UNDERSTANDING HEAT RESISTANT STRUCTURE FORMATION
IN WHITE CHOCOLATE

A Thesis in
Food Science
by
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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2013
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Abstract

Heat resistant chocolate is of much interest to confectionery companies for marketing in tropical areas where the weather is not conducive to the sale and consumption of regular chocolate. Patents describe methods to produce heat resistant chocolate by exposing the product to high relative humidity (RH) and increased temperatures, but there is no literature on how this heat resistant structure forms. The overall goal of this research was to explore different instrumental and imaging techniques for studying heat resistant structure formation in white chocolate cured at 83% RH and 29°C.

Screening studies were performed to determine curing conditions for standard white chocolate (30% whole milk powder, 44% sucrose, and 26% cocoa butter) and for the individual ingredients including amorphous lactose, whole milk powder, sucrose, and milk protein isolate. Chocolate bars were cured at 83% RH and 29°C for 7 days, while individual ingredients were cured at the same RH and temperature for 4 hours. In order to understand the role of different emulsifiers in structure formation, white chocolate was made with 0.1% polyglycerol polyricinoleate (PGPR), 0.3% lecithin, or no added emulsifier.

Instrumental techniques measured physical and textural properties of individual ingredients and white chocolate bars before and after curing. Physical properties of white chocolate samples measured were moisture content using Karl Fischer, fat content using Mojonnier method, and force required to penetrate at 29°C using a texturometer. The changes in individual ingredients after curing were evaluated by monitoring moisture content using Karl Fischer as well as phase changes using X-ray diffraction (XRD) and
mid-Fourier transform infrared (FTIR) spectroscopy. Image analysis was performed using scanning electron microscopy (SEM).

Texturometer results showed that post-cured bars required significantly more force to penetrate than pre-cured bars, indicating structure formation had occurred. XRD patterns showed crystallization of lactose by itself and within the whole milk powder particles after curing. Milk protein isolate and sucrose did not exhibit any major phase changes. FTIR spectra corresponded well with XRD patterns.

Identification of individual ingredients in SEM images of white chocolate samples was difficult, so structure formation was inferred based on information from SEM images of individual ingredients. SEM images showed lactose transitioned from its glassy state, became sticky enough to ‘glue’ molecules together, and crystallized into a welded mass. Whole milk powder SEM images also showed lactose crystallization, while milk protein isolate and sucrose SEM images did not show any structural differences after curing. No discernable visual differences in structure were detected based upon emulsifiers used.

This study showed that high humidity is useful in creating heat resistant structure in white chocolate. Analyses of individual ingredients showed that the stickiness observed in lactose within whole milk powder provided the adhesion necessary between lactose molecules and other ingredients to establish a structure in white chocolate. The complimentary nature of XRD and FTIR measurements with SEM showed the usefulness of combining instrumental and imaging techniques to better understand heat resistant structure formation in white chocolate.
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Acknowledgements

I would like to thank the Penn State University Food Science Department and Hershey Co. for sponsoring me and providing facilities, ingredients, and equipment for my thesis. I thank my advisors Dr. Swamy Anantheswaran and Dr. Doug Brown for their mentorship and guidance throughout the whole process. I would also like to extend my appreciation to Dr. John Coupland and Dr. John Floros for serving as members of my committee.

I would like to thank my lab mates, Anneliese Luttmann, Minal Lalpuria, Radhika Ramachandran, Sneha Karthikeyan, and Vaishnavi Chandrasekar for their friendship and support. You girls are amazing and I know you will do many great things in the future. Special thanks to Anneliese and Minal for opening their homes to me whenever I needed a place to stay and a friend to listen.

Thank you to Dr. Anantheswaran, Ms. Lata Anantheswaran, Rohit and Nikhil for the many wonderful dinners, talks, advice, and laughs. A special note to Nikhil: as I know you will stay up late in the night to read my exhilarating thesis, I thought now would be a good time to let you know that upon my graduation, I am released from any obligation to get you a dog. But I do thank you for keeping your end of the bargain.

A special gratitude is due to Mr. Dave Stumpf for his mentorship, guidance, and expertise in imaging techniques. Thanks for the laughs and advice and being my champion through it all. Many thanks to the Penn State Materials Research Institute, in particular Dr. Josh Stapleton, for training me to use FTIR spectroscopy and other imaging techniques.
Thank you to everyone in the Hershey lab including Brenda Sandsjo, Brian Baker, Bruce Kiefer, Linda Wright and the many, many others who always made me feel a part of the family.

Much gratitude is due to the many special people at my undergraduate school, the University of Georgia, including Ms. Anne Nielson, Dr. Robert Shewfelt, Dr. Louise Wicker, and Dr. Aaron Brody. For your support and belief in me, I thank you.

Thank you to the crew in the PepsiCo. international Gatorade lab in Valhalla, NY for allowing me to intern and expand my horizons. Thank you also to my classmates, friends, and roommates at Penn State. I enjoyed getting to know you and look forward to crossing paths again soon. Good luck to you all.

Of utmost importance, I extend my deepest appreciation to my mom and dad, Lee and John Laughter, and my sister, Amy, as well as our many family friends in Macon, GA, for letting me spread my wings and move up north. Much gratitude to Mom, Dad, and Amy for the many research papers you read and did not understand, but still believed I was doing something worthwhile and scientific. Thank you for guiding me through the good times and the rough ones too. I could not have asked for a better family. I think I’ll keep you guys around for a while.
Chapter 1 Introduction

Chocolate is a dispersion of solids in a continuous, semi-crystalline fat phase of cocoa butter. The main types of chocolate are milk, dark, and white. Milk chocolate is a suspension of cocoa solids, milk solids and sugar in cocoa butter. White chocolate also has milk solids and sugar but does not contain non-fat cocoa solids, while dark chocolate includes cocoa solids and sugar but no or very little milk ingredients. The melting profile of chocolate is determined by the fatty phase, most of which is composed of cocoa butter. As the temperature approaches 28-32°C, chocolate begins to soften and lose shape, finally melting at temperatures between 34-36°C. At its melting point, chocolate adheres to wrappers and sticks to hands (Beckett, 2008).

In tropical countries that do not have adequate storage conditions for regular chocolate, heat resistant chocolate has become of interest. Heat resistant chocolate should resist adhering to wrappers at temperatures exceeding 30°C, maintain shape at temperatures above 35°C and not stick to hands at 40°C (Schenk and Peschar, 2004; Beckett, 2008; Best et al., 2007). The finished product should have a flavor comparable to that of regular chocolate (Best et al., 2007).

Many patents describe methods to produce heat resistant chocolate, including exposing chocolate to high relative humidity (RH) and high temperatures (Kempf 1958, O’Rourke 1959). However, there is currently no established methodology to measure and visualize the structure in soft materials such as chocolate, and therefore, to understand the mechanism(s) of heat resistance. This research was conducted using a combination of instrumental and imaging techniques to understand how heat resistance structure forms in white chocolate.
1.1 Objectives

The overall goal of this research was to explore a variety of instrumental and imaging techniques for studying heat resistant structure formation in white chocolate. The specific objectives were:

a. Develop a standard protocol for producing heat resistant white chocolate by curing at:
   i. 50% RH at 29°C
   ii. 83% RH at 29°C

b. Explore the following instrumental and imaging techniques for characterization of heat resistant structure formation in white chocolate:
   i. X-ray diffraction
   ii. Texturometer measurements
   iii. Mid-Fourier transform infrared spectroscopy
   iv. Scanning electron microscopy

c. Investigate the role of the individual ingredients in creating a heat resistant structure.
1.2 References


Chapter 2 Literature Review

2.1 Chocolate Processing

Prior to chocolate processing, cocoa pods are harvested, fermented, dried, cleaned, and roasted as shown in Figure 2.1. The shells are removed to obtain cocoa nibs that are then roasted and pressed. This step results in cocoa liquor and cocoa cake, which is further milled and refined into cocoa powder (Beckett, 2008).

During chocolate production, cocoa liquor, sugar, and part of the cocoa butter are initially mixed together as shown in Figure 2.2. Subsequent refining reduces the particle size of ingredients until the desired smoothness is achieved. Particle sizes between 17-25µm allow for very little to no grittiness within the final chocolate product (Beckett, 2008).

The mixture then enters the conching phase during which undesirable flavors and moisture are removed and optimum viscosity is attained. During this stage, sugar particles and non-fat solids are coated with fat while constantly moving past one another, altering the flow properties of chocolate. Emulsifiers such as lecithin and PGPR are added as lubricants to reduce viscosity. Once the desired viscosity is reached, chocolate is tempered, a technique used to control fat crystallization by forming the most stable crystalline configurations. Cocoa butter can form six crystalline structures with different melting characteristics (Table 2.1). Crystalline form V has the second highest melting point of six crystalline forms and is desired for chocolate to withstand temperatures up to 28-30°C without softening. This critical step greatly affects the final melting point, snap, gloss, and mouth-feel of chocolate (Beckett, 2008).
Table 2.1 Melting point ranges of cocoa butter.

<table>
<thead>
<tr>
<th>Polymorphic Forms</th>
<th>Melting Point Ranges (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form I</td>
<td>16-18</td>
</tr>
<tr>
<td>Form II</td>
<td>21-22</td>
</tr>
<tr>
<td>Form III</td>
<td>25.5</td>
</tr>
<tr>
<td>Form IV</td>
<td>27-29</td>
</tr>
<tr>
<td>Form V</td>
<td>34-35</td>
</tr>
<tr>
<td>Form VI</td>
<td>36</td>
</tr>
</tbody>
</table>

Once tempered, chocolate is molded into tablets or used for enrobing, with excess product removed by shaking or blowing. Finally, the finished product is packaged to protect it from moisture, breakage, dust, animals, and insects (Beckett, 2008).
Figure 2.1 Processing of cocoa beans to cocoa ingredients used in chocolate manufacturing (Beckett, 2008).
Figure 2.2 Manufacturing of milk chocolate (Beckett, 2008).
2.2 Heat Resistant Chocolate

2.2.1 Defining Heat Resistant Chocolate

Heat resistant chocolate and the mechanism(s) enabling it to withstand tropical climates has become of interest to manufacturers wanting to expand their markets. Heat resistant chocolate is defined as having the capability to resist adhering to wrappers at temperatures exceeding 30°C, maintain shape at temperatures above 35°C, and not stick to hands at 40°C (Schenk and Peschar, 2004; Beckett, 2008; Best et al., 2007). Table 2.2 shows three main categories of heat resistant chocolate: mild, intermediate, and extreme. Chocolates classified in these groups maintain their shape and resist sticking to wrappers within the specified temperature ranges (Dicolla, 2009).

<table>
<thead>
<tr>
<th>Category</th>
<th>Temperature (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild heat resistance</td>
<td>26.7-32.2</td>
</tr>
<tr>
<td>Intermediate heat resistance</td>
<td>32.3-37.8</td>
</tr>
<tr>
<td>Extreme heat resistance</td>
<td>37.9 and up</td>
</tr>
</tbody>
</table>

2.2.2 Measuring Heat Resistance of Chocolate

Ether immersion, as described in British Patent 1,219,996 (Pirsch et al., 1971), can be used to establish whether or not chocolate retains its shape. Immersing chocolate in ether for at least 24 hours extracts enough fat that the fat no longer maintains the product’s shape. Heat-resistant chocolate will retain a structure made of non-fat solids, while regular chocolate does not. Hexane may also be used in place of ether for this test.

Best and others (2007) measured the width of a chocolate bar, placed it on a tray and exposed it to 40˚C for 1 hour, after which the tray was dropped onto the floor. The width of the bars was measured again, with heat resistant chocolate showing less deformation than non-heat resistant chocolate. Others used cone penetrometry, vertical
needle, or a Gallenkamp melting point instrument to measure heat resistance of chocolate (Schubiger and Rostagno, 1965; Giddey and Dove, 1984; Ogunwolu and Jayeola, 2006).

Other instrumental measurements provide information about textural characteristics of heat resistant chocolate (Dicolla, 2009). During dynamic mechanical analysis, a static force is applied to a sample while increasing the temperature at a constant rate. This test measures the amount of strain, or deformation, experienced by the sample, with heat resistant chocolate capable of withstanding more stress. It also measures the temperature at which chocolate collapses.

Texture Profile Analysis simulates the movement of jaws when biting and allows for the quantification of texture. A force-time graph provides information about attributes of texture such as gumminess, adhesiveness, cohesiveness, chewiness, and resilience. The three-point bend test is also used to analyze the texture of chocolate. Blades are pressed into the sample at a constant speed, and the resistive force given by the sample is measured. A force versus distance graph indicates snap while a force versus penetration graph shows hardness of the sample (Dicolla, 2009).

Sensory evaluation is another method to characterize heat resistant chocolate and how it is perceived by consumers. Panelists were trained in rating chocolate on several degrees of intensity for 13 attributes including time to melt, firmness to touch, and number of particles, among others (Dicolla, 2009). This information can be useful for the chocolate industry in determining if consumers perceive heat resistant chocolate differently than non-heat resistant chocolate.
2.2.3 Making Heat Resistant Chocolate

Several methods have been created to produce heat resistant chocolate, including the incorporation of higher melting point fats, the addition of moisture, the inclusion of amorphous sugars, the use of polyols, and the application of heat treatments.

