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Finding Relationships Between Physical Properties of Butter and Water Loss

Annalisa Jones
Utah State University

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FINDING RELATIONSHIPS BETWEEN PHYSICAL PROPERTIES OF BUTTER AND WATER LOSS

by

Annalisa Jones

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTERS OF SCIENCE in
Nutrition and Food Sciences

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2022
ABSTRACT

Finding Relationships Between Physical Properties of Butter and Water Loss

by

Annalisa Jones, Master of Science
Utah State University, 2022

Major Professor: Dr. Silvana Martini
Department: Nutrition, Dietetics, and Food Sciences

Butter is a desirable fat rich product for laminated pastries because of its flavor and consumer acceptance but its use can be affected by defects in its functionality. One defect that limits its use is water loss, which is seen in the manufacturing of butter for laminated pastry use. During this process, a large block of butter is extruded into a thin sheet. Water can be seen dripping from the extruder as a result of weakening of the water-in-oil emulsion. This study investigates the impact of physical properties of butter on water loss during processing for lamination. The physical properties evaluated included solid fat content (SFC), water content, water droplet size, viscoelastic behavior, melting profile, hardness, and water loss. Commercial butters were analyzed first to understand their physical properties and how they relate to water loss. Water loss in high fat products increased when there was an increase in SFC or water content and negatively correlated with $G'$, $G''$ ($p<0.05$). In a second experiment, the fat content of the cream was adjusted (38-48%) by addition of anhydrous milk fat (AMF) to see the effect on water loss and physical properties. Water loss was positively correlated with fat content of...
cream, enthalpy, water content, and delta, and negatively correlated with $G'(p<0.05)$. Lastly, the AMF was fractionated (20°C, 25°C and 30°C) and added in two different steps to allow for identification of how AMF can best be incorporated to reduce water loss. Addition of AMF to cream resulted in positive correlation between water loss and water content, crossover point and oleic acid and a negative correlation with hardness, SFC, enthalpy, and palmitic acid ($p<0.05$). The addition of AMF in the working step identified a positive correlation with hardness, SFC, and myristic acid ($p<0.05$). Overall, the processing methods and composition, especially water content, impacted the effect of physical properties on water loss. In the end it is suggested that lower fat cream (38-46%) with a high melting fraction incorporated in the cream before churning is used for water loss reduction.
PUBLIC ABSTRACT

Finding Relationships Between Physical Properties of Butter and Water Loss

Annalisa Jones

Butter is a desirable fat rich product used for laminated pastries, like croissants, because of its flavor and consumer acceptance. However, butter has some functional aspects that reduce its performance and quality. In manufacturing of butter for laminated pastries, large blocks of butter are pushed through a rectangular opening to form a thin sheet. In this process it is not unusual to see water dripping, indicating water loss in the butter. The purpose of this study was to understand the properties of butter and their role in water loss during processing.

The properties of commercial butters were tested to understand their relation to water loss. Water content was found to play a key role in water loss. Butters with less water and a lower amount of solid fat at low temperatures had less water loss. Changes in fat content of cream were tested to see the impact on butter properties and water loss. Water content and fat content of the cream led to a higher amount of water loss. This study indicated that the butter needed to be pliable without losing its structure to reduce water loss. Lastly, three fats with different melting properties were added to the cream for butter making. The fat that was solid at higher temperatures made a harder butter with a lower water content. This butter had the lowest amount of water loss in this method. A similar method was performed adding the butter fat to the end of the butter making
process. This resulted in a lower water loss in the softer butter. However, all the butters made in that method had large crystals that would not be desirable for consumers. These studies indicate that the properties of the butter play a role in water loss and that they can be manipulated by the water content and processing method. For best results in water loss reduction a lower fat cream (38-46%) with a butter fat that is solid at higher temperatures and that is incorporated in the cream before churning is recommended.
Dedicated to my parents, Jeff and Mary Jones, for their constant support, love and sacrifice
I would like to thank my mentor professor, Dr. Martini. I would not have been able to get to this point without her. Her guidance and help have inspired me in my research. She has spent countless hours looking over my papers, guiding me in my edits, working through data, and teaching me as we have made discoveries together. I would also like to thank my committee members, Karin Allen and Prateek Sharma, for their guidance and help throughout this process. Their expertise has been invaluable in making decisions on how to proceed in my research. I would also like to thank Juhee Lee, Melissa Marsh, and Weston Christensen, who have been a great support in training me in the lab and encouraging me in my research. Weston and Melissa also put in hours of work to help produce enough AMF to use in this project.

I would also like to thank BUILD Dairy for the support in funding my research and connecting me with people who have helped me succeed in this project and will help me succeed in the future. I have felt taken care of by all those associated with BUILD Dairy. I would also like to thank High Desert Milk who contributes to the BUILD dairy funding and provided me with cream for my experimentation. Shawn and Brandon from High Desert have also been a great help in understanding the production side of butter.

Lastly, I would like to thank my family for the great support and encouragement they have provided over the last two years. They have encouraged me to be my best and have rejoiced with me in every step of this journey. I would especially like to thank my husband, Dirk, who has been my greatest support.
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Rheological properties for butters obtained using creams with various fat contents including G’ (A), G” (B), delta (C), crossover point (D), and yield stress (E)
LIST OF ABBREVIATIONS

- $\Delta H$ – Enthalpy
- $\Delta H_2$ - Enthalpy of the second peak
- AMF f- Anhydrous Milk Fat Fraction
- AMF- Anhydrous milk fat
- AMF20- butter with 20°C AMF fraction
- AMF25- butter with 25°C AMF fraction
- AMF30- butter with 30°C AMF fraction
- AOCS- American Oil Chemist Society
- CFR- Code of Federal Regulations
- DSC- Differential scanning calorimeter
- FA- Fatty acid
- FAO- Food and Agriculture Organization
- HMF- High melting fraction
- LC-MS- Liquid Chromatography mass spectrometry
- LMF-Low melting fraction
- MMF-Medium melting fraction
- NMR-Nuclear Magnetic Resonance
- PFG- Pulsed field gradient
- SFA- Saturated fatty acid
- SFC- Solid fat content
- SNF- solids non-fat
- TAG- Triacylglycerol
$T_{on}$ - Onset Temperature

$T_p$ - Peak temperature

TPA - texture profile analyzer

UFA - Unsaturated Fatty acid

UHPLC - Ultra-High-performance Liquid Chromatography

WC - Water Content

WD - Water Droplet

WHO - World health organization

WL - Water loss

WL - Water loss

$\delta$ - delta/rheological phase shift angle
Butter is a desirable fat rich dairy product in pastry use because of its flavor profile and consumer acceptance. As a water-in-oil emulsion, butter has droplets of water distributed throughout a continuous fat phase (Ooms et al., 2016). The water droplets play a functional role in the structure of the butter and also in the baking of laminated pastries. The water droplets vaporize in the heat of baking, being released from the emulsion, contributing to the water in the dough and increasing dough lift (Kaylegian and Lindsay, 1995; Bockisch, 1998). Water is a key component of both the pastry and the butter. However, the water-in-oil emulsion can become unstable from the shear force used to roll out the layers of laminated dough, resulting in water escaping the butter emulsion and being absorbed in the dough (Ghotra et al., 2002). This loss of water has been identified as a defect called leaking (Bradley and Smukowski, 2009). Butter for pastry use needs to be able to withstand the shear forces of lamination (Bockisch, 1998).

There has been very little research performed on the effect of leaking on laminated dough. Fessas and Schiraldi studied the effect of moisture content on the water loss of doughs in baking. A higher rate of water loss was evident in doughs that incorporated more water (Fessas and Schiraldi, 2001). If the emulsion is not stable a greater amount of water may be incorporated into the dough, allowing for a higher rate of water loss.
In manufacturing of laminated dough, butter is often taken from large blocks, approximately 50 lbs., and extruded into a ribbon of butter less than an inch thick. The ribbon is then laid on top of the dough to begin lamination. It has been observed that water can be lost in the processing of butter in preparation for lamination. When the ribbon of butter comes out of the extruder there can be excess water dripping from the extruder. It is evident that the emulsion of the butter is not strong enough to withstand the shear forces of milling and extrusion. Excess water dripping from the equipment results in less water available in the butter to increase pastry lift. It also can contribute to water dripping on the pastry dough, increasing water content of the dough. Free moisture in butter is not a new problem. The working step of the butter making is where the water droplet size and water content can be most affected. According to Wibley, free moisture is more common in butters produced with a single working unit (Wibley, 2009). Many manufacturers now use two working units that can be individually controlled to reduce this loss of water (Wibley, 2009). While working of butter has been better optimized there is still a need for increased understanding in the loss of water recognized in butter preparation for lamination.

The overall goal of this research is to understand the physical properties that minimize water loss in butter.

Hypothesis

Physical properties such as water content, solid fat content, viscoelasticity, hardness, water droplet size, and melting profile of butter affect water loss.

Objectives

To test this hypothesis, the following objectives are proposed:
1. Characterize various butter and margarine products currently in the market for their physical properties and their water loss.

2. Evaluate the effect of fat content of the cream on water loss and other physical properties of butter.

3. Obtain anhydrous milk fat and high melting fractions of milk fat from cream and incorporate the melting fractions in butter making (A) in the cream and (B) in the working step.
CHAPTER 2:

LITERATURE REVIEW

Function of Fat in Laminated Dough

Dough lamination is a technique used in pastry making that allows for a layered, flaky structure. Pastries such as danishes, croissants, or puff pastry all use this technique. The structure of these pastries is developed by sheeting out the dough and a layer of fat (typically shortening, margarine, or butter). The fat is laid on top of the dough and the dough and fat are folded multiple times to create a distinct layered structure. English, French, and Scottish methods of lamination are the most common (Wickramarachchi et al., 2015). The three methods differ in their folding techniques. The French and English methods incorporate the butter by creating a rectangle of dough and laying a sheet of fat over either half (French) or two thirds (English) of the dough. The Scottish method adds big lumps of fat to the mixer and mixes for a short amount of time to prevent the mixture from becoming homogenous (Bennion et al., 1997). While there may be multiple methods for lamination, the mechanism for rise and flakiness in each of these methods remains the same. The fat is a key factor in the structure of the pastry since it interrupts the gluten network formed by the water and flour in the dough and allows the formation of characteristic horizontal layers (Baardseth et al., 1995) (Figure 2-1). Some sources say that this fat creates an impervious layer trapping any steam in the dough matrix (Deligny et al., 2015). Other sources indicate that the fat simply creates a barrier to prevent the dough layers from touching and, when melted, creates a space for the water to expand.
into steam and the gluten network in the dough keeps the steam from escaping (Baardseth et al., 1995; Mattice and Marangoni, 2017). Regardless of the specific mechanism, the fat plays a major role in the structure and rise of the dough as it creates a space for the water in the pastry to expand into water vapor. The steam created from evaporation expands the space created by the fat and causes dough lift (Deligny et al., 2015; Rønholt et al., 2013).

