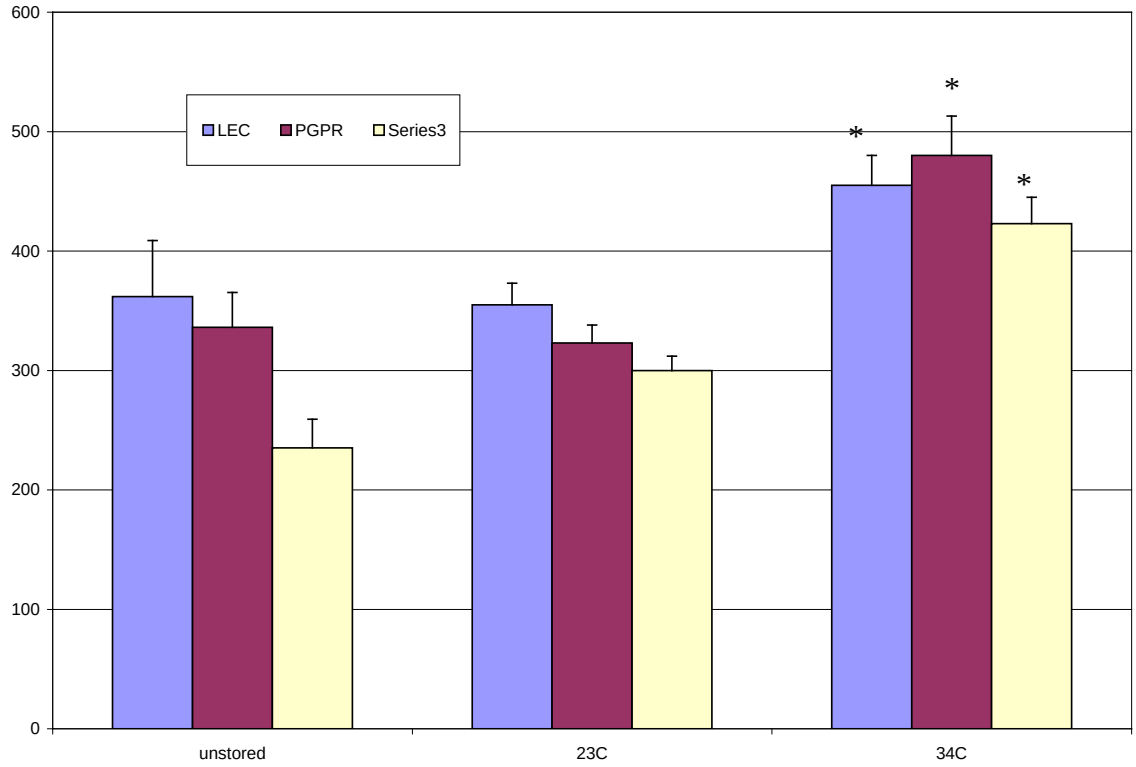


Figure 3.7 Impact of long term storage on surface roughness of dark chocolate stored at 23°C, 34°C, and unstored control samples formulated with 0.2% emulsifiers.[a]



[a]Emulsifer type was not significant for surface roughness ($p<0.05$). *Storage temperatures caused significant differences ($p<0.05$) in surface roughness.

Figure 3.8 Cluster diagram of sensory attributes

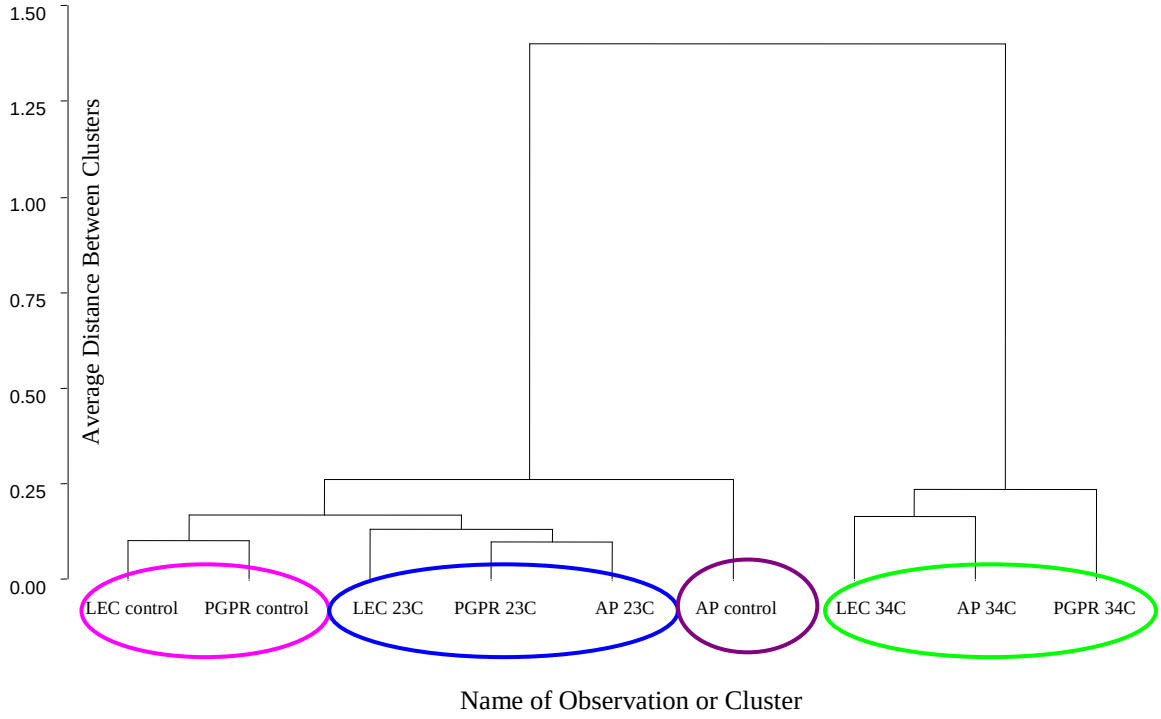


Figure 3.9 Principal component analysis (PCA) plot for all sensory attributes

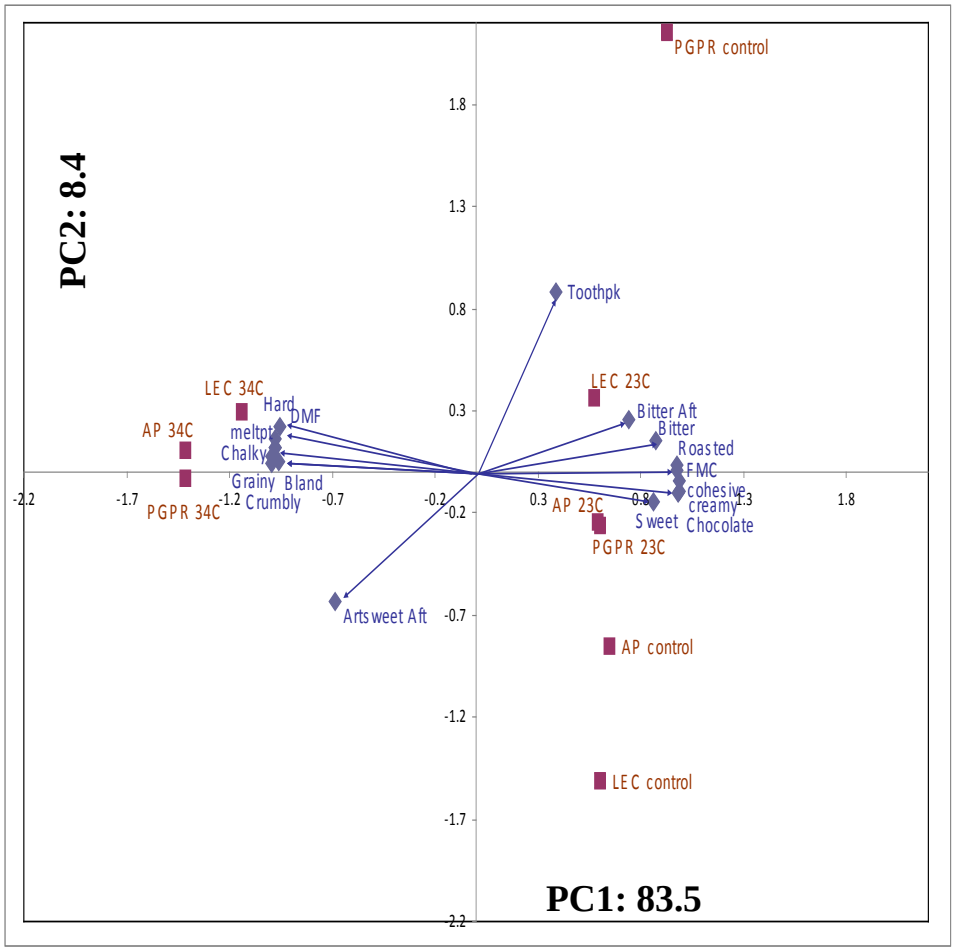


Figure 3.10 Cluster diagram for sensory versus instrumental results

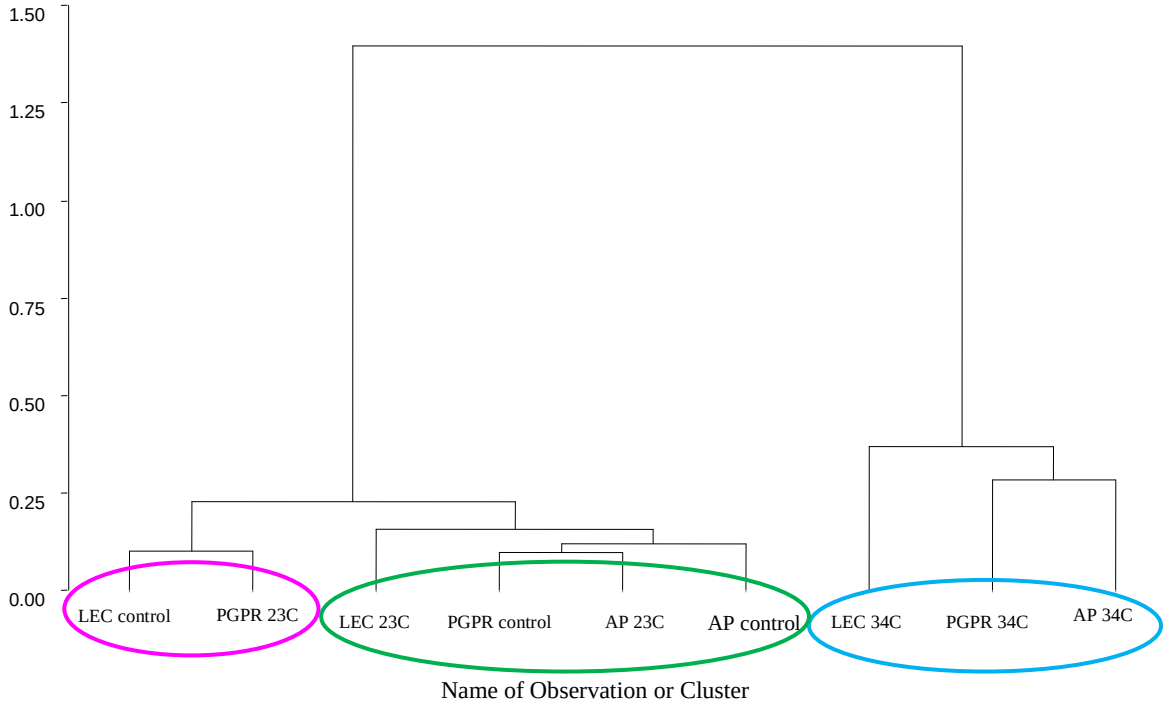


Figure 3.11 Principal component analysis (PCA) biplot for instrumental texture data

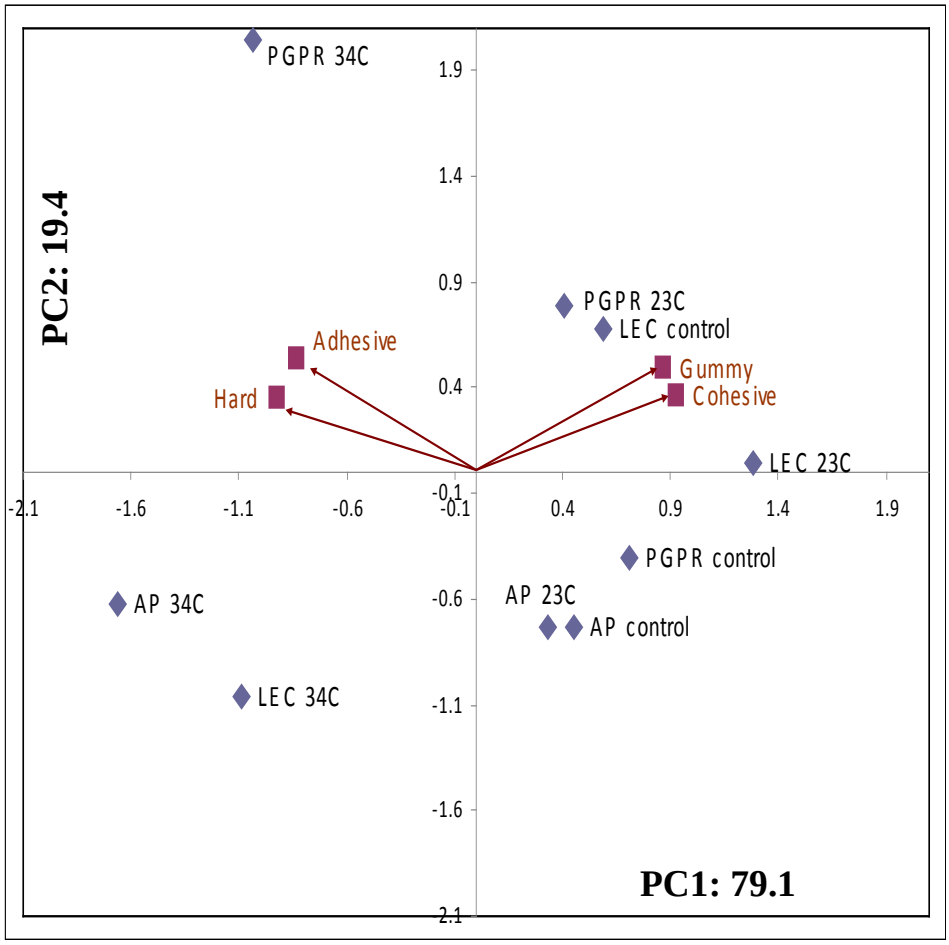


Figure 3.12 Principal component analysis (PCA) biplot for instrumental color, AFM roughness, DSC data

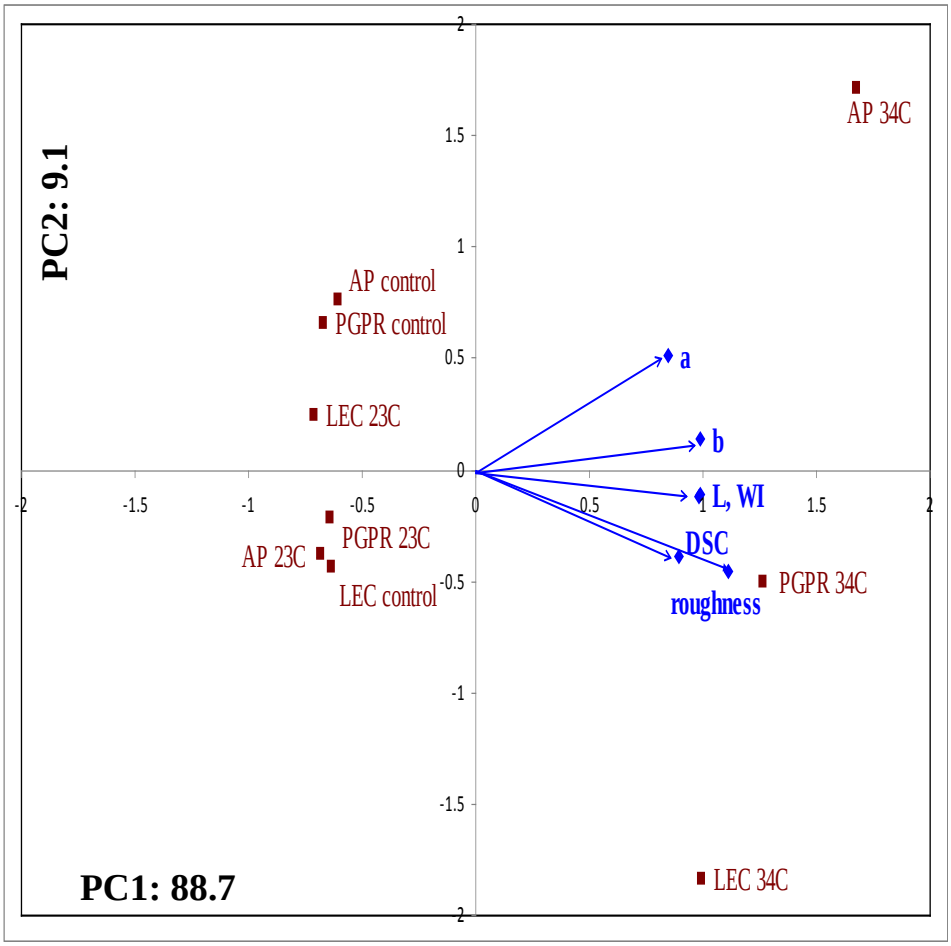


Figure 3.13 Partial Least Squares (PLS2) biplot for sensory versus instrumental results