Higher Melting Point Fats

Cocoa butter from various parts of the world differs in melting points but is still insufficient in withstanding temperatures over 38°C. Other vegetable fats with higher melting points are sometimes added to chocolate to help maintain shape (Beckett, 2008). A method by Jeyarani and Reddy (1999) incorporates fractionated muhua and kokum fats to increase the solid fat content (SFC) of a cocoa butter blend. While the finished chocolate has higher initial SFC as compared to regular chocolate, the SFC decreases to less than 20% of the total fat phase once the temperature reaches 37.5°C, indicating the product is not able to withstand temperatures much higher than 38°C.

Maheshwari and Reddy (2005) also used kokum fat to increase the melting point of the fatty phase. In this investigation, kokum is not fractionated, but rather refined and blended with cocoa butter to increase SFC. Even with the addition of 5% kokum fat, chocolate is only heat resistant up to 34.8°C.

Bruse, Wallecan and Arruda (2008) describe a method in which no other vegetable fat besides cocoa butter is used to increase heat resistance. Cocoa butter is enzymatically interesterified to achieve a higher melting point than conventional cocoa butter. The interesterified cocoa butter is then added to the fat phase of regular chocolate before conching. Chocolate production follows in the usual manner, with the finished
product being heat resistant up to 42.5°C. The final fat content consists of 1-30% interesterified cocoa butter, 45-99% regular cocoa butter, and 0-20% milk fat.

Nalur and Napolitano (2002) suggest incorporating a higher melting point emulsifier to chocolate to increase heat resistance. The emulsifiers have a melting point between 50-90°C and are added 1-6% w/w to chocolate. The amount of emulsifier depends upon the main fat phase, desired heat resistance, and permitted ingredients.

While incorporating higher melting point fats may increase heat resistance, many countries do not allow fats other than cocoa butter to be used in chocolate. Higher-melting point fats often impart a waxy mouth-feel uncharacteristic of the smooth texture expected in chocolate (Takemori et al., 1992).

**Direct Addition of Moisture**

Moisture is often avoided in chocolate because of its tendency to increase viscosity and impart a gritty mouth-feel to the finished product; however, several methods propose adding small amounts of moisture to chocolate to disrupt the continuous cocoa butter phase. The addition of 1-4% moisture of the total weight of chocolate is optimal. A percentage lower than 1% does not significantly increase heat resistance, while percentages higher than 4% increase viscosity so much that chocolate becomes difficult to process (Beckett, 1999).

The proposed mechanism of moisture addition mainly affects the sugars in chocolate. Sugars dissolve and re-crystallize with neighboring sugar molecules, creating a supporting framework that maintains shape up to 50°C. As cocoa butter melts, the heat resistant sugar skeleton keeps its shape (Beckett, 2008).
One method to incorporate moisture into chocolate is to add small droplets of water to chocolate right before molding, being careful to do this quickly so as not to increase viscosity and encourage hardening of chocolate before the molding process is finished. Using a moist center, such as nougat, is also a way to incorporate moisture into chocolate. Moisture from the nougat migrates outwards towards the chocolate, dissolving and sticking sugar particles together, eventually forming a sugar framework (Beckett, 2008).

Lataner (1949) claims 7-15% moisture can be directly added to chocolate after initial mixing of the main ingredients. During conching, the mass is heated to 66°C (150°F) to dissolve sugar and reduce water content below 2-3%. Chocolate is molded and cooled as usual, after which it becomes heat resistant.

**Indirect Addition of Water**

Water is incorporated into chocolate indirectly by water-in-oil (w/o) emulsions. These emulsions slowly release moisture to avoid rapid direct contact with hygroscopic ingredients so viscosity does not dramatically increase (Beckett, 2008).

US Patent 5,149,560 (Kealey and Quan, 1992) details a process in which 1-3% of a hydrated lecithin w/o emulsion is added to conventionally prepared chocolate to create a heat resistant structure. US Patent 5,486,376 (Alander et al., 1996) describes a similar method in which a w/o micro-emulsion consisting of vegetable fat or cocoa butter, an emulsifying agent, and water is incorporated into conventionally made chocolate at 0.4-3.4% w/w. After molding, the final product holds its shape up to 40°C.

Takemori et al. (1992) explains a process that creates a w/o emulsion consisting of 20-60% hydrophilic substances (i.e. sugar, grape sugar, fruit sugar, malt sugar, starch...
syrup, sugar alcohols, sorbitol, mannitol), 0.1-3% of an emulsifying agent (i.e. lecithin, glycerol fatty acid ester, PGPR, sucrose fatty acid ester), 15-25% water, and 30-60% oil and fats. This emulsion is added to tempered chocolate between 4-10% w/w. After molding and cooling, chocolate is stored for several days during which a heat resistant structure forms.

In US Patent 2,760,867 (Kempf and Downey, 1951), a w/o emulsion is added to chocolate containing skim or non-fat milk solids. The amount of w/o emulsion incorporated depends upon the amount of skim milk solids in the chocolate. Smaller amounts of milk solids require more moisture to encourage milk solids swelling and attaching to one another, creating a heat resistant structure.

US Patent 4,081,559 (Jeffery et al., 1978) describes a w/o emulsion containing at least 15-35% cocoa butter, no more than 5% water, 40% sugar, and up to 1% emulsifier such as lecithin. After exposure to high shear mixing, the emulsion paste is sent to a vacuum belt dryer where it is exposed to temperatures up to 85°C. Afterwards, the material is subjected to microwave heat treatment and becomes heat resistance up to 40-50°C.

European Patent 1,673,977A1 (Simbürger, 2006) suggests using 20-70% water, 20-60% cocoa butter or soybean oil, 30-75% hydrophilic substances (i.e. saccharides, sugar alcohols), and 1-5% emulsifying agents (i.e. lecithin fatty acid esters, PGPR) to create a w/o emulsion. Approximately 5-15% of this emulsion is added to tempered chocolate which is then mixed, molded, and cooled. Frequencies between 2.45 and 5.8GHz from a 3.3kW microwave are supplied to chocolate in two heating cycles of 40-50 seconds each to encourage moisture in the emulsion to interact with ingredients. The
authors state that microwave heating is responsible for producing chocolate instantly heat resistant up to 40-45°C.

In another study, a cross flow membrane emulsification system was used to make 30% water-in-soybean oil emulsions (Killian and Coupland 2012). These emulsions were incorporated into model chocolate made from dispersions of sugar crystals in oil. Liquid bridging occurred between sugar molecules, creating a heat resistant structure.

European Patent 2,186,420 A1 (Hennen et al., 2008) calls for high shearing of cocoa liquor suspended in aqueous solution to form micro-droplets of cocoa butter. Upon freeze- or spray-drying, the non-fat cocoa solids precipitate on the surface of the cocoa butter micro-droplets, creating a strong network that encapsulates cocoa butter, disabling it from secreting out of the network. This paste is added to typical chocolate ingredients, refined, conched, and standardized in the usual manner. Once molded, chocolate is subjected to microwave radiation, forming a chocolate that is heat resistant up to 40-50°C.

Curing chocolate at a high relative humidity indirectly adds water to the system by allowing interactions between hygroscopic ingredients and moisture in the atmosphere. Kempf and Downey (1956) describe a method of adding 2-3% moisture by exposing the finished product to 90-95% RH at 36.7°C for several days. The patent claims the bars are heat resistant up to 48.9°C; however, sugar and fat bloom appear on the bar surface. O’Rourke (1959) also notes keeping bars at 50-70% RH for 2-4 weeks improves heat stability in the end product.
Addition of Humectants

Humectants are hygroscopic materials used to make heat resistant chocolate by encouraging structure formation between hydrophilic ingredients. US Patent 5,108,769 (Kincs, 1992) states polyols, such as glycerin, are effective in imparting heat and bloom resistance to chocolate coatings. Glycerin is particularly useful because it does not impart undesirable flavor to chocolate. Disadvantages of glycerin include its high viscosity and tendency to quickly harden chocolate. To overcome these difficulties, polyols are usually added at the latest possible moment before molding.

US Patent 6,841,186 B2 (Davila and Finkel, 2005) describes adding glycerin to chocolate anywhere between 0.5-20% of the total weight during the conching phase, causing an initial increase in viscosity. With further mixing, viscosity decreases and chocolate is manufactured in the usual manner. The finished product becomes more heat tolerant than regular chocolate within 24 hours.

O’Rourke (1956) explains a method to make heat resistant chocolate by mixing compatible humectants (i.e. corn syrup solids, glucose syrup solids, dextrose, maltose, invert sugar, levulose, xylose, amorphous sugar, glycerin, mannitol, PGPR, sorbitol) and protein (i.e. casein in milk solids, albumen). After tempering, molding, and cooling, chocolate is packaged in moisture-permeable bags and stored in 50-70% relative humidity for 2-4 weeks in order to increase heat resistance (US Patent 2,904,438).

US Patent 5,523,110 (Mandralis and Weitzenecker 1996) explains a method of improving heat resistance in chocolate through the addition of 0.5-10% w/w polyol while maintaining a similar setting rate to that of conventional chocolate. The authors propose freezing a prepared polyol, grinding it in a cold grinder, and adding it before, during, or
after tempering as long as the material remains solid throughout the process. The polyol gradually diffuses throughout the chocolate during storage and imparts heat resistance to the product. Glycerol, mannitol, sorbitol, corn syrup, or combinations therein, are classified as useful polyols in this invention.

US Patent 5,126,160 (Giddey and Dove, 1991) describes incorporating foam made of egg albumin and water to make heat resistant chocolate. Upon initial addition of the foam into chocolate, viscosity greatly increases. With further mixing, chocolate gains pliability and processing continues in the usual manner. This invention claims to not lengthen the time of milk chocolate production.

Ogunwolu and Jayeola (2006) researched the addition of gelatin or starch to increase heat tolerance in chocolate. Starch is used during mixing while gelatin is added towards the end. Chocolate is then processed in the usual manner. The patent claims to increase heat resistance to 50°C with 10% corn starch and 45°C with 10% gelatin. The authors propose that the starch and gelatin increase viscosity so much that melted cocoa butter cannot flow out of the matrix.

**Addition of Amorphous Sugars**

Adding other hygroscopic ingredients such as amorphous sugars is another way to initiate heat resistant structure formation in chocolate. US Patent 3,218,174 (Shubiger and Rostagno, 1965) proposes a method to create a sugar skeleton by mixing a mass of non-conched chocolate containing mostly amorphous sugar with another mass of conventional chocolate containing mostly crystalline sugar. Moisture interacts with the amorphous material that subsequently undergoes its glass transition and eventually crystallizes. Once a crystalline lattice is made, water is released back into the chocolate matrix, and
the process repeats itself. During 10-60 days in storage, this same reaction takes place repeatedly, producing an intact sugar framework.

Another patent, British Patent 1,219,996 suggests a similar procedure as described above except this method calls for the incorporation of 1-10% finely-ground (<20µm) amorphous sugar into conched chocolate containing crystalline sugar. This patent claims that particle size of amorphous sugar directly relates to the resulting heat resistance, with smaller particle size causing greater shape retention at high temperatures (Pirsch et al., 1969).

2.2.4 Role of Moisture in Heat Resistant Structure Formation

Moisture addition has been suggested as a way to make heat resistant chocolate primarily through the formation of a sugar skeleton. The proposed mechanism of a heat resistant sucrose skeleton begins with moisture dissolving sugar. Upon re-crystallization, the sucrose molecules weld together and form a structure. Sucrose crystallization releases moisture, so the dissolution and re-crystallization process repeats itself (Beckett, 2008).

Moisture can play another role in influencing heat resistant structure formation by acting as a plasticizer for glassy materials such as amorphous lactose and protein in milk powders. Although not directly noted in patents, glass transition (Tg) and stickiness can promote structure formation in chocolate. Glass transition is a phenomenon observed when materials transition from their glassy state to their viscous phase. The brittle glass is non-crystalline, highly disordered, and in an out-of-equilibrium state. Only short-range movements are allowed such as vibrations of molecules or small-scale reorientation of atoms, and the molecules move in localized regions. As the system’s temperature approaches and exceeds its Tg, molecules gain enough energy to move independently
from each other, inducing a relaxation capable of molecular backbone translational rotation (Champion et al., 2000).

Plasticizers, such as water and small sugar molecules, wedge their way between molecules in the glassy state and encourage molecular mobility, decreasing Tg. These plasticizers eventually cause enough molecular movement that amorphous ingredients transition into a phase between the glassy and crystalline state known as stickiness. As the molecules continue to gain flexibility, they are capable of interacting with one another and forming inter-particle bridges between adjacent particles (Roos and Karel, 1992).