**Figure 2-1:** Lamination process of sheeting and folding. Individual fat and dough layers are identified (Ooms et al., 2016 reproduced with permission from Taylor and Francis)

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**Cream used in butter making**

Butter is made from cream. Cream for butter making is typically 35-40% fat for batch churning or 40-48% for continuous butter making processes (Wibley, 2009). The high fat content can be achieved in a centrifugal separator (Fearon, 2011). After
separation, the cream is typically pasteurized to at least 185°F for 15 seconds and cooled (Cream for Butter Making, 2022). Aging (holding the cream at a low temperature) is a critical part of cream preparation to induce fat crystallization (Lee and Martini, 2018). The crystallization of the fat will affect the churning process, and the number and size of the crystals in the butter (Fearon, 2011). Higher cooling rates and higher agitation promote smaller crystallization size which can increase hardness of the final product (Lee and Martini, 2018).

The diet of the cows can have a great impact on the chemical composition of the cream. It is understood that winter milk is higher in short chain fatty acids and has lower levels of oleic acid (Wibley, 2009). These changes make for a harder butter because of the increased proportion of high melting triacylglycerols. Temperature and time combinations of cooling can help to account for the change in physical properties. The high melting fractions of anhydrous milk fat can also be added to prevent the butter from being too soft (Wibley, 2009).

**Anhydrous Milk Fat (AMF)**

Anhydrous milk fat (AMF) is by definition 99.8% milk fat (FAO and WHO, 2019). AMF is very stable product because the low water content restricts microbial growth. For this reason, AMF is mainly sent to countries with little availability of fresh milk. AMF can also be used for adding to milk products to increase fat content or adjusting the composition of milk by adding fractionated AMF (Mortensen, 2011).

AMF can be prepared from cream or butter (Figure 2-2). When cream is used for AMF production, the cream is separated to be 70-80% fat. The high fat cream is
homogenized which causes a phase inversion. This allows for the water still in the cream to be drained. The fat phase is then washed and neutralized with sodium hydroxide. The fat phase is then heated to 90-95°C and pumped into a vacuum chamber in a thin film or spray (Mortensen 2011). AMF can also be obtained from butter. In this case, the butter is melted completely to approximately 60°C. After melting the butter is pumped to a holding tank to ensure complete melting and to allow aggregation of proteins in the water phase. The water phase is then separated from the fat phase and the fat should be at about 99.5%. Once the fat is separated from the water it follows the same process of the cream; that is, it is being heated and sent though the vacuum chamber (Bylund et al., 2003).
**Figure 2-2:** AMF processing production flow chart. Both the cream and butter method of AMF production are represented in the flow chart (Bylund *et al.*, 2003).

**Melting Fractions**

Milk fat is a complex fat because it contains a wide variety of fatty acids. About 400 different fatty acids have been identified in milk fat. Palmitic acid (22-35%), stearic acid (9-14%), myristic acid (8-14%), and oleic acid (20-30%) are among the most prevalent of the fatty acids found in milk (Buldo and Wilking, 2016). The variation in the composition of the milk fat is greatly affected by the diet of the cows. However, adjusting the composition of the milk fat can also be achieved by fractionation. There are three
main identified melting fractions of milk fat between -40°C and 40°C. The melting fractions include a low melting fraction (LMF) that melts below 10°C, a medium melting fraction (MMF) that melts between 10-19°C, and a high melting fraction (HMF) that melts above 20°C (Figure 2-3) (Rønholt et al., 2013).

**Figure 2-3:** Melting profile of anhydrous milk fat. Three distinct peaks are represented showing the low, medium, and high melting fractions (Rønholt et al., 2013 reproduced with permission from John Wiley and Sons).

Fractionation is the process of isolating triacylglycerols with varying melting points into the previously mentioned melting fractions. In isolating these fractions, the overall fat composition of the milk, cream, or butter can be changed by adding back the desired amount of each fraction. This technique has been used to adjust the hardness of refrigerated butter (Kaylegian and Lindsey, 1992) or improve product quality (Vanhoutte et al., 2002). Fractionation is performed by selecting a series of set temperatures for
desired crystallization. In particular, the fat, anhydrous milk fat (AMF), is melted and
placed in an oven to erase crystal memory. The AMF is then cooled to the desired
crystallization temperature. A test using fractions from 34, 30, 25, 20, 16, and 13°C was
performed allowing for greater understanding of the effect of fractions from both MMF
and HMF (Kaylegian and Lindsey, 1992). The fractions from specific temperatures can
be done in a single step or multiple step fractionation. Single step fractioning simply
cools the AMF to the desired crystallization temperature. Typically, the AMF is held at
that temperature for at least 24 hours to ensure the fat has crystallized. The crystals can
then be filtered from the liquid using a vacuum filter. The multiple step fractionation
involves filtering a sample to obtain a high melting fraction (crystals) and then cooling
the liquid (olein) to a lower crystallization temperature. This allows for fractionation of
only that temperature set rather than including the higher melting fractions.

**Butter Making**

Butter is defined as a product made from only milk of cream, with or without the
addition of butter and coloring, and with at least 80% milkfat (Federal Food, Drug, and
Cosmetic Act, 1906). In the Codex Alimentarius it identifies that butter must have at least
80% fat and a maximum water content of 16% (Codex Alimentarius 2018). Butter
consumption has increased over the past 10 years. In 2010 consumption was about 4.9 lbs
per person. In 2020, butter consumption increased to 6.3 lbs per person (US Department
of Agriculture; Economic Research Service). The butter market is growing and there is
room for greater utilization of it.
Butter can be produced in two different methods, batch and continuous. Industrial continuous butter making is the most common method. The continuous method requires the cream to be consistent in fat content, temperature, and physical characteristics throughout the run to allow for a consistent product. Often this is done by drawing cream from a silo or a balance tank. The temperature of the cream in the silo is dependent on the time of year and the fatty acid content of the cream. In the winter the cream has a higher amount of saturated fat and will need a higher temperature. The optimal temperature range for winter cream is 10-13°C and 7-10°C for summer cream (Tondhoosh et al., 2016). The temperature should be such that the 50% of the fat in the cream is liquid (Wibley, 2009).

Once the cream has been aged and stabilized at the proper temperature, the churning process can begin. The purpose of churning is to incorporate air into the cream, making a foam (Mortensen, 2011). The cream used in butter making has been cooled to allow for the milk fat globules to have both liquid and solid fat (Figure 2-4). The solid fat crystals must be present for churning to be possible. Without the crystals a high amount of fat would be lost in the butter milk (Walstra et al., 2006). When there is 50% solid fat in the cream the flexibility of the fat globules is reduced by the fat crystals (Wibley, 2009). The shear created in the making of the foam damages the fat globule membranes by penetration of the crystalline fat allowing for liquid fat to be released. The liquid fat is hydrophobic and will collect at the interface of the air cells. The movement of the liquid fat to the interface displaces the proteins and increases the rate of coalescence of air cells. This creates an unstable foam, and the air cells collapse (Wibley, 2009). When the air cells collapse, the fat will agglomerate creating small butter grains. The size of the butter
grains is of great importance because it can affect the moisture. When the grains are too large or too small, they will retain excess moisture (Fearon, 2011).

**Figure 2-4:** Transition of fat globules in milk to butter grains. Step one indicates milk or cream. Step two shows the phase inversion from a water-in-oil emulsion to an oil-in-water emulsion. Step three shows the agglomeration of fat to create butter grains. (Ronholt *et al.*, 2013 reproduced with permission from John Wiley and Sons).

After the butter grains begin to form the speed of churning is decreased. The buttermilk is drained, and the decreased speed allows for the grains to release excess buttermilk through the force of grains falling on each other. A high speed is not used because the butter grains would pack together which would increase the retention of buttermilk, increasing the water content of the butter (Wibley, 2009). The working step follows the draining. In industry the working step is typically performed by two augers
rotating in opposite directions. The augers will push the butter forward while squeezing the remaining buttermilk out of the grains (Mortensen, 2011). The working step plays a large role in water content and water droplet size of the butter. If the water droplet size is too big there is a greater chance of microbial growth and therefore, this process is targeted to reduce water droplet diameter to less than 10 µm (Wibley, 2009).

*Physical Properties of Butter*

Important physical properties of butter include solid fat content, melting profiles, rheology, water content, hardness, and water droplet size. These properties are of great importance because they determine the functionality of the butter (Wassell, 2014). Butter is made up of fat globules, fat crystals, air bubbles, and water droplets that all play a part in the physical properties (Mortensen, 2011). Appearance and texture are two of four principal quality factors (Bourne 2002). Both of these factors are greatly impacted by the physical properties that will be discussed below.

Specific pieces of equipment are used to quantify important physical properties of butter. These instruments include nuclear magnetic resonance (NMR), differential calorimeter (DSC), rheometer, and texture profile analyzer (TPA). NMR can measure both solid fat content and water droplet size (McClements, 2005; van Lent, 2007). DSC can determine the crystallinity of a sample identifying its specific melting profile (Hartel 2001). The rheometer evaluates the storage and loss modulus of the sample through small deformation oscillatory testing (da Silva Lannes and Ignácio, 2013). TPA can be used to measure the hardness of the product (Rosenthal, 2010). All these instruments and characteristics will be discussed in further detail below.
Solid Fat Content

While butter appears solid or plastic, not all the fat in the system is solid. The amount of solid fat is driven by the chemical composition of the triacylglycerols in the butter sample and by its temperature. Solid fat content (SFC) is the measurement of solid fat as a percentage of all the fat in the system. This information can be very useful in understanding brittleness. The solid fat content is measured using nuclear magnetic resonance (NMR). This technique uses radio frequency magnetic radiation within a magnetic field to excite molecules to a higher spin state. When the pulse of radiation has concluded, the molecules will return to their normal spin state. As they return to their normal spin state nonradiative energy is released. The energy released is then characterized to understand the molecular state (Hartel, 2001). This technology can be used for many kinds of food analysis including solid fat content and isothermal crystallization rates. The difference in the relaxation rates of solid and liquid parts of the sample allow for understanding the percent solid fat (Bakota, 2012).

Differential Scanning Calorimeter (DSC)

Measuring the melting profile of a fat product is crucial in understanding the composition of the fat and the way it reacts to temperature changes. Within production there is capacity for the temperature to change affecting the properties of the fat. One of the challenges of using butter in a laminated pastry dough is the changes that take place in working and extruding the butter for dough lamination. A differential scanning calorimeter (DSC) has the capacity to measure the changes in a product over a range of temperatures. This includes recording melting profiles, crystallization profiles, and
finding the melting point. Advantages of DSC include small sample size requirements (1-40 mg) and high sensitivity. However, the small sample size and conditions may not be perfectly applicable to operation conditions (Hartel, 2001).

The DSC uses a reference pan and a pan containing the sample. The DSC continually establishes a consistent temperature in both the reference pan and the sample pan. The energy required to keep the sample pan at the same temperature as the reference pan is recorded (McClements, 2005). When a phase transition happens, heat is absorbed or released. The DSC will counteract the absorption or release by adjusting the energy needed to each of the pans to ensure they are at the same temperature. (McClements, 2005).

**Rheology**

Rheology studies the reaction of a material to deformation. According to Schramm, ideal solids have elastic deformation while ideal fluids have irreversible deformations because they will flow (Schramm, 2001a). Many food materials are far from being ideal solids or fluids, but rheology helps to understand the deformation in the sense of the elastic or storage modulus (G’) and the viscous or loss modulus (G’`). This small deformation testing allows for elastic and viscous properties to be identified with only a small amount of sample (Wright, 2001).