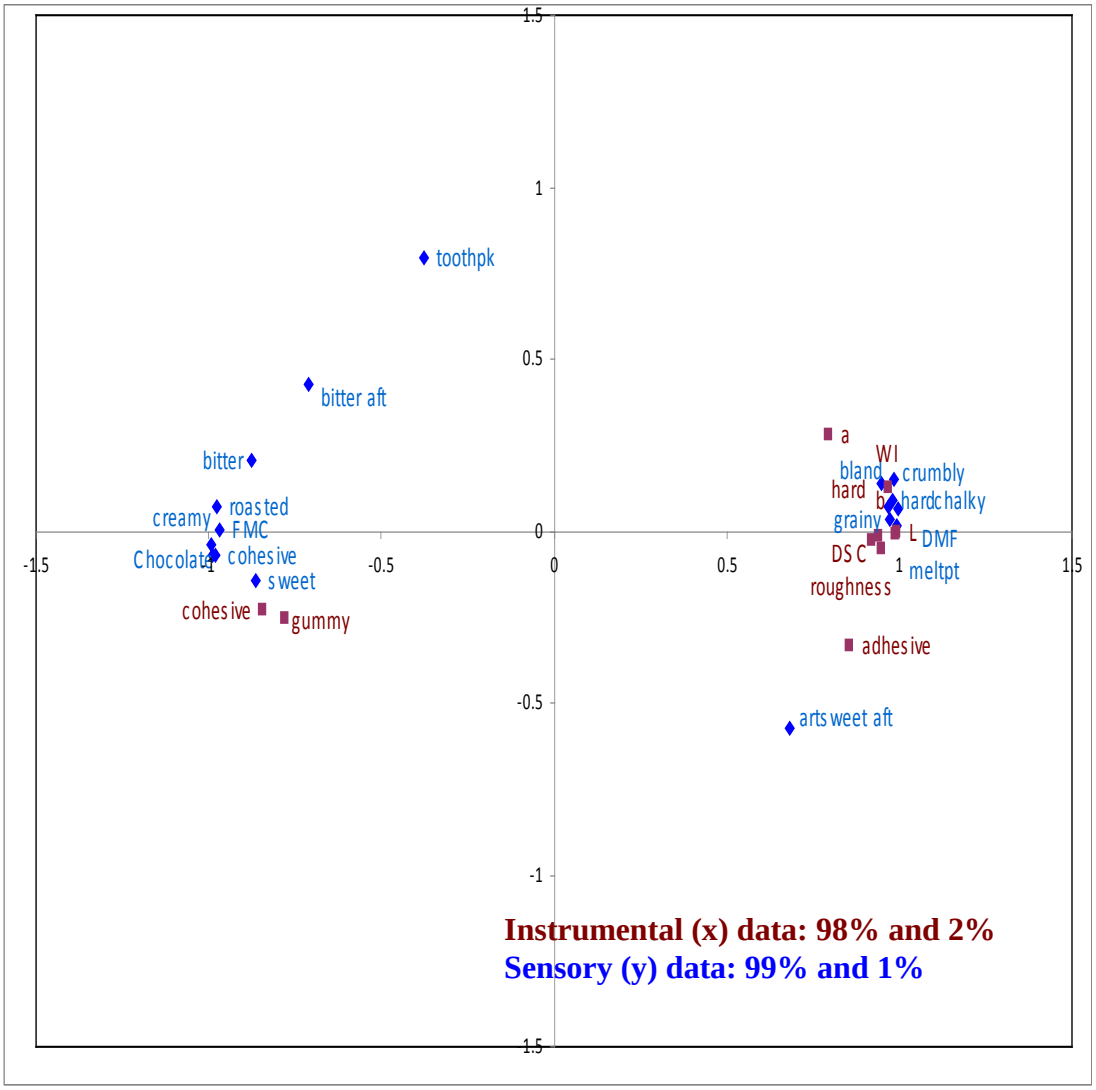


Figure 3.14 Partial Least Squares (PLS) biplot of sensory versus color data

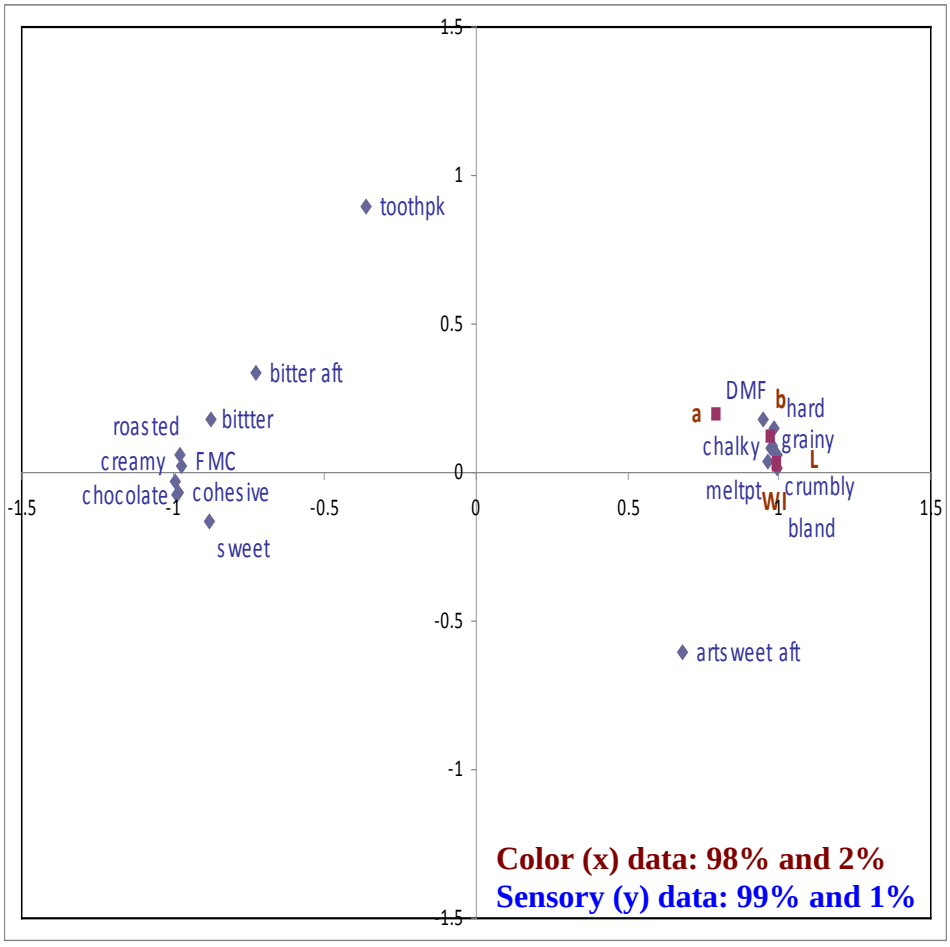


Figure 3.15 Partial Least Squares (PLS) biplot of sensory versus DSC melting point data

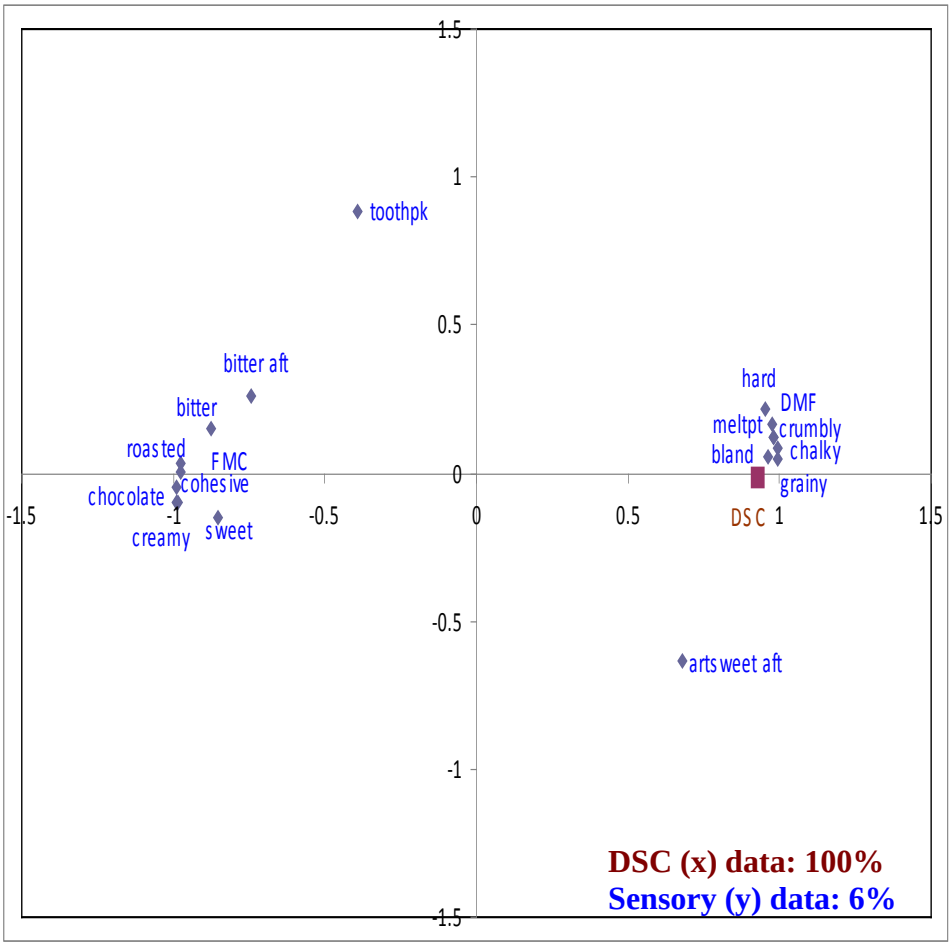
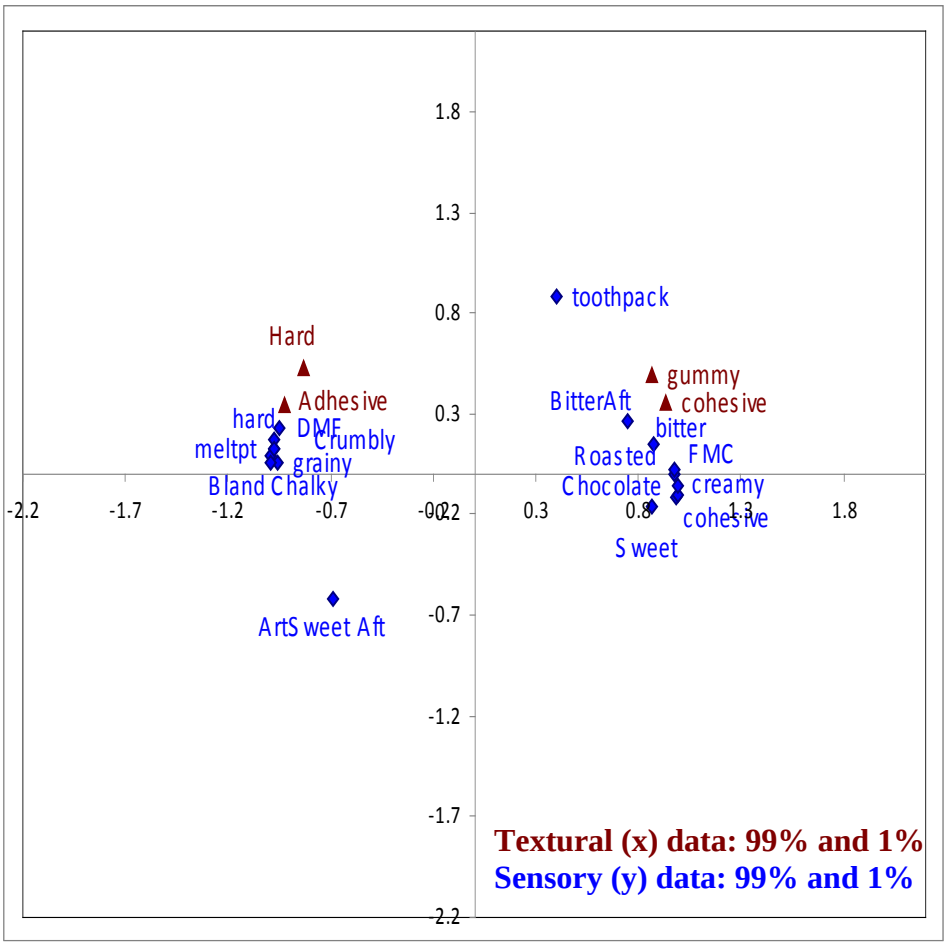


Figure 3.16 Partial Least Squares (PLS) biplot of sensory versus texture analyzer data



3.6 Tables

Table 3.1 Chocolate storage conditions and experimental definitions.

Condition	Sample Conditions Definition	Storage Temperature
Control	Freshly made samples (tested with in a few days)	unstored
Room temperature	Chocolate stored in an ambient temperature room for 8 weeks	23°C
High temperature	Chocolate stored in plastic containers in a water bath for 8 weeks	34°C
Emulsifiers Used		
PGPR	Polyglycerol Polyricinoleate	unstored, 23°C, 34°C
AP	Ammonium Phosphatide	unstored, 23°C, 34°C
Lecithin (LEC)	Soy lecithin	unstored, 23°C, 34°C

Table 3.2 Sensory attributes, definitions, and associated references generated for sample texture, mouthfeel, and time intensity melting.

Attribute	Definition	Reference
Hardness	*Initial fracture of a carrot*	Baby Carrot
Grainy	*Graininess of ground cinnamon*	Ground Cinnamon
Crumbly	*Initial crumbly feeling of fresh feta cheese after chewing 5 times*	Athens® Plain Feta Cheese
Cohesive	*Cohesiveness of whipped vanilla frosting*	Betty Crocker® Whipped Vanilla Frosting
Creamy	*Creaminess of chocolate pudding*	Kostas® Chocolate Pudding
Tooth Packing	*Tooth packing feel of animal crackers*	Barnahs® Animal Crackers
Fatty Mouth coating	*Fatty mouth coating of whipped cream and 2% milk (2:1 ratio)*	Deans® Whipped Cream and Deans® 2% milk Solution
Dry Mouthfeel	*Dry feeling of unsweetened cocoa powder*	Hershey® Cocoa Powder
Time Intensity Melting Point	*Time it takes for 0.25-0.3g sample to completely melt in mouth.*	Dark Chocolate Dove Promises®

Table 3.3 Sensory attributes, definitions, and references generated for sample flavor.

Attribute	Definition	Reference
Roasted	*Roasted flavor of unsalted almonds*	Natural Unsalted Almonds
Chocolate	*Chocolate flavor of unsweetened cocoa powder*	Hershey® Unsweetened Cocoa Powder
Bitter	*Bitter flavor of a 0.06% caffeine solution*	Caffeine Solution
Sweet	*Sweet flavor of a 5% sucrose solution*	Granulated Sugar Solution
Bland	*Bland flavor of an unsalted rice cracker*	Unsalted Rice Cracker
Chalky	*Initial chalky flavor of cornstarch powder after chewing 4 times*	Cornstarch
Artificial Sweet Aftertaste	*Artificial sweet aftertaste of a 0.2% Splenda® solution 5 sec after expectorating sample*	Splenda® Solution
Bitter Aftertaste	*Bitter aftertaste of a 0.06% caffeine solution 5 sec after expectorating sample*	Caffeine Solution

Table 3.4 Whiteness Index (WI) for all samples with 0.2% (w/w) emulsifier.[a,b]

Emulsifier	Storage condition	L	a	b	WI
Soy Lecithin	unstored	16.96±0.1	4.96±0.3	3.20±0.1	16.74±0.2[c]
	23°C	17.58±0.1	4.75±0.2	2.81±0.0	17.41±0.2[c]
	34°C	38.97±0.3	5.74±0.0	9.05±0.3	37.37±0.4[d]
Polyglycerol Polyricinoleate	unstored	15.75±0.2	5.33±0.0	3.74±0.2	15.49±0.3[c]
	23°C	16.77±0.2	4.88±0.0	2.98±0.2	16.57±0.3[c]
	34°C	45.79±0.4	6.33±0.2	12.24±0.3	40.07±0.3[d]
Ammonium Phosphatide	unstored	15.94±0.2	4.75±0.1	2.95±0.0	15.76±0.2[c]
	23°C	16.46±0.4	5.52±0.1	3.76±0.0	16.19±0.4[c]
	34°C	43.05±0.4	8.72±0.2	14.52±0.3	44.52±0.5[d]

[a]Results stated as mean±SD (n=3). [b]L: 100=white, 0=black; a:100=red, -100=green; b: 100=yellow, -100=blue; Whiteness Index (WI). [c,d]Numbers with same letters were not significantly different at p<0.05.

Table 3.5 Average texture data for samples with formulated with 0.2% (w/w) emulsifier.[a]

Sample	Hardness ($x10^3$)[b]	Cohesiveness ($x10^{-1}$)[b]	Adhesiveness[b]	Gumminess ($x10^3$)
Soy Lecithin				
unstored	17.65±1.3[c]	1.96±0.3[c]	10.50±1.1[c,d,e]	3.45±0.6[c]
23°C	16.10±1.5[c]	2.28±0.2[c]	3.30±1.8[c]	3.70±0.4[c]
34°C	20.25±1.7[d]	0.69±0.1[d]	13.20±1.4[d,e,f]	1.37±0.3[d]
Polyglycerol Polyricinoleate				
unstored	17.69±2.0[c]	1.79±0.5[c]	3.40±0.5[c]	3.17±0.6[c,d]
23°C	18.41±1.3[c,d]	1.85±0.4[c]	11.50±1.0[c,d,e]	3.40±0.5[c]
34°C	23.96±1.5[e]	1.03±0.2[c,e]	21.00±0.8[f]	2.39±0.5[c,d]
Ammonium Phosphatide				
unstored	17.37±0.7[c]	1.60±0.4[c,e]	5.40±0.6[c,d]	2.76±0.9[c,d]
23°C	17.59±0.4[c]	1.52±0.3[c,e]	6.20±0.6[c,d]	2.67±0.7[c,d]
34°C	21.79±1.4[d]	0.33±0.1[d]	15.7±0.3[e,f]	0.84±0.1[d]

[a]Results stated as mean±SD (n=2). [b]Storage conditions were significant at $p<0.05$. [c-f]Numbers with same letters in separate columns were not significantly different ($p<0.05$) for emulsifier type.

Table 3.6 Melting point averages, by Differential Scanning Calorimetry for all samples with 0.2% (w/w) emulsifier.[a]

Emulsifier	Storage condition	Melting Point (°C)
Soy Lecithin	unstored[g]	33.86±0.3[d,e]
	23°C[i]	33.13±0.4[e,f]
	34°C[k]	37.00±0.3[b]
Polyglycerol Polyricinoleate	unstored[h]	33.14±0.6[f]
	23°C[j]	33.79±0.4[d,e]
	34°C[l]	35.55±0.6[c]
Ammonium Phosphatide	unstored[g,h]	33.20±0.3[e,f]
	23°C[j]	33.89±0.5[d]
	34°C[l]	35.88±0.5[c]

[a]Results stated as mean±SD. [b-f]Numbers with same letters in columns were not significantly different at p<0.05. [g-l]Storage conditions with same letters in columns were not significantly different (p<0.05) for emulsifier type.

Tables 3.7 Polymorph *d*-spacing and polymorph state for each storage condition with 0.2% (w/w) emulsifier.