Several factors can affect the ability of moisture to migrate throughout chocolate and interact with hydrophilic ingredients. Some of these factors include temperature, water activity gradients, and the amount of hydrophilic ingredients (Biquet and Labuza, 1988; Ghosh et al., 2002). Biquet and Labuza (1988) observed that raising the temperature from 20°C to 26°C tripled the water vapor transmission rate in dark chocolate films, while increasing the temperature from 10°C to 20°C did not significantly affect this rate. They postulated that the temperature increase from 20°C to 26°C increased the liquid fat content. Moisture was able to migrate more easily through liquid fat than solid fat. Ghosh et al. (2002) stated that the chemical potential gradient between areas with higher water activity to those with lower water activity is a driving force for moisture migration. The moisture migrates throughout the chocolate until hydrophilic ingredients such as sugars adsorb it.
2.3 Influence of Ingredients on Heat Resistance of Chocolate

2.3.1 Cocoa Ingredients

Cocoa Butter

The composition of cocoa butter contains several different triglycerides, with palmitic-oleic-palmitic, stearic-oleic-stearic, and palmitic-oleic-stearic as the main configurations. The unsaturated chain from the oleic acid is situated in the 2-position on the glycerides. This unique composition gives cocoa butter its sharp melt in the mouth (Beckett, 2008).

The unsaturated fats are not capable of packing as closely together as compared to saturated fats, so they are less heat resistant when introduced to elevated temperatures. As the degree of unsaturation increases, the chocolate becomes more susceptible to melting at lower temperatures. Cocoa butter with higher percentages of saturated fats experiences more efficient molecular packing, and subsequently, higher melting temperatures.

Cocoa Powder

Cocoa powder swells in the presence of sufficient moisture and heat, increasing viscosity and potentially creating structure. Cocoa powder tends to gelatinize around 60-65°C, temperatures sometimes seen during milk and dark chocolate conching (Geilinger et al., 1981). During gelatinization, starch granules swell and burst, releasing starch into the surrounding environment. The bonds between crystalline starch molecules are broken, and the starch granules become sticky, potentially creating a structure.
2.3.2 Sugars

Sucrose

Sucrose is usually added to chocolate in its crystalline state. When it comes in contact with moisture, it dissolves and re-crystallizes. Upon re-crystallization, sucrose molecules stick to their neighboring sucrose molecules, creating a sugar skeleton structure (Killian and Coupland, 2012). During the crystallization process, molecules of moisture are expelled, encouraging the dissolution and re-crystallization process to repeatedly occur (Beckett, 2008).

According to Schmidt (2012), the critical water activity for crystalline sucrose dissolution is between 84.4-85.9%RH at 25°C. In this RH range, sucrose crystals adsorb enough moisture and dissolve forming liquid bridges between themselves. Lowering the RH results in solid bridges and caking once the liquid bridges form. Yuan et al. (2009) also found that sucrose adsorbed very little moisture and remained in its crystalline state when exposed to water activity below its deliquescence point of 85% RH at 25°C.

Lactose

Lactose is the most abundant component in milk products. It is mostly found in amorphous form in chocolate through the addition of milk powders. Amorphous lactose is thermodynamically meta-stable, highly hygroscopic, and can transition to its crystalline state by exceeding its glass transition temperature. Exposure to moisture and high temperatures plasticize glassy, amorphous molecules, allowing for increased molecular mobility. If given enough time, lactose molecules become sticky and form inter-particle bridges (Champion et al., 2000). Eventually, amorphous lactose transitions from this
sticky phase to its more stable, less hygroscopic crystalline state, expelling moisture during the process (Aguilar et al., 1994). This moisture continues to interact with other amorphous lactose molecules, as well as other ingredients, potentially leading to a sticky lactose network.

2.3.3 Milk Ingredients

Milk Fat

Milk fat consists of 26.3% palmitic, 29.8% oleic, 14.6% stearic fatty acids and other fatty acids. It is often incorporated into chocolate as a softening agent and flavor component. Chocolate with both cocoa butter and milk fat melts at lower temperatures because the combination exhibits a eutectic effect (Beckett, 2008). Milk fat can be fractionated into three melting temperature ranges: high, medium and low. One study shows that milk chocolate containing anhydrous milk fat or any of its fractions experiences a decrease in melting point, hardness, melting enthalpy, and solid fat content, with these effects increasing as the amount of milk fat in the samples increases. Milk fats with high melting point fractions, however, contained residual solids above body temperature (Full et al., 1996). Using the highest melting point fraction could increase heat tolerance in chocolate, compared to those containing medium or low melting point fractions, while still maintaining the characteristic flavor and smoothness of chocolate.

Milk Powder

Milk powders are spray-dried or roller dried with both processes producing milk powder particles containing amorphous lactose and protein, as well as encapsulated globules of fat and air. For spray-dried milk powders, milk enters a spray tower through an atomizer that controls droplet size, upon which it is then rapidly dried with hot gas. As
the particles dry, they drop to the bottom of the spray tower where they are expelled (Singh and Heldman, 2009). Lactose molecules in the milk are not given enough time to position themselves in an organized, crystalline fashion (Goff, 2011). They create a continuous, ‘glassy’ matrix in which proteins, fat, and air vacuoles are dispersed and trapped (Ziegler and Langiotti, 2003). This amorphous lactose is unstable and highly hygroscopic when in the presence of moisture.

Roller dried powders are produced by directly heating a thin layer of milk powder on a rotating drum, resulting in a compact powder with little occluded air and much more free fat than spray-dried milk powders (Ziegler and Langiotti, 2003). Free fat could dilute chocolate, reducing heat resistance in terms of viscosity; however, more free fat can also facilitate increased molecular mobility and subsequent ingredient interactions.

Powders with higher vacuole volumes occupy larger spaces of the continuous fat phase, and therefore, increase the viscosity and yield value of chocolate (Keogh et al., 2003). A higher viscosity could lead to a more temperature robust chocolate by entrapping cocoa butter.

Moisture and temperature play an integral role in powder stability and structure. Both parameters encourage lactose to exceed its Tg and become sticky. If given enough time, lactose crystallization can occur, holding the sticky structure into place.

Powder type may also affect heat resistance. Table 2.3 illustrates the different compositions of whole milk powder and skim (non-fat) milk powder. Both powders contain amorphous lactose that can play a role in structure formation if exposed to moisture. Skim milk powder has more amorphous lactose to take part in creating a heat resistant structure than whole milk powder (Anonymous, 2005).
Table 2.3 Compositions of milk powders. (Anonymous, 2005).

<table>
<thead>
<tr>
<th>Whole Milk Powder</th>
<th>Skim Milk Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.5-27% protein</td>
<td>34-37% protein</td>
</tr>
<tr>
<td>36-38.5% amorphous lactose</td>
<td>49.5-52% amorphous lactose</td>
</tr>
<tr>
<td>26.6-40% fat</td>
<td>0.6-1.25% fat</td>
</tr>
<tr>
<td>5.5-6% ash</td>
<td>8.2-8.6% ash</td>
</tr>
<tr>
<td>2-4.5% moisture</td>
<td>3-4% moisture</td>
</tr>
</tbody>
</table>

Milk Proteins

Proteins within milk powders constitute about 25-38% of the total non-fat solids, with 80% of the proteins consisting of casein and the rest consisting of whey protein. Amorphous protein in combination with reducing sugars such as lactose can undergo a browning process known as the Maillard reaction at elevated temperatures. Water is generated from this reaction, leading to an increase in the moisture content and potentially promoting structure formation (Wursch et al., 1984).

Proteins can also delay lactose crystallization, a process that can impart a gritty texture to chocolate. Non-covalent hydrogen bonds form between proteins and sugars presenting competition to lactose reacting with itself during nucleation (Morgan et al., 2004). According to Kalichevsky et al. (1993), casein acts as a physical barrier, interrupting lactose crystallization. The inhibition of crystallization, however, does not mean lactose cannot transition into its sticky phase, causing structure formation.
2.3.4 Emulsifiers

Lecithin and polyglyceryl polyricinoleate (PGPR) are the most common emulsifiers used to standardize viscosity and yield value of chocolate respectively (Holdgaard 2012). Lecithin significantly reduces viscosity when used between 0.35-0.4%; however, beyond that level, the yield value of chocolate increases. PGPR is effective in decreasing yield value, but it has little effect on the viscosity. The two emulsifiers are often used in synergistic combination to achieve the optimal flow properties (Holdgaard 2012).

Ziegler et al. (2003) found that lecithin and PGPR behave in different manners when added to sugar dispersions. The polar heads of lecithin molecules adsorb to the surface of sugars in a multi-layer domain. At water activities above 0.7, lecithin can form reverse micelles, adding to its structural complexity. The polar portions of PGPR also adsorb to the surface of hydrophilic ingredients, but these polar parts are interspersed along the highly branched PGPR molecule. Instead of a multi-layer domain, the absence of a polar head causes the hydrophilic groups on PGPR to form a loosely bound film around sugar molecules while the hydrophobic portions protrude into the fatty phase. According to Rector (2000), these hydrophobic portions are primarily composed of polyricinoleic acid chains that repel each other, keeping the solid particles separated.
2.4 Influence of Unit Operations on Formation of Heat Resistant Structure

2.4.1 Mixing

Sugar, cocoa solids, milk solids, and a portion of cocoa butter are initially mixed together in preparation for refining. During this step, any moisture introduced by ingredients can alter the behavior of amorphous materials by acting as a plasticizer, leading to agglomeration and stickiness.

2.4.2 Refining

The mixed mass is then sent through a refiner to reduce particle size. During refining, solid particles travel through a series of rolls heated to about 40°C and running at various speeds and pressure. Adjusting the refiner settings to achieve smaller particle sizes increases the overall surface area for inter-particle interactions to occur and form structure.

2.4.3 Conching and Standardizing

Conching allows for flavor development, moisture evaporation, and lubrication of non-fat solids with cocoa butter. The duration of conching is important in that if it is not long enough, moisture remains in the system and interacts with hygroscopic ingredients. Additionally, if emulsifiers are added too soon, they can trap moisture in chocolate, causing a higher than desired viscosity and inter-particle friction (Ziegler et al., 2003).

2.4.4 Tempering

Tempering is a process during which chocolate is thermally treated to produce highly stable fat crystals of desired type and size. Long chain tri-saturated
triacylglycerols crystallize first, increasing viscosity because less liquid fat is available. Saturated-unsaturated-saturated triacylglycerols crystallize next, giving chocolate its temper and providing stable polymorphic crystals. Proper tempering promotes the formation of crystal form V, the more heat tolerant polymorph (Beckett, 2008).

2.4.5 Molding

After tempering, chocolate is poured into molds to create shaped products, or, it is used for enrobing centers.

2.4.6 Packaging

Moisture permeable packages allow atmospheric moisture to contact chocolate products, perhaps leading to structure formation over time. Moisture impermeable packages do not let moisture in direct contact with chocolate, but they do not inhibit moisture within chocolate playing a role in structure formation.

2.5 Instrumental Techniques to Characterize Structure

Instrumental measurements can be useful in understanding how individual ingredients create a heat resistant structure.

2.5.1 X-ray Diffraction

Drapier-Beche et al. (1996) used X-ray diffraction to detect changes in amorphous and crystalline material when exposed to high relative humidity. It was particularly useful in monitoring changes from amorphous to crystalline state in amorphous lactose and milk powders. Many other studies have also used X-ray diffraction to study the crystallization of amorphous material (Barham and Hodnett, 2005; Elamin et al., 1995; Haque and Roos, 2005; Roos and Karel, 1992).
2.5.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) spatially resolves chemical and structural details in food. It can identify unknown samples, quantify the amount of constituents in a mixture, and measure the quality of a sample. There are two main modes of FTIR: transmission and attenuated total internal reflectance (ATR). During transmission, a thin layer of sample is spread onto a crystal. Infrared radiation is transmitted through the sample, with some of the IR radiation absorbed and the rest of it passing through the sample, resulting in a spectrum. This information represents the absorption and transmission unique to a particular molecule (Anonymous, 2001).

During ATR, a germanium or diamond crystal comes in direct contact with a sample and infrared light is reflected back and forth between the crystal and the sample, penetrating into the sample between 0.5-2 µm, depending on the properties of the sample. The beam exits the crystal and is collected by a detector. Since the ATR crystal remained in direct contact with the sample during the analysis, distortion of images is reduced because the IR beam did not travel through air; however, sample structural integrity is compromised (Anonymous, 2001).

FTIR provides several advantages over other dispersive instruments, one of which is faster scanning times. Most FTIR measurements occur within seconds while other techniques require several minutes. This speed is due to a simple optical device known as an interferometer that produces a certain type of signal encoded with all of the infrared frequencies, resulting in a signal that can be measured rapidly. FTIR is highly sensitive, less prone to mechanical breakdowns, and incredibly reliable in positively identifying samples (Anonymous, 2001).
FTIR spectroscopy identified different crystalline forms of lactose as well as distinguished between amorphous and crystalline lactose (Islam and Langrish, 2010). It has been used to determine the amount of carbohydrate, fat, protein, moisture, and cocoa content in chocolate (van de Voort et al., 1993).

2.6 Imaging Techniques

Currently, there are no proposed methods to visualize the structure of heat resistant chocolate. However, many imaging techniques have been used to understand structural developments in food, including those in chocolate.

Light microscopy transmits or reflects visible light from samples through lenses to create a magnified image. Researchers determined the effects of tempering and fat crystallization on the microstructure of dark chocolate (Afoakwa et al., 2008) as well as detected composition and structure of fat bloom in un-tempered chocolate (Kinta and Hatta, 2005). Light microscopy images showed differences in crystalline networks in dark chocolate refined to various particle sizes (Afoakwa et al., 2009).