Oscillatory testing inputs oscillatory strain or stress and measures the oscillatory strain or stress output. This is a form of small deformation rheology. The G’ response is representative of a solid like component. The G’` response is representative of the liquid like response (da Silva Lannes and Ignácio, 2013). Within the elastic and viscous
modulus, we can also determine the shift angle (\(\delta\)). A completely viscous sample would be a 90° shift angle while a completely elastic modulus would be 0° shift angle (Shramm, 2001b). The \(\delta\) is determined by the ratio of \(G''\) to \(G'\). When \(G''\) higher than \(G'\) the sample will behave in a liquid manner. When \(G'\) is larger than \(G''\) the sample will behave in a more solid manner (da Silva Lannes and Ignácio, 2013).

**Water Content**

Butter is not a pure fat product. In this product, water is trapped in small droplets within the fat matrix forming a water-in-oil emulsion. The amount of water in the product can affect other physical properties. To control the water content there are a few factors that can affect water loss. According to Mortensen, low fat content of cream, high churning temperature, low and high churning and working speeds, small and large butter grains, and high working temperature can all lead to an increased water content (Mortensen, 2011). Measuring this water content can be performed in a few different methods.

One of the most common methods is done by placing the sample in a crucible in a vacuum oven and determining the difference in weight (Rønholt et al., 2014b). The heat from the oven evaporates the water over a period of hours. The difference in the weight accounts for the water content. A similar method is performed using a moisture analyzer. The same technique, evaporating the water from the sample, is used in this rapid method. The sample is weighed on a pan and the apparatus heats up. The weight change is recorded as the sample is heated. When the weight stabilizes, the water content is reported.
**Water Droplet Size**

Butter is a water-in-oil emulsion meaning that water is suspended in the continuous fat phase. In a water-in-oil emulsion the water is entrapped in the form of droplets within the fat phase. The water droplet size is a key factor in microbial stability, physical stability, rheological properties, and sensory properties (Voda and van Duynhoven, 2009; Nelis *et al.*, 2021). According to van Dalen, a smaller water droplet size (below 5 µm) will not contain enough nutrients for microbes to survive, reducing the microbial threats (van Dalen, 2002). The size of water droplets varies greatly depending on processing. Van Lent identified a water droplet size of 2.3-6.4 µm in 82% fat commercial butter (Van lent *et al*., 2008). van Dalen reported a similar range of water droplet size in butters with 40-80% fat recording 2.6–10.6 µm water droplet sizes (van Dalen, 2002). Rønholt *et al.* performed a study in which AMF was added to skim milk reporting much higher water droplet sizes of 28-35 µm. Rønholt’s study used small scale manufacturing which is assumed to be the reason for larger water droplet size (Rønholt, 2012).

The droplet size distribution can be measured using pulsed field gradient (PFG) NMR. This method is relatively cheap, fast, and non-destructive to the sample (van Dalen, 2002). The PFG-NMR droplet size distribution test consists of two equal gradient pulses with the same magnitude and duration with a 180° pulse between these two pulses. The first gradient pulse dephases the proton precession frequency. The second gradient pulse refocuses the proton phases. However, not all the initial phases will be recovered by the second pulse. The difference in the initial signal and the signal after the second
gradient pulse is proportional to the root mean square displacements between the pulses (Voda 2009). This process was explained in an equation by Fourel et al. (1995):

\[ \ln R = \ln \frac{I_g(2\tau)}{I_0(2\tau)} \]  

[1]

Where \( I_g(2\tau) \) is the echo intensity with the gradient, \( I_0(2\tau) \) is the intensity of the echo without the gradient and \( R \) is the radius of the droplet (Fourel et al., 1995).

**Hardness**

Hardness is one of the key characteristics in pastry butter (Wickramarachchi et al., 2015; Pajin et al., 2010). Hardness can be affected by many different factors.

According to Rønholt, hardness is determined by crystal size, amount of butter grains, and strength of the bonds (Rønholt et al., 2014a). Lee and Martini identified amount of crystalline material in the sample and crystal size as determining factors (Lee and Martini, 2018). Increased water droplet size has the capacity to reduce crystal interactions decreasing overall hardness (Rønholt et al., 2012). There are many factors playing a part in the hardness. Hardness can be measured using a texture profile analyzer (TPA).

The TPA can measure hardness, adhesiveness, cohesiveness, elasticity, and brittleness. The testing can be used to test solid and semisolid materials (Rosenthal, 2010). The test has a two-compression test designed to mimic a chewing motion (Rosenthal, 2010). The test produces a curve based on the force produced by the TPA against the sample.
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CHAPTER 3

RELATIONSHIP BETWEEN BUTTER PHYSICAL PROPERTIES AND WATER LOSS

ABSTRACT

Butter provides ideal flavor and texture in laminated pastries. During industrial production of butter for pastries, the butter structure can break due to shear forces during lamination resulting in water loss (WL). This WL in during lamination can affect the desired qualities of the pastry. There is a need to unveil the physical properties that drive WL in butter processing. The objective of this study was to characterize the physical properties of seven commercial butter products and correlate these properties to WL during processing. The physical properties evaluated included solid fat content (SFC), water content, water droplet size, viscoelastic behavior, melting profile, hardness, and WL. Water content is the only characteristic that was significantly correlated to WL. Regression analysis showed that an increase in water content, high melting fraction enthalpy, and water droplet size, and a decrease in the viscoelastic phase shift angle

increased WL. Correlation analysis was also performed in high fat content butter (>83% butterfat) and regular fat content butter (<83% butterfat) separately. The WL in high fat butters had significant negative correlations with $G'$ and $G''$ and significant positive correlations with SFC at 5, 10, and 15°C ($\alpha = 0.05$). The WL of regular fat butters had significant positive correlations with hardness, water droplet size, and SFC at 30°C ($\alpha = 0.05$). No other significant correlations were identified with WL in these two groups. Overall, these results show that various physical properties can affect butter quality for lamination purposes including water content, water droplet size, viscoelasticity, and melting behavior.

INTRODUCTION

Dough lamination is a specific technique used in baking to create a pastry with a characteristic airy and flaky appearance. Puff pastry, danish pastry, and croissants all use this method to achieve their typical flaky texture. Multiple methods of creating a laminated dough are available including the English, French, and Scottish methods (Wickramarachchi et al., 2015). The three methods differ in their folding techniques. The French and English methods incorporate the butter by creating a rectangle of dough and laying a sheet of fat over either half (French) or two thirds (English) of the dough. The Scottish method adds big lumps of fat to the mixer and mixes for a short amount of time to prevent the mixture from becoming homogenous (Bennion et al., 1997). Each method creates alternating layers of dough and fat. These layers are created by folding and sheeting the dough and fat layers. While there may be multiple methods for lamination, the mechanism for rise and flakiness in each of these methods remains the same. The
major components of any laminated dough include flour, salt, water, and fat (Ooms et al., 2016). The flour contributes to the overall structure by creating a gluten network across each layer but does not create a continuous gluten network throughout the pastry since the network is interrupted by the layers of fat. The fat is crucial in the structure of the dough. The interruption of the gluten network allows for the horizontal layers that are characteristic of laminated doughs (Baardseth et al., 1995). Some sources say that this fat creates an impervious layer trapping any steam in the dough matrix (Deligny & Lucas, 2015). While other sources indicate that the fat simply creates a barrier to prevent the dough layers from touching and, when melted, creates a space for the water to expand into steam and the gluten network in the dough keeps the steam from escaping (Baardseth et al., 1995; Mattice & Marangoni, 2017). Regardless of the specific mechanism, it is determined that the water in the dough and the fat are the major cause of rise in the system. When the pastry is cooked, some of the water in both the fat and the dough evaporates and is trapped in the dough. The steam created from evaporation expands the space created by the fat and causes dough lift (Deligny & Lucas, 2015; Rønholt et al., 2013).

The fat used in the laminated dough has been a topic of interest because the characteristics of fat are important for creating a high-quality lamination. Butter is a commonly used fat that has a high consumer acceptance due to its flavor profile. It is a water-in-oil emulsion, meaning it has droplets of water dispersed in a continuous fat phase (Ooms et al., 2016). The water droplets have potential to help in dough lift when the fat melts and the water is released from the emulsion. This release allows for the water in the fat to vaporize with the water in the dough creating the “puff” needed in
laminated doughs (Bockisch, 1998). According to Ghotra et al. (2002), the shear force used to roll out the layers of the laminated dough can break the water-in-oil emulsion where the water entrapped in the butter will be released and absorbed by the dough. Therefore, butters used as laminating fats must be able to withstand these shear forces while creating a thin layer of fat between the dough layers (Bockisch, 1998). Little research has been performed on effect of water loss in a water-in-oil emulsion on dough properties. However, a study by Fessas and Schiraldi (2001) tested the water loss of doughs with different moisture levels. They identified that the dough samples with a higher moisture content had a much faster rate of water loss. This may be due to oversaturation of the gluten network allowing for water to flash off (Fessas & Schiraldi, 2001). When the emulsion of butter breaks it has potential to incorporate more water into the gluten matrix than intended. In addition, when the emulsion breaks less water will be available to create the desired “puff” in the dough.

In manufacturing, the dough is rolled and the butter is extruded onto dough before lamination. In this extrusion process, large blocks of butter are extruded to form a thin ribbon of butter. This produces a consistent amount of butter to be added to the dough to allow for proper lamination. However, it has been observed that water is lost in the extrusion process. Water can be seen dripping from the extruder where the butter comes out. This is evidence of the water in oil emulsion breaking. It is assumed that the shear force of the extrusion processes breaks the water-in-oil emulsion resulting in the loss of water before the butter is incorporated to the dough. As discussed previously, excess water dripping and loss of water in the butter could contribute to a decreased amount of available water to produce the desired puff in the pastries.
Overall, it is known that butters used in laminated dough must be plastic so that they can withstand shear forces and must retain the encapsulated water to achieve the right “puff.” However, it is still unknown if SFC or plasticity are the only characteristics that drive water loss. There is a need to understand the role of butter physical properties on water retention during lamination. It is the purpose of this study to determine physical properties of butter products that are already on the market that drive water retention. Each of the butter products tested had a slightly different formula. The characteristics that will be identified include solid fat content (SFC), melting profiles, water droplet size, hardness, elasticity, water content, and water loss of butter when sheeted for lamination. Water loss will be tested for correlation with the other characteristics to gain a greater understanding of a potential compromised emulsion.

**MATERIALS AND METHODS**

**Sample preparation**

Three butter samples, with different lot/numbers, of each brand were purchased from a local grocery store. Samples were identified with a number, representing the brand (samples 1–7, Table 3-1), and within each sample replicates were identified with a letter, representing the lot number (A, B, and C). Each sample was stored at 5°C for 24 h before testing commenced. A storage temperature of 5°C was maintained throughout testing. Reported fat content and ingredient statements from the nutrition label of each sample were recorded (Table 3-1). The variety of butter samples include a range of fat content reported to be between 80% and 86%. Although many of the butter samples have similar
fat content claims, characteristics may differ depending on the processing, storage, or distribution. The characteristics of each butter were determined by the following tests.

**Table 3-1** Identification of sample numbers and composition of each butter sample.

Amount of fat in butter type column is determined from the information provided in the nutritional facts panel. Calculated fat content is based on water content and nonfat solids.