Sample, Storage Condition	2-theta	*d*-spacing (Å)	Polymorph
Soy Lecithin			
unstored	22.33, 23.05, 23.74, 24.28	3.96, 3.86, 3.74, 3.66	V
23°C, 8 weeks	22.16, 22.88, 23.55, 24.12	4.01, 3.88, 3.78, 3.69	V
34°C, 8 weeks	21.00, 22.87, 23.89	4.10, 3.89, 3.71	VI
Polyglycerol Polyricinoleate			
unstored	22, 27, 22.94, 23.68, 24.18	3.99, 3.87, 3.75, 3.68	V
23°C, 8 weeks	22.23, 22.9, 23.63, 24.04	3.99, 3.87, 3.75, 3.69	V
34°C, 8 weeks	21.99, 22.99, 24.03	4.04, 3.86, 3.72	VI
Ammonium Phosphatide			
unstored	22.34, 23.01, 23.68, 24.24	3.96, 3.86, 3.75, 3.67	V
23°C, 8 weeks	22.29, 23.00, 23.65, 24.23	3.98, 3.86, 3.76, 3.67	V
34°C, 8 weeks	21.87, 22.89, 23.89	4.05, 3.88, 3.71	VI

Table 3.8 Average descriptive panel texture ratings for all emulsifier formulations.

Sample	Hardness[a,b]	Grainy[a,b]	Crumbly[a,b]	Cohesive[a,b]	Creamy[a,b]	TPk[a]	FMC[a,b]	DMF[a,b]	T-I meltpt[b,c]
Lecithin control	7.96	3.94	5.84	6.76	7.19	6.11	6.36	4.17	50.91
Lecithin 23°C	8.48	4.12	6.23	6.05	6.71	6.37	5.91	4.43	53.93
Lecithin 34°C	10.09	6.38	8.79	3.73	3.48	6.17	4.88	6.94	71.61
PGPR control	8.41	4.04	6.12	6.63	6.99	6.67	6.74	4.75	51.13
PGPR 23°C	8.54	4.78	6.56	6.11	6.61	6.26	6.53	4.60	53.07
PGPR 34°C	10.33	6.58	8,72	3.58	3.47	6.19	4.83	7.44	74.13
AP control	7.87	4.25	6.09	6.56	6.70	6.17	6.36	4.48	48.35
AP 23°C	8.67	4.98	6.55	5.95	7.01	6.16	6.27	4.95	51.86
AP 34°C	10.01	7.02	8.76	3.55	3.29	6.19	4.46	7.58	69.82

[a]Results rated on a 15 point scale. [b]Storage treatments caused significant changes ($p<0.05$) in attributes. [c]Results measured in seconds. DMF=dry mouth feel; FMC=Fatty mouth coating; TPk=Tooth packing; T-I meltpt= time intensity melting point.

Table 3.9 Average descriptive panel flavor ratings for all emulsifier formulations[a].

Sample	Roasted[a,b]	Chocolate[a,b]	Chalky[a,b]	Bitter[a,b]	Sweet[a,b]	Bland[a,b]	Artificial Sweet Aftertaste[a]	Bitter Aftertaste[a]
Lecithin control	5.66	8.79	4.23	6.12	7.41	3.11	5.35	6.02
Lecithin 23°C	6.05	8.88	4.27	6.80	6.52	2.95	5.11	6.04
Lecithin 34°C	4.85	5.84	7.64	5.73	6.46	6.54	5.20	5.59
PGPR control	6.14	9.24	4.27	6.70	7.01	2.89	4.78	6.48
PGPR 23°C	6.20	8.99	4.16	6.61	7.36	3.10	5.25	6.27
PGPR 34°C	4.61	6.00	7.85	5.72	5.24	6.62	5.49	5.43
AP control	6.17	9.20	4.28	6.54	6.75	3.26	5.23	6.00
AP 23°C	5.98	9.00	4.46	7.05	7.06	2.94	5.19	6.46
AP 34°C	4.59	5.21	8.13	5.73	5.12	6.67	5.58	6.05

[a]Results rated on a 15 point scale. [b]Storage treatments caused significant changes ($p<0.05$) in attributes.

Table 3.10 Analysis of Variance (ANOVA) results for all sensory attributes.[a,b]

Attribute	Judge (J)	Treatment (T)	Replication (R)	J*T	J*R	T*R
Texture						
Hardness	**14.33***	**21.29***	**0.17**	**2.10****	**1.19**	**1.43**
Grainy	**44.47***	**26.92***	**8.34****	**1.52*****	**0.87**	**0.62**
Crumbly	**46.16***	**25.27***	**2.82**	**1.32**	**1.20**	**0.89**
Cohesive	**27.84***	**36.28***	**0.01**	**1.64****	**1.84**	**0.44**
Creamy	**14.38***	**54.48***	**0.16**	**1.76****	**1.02**	**0.71**
Tooth Packing	**35.87***	**2.87**	**2.18**	**1.42*****	**1.27**	**1.29**
Fatty Mouth Coating	**20.95***	**20.26***	**0.27**	**3.79***	**0.57**	**1.06**
Dry Mouthfeel	**38.36***	**39.81***	**9.30****	**3.84***	**0.63**	**2.19*****
T-I melting point	**20.85***	**71.49***	**3.69**	**1.48**	**8.14*****	**1.07**
Flavor						
Roasted	**15.33***	**8.66***	**0.25**	**2.07****	**1.75**	**0.49**
Chocolate	**19.52***	**101.10***	**0.61**	**4.55***	**2.61****	**1.61**
Bitter	**22.30***	**3.78****	**0.65**	**1.54*****	**1.84**	**1.38**
Sweet	**29.07***	**26.12***	**0.18**	**2.83***	**1.53**	**1.20**
Bland	**25.31***	**84.70***	**2.30**	**3.41***	**1.80**	**0.38**
Chalky	**25.58***	**56.45***	**7.17****	**2.58***	**1.79**	**0.58**
Artificial Sweet Aftertaste	**21.36***	**1.37**	**0.41**	**1.53**	**2.14**	**2.36**
Bitter Aftertaste	**15.55***	**1.37**	**1.77**	**1.10**	**4.23****	**0.28**

[a]F ratios used for variation results. [b]Significant at * (p<0.001), **(p<0.01), and *(p<0.05), respectively.**

Table 3.11 Principal component (PC) loading factors for sensory data.[a]

Attribute	PC1	PC2
Texture		
Hardness	**-0.95148**	0.21931
Grainy	**-0.96159**	0.05082
Crumbly	**-0.97954**	0.12119
Cohesive	**0.98428**	-0.10033
Creamy	**0.98908**	-0.09805
Tooth Packing	0.38901	**0.88215**
Fatty Mouth Coating	**0.97832**	0.00741
Dry Mouthfeel	**-0.9766**	0.16349
T-I Melting	**-0.97952**	0.12388
Flavor		
Roasted	**0.97536**	0.03573
Chocolate	**0.98958**	-0.04486
Bitter	**0.87474**	0.15304
Sweet	**0.86541**	-0.14339
Bland	**-0.99283**	0.04555
Chalky	**-0.99299**	0.08086
Artificial Sweet Aftertaste	-0.68287	-0.63477
Bitter Aftertaste	**0.74473**	0.26106

[a]Factors in **bold** are loaded high for each respective PC.

Table 3.12 Correlation matrix between sensory and instrumental data.[a]

	Hard	Grainy	Crumbly	Cohesive	Creamy	Toothpk	FMC	DMF	Roasted	Chocolate	Chalky
Hard	1.00										
Grainy	0.954*	1.00									
Crumbly	0.981*	0.980*	1.00								
Cohesive	-0.983*	-0.978*	-0.995*	1.00							
Creamy	-0.955*	-0.957*	-0.988*	0.984*	1.00						
Toothpk	-0.209	-0.377	-0.377	0.324	0.300	1.00					
FMC	-0.915**	-0.928	-0.944*	0.966*	0.961*	0.390	1.00				
DMF	0.971*	0.976*	0.984*	-0.981*	-0.979*	-0.253	-0.941**	1.00			
Roasted	-0.910**	-0.911**	-0.931**	0.937*	0.946*	0.394	0.949*	-0.945*	1.00		
Chocolate	-0.940**	-0.954*	-0.973*	0.974*	0.985*	0.345	0.975*	-0.971*	0.976*	1.00	
Chalky	0.954*	0.956*	0.981*	-0.980*	-0.989*	-0.319	-0.966*	0.988*	-0.976*	-0.993*	1.00
Bitter	-0.723**	-0.755**	-0.805**	0.789**	0.861**	0.396	0.817**	-0.802**	0.909*	0.883*	0.8712**
Sweet	-0.812**	-0.825**	-0.819**	0.852**	0.855**	0.162	0.889**	-0.878**	0.828**	0.843**	-0.862**
Bland	0.943*	0.950*	0.979*	-0.974*	-0.995*	-0.345	-0.957*	0.978*	-0.968*	-0.988*	0.995*

[a]Results significant at, *p<0.01, **p<0.001, ***p<0.05. DMF=dry mouth feel; FMC=Fatty mouth coating; ToothPk=Tooth packing.

Table 3.12 (continued): Correlation matrix between sensory and instrumental data[a].

	Bitter	Sweet	Bland	ASA	BA	T-I MP	L	a
Hard				0.504	-0.639	0.982*	0.951*	0.667***
Grainy				0.659***	-0.575	0.937	0.945*	0.779**
Crumbly				0.577	-0.672***	0.980*	0.971*	0.731***
Cohesive				-0.607	0.684***	-0.982*	-0.973*	-0.737***
Creamy				-0.599	0.726**	-0.978*	-0.985*	-0.775**
Toothpk				-0.792***	0.464	-0.272	-0.323	-0.137
FMC				-0.679***	0.713***	-0.945*	-0.957*	-0.787**
DMF				0.581	-0.627	0.966*	0.979*	0.799**
Roasted				-0.686***	0.726***	-0.952*	-0.977*	-0.746***
Chocolate				-0.648	0.685***	-0.967*	-0.981*	-0.802**
Chalky				0.619	-0.701***	0.975*	0.992*	0.789**
Bitter	1.00			-0.655	0.791**	-0.841**	-0.887**	-0.663
Sweet	0.667***	1.00		-0.608	0.582	-0.825**	-0.877**	-0.859**
Bland	-0.896**	-0.841**	1.00	0.626	-0.747***	0.975*	0.991*	0.765**

[a]Results significant at, *p<0.01, **p<0.001, ***p<0.05. DMF=dry mouth feel; FMC=Fatty mouth coating; ASA=Artificial aftertaste; BA= Bitter aftertaste; ToothPk=Tooth packing; T-I MP= time intensity melting point.

Table 3.12 (continued): Correlation matrix between sensory and instrumental data[a].

	b	WI	DSC	t.hard[b]	t.cohesive[b]	t.adhesive[b]	t.gummy[b]
Hard	0.902*	0.952*	0.899**	0.896**	-0.755**	0.800**	-0.703***
Grainy	0.936**	0.942*	0.904*	0.919*	0.874**	0.824**	-0.811**
Crumbly	0.932**	0.969*	0.939**	0.907**	-0.841**	0.815**	-0.795**
Cohesive	0.937*	-0.972*	-0.919**	-0.902**	0.812**	-0.807**	0.765**
Creamy	-0.955*	-0.983*	-0.922**	-0.913**	0.832**	-0.814**	0.783**
Toothpk	-0.245	-0.324	-0.429	-0.293	0.337	-0.482	0.334
FMC	-0.939*	-0.954*	-0.879**	-0.854**	0.793	-0.753**	0.753**
DMF	0.970*	0.976*	0.893**	0.944*	-0.849**	0.812**	-0.787**
Roasted	-0.940*	-0.976*	-0.907**	-0.918**	0.784**	-0.858**	0.721***
Chocolate	-0.961*	-0.978*	-0.934*	-0.906*	0.853*	-0.817**	0.799**
Chalky	0.968*	0.989*	0.926**	0.928**	-0.848**	0.823**	-0.795**
Bitter	-0.833**	-0.887**	-0.841***	-0.830***	0.690***	-0.846**	0.624
Sweet	-0.934**	-0.874***	-0.618	-0.859***	0.650	-0.664	0.571
Bland	0.955*	0.990*	0.934*	0.923**	-0.837***	0.838**	-0.787**

[a]Results significant at, *p<0.01, **p<0.001, ***p<0.05. [b]Textural instrument data. DMF=dry mouth feel; FMC=Fatty mouth coating; ToothPk=Tooth packing.

Table 3.12 (continued): Correlation matrix between sensory and instrumental data[a].

	t.hard[b]	t.cohesive[b]	t.adhesive[b]	t.gummy[b]	ASA	BA	T-I MP	L	a	b	WI	DSC
t.hard[b]	1.00											
t.cohesive[b]	-0.735**	1.00										
t.adhesive[b]	0.925*	-0.586	1.00									
t.gummy[b]	-0.621	0.979*	-0.465	1.00								
ASA	0.692***	-0.507	0.779**	-0.402	1.00							
BA	-0.630	0.351	-0.698***	0.345	-0.539	1.00						
TI MP	0.908*	-.741***	0.841**	0.688***	0.567	0.751**	1.00					
L	0.952*	-0.784**	0.868**	-0.717***	0.650	0.751**	0.982*	1.00				
a	0.779**	-0.814**	0.571	0.722***	0.598	-0.297	0.676**	0.766**	1.00			
b	0.950*	-0.829**	0.804**	-0.744***	0.657	-0.605	0.919*	0.967*	.901*	1.00		
WI	0.952*	-0.774**	0.873**	-0.71***	0.648	-0.760**	0.983*	0.998*	.754**	0.962*	1.00	
DSC	0.791**	-0.849**	0.774**	-0.836**	0.542	-0.677*	0.919*	0.898*	0.593	0.815**	0.897*	1.00

[a]Results significant at, *p<0.01, **p<0.001, ***p<0.05. [b]Textural instrument data. DMF=dry mouth feel; FMC=Fatty mouth coating; ASA=Artificial aftertaste; BA= Bitter aftertaste; ToothPk=Tooth packing; T-I MP= time intensity melting point.

Table 3.13 Parameter estimates for regression equations for sensory and instrumental analyses.

Variable	β_0	x_1	x_2	x_1^2	x_2^2	x_1x_2	Adj R^2
Hardness	7.30	0.96	-0.113	-0.25	0.006	0.002	0.96
Grainy	3.66	0.25	-0.16	-0.02	0.007	0.007	0.98
Crumbly	5.42	0.51	-0.18	-0.09	0.009	-0.003	0.99
Cohesive	6.81	-0.07	0.17	-0.007	-0.009	0.0007	0.99
Creamy	7.49	-0.36	0.28	0.04	-0.013	0.008	0.98
Toothpk	5.7	0.724	0.07	-0.18	-0.0002	-0.0018	**0.58**
FMC	5.3	1.36	0.13	-0.327	-0.006	-0.004	0.92
DMF	3.59	0.84	-0.24	-0.17	0.01	0.005	0.96
Roasted	5.15	0.65	0.16	-0.1	-0.006	-0.012	0.97
Chocolate	7.73	1.27	0.32	-0.26	-0.01	-0.02	0.98
Bitter	5.96	0.28	0.14	-0.02	-0.005	-0.006	**0.80**
Sweet	7.5	-0.26	0.13	0.017	-0.005	-0.002	**0.77**
Bland	3.1	-0.103	-0.34	0.04	0.015	-0.001	0.99
Chalky	4.67	-0.41	-0.35	0.09	0.01	0.008	0.99
ArtSweet Aft	5.6	-0.49	-0.03	0.1	0.0009	0.007	**0.59**
BitterAft	6.06	0.133	0.04	-0.034	-0.002	0.008	**0.75**
T-I Melting	47.04	5.50	-1.56	-1.70	0.07	0.012	0.98
DSC	34.89	-1.52	-0.22	0.33	0.01	0.001	0.91
L	14.09	4.22	-2.55	-1.27	0.11	0.07	0.97
a	5.58	-0.70	-0.26	0.22	0.009	0.02	**0.80**
b	2.29	1.21	-0.96	-0.28	0.036	0.05	0.95
WI	13.48	4.65	-2.37	-1.37	0.10	0.06	0.96
t.hardness	13.02	5.72	-0.65	-1.48	0.02	0.05	0.92
t.cohesiveness	1.27	0.91	0.11	-0.28	-0.005	-0.002	**0.87**
t.adhesiveness	1.11	9.91	-1.13	-3.07	0.04	0.038	**0.82**
t.gumminess	2.01	1.92	0.127	-0.58	-0.01	-0.007	**0.83**

Chapter 4: Educational program designed to increase exposure of high school students to food science through the study of chocolate

To maintain global competitiveness, the National Academy of Science emphasized US enhancement of education in K-12 science and engineering. This was addressed by extending research on chocolate lipid polymorphism to creation of educational workshops for high school students. The goal is to attract bright science-minded students to food science through study of chocolate. Chocolate has widespread appeal and is promoted for positive impacts on human health; this together with exciting technologies existing to analyze its properties, makes a great subject for a summer educational program.

The Department of Food Science and Human Nutrition at the University of Illinois hosted a 2 ½ week chocolate educational program for six high school students (June 2009). The program incorporated experiential learning, professional guest speakers, and classroom lectures focused on many facets of food science, with the overall theme of chocolate. Capstone concentrations taught during the program included microbiology, chemistry, safety, processing, product development, sensory science, nanotechnology, and nutrition. Students refined team building skills, public speaking, conducted a mock human intervention study, and learned how to use scientific techniques to solve applied problems in food science.

Surveys were administered to participating students in order to evaluate individual knowledge about the field of food science prior to and at completion of the program. The program increased overall knowledge about food science and increased overall interest in food science career options. Students indicated that the most beneficial elements of the program were laboratory work, discussions after experiments, and exploring other current food science research at the University. Success of this experiential learning program will serve as a model for creative efforts of recruitment to fields that need more visibility at the high school level. Through this program development we are also able to test strategies for teachers to use in the classroom for educating scientific principles of food.

4.1 Introduction

It is well established that learning through experience is a motivational force for students (Sadler, 2009). The idea that students learn from hands-on experiences applicable to everyday situations is an excellent approach that should be recognized by teachers across the nation. It is a concern that science education delivered in highly structured classroom environments will become limited to those formal settings and wield a constrained influence on students' experiences and thinking (Greeno, et al., 1993; Cobb, 1994). Bauer and Bennett (2003) explained the use of experiential learning techniques in undergraduate education as being highly influential in the continuation of the student's education into graduate programs. Reforms in science education stress the importance of pre-college students developing an understanding of the nature of science and of the scientific inquiry process (AAAS, 1989; NRC, 1996). Educators have been encouraged to provide students with opportunities to experience science through in-class science projects or extracurricular work with scientists such as camps, summer programs, or apprenticeships (Bell et al., 2003). Specifically, apprentice learning experiences, where a student works with an 'expert' mentor in realistic contexts, offer a different model for education especially as compared to traditional classrooms. Apprenticeship is to learn a trade and expertise through direct participation under guidance (Barab and Hay, 2001). Recently, there has been a surge of experiential interactive based programs as a previously limited method for learning and for recruitment of younger students into scientific fields (Bell, 2003).