X-ray micro-computed tomography is a non-invasive, non-destructive technique that uses x-rays to image cross-sections of a sample to create 3-dimensional images. Frisullo et al. (2010) imaged the microstructure of Italian aerated chocolate containing different amounts of cocoa solids, with air bubbles being the most distinct structural component. In another study, X-ray micro-CT imaged the distribution of gas bubbles in chocolate to understand the effects of carbon dioxide, nitrogen, nitrous oxide, and argon on sensory properties of milk chocolate (Haedelt et al. 2007).

Atomic force microscopy (AFM) is a useful tool in understanding structure in chocolate, most extensively fat bloom formation. AFM is a high-resolution scanning
microscopy technique that consists of a probe scanning sample surfaces and measuring contact forces such as van der Waals and chemical bonding. Researchers have analyzed the formation of fat bloom in dark and milk chocolate during long storage time, imaged effects of temperature fluctuations on fat bloom formation in dark chocolate filled with hazelnut oil, and determined effects of temperature abuse on the surface topography of chocolate (Svanberg et al., 2011; Sonwai and Rousseau, 2008; Hodge and Rousseau, 2002).

Confocal laser scanning microscopy (CLSM) images a sample over a depth of field, which is later reconstructed into a 3-dimensional image. Fluorescent dyes are often used to make selected items more visible. CLSM images showed the effects of sucrose, cocoa particles, and lecithin during fat crystallization in seeded and non-seeded chocolate model systems (Svanberg et al., 2011). Auty et al. (2001) imaged the distribution of fat and protein in cheeses, milk chocolate, and milk powders, while Altimiras et al. (2007) imaged changes in microstructure of cocoa butter model bars during storage.

Scanning electron microscopy (SEM) has been used extensively in food research because of its ability to provide detailed images of structure with resolution as low as 1-50nm (Bogner et al., 2007). Samples are bombarded with a high-energy beam of electrons that interact with atoms on the surface of the sample. A computer then produces images of surface topography from the electron excitation feedback. Samples are either coated in gold to improve resolution in traditional SEM procedures. If they are left uncoated and imaged in a gaseous environment in environmental SEM (ESEM), with the main disadvantage being reduced resolution. Dahlenborg et al. (2011) investigated the use of low-vacuum SEM in imaging fat bloom after chocolate samples were subjected to
cycled temperature storage for 36 weeks. James and Smith (2009) imaged the evolution of bloom formation on well-tempered, poorly tempered, and un-tempered dark chocolate, including detailed characteristics of surface roughening.

In another study, processing effects on cheeses were investigated using cryo-SEM and ESEM. Microstructure was elucidated by both SEM techniques; however, ESEM was more useful in determining the ‘true’ structure in cheese because it allowed moisture to remain in the food matrix (Noronha et al., 2008). Environmental SEM was also used to uncover the surface structural evolutions of milk powders containing different amounts of sucrose and refined at various temperatures (Lay Ma et al., 2008). Wursch et al. (1984) used traditional SEM to image different crystalline forms of lactose.

Recent developments in FTIR spectroscopy use spectral measurements and image analysis to produce pictures of microstructure. Noronha et al. (2008) imaged the spatial distribution of fat, protein, and starch in cheeses containing native, pre-gelatinized, resistant, or waxy corn starches. Compared to SEM and light microscopy, FTIR provided more information about the processing effects on the cheeses and positively identified the different ingredients used during cheese manufacture. Cremer and Kaletunç (2003) also used FTIR imaging to study the distribution of starch, protein and lipid in the microstructure of extrudates prepared from corn and oat, while Kirschner et al. (2004) analyzed the denaturation processes in beef loin undergoing the aging process.
2.7 References


Kempf NW and Downey PJ, inventors; General Foods Corporation, assignee. 1956. Finished chocolate product. US 2760867.


Chapter 3 Materials and Methods

3.1 Materials

Sucrose and lactose were obtained from Domino Sugar (Arabi, LA). Non-fat milk powder and whole milk powder came from Land O’ Lakes (Carlisle, PA). The American Casein Company provided milk protein isolate (Burlington, NJ). Cocoa butter was provided by Hershey Foods Company (Hershey, PA). To prepare amorphous lactose, 200g of crystalline lactose were dissolved in 800g of DI water heated to 60°C. This solution was poured drop wise into an isolated container filled with liquid nitrogen to flash freeze the lactose. The amorphous lactose was then placed into aluminum boats inside a Virtis Freezemobile 6 freeze dryer (Gardner, NY) set at 20mmTorr and -58°C. The lactose remained in the freeze-dryer for 5 days, after which it was placed into 0% RH in a desiccator containing P₂O₅ (J.T. Baker, Phillipsburg, NJ). X-ray diffraction and polarized optical microscopy were used to characterize the lactose as amorphous.

3.2 Manufacture of White Chocolate

White chocolate production began with initial mixing of sugar, non-fat milk solids, and cocoa butter in a Hobart 12-qt. bowl and mixer. Formulas for the various white chocolate samples made are listed in Table 3.1. Heat was applied during mixing until the temperature of the chocolate was 40±3°C. After the mixed mass became uniform, it was double refined using a Buhler 3-roll refiner to achieve a particle size of 18±2µm. A small sample of flakes was mixed with mineral oil in equal volume amounts with a spatula until the flakes were dispersed in the oil. Particle size was measured with a handheld Mitutoyo IP 65 micrometer (Mitutoyo Corporation, Kawasaki, Kanagawa, Japan) to determine if adjustments needed to be made to the refiner settings to reach the
desired measurement. The refined flake was then mixed with cocoa butter so the total fat in the chocolate mass was 26-28%. Conching followed and continued for three hours at 50°C. The mass was then standardized by adding the remaining cocoa butter and lecithin.

Table 3.1 Formulas for white chocolate samples with whole milk powder.

<table>
<thead>
<tr>
<th>Lecithin</th>
<th>PGPR</th>
<th>No added emulsifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.9% whole milk powder</td>
<td>29.97% whole milk powder</td>
<td>30% whole milk powder</td>
</tr>
<tr>
<td>43.9% sucrose</td>
<td>43.96% sucrose</td>
<td>44% sucrose</td>
</tr>
<tr>
<td>25.9% cocoa butter</td>
<td>25.97% cocoa butter</td>
<td>26% cocoa butter</td>
</tr>
<tr>
<td>0.3% lecithin</td>
<td>0.1% PGPR</td>
<td>no emulsifier added</td>
</tr>
</tbody>
</table>

Finally, chocolate was tempered using Sinsation K5 tempering machines (Chocovision, Poughkeepsie, NY). The standardized chocolate was placed in the tempering machine where it was allowed to heat to 40°C. During this time, seed was made by spreading a thin layer of 15% of standardized chocolate onto a marble table at 21°C in a darkened room. The layer was cut into small pieces, becoming seed added to chocolate as it slowly cooled to 31°C. Once the temperature became constant and seed was thoroughly mixed into the chocolate, a Tricor Systems Inc. chocolate temper meter (Elgin, IL) was used to determine whether or not the chocolate was properly tempered as determined by a slope of 0±1. A small cup was filled with the prepared chocolate and placed inside the Tricor sample chamber. After seven minutes, the slope and chocolate tempering units of the sample were obtained.

Chocolate was then poured into tablet molds on a vibrating table to remove air bubbles. The bars were placed in a cooler at 10°C for 15 minutes. Once set, the bars were
taken out of their molds and packaged either in heat sealed aluminum moisture impermeable bags or heat-sealed plastic films. The bars were stored at 18°C until used for testing. All bars were made in triplicate.

3.2.1 Emulsifier Study

A study was performed to understand how common percentages of emulsifiers used in industrial manufacturing affected heat resistant structure formation in white chocolate. The levels of emulsifier are shown in Table 3.1.

3.2.2 Curing Conditions

An initial screening study was performed with white chocolate samples placed in either 18°C or 29°C both at 50% RH for 12 weeks. Samples at both temperatures indicated structure formation, with those at 29°C exhibiting more.

To expedite the process, white chocolate bars were placed into a jar containing 83% RH KCl salt slush at 29°C for 7 days (Greenspan 1977). The KCl salt slush provided a suitable relative humidity environment to promote heat resistant structure formation without causing deliquescence of sucrose, which occurs at ~85% RH and 25°C (Listiohadi et al., 2005). Small crystals of thymol were placed in the curing jars to inhibit mold growth. The samples were then placed onto an aluminum stage and into a beaker containing hexane for fat extraction.

For the analysis of individual ingredients, several samples of 3.0g of white chocolate were spread into a 1.3cm tall and 4.4cm diameter cups sufficient to cover the bottom and cured for various times until structure formation was determined by hexane
immersion (Schubiger and Rostagno, 1965). After 4 hours at 83% RH and 29ºC, the smear exhibited structure formation. Individual ingredients were then placed in the same curing conditions for 4 hours and analyzed for changes pre- and post-cure.

3.3 Instrumental Measurements

3.3.1 Moisture Content

In order to evaluate the amount of moisture in samples, Karl Fischer was used. The instrument comprised of a 784 KFP Titrino titrator with a 703 Titration stand (Brinkmann, Westbury, NY). This method involves a reaction between sulfur dioxide, iodine, and water within a solvent made of methanol:formamide:chloroform (2:2:1) as shown below.

\[
I_2 + SO_2 + H_2O + CH_3OH + 3py = 2pyH+I^- + pyHSO_3OCH_3
\]

Through the use of electrodes monitoring the titration, the reaction takes place under controlled conditions. Once the reaction is complete, no free moisture should remain in the sample. Using the weight of the sample and the amount of reagent used, a percentage of moisture contained within the food before the Karl Fisher method was applied was calculated using the equation below.

\[
\text{Factor} = \left( \frac{\text{weight of water/mL titrated}}{\text{mL titrated}} \right) \times 100
\]

\[
\%H_2O = \frac{\text{mL titrated} \times \text{factor}}{\text{weight of sample}}
\]
3.3.2 Fat Content

Through a series of extractions, the Mojonnier procedure allows for the quantification of fat in chocolate. The sample undergoes its first fat extraction using 1.5mL ammonia, 10mL ethyl alcohol, 25mL ethyl ether, and 25mL petroleum ether. The next extraction involves 5mL ethyl alcohol, 15mL ethyl ether, and 15mL petroleum ether. Finally, the third extraction calls for 15mL ethyl ether and 15mL petroleum ether. The extractions are used to induce a phase separation between a mixture of ethers and the sample in aqueous phase. After all three extractions, the fat is dried, weighed, and calculated as percent fat of the sample. The following equation is used to calculate fat content:

\[
\text{Fat weight} = (\text{pan} + \text{residue}) \text{ – empty pan}
\]

\[
\% \text{ Fat} = \left( \frac{\text{fat weight}}{\text{sample weight}} \right) \times 100
\]

3.3.3 X-ray Diffraction

X-ray diffraction was used to observe a change in phase of structure in individual ingredients before and after curing. It was also used to confirm the amorphous state of lactose. Samples were packed and leveled into a 2cm x 2cm x 0.5cm cell, then placed into the goniometer stage. The Phillips X-ray diffractometer (Westborough, MA) was set to measure between 10 to 26 2\(\Theta\). The X-rays from copper emissions passed through slits, collimated, and became finer, more intense beams. X-rays hit the sample, diffracted, and traveled to another set of slits where they were further collimated in the detector. The alignment of molecules within the ingredients were measured and illustrated in an
intensity vs. 2Θ graph. Static noise with no distinguishable peaks indicated the presence of amorphous material. All measurements were performed in triplicate.

3.3.4 Texturometer

Stable Micro Systems Texture Analyzer TA-XT2 (Surrey, UK) and the software Texture Exponent 32 (Stable Micro Systems, Surrey, UK) were used to measure the amount of force required to penetrate pre- and post-cured white chocolate samples. The instrument was calibrated for force and probe position. Molded white chocolate samples (9cm x 3cm x 1.5cm) were placed on the sample stage underneath a 2mm diameter blunt stainless steel probe. Figure 3.1 shows a white chocolate sample during a texturometer test.

![Figure 3.1 Typical texturometer setup.](image)

Once curing was completed, samples were immediately measured with the texturometer to capture structure formation at the curing temperature of 29ºC. The probe
was lowered onto the sample at 1mm/s to a distance of 10mm below the surface. The probe was then returned to starting position at 10mm/s, ending the test. Measurements were performed in triplicate. The equipment and textural parameters were controlled and calculated using the software Texture Exponent 32 from the force-time graph generated by running a programmed macro. Settings for the test are listed in Table 3.2.