<table>
<thead>
<tr>
<th>Butter Number</th>
<th>Butter Type</th>
<th>Ingredients</th>
<th>Calculated Fat Content</th>
<th>Category for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unsalted butter 11g of fat per 14g butter (~79% fat)</td>
<td>Pasteurized Cream (Milk), Natural Flavorings</td>
<td>81.8%</td>
<td>Regular fat</td>
</tr>
<tr>
<td>2</td>
<td>Unsalted butter 12g fat per 14 grams butter (~85.7% fat)</td>
<td>Cream (milk), natural flavor</td>
<td>85.8%</td>
<td>High fat</td>
</tr>
<tr>
<td>3</td>
<td>Unsalted European style butter 12g fat per 14 grams butter (~85.7% fat)</td>
<td>Pasteurized cream (milk) and lactic acid</td>
<td>87.4%</td>
<td>High fat</td>
</tr>
<tr>
<td>4</td>
<td>Unsalted butter 12g of fat per 14g butter (~85.7% fat)</td>
<td>Cream, Natural Flavor</td>
<td>84.3%</td>
<td>High fat</td>
</tr>
<tr>
<td>5</td>
<td>Unsalted butter 11g of fat per 14g butter (~79% fat)</td>
<td>Cream (milk), natural flavor</td>
<td>82.9%</td>
<td>Regular fat</td>
</tr>
<tr>
<td>6</td>
<td>Unsalted butter 11g of fat per 14g butter (~79% fat)</td>
<td>Pasteurized cream (milk), natural flavoring</td>
<td>82.1%</td>
<td>Regular fat</td>
</tr>
<tr>
<td>7</td>
<td>Unsalted butter 11g of fat per 14g butter (~79% fat)</td>
<td>Sweet Cream, natural flavorings</td>
<td>82.3%</td>
<td>Regular fat</td>
</tr>
</tbody>
</table>

*Note:* Brand names are not used to maintain confidentiality.
**Water Content and Fat Content**

Water content of the sample was tested using a moisture analyzer (Sartorius MA 150 Moisture Analyzer Sartorius, Weender Landstrasse, Goettingen, Germany) using the rapid moisture analyzer method described by Bradley Jr. (2010). The moisture analyzer was set on a “standard drying” setting at 110°C. A weighing pan (102 mm × 8 mm, 60 ml) was tared, and approximately 5 g of sample was placed on the pan. Sample was cut into small pieces at room temperature and spread across the pan to increase accuracy of reading. The moisture analyzer was closed, and the percent water content was determined. Samples were tested in triplicate.

Fat content was identified by an indirect method. The fat content was determined by subtracting the water content, salt content, and solids-non-fat (SNF) from the whole. The amount remaining is determined to be fat content. Water content was accounted for as described previously. Salt determination was not necessary recognizing that all butters used were unsalted. A standard value of 1.5 g/100 g was used to account for SNF. A 1.5 g/100 g is a value commonly used in industry for SNF in cream. Fat content was not used as a factor for correlation analysis. It was only used in determining general fat levels.

**Water droplet size distribution**

Determination of water droplet size was executed using a method similar to van Lent et al. (2008). Nuclear Magnetic Resonance (NMR) Spectroscopy (Minispec mq-20, Bruker Inc., Billerica, MA) was used to determine the water droplet size. A core of each butter sample was taken using the back end of a pipette (9” disposable Pasteur pipette).
Each pipette was put inside an NMR tube (10 mm in diameter and 180 mm in height) and stored at 5°C for 24 h. The NMR was set to 5°C using a water bath. The settings were such that a 90° and 180° pulse length was checked, and calculations were conducted at the end of the measurement. Results for D3_3 were reported. Each sample was tested in triplicate.

**Solid fat content**

The solid fat content (SFC) of each sample was determined using Nuclear Magnetic Resonance (NMR) Spectroscopy (Minispec mq-20, Bruker Inc., Billerica, MA) following the AOCS Cd 16b-93 method (AOCS, 2009). Each sample was prepared by taking a core of the sample with the back end of a pipette (9” disposable Pasteur pipette). The end containing the sample was placed in an NMR tube (10 mm in diameter and 180 mm in height). All samples were placed in a water bath at 5°C for 1 hour and inserted into the NMR for reading. Results were recorded and the samples were then put in a water bath at 10°C for 1 h. This process was repeated at 5°C intervals from 5 to 40°C. The SFC of the sample at each temperature was recorded to identify the melting trend and amount of solid fat at each temperature. Measurements were performed in triplicate. All results were adjusted for water content.

**Melting behavior**

Melting behavior was determined using a method similar to that described in Lee and Martini (2018). Using a differential scanning calorimeter (DSC) (Q20, TA Instruments, New Castle, DE), 8–15 mg of each sample was put into an aluminum
hermetic pan. Each pan was sealed and placed in the DSC set to 5°C. An empty pan was used as a reference. The pan was held at 5°C for 1 min before temperature change. The DSC tracked melting behavior as the temperature increased from 5°C to 60°C at a ramp rate of 5°C per minute. The reported values include the change in enthalpy associated with the melting process, onset temperature, and peak temperature. Each of these parameters were determined using TA Universal Analysis software. Enthalpy measurements were adjusted for water content. Three measurements were performed for each sample with four measurements for samples with high variability.

**Texture/hardness**

Texture was analyzed by determining the hardness with a texture profile analyzer (TA-XT Plus texture analyzer, Texture Technologies Corp., Hamilton, MA) using a 1.5-inch diameter cylinder probe TA-4 (Texture Technologies Corp.). The sample was prepared by taking a core out of the butter with a plastic culture tube (12 × 75 mm, 5.0 ml). The end of the culture tube was cut off to allow for extrusion of the sample. Sample was refrigerated overnight at 5°C in the culture tube. Pushing the sample out of the tube, a 1 x 1 cm cylinder was cut and placed on the stage, resting on the flat side. A compression method was used to determine the hardness of the sample. The probe was calibrated to a starting position of 20 mm. The probe was lowered until the normal force just became positive, indicating the probe was barely touching the sample. At this level, the displacement was zeroed out and the test was initiated. The test was set to compress down 6 mm at a rate of 60 mm/s with a load cell of 50 N. After this step, the probe
returned to the zeroed-out position and the 6 mm compression was repeated. Measurements were performed in triplicate.

**Viscoelasticity**

Small deformation testing has been used in many studies on both milkfat and butter to understand materials' viscoelastic behavior. Wright and Marangoni (2006) outlined the procedure used for identification of viscoelastic properties. The viscoelastic behavior of the samples was determined using a rheometer (AR-G2, TA instruments New Castle, DE) with an 8 mm Plate SST Smart Swap geometry (TA Instruments). The instrument was set to 5°C with a frequency of 1 Hz and a strain between $8.0 \times 10^{-4}$ and 10% and a gap of 1000–1500 μm. The sample was prepared by taking a core of the butter sample with a culture tube (12 × 75 mm, 5.0 ml). The sample was refrigerated for 24 h at 5°C. Similar to the hardness measurement, the end of the culture tube was cut off to allow for the sample to be pushed out of the tube. The butter was pushed out of the tube and a 1 cm cylinder, 1-2 mm in height, was cut from the sample. The cylinder was placed, flat side down, on the stage. The geometry was lowered until it the normal force was just barely positive. The test began and the $G'$, $G''$, and phase shift angle ($\delta$) were recorded. The crossover point was calculated from this data as the strain values where $G' = G''$. Three measurements for each sample were performed.

**Water loss**

A method to measure the amount of water lost during the lamination process was developed in-house. The goal of measuring water loss was to mimic the water loss
observed in industry caused by the extrusion of the butter into a thin ribbon. All water loss tests were performed at room temperature. Butter at 5°C was used to measure water loss. This temperature was used to mimic the process performed in an industrial scale and to reduce oil loss during the extrusion. Five grams of sample were placed in the center of a rectangular, grade 1 filter paper (11 cm x 27 cm) and covered with a similar piece of filter paper. The sample and filter paper were rolled through a pasta roller (Atlas 150 Macchina per pasta, Campodarsego, Italy) at level 0 once. This rolling was repeated at each consecutive level once up to level 6 (1.2 mm). The purpose of this process is to absorb the water lost during the extrusion in the filter paper. After the rolling process, the sample and filter paper were cooled at 5°C for 30 min to mimic a resting step. This resting step also acted as a crystallization step to allow any fat melted by the friction or shear to harden in an effort to reduce oil loss. After 30 min the filter papers were gently pulled apart. Butter was scraped off the surface of each filter paper using a flat sided spatula. All butter scraped off was placed on a tared pan (102 × 8 mm, 60 ml) in the moisture analyzer (Sartorius MA 150 Moisture Analyzer) to measure the water content. The sample was evenly distributed across the surface of the pan. The moisture analyzer was set on a “standard drying” setting at 110°C and the analysis was started. Water loss was calculated by determining the difference between water content of butter before and after the extrusion. Measurements were performed in triplicate. A two-way ANOVA was used to determine significant differences between the rolled sample and the control (water content) sample. A one-way ANOVA was also used to determine significant differences between the amount of water lost between samples. This test was performed in triplicate.
Statistical analysis

Data was analyzed using Prism 9.0 (GraphPad Software, San Diego, CA). One-way ANOVA was used with Tukey's multiple comparison to analyze results of moisture analysis, water droplet size distribution, SFC, melting behavior, texture/hardness, and elasticity. A two-way ANOVA with Bonferroni multiple comparisons test was used for water loss. Correlation of all characteristics was determined in relation to water loss. A $\alpha = 0.05$ level of significance was used in statistical evaluation. A regression analysis was performed using SAS studio.

RESULTS AND DISCUSSION

Physical Properties

Water content

The water content of the butters evaluated ranged between 9% and 18% (Figure 3-1). The lowest water content was observed for sample 3A and was not significantly different from the results obtained for sample 3B ($p > 0.05$). The highest water content was observed for sample 7B and was not significantly different from the results obtained for samples 1A, 1B, 1C, 5B, 5C, 6A, 6B, 6C, and 7A ($p > 0.05$). Butters 2, 3, and 4 all had a water content below 15% while all other butter samples were generally above 15% water content. Butters 2, 3, and 4 are higher fat content butters (Table 3-1). We would expect a significant difference between the high fat content and regular fat content samples. Figure 3-1 shows that all lot numbers of samples 1, 6, and 7 are significantly different from all lot numbers of samples 2 and 3. These results are
consistent with our expectations that incorporating more fat would reduce the amount of water that can be included in the formulation. We would not expect to see a significant difference in physical properties within brands of butter. However, in both sample 3 and 7 we see a significant difference. While processing should be the same and ingredients should not differ, parameters of acceptance are often defined by a range that they must fall within. Water content can be changed by slight variations from the standard processing due to original fat composition, slight changes in temperature during churning or working, rotation speed, or the size of the butter grains (Mortensen, 2011). While the processing plants have set standards for all of these parameters, the range of acceptance for each parameter may allow for some slight variation.
**Figure 3-1** Water content of butter samples evaluated in this study. Samples sharing the same letter (a-j) are not significantly different (α=0.05). Error bars indicate standard error of the mean. Dotted lines indicate the mean of the lot numbers within a brand. On axis labels, sample brands represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.