At the University of Illinois, there have been many successful secondary educational programs implemented to introduce bright, young science-minded students to possibilities of science careers and majors. One program that has received credible notice is the Nanoscale Chemical-Electrical-Mechanical Manufacturing Systems (Nano-CEMMS) workshops. These workshops have been documented to be an outstanding success in the attraction of high school students into the field of nanotechnology by usage of an interesting approach with computer-assisted educational efforts (Destefano et al., 2004/05). Another program is the Research Apprentice Program (RAP; summerprograms.aces.illinois.edu/rap), that focuses on integrating underrepresented high

school students into the food, human, and environmental sciences through an intensive seven week laboratory and academic summer program. The RAP program helps build skills in areas of math, science and communication, utilizing computers and technology to enhance critical thinking abilities of participants.

One residential program conducted at the University for 8th and 9th graders is the Girls' Adventures in Mathematics, Engineering, and Science (G.A.M.E.S.) Camp (wiki.engr.illinois.edu/display/games) G.A.M.E.S. is conducted through the College of Engineering and is focused on giving academically talented young women an opportunity to explore math, engineering, and science through demonstrations, classroom presentations, hands-on activities, and direct contact with female professionals from technical fields. All of these programs focus on a balance between lecture based and interactive learning as a method to attract students into the fields of science and show them the endless opportunities available.

It is important to expose more students with interest in basic sciences into fields of agricultural importance that are widely applicable, such as food science. It is generally recognized that food science does not have great exposure in K-12 education. Thus, food scientists are in a perfect position to investigate the development of new tools and recruitment through educational programming. Through exposure to food science, young students will directly gain insight to relevant research applications and technologies, such as the rapidly expanding field of nanotechnology. They will also be encouraged into solving critical problems in food systems and to using creative strategies to develop novel food products. In order for this to occur it is necessary to assure that there will be a consistent supply of well-trained students who seek to pursue advanced degrees in the food sciences. It is our goal to utilize creative approaches to awaken bright, young scientists to the possibilities of applying basic scientific principles to research foods that are nutritious, complex, creative, and appealing. This was accomplished through the development of the Food Science Chocolate Education Program that exposed students to all disciplines of food science through the study of chocolate. This program was offered to high school students with science minded interests and did not discriminate against race or gender. This educational effort follows the idea of experimental learning, through hand-on lessons and interactive lectures and essentially allowed students, in a short

period of time, to experience the integration of food science with state of art technology to a fun and interesting food product, chocolate. This program and other future educational efforts like these will promote understanding of scientific ideals and principles, foster intellectual development and inspire young scientists to pursue opportunities available in food science.

4.2 Materials and Methods

4.2.1 Experimental Design

Our laboratory has conducted pioneering research on the relationship of instrumental analysis and sensory evaluation of the impact of chocolate storage on lipid polymorphism and how alterations in cocoa butter polymorphs affect flavor and textural attributes (Andrae, 2006; Engeseth and Nightingale, 2007; Andrae-Nightingale et al., 2009). Our research led to incorporation of a chocolate theme into summer workshops on nanotechnology, coordinated through the University of Illinois Nano-CEMMS. A previous graduate student in our laboratory, Dr. Lia Andrae-Nightingale, interacted with approximately 300 students from middle and high schools over a series of summer workshops (Summer, 2006). This series of workshops was followed by an evaluative tool (Destefano et al., 2004/05) indicating that the chocolate themed portion of the workshops was favored by many. A USDA CSREES NRI Competitive Integrated Grant was obtained to continue our research on lipid polymorphism during chocolate storage and build into this a Summer Chocolate Education Program, designed to bring 6 bright, science-minded high school students to campus for approximately 3 weeks to learn all about food science through the study of chocolate. Students would be introduced to the University of Illinois, College of Agricultural, Consumer and Environmental Sciences (ACES), Department of Food Science and Human Nutrition, and the field of food science through the study of something they all identified with, chocolate. Participants would learn fundamental aspects of team work, how to conduct scientific experiments, how to analyze data collected in research, and how to translate basic science knowledge to solving problems and answering questions in food and agricultural sciences.

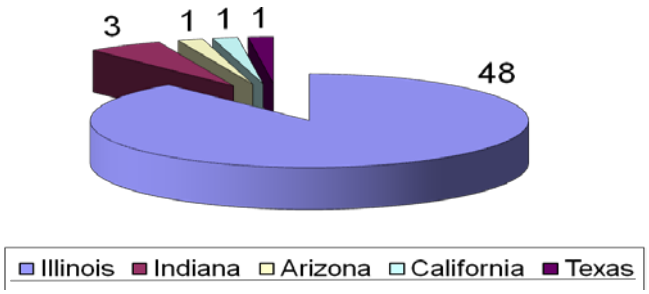
4.2.2 Recruitment

Students were recruited for the 2009 Food Science Chocolate Education Program using various forms of communication. Letters with brochures and applications (**Appendix C.1**) were sent to several high schools across the State of Illinois and a webpage (www.chocolate.illinois.edu) was later created with information for recruitment to the program. Additionally, Phyllis Picklesimer, Media Communication Specialist, College of ACES, issued a press release to publicize the program which was picked up by several newspapers around the country (**Appendix C.2**). An application form was developed that could be sent along with these materials (**Appendix C.3**).

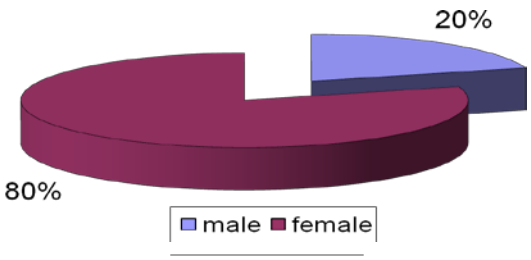
High school students with strong science backgrounds/interests were encouraged to apply. Submission of application included two letters of reference from high school science teachers and/or guidance counselors, transcripts, a resume, and a personal statement. After a two month recruitment period, a total of 54 students applied from across the country (**Figure 4.1**).

Figure 4.1: Demographic regional data (a), and gender data (b) of applicants.

(a)



(b)



4.2.3 Selection

In order to select 6 students from this pool of applicants, a selection committee was established which consisted of several graduate students, an undergraduate student, a postdoctoral fellow, and the Principal Investigator, Dr. Nicki Engeseth. Each committee member was familiar with the goals of the project and the grant that established the funding for this project. Most have been actively involved in various aspects of creating educational materials for this program. The major qualification was for the applicant to be a high school student with an intense interest in science. Students were selected on the basis of the application and chosen based on academic performance, creativity, exposure to and aptitude for high school science courses and involvement in meaningful extracurricular activities. Once the 6 students were selected they were notified via formal email letters and asked to respond by email as to whether or not they accepted the position. Alternates were identified in case someone did not accept.

4.2.4 Program Details

Consent and assent forms were distributed to parents and students, respectively, ahead of their visit (**Appendix C.4 & C.5**), giving them time to read them and ask questions prior to signing. As stated in the rules for human subject (particularly children) research, participants were notified that the program was voluntary and that they could withdraw at any time. All protocols were approved by the University of Illinois Institutional Review Board (IRB).

The program included support and collaboration from many individuals, faculty, coordinators, and industry professionals. Two graduate student resident assistants were hired to mentor, assist, and live in the residence halls along with the participants for the duration of the program. Students were housed in University of Illinois Housing (Illinois Street Residence Hall) and provided resident meals for the duration of the program. Student's daily activities were held at Bevier Hall Monday-Friday; while weekend activities took place in the Champaign-Urbana community and surrounding areas. The program was coordinated by Melissa Tisoncik, a MS student, and overseen by Dr. Nicki Engeseth. Throughout the program, various guest speakers from the University, alumni, business and food industry representatives presented lectures on food science related topics (**Table 4.1**). The program was held from June 15th-July 2nd, 2009.

Table 4.1: Guest lectures, topics, educational trips conducted throughout the program.

Guest Speaker	Topic	Concentration
Neil Widlak, MS *Director, Strategic Technical Development, ADM Cocoa; Milwaukee, WI*	Industrial chocolate processing and production	Food Processing
Kelly Van Haren, MS *Scientist/Engineer, Proctor & Gamble; Cincinnati, OH*	Product development in an industrial company	Product Development
Scott MacLaren, Ph.D. *Research Scientist University of Illinois, Center for Microanalysis of Materials*	Applications of nanotechnology	Nanotechnology
Deborah Black *Research Specialist in Life Science,University of Illinois*	Plant species at the conservatory	Plant Science
Crystal Goshorn, MS *University of Illinois Graduate Student*	SOYFACE research	Food Chemistry
Aaron Rasmussen *University of Illinois Graduate Student*	Basics concepts of product development	Product Development
Professional Representative *Eli's Cheesecake; Chicago, IL*	Chocolate use in an industrial corporation	Industry and Business
Leslie Cooperband, Ph.D. *Prairie Fruits Farm Owner*	Local foods/processing and production	Food Processing/Local Foods
Hun Kim, Ph.D. *University of Illinois Post Doctoral Research Associate*	Use of Gas Chromatography-Olfactometry to detect flavor compounds	Flavor Chemistry
Terri Cummings *Director of Student Services, Dept. Food Science and Human Nutrition*	Admission and applying to FSHN/undergraduate options	UIUC opportunities
Dr. Andrea Bohn *Assistant Dean Academic Programs, College of ACES, Study Abroad Coordinator*	College of ACES study abroad programs	UIUC opportunities

4.3 Lessons

Lessons presented were designed to introduce students to capstone concentrations in food science through the study of chocolate. These concentrations included: food

chemistry, food microbiology, food processing, product development, sensory science, nanotechnology, and nutrition. These lessons give an introduction to above concentrations and involve key concepts associated with these food science topics. The lessons were presented using hands-on learning, laboratory experiments, accompanying handouts, and lectures associated with each topic. Specific highlights from program lessons are presented below:

4.3.1 Lesson 1: Human Health Intervention Study (Nutrition)

We covered the importance of chocolate used in ongoing research studies due to its potential health benefits. This lesson allowed students to experience the processes involved in conducting a human study and learn how to apply the scientific method, collect and evaluate data, and present research in proper presentation format. Students were initially asked to find health claims in the popular press about chocolate in order to understand the potential health benefits of chocolate. This task helped students sort out claims made by popular press and helped demonstrate the importance of research in substantiating health claims; specifically, most of the focus was on antioxidants and their role in prevention of heart disease. A lecture was given on the potential health benefits of chocolate and the topic of human intervention studies was also introduced. Two previous human health intervention studies conducted on chocolate consumption and its effect on blood pressure were used as references for the students to model their experimental design (Taubert et al., 2003; Grassi, et al., 2005). Students were introduced to IRB protocols, were expected to formulate a hypothesis and design an experiment to feed dark chocolate to 6 human subjects and monitor blood pressure over a 10 day period, using white chocolate as a control. At the end of the study, students presented their findings in groups with a formal poster presentation.

4.3.2 Lesson 2: Product Development

Students learned basic concepts of product development and the steps involved in creation, formulation, and successful marketing of a new product in both culinary and industrial settings (**Figure 4.2**). This was a two part lesson conducted over two days. The first day involved preparation for both the “Iron Chef” contest as well as lectures

introducing students to the product development concentration in food science. Lectures covered both academic and industrial elements of product development. The activity associated with the lectures was a "drawing board" step of development, allowing students to create ideas for a new chocolate product with a partner. Their task was to create a design that included: a creative slogan, expected target market, benefits of product, an illustration, and development steps specific to their product. Students presented their product idea and were asked a series of questions pertaining to development issues. This helped students focus on the planning and collaboration needed to create a chocolate product in both academic and industrial settings.

The second part of this lesson was conducted in an experimental teaching kitchen in Bevier Hall. We conducted an "Iron Chef" product development contest. Paired students were required to design a culinary dessert, based upon a recipe selected beforehand; followed by judging on creativity, taste, appearance, team work, and the ability to incorporate unique ingredients and two types of chocolate. In this lesson, students refined basic kitchen skills, while gaining an understanding of the importance of following a recipe, team collaboration, and timing associated with preparation. Students were also asked to be creative in combining flavors. Scores and critiques were given based upon three judges' response to each team's efforts. This part of the lesson was essential in teaching students hands on techniques and the importance for a food scientist to be familiar with food not only in research, but also in a kitchen setting. The friendly competitive aspect was fun and a motivational force for the students as well.

Figure 4.2: Pictures capturing moments during the "Iron Chef" product development contest.

4.3.3 Lesson 3: Mock Descriptive Analysis Panel (Sensory Science)

Students participated in a "mock" sensory descriptive analysis panel, in which they learned the essential aspects of sensory studies and generated terminology for evaluation of chocolate. Students learned elements of planning, organizing, and implementing a sensory descriptive analysis panel. It was an open discussion panel set up similarly to a training session, where students were led through a descriptive analysis panel as the participating "subjects" tasting chocolate samples. They were taught the importance of initial IRB approval, rinsing protocols, generating terminology, and reference matching for evaluating attributes. They rated chocolate samples using an example 15 cm line scale (**Appendix C.6**) and matched the intensity of each attribute to the appropriate reference presented (**Table 4.2**). This lesson helped students understand the methods used to conduct a descriptive analysis sensory study, as well as understand the purpose and importance of sensory science in both an academic and industrial setting.

Table 4.2: Example of texture and flavor attributes generated and associated references used during mock descriptive analysis panel (adapted from Andrae, 2006).

Attribute Generated	References Selected/Utilized
Hardness	Carrot, Parmesan Cheese
Fracture/Cohesive	Parmesan, Mozzarella Cheese
Chewiness	Rice Cake, Mozzarella Cheese
Fatty Mouth Coating	Sweetened Condensed Milk
Dry Mouthfeel	Tannin solution
Toothpacking	Animal Crackers
Bitterness	Caffeine Solution
Sweetness	Sugar Solution
Chocolate	Cocoa Powder
Cream	2% Milk
Roasted	Light Roasted Coffee

4.3.4 Lesson 4: Lipid Extraction Laboratory (Food Chemistry)

Students learned basic lipid food chemistry and associated laboratory techniques related to extraction of lipids and basic principles of lipid behavior in chocolate. This

lesson was modeled after a teaching lab from the IFT Educational Food Chemistry website (Watkins, 2009). Students were required to observe and report their findings after extracting lipids from various lipid-containing food substances in this independent experiment. Students refined their basic laboratory skills. Students were challenged to follow standard laboratory protocols and think critically about the approach and the scientific principles involved. Discussion following revisited the basics of lipid chemistry in chocolate, in addition to information about unsaturated, saturated, and trans fats in foods.

4.3.5 Lesson 5: Formulating, Making, and Molding Chocolate (Food Science Research)

Students were introduced to graduate level food science research and instrumental techniques used to analyze data. Specifically, students gained knowledge on lipid polymorphism, the importance of emulsifiers in chocolate, their role associated with fat bloom formation, formulation and preparation of chocolate on a laboratory scale, and impact of storage on chocolate quality. Students were taught principles behind formulating and preparing chocolate. As a large group, students learned how to mathematically manipulate formulations, measure/prepare ingredients, conche/refine, temper, and finally mold chocolate properly (**Figure 4.3**). Students learned the chemistry of emulsifiers and their role in chocolate production. Students were introduced to bloom formation (fat and sugar), how it occurs and the proper methods of processing, handling and storage to avoid chocolate bloom. Students were introduced to various instrumental techniques used to analyze chocolate samples; such as atomic force microscopy (AFM), texture analyzer, colorimetry, powder x-ray diffraction (XRD), gas chromatography, and oxygen radical absorbance capacity (ORAC) was discussed.

Figure 4.3: Pictures capturing moments during making and molding chocolate.

4.4 Results

Primary assessment instruments used to evaluate this program were anonymous confidential questionnaires administered to students. Pre- and post-program questionnaires (**Appendix C.7 & C.8**) approved by the University of Illinois IRB were used to determine the level of knowledge and interest gained about the field of food science. They also served as a method to evaluate the program to make improvements for the following program in 2010. Questionnaires consisted of open-ended questions in order to probe student thinking and elicit responses that would provide details about views on food science. In the pre-program questionnaire, students were asked to answer questions concerning their initial interest in the program, their fears and anticipations for the program, and their previous science background. They were also asked science-related questions about food science and chocolate. After the program concluded, students were asked to complete a post-questionnaire answering questions about their overall experience, likes/dislikes of the program, suggestions for improvement, level of interest in food science, and evaluation of lectures. Students were again asked questions pertaining to basic scientific principles about chocolate, ideas learned throughout the program, and food science in general.

4.4.1 Evaluation of Program

4.4.1.1 Pre-Program Questionnaire

This questionnaire focused on issues prior to starting the program. Student participants initially heard about the workshop from the following forms of communication: the internet, local newsletters (e.g., 4-H, ACES, Daily Illini and Rockford Star) and current high school science teachers. Students were interested first in the chocolate theme and then food science studies. They indicated that the most appealing aspects of the program were planned trips, tours, and taste testing of chocolate; the least being the lectures. Students stated they were excited about living on campus in University housing. They explained the skills previously learned in their high school science classes (lab safety skills, basic chemistry) would be most useful to them during the program. Some fears that students anticipated were being in a foreign environment, laboratory write ups, and staying focused throughout the program. The student's excitement for learning about chocolate, obtaining lab experience, gaining knowledge about the University and food science as a career outweighed the fears prior to the program start. Previous courses included high school level biology and chemistry; some also had taken physics, organic chemistry, and anatomy. Students enjoyed the laboratory work and challenges associated with these science courses. Many had never attended a similar type of educational program previously. Many were still undecided about their college major, but were curious about science-related disciplines at the University of Illinois. They were all talented, bright students involved in many extracurricular activities.

Prior Chocolate Food Science Knowledge

Students were asked basic food science questions related to chocolate to gauge previous knowledge. Students were also asked to define food science in their own words. Most answers were vague explaining food science was the study of "food and its properties…chemistry of edibles…what can be consumed for survival". Student's knowledge on chocolate food science related questions was similarly vague. Students were not familiar with the origins of the cacao bean and the prime export countries. Most students understood chocolate contained antioxidants, but did not know why antioxidants were beneficial. Students could only differentiate between chocolate (white, milk, dark)

by taste and color. Students were unable to estimate caffeine concentrations in chocolate, explain fat bloom formation, or describe the purpose of an emulsifier in chocolate. Overall, students had little insight to the chemistry of chocolate and were unaware of the potential benefits of chocolate consumption.