Table 3.2 Texturometer parameters of the TA-XT2 Texture Analyzer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test speed</td>
<td>1.0 mm/sec</td>
</tr>
<tr>
<td>Target mode</td>
<td>Force</td>
</tr>
<tr>
<td>Distance</td>
<td>10mm</td>
</tr>
<tr>
<td>Trigger type</td>
<td>Auto (force)</td>
</tr>
</tbody>
</table>

3.3.5 Fourier-Transform Infrared Spectroscopy

FTIR spectral measurements were obtained using a Bruker Optics (Billerica, MA) as seen in Figure 3.2 and analyzed with OPUS software (Bruker Optics, Billerica, MA). The samples were placed in contact with the ATR element at room temperature. FTIR data were collected over the region 4000-400 cm\(^{-1}\) by collecting 100 scans at a resolution of 6 cm\(^{-1}\). All spectra were ratioed against a background air spectrum and stored as absorbance values at each data point. All samples were scanned in triplicate. Each ingredient was allocated a specific absorption band for identification. Wave numbers between 1720-1480 were used to characterize proteins, and wavenumbers between 1200-800 cm\(^{-1}\) were used to identify any changes in sugars (Hogan and O’Callaghan, 2010). Wavenumbers 916, 899, and 876 cm\(^{-1}\) were used to specifically identify the presence of \(\alpha\)-
lactose monohydrate, while vibrations at 948, 892, 877, and 833 cm\(^{-1}\) identified β-lactose (Kirk et al., 2007).

Figure 3.2 ATR equipment setup for spectral measurements.

3.4 Imaging Techniques

3.4.1 Sample Preparation for Scanning Electron Microscopy

White chocolate samples (9cm x 3cm x 1.5cm) were placed into a jar containing 83% RH KCl saturated salt slush at 29°C for 7 days. The samples were then placed onto an aluminum stage and into a beaker containing hexane for fat extraction for 24-hour immersion.

3.4.2 Scanning Electron Microscopy Imaging

A Philips SEM (Portland, OR) was used to examine the structure of heat resistant chocolate. After 24 hours hexane extraction, the remaining skeleton was coated in gold.
and placed into the microscope sample chamber. Images were performed at full vacuum and 8kV at various magnifications.

3.4.3 Polarized Optical Microscopy

Sample material was mixed with lecithin and spread into a thin smear on a light microscope slide and evaluated using a polarized optical microscope (Leica Laborlux D, Buffalo Grove, IL). Under the 100x objective lens, the freeze-dried lactose and milk powder samples were examined for any birefringent material indicating the presence of crystals.

3.5 Statistical Data Analysis

Results from fat content, moisture content, and texturometer results of bars were analyzed using one-way ANOVA and Tukey’s post-hoc test. The moisture content of individual ingredients was analyzed using paired t-tests. All statistics were performed using MINITAB Student Release 14 (version 14.11.1, State College, PA).
3.6 References


Chapter 4 Results and Discussion

4.1 Screening Study

The combination of high relative humidity and increased temperatures can promote the formation of heat resistant structure in chocolate (Kempf and Downey 1956, O’Rourke 1959). In this screening study, white chocolate bars were cured at 18ºC/50% RH or 29ºC/50% RH for 12 weeks. After curing, samples were immersed in hexane for 24 hours, and the de-fatted structures were imaged using SEM. Compared to control bars that were not cured, these samples exhibited a skeleton, indicating that both curing conditions were adequate in creating a heat resistant structure.

Figure 4.1 shows samples before and after hexane immersion. White chocolate cured at 18ºC/50% RH showed slight collapse in the middle as compared to samples cured at 29ºC/50% RH. Since both samples were stored at 50% RH, curing at 29ºC may have increased moisture migration into the samples, leading to more interactions between ingredients and creating a sturdier structure than samples cured at 18ºC which showed collapse in the middle.

Figure 4.1 White chocolate cured for 12 weeks at 18ºC/50% RH A) before hexane immersion and C) after hexane immersion. White chocolate cured for 12 weeks at 29ºC/50%RH B) before hexane immersion and D) after hexane immersion.
After hexane immersion, the outer shells, presumably the most heat resistant portion, were imaged using SEM. Figure 4.2 shows structure formation at two different locations in the outer shell of the sample.

![Figure 4.2 SEM images (different views) of hexane immersed white chocolate cured at 29°C/50%RH for 12 weeks. Each tic mark represents 0.5µm.](image)

While curing at 29°C/50%RH for 12 weeks was sufficient in creating a heat resistant structure, the effect of increasing relative humidity in expediting the curing process was investigated. White chocolate made with 0.3% lecithin, 0.1% PGPR, or no added emulsifier was cured at 29°C/83%RH for 7 days. All cured samples exhibited structure after 24 hours in a hexane immersion (Figure 4.3). In Figure 4.4, SEM images of samples with 0.3% lecithin show structure formed between ingredients.
Figure 4.3 Hexane immersed white chocolate made with different emulsifier types. A) Control white chocolate and white chocolate cured at 29°C/50%RH for 7 days with B) 0.3% lecithin, C) 0.1% PGPR, and D) no added emulsifier.

Figure 4.4 SEM images (different views) of white chocolate with 0.3% lecithin cured at 29°C/83%RH for 7 days. Each tic mark represents 5µm.

For a more in-depth understanding of how this structure was formed, the role of individual ingredients was investigated. Because the individual ingredients were directly exposed to the curing temperature and RH, as opposed to being within dispersion, less time was used to not exaggerate the effects of the curing process. In order to decide this time, another screening study was performed during which 3g of white chocolate were poured into a 1.3cm tall and 4.4cm diameter cup to create a 3mm thick layer and were cured for different lengths of time. The sample cured for 4 hours at 29°C and 83% RH
had structure and could be removed from the sample cup as shown in Figure 4.5. Using this information, individual ingredients were placed into similar sample cups and cured at 29°C/83% RH for 4 hours.

![Image of white chocolate samples](image)

**Figure 4.5 Hexane immersion of 5mm thick cured white chocolate. A) Control white chocolate after 24 hours hexane immersion; B) white chocolate after 3 hours at 29°C/83%RH followed by 24 hour hexane immersion; C) white chocolate after 4 hours at 29°C/83%RH followed by 24 hour hexane immersion (sample has been removed from sample cup).**
4.2 Analysis of Instrumental Measurements

4.2.1 Fat Content

The amount of fat can influence the rate of moisture migration into chocolate (Ghosh et al., 2004). Table 4.1 shows the fat content of white chocolate bars made with lecithin, PGPR, or no added emulsifier. There were no significant differences in fat content between samples measured.

Table 4.1 Fat content measured by OICC, AOAC official Mojonier fat extraction method for white chocolate samples. Same letter implies no significant difference ($\alpha=0.05$).

<table>
<thead>
<tr>
<th>White chocolate samples</th>
<th>Fat Content (g fat/100g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>$35\pm1.1^a$</td>
</tr>
<tr>
<td>PGPR</td>
<td>$35\pm0.72^a$</td>
</tr>
<tr>
<td>No added emulsifier</td>
<td>$35\pm1.4^a$</td>
</tr>
</tbody>
</table>

4.2.2 Moisture Content

Moisture content of individual ingredients and white chocolate samples were measured using Karl Fischer titration. Table 4.2 shows the moisture content of white chocolate bars containing lecithin, PGPR, or no added emulsifier. The samples were not significantly different before curing; however, after curing at 83% RH and 29°C for 7 days, the samples experienced a significant increase in moisture content.
Table 4.2 Moisture content measured by Karl Fischer in wet and dry basis of white chocolate samples before and after curing. Same letter implies no significant difference (α=0.05).

<table>
<thead>
<tr>
<th>White chocolate samples</th>
<th>Moisture Content (g moisture/100g wet basis)</th>
<th>Moisture Content (g moisture/100g dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin pre-cure</td>
<td>0.92±0.086&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93±0.086&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lecithin post-cure</td>
<td>4.6±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PGPR pre-cure</td>
<td>0.77±0.067&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.067&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PGPR post-cure</td>
<td>5.1±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.4±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No added emulsifier pre-cure</td>
<td>0.82±0.038&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83±0.038&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>No added emulsifier post-cure</td>
<td>4.2±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Ghosh et al. (2002) stated that the rate of moisture migration is affected by several factors including the chemical potential gradient between areas with higher water activity to those with lower water activity. When moisture migrates to hydrophilic ingredients, adhesive forces between the polar groups and moisture favor adsorption until equilibrium is reached. In the current study, subjecting samples to 83% RH may have provided the driving force for moisture migration into the bars. This moisture was adsorbed by hydrophilic ingredients, as evidenced by the significant gain in moisture content after curing.

Temperature also influences moisture migration. Biquet and Labuza (1988) observed that raising the temperature from 20°C to 26°C tripled the water vapor transmission rate in dark chocolate films, while increasing the temperature from 10°C to 20°C did not significantly affect this rate. They postulated that the temperature increase from 20°C to 26°C increased the liquid fat content, making moisture migration easier throughout the dark chocolate films. Samples in the current study were cured at 29°C,
perhaps increasing the liquid fat content, resulting in increased diffusion rate of moisture into the chocolate.

Table 4.3 shows the moisture content of the individual ingredients before and after curing at 29°C and 83% RH for 4 hours. Sucrose was the only ingredient that did not experience a significant increase in moisture content. This lack of significant moisture gain can be attributed to sucrose being cured in its stable, crystalline form below its deliquescence point of 85%RH at 25ºC (Schmidt, 2012). Yuan et al. (2009) found that sucrose adsorbed very little moisture and remained in its crystalline state at water activity gradients between 0.75-0.80. Moisture traveled along the surfaces of the sucrose molecules but did not dissolve them. When exposed to water activity above its deliquescence point of 0.85, sucrose began to adsorb moisture quickly and dissolve.

Table 4.3 Moisture content measured by Karl Fischer of individual ingredients before and after curing. Same letter implies no significant difference (α=0.05) between pre- and post-cured for each ingredient.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Moisture Content (g moisture/100g wet basis)</th>
<th>Moisture Content (g moisture/100g dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose pre-cure</td>
<td>0.20±0.040&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20±0.040&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrose post-cure</td>
<td>0.24±0.071&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24±0.071&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amorphous lactose pre-cure</td>
<td>2.3±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amorphous lactose post-cure</td>
<td>6.8±0.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3±0.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk protein isolate pre-cure</td>
<td>8.1±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.8±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk protein isolate post-cure</td>
<td>11±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WMP pre-cure</td>
<td>3.4±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WMP post-cure</td>
<td>8.9±0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8±0.015&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Amorphous lactose and whole milk powder significantly gained moisture during the curing process. When exposed to high RH, highly hygroscopic amorphous lactose readily adsorbs moisture. Milk powders containing amorphous lactose are similarly affected. If enough moisture is adsorbed, both ingredients undergo their glass transition and become sticky (Joupilla and Roos, 1994). This phenomenon leads to inter-particle bridging that could be potential in creating a heat resistant structure.

Joupilla and Roos (1994) measured sorption isotherms of amorphous lactose and milk powders. Lactose stored at 24°C crystallized at a critical water activity of 0.37. Milk powders with lactose also experienced crystallization at a similar water activity. The critical water contents for lactose crystallization was 6.8g water/100g solids in pure lactose and 7.6g water/100g solids for milk powders containing lactose. The authors concluded that enough moisture was adsorbed, plasticizing the amorphous material, depressing the glass transition temperature below ambient temperature, and causing lactose crystallization. The current research shows post-cured amorphous lactose and WMP had moisture contents similar to or exceeding those found in the previous study, indicating conditions were favorable for lactose stickiness to occur followed by crystallization.

Milk protein isolate experienced a significant increase in moisture content during the curing process. It is composed of about 80% casein and 20% whey protein. Caseins contain both hydrophilic and hydrophobic constituents (Goff, 2011); however, it is mostly considered a hydrophobic entity. Whey protein is slightly soluble and may adsorb moisture as would milk salts present in the powder.
4.2.3 Texturometer

Figure 4.6 Typical texturometer graph.

Figure 4.6 shows a typical texturometer graph of cured white chocolate.

Texturometer data was used to compare post-cured white chocolate bars containing 0.3% lecithin, 0.1% PGPR, or no added emulsifier to a pre-cured control bar of the three samples. All samples were measured at 29°C. Table 4.4 shows the force required to penetrate post-cured white chocolate samples containing different emulsifiers. Results reveal that all three post-cured white chocolate bars required significantly more force to penetrate than their respective controls. This indicates that the curing process of 83% RH and 29°C for 7 days was sufficient in creating structure.
Table 4.4 Average maximum force (g) required penetrating post-cured white chocolate containing lecithin, PGPR, or no added emulsifier compared to controls. Same letter implies no significant difference (α=0.05).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pre-cured average max force (g)</th>
<th>Post-cured average max force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>240±19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300±23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PGPR</td>
<td>260±37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>380±28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No added emulsifier</td>
<td>270±37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>420±81&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The samples were exposed to conditions favorable for lactose stickiness and crystallization. Ziegleder et al. (2004) measured the moisture sorption isotherm of white chocolate pastes when exposed to high relative humidity. They found that at 60% RH and 25°C, a break occurred in the moisture sorption isotherm, indicating lactose in the milk powder had crystallized. Crystallization of lactose in chocolate exposed to 25°C/60% RH corresponded to a thickening of the pastes caused by the inter-particle bridging between lactose molecules (Ziegleder et al., 2004). While thickening of chocolate is not generally thought of as ideal for manufacturing, this increase in thickness might be influential in producing a heat resistant structure in chocolate.