**Water loss**

The difference in water content between the original samples and the rolled (or extruded) samples ranged from 0.7% to 6.0% (Figure 3-2). All samples were analyzed to identify a significant difference between the original sample and the rolled sample.

Samples 2A (p-value: 0.5670), 3A (p-value: 0.9908), and 4B (p-value: 0.0552) were the only samples that did not have a significant difference between the control and rolled samples. This means that the extrusion process did not result in any significant loss in
water. All other samples showed a significant change in water content between control and rolled samples ($\alpha = 0.05$). The percent change between each original sample and the treated sample is represented in Figure 3-2. The loss of water is a result of the breaking of the water-in-oil emulsion due to the shear force of the rolling process (Ghotra et al., 2002). Since no research has been performed to explain water loss in butter during lamination physical properties were tested in the butter samples to identify the properties that drive water loss. Characteristics correlated with water loss can help in understanding formulation needed to increase water retention during the lamination process.

**Figure 3-2** Water loss of samples. Samples sharing the same letter (a-l) are not significantly different ($\alpha=0.05$). Error bars indicate SEM. Dotted line indicates the mean of the lot numbers within a brand. On axis labels, sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.
Water droplet size

All samples had water droplet sizes between 2.6 and 7.5 μm (Figure 3-3). The results from all samples are consistent with results from other literature. Van Lent et al. conducted an experiment comparing the results of NMR and confocal scanning laser microscopy in determining water droplet size. The NMR results reported for the water droplet size of 82% fat commercial butter were 2.3–6.4 μm (van Lent et al., 2008). All the samples tested in this experiment have similar fat content (~81.8–87.4% fat) to those tested in van Lent’s study. Van Dalen published study identifying water droplet size in commercial spreads with 40%–80% fat. He reported a 2.6–10.6 μm water droplet size (van Dalen, 2002). In our study sample 7B tended to have smaller droplets, averaging around 2.67 μm, which were not significantly different from the water droplets in samples 1B, 1C, 2A, 2B, 4B, 4C, 6A, 6B, 6C, 7A, and 7C (p > 0.05).
**Figure 3-3** Water droplet size of butter samples evaluated in this study. Error bars represent standard error of the mean. Samples sharing the same letter (a-e) are not significantly different ($\alpha=0.05$). Dotted line indicates the mean of the lot numbers within a brand. On axis labels, sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.

In relation to the water loss, our hypothesis was that the size of the water droplets would have a significant effect on water loss. We thought that a larger water droplet would increase the likelihood of breaking the emulsion under pressure. However, there was no significant difference in water loss between the sample with the largest (sample 2C) and smallest (sample 7B) water droplet sizes (Figure 3-2). As visible in Figure 3-3, there are significant differences between the samples, but it does not seem to have a
significant effect on the water loss. The correlation analysis revealed no significant correlation between water droplet size and water loss when analyzing all the samples ($p = 0.881, r = 0.032$). Analysis of the high fat ($p = 0.264, r = 0.417$) also showed no significant correlation.

**Solid fat content**

The solid fat content (SFC) has been identified as one of the key characteristics to determine pastry fat quality (Lefebure et al., 2013). The SFC was tested to develop a curve of SFC across a temperature scale of 0–40°C (Figure 4). As expected, the SFC consistently decreased as the temperature increased. de la Horra et al. (2017) performed a similar study identifying the SFC of three different fats including shortening, oleomargarine, and refined bovine fat. In this study the SFC also consistently declined with increase of temperature. Both our results and the results of de la Horra et al. follow the expected effect that increased heat would melt the fat. The curve created by de la Horra et al. (2017) shows that the shortening and the bovine fat was comparable to the butter samples that were tested. The bovine fat followed a very similar trend to the measurements we recorded with a SFC in 45%–49% range at 10°C and approaching zero at 45°C. As depicted in Figure 4-4, all butter samples followed a similar curve. Even with a very similar trend, significant differences were identified between temperature levels within a sample, and samples within a temperature level (Table A-1). All temperature levels showed a significant difference between samples except 30°C.
Figure 3-4 Solid fat content (SFC) of samples at temperatures ranging from 5°C-40°C.

Sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.
In a study by Pajin et al. (2010) it is stated that SFC of pastry margarine should be between 11% and 16% at 40°C. With this understanding it could be assumed that high SFC at temperatures above room temperature would be associated with a decrease in water loss. In our testing the samples at 40°C ranged from 0.9% to 5.7% SFC. This is much lower than the desired range. Water loss and SFC did not have a significant correlation at any temperature when analyzing all samples (Table A-2).

*Melting behavior*

The melting behavior exhibited two peaks, one at approximately 12–16°C (peak 1) and a second one at approximately 23–27°C (peak 2). Figure A-1 shows the melting profile of sample 6C as a representation of all the samples. Similar results were recorded by Lee and Martini (2018). In their study they identified peak 1 as the medium-melting-fraction (MMF) and peak 2 as the high-melting-fraction (HMF) one. Onset temperatures ($T_{on}$), peak temperatures ($T_p$), and melting enthalpy ($\Delta H$) obtained for the individual peaks and enthalpy for the whole melting profile were determined. $T_{on}$ was not able to be identified in many of the peak 1 measurements due to immediate melting upon heating of the sample. For this reason, peak 1 onset temperature data is not included. Peak 1 enthalpy had no significant difference between samples with an overall average of $10.8 \pm 5.0$ J/g. The enthalpy of the second peak also had no significant difference between samples with an average of $25.2 \pm 7.6$ J/g. The enthalpy of the whole curve only identified a significant difference between the lowest result, 1A, and samples 2A, 2B, 3B, 3C, 4B, 4C, 5A, and 7A ($p < 0.05$) (Figure 3-5). The $T_p$ of the first peak also did not have any significant differences with any of the samples with an average of $13.1 \pm 2.8$ °C.
The $T_p$ of the second peak was highest in sample 3A with no significant difference from samples 1A, 2A, 2B, 2C, 4A, 4B, 4C, 5B, 5C, and 6C (Figure 3-6). Sample 1B was the lowest observed value for $T_p$ and was not significantly different from any of the samples except 2A and 3A ($p > 0.05$). The $T_{on}$ of the second peak did not have any identifiable significant differences among samples. It had an average onset temperature of $21.5 \pm 2.0^\circ C$ ($p > 0.05$).

**Figure 3-5:** Enthalpy of the whole in the melting profile identified by the DSC. Error bars represent standard error of the mean. All columns with results sharing the same letter (a-b) within the same graph are not significantly different ($\alpha=0.05$). Dotted line indicates the mean of the lot numbers within a brand. Sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.
Figure 3-6: Peak temperature peak 2 in the melting profile identified by the DSC. Error bars represent standard error of the mean. All columns with results sharing the same letter (a-b) within the same graph are not significantly different (α=0.05). Dotted line indicates the mean of the lot numbers within a brand. Sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.

While very few significant differences were identified between samples, the variation between samples did not correlate with water loss on any of the measurements. Enthalpy was expected to be an indicator correlated with water loss because it identifies the energy needed to melt the sample. While no heat is applied in the lamination process, the friction of the extrusion process could create heat. Butter can have also moisture.
migration at room temperature (Wright et al., 2001) indicating even a slight increase in temperature would be expected to increase water loss. However, total enthalpy \( (p = 0.297, r = -0.239) \), first peak enthalpy \( (p = 0.380, r = -0.202) \), and second peak enthalpy \( (p = 0.723, r = 0.082) \) all had no significant correlation. Onset temperature and peak temperature of both MMF and HMF did not show any significant correlation in results as a whole or within the high fat content group and regular fat content group.

**Hardness**

The hardness of the samples ranged from 4.7 to 9.6 N. According to Lee and Martini (2018), the hardness can be a result of the amount of crystalline material in the sample (SFC) but also is dependent on the crystal sizes. It has also been identified that the amount of water and the water droplet size as determining factors in hardness (Rønholt et al., 2012). Water is less viscous than fat and could decrease the hardness. Large water droplets reduce the crystal interactions also decreasing hardness (Rønholt et al., 2012). In our study there was a significant correlation between hardness and SFC at 20°C \( (p = 0.002, r = 0.625) \), 25°C \( (p = 0.001, r = 0.651) \), and 30°C \( (p = 0.0004, r = 0.697) \). Water droplet size \( (p = 0.357, r = 0.212) \) and water content \( (p = 0.090, r = -0.379) \) did not have a significant correlation with hardness \( (p > 0.05) \). While our data does not show water droplet size having a large effect on the hardness of our butter, the SFC at room temperature does have an effect. The butter hardness was measured at room temperature, which helps to understand the correlation between hardness and SFC at 20–30°C. We can conclude that, like Lee and Martini (2018) identified, hardness is highly affected by the amount of crystalline material.
The hardness values between lot numbers within each brand are consistent. The highest hardness value is found in sample 4C but is not significantly different from 1B, 1C, 2A, 2B, 2C, 3A, 3C, 4A, 4B, 5A, 5B, 5C, and 7A ($p > 0.05$). The lowest hardness value is found in sample 6C which is not significantly different from samples 1A, 1B, 2B, 3B, 6A, 6B, 7A, 7B, and 7C ($p > 0.05$; Figure 3-7). When comparing trends of hardness with water loss, hardness values do not follow any trend in the water loss values. A correlation analysis confirms the visual analysis indicating there is not a significant correlation with water loss and hardness ($p = 0.909$, $r = -0.007$) ($p > 0.05$). Many studies have identified hardness as a key characteristic in the quality of pastry butter (Haegens, 2014; O'Brien, 1998; Pajin et al., 2010). According to Haegens (2014) the hardness of a fat is crucial in determining its quality for use in pastry lamination. In lamination the butter must have a specific hardness to mimic the dough. If the hardness is too high the butter will break through the dough. If the hardness is too low it will squeeze out the sides. While these are important in pastry making, these concerns would not have a great effect the stability of the emulsion in extrusion.
**Figure 3-7** Hardness of butters stored at 5°C. Error bars represent standard error of the mean. All columns with results sharing the same letter (a-f) are not significantly different ($\alpha=0.05$). Dotted line indicates the mean of the lot numbers within a brand. Sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.

Viscoelasticity

$G'$ values are depicted in Figure 3-8. Sample 7A had the largest $G'$ results and was significantly different from all samples except 3A, and 5A ($p < 0.05$). The $G'$ results for sample 3B were the lowest but were not significantly different from many samples including 1B, 1C, 2B, 2C, 3C, 4A, 4C, 5B, 5C, 6B, 6C, 7B, and 7C ($p > 0.05$). Values of
all other rheological results are presented in Table A-3. $G''$ followed a similar pattern with sample 7A as the highest value with no significant difference from samples 1A, 2A, 3A, 4B, and 5A ($p < 0.05$). Sample 3B was again the lowest value and was not significantly different from samples 1B, 1C, 2B, 2C, 3C, 4A, 4C, 5B, 5C, 6A, 6B, 6C, 7B, and 7C ($p > 0.05$). The phase shift angle ($\delta$) is related to the ratio of $G''$ to $G'$. The phase shift angle did not follow the same pattern as $G'$ and $G''$. The highest value was found in 3C which was significantly different from samples 1A, 3A, 4A, 6B, and 7A with 4A being the lowest ($p < 0.05$). The crossover point is the strain at which the $G'$ and $G''$ are equal. After that strain the structure becomes more liquid like. The crossover point identified sample 1B with the highest results but there is no significant difference with any of the samples except 5A ($p < 0.05$).
**Figure 3-8** G’ values obtained for the butter samples. G’ indicates the elasticity or solid-like behavior of the sample. Results sharing the same letter (a-f) are not significantly different (α=0.05). Highest average G’ in sample 7. Dotted line indicates the mean of the lot numbers within a brand. Sample brands are represented by numbers 1-7 and lot numbers within each brand are represented by letters A-C.