4.4.1.2 Post-Program Questionnaire

At the end of the program, students were asked to evaluate their overall experience of the program. **Tables 4.3 & 4.4** summarize the lessons employed throughout the program and feedback associated with each lesson. In general, students believed the program was "fun and enjoyable." The program taught students basic food science principles and gave an insight to college living at the University. It was noted that "there was a good balance between lectures and fun activities". The most cited enjoyable portion of the program was the laboratory work and interactive experiments. Some notable aspects highlighted were the educational trips, the 'mock' health intervention study, food product development activities and lectures on the health benefits of chocolate. The program incorporated all aspects of food science and most lectures were well received. Students recorded specific concentrations of food science that were most appealing to them after the program such as: food chemistry, nutrition, food processing, sensory science, and nanotechnology. Students were most engaged in hands-on experiments, and interested to hear about future college opportunities.

Many activities had lectures associated with them. **Table 4.5** categorizes the lectures into three main groups. Some lectures were less significant than others, others needed further development, and others were highly influential. Laboratory work was viewed most beneficial for students, but it was recorded that "there should have been more of a discussion after the labs." Student's interest in the field of food science did increase and "opened [my] eyes to the options a food science career can bring". In general, the program gave a good overview of the food science field. Many students benefited to working both in groups (Iron Chef; Human Intervention Study) and as individuals (Food Chemistry labs; 3-slide presentations). The most notable challenging aspect for students was group dynamics and presenting in front of a group.

Table 4.3: Summary of basic food science principles taught, methods for teaching, and associated feedback from students.

Food Science Concentration	Basic Principles Taught	Method for Teaching	Response From Students[a]
Food Chemistry	Lipid chemistry, analytical laboratory techniques	**Lab:** Extracting lipids from foods	(±) "Loved the hands on experiment and working alone". Needs more of a lab discussion and models to describe fats
Food Safety	Food borne outbreaks and illness	**Lecture**	(-) Misunderstood. Lecture was not detailed and unrelated.
Food Processing	From bean to bar: chocolate production	**Guest Speaker Lecture**	(±) Presentation style was long, but extremely informational
Product Development	Basic academic and industrial product development principles	**Activity:** Designing own products **Guest Speaker Lectures**	(+) Fun and a creative outlet (+) Informational and engaging
Sensory Science	Basic descriptive analysis sensory panel principles	**Lab:** Mock Descriptive Analysis Panel	(+) Interactive panel activity
Nutrition	Conducting a study, presenting research, teamwork	**Lab/Research:** Human Health Intervention Study	(+) Students agreed this topic was "one that really intrigued me even before I applied."
Graduate Research	Research oriented learning in laboratory	**Lab:** Formulating, making, molding chocolate	(±) Fun, interesting, but students wanted to learn more about research
Food Chemistry	Fermentation	**Activity:** Bottle fermentation	(0) Indifferent. No comment.
Nanotechnology	Instrumental applications	**Guest Speaker/ Demonstration**	(+) Discussing the applications of nanotechnology was cool and "blew my mind".

[a]Responses complied from all 6 participants unless noted by direct quotation. (+) Positive Reaction; (-) Negative Reaction; (±) Both Positive and Negative Reactions; (0) Indifferent Reaction.

Table 4.3(cont): Summary of basic food science principles taught, methods for teaching, and associated feedback from students.			
Food Science Concentration	**Basic Principles Taught**	**Method for Teaching**	**Response From Students[a]**
Chemistry in the Kitchen	Applications in the kitchen, teamwork, basic kitchen skills	**Lab:** Making Ancient Hot Coco **Lab:** Iron Chef Contest	(+) "Fun!" Good introduction to test kitchen (+) "It was a lot of fun" "It was great to work in the kitchens and be creative"
Food Chemistry	Fat globule interaction	**Activity:** Whipping cream to butter	(+) Short, quick, and exciting
Food Chemistry	Polymorphism	**Lecture/Discussion**	(0) Indifferent: No comment.
Flavor Chemistry	Basic flavor compounds and chocolate flavor, learning GC-O techniques	**Lecture** **Lab:** Creating personal flavors **Demonstration/Field Trip:** visit flavor lab and use GC-O	(0) Indifferent: No comment. (±) "cool, but seemed a little off target" (±) Fun, but "needs more time in lab." Students "learned a lot about how to break different compounds apart"
Gradutate Reserach	SOYFACE Research	**Lecture/Field Trip:** visit to the soy bean plots	(+) It was fun and interactive. Students "want to learn and hear more about it" and "liked seeing the experiments in progress."
Plant Science	Informational plant science tour	**Field Trip** : visit to Plant Science Conservatory	(0) Indifferent: No comment. Informational tour.

[a]Responses complied from all 6 participants unless noted by direct quotation. (+) Positive Reaction; (-) Negative Reaction; (±) Both Positive and Negative Reactions; (0) Indifferent Reaction.

Table 4.4: Summary of other chocolate related topics presented, method of teaching, and associated feedback

Topic	Basic Principles Taught	Method of Teaching	Response from students[a]
Chocolate Flavor	Flavor differences between white, milk, dark chocolate	**Lecture** **Activity:** tasting different chocolate brands	(±) Informational, but long and somewhat boring (+) Enjoyable
History	History of chocolate	**Lecture**	(+) Interesting and a "great way to open up program with interesting facts"
Chocolate Flavor	Differences between flavored chocolate	**Activity:** tasting different flavored chocolate	(+) Enjoyable
Single Origins, Fair Trade, and Organic Chocolate	Issue of chocolate politics, labeling, and bean sourcing	**Lecture** **Activity:** Tasting different chocolate brands	(+) Informational (+) Enjoyable
Marketing	Chocolate marketing schemes	**Lecture**	(-) Lacking significance and too rushed
Presentations	Communication, public speaking, presenting data, answering questions, team collaboration	**Student Presentations:** 3-Slide PowerPoint Final Human Health Intervention Study Presentation	(+) Learned a lot about something new they independently researched (±) Some students "didn't like public speaking on big topics." Others, thought it was the "most challenging aspect, but overall worth it"
UIUC Opportunities	Introduction to study abroad program, FSHN majors, and applying/admissions	**Lecture**	(±) This lecture "really helped me decide what I can do/major in." However, a student mentioned, "I already knew the stuff about applying to college"

[a]Responses complied from all 6 participants unless noted by direct quotation. (+) Positive Reaction; (-) Negative Reaction; (±) Both Positive and Negative Reactions; (0) Indifferent Reaction.

Table 4.5: Specific lectures categorized into groups after evaluating feedback from students.

Lectures Lacking Significance	Lectures Needing Development	Influential Lectures
1) IRB task **2)** Food safety **3)** Marketing	**1)** Flavor chemistry **2)** Basic nutrition lecture **3)** Chocolate emulsifier research **4)** Chocolate processing	**1)** Food chemistry **2)** History of chocolate **3)** SOYFACE research **4)** UIUC opportunities **5)** Health benefits of chocolate **6)** Nanotechnology **7)** Sensory science **8)** Product development

Post Chocolate Food Science Knowledge

This part of the questionnaire was to assess student knowledge gained on basic chocolate food science principles taught throughout the program. Overall, students were more specific than on the pre-questionnaire when defining food science, but the answers were still quite general. In some cases, the unfortunate result was that students left some open-ended questions blank. The topics left blank by more than one student were instrumental analyses used to identify fat bloom, and the definition of the fair trade label. On the other hand, students explained the definitions and distinctions between fat bloom and sugar bloom in chocolate correctly. It was understood that fat bloom is both unappealing for flavor and appearance in chocolate, but it is not detrimental to human health and can be consumed. After the mock human intervention study, students were able to identify the health benefits associated with dark chocolate and tell the difference between white and dark chocolate benefits. Students were able to explain the importance

of tempering, the role of an emulsifier, and what conching means in chocolate production. The program helped students identify what they love about science and for some it increased or potentially increased their interest in the food science field as a future career choice (average 83.3% of students). A range of answers captured students' thoughts on the most valuable aspect of the program such as "the experience and knowledge gained throughout the program…the introduction to college life…the affirmation of career choices…and the fun activities throughout the program."

4.5 Discussion

"Telling children how scientists do science does not necessarily lead to far-reaching changes in how children do science" (Papert and Harel, 1991). This is an important fundamental concept that can lead to changes in teaching methodology to more experiential learning techniques. Students need to be immersed in science to fully understand and gain knowledge from their experience (American Association of Science, 1993). At a higher educational level, research has shown that graduate students learn through hands-on apprentice techniques at the start of their research (Sadler, 2009). This idea of immersing students through apprenticeship learning can be directly applied to K-12 education. Specifically, National Academy of Science emphasized US enhancement of education in K-12 science and engineering (NAS, 2005). In apprentice programs, students will learn from experiencing the hands-on-techniques by a "master or expert" of a specific field. The concepts associated with such teaching techniques have been utilized by many programs aimed at recruiting K-12 students into the sciences (Ritchie and Rigano, 1996; Hay and Barab, 2001; Stakes and Mares, 2001; Bell et al., 2003).

In the present study, recruitment efforts to expose high school students to food science was manifested in an intensive summer program. The Chocolate Food Science Education Program focused on experiential learning and introduced students to food science education. Sadler (2009) conducted an extensive review of literature on educational research programs targeting students in secondary education. The paper suggested there are four main goals associated with educational programs such as this. The goals of the educator included: 1) encouraging career aspirations, 2) promoting

understanding of scientific ideas and principles, 3) instilling confidence and self efficacy, 4) developing intellect and scientific skills.

Firstly, this program exposed students to academic and industrial professionals in the field of food science to educate students in the many career opportunities available. These interactions demonstrated the different challenges, processes, and rewards associated with academic and industrial careers. Out of 6 participants, 3 increased interest in the field of food science, 2 wanted to explore other areas of science, and 1 was interested only in computer science. Promoting career aspirations via summer programs has been reported by many studies across the nation. Stake and Mares (2001) documented that most participants began their program with scientific career aspirations, but the experience introduced more career options within science. They reported a significant shift away from medical concentrations to other opportunities.

The second goal in educational programs is to promote educational understanding of the nature of science. In our program, students were able to identify key concepts at the end of the program, documented in the post-questionnaire. Charney et al. (2007) noted a significant increase in discipline-specific content knowledge as measured through open-ended essay questions; however, most surveys do not provide a well-documented account of the quantity of learning. This is a drawback to assessing educational programs and more quantitative evaluation is necessary. The basis of this learning stems from the time dedicated to teaching the material and opportunities available for learning. The duration of education programs is crucial to the method of presentation of materials as well as retention of knowledge. Ritchie and Rigano (1996) confirm this idea and suggest that conceptual gains result only after students become immersed in their learning environment and attain the technical skills necessary to function competently within that environment. These apprenticeships were for 6 months versus a few weeks like normal high school student programs. Although the chocolate program only lasted 2 ½ weeks, it gave ample time to grasp introductory concepts of all food science concentrations; that this was a small group of students resulted in quick acclimation to the laboratory and to each other, for easy assimilation of information. The idea of the program was to ensure students gained comprehensive knowledge of several areas of food science to broaden their horizons and expose them to numerous opportunities within the field.

The third goal of many educational research programs is to instill confidence and self efficacy in students (Sadler, 2009). Students in our program exhibited such behavior by performing tasks such as presenting their final research on the human health intervention study to the group at the end of the program. Students practiced public speaking, group collaboration, and exhibited confidence in their research. The overall design of the program challenged students outside their comfort zones by fostering an independent college environment. Students lived in residence halls, attended daily lectures, and were expected to perform all activities to the best of their ability. One student mentioned "the informality of the program helped me ask more questions," an essential fundamental to learning. Previous research experience has been demonstrated to increase student confidence levels for participation in science (Bell et al., 2003). The author states that student perceptions of the benefits of summer research opportunities are positively mediated by experiences students have after returning to their schools. Two students from the 2009 Chocolate Food Science Education Program went on to submit their research learned from the program to 4-H state fair competitions. Another student also encouraged friends to apply for our second program based upon the experience. This demonstrates that students pass on information to their peers when they feel they have retained knowledge; this can be seen as a positive artifact of an educational program.

Another important aspect of all educational programs is associated with creating an environment to help refine skills and increase intellect. According to Hay and Barab (2001), experiential hands-on opportunities foster intellectual development through critical thinking and scientific reasoning. This suggests that the more opportunities given in a longer time frame (2 weeks) will demonstrate stronger outcomes as compared to students with more limited opportunities such as day programs (Berkes and Hogrebe, 2007; Russell et al., 2007). Gaining skills through research experiences is beneficial and useful for young scientists. Skills achieved by students in our program were: basic communication (oral and written), technical skills (using lab equipment), working independently, basic research principles, understanding primary literature, scientific ethics and teamwork. "If participants' experiences do not evolve to include a wider range of demanding practices such as data analysis, hypothesizing and developing research questions, then learning gains on higher order outcomes (critical thinking, content

knowledge, discourse practices) will likely be limited" (Hay and Barab, 2001). Lastly, satisfaction from research experiences is important to program development as well. Most participants in our program commented on their experiences with positive appraisals and cited numerous benefits of the program. The satisfaction of a participant helps ensure that the program was at least beneficial to the student on a personal level and leads to the potential of future programs to be developed.

4.6 Future programs

Overall, the initial Chocolate Food Science Education Program was a success. The grant was funded to support two programs for high school students; the second will occur in summer 2010. Participant feedback will be used to enhance the second program. The key element of learning basic food science principles is present throughout the duration of the program. Certain elements perceived as "boring" will be eliminated or reconfigured. We will incorporate additional experiential learning bench top experiments based upon student feedback. For example, the lipid extraction experiment associated with the food chemistry lecture helped improve the overall quality of the lesson being taught because it was interactive. The flavor chemistry lab will be improved so as to enhance students' understanding of flavor analysis by gas chromatography–olfactometry. Also, we will clarify linkages and collaborative efforts between the analytical focus on flavor with sensory science. Certain lessons required team collaboration, which students enjoyed; however, some also suggested "there be more individual tasks" throughout the program.

Improvements will be made to questionnaires as well. Specifically, numerical rankings will be introduced for detailed questions from certain key lectures. This will allow quantitative assessment of student learning and impressions from each lecture, which will provide feedback for the program and raise questions for overall food science education programs. Our program will also maintain a database for students after leaving so as to track their areas of focus. One student from the 2009 summer program has applied to and been accepted to the Food Science and Human Nutrition Department for undergraduate studies at the University of Illinois for fall 2010. Success of these

experiential learning programs will serve as a model for creative efforts of recruitment to fields that need more visibility at the high school level.

4.7 Conclusions

The 2009 Chocolate Food Science Education Program was a success. The goal was to attract bright, science-minded students who may not have been exposed to food science and teach them about the many facets of this exciting field through the study of chocolate. Chocolate as the overall theme was an excellent recruiting tool and a food product that all participants identified with. Students were exposed to many industrial and academic professionals in the field of food science. Students learned about the concentrations of food science through means of lectures, demonstrations, activities, field trips, research, and interactions with faculty, staff, graduate students, and industry professionals. Students refined team building skills, public speaking, conducted a mock human intervention study, and learned how to use scientific techniques to solve applied problems in food science. Evaluation of the program documented that this was a beneficial program for participants and has great potential as a recruitment tool for future food scientists. This program is also laying the foundation for creation of another educational workshop (2010) for high school teachers, to provide them materials and ideas of creative ways to introduce scientific concepts in a way that draw attention to food science as a possible career option.

Concluding Remarks and Future Studies

The studies presented in this thesis show the impact of emulsifiers on physical, sensory, and microstructural properties in formulated dark chocolate with an innovative educational approach. Results from Chapter 2 illustrate the impact of varying emulsifier concentrations on fat bloom formation in stored dark chocolate undergoing two temperature cycling regimes. Cycling at 34°C increased roughness, melting point, textural hardness, and whiteness index for all samples. These samples transitioned to polymorph VI. Cycling at 37°C increased roughness and whiteness for all samples. These samples were in polymorph V. Increased concentrations of emulsifier lead to increased dimensions (width) for all samples cycled at 37°C. Specifically, samples formulated with PGPR had the greatest increase in texture hardness and had the highest melting points. Samples formulated at 0.5% with PGPR may have experienced steric hindrance and agglomeration of intermolecular particles. Samples formulated with 0.5% soy lecithin or ammonium phosphatide had a decrease in melting point; these samples may have experienced intermolecular mobility of particles. Bloom was apparent in all samples cycled at 34°C and 37°C, although the composition of bloom formation was undetermined. Future studies should focus on the compositional characteristics of these samples after temperature cycling by performing a DSC melting point analysis on various areas of chocolate samples. The use of microscopic techniques such as PLM or SEM can help determine compositional differences on areas of bloomed chocolate and identify changes in crystalline structure as well. This future data should be complimented with rhelogical analysis; both particle size and viscosity need to be measured and results compared between chocolates formulated with different emulsifiers.

Results from Chapter 3 demonstrate the effects of long term storage on fat bloom formation, textural properties, and sensory attributes in dark chocolate samples formulated with different emulsifiers at 0.2% (w/w). Specifically, 8 week storage had impact on texture and flavor of dark chocolate. PGPR had increased textural hardness, cohesiveness, and gumminess, as well as increased melting point. Samples stored at 34°C experienced fat bloom formation, transitioned to polymorph VI, and had increased surface roughness. AFM results revealed that ‘spike and needle’ crystal formations occurred on lighter surface area of the chocolate samples. Both chocolate samples

freshly prepared and those stored at 23°C for 8 weeks did not experience fat bloom formation, increased surface roughness, or a polymorphic transition. However, samples stored at 23°C exhibited more intense flavor notes as described by panelists during descriptive analysis. Samples stored at 23°C were described as cohesive, creamy, fatty mouth coating, and had a roasted, chocolate, bitter, and sweet flavor. These results scientifically prove that controlled 'aging' of chocolate, a technique used by chocolatiers, does result in more flavorful chocolate. While chocolate experiencing fat bloom (storage 34°C) was harder, grainy, crumbly, left a dry mouthfeel, and had a bland, chalky flavor. The addition of different emulsifiers did not have any affect on flavor and texture attributes of chocolate. Future studies are necessary to confirm compositional and structural rearrangement of TAGs in dark chocolate formulated with soy lecithin, PGPR and ammonium phosphatide. Another descriptive analysis panel should be conducted to determine the degree of difference in flavor and textural sensory attributes throughout various points in long term storage of chocolate formulated with different emulsifiers.