Chocolate with no added emulsifier exhibited significantly higher forces to penetrate than chocolate with lecithin; however, moisture adsorption was not significantly different between the two samples. Emulsifiers decrease viscosity of chocolate, so samples with no emulsifiers are inherently more viscous, influencing the overall force required for penetration. It should be noted that because of this lack of emulsifier, the feasibility of efficiently manufacturing this product is low.
Since emulsifiers are used to standardize the rheological properties of chocolate, the effects of lecithin and PGPR on the force required for penetration was studied. Bars with lecithin and PGPR adsorbed a significant amount of moisture during the curing process and required more force to penetrate than their controls. Due to their amphiphilic nature, lecithin and PGPR have hydrophilic and hydrophobic portions with the polar parts binding incoming moisture (Zielger et al. 2003). Lecithin has been noted as increasing moisture permeability into fat-based films (Yuan et al., 2009). This moisture can interact with hydrophilic ingredients and helps create structure between ingredients, explaining why more force was required for post-cured samples versus pre-cured ones.

Bars with PGPR required significantly more force than those made with lecithin. Ziegler et al. (2003) found that lecithin and PGPR behave in different manners when added to sugar dispersions. The polar heads of lecithin molecules adsorb to the surface of sugars in a multi-layer domain. At water activities above 0.7, lecithin can form reverse micelles, adding to its structural complexity.

The polar portions of PGPR also adsorb to the surface of hydrophilic ingredients, but these polar parts are interspersed along the highly branched PGPR molecule. Instead of a multi-layer domain, the absence of a polar head causes the hydrophilic groups on PGPR to form a loosely bound film around sugar molecules while the hydrophobic portions protrude into the fatty phase (Ziegler et al., 2003).

While both emulsifiers have water-binding properties, lecithin creates a more compact structure surrounding sugar molecules. Moisture can travel through the reverse micelles and multi-layer system, but this complex matrix could be obstructing moisture.
from reaching the hydrophilic ingredients. The loosely bound structure of PGPR may provide less of a hurdle for moisture migrating to and interacting with sugar molecules. These structural differences between emulsifiers might explain why bars with PGPR gained significantly more moisture and required significantly more force to penetrate than those made with lecithin.
4.2.4 X-ray Diffraction

Lactose

Figure 4.7 shows XRD patterns of freeze-dried lactose before and after exposure to curing conditions. The starting material did not show any distinct peaks, but after curing, crystallization was apparent. Based upon previous research, the presence of this crystalline state was preceded by amorphous lactose adsorbing enough moisture to transition from its glassy state into a sticky phase (Haque and Roos 2005; Haque and Roos 2006; Kirk et al. 2007; Listiohadi et al. 2005; Roos and Karel 1992). This sticky phase could provide the main structural component in heat resistant white chocolate.

Figure 4.7 XRD of amorphous lactose A) pre-cure represented by the red line and B) post-cure represented by the blue line. Post cured amorphous lactose showed peaks associated with crystallization of \( \alpha \)-lactose monohydrate and anhydrous \( \beta \)-lactose. ALM corresponds to \( \alpha \)-lactose monohydrate peak at \( 2\Theta =12.5^\circ \) and \( 16.4^\circ \) and BL corresponds to anhydrous \( \beta \)-lactose peak at \( 2\Theta =10.5^\circ \).

After 4 hours at 83% RH and 29°C, lactose showed features of both \( \alpha \)-lactose monohydrate at \( 2\Theta =12.5^\circ \) and \( 16.4^\circ \) and \( \beta \)-lactose crystals at \( 2\Theta =10.5^\circ \). For identification of specific peaks, Barham and Hodnett (1997) observed anhydrous \( \alpha \)- and
α-lactose monohydrate as sharing a peak at 2θ=12.5°. They included 2θ=16.4° to distinguish between the two as only α-lactose monohydrate exhibits this peak. Several other intense peaks became evident between 2θ=19-21°, but according to this same study, these angles are indicative of all forms of lactose crystals, making specific identification difficult in this region.

According to Haque and Roos (2004), amorphous lactose crystallizes as a mixture of α-lactose monohydrate and anhydrous β-lactose when stored at conditions above 50% RH at 25°C with crystallization of lactose occurring more rapidly at higher RH. The extent of lactose crystallization increased with increasing storage RH and time. Barham and Hodnett (1997) also found that after prolonged exposure to high humidity conditions, only α-lactose monohydrate and anhydrous β-lactose were observed. The current study agrees with these observations.

The way in which amorphous lactose was made affects the crystal formation during curing. In this study, crystalline lactose was dissolved in water at 60°C and immediately freeze-dried. As lactose crystallized, a mixture of the two isomers was observed. According to Listiohadi et al. (2005), processing lactose at temperatures between 50-70°C results in β/α ratios of 1.52 to 1.46.
Whole Milk Powder

WMP did not contain crystalline material prior to curing at 83% RH and 29°C for 4 hours as determined by the lack of peaks in Figure 4.8A; however, crystallization was observed after curing, indicating that lactose had gone through its glass transition and sticky phase. Joupilla and Roos (1994) explained that when exposed to high humidity, milk powders adsorb moisture and become sticky, forming inter-particle bridges between molecules before finally crystallizing. This sticky transition might be the structural component creating heat resistant chocolate.

Haque and Roos (2005) showed freeze-dried lactose crystallized similarly to spray-dried lactose when stored at RH>54%; therefore, comparisons were made between the freeze-dried amorphous lactose and spray-dried ingredients containing it in this present study. Peaks for α-lactose monohydrate did not occur at 2Θ=12.5° or 16.4° as they had in post-cured lactose; however, a distinct peak at 2Θ=10.5° corresponded to the presence of β-lactose. No conclusions regarding protein in the powder could be determined from the XRD patterns.
Figure 4.8 XRD of whole milk powder A) pre-cure represented by the red line and B) post-cure represented by the blue line. Post cured whole milk powder shows peaks associated with crystallization of anhydrous $\beta$-lactose.

Similarly to lactose, the crystalline forms present in milk powder are affected by how the material was initially processed. Milk powders are usually pre-processed and spray-dried at temperatures above the pasteurization temperature (Goff, 2011). Many spray-dryers use hot gas heated to temperatures between 60-100°C, depending on the product made (Crossley, 1960). As the milk slurry is dried, the lactose becomes amorphous and a mixture of $\alpha$- and $\beta$- lactose forms are ‘locked’ into a certain ratio. By processing at these high temperatures, $\beta$-lactose dominates the isomer configuration with ratios ranging from 1:1.46 at 75°C to 1:1.37 $\alpha/\beta$ at 100°C (Listiohadi et al., 2005).
Milk Protein Isolate

The milk protein isolate XRD pattern in Figure 4.9 shows amorphous material before and after curing at 83% RH and 29ºC for 4 hours. In each XRD pattern, post-cured milk protein isolate appeared to have a lower intensity overall as compared to pre-cured material. Perhaps the adsorption of moisture by hydrophilic portions dampened the XRD signal; however, more work would need to be done to verify this hypothesis. XRD patterns did not indicate any structural organization of milk proteins.

Figure 4.9 XRD of milk protein isolate A) pre-cure represented by the red line and B) post-cure represented by the blue line. Post-cured milk protein isolate shows a decrease in intensity signal.
Sucrose

Defined peaks were present in sucrose before and after curing (Figure 4.10), indicating sucrose remained crystalline. XRD patterns, however, were not the same between pre- and post-cured material. Sucrose was stored below its deliquescent point, so complete dissolution was not expected nor observed; however, if the outer portions of the particles adsorbed enough moisture, the dissolution and re-crystallization process could have occurred as a surface event, thus leading to variations in XRD patterns.

Kawakami et al. (2006) studied the crystallization of sucrose exposed to 30°C at 83% RH for 12 hours using X-ray diffraction. XRD patterns of sucrose were measured at various time intervals during the 12 hours period, and they all showed different placement of peaks. The authors surmised that these differences in XRD patterns resulted from variations in molecular orientation based on the rate of moisture adsorption over time. XRD patterns in the current research did not match those found by Kawakami et al. More research would need to be performed to understand these variations.
Figure 4.10 XRD of sucrose A) pre-cure represented by the red line and B) post-cure represented by the blue line.
4.2.5 FTIR spectral measurements

Lactose

FTIR spectral measurements are capable of representing the transition from the glassy phase to the crystalline state by measuring changes in free volume and increases in the average intermolecular distance between molecules (Listiohadi et al. 2005). Peaks of amorphous materials appear smooth while peaks of crystalline material show a definite and jagged shape.

In the current research, considerable spectral changes occurred during the curing of amorphous lactose at 83% RH and 29°C for 4 hours. Figure 4.11 shows lactose spectra transitioned from smooth, less defined peaks to more jagged shapes. As lactose adsorbed moisture from the high RH atmosphere, the molecules gained mobility and eventually ordered themselves into a crystalline state.

For identifying the different crystalline forms, Kirk et al. (2007) concluded that α-lactose monohydrate vibrations occur at 916, 899, and 876 cm\(^{-1}\) wave numbers, while β-lactose vibrations appear at 948, 892, 877, and 833 cm\(^{-1}\). In Figure 4.12, the presence of β-lactose was observed at ~891 and ~948 cm\(^{-1}\) as was α-lactose monohydrate at 899 and 915 cm\(^{-1}\) in agreement with Kirk et al. (2007).
Figure 4.11 Mid-FTIR spectra of amorphous lactose. Pre-cured material is represented by the black line and post-cured is represented by the purple line.

Figure 4.12 Mid-FTIR spectra of amorphous lactose peak assignments. Pre-cured material is represented by the blue line and post-cured is represented by the red line. The peak at 875 cm$^{-1}$ can be indicative of both $\alpha$-lactose monohydrate and $\beta$-lactose. Peaks at $\sim$891 and 948 cm$^{-1}$ are specific for $\beta$-lactose, while the peaks at $\sim$899 and 915 cm$^{-1}$ represent $\alpha$-lactose monohydrate (Kirk et al., 2007).
**Whole milk powder**

Figure 4.13 shows spectral measurements of WMP before and after the curing process. The post-cured spectra showed more defined peaks than the pre-cured sample, suggesting lactose had crystallized. For a more in-depth analysis, Lei et al. (2010) used a series of wave numbers to study milk powders. The researchers concluded peaks at 1658 and 1544 cm\(^{-1}\) were indicative of vibrations in proteins while peaks between 1250-800 cm\(^{-1}\) characterized the various C-O vibrations found in carbohydrates. Hogan and O’Callaghan (2010) broadened the range of wave numbers between 1720-1480 and 1200-800 cm\(^{-1}\) to characterize protein and lactose in milk powders, respectively.

The present research shows there are no distinct differences in spectral shape in the protein region of 1720 to 1480 cm\(^{-1}\) as identified by Hogan and O’Callaghan (2010). This may indicate that casein, the primary protein in milk powders, is not affected by moisture adsorption due to its high hydrophobicity. Similar spectra between post-cured lactose and post-cured WMP showed that the amorphous lactose in WMP crystallized when exposed to 83% RH at 29°C for 4 hours (Figure 4.14). The peaks at ~899 and 915 cm\(^{-1}\) representative of \(\alpha\)-lactose monohydrate do not appear; however, peaks at ~891 and 948 cm\(^{-1}\) specify the formation of \(\beta\)-lactose.
Figure 4.13 Mid-FTIR spectra of whole milk powder. Pre-cured material is represented by the black line and post-cured is represented by the purple line.

Figure 4.14 Mid-FTIR spectra of whole milk powder peak assignments. Post-cured amorphous lactose is represented by the black line and post-cured whole milk powder is represented by the red line. The peak at 875 cm\(^{-1}\) can be indicative of both α-lactose monohydrate and β-lactose. Peaks at ~889 and 948 cm\(^{-1}\) are specific for β-lactose. The peaks at ~899 and 915 cm\(^{-1}\) representative of α-lactose monohydrate do not appear.
Milk protein isolate

No spectral changes appeared that could elucidate the role of milk protein in structure formation (Figure 4.15). Only moisture adsorption was observed at 3400 cm$^{-1}$. According to Vega and Roos (2007), casein behaves as an inactive and hydrophobic polymer that does not interact with moisture or other hydrophilic ingredients such as sugar molecules. In their research, combinations of sugar and casein micelles did not differ in glass transition from those of pure sugar systems. This finding suggests that casein and sugars are not miscible because of the hydrophobic nature of casein.

![Figure 4.15 Mid-FTIR spectra of milk protein isolate. Pre-cured material is represented by the black line and post-cured is represented by the purple line.](image)
Sucrose

Sucrose did not show any discernible differences in spectral shapes in Figure 4.16. FTIR spectral measurements were only able to confirm the ingredients remained crystalline throughout curing. XRD patterns in Figure 4.10, however, vary between pre- and post-cured material, indicating curing conditions might be affecting sucrose more than what was detected by mid-FTIR. More work must be done to understand the discrepancies between XRD patterns and FTIR spectra.