While there was some significance in the differences between the samples, the differences did not correlate with the water loss of all the samples in any of the measurements. Both the samples with the highest and lowest values in G’ and G” were not among the highest or lowest values of water loss. Similarly, delta and crossover point did not follow the trend of water loss. The elasticity (G’) of all the samples did not have any correlation with the water loss ($p = 0.306$, $r = -0.217$). The G” also did not show a
significant correlation to water loss ($p = 0.258, r = -0.208$). The correlation of water loss to delta ($p = 0.661, r = -0.008$) and the crossover point ($p = 0.772, r = -0.308$), similarly, did not show significant results.

**Correlations**

The aim of this study was to find correlations between water loss and physical properties of butter. However, correlations were low among many of the characteristics tested. This may be due to a high amount of variation in the samples. The high variation allows for us to understand the effects of physical properties at a wide range of levels. However, variation may mask some of the effects of physical properties on water loss due to the influence of one physical property on another. To better understand the data, correlations were analyzed first as a whole, followed by separation into smaller groups as determined by fat content. Separating the samples allowed for a reduction in variation of water content to help understand correlations. The groups were split into high and regular fat content. All samples with fat levels below 83\% were included in the regular fat content group (Table 3-1). This includes samples 1, 5, 6, and 7. High fat butters were determined to be any with levels of fat reported above 83\% fat (Table 3-1). In the analysis of correlations as a whole it was expected for hardness, SFC, water droplet, and water content to play a major role in the stability of the emulsion. However, after data analysis it was apparent that many of these characteristics had very little correlation with water loss ($p > 0.05$) when all the samples were considered. Water content was the only characteristic correlated with water loss ($p$-value $= 0.009, r = 0.519$) (Figure 3-9). It is likely that the variation in the samples reduces the correlation between factors.
**Figure 3-9:** Correlation of water loss and physical properties with all samples. Water content is the only significant correlation. Values are the Pearson R values. Dotted line indicates level of significance.

It is also important to note that some physical properties may not play a significant role in prediction on their own. However, combining the effect of multiple physical properties gives us a better idea of how to predict water loss. A regression analysis was done on the data to identify what combination of factors could relate to the overall water loss. In analysis the following model was identified to account for 76% of variation:

\[
\text{WL} = -4.49 + 0.65\text{WC} + 0.08\Delta H_2 - 0.56\delta + 0.57\text{WD}
\]  \[2\]

In this regression the water content (WC), enthalpy of the second peak (\(\Delta H_2\)), phase shift angle (\(\delta\)), and water droplet (WD) are influential factors in the variation of water loss.
(WL) \((p\text{-value} < 0.001, r = 0.898)\). This equation helps us to understand the effect of each individual variable on the whole. For example, if we hold all other variables constant and increase water content 1\% we will see a 0.65\% increase in water loss. Similarly, if the enthalpy of the second peak increases one unit and all other variables are held constant, the water loss is predicted to increase by 0.08\%. All other coefficients and variables can be understood in a similar manner. While we may not see the influence in correlation a significant linear relationship exists in combination with the other properties. Validation of this model can be observed in Figure 3-10.

A regression equation was not performed on the high and regular fat groups because there was not enough data fulfil the degrees of freedom necessary for analysis.

**Figure 3-10:** Validation plot of linear regression. Predicted water loss vs. actual water loss. Positive linear trend indicates that the actual and predicted models follow a similar trend.
In the separated groups the high fat content group showed correlations with $G'$, $G''$, water content, and SFC at 5, 10, and 15°C (Figure 3-11). According to these correlations we can deduct that a higher elastic ($G'$) ($p = 0.010, r = -0.800$) and viscous ($G''$) ($p = 0.012, r = -0.786$) modulus will result in a lower amount of water loss in butter with a fat content higher that 83%. Viscoelastic values like $G'$ and $G''$ give us a sense of how the butter will react to small deformation. $G'$ and $G''$ only have a significant correlation with water loss in the high fat samples. The higher amount of fat increases the effect of viscoelasticity on water loss. These results suggest that water loss is driven by the molecular interactions of the crystalline network that help entrap the water droplets within the matrix. Low $G'$ values are also associated with larger crystal sizes, often due to a slow cooling rate (Rønholt et al., 2013). We can assume the large crystal size in higher fat products has a greater effect on water loss than low fat products. Our correlation analysis also indicated that a lower amount of water content will decrease the water loss in the sample with high fat products. A significant positive correlation between SFC and water loss in the high fat samples was also observed at 5°C ($p = 0.015, r = 0.769$), 10°C ($p = 0.026, r = 0.728$), and 15°C ($p = 0.036, r = 0.699$).
The water loss in butters with regular fat levels had a positive correlation with hardness, water droplet size, and SFC at 30°C (Figure 3-12). No correlation was found in this group between water loss and $G'$ and $G''$, and surprisingly, as mentioned above, there is a positive correlation with hardness, indicating that a harder butter will result in greater water loss. It is our hypothesis that for butters with high fat content molecular interactions in the fat matrix (measured by $G'$ and $G''$) drive water retention and that these molecular interactions allow the butter to withstand the shear forces created during lamination. In the case of butters with regular fat content, these molecular interactions are not as significant, and hardness and droplet size become the factors that control water loss. When butter is laminated, shear forces deform the butter creating a thin sheet of
material. As the hardness increases, the butter becomes less prone to deformation during the lamination process and therefore will break more easily resulting in a higher water loss. Similarly, the regular fat content samples indicated a positive correlation with SFC 30°C \( (p = 0.007, r = 0.730) \). This positive correlation is somehow surprising since we would have expected that a higher SFC would result in a lower water loss. It is likely that this positive correlation is associated with the crystalline structure of the sample. A higher SFC, especially at lower temperatures might result in a crystalline structure that might easily break when subjected to strain. In the case of butter, this breakage could result in the release of water during the extrusion process. Analysis of regular fat butter did have a positive correlation \( (p = 0.042, r = 0.593) \) between water loss and water droplet size. As described above, the water content of this group is not significantly different with a few exceptions (figure 3-1). When the water content is similar between samples, the water droplet has a greater effect on the water loss. This indicates that the amount of water has a greater effect on water loss than the water droplet size. When water content is high with no significant difference between samples the water droplet does influence water loss.
**Figure 3-12:** Correlation of water loss and physical properties with regular fat samples.

Values are the Pearson r values. Dotted lines indicate level of significance.

**CONCLUSION**

In this study water loss was found to be positively correlated with water content indicating that a higher fat product will have less water loss. In addition, regression analysis showed that water loss can also be affected by the melting enthalpy of the second peak, the phase shift angle value, and the water droplet of the butters. In short, an increase in water content, melting enthalpy of the second peak, and water droplet will increase water loss; while an increase in $\delta$ will decrease water loss. When only the high fat products are considered more correlations can be discovered. The high fat products had a negative correlation with $G'$ and $G''$. This correlation shows that higher elastic and
viscous values will decrease water loss in high fat products. Solid fat content had a positive correlation with water loss in high fat products at temperatures from 5°C to 15°C. This could be an indication that a crystal structure associated with high SFC will result in a greater amount of water loss. A similar positive correlation was found in water loss and SFC 30°C in the regular fat levels. The regular fat content products also had a positive correlation with hardness and water droplet size. Understanding how to control these characteristics is crucial to strengthen the water-in-oil emulsion to withstand shear forces caused by rolling. In short, to minimize water loss in butters used for lamination is important to understand that water loss is mainly affected by the water content of the butter but also by the melting enthalpy of the second peak, the δ, and the water droplet size. In addition, if producers are dealing with butters with high fat content, viscoelastic properties such as $G'$ and $G''$, and solid fact content must be also considered. If producers are working with butters with low fat contents, then hardness, water droplet size, and solid fat content are the main properties that must be considered. This research provides important information for butter producers that are looking for ways of improving the physical properties of their butters to be used for lamination purposes.

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CHAPTER 4

FAT CONTENT OF CREAM AFFECTS THE WATER HOLDING CAPACITY OF BUTTER

ABSTRACT

Extrusion of butter for laminated dough can result in water loss. The degree of water loss can be affected by butter physical properties which, in turn, can be affected by the fat content in the cream. In this study, butter was made from cream with six different levels of fat (38%–48%). Correlation analysis showed that the fat content of the cream had a positive correlation with water loss and water content and that water loss increases with a higher fat content of cream ($r$: 0.918), water content ($r$: 0.971), enthalpy ($r$: 0.950), delta ($r$: 0.975) and a decreased $G'$ ($r$: 0.826).

INTRODUCTION

Butter production is the process of changing cream, an oil-in-water emulsion, to butter, a water-in-oil emulsion. This phase inversion takes place when fat globule

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membranes are damaged during churning allowing for oil to leak out. The liquid fat that has leaked out makes up about two-thirds of the total fat of the cream. The fat globules make up the other third of the overall fat. Fat leakage and the coalescence of the fat globules form a continuous phase (Wright et al. 2001), entrapping water in the form of droplets and suspending the remaining milk fat globules in the fat phase. The buttermilk containing most of the water can then be drained and the butter will be worked to release more of the water.

There are many factors in the process of butter making that can affect the overall structure and quality of the butter. According to Rønholt et al. (2012), the cooling method used on the cream, or the mechanical treatment applied during the churning process, can have a large effect on the properties of the end product. Panchal et al. (2017) identified the importance of small fat globule size in reducing fat loss in the buttermilk and reducing churning time. Different temperature treatments before churning have reduced butter hardness and increased spreadability (Tondhoosh et al. 2016). Even though the effect of various processing conditions on the physical properties of butter has been previously studied, the effect of the fat content of the cream has not been explored. Therefore, the objective of this study is to understand the effect of the fat content of the cream on the physical properties of butter and relate these with butter water loss during lamination.

In laminated pastry making, high-quality butter is essential. Butter acts as a barrier between layers of dough creating individual flaky layers. When cooked, the butter melts and any water in the dough or butter evaporates into water vapor. This water vapor fills the space in between the dough layers created by the butter. The expansion from a
liquid to gas causes the rise of the pastries (Deligny and Lucas 2015). One quality defect seen in butter during the manufacturing of laminated doughs is the loss of water in the butter. In industrial manufacturing of laminated dough, butter is taken from a 55-pound block and milled and extruded to a thin ribbon. Water loss can be observed as water dripping from the extruder onto the dough or a conveyer belt below. Bradley and Smukowski (2009) identified this as a body or texture defect called leaking. This defect can be detrimental to the processing of laminated pastries. Since water is an essential part of the rise in laminated pastries, a loss in water during extrusion will result in a decrease in rise. If the water drips onto the pastry, it can increase the water content of the dough to a point that the dough cannot contain the water properly in cooking and the water will flash off instead of expanding in between the layers of dough (Fessas and Schiraldi 2001). Understanding the physical properties of butter may help in understanding what causes this defect in butter processing for laminated dough. The purpose of this study is to identify how the fat content of the cream is correlated with the physical properties of butter, specifically water loss in lamination. We also aim to understand how the physical properties of butter are correlated with water loss to help understand how processing can be adjusted to optimize water retention.