Chapter 4 results illustrate the impact the 2009 Chocolate Food Science Education Program had on teaching high school students about the food science field. Chocolate as the overall theme was an excellent recruiting tool and a food product that all participants identified with. Students learned about the concentrations of food science through means of lectures, demonstrations, activities, field trips, research, and interactions with faculty, staff, graduate students, and industry professionals. Students refined team building skills, public speaking, conducted a mock human intervention study, and learned how to use scientific techniques to solve applied problems in food science. Evaluation of the program documented that this was a beneficial program for participants and has great potential as a recruitment tool for future food scientists. This program will lay the foundation for creation of another educational workshop (2010) for high school teachers, to provide them materials and ideas of creative ways to introduce scientific concepts in a way that draw attention to food science as a possible career option. Future work involves the creation and implementation of another program (summer 2010) and will allow for a second year of assessment. Overall, this program will serve as a model for creative efforts of recruitment to fields that need more visibility at the high school level.

References

21 Code of Federal Regulations. Part 163. 2003. U.S. Government Printing Office. Washington, D.C.

21 Code of Federal Regulations. Part 172. 2010. U.S. Government Printing Office. Washington, D.C.

Adenier H, Chaveron H, Ollivon M. 1993. Mechanism of fat bloom development on chocolate. In: Charalambous G, editor. Shelf Life Studies of Foods and Beverages. London: Elsevier. p 353-389.

Afoakwa EO, Paterson A, Fowler M. 2007. Factor influencing rheological and textural qualities in chocolate-a review. Trends in Food Science and Technology. 18:290-298.

Afowaka EO, Paterson A, Fowler M, Vieira J. 2008a. Particle size distribution and compositional effects on textural properties and appearance of dark chocolates. European Food Research and Technology. 87:181-190.

Afowaka EO, Paterson A, Fowler M, Vieira J. 2008b. Relationship between rheological, textural and melting properties of dark chocolate as influenced by particle size distribution and composition. European Food Research and Technology. 227:1215-1223.

Afowaka EO, Paterson A, Fowler M, Vieira J. 2008c. Effects of tempering and fat crystallization behavior on microstructure, mechanical properties and appearance in dark chocolate systems. Journal of Food Engineering. 89:128-136.

Afoakwa EO, Paterson A, Fowler M, Vieira J. 2009a. Comparison of rheological models for determining dark chocolate viscosity. International Journal of Food Science and Technology. 44:162-167.

Afoakwa EO, Paterson A, Fowler M, Vieira J. 2009b. Fat bloom development and structure-appearance relationships during storage of under-tempered dark chocolates. Journal of Food Engineering. 91:571-581.

Aguilera JM, Michel M, Mayor G. 2004. Fat migration in chocolate: diffusion or capillary flow in a particulate solid?- a hypothesis paper. Journal of Food Science. 69(7):R167-R174.

Ali A, Selamat J, Che Man YB, Suria AM. 2001. Effect of storage temperature on texture, polymorphic structure, bloom formation and sensory attributes of filled dark chocolate. Food Chemistry. 72:491-497.

American Association for the Advancement of Science (AAAS). 1989. Project 2061: Science for all Americans. Washington, DC: Author.

American Association for the Advancement of Science (AAAS). 1993. Benchmarks for science literacy: A Project 2061 Report. New York: Oxford University Press.

Amey J, Fletcher M. 2008. Meet the molecules in chocolate: Informal opportunities for building thematic molecular models with children. Journal of Chemical Education. 85(10):1361-1364.

Andrae LM. 2006. Doctoral Dissertation. Physical, perceptual, and bioactive components of chocolate. Food Science and Human Nutrition Department. University of Illinois.

Andrae-Nightingale LM, Lee SY, Engeseth NJ. 2009. Textural changes in chocolate characterized by instrumental and sensory techniques. Journal of Texture Studies. 40:427-444.

Andrae-Nightingale LM, Lee S-Y, Engeseth NJ, 200X. Impact of storage on dark chocolate: I. Texture and polymorphic changes. submitted.

Baker B, Brown D, Anantheswaran R. 2006. Measurement of yield stress in dark chocolate using controlled stress van method. Journal of Texture Studies. 37:655-667.

Bamford H, Gardiner K, Howat GR, Thomson AF. 1970. The use of polyglycerol polyricinoleate in chocolate. Confectionery Production. 36(6):359-361.

Barab S, Hay K. 2001. Doing science at the elbows of experts: Issues related to the science apprenticeship camp. Journal of Research in Science Teaching. 38(1):70-102.

Bartusch W. 1974. Effect of the agglomerate structure″instant″ properties of agglomerated cocoa-sugar mixtures. In: 1st International Congress on Cocoa and Chocolate Research. Food Science and Technology Abstracts. Munich. p 308-317.

Bauer KW, Bennett JS. 2003. Alumni perceptions used to assess undergraduate research experience. The Journal of Higher Education. 74: 210–230.

Beckett ST. 1999. Industrial chocolate manufacture and use. 3rd ed. Oxford, UK: Blackwell. p 192.

Beckett, ST. 2000. The science of chocolate. The Royal Society of Chemistry. Cambridge, UK. p 1-170.

Beckett, ST. 2008. The science of chocolate. The Royal Society of Chemistry. 2nd ed. Cambridge, UK. p 1-234.

Bell R, Blair L, Crawford B, Lederman N. 2003. Just do it? Impact of a science apprenticeship program on high school students' understanding of the nature of science and scientific inquiry. Journal of Research in Science Teaching. 40(5):487-509.

Berkes E, Hogrebe M. 2007. Undergraduate laboratory research, persistence in science, and the effect of self-efficacy beliefs: A quantitative study. Paper presented at the American Educational Research Association, Chicago, IL.

Bolenz S, Amtsberg K, Lipp E. 2005. New concept for fast continuous conching of milk chocolate. European Food Red Technology. 220:47-54.

Bolliger S, Breitschuh B, Stranzinger M, Wagner T, Windhab EJ. 1998. Comparison of precrystallization of chocolate. Journal of Food Engineering. 35:281-297.

Bomba PC. 1993. Shelf life of chocolate confectionery products. In: Charalambous G, editor. Shelf Life Studies of Foods and Beverages: Chemical, Biological, Physical and Nutritional Aspects. Amsterdam: Elsevier. p 341-351.

Bricknell J, Hartel RW. 1998. Relation of fat bloom in chocolate to polymorphic transition of CB. Journal of the American Oil Chemists' Society. 75(11):1609-1615.

Briones V, Aguilera JM. 2005. Image analysis of changes in surface color of chocolate. Food Research International. 38:87-94.

Cadwallader, K. 2008. Isolation of Volatile Techniques Lecture. Flavor Chemistry Course. FSHN: 595. University of Illinois, Urbana-Champaign.

Cambrai A, Marcic C, Morville S, Houer P, Bindler F, Marchioni E. 2010. Differentiation of chocolates according to the cocoa's geographical origin using chemometrics. Journal of Agricultural and Food Chemistry. 58:1478-1483.

Case, CL. The microbiology of chocolate. March 29, 2010. www.smccd.edu.

Cebula Ziegleder. 1993. Studies of bloom formation using x-ray diffraction from chocolates after long-term storage. FSTech. 95(9):340-343.

Chaisier S, Dimick P. 1995. Dynamic crystallization of cocoa butter. II. Morphological, thermal, and chemical characteristics during crystal growth. Journal of the American Oil Chemists' Society. 72(12):1497-1504.

Chapman GM, Akehurst EE, Wright WB. 1970. Cocoa butter and confectionery fats. Studies using programmed temperature x-ray diffraction and differential scanning calorimetry. Journal of the American Oil Chemists' Society. 48:824-830.

Charney J, Hmelo-Silver CE, Sofer W, Neigeborn L, Coletta S, Nemeroff M. 2007. Cognitive apprenticeship in science through immersion in laboratory practices. International Journal of Science Education. 29:195–213.

Chatterjee, S. Williamson, V, McCann K, Peck L. 2009. Surveying students' attitudes and perceptions toward guided-inquiry and open-inquiry laboratories. Journal of Chemical Education. 86(12):1427-1432.

Chevalley J. 1999. Chocolate Flow Properties, in Industrial Chocolate Manufacture and Use. Beckett ST, editor. 3rd ed. Oxford, UK: Blackwell.

Choi Y, McCarthy K, McCarthy M. 2005. Oil migration in a chocolate confectionery system evaluated by magnetic resonance imaging. Journal of Food Science. 70(5):312-318.

Cidell JL, Alberts HC. 2006. Constructing quality: the multinational histories of chocolate. Geoforum. 37:999–1007.

Cobb P. 1994. Where is the mind? Constructivist and sociocultural perspectives on mathematical development. Educational Researcher. 23(7):13–20.

Cohen KO, Luccas V, Jackix MNH. 2004. Review: tempering or precrystallization of chocolate. Brazilian Journal of Food Technology. 7:23–29.

Counet C, Callemien D, Ouwerx C, Collin S. 2002. Use of GC-O to identify key odorant compounds in dark chocolate. Comparison of samples before and after conching. Journal of Agriculture and Food Chemistry. 50:2385-2391.

Davis TR, Dimick PS. 1989. Lipid composition of high melting seed crystals formed during cocoa butter solidification. Journal of the American Oil Chemists' Society. 66(10):1494-1498.

Deibler KD, Acree TE, Lavin EH. 1999. Solid phase microextraction application in gas chromatography/olfactometry dilution analysis. Journal of Agricultural and Food Chemistry. 47:1616-1618.

Dedinaite A, Campbell B. 2000. Interactions between mica surfaces across triglyceride solution containing phophoslipid and polyclycerol polyricinoleate. Langmuir. 16:2248-2253.

DeMan JM, DeMan L. 2001. Polymorphism and texture of fats. In: Widlak N, Hartel R, Narine S, editors. Crystallization and Solidification Properties of Lipids. Champaign, IL: AOCS Press. p 225-235.

Destefano L, Burke M, Lemons R. 2004/05. An external evaluation of University of Illinois at Urbana-Champaign Nano-CEMMS human resource development and educational outreach programs: year two repot. p1-113.

Dhonsi D, Stapley, AGF. 2006. The effect of shear rate, temperature, sugar and emulsifier on the tempering of cocoa butter. Journal of Food Engineering. 77:936-942.

Dickinson E, Golding M. 1992 Influence of Alcohol on Stability of Oil-in-Water Emulsions Containing Sodium Caseinate. Journal of Colloid and Interface Science. 197(1):133-141.

Duck WN. 1964. Determination of solid fat in melted fat, their role in formation and polymorphic form by viscometry. Masters Thesis. Franklin and Marshall College, Lancaster, PA. 72p.

Engeseth, N.J. and Nightingale, L.M. 2007. Impact of storage on flavor and texture perception of chocolate. Manufacturing Confectioner. 87(5):50-56.

European Synchrotron Radiation Facility Site. 2010. www.esrf.eu.

Fennema OR. 1996. Food Chemistry (Food Science and Technology). 3rd ed. New York: CRC Press. p 767-825.

Fletcher A. 2006. Emulsifiers vital for chocolate coating. Palsgaard Press Release. FoodNavigator.com-Europe.

Franke K, Tscheuschner HD. 1991. Modeling of the continuous high shear rate conching process for chocolate. Journal of Food Engineering. 14(2):103-115.

Fryer P, Pinschower, K. 2000. The materials science of chocolate. MRS Bulletin. p 25-29.

Full NA, Yella Reddy S, Dimick PS, Ziegler GR. 1996. Physical and sensory properties of milk chocolate formulated with anhydrous milk fat fractions. Journal of Food Science. 61(5):1068-1072,1084.

Garti N, Schlichter J, Sarig S. 1986. Effect of food emulsifiers on polymorphic transitions of cocoa butter. Journal of the American Oil Chemists' Society. 63(2):1558-9331.

Garti N, Sato K. 1988. Crystallization and Polymorphism of Fats and Fatty Acids. New York: Marcel Dekker Publishers. p 450.

Garti, N. 2001. In: Sjoblom J, editor. Encyclopedic Handbook of Emulsion Technology. New York: Marcel Dekker Publishers. p 407.

Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. 2005. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. American Journal of Clinical Nutrition. 81(3). 611-614.

Greeno JG, Moore JL, Smith DR. 1993. Transfer of situated learning. In: D.K. Detterman and R.J. Sternberg, editors. Transfer On Trial: Intelligence, Cognition, and Instruction. Norwood, NJ: Ablex Publishing. pp. 99–167.

Guinard J, Mazzucchelli R. 1999. Effects of sugar and fat on the sensory properties of milk chocolate: descriptive analysis and instrumental measurements. Journal of the Science of Food and Agriculture. 79:1331-1339.

Guittard G. 2009. Cocoa bean types and characteristics. Manufacturing Confectioner. 89(9):77-80.

Hansen AP, Keeney PG. 1970. Comparison of carbonyl compounds in moldy and non-moldy cocoa beans. Journal of Food Science. 35(1):37-40.

Hartel RW. 2001. Crystallization in foods. Gaithersburg, Maryland. USA, Aspen Publishers. p 325.

Hay KE, Barab SA. 2001. Constructivism in practice: A comparison and contrast of apprenticeship and constructionist learning environments. Journal of the Learning Sciences. 10:281–322.

Hodge SM, Rousseau D. 2002. Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. Journal of American Oil Chemists' Society. 79(11):1115-1121.

Hoskin JC, Dimick PS. 1983. Role of nonenzymatic browning during the processing of chocolate-a review. Process Biochemistry. 19:150-156.

Hoskin JC. 1994. Sensory properties of chocolate and their development. American Journal of Clinical Nutrition. 60:1068S-1070S.

International Cocoa Organization (ICCO). 2006/07. Annual Report. ICCO Quarterly Bulletin of Cocoa Statistics, Vol XXXIII, No. 4 2006/07.

Information Resources Inc. 2010. U.S. Confectionery Sales 2009. Manufacturing Confectioner. 90(1):13-20.

James B, Smith B. 2009. Surface structure and composition of fresh and bloomed chocolate analyzed using X-ray photoelectron spectroscopy, cryo-scanning electron microscopy and environmental scanning electron microscopy. Food Science and Technology. 42:929-937.

Jensen HR. 1931. The Chemistry, Flavouring and Manufacture of Chocolate Confectionery and Cocoa. London: J&A Churchill. p 406.

Jinap S, Dimick PS, Hollender R. 1995. Flavor evaluation of chocolate formulated from cocoa beans from different countries. Food Control. 6(2):105-110.

Johansson D, Bergenstahl B. 1992a. The influence of food emulsifiers on fat and sugar dispersions in oils-I. adsorption, sedimentation. Journal of the American Oil Chemists' Society. 89(8):705-717.

Johansson D, Bergenstahl, B. 1992b. The influence of food emulsifiers on fat and sugar dispersions in oils. II. Rheology, Colloidal Forces. Journal of the American Oil Chemists' Society. 69(8):718-727.

K&K Chocolate Confectionery Machinery Site. 2009. www.kurtmakina.com.

Karnjanolarn R, McCarthy K. 2006. Rheology of different formulations of milk chocolate and the effect on coating thickness. Journal of Texture Studies. 37:668-680.

Kinsella JE. 1990. Flavor perception and binding. Inform 1:215-226.

Kinta Y, Hatta T. 2005. Composition and structure of fat bloom in untempered chocolate. Journal of Food Science. 70(7):S450-S452.

Kinta Y, Hatta T. 2007. Composition, structure, and color of fat bloom due to partial liquefaction of fat in dark chocolate. Journal of the American Oil Chemists' Society. 84:107-115.

Kinta Y, Hartel RW. 2010. Bloom formation on poorly tempered chocolate and effects of see addition. Journal of the American Oil Chemists' Society. 87:19-27.

Knoth A, Scherze I, Muschiolik G. 2005. Effect of lipid type on water-in-oil-emulsions stabilized by phosphatidylcholine-depleted lecithin and polyglycerol polyricinoleate. European Journal of Lipid Science and Technology. 107:857-863.

Langevelde A, Driessen R, Molleman W, Peschar R, Schenk H. 2001. Cocoa butter long spacings and the memory effect. Journal of the American Oil Chemists' Society. 78(9):911-918.

Lee S, Heuberger M, Rousset P, Spencer ND. 2002. Chocolate at a sliding interface. Journal of Food Science. 67(7):2712-2717.

Liang B, Hartel RW. 2004. Effects of milk powder in milk chocolate. Journal of Dairy Science. 87(1):20-31.

Lipp M, Anklam E. 1998. Review of cocoa butter and alternative fats for use in chocolate—Part A. compositional data. Food Chemistry. 62(1):73-97.

Lipp M, Simoneau C, Ulberth F, Anklam E, Crews C, Brereton P, de Greyt W, Schwack W, Wiedmaier C. 2001. Fat crystal growth and microstructural evolution in industrial chocolate. Journal of Food Composition and Analysis. 14:399-408.

Loisel C, Lecq G, Ponchel G, Keller G, Ollivon M. 1997. Fat bloom and chocolate structure studied by mercury porosimetry. Journal of Food Science. 62(4):781-788.

Loisel C, Lecq G, Keller G, Ollivon M. 1998. Dynamic crystallization of dark chocolate as affected by temperature and lipid additives. Journal of Food Science. 63(1):73-79.

Lonchampt P, Hartel RW. 2006. Surface bloom on improperly tempered chocolate. European Journal of Lipid Science and Technology. 108:159-168.

Lucisano M, Casiraghi E, Mariotti M. 2006. Influence of formulation and processing variables on ball mill refining of milk chocolate. European Food Research and Technology. 233(6):797-802.

Manning DM, Dimick PS. 1983. Interpreting the thermal characteristics of cocoa butter using the differential scanning calorimeter. Manufacturing Confectioner. 63:73-80.

Manning DM, Dimick PS. 1985. Crystal morphology of cocoa butter. Food Microstructure. 4:249-265.

Markov E, Tscheuschner HD. 1989. Instrumental texture studies on chocolate IV: comparison between instrumental and sensory texture studies. Journal of Texture Studies. 20:151-160.

Martelli HL, Dittmar HFK. 1961. Cacao fermentation. Yeast isolated from cacao beans during the curing process. Applied Environmental Microbiology. 9(5):370-371.