Figure 4.16 Mid-FTIR spectra of sucrose. Pre-cured material is represented by the black line and post-cured is represented by the purple line.
4.3 Imaging Techniques

4.3.1 Scanning electron microscopy

Individual ingredients

Lactose

Amorphous lactose in Figure 4.17A appeared as shards of broken glass, while crystallized lactose in Figure 4.17B showed needle-like structures welded together. This structure appears to support the stickiness of amorphous lactose upon moisture adsorption. Haque and Roos (2006) observed freeze-dried lactose resembled broken glass or had a flake-like structure. They found that after curing at 76.1% RH and 25°C for 72 hours, needle-like or rod-shaped structures formed in freeze-dried lactose. Millqvist-Fureby et al. (1999) also showed flake-like morphology was obtained in freeze-dried amorphous lactose while a long rod-like structure corresponded with lactose crystallization. Neither study mentioned a welded structure.

Figure 4.17 SEM images of amorphous lactose. A1 and A2) pre-cured; B1 and B2) post-cured at 1000x magnification. Each tic mark represents 5μm.
Whole Milk Powder

SEM images of pre-cured WMP in Figure 4.18A showed a smooth, regular surface while post-cured WMP particles in Figure 4.18B showed lactose crystallization disrupted the particles causing protein to be released, as evidenced by the spongy material around the lactose crystals. Ziegleder et al. (2004) observed white chocolate hardening when lactose in milk powers crystallized. As the amorphous lactose adsorbed moisture, it became sticky and formed inter-particle bridges with neighboring molecules. The resulting structure caused overall thickening of the white chocolate paste. In the present study, this lactose stickiness and crystallization in WMP may be creating structure and thickening the white chocolate samples, leading to heat resistance.

Figure 4.18 SEM images of whole milk powder. A1 and A2) pre-cured; B1 and B2) post-cured at 1000x magnification. Each tic mark represents 5µm.
Milk protein isolate

Milk protein isolate did not exhibit any changes in structure during the curing process as shown in Figure 4.19. XRD patterns and FTIR spectral measurements corresponded well with SEM images.

Figure 4.19 SEM images of milk protein isolate. A1 and A2) pre-cured; B1 and B2) post-cured at 1000x magnification. Each tic mark represents 5µm.
Sucrose

Sucrose remained crystalline during the curing process of 83% RH at 29°C for 4 hours. Beckett (2008) states sucrose adsorbs moisture, dissolves, and re-crystallizes with neighboring sucrose molecules to create a sugar network capable of encasing melted cocoa butter at elevated temperatures. As shown in Fig. 4.20, the crystals remained unaffected by humidity since sucrose was exposed to humidity below its deliquescent point of 85%RH at 25°C.

Figure 4.20 SEM images of sucrose. A1 and A2) pre-cured; B1 and B2) post-cured at 100x magnification. Each tic mark represents 50µm.
White chocolate bars

SEM images in Figures 4.21, 4.22, and 4.23 show post-cured hexane immersed white chocolate bars with no added emulsifier, 0.1% PGPR, or 0.3% lecithin, respectively. Pre-cured samples could not be imaged because no structure remained after hexane immersion.

Figure 4.21 SEM images of white chocolate bars cured at 29°C/83%RH for 7 days with no added emulsifier at 1000x magnification. Each tic mark represents 5µm.

Figure 4.22 SEM images of white chocolate bars cured at 29°C/83%RH for 7 days with 0.1% PGPR at 1000x magnification. Each tic mark represents 5µm.
Identification of individual ingredients was difficult in SEM images of chocolate, so measurements of individual ingredients were used to provide information about structure development. SEM images of WMP showed disruption of particles because of lactose crystallization, while lactose by itself showed a welded crystalline mass after curing. Many researchers have observed amorphous lactose in milk powders transitioning from a glassy state to a crystalline state by way of a sticky phase (Haque and Roos 2005; Haque and Roos 2006; Kirk et al. 2007; Listiohadi et al. 2005; Roos and Karel 1992). The present study suggests that this sticky lactose within whole milk powder provided the ‘glue’ necessary between ingredients to make heat resistant structure in white chocolate.

SEM images (Fig. 4.21-23) did not reveal any differences attributable to emulsifiers; however, texturometer results in Table 4.4 measured differences between bars, with PGPR and no added emulsifier exhibiting more force to penetrate than samples made with lecithin. More research is necessary to understand and visualize the role of emulsifiers in creating this structure.
4.4 Handling of Heat Resistance Chocolate

Instrumental and imaging techniques provide details about the behavior of individual ingredients during curing, but they do not fully describe the finished product. Dicolla (2009) categorized temperature tolerance of chocolate by mild, intermediate, and extreme heat resistance, with the most heat resistant chocolate withstanding temperatures exceeding 37.9°C. In order to understand the heat resistance of the current samples, cured white chocolate containing either PGPR or lecithin were exposed to various temperatures and time intervals. Figure 4.24 shows cured samples with PGPR or lecithin maintained their shape after exposure to 33°C for 1.5 hours (Fig. 4.24 A, C) and 16 hours (Fig. 4.24 B, D). Figure 4.25 shows handling of post-cured white chocolate with PGPR or lecithin after exposure to 55°C for 1.5 hours. The samples were capable of withstanding extreme temperatures.

Figure 4.24 Post-cured heat resistant white chocolate “handleability” after exposure to 33°C for various time intervals. A) PGPR sample after 1.5 hours exposure; B) PGPR sample after 16 hours exposure; C) lecithin sample after 1.5 hours exposure; and D) lecithin sample after 16 hours exposure.
Bars made with lecithin showed the most difference in handling in these tests. After 1.5 hours at 33°C, the bar bent under the force of holding it. In contrast, after 16 hours at 33°C, the same bar was rigid. Bars at 55°C for 1.5 hours exhibited the most “handleability”. Both time at 33°C and increased temperature of 55°C resulted in further strengthening of the heat resistant structure. Untreated bars melted at these same elevated temperature and time conditions and could not be handled.
4.5 Exploratory Milk Powder Study

White chocolate was made with non-fat dry milk (NFDM) to investigate its ability to create structure in samples cured at 83% RH and 29°C for 7 days. Table 4.5 shows the formula used. The individual ingredient was cured at 83% RH and 29°C for 4 hours and studied using XRD, FTIR, and SEM.

Table 4.5 Formula for white chocolate with non-fat dry milk.

<table>
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<th>NFDM</th>
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<tr>
<td>29.9% non-fat dry milk</td>
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<tr>
<td>43.9% sucrose</td>
<td></td>
</tr>
<tr>
<td>25.9% cocoa butter</td>
<td></td>
</tr>
<tr>
<td>0.3% lecithin</td>
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</table>

4.5.1 Karl Fischer moisture content

Table 4.6 shows that NFDM gained a significant amount of moisture during the curing process. Table 4.7 shows white chocolate bars containing NFDM significantly gained moisture after curing.

Table 4.6 Moisture content measured by Karl Fischer of non-fat dry milk before and after curing. Same letter implies no significant difference between pre and post-cured chocolate (α=0.05).

<table>
<thead>
<tr>
<th>Individual Ingredient</th>
<th>Moisture Content (g moisture/100g wet basis)</th>
<th>Moisture Content (g moisture/100g dry basis)</th>
</tr>
</thead>
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<tr>
<td>NFDM pre-cure</td>
<td>4.4±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>NFDM post-cure</td>
<td>9.4±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 4.7 Moisture content measured by Karl Fischer of white chocolate samples with non-fat dry milk before and after curing. Same letter implies no significant difference ($\alpha=0.05$).

<table>
<thead>
<tr>
<th>White Chocolate Bar</th>
<th>Moisture Content (g moisture/100g wet basis)</th>
<th>Moisture Content (g moisture/100g dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFDM pre-cure</td>
<td>1.1±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NFDM post-cure</td>
<td>3.5±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**4.5.2 X-ray Diffraction**

XRD patterns did not show any apparent phase change between pre- and post-cured samples of NFDM. The sample remained amorphous throughout the curing process in this study as shown in Figure 4.26.

![Figure 4.26 XRD of non-fat dry milk powder A) pre-cured represented by the red line and B) post-cured represented by the blue line.](image)
XRD cannot distinguish between lactose in its glassy or sticky phase but can only detect the crystalline state. The amorphous lactose did not crystallize, but it may have extended into its sticky phase, potentially leading to structure formation in the final product. More research is needed to confirm this hypothesis.

4.5.3 FTIR spectral measurements

The same regions analyzed for spectral changes in WMP were used for NFDM. Figure 4.27 shows no change in the spectral region 1720-1480cm⁻¹ between pre- and post-cured non-fat dry milk, indicating no significant changes in protein molecular bonds. There are no specific peaks corresponding to lactose crystals, but there is a shift towards a more ordered state in the post-cured NFDM spectrum after curing. It is important to note that no studies have used FTIR to determine stickiness in powders. Additional work is needed to determine if FTIR can be used to study this transitional step.
Figure 4.27 Mid-FTIR spectra of non-fat dry milk. Pre-cured material is represented by the black line and post-cured is represented by the purple line.

4.5.4 Scanning electron microscopy

SEM images of NFDM does not show differences between pre- and post-cured material as shown in Figure 4.28 A and B; however, chocolate bars containing NFDM left a structure behind after hexane immersion and were capable of being imaged (Figure 4.29). FTIR spectra showed a transition of lactose in NFDM to a more ordered state, but XRD patterns revealed lactose remained amorphous after curing; therefore, it is hypothesized that amorphous lactose in NFDM was transitioning through its sticky phase and had not yet crystallized at the end of curing.
Figure 4.28 SEM images of non-fat dry milk. A1 and A2) pre-cured; B1 and B2) post-cured at 1000x magnification. Each tic mark represents 5µm.

Figure 4.29 SEM images of white chocolate bars with non-fat dry milk at 1000x magnification. Each tic mark represents 5µm.
4.6 Conclusion

Curing at 83%RH and 29ºC for 7 days created an environment conducive for structure development by absorption of moisture in the chocolate samples. Moisture acted as a plasticizer for the glassy lactose molecules, increasing their mobility and allowing them to become sticky. This sticky phase provided adhesion between ingredients. Even as amorphous lactose crystallized, it remained in this ‘glued’ state, creating structure between itself and other ingredients.

Instrumental tools and imaging techniques provided insight into structural development of ingredients in heat resistant white chocolate. Food companies can use the techniques explored in this research to commercialize products based on their consumer needs. By understanding structure formation, confectionery companies can design innovative products for their consumers, expanding further into the worldwide market.
4.7 References


Crossley EL. 1962. Dried milk. Milk Hygiene: hygiene in milk production processing, 
and distribution. World Health Organization Monograph Series No. 48. Geneva, 
Switzerland.

Pennsylvania State University.

crystalline forms by nondestructive analysis. Journal of Dairy Science 80(3): 457- 
463.

Faldt P and Bergenstähl B. 1996. Changes in surface composition of spray-dried food 
powders due to lactose crystallization. Lebensm-Wiss u-Technol 29: 438-446.

Frisullo P, Conte A, and Del Nobile MA. 2010. A novel approach to study biscuits and 
breadsticks using x-ray computed tomography. Journal of Food Science 75(6): 
E353-358.

Frisullo P, Licciardello F, Muratore G, and Del Nobile MA. 2010. Microstructural 
characterization of multiphase chocolate using x-ray microtomography. Journal of 
Food Science 75(7): E469-476.

starch from cocoa beans. Starch 33(3):76-79.

Ghosh V, Ziegler GR, Anantheswaran RC. 2002. Fat, Moisture, and Ethanol Migration 
through Chocolates and Confectionary Coatings. Critical Reviews in Food 
Science and Nutrition 42(6): 583-626.

Goff D. Dairy Chemistry and Technology Education Series. 2011. The University of 


Haque MK and Roos YH. 2005. Crystallization and X-ray diffraction of spray-dried and 


Kempf NW and Downey PJ, inventor; General Foods Corporation, assignee. 1956. Finished chocolate product. US patent 2760867.


Ziegler GR, Garbolino C, Coupland JN. 2003. The influence of surfactants and moisture on the colloidal and rheological properties of model chocolate dispersions. 3rd International Symposium on Food Rheology and Structure.
Chapter 5 Conclusions and Suggestions for Future Research

The overall goal of this research was to explore the various instrumental and imaging techniques for studying heat resistant structure formation in white chocolate. The three main sub-objectives were:

1. Develop a standard protocol for producing heat resistant chocolate.

An 83% RH environment caused heat resistant structure formation in white chocolate. Mold growth was seen on some samples, showing that the high water activity fostered the growth of microorganisms. Thymol crystals were then added to the curing jars to eliminate mold. Nonetheless, this type of curing process could potentially lead to microbial safety concerns not only in heat resistant chocolate but in other products made within the same facility.

The curing process most likely caused lactose crystallization in white chocolate containing WMP. This crystallization punctured the WMP particles, plausibly releasing milk fat into the white chocolate samples. Increased amounts of milk fat have been attributed to increased rate of fat oxidation. Lactose crystallization has also been noted as leaving a gritty texture in finished products.

Therefore, it is important to note, that while the curing process used in this present study was useful in elucidating potential mechanisms of heat resistant structure formation in chocolate, it is in no way suitable for commercial use. By understanding the development of structure via exposure to moisture, manufacturers can develop appropriate curing methods for making heat resistant chocolate that is safe for consumers.

Suggested research:

- Explore other methods to make heat resistant chocolate for their viability in industrial production. Lower RH and longer curing times should be investigated for their
ability to promote structure formation in chocolate while still maintaining a safe product. Extending the understanding of heat resistant structure formation to include milk and dark chocolate is also of importance.