MATERIALS AND METHODS

Anhydrous milk fat preparation

Anhydrous milk fat (AMF) can be made from butter or a high-fat cream (Fearon 2011). The AMF used in this study was made from pasteurized cream (42% fat,
High Desert, Burley, ID) and processed into butter to allow separation of the fat. The cream was stabilized between 15 and 18°C. Approximately 4 liters of cream were added to the butter churn (Armfield FT21 Butter Churn, Hampshire, England) and churned at level 3 for 25 min. The speed was lowered to level 2 and churned for 2 min. The speed was then reduced to level 1 for 2 min or until a significant amount of buttermilk had been collected and small grains of butter were observed. The buttermilk was drained, and the butter was washed with distilled tap water. The churn was turned at low speed (setting 1) for 30 seconds and water was drained. Next, a 50:50 distilled to ice-water wash was added to the churn. Again, the butter and water were churned for 1 min at low speed. The water was drained and replaced with an ice-water wash and churned for 1 min. Water was drained, and the butter was scraped out of the churn and placed on multiple layers of cheesecloth. After wrapping the butter in cheesecloth, the butter was squeezed to remove any excess water. Butter was then stored in the fridge at 5°C until making AMF.

The procedure of obtaining AMF from butter was similar to the one described by Shukla et al. (1994). Butter was removed from the refrigerator and placed in a beaker in the oven at 65°C until the butter was completely melted. Once melted, the butter was poured into a separatory funnel in the oven. The fat and water got separated in the funnel and the water was drained from the bottom. Sodium sulphate was added to the fat fraction at a level of 1% to remove any remaining water. The salt was stirred, and the mixture sat in the oven for 5 min at 65°C allowing for the salt to settle. The solution was stirred again and set in the oven for another 10 min. The solution was stirred one more time and filtered using vacuum filtration and a glass microfiber filter. The filtered fat was collected and stored at 5°C. The final AMF had a melting point of 33.55 ± 0.56.
Sample preparation

Organic pasteurized cream (30% fat; Kalona Organics, Kalona, IA, USA) with no additives was purchased from a local grocery store. The cream was stored at 5°C for 24 h before use. Cream fat content was measured using the Babcock method (described below). Once the fat content is determined, the cream can be standardized by adding AMF to reach fat levels of 38%, 40%, 42%, 44%, 46% and 48%. Proportions of AMF and cream were determined using the following formulas:

$$100x + (\text{cream fat content})y = \text{desired percent fat} \quad [3]$$

$$x + y = 1 \quad [4]$$

In these formulas, $x$ represents the fraction of AMF needed in the mixture and $y$ represents the fraction of cream. The cream and AMF mixture was prepared (1.1 L) for each fat level in triplicate. The cream was heated to 15–18°C in a water bath and the melted AMF was added mixing with an immersion blender (Hamilton Beach 225-Watt motor, high speed, Glen Allen, VA, USA) for 30 s. Samples of the mixture were taken and a Babcock test was performed to quantify fat content of the mixture.

The cream/AMF mixture was stabilized between 15 and 18°C. One liter of cream/AMF mixture was added to the butter churn (Armfield FT21 Butter Churn, Blashford, Ringwood, UK) and turned on level 3 for 7 min. The speed was lowered to setting 2 and ran for 2 min. Again, the speed was lowered to setting 1 and ran for 2 min. The buttermilk was drained and collected. Fat content analysis was performed on the buttermilk to understand the amount of fat lost. Distilled water was added to the butter in the churn to wash the butter. The churn was turned on low speed (setting 1) for 1 min and
water was drained. Half a liter of distilled water and half a liter of ice water were added to the churn. Again, the butter and water were churned for 1 min at low speed. Water was drained and replaced with 1 L ice water and churned for 1 min. Water was drained and the butter was scraped out of the churn and placed on multiple layers of cheesecloth. After wrapping the butter in cheesecloth, the butter was squeezed to remove any excess water. Butter was formed into blocks and stored in the fridge at 5°C for at least 24 h before testing. Three replicates were performed at each cream fat content level.

**Fat content of cream**

The fat content of cream, cream/AMF and buttermilk was measured using the Babcock method (Lynch *et al.* 1996). In this method, 9 grams of sample was placed in a cream Babcock bottle. Sulphuric acid (17.5 mL) was added to the bottle and the sample was mixed. Bottles were placed in the centrifuge for 10 min, filled with water to bring the solution up into the neck of the bottle and centrifuged again for 5 min. Measurements were made by using the scale on the neck of the bottle. Measurements were done in triplicate for each sample.

**Water content**

Water content was determined by a rapid moisture analyzer (Sartorius MA 150 Moisture Analyser, Sartorius, Weender Landstrasse, Goettingen, Germany) using the rapid moisture analyzer method described by Bradley (2010). Using the ‘standard drying’ setting at 110°C, the sample was dried to remove all water. Approximately 5 g of sample were placed on a tared weighing pan (102 mm × 8 mm, 60 mL). Small amounts of butter
sample were placed across the pan to ensure even melting and water reduction. The water content was then determined as the moisture analyzer heated the sample and recorded water loss. Samples were tested in triplicate.

**Water droplet size**

Water droplet size was determined using a method similar to van Lent *et al.* (2008). Nuclear Magnetic Resonance (NMR) Spectroscopy (Minispec mq-20; Bruker Inc., Billerica, MA, USA) was used to determine the water droplet size. A core of each butter sample was taken using the back end of a pipette (9” disposable Pasteur pipette). Each pipette was put inside an NMR tube (10 mm in diameter and 180 mm in height) and stored at 5°C for 24 h. The NMR was set to 5°C using a water bath. The settings were such that a 90- and 180-degree pulse length was checked, and calculations were conducted at the end of the measurement. Results for D3_3 were reported. Each sample was tested in triplicate.

**Solid fat content**

The solid fat content (SFC) of each sample was determined using NMR Spectroscopy (Minispec mq-20; Bruker Inc.) following the AOCS Cd 16b-93 method (AOCS 2009). Each sample was prepared by taking a core of the sample with the back end of a pipette (9” disposable Pasteur pipette). The end containing the sample was placed in an NMR tube (10 mm in diameter and 180 mm in height). All samples were placed in a water bath at 5°C for 1 h and inserted into the NMR for reading. Results were recorded and the samples were then put in a water bath at 10°C for 1 h. This process was
repeated at 5°C intervals from 5°C to 35°C. The SFC of the sample at each temperature was recorded to identify the melting trend and amount of solid fat at each temperature. Measurements were performed in triplicate. All results were adjusted for water content.

**Melting behavior**

Melting behavior was determined using a method similar to that described in Lee and Martini (Lee and Martini 2018). Using a differential scanning calorimeter (Q20; TA Instruments, New Castle, DE, USA), 8–11 mg of each sample was put into an aluminum hermetic pan. Each pan was sealed and placed in the DSC set to 5°C. The pan was held at 5°C for 1 min before temperature change. The DSC tracked melting behavior as the temperature increased from 5°C to 60°C at a ramp rate of 5°C per min. The reported values include the change in enthalpy associated with the melting process, onset temperature and peak temperature. Each of these parameters was determined using TA Universal Analysis software. Enthalpy measurements were adjusted for water content. Measurements were performed in triplicate.

**Texture/hardness**

Texture was analyzed by determining the hardness with a texture profile analyzer (TA-XT Plus texture analyzer; Texture Technologies Corp., Hamilton, MA, USA) using a cylinder probe TA-4 (Texture Technologies Corp.). The sample was prepared by taking a core out of the butter with a plastic culture tube (12 × 75 mm, 5.0 mL). The sample was refrigerated overnight at 5°C in the culture tube. A compression method was used to determine the hardness of the sample as described by Wright et al. (2001). The probe was
calibrated to a starting position of 20 mm. The probe was lowered until the normal force just became positive, indicating the probe was barely touching the sample. At this level, the displacement was zeroed out and the test was initiated. The test was set to compress down 6 mm at a rate of 60 mm/s. After this step, the probe returned to the zeroed-out position and the 6 mm compression was repeated. Measurements were performed in triplicate.

**Viscoelasticity**

The viscoelastic behavior of the samples was determined in a method similar to de la Horra *et al.* (2017) using a rheometer (AR-G2; TA instruments) with an 8 mm Plate SST Smart Swap geometry (TA Instruments). The instrument was set to 5°C with a frequency of 1 Hz and a strain between $8.0 \times 10^{-4}$ to 10%. The sample was prepared by taking a core of the butter sample with a culture tube (12 × 75 mm, 5.0 mL). The sample was refrigerated for 24 h at 5°C. A 1–2 mm cylinder was cut from the sample and placed, flat side down, on the stage. The geometry was lowered until the normal force was just barely positive with a gap of 1000–1600 μm. The test began and the $G'$, $G''$ and delta were recorded. The 12th measurement was recorded because the reading had stabilized at this point. The crossover point was calculated from these data as the strain values were $G' = G''$. The data were reported in triplicate.

**Water loss**

A method to measure the amount of water lost during the lamination process was developed in-house. The goal of measuring water loss was to mimic the water loss
observed in the industry caused by the extrusion of the butter into a thin ribbon. Five grams of sample were placed in the center of a rectangular, grade 1 filter paper (11 cm × 27 cm), and covered with a similar piece of filter paper. The sample and filter paper were rolled through a pasta roller (Atlas 150 Macchina per pasta, Campodarsego, Italy) at level 0 once. This rolling was repeated at each consecutive level once up to level 6 (1.2 mm). The purpose of this process is to absorb the water lost during the extrusion in the filter paper. After the rolling process, the sample and filter paper were cooled at 5°C for 30 min to mimic a resting step. This resting step also allowed the fat to harden after being handled in an effort to reduce fat loss. After 30 min, the filter papers were gently pulled apart. Butter was scraped off the surface of each filter paper using a flat-sided spatula. All butter scraped off was placed on a tared pan (102 mm × 8 mm, 60 mL) in the moisture analyzer (Sartorius MA 150 Moisture Analyzer) to measure the water content. The sample was evenly distributed across the surface of the pan. The moisture analyzer was set on a ‘standard drying’ setting at 110°C and the analysis was started. Water loss was calculated by determining the difference between the original water content and the water content of the sample after rolling it out. Measurements were performed in triplicate. This test was performed in triplicate.

**Statistical analysis**

Data were analyzed using Prism 9.0 (GraphPad Software, San Diego, CA, USA). One-way ANOVA was used with Tukey’s multiple comparisons to analyze results of moisture analysis, water droplet size, SFC, melting behavior, texture/hardness and between the amount of water lost between samples and elasticity. A two-way ANOVA
with Bonferroni multiple comparisons test was used for water loss. The correlation of all characteristics was determined in relation to water loss. An $\alpha = 0.05$ level of significance was used in statistical evaluation represented by a p-value of less than 0.05 to be considered significant.