Mazzanti G, Guthrie S, Sirota E, Marangoni A, Idziak S. 2003. Orientation and phase transitions of fat crystals under shear. Crystal Growth and Design. 3(5):721-725.

McClements DJ, Dungan SR, German JB, Simoneau C, Kinsella JE. 1993. Droplet size and emulsifier type affect crystallization and melting of hydrocarbon-in-water emulsions. Journal of Food Science. 58:1148-1152.

McClements DJ. 2005. Food Emulsions: Principles, Practices, and Techniques. 2nd ed. Florida: CRC press. p 21-122.

McGee H. 1999. On food and cooking: the science and lore of the kitchen. New York: Simon & Schuster. p 112.

Mentink L, Serpelloni M. 1994. Low-calorie chocolate. US Patent 5, 360,621.

Merken GV, Vaeck SV. 1980. Etude du polymorphisme du beurre de cacao par calorimetrie DSC. Food Science and Technology. 13:314-317.

Metin S, Hartel RW. 2005. Crystallization of fats and oils. In: Sahidi F, editor. Bailey's Industrial Oil and Fat Products. New York: Wiley. p 45–76.

Min D. 2003. Polymorphism. Ohio State University lecture. class.fst.ohio-state.edu/fst821/lect/polymer.pdf.

Minifie BW. 1989. Chocolate, cocoa, and confectionery: Food Science and Technology. 3rd ed. New York: Chapman & Hall. p 233-287.

Miquel ME, Carli S, Couzens PJ, Wille HJ, Hall LD. 2001. Kinetics of the migration of lipids in composite chocolate measured by magnetic resonance imaging. Food Research International. 34:773-781.

Misnawi, Jinap S, Jamilah B, Nazamid S. 2004. Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting. Food Quality and Preference. 15:403-409.

Mont Blanc Gourmet Site. January 2010. www.montblancgourmet.com.

Morgan J. 1994. Chocolate: a flavor and texture like no other. American Journal of Clinical Nutrition. 60:1065S-1067S.

Narine SS, Marangoni AG. 1999. Relating structure of fat cystal networks to mechanical properties-a review. Food Research International. 32(4):227-248.

National Academy of Science (NAS). 2005. Letter to Academy members from President Bruce Alberts. www.nasonline.org

National Confectioners Association. 2005. Global confectionery market. Manufacturing Confectioner. 85(3):27-31.

National Research Council. 1996. National science education standards. Washington, DC: National Academic Press.

Nebesny Ewa, Zyzelewicz D. 2005. Effect of lecithin concentration on properties of sucrose-free chocolate masses sweetened with isomalt. European Food Research and Technology. 220:131-135.

Nieuwenhuyzen W, Szuhaj B. 1998. Effects of lecithins and proteins on the stability of emulsions. Fett/Lipid. 100(7):282-291.

Papert S, Harel I. 1991. Situating Constructionism. In: Constructionism. Papert S, Harel I, editors. Ablex Publishing Corpation. p238-244.

Pastor C, Santamaria J, Chiralt A, Aguilera JM. 2007. Gloss and colour of dark chocolate during storage . Food Science and Technology International. 13(1):27–34.

Patric A. 2006. Patric Chocolate interview with Alan Patric. Cacaolab. www.cacaolab.com.

Pelchat ML, Beauchamp GK. 1999.Sensory and taste preferences of chocolate. In: Knight I, editor. Chocolate and Cocoa: Health and Nutrition. Massachusetts: Blackwell. p 310-320.

Pollard A. 2007. Amano Chocolate interview with Art Pollard. Cacaolab. www.cacaolab.com.

Prawira M. 2009. Effects of conching time and ingredients on preference of milk chocolate. Journal of Food Processing and Preservation. 33:571-589.

Presilla ME. 2001. The New Taste of Chocolate. California: Ten Speed Press. p 1-176.

Quevedo R, Brown C, Bouchon P, Aguilera J. 2005. Surface roughness during storage of chocolate: fractal analysis and possible mechanisms. Journal of the American Oil Chemists' Society. 82(6):457-462.

Rector R. 2000. Chocolate-controlling the flow, benefits of polyglycerol polyricinoleic acid. Manufacturing Confectioner. 80(5):63-70.

Reverend B, Fryer PJ, Coles S, Bakalis S. 2010. A method to qualify and quantify the crystalline state of cocoa butter in industrial chocolate. Journal of the American Oil Chemists' Society. 87:239-246.

Ritchie SM, Rigano DL. 1996. Laboratory apprenticeship through a student research project. Journal of Research in Science Teaching. 33:799–815.

Rosenbum M. 2005. Chocolate: A Bittersweet Saga of Dark and Light. New York: North Point Press. p 84.

Rosenthal AJ. 1999. Relation between instrumental and sensory measures of food texture. In: Rosenthal AJ, editor. Food Texture, Measurement, and Perception. Gaithersburg, MD: Aspen Publishers, Inc. p 1-17.

Rousseau D, Sonwai P. 2008. Microstructure of fat bloom development in plain and filled chocolate confections. Soft Matter. 4:1706-1712.

Rousset P, Sellappan P. 2002. Effect of emulsifiers on surface properties of sucrose by inverse gas chromatography. Journal of Chromatography. 969:97-101.

Russell SH, Hancock MP, McCullough J. 2007. The pipeline: Benefits of undergraduate research experiences. Science. 316:548–549.

Sadler T, Burgin S, McKinney L, Ponjuan L. 2009. Learning science through research apprenticeships: A critical review of the literature. Journal of Research in Science Teaching. p 1-23.

Sato K. 1999. Solidification and phase transformation behaviour of food fats-a review. Fett/Lipid. 101(12):467-474.

Sato, K. 2001. Molecular aspects in fat polymorphism. In: Widlak N, Hartel R, Narine S, editors. Crystallization and Solidification Properties of Lipids. Champaign, IL: AOCS Press. p 1-16.

Sato K, Koyano T. 2001. Crystallization processes of cocoa butter. In: Garti N, Sato K, editors: Crystallization Processes in Fats and Lipid Systems. New York: Marcel Dekker. p 429-456.

Scharffenberger J, Steinberg R. 2006. The Essence of Chocolate. New York: Hypersion Press. p 122.

Schantz B, Rohm H. 2005. Influence of lecithin-PGPR blends on the rheological properties of chocolate. Lebensm-Wiss. u-Technology. 38:41-45.

Schenk H, Peschar R. 2004. Understanding the structure of chocolate. Radiation Physics and Chemistry. 71:829-835.

Schlichter-Aronhime J, Sarig S, Garti N, 1988. Reconsideration of polymorphic transformations in cocoa butter using the DSC. Journal of the American Oil Chemists' Society. 65(7):1140.

Scholfield CR. 1980. Composition of soybean lecithin. Journal of the American Oil Chemists' Society. 58(10):889-892.

Schneider M. 1986. Emulsifying agents. Confectionery Manufacture and Marketing. 23(4):1-14.

Schnermann P, Schieberle P. 1997. Evaluation of Key Odorants in Milk Chocolate and Cocoa Mass by Aroma Extract Dilution Analyses. Journal of Agricultural and Food Chemistry. 45(3):867–872.

Schumacher AB, Brandelli A, Schumacher EW, Macedo FC, Pieta L, Klug TV. 2009. Development and evaluation of a laboratory scale conch for chocolate production. International Journal of Food Science and Technology. 44:616-622.

Schmitt H. 1995. Lecithin - the emusifier in the chocolate industry. Food Consumption. 48(7/8)310-312.

Schwan RF, Wheals AE. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Critical Reviews in Food Science and Nutrition. 44(4):205-221.

Seguine E S. 1988. Casson plastic viscosity and yield value. What they are and what they mean to the confectioner. Manufacturing Confectioner. 68:57-63.

Sondergaard C. 1987. Conference. Wiesbaden: Germany. Proceedings. p 3–10.

Stake JE, Mares KR. (2001). Science enrichment programs for gifted high school girls and boys: Predictors of program impact on science confidence and motivation. Journal of Research in Science Teaching. 38:1065–1088.

Steir R. 2009. Fats: from nutritional nuances to physical functionality. Prepared foods.com. R&D Applications Seminar. p 50-56.

Sune F, Lacroix P, Huon de Kermadec F. 2002. A comparison of sensory attribute use by children and experts to evaluate chocolate. Food Quality and Preference. 13:545-553.

Surh J, Vladisavljevic G, Mun S, McClements DJ. 2007. Preparation and characterization of water/oil and water/oil/water emulsions containing biopolymer-gelled water droplets. Journal of Agricultural and Food Chemistry. 55:175-184.

TA Instruments. 2009. Advanced DSC Training Course. November 10, 2009.

Talbot G. 1995. Chocolate fat: the cause and the cure. Internatinal Food Ingredients. 1:40-45.

Taubert D, Berkels R, Roesen R, Klaus W. 2003. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. Journal of American Medical Association. 290:1029 –1030.

Thamke I, Durrschmid K, Rohm H. 2009. Sensory description of dark chocolates by consumers. LWT-Food Science and Technology. 42:534-539.

Tscheuschner HD, Markov E. 1986a. Instrumental texture studies on chocolate I. Methods of measurement and texture characteristics. Journal of Texture Studies. 17:37-50.

Tscheuschner HD, Markov E. 1986b. Instrumental texture studies on chocolate II: processing conditioned factors influencing the texture. Journal of Texture Studies. 17:37-50.

Tscheuschner HD, Markov E. 1989. Instrumental texture studies on chocolate III: compositional factors influencing texture. Journal of Texture Studies. 20:335-345.

Urbanski J. 2001. Dark chocolate: what affects the flavor? Manufacturing Confectioner. 81(6):67-72.

Vaeck SC. 1951. The polymorphism of certain natural fats: cocoa butter. Review of International Chocolate. 6:100.

Vaeck SC. 1960. Cacao butter and fat bloom. Manufacturing Confectioner. 40:35-74.

Vernier F. 1997. Influence of emulsifiers on the rheology of chocolate and suspensions of cocoa or sugar particles in oil. Doctoral thesis. Reading University.

Van Malssen K, Peschar R, Schenk H. 1996. Real-time x-ray powder diffraction investigations on cocoa butter. I. temperature-dependent crystallization behavior. Journal of the American Oil Chemists' Society. 73(10):1209-1215.

Van Malssen K, Langevelde A, Peschar R, Schenk H. 1999. Cocoa butter investigated with real-time x-ray powder diffraction. Journal of the American Oil Chemists' Society. 76(6):669-677.

Walstra P. 1996. Dispersed Systems: Basic Consideration. In: Fennema, OR, editor. Food Chemistry. 3rd ed. p 96-151.

Walter P, Cornillon P. 2001. Influence of thermal conditions and presence of additives on fat bloom in chocolate. Journal of the American Oil Chemists' Society. 78(9):927-93.

Watkins BA. 2009. Food chemistry experiment: Lipids. Institute of Food Technologists site. www.ift.org/IFT/Education/EduResources/experiments

Wille RL, Lutton ES. 1969. Polymorphism of cocoa butter. Journal of the American Oil Chemists' Society. 43:491-496.

Wilson R, Van Schie B, Howes D. 1998. Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR). Food and Chemistry Toxicology. 36:711-718.

Young AM. 1994. The Chocolate Tree: A Natural History of Cacao. Washington: Smithsonian Institution Press. p 19-24.

Zarringhalami S, Sahari M, Barzegar M, Hamidi-Esfeshani Z. 2010. Enzymatically modified tea seed oil as cocoa butter replacer in dark chocolate. International Journal of Food Science and Technology. 45:540-545.

Ziegleder G. 1997. Fat bloom and migration. Manufacturing Confectioner. 77(2):43-44.

Appendix A.1 Chocolate Tempering Protocol (adapted from Revolution2 Manual)

Prior to tempering:

1) Chop chocolate into medium size chunks.
2) Weigh out seed chocolate (~ 8% of total tempering sample). Set aside.
   NOTE: Do not exceed m*aximum capacity for tempering, 1.5 lbs (680.38g).*
3) Prime bowl by taking a piece of chocolate and rub it along the inside of the bowl. Do the same for the plastic scraper.
4) Set up temper machine according manual instructions.
5) Select type of chocolate sample to temper (white, milk, dark).

During tempering:

6) Place chopped chocolate behind baffle in order to melt. Add chocolate in portions. Do not overload the bowl.
7) If desired, manually adjust temperature according to chocolate type.
   NOTE: Best melting temperature for dark chocolate is 113ºF.
8) Once machine starts beeping/flashing temper button, the chocolate is completely melted. Select the Temper #2 method. Gradually add seed chocolate behind baffle and allow seed chocolate to melt.
9) Once the machine starts beeping/flashing 'seed out' button, hold down button. Then, take out remainder seed chocolate that did not melt. Set aside.
10) Allow machine to continue the tempering process. At this time, prepare molds. Put molds into an oven set at 32ºC. Clamp plastic piping bag onto foil ring stand.
11) When the machine beeps a final 3rd time, the chocolate is fully tempered; push reset button (R) to stop bowl from spinning.
12) Take out mold from oven and put on tray.

Molding chocolate:

13) Using a metal scrapper, scrape melted tempered chocolate into piping bag from bowl.
14) Remove piping bag from ring stand, seal it, and cut off tip on bottom to allow chocolate to flow out the bottom of bag in a steady stream.
15) Take bag, applying pressure from the top, and squeeze chocolate into well. The tip should touch the bottom of the well first and then slowly pull upward to completely fill each individual well. Repeat this procedure in all mold wells. *NOTE: Do not place hands on outer surface of bag when piping because it will add additional heat. Hold at base and tip of bag. You must mold quickly.*
16) After liquid chocolate has been piped into wells, take dipping tool and mix chocolate in individual wells.
17) Scrape off excess chocolate from mold using metal spatula until chocolate fills each well completely.
18) Moderately tap molds on bench surface to release air bubbles.
19) Place molded chocolate in ambient control temperature room. Allow chocolate to set overnight.
20) Clean equipment with warm water and rinse with deionized water.

Appendix B.1 Informed consent for sensory panel

INFORMED CONSENT FORM FOR SENSORY EVALUATION PANELISTS TO PARTICIPATE IN: Descriptive Analysis for Chocolates Study

---

You are invited to participate in a study involving chocolate sensory evaluation. The overall objective of this study is to develop a descriptive list of terms for the chocolates to be tested. These chocolates will be evaluated using a sensory evaluation method known as descriptive analysis. You will be trained to identify, name and classify a range of flavor, taste and texture characteristics of these samples. You will be asked to taste and expectorate the samples, and to rate the samples for intensity of each characteristic. There are minimal risks or discomforts expected as a result of your participation. If you have prior experience of any allergic reactions to chocolates, you should not participate in this study. If you experience allergic reactions any time during the study, you should discontinue the study. The chocolates do not contain nuts. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason. We also reserve the rights to terminate your participation of the study at any time and for any reason.

The study will be conducted at Bevier Hall Room # 376 (Sensory laboratory). You will participate in 4 weeks of training sessions (approximately 4 to 5 hours a week, 1 hour per day) and 1 week of analysis (1 hour per day). Participation of this study is voluntary, and you will not be compensated monetarily for your participation.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance. Names or other identifiable information will not be disclosed or published.

You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. However, answers which could influence the outcome of the study will be deferred to the end of the experiment. Questions can be addressed to Dr. Nicki Engeseth (217-244-6788, engeseth@illinois.edu). If you have any questions about the rights of research subjects, please feel free to contact the IRB Office (217-333-2670, irb@illinois.edu).

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of this consent form.

Signature Date

Appendix B.2 Sensory panel questionnaire.

Panelist # _____

Chocolate Panel Questionnaire

Personal Info

1. Sex

☐Male ☐Female

2. Age

☐<20 ☐21-30 ☐31-40 ☐41-50 ☐51-60 ☐>61

Health

1. Do you have any of the following?

☐Diabetes ☐Hypoglycemia ☐Hypertension ☐Other Medical Problems

2. Do you have any food allergies? If so, please list.

_____
_____

3. Do you take any medications, which may affect your senses, especially taste and smell?

_____
_____

4. Are you currently on a restricted diet? If so, please explain.

_____
_____

Eating Habits

1. How often do you consume chocolate per week?

☐0 ☐1-3 ☐4-6 ☐>7

2. Which do you prefer?

☐Dark Chocolate ☐Milk Chocolate ☐White Chocolate ☐No Preference

Appendix B.3 Sample ballot for descriptive analysis panel[a]

Chocolate Descriptive Panel
RATING SAMPLES

Sample #: ____**209**_____

Texture Attributes

Hardness (Carrot)

0 1 2 3 4 5 6 7 **8** 9 10 11 12 13 14 15

Grainy (Ground Cinnamon)

0 1 2 3 4 5 6 7 **8** 9 10 11 12 13 14 15

Crumbly (Feta Cheese)

0 1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15

Cohesive (Frosting)

0 1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15

Creamy (Pudding)

0 1 2 3 4 5 6 7 8 9 **10** 11 12 13 14 15

Tooth Packing (Animal Cracker)

0 1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15

Fatty Mouth Coating (Diluted Cream)

0 1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15

Dry Mouth feel (Cocoa Powder)

0 1 2 3 4 5 6 7 8 9 10 **11** 12 13 14 15

---

[a]Anchored reference ratings are **bolded** on scale and associated references are in parentheses.

Appendix B.4 Sample Melting Time Intensity Test scorecard during training weeks.

Melting Time Intensity Test

DIRECTIONS: Place sample on top of tongue in your mouth. Press sample to the roof of your mouth and record how long it takes to completely melt the sample. Record the final melting time and comment.

Sample # __843_____ Melting Time: _____Comment:_____

Sample # __114_____ Melting Time: _____Comment:_____

Sample # ___836__ Melting Time: _____Comment:_____

Sample # __589_____ Melting Time: _____Comment:_____

Sample # ___672____ Melting Time: _____Comment:_____

Sample # __423___ Melting Time: _____Comment:_____

Sample # _110_____ Melting Time: _____Comment:_____

Appendix C.1 Chocolate Food Science Education brochure (front and back) for recruitment purposes.

This Program Will Focus On:

- Experimental learning in a lab setting.
- Introducing the concept of lipid polymorph changes in chocolate
- Using scientific techniques to solve applied problems in food science
- Learning about exciting new technologies incorporated in our research
- Conducting experiments, doing background research, analyzing data, and presenting your findings

The Chocolate Idea

Your text here.

Example Workshops

Through these workshops, we will explore the many facets of food science, including food chemistry, food processing, food microbiology, food product development, nanotechnology, sensory science, and nutrition.

...Did Someone Say Chocolate?

YES, chocolate!!!