- Perform sensory evaluations on heat resistant chocolate cured in various RH and temperature combinations to understand how the formation of a heat resistant structure affects consumer perception of the finished product.

2. Explore the following instrumental and imaging techniques for characterization of heat resistant structure formation:

   - X-ray diffraction
   - Texturometer measurements
   - Mid-Fourier transform infrared spectroscopy
   - Scanning electron microscopy

Many different instrumental and imaging techniques were explored for their ability to elucidate the role of individual ingredients in structure development and to characterize the finished product. There is certainly more opportunity to use these techniques to provide more details about structure development in heat resistant chocolate than what was currently assessed.

Suggested Research:

- Study the differences in XRD patterns for sucrose after exposure to moisture.

  - Expand the use of texturometer measurements beyond the force required to penetrate samples to better understand how heat resistant structure formation affects more physical properties of the finished product.
- Determine if FTIR spectral measurements can be used to detect the glass transition and sticky phase of amorphous materials.

- Investigate ATR FTIR imaging for its ability to provide visual information about the molecular changes occurring in pre- and post-cured chocolate samples.

- Analyze heat resistant chocolates using confocal scanning laser microscopy with a 583nm or 745nm laser.

3. Investigate the role of the individual ingredients in creating this heat resistant structure.

Individual ingredients were cured at 83% RH at 29ºC for 4 hours. It was revealed that these curing conditions led to lactose transitioning from its glassy to its crystalline phase via a sticky state. This stickiness allowed lactose to serve as an adhesive material between itself and other ingredients.

A basic formula for white chocolate was used in this study, but manufacturers have their own unique formulas. Therefore, different results may occur based on variations in formulas. By understanding the role of individual ingredients in heat resistant structure formation, manufacturers can better predict the performance of their products.

Suggested research:

- Examine the effects of using non-fat dry milk in creating a heat resistant structure in white chocolate.

- Expand research to include other ingredients found in white, milk and dark chocolate.

- Elucidate the role of emulsifiers in heat resistant structure formation by incorporating various amounts of emulsifiers and combinations therein in chocolate.
Appendix Instrumental Measurements

A. Moisture content of white chocolate samples measured by Karl Fischer.

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B. Fat content of white chocolate samples measured by IOCC.

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C. Texturometer force measurements calculated by Texture Expert 32.

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<tr>
<td>Go to...Distance</td>
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<tr>
<td>Mark Value Force</td>
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<tr>
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<td></td>
<td>X</td>
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</tr>
<tr>
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<tr>
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<td></td>
<td>X</td>
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<tr>
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<td>sec</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Area</td>
<td></td>
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<td></td>
<td>*</td>
<td></td>
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<tr>
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<td>X</td>
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<tr>
<td>Mark Value Force</td>
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<td>X</td>
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<tr>
<td>Go to Abs. -ve Value</td>
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<tr>
<td>Mark Value Time</td>
<td></td>
<td></td>
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<td>X</td>
<td></td>
</tr>
</tbody>
</table>
E. ANOVA Tables

One-way ANOVA: Moisture content of white chocolate bars pre- and post-cured

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>7</td>
<td>76.8021</td>
<td>10.9717</td>
<td>309.57</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>0.5671</td>
<td>0.0354</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>77.3692</td>
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</tbody>
</table>

S = 0.1883  R-Sq = 99.27%  R-Sq(adj) = 98.95%

<table>
<thead>
<tr>
<th>Level</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin pre</td>
<td>3</td>
<td>0.9233</td>
<td>0.0862</td>
<td></td>
<td>(*)</td>
</tr>
<tr>
<td>Lecithin post</td>
<td>3</td>
<td>4.6067</td>
<td>0.1102</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>No emulsifier post</td>
<td>3</td>
<td>4.2167</td>
<td>0.3053</td>
<td>(*)</td>
<td></td>
</tr>
<tr>
<td>No emulsifier pre</td>
<td>3</td>
<td>0.7667</td>
<td>0.0666</td>
<td>(*)</td>
<td></td>
</tr>
<tr>
<td>PGPR post</td>
<td>3</td>
<td>5.1333</td>
<td>0.2082</td>
<td></td>
<td>(*)</td>
</tr>
<tr>
<td>PGPR pre</td>
<td>3</td>
<td>0.8167</td>
<td>0.0379</td>
<td></td>
<td>(*)</td>
</tr>
</tbody>
</table>

Individual 95% CIs For Mean Based on Pooled StDev

<table>
<thead>
<tr>
<th>Level</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin pre</td>
<td>3</td>
<td>0.9233</td>
<td>0.0862</td>
<td></td>
<td>(*)</td>
</tr>
<tr>
<td>Lecithin post</td>
<td>3</td>
<td>4.6067</td>
<td>0.1102</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>No emulsifier post</td>
<td>3</td>
<td>4.2167</td>
<td>0.3053</td>
<td>(*)</td>
<td></td>
</tr>
<tr>
<td>No emulsifier pre</td>
<td>3</td>
<td>0.7667</td>
<td>0.0666</td>
<td>(*)</td>
<td></td>
</tr>
<tr>
<td>PGPR post</td>
<td>3</td>
<td>5.1333</td>
<td>0.2082</td>
<td></td>
<td>(*)</td>
</tr>
<tr>
<td>PGPR pre</td>
<td>3</td>
<td>0.8167</td>
<td>0.0379</td>
<td></td>
<td>(*)</td>
</tr>
</tbody>
</table>

Pooled StDev = 0.1883

Tukey’s Test

Moisture Content

<table>
<thead>
<tr>
<th>Samples</th>
<th>N</th>
<th>Subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>conL</td>
<td>9</td>
<td>0.9233</td>
</tr>
<tr>
<td>conP</td>
<td>9</td>
<td>0.8167</td>
</tr>
<tr>
<td>conNE</td>
<td>9</td>
<td>0.7667</td>
</tr>
<tr>
<td>lecithin</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>No emul</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>PGPR</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td>.225</td>
<td>.376</td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 9.000.
**Paired t-test for moisture content of individual ingredients before and after curing.**

**Paired T for amor lactose**

<table>
<thead>
<tr>
<th>Test type</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amor lactose pre</td>
<td>3</td>
<td>2.287</td>
<td>0.287</td>
</tr>
<tr>
<td>Amor lactose post</td>
<td>3</td>
<td>6.753</td>
<td>0.825</td>
</tr>
</tbody>
</table>

Difference = \(\mu(1) - \mu(2)\)

Estimate for Difference: -4.46600

95% CI for difference: (-5.8662, -3.06580)

T-test of difference = 0 (vs not =): T-Value = 8.865

\[\text{p-value} = 0.0009 \ \text{DF}=4\]

**Paired T for milk protein**

<table>
<thead>
<tr>
<th>Test type</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk protein pre</td>
<td>3</td>
<td>11.177</td>
<td>0.532</td>
</tr>
<tr>
<td>Milk protein post</td>
<td>3</td>
<td>8.093</td>
<td>0.530</td>
</tr>
</tbody>
</table>

Difference = \(\mu(1) - \mu(2)\)

Estimate for Difference: 3.08400

95% CI for difference: (1.88024, 4.28776)

T-test of difference = 0 (vs not =): T-Value = 7.1132

\[\text{p-value} = 0.0021 \ \text{DF}=4\]

**Paired T for nfdm**

<table>
<thead>
<tr>
<th>Test type</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>nfdm pre</td>
<td>3</td>
<td>9.363</td>
<td>0.134</td>
</tr>
<tr>
<td>nfdm post</td>
<td>3</td>
<td>4.440</td>
<td>0.174</td>
</tr>
</tbody>
</table>

Difference = \(\mu(1) - \mu(2)\)

Estimate for Difference: 4.923

95% CI for difference: (4.57096, 5.27504)

T-test of difference = 0 (vs not =): T-Value = 38.826

\[\text{p-value} = 0.0001 \ \text{DF}=4\]
Paired T for sucrose

<table>
<thead>
<tr>
<th>Test type</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>sucrose pre</td>
<td>3</td>
<td>0.237</td>
<td>0.071</td>
</tr>
<tr>
<td>sucrose post</td>
<td>3</td>
<td>0.200</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Difference = \( \mu(1) - \mu(2) \)

Estimate for Difference: 0.037

95% CI for difference: (-0.09363, 0.16763)

T-test of difference = 0 (vs not =): T-Value = 0.7864

\( p \)-value = 0.052 DF=4

Paired T for wmp

<table>
<thead>
<tr>
<th>Test type</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>wmp pre</td>
<td>3</td>
<td>8.927</td>
<td>0.015</td>
</tr>
<tr>
<td>wmp post</td>
<td>3</td>
<td>3.353</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Difference = \( \mu(1) - \mu(2) \)

Estimate for Difference: 5.574

95% CI for difference: (5.33235, 5.81565)

T-test of difference = 0 (vs not =): T-Value = 64.0436

\( p \)-value = 0.0001 DF=4
One-way ANOVA for fat content for all white chocolate samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>emulsifier</td>
<td>3</td>
<td>148.242</td>
<td>49.414</td>
<td>55.21</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>7.160</td>
<td>0.895</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>155.402</td>
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<td></td>
</tr>
</tbody>
</table>

\[ S = 0.9460 \quad R^2 = 95.39\% \quad R^2(\text{adj}) = 93.66\% \]

Individual 95% CIs For Mean Based on Pooled StDev

<table>
<thead>
<tr>
<th>Level</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>-------------------</th>
</tr>
</thead>
<tbody>
<tr>
<td>lecithin</td>
<td>3</td>
<td>35.267</td>
<td>1.097</td>
<td>(--*----)</td>
</tr>
<tr>
<td>NFDM</td>
<td>3</td>
<td>26.800</td>
<td>0.100</td>
<td>(--*----)</td>
</tr>
<tr>
<td>No emul</td>
<td>3</td>
<td>34.667</td>
<td>0.723</td>
<td>(--*----)</td>
</tr>
<tr>
<td>PGPR</td>
<td>3</td>
<td>34.767</td>
<td>1.358</td>
<td></td>
</tr>
</tbody>
</table>

27.0       30.0       33.0       36.0

Tukey’s test

**Fat**

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>N</th>
<th>Subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NFDM</td>
<td>3</td>
<td>26.800</td>
</tr>
<tr>
<td>No emul</td>
<td>3</td>
<td>34.667</td>
</tr>
<tr>
<td>PGPR</td>
<td>3</td>
<td>34.767</td>
</tr>
<tr>
<td>Lecithin</td>
<td>3</td>
<td>35.267</td>
</tr>
<tr>
<td>Sig.</td>
<td>1</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>.863</td>
<td></td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.
One-way ANOVA: Force (g) versus White Chocolate Sample

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Chocolate Sample</td>
<td>5</td>
<td>238225</td>
<td>47645</td>
<td>26.07</td>
<td>0.000</td>
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<tr>
<td>Error</td>
<td>48</td>
<td>87720</td>
<td>1828</td>
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</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>325946</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 42.75  R-Sq = 73.09%  R-Sq(adj) = 70.28%

Individual 95% CIs For Mean Based on Pooled StDev

<table>
<thead>
<tr>
<th>Level</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>control L</td>
<td>9</td>
<td>234.96</td>
<td>19.00</td>
</tr>
<tr>
<td>control NE</td>
<td>9</td>
<td>272.89</td>
<td>37.49</td>
</tr>
<tr>
<td>control P</td>
<td>9</td>
<td>255.46</td>
<td>37.26</td>
</tr>
<tr>
<td>lecithin</td>
<td>9</td>
<td>299.48</td>
<td>22.71</td>
</tr>
<tr>
<td>no emulsifier</td>
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<td>417.82</td>
<td>80.62</td>
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<tr>
<td>PGPR</td>
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<td>378.93</td>
<td>28.18</td>
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</tbody>
</table>

Tukey’s test

Force (g)

<table>
<thead>
<tr>
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<th>N</th>
<th>Subset for alpha = 0.05</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>conL</td>
<td>9</td>
<td>234.9633</td>
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<td></td>
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</tr>
<tr>
<td>conP</td>
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<td>255.4278</td>
<td>255.4278</td>
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</tr>
<tr>
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<td>284.0011</td>
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<td>lecithin</td>
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<td>299.4789</td>
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<td></td>
<td></td>
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<tr>
<td>PGPR</td>
<td>9</td>
<td>378.9278</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No emul</td>
<td>9</td>
<td>417.8222</td>
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</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 9.000.
F. X-ray diffraction patterns

Amorphous lactose pre-cured

![Graph showing X-ray diffraction pattern for amorphous lactose pre-cured](image)

Amorphous lactose post-cured

![Graph showing X-ray diffraction pattern for amorphous lactose post-cured](image)
Whole milk powder pre-cured

Whole milk powder post-cured
Milk protein isolate pre-cured

Milk protein isolate post-cured
Sucrose pre-cured

Sucrose post-cured
Non-fat dry milk pre-cured

Non-fat dry milk post-cured
G. FTIR spectra

Amorphous lactose

Whole milk powder
Sucrose

Milk protein isolate
Non-fat dry milk