This summer the Food Science and Human Nutrition Department at the University of Illinois will be hosting six high school students to participate in a chocolate program with graduate students.

The various angles of food science taught at the University will reveal the mysteries behind the science of chocolate. During the program, you'll have the opportunity to be immersed in the campus, work in a laboratory, and find out what it is like to do research on a fun topic like chocolate!

Chocolate program conducted by Illinois graduate student Melissa Tisoncik and overseen by Professor Nicki Engeseth. This program has been made possible by a grant from the USDA.

Program from June 15-July 2, 2009 provided for six high school students. Costs covered by the program include:

-15 days of workshops at Illinois campus
-University of Illinois Housing
-University of Illinois Meals

Students will need to arrange their own transportation to and from the University .

Applications Due: March 2
Decisions Made By: March 23

For more information contact:
uiuc.chocolate@gmail.com

Interested in Food Science?

Food Science incorporates the study of the physical, microbiological, chemical and biochemical properties of food to ensure maximum safety and optimal quality for the everyday consumer.

~~~~~~~~

Food scientists focus on basic research, product development, quality control, processing, engineering, packaging, and technical sales of food.

This summer YOU could have the opportunity to learn about all aspects of food science. Apply for the Chocolate Education Summer Program at the University of Illinois-Urbana Champaign and you will learn the capstone concentrations of food science through the study of
~~~~~~~~

Appendix C.2 College of ACES Press Release for program (January 2009).

U of I Summer "Science of Chocolate" Internships Available to Teens

Source: Nicki Engeseth, (217) 244-6788, engeseth@illinois.edu

URBANA - Science-minded high-school students should consider applying for a 15-day summer internship at the University of Illinois, especially if they're interested in chocolate.

"Our goal is to introduce the students to many aspects of food science through the study of chocolate. We will teach them to monitor quality changes in chocolate and use scientific principles to investigate changes in chocolate during storage," said Nicki Engeseth, an associate professor of food chemistry in the U of I Department of Food Science and Human Nutrition.

Interns will tour the U of I Center for Microanalysis of Materials where U of I food scientists have used nanotechnology to analyze grain size, crystal structure, and roughness parameters of chocolate, all factors that studies have shown influence taste, texture, and the release of flavor compounds, the scientist said.

"The students will also learn about the history of chocolate, including fair-trade issues that are relevant today. We'll cover its production all the way from its beginning in the cacao pod to the final product, either milk, white, or dark chocolate, and talk about the differences between them," she said.

"Nutritionists and food scientists are also interested in the health benefits of chocolate," she said. "We will demonstrate a nutritional intervention study on the effects of consuming chocolate comparing students to illustrate how such studies are conducted and analyzed."

Engeseth's laboratory also contains tools for making chocolate, including conching, tempering, and molding machines. In the teaching laboratory, students will learn why chocolate behaves as it does during food preparation and compete in an Iron Chef competition.

Also planned are a field trip to a local chocolatier to learn how the experts mold some of their more fanciful and fun chocolate creations as well as participation in a sensory panel in which students will evaluate such qualities as flavor, mouth-feel, and graininess, record their impressions, and relate these to other instrumental analyses.

As part of the project, Engeseth and Melissa Tisoncik, a graduate student in her laboratory, will conduct a short workshop for high-school science teachers, giving them hands-on experience so they can bring some of the activities back to their own classrooms.

Six interns will be chosen to participate in the program. Housing and meals will be provided through support from a USDA CSREES-sponsored integrated grant proposal, but students will need to arrange their own transportation to and from the university, Engeseth said.

Interested students should apply by March 2, and a decision will be reached by March 23, according to Engeseth. For more information, interested students should contact Melissa Tisoncik at uiuc.chocolate@gmail.com.

Appendix C.3 2009 Chocolate Food Science Education Program application

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Summer 2009 Chocolate Education Program Application

The application and all attachments should be filled out fully and submitted by **March 2, 2009**.
Please submit only one application, typed or neatly hand written.

I. Basic Information

Name (last, first, middle):_____
Home Address (street, city, state, zip code):
_____
_____
Home Phone:_____ Cell Phone:_____
Email:_____
High School Currently Attending: _____Current Grade:_____
What is your intended field of study for your future career? _____

II. Additional Information

A. **Grades**- Please attach a current high school transcript (required).

B. **Two Letters of Recommendation** (please enclose in separate sealed envelopes, with signature across the seal)-from either Science Teachers or Guidance Counselors, or both. These letters should include: student's academic record, ability to cooperate, character and personality, capacity for work, and involvement in extracurricular activities.

C. **Resume**-Please enclose a resume that includes all extracurricular activities, leadership positions, awards, work experience/internships, and any other notable credentials.

III. Essay (typed and enclosed with application)

The essay should address the following question:

Why should you be considered for this program?

IV. If there is anything else that should be considered when reviewing this application please indicate it in this section:

Signature:_____ Date:_____

Applications due **March 2, 2009**. Please send letters of recommendation, transcripts, resume, and application in ONE envelope together. Send to **Melissa Tisoncik, 208 Bevier Hall, 905 S. Goodwin Ave., Urbana IL 61801**. Please contact Melissa Tisoncik at uiuc.chocolate@gmail.com with further questions.

Appendix C.4 Informed consent form for parents of program participants

INFORMED CONSENT FORM FOR PARENTS OF CHILDREN PARTICIPATING IN: Summer Chocolate Education Program at the University of Illinois

Your child has been selected and has accepted our invitation to join us for an exciting 2 ½ weeks of immersed education in the Summer Chocolate Program at the University of Illinois, conducted by Associate Professor Dr. Nicki Engeseth (Department Food Science & Human Nutrition) and her graduate students. The six selected high school students will be getting an introductory look into the Food Science and Human Nutrition Program through the study of chocolate. We are very excited and look forward to working with your child.

The program will include activities introducing your student to the University of Illinois, College of ACES and Department of Food Science & Human Nutrition (FSHN). Most activities will take place in FSHN and will include presentations on history, health benefits and production of chocolate as well as demonstrations into sensory evaluation studies and how to conduct human dietary intervention studies. Many facets of food science research will be highlighted.

Possible Risks. The risks associated with this workshop to program participants are not greater than those experienced in daily life. The students will be encouraged to sample foods as part of the process – thus, we have asked that you indicate in the space below any known food allergies.

Benefits. Your child will gain from this experience in many ways. Initially, this will be an exciting, fun, educational summer program. They will be exposed to life at the University of Illinois and will gain exposure to many faculty, staff and students in the University as well as gaining an appreciation for the breadth of programs offered in FSHN and also in the College of ACES. There will also be significant exposure to research in food science and the scientific principles that dictate food quality and consumer perception of foods.

Compensation/Costs associated with the workshop. For the duration of the program, your child will stay in a University Residence Hall. University meals, housing, and fifteen days of workshops will be provided for by a USDA-CSREES NRI competitive grant. The only cost you are responsible for is the travel to and from the University.

Confidentiality. The workshop will be well documented by our laboratory; the University may include your child in possible printed material. This could be printed onto a University affiliated website or brochures and possible educational publications. This material may include pictures of your child. Any information obtained during the Summer Chocolate Program will be kept

strictly confidential and your child's name or any other close personal information will not be identified in any of these materials.

Rights and Responsibilities. Participation by your child is voluntary and they may withdraw from any of the activities, or the program itself if so desired, without any penalty to the child or without any compromise to future relationships with the University of Illinois. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this workshop, nor will the university will not provide compensation for any injury sustained during participation in this research activity, except as required by law.

Whom to Contact/Questions. If you have any questions pertaining to any information stated in this consent form or about the study, please feel free to contact:

Melissa Tisoncik
Chocolate Program Coordinator
MS Graduate Student
University of Illinois
(217) 244-5760
mtisonc2@illinois.edu

or

Nicki J. Engeseth, Ph.D.
Associate Professor
Dept. Food Sci & Human Nutr.
University of Illinois
(217) 244-6788
engeseth@illinois.edu

Cell phone numbers of the resident assistants who will be staying in the residence hall with the students will also be provided along with residence hall contact information in a separate document. If you have any questions regarding subject rights, please feel free to contact the Institutional Review Board office at (217) 333-2670 (irb@illinois.edu). You will be given a copy of this consent form.

Please return this note with your child when they arrive to participate in the summer program on June 15th. You will be given a second copy of this consent form for your records

Parental Consent. I have read and understand the above consent form and voluntarily agree that my child may participate in this study.

_____
(Print) Parent(s) Name(s)

_____ _____
Parent(s) Signature(s) Date

I do/do not (circle one) give permission for **photos** to be taken and published of my child _____ during the program described above.

_____ _____
Parent Signature Date

If there are any known **food allergies** your child has please indicate them below:

Appendix C.5 Informed assent form for program participants

INFORMED ASSENT FORM FOR STUDENTS PARTICIPATING IN: Summer Chocolate Education Program at the University of Illinois

You have accepted and been approved to join us for an exciting 2 ½ weeks of immersed education in the Summer Chocolate Program at the University of Illinois, conducted by Associate Professor Dr. Nicki Engeseth (Department of Food Science & Human Nutrition) and her graduate students. You and five other selected high school students will get an introductory look into the Food Science & Human Nutrition Program through the study of chocolate. We are very excited and look forward to working with you.

The program will include activities introducing you to the University of Illinois, College of ACES and Department of Food Science & Human Nutrition (FSHN). Most activities will take place in FSHN and will include presentations on history, health benefits and production of chocolate as well as demonstrations into sensory evaluation studies and how to conduct human dietary intervention studies. Many facets of food science research will be highlighted.

Possible Risks. The risks associated with this workshop to program participants are not greater than those experienced in daily life. You will be encouraged to sample foods as part of the process – thus, we have asked that you indicate in the space below any known food allergies.

Benefits. You will gain from this experience in many ways. Initially, this will be an exciting, fun, educational summer program. You will be exposed to life at the University of Illinois and will gain exposure to many faculty, staff and students in the University as well as gaining an appreciation for the breadth of programs offered in FSHN and also in the College of ACES. There will also be significant exposure to research in food science and the scientific principles that dictate food quality and consumer perception of foods.

Compensation/Costs associated with the workshop. For the duration of the program, you will stay in a University Residence Hall. University meals, housing, and fifteen days of workshops will be provided for by a USDA-CSREES NRI competitive grant. The only cost you are responsible for is the travel to and from the University.

Confidentiality. The workshop will be well documented by our laboratory; the University may include you in possible printed material. This could be printed onto a University affiliated website or brochures and possible educational publications. This material may include pictures of you. Any information obtained during the Summer Chocolate Program will be kept strictly

confidential and your name or any other close personal information will not be identified in any of these materials.

Rights and Responsibilities. Your participation is voluntary and you may withdraw from any of the activities, or the program itself if so desired, without any penalty to yourself or without any compromise to future relationships with the University of Illinois. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this workshop, nor will the University provide compensation for any injury sustained during participation in this research activity, except as required by law.

Whom to Contact/Questions. If you have any questions pertaining to any information stated in this consent form or about the study, please feel free to contact:

Melissa Tisoncik	or	Nicki J. Engeseth, Ph.D.
Chocolate Program Coordinator		Associate Professor
MS Graduate Student		Dept. Food Sci & Human Nutr.
University of Illinois		University of Illinois
(217) 244-5760		(217) 244-6788
mtisonc2@illinois.edu		engeseth@illinois.edu

Cell phone numbers of the resident assistants who will be staying in the residence hall with you will also be provided along with residence hall contact information in a separate document. If you have any questions regarding subject rights, please feel free to contact the Institutional Review Board office at (217) 333-2670 (irb@illinois.edu). You will be given a copy of this consent form.

Please return this note when you arrive to participate in the summer program on June 15th. You will be given a second copy of this consent form for your records

Student Assent. I have read and understand the above consent form and voluntarily agree that I will participate in this study.

_____

(Print) Student(s) Name(s)

_____ _____

Student(s) Signature(s) Date

I do/do not (circle one) give permission for **photos** to be taken and published of myself during the program described above.

_____ _____

Student Signature Date

_____

If there are any known **food allergies** you have please indicate them below:

_____

_____

Appendix C.6 An example 15 cm line scale used during the mock sensory study lesson

Chocolate Descriptive Panel
RATING SAMPLES

Sample: Dove Dark Chocolate Promise™

Texture Attributes

Hardness (Carrot, Parmesan Cheese)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Fracture/Cohesive (Parmesan Cheese, Mozzarella Cheese)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Chewiness (Rice Cake, Mozzarella Cheese)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Fatty Mouth Coating (Sweetened Condensed Milk)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Dry Mouthfeel (Tannin Solution)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Tooth Packing (Animal Cracker)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

_____

Appendix C.7 Pre-program questionnaire approved by Illinois Institutional Review Board (IRB) and used for program assessment

Summer Chocolate Program 2009 Questionnaire

We are excited to have you in our Summer Chocolate Program. In order to obtain feedback about our program we would greatly appreciate you taking time to fill out this questionnaire. Answering the following questions is completely voluntary. Your answers will be kept confidential and will not be subject to any judgments or consequences. Please answer the following questions as openly and as thorough as possible. This questionnaire will remain anonymous and is solely for feedback about the program.

1. How did you first hear about our Summer Chocolate Program?

2. What interested you the most about the program?

3. After reading the information packet, what activities do you think you will enjoy the most?

4. What do you think will be the least interesting part of the program for you?

5. How do you feel about living at the University of Illinois for 2 ½ weeks with other students with similar interests?

6. Is this the first time you have been away from home for more than a couple days without your family?

7. How supportive were your parents about your participation in this workshop?

8. Describe briefly what you have learned from your high school curriculum that you think will be applied in this program.

9. What do you hope to learn from your participation in the Summer Chocolate Program?

10. What do you think will be a challenge for you in this program? Why?

11. List the science classes you have had previously in high school or other important educational studies.

12. What areas are you currently most interested in for your future career or field of study?

13. Have you ever attended another educational summer program? If yes, please list subject and location.

14. What do you like most about science?

15. Have you worked in teams to conduct science experiments? If yes, what do you think were the biggest challenges? What was the best about working in a team environment?

16. What extracurricular activities are you involved in? Which ones are your favorites?

17. Are you considering applying to the University of Illinois for undergraduate education? If so, indicate what major, if known.

18. Were the materials in the information packet that was provided to you prior to the program useful? If not, please comment on those issues that you did not like or understand.

Food Science Oriented Questions:

19. Define *food science* in your own words.

20. What is cacao? Which country produces the largest amount of cacao per year?

21. What are the health benefits of consuming chocolate?

22. Explain the differences between white, milk, and dark chocolate.

23. If you were craving your favorite chocolate chip cookies, would you use the pictured chips to make your favorite recipe? Explain why or why not…

24. Does chocolate contain caffeine? If so, how much compared to coffee, soda, and tea?

25. What is an emulsifier? Which emulsifiers are used in chocolate?

26. What is your favorite chocolate recipe?

Thank you for your comments!

Appendix C.8 Post-program questionnaire approved by Illinois Institutional Review Board (IRB) and used for program assessment

Summer Chocolate Program 2009 Questionnaire

Thank you for participating in the Summer Chocolate Program!! It was a pleasure to have you here and we hope you are leaving with valuable information. In order to obtain feedback about our program we would greatly appreciate you taking time to fill out this questionnaire. Answering the following questions is completely voluntary. Your answers will be kept confidential and will not be subject to any judgments or consequences. Please answer the following questions as openly and as thorough as possible. This questionnaire will remain anonymous and is solely for feedback about the program.

1. Describe your overall experience participating in the Summer Chocolate Education Program?
2. What did you like best about the program?
3. What did you like the least about the program?
4. What was your favorite lecture/topic covered in the Program? Why?
5. What was your **least** favorite lecture/topic? Why?
6. What educational trip did you like the best? What did you learn while on the trip?
7. Was there a topic presented that you would have liked to have covered in more detail? If so explain.
8. Did this program change your knowledge of/increase your interest in the field of food science? Explain.
9. How did you like living in the University residence hall? What did you like most about it? Least?
10. Were the Residence Assistants helpful answering questions and planning events? Explain.
11. Were there enough activities planned for you to participate in during the Program? Too many?

12. Did you enjoy the interaction, planning and working together with other peers in groups? Explain.

13. Which assignment given throughout the program did you enjoy the most? Which was least enjoyable to you? Explain.

14. What was the most challenging aspect of your participation in the Summer Chocolate Program? Explain.

15. After learning about the different fields within food science (food chemistry, food microbiology, nutrition, food processing and sensory analysis), which of these was most attractive to you? Would you have liked more information on any of these? Explain.

16. Was the laboratory work enjoyable for you?

17. If you could attend another educational summer program, what would be something of interest to you?

Food Science Oriented Questions:

18. Define Food Science in your own words.

19. What is fat bloom? What is sugar bloom?

20. Can you eat chocolate that has bloom on it? Explain.

21. What are the main ingredients in dark chocolate?

22. Name three instrumental analyses used to study the fat bloom on dark chocolate? Explain what each is useful for.

23. Who first discovered chocolate?

24. What are the health benefits of consuming dark chocolate and white chocolate?

25. What does the label “Fair Trade” chocolate mean?

26. Why do manufactures need to temper chocolate?

27. What is an emulsifier? What role does it play in chocolate quality?

28. Would you consider pursuing a career in food science? Explain.

29. What do you like most about science?

30. What do you think was most valuable to you as you leave this program?

Additional Comments/ Suggestions: (here we would appreciate any comments/suggestions you have that would help make the future Summer Chocolate Programs more enriching for high school students)

Thank you for your comments!
We hope you enjoyed yourself and had fun in the Chocolate Education Program!

Author's Biography

Melissa Tisoncik was born in Denver, Colorado and later moved to Barrington, Illinois where her family currently resides. She has been very passionate about chemistry and food her whole life; at the age of 17 she discovered the area of food science while applying to colleges. Since then, she realized that was the career path she wanted to pursue. She attended the small private school Illinois Wesleyan University in Bloomington, IL. In 2007, she earned her Bachelor of Science degree studying chemistry. Following her undergraduate studies, she came to the University of Illinois Urbana-Champaign to pursue a Masters degree in food science in the College of ACES. For her graduate research she worked in Dr. Nicki Engeseth's laboratory and studied her dream subject—dark chocolate. She plans to graduate with a Masters of Science in May 2010. She wants to pursue a career in the confectionery industry and work specifically for a chocolate company. Additionally, in her future quest she plans to obtain a Culinary Certificate in the Pastry Arts and combine this with her research experience and knowledge in the study of chocolate to the confectionery industry.