RESULTS AND DISCUSSION

Physical properties

All physical properties of butter made with creams with different fat content were recorded and analyzed. Significant differences were not identified in most of the properties. Since these properties were not affected by the fat content of the cream, values obtained for the physical properties of butter formulated using creams with various fat content were averaged and reported in Table 4-1.
Table 4-1: Mean values and Standard deviation of physical properties of all cream fat content levels.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content (%)</td>
<td>41.6 ± 3.4</td>
</tr>
<tr>
<td>SFC 5°C (%)</td>
<td>52.7 ± 0.5</td>
</tr>
<tr>
<td>SFC 10°C (%)</td>
<td>47.0 ± 0.4</td>
</tr>
<tr>
<td>SFC 15°C (%)</td>
<td>37.6 ± 0.5</td>
</tr>
<tr>
<td>SFC 20°C (%)</td>
<td>26.0 ± 0.5</td>
</tr>
<tr>
<td>SFC 25°C (%)</td>
<td>15.1 ± 0.6</td>
</tr>
<tr>
<td>SFC 30°C (%)</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>SFC 35°C (%)</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>Enthalpy (J/g)</td>
<td>57.4 ± 3.1</td>
</tr>
<tr>
<td>Onset Temperature (°C)</td>
<td>21.5 ± 2.0</td>
</tr>
<tr>
<td>Peak Temperature (°C)</td>
<td>22.5 ± 1.1</td>
</tr>
<tr>
<td>G' (Pa)</td>
<td>387,335 ± 92,809</td>
</tr>
<tr>
<td>G'' (Pa)</td>
<td>89,273 ± 17,001</td>
</tr>
<tr>
<td>Delta (°)</td>
<td>13.3 ± 0.9</td>
</tr>
<tr>
<td>Rheological Crossover Point</td>
<td>0.0110 ± 0.0007</td>
</tr>
<tr>
<td>Water Droplet Size (µm)</td>
<td>26.7 ± 1.2</td>
</tr>
<tr>
<td>Water Content (%)</td>
<td>13.7 ± 0.7</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Water Loss (%)</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Rheology Yield Strain</td>
<td>0.0030 ± 0.0003</td>
</tr>
</tbody>
</table>

Fat content of the cream

A sample of each cream was collected just prior to churning to allow for the most accurate representation of the cream fat content. While the cream fat content was calculated for each formulation, the Babcock test allowed for a precise measurement to validate the calculations. The fat content ranged from an average of 36.8 to 46.1% fat. Each fat level was significantly different from the others (Figure 4-1). Each sample was created to determine how a range of fat contents affects the other properties of the butter.
**Figure 4-1**: Measured fat content of the cream at each calculated cream fat content.

Results sharing the same letter (a-f) are not significantly different ($\alpha=0.05$).

![Bar graph showing measured fat content at each calculated cream fat content.](image)

**Water droplet size**

Water droplet size in our study ranged from 24.5 to 28.0 $\mu$m which is higher than the droplet sizes of commercial butter (Figure B-1). van Lent *et al.* (2008) reported droplet sizes between 2.3 and 6.4 $\mu$m; while van Dalen (2002) reported droplet sizes from 2.6 to 10.6 $\mu$m. These values are both from commercial butter spreads. However, Rønholt *et al.* performed a study in which AMF was added to skim milk where water droplet was reported to be between 8 and 35 $\mu$m. The higher water droplet was a result of small-scale manufacturing as opposed to industrial manufacturing (Rønholt *et al.* 2012). The average water droplet for the whole data set is 26.9 ± 0.6 $\mu$m and no significant differences ($P > 0.05$) were found for droplet sizes among the butter made with creams with different fat contents. Our water droplet size is much higher than the commercial
butter results reported in van Lent and van Dalen's studies. However, it is much closer to the range of small-scale manufacturing (Rønholt et al. 2012). Water droplet size does not have a significant correlation with any other characteristics indicating that water droplet size affects the structure. Similar results were found in Chapter 3 (Jones and Martini 2022).

**Solid fat content**

The solid fat content follows a similar curve to that reported by Wright et al. Wright describes that the triacylglycerols in milk fat have melting points between −40 and 40°C. Her report shows a starting SFC around 55 at 0°C and decreases until it levels out close to zero at around 40°C (2008). All samples followed a very similar curve (Figure B-2). All samples showed a significant difference between temperature levels ($P < 0.05$). However, there were no significant differences between samples at each temperature. The average SFC values obtained for the butter made with creams with different fat contents are reported in Table 4-1.

**Water content**

Water content of the butter ranged from 13.7 to 14.3%. The water content slightly increased with the amount of fat content in the cream (Figure B-3). However, the increase was not significant between the samples ($P = 0.25$). All of the water content values in the butter were within the regulation for the standard of identity in both the US Code which indicates that butter must have 80% fat (Federal Food, Drug, and Cosmetic Act, 1906)
and the Codex Alimentarius which identifies that butter must have 80% fat and a maximum water content of 16% (Codex Alimentarius 2018).

**Melting profile**

The melting of each of the samples followed a similar pattern. The melting profile of each of the cream fat content levels can be seen in Figure B-4. The onset temperature is pretty steady across all the cream fat content levels with no significant difference between any of the levels ($P > 0.05$) and an average onset temperature across all the fat levels of $21.5 \pm 2.0\,^\circ C$ (Table 1). Similarly, the peak temperature is also not significantly different among the various butter ($P > 0.05$) (Figure B-4) with an average value of $23.9 \pm 2.3\,^\circ C$ (Table 1). We would expect the onset and peak temperatures of the butter to be similar across all the fat levels since the AMF added to the cream was all from the same sample giving it a similar melting profile.

The enthalpy of the samples has a steadily increasing pattern (Figure B-5A). The enthalpy ranges from 51.3 to 61.9 J/g, increasing with the amount of fat in the cream. While the pattern follows the increase in fat content, many of the levels did not have a significant difference ($P > 0.05$). The only significant difference was identified between the lowest fat content, 38% fat cream, and the two highest fat contents, 46% fat and 48% fat creams. While both the onset temperature and peak temperature do not show any significant difference, it is not unusual that we would see some significant difference with the enthalpy. Since the water content of the butter was not significantly different, we can assume that the fat content is not significantly different either. Enthalpy is the measure of energy required to melt the sample. The increase in energy consumption is most often
related to an increased amount of crystalline fat or increased interactions. Densely packed crystals will take more energy to melt than individual crystals. The increase in enthalpy observed for butter made with high-fat creams suggest a stronger and more organized crystalline network that required more energy to melt the sample.

**Hardness**

Hardness levels were consistent between samples ranging from 3.5 to 4.1 N (Figure B-6). No significant difference was found between samples \( P > 0.05 \). There are many factors that can play a part in hardness. Crystalline network, water content and water droplet size can contribute to the overall hardness (Rønholt et al. 2012; Lee and Martini 2018). There is no significant difference in water content or water droplet size and therefore it is expected not to see any differences in hardness. The difference found in enthalpy values for butter made with high-fat creams might not be sufficient to result in a significant change in hardness.

**Viscoelasticity**

The properties related to viscoelasticity include \( G' \), \( G'' \), delta, crossover point and yield strain. \( G' \) represents the elasticity of the sample and is known as the storage modulus. \( G'' \) represents the viscous behavior of the sample and is known as the loss modulus. Delta (phase angle) is calculated by the ratio of these two moduli. All four of these factors did not have a significant difference between the different cream fat levels \( P > 0.05 \) (Figure B-7). Average values obtained across the various butter are reported in Table 4-1. The crossover point is the strain at which the \( G' \) and \( G'' \) are the same. After
this point the sample has a more liquid-like behavior instead of a more solid-like behavior. As seen in Table 4-1, there is very little variation in the crossover point indicating the deformation pattern is very similar between all samples. The yield strain is the strain where the solid-like behavior begins to diminish. Before this point the \( G' \) values follow a steady pattern. Similar to the other rheological properties, the yield strain did not have a significant change between fat content levels.

**Water loss**

One of the major correlation factors we wanted to identify is the correlation of physical properties to water loss. Understanding the fat content of cream that results in the lowest amount of water loss allows for direction to what fat content of cream to use in butter production. Butter that were laminated in the lab showed a significant amount of water loss (Figure 4-2a). However, the amount of water lost between butter was only significantly different between the lowest and highest fat contents (Figure 4-2b). The average water loss ranged from 3.8 to 6.2.
Figure 4-2: (a) Water content of butter (control) compared to water content of butter after lamination (Rolled). Significant difference between the control and rolled samples is indicated by a * above the control bar (p<0.05). (b) Measured water loss of butter at each calculated cream fat content. Results sharing the same letter (a-b) are not significantly different (α=0.05).
Correlations

The correlations between the physical properties can be an indicator of changes influencing water loss. Correlations related to water loss and fat content of the cream are of greatest interest. Understanding what physical properties were affected by the cream fat content can help us to identify potential changes to the process of butter making.

Table 4-2 includes all the correlation factors relating to both of these physical properties (fat content and water loss).

Table 4-2: Correlation factors of physical properties to fat content and water loss.

<table>
<thead>
<tr>
<th></th>
<th>Fat Content</th>
<th>Water Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
</tr>
<tr>
<td>Fat content</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>SFC 5</td>
<td>-0.176</td>
<td>0.739</td>
</tr>
<tr>
<td>SFC 10</td>
<td>-0.745</td>
<td>0.089</td>
</tr>
<tr>
<td>SFC 15</td>
<td>-0.858</td>
<td>0.029</td>
</tr>
<tr>
<td>SFC 20</td>
<td>0.532</td>
<td>0.277</td>
</tr>
<tr>
<td>SFC 25</td>
<td>0.595</td>
<td>0.213</td>
</tr>
<tr>
<td>SFC 30</td>
<td>0.256</td>
<td>0.624</td>
</tr>
<tr>
<td>SFC 35</td>
<td>0.167</td>
<td>0.752</td>
</tr>
<tr>
<td>DSC (Enthalpy)</td>
<td>0.828</td>
<td>0.042</td>
</tr>
<tr>
<td>DSC Onset Temperature</td>
<td>-0.077</td>
<td>0.885</td>
</tr>
<tr>
<td>DSC Peak Temperature</td>
<td>0.112</td>
<td>0.832</td>
</tr>
<tr>
<td>Rheology (G’)</td>
<td>-0.581</td>
<td>0.227</td>
</tr>
<tr>
<td>Rheology (G”)</td>
<td>-0.334</td>
<td>0.518</td>
</tr>
<tr>
<td>Rheology (Delta)</td>
<td>0.883</td>
<td>0.020</td>
</tr>
<tr>
<td>Rheology Crossover Point</td>
<td>0.022</td>
<td>0.967</td>
</tr>
<tr>
<td>Water Droplet (NMR)</td>
<td>0.332</td>
<td>0.520</td>
</tr>
<tr>
<td>Water Content</td>
<td>0.948</td>
<td>0.004</td>
</tr>
<tr>
<td>Hardness</td>
<td>-0.603</td>
<td>0.205</td>
</tr>
<tr>
<td>Water Loss</td>
<td>0.918</td>
<td>0.010</td>
</tr>
<tr>
<td>Rheology Yield</td>
<td>-0.347</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Fat content of cream

The fat content of the cream is positively correlated with enthalpy of the melting profile \( (P = 0.049, r = 0.813) \). The enthalpy measures the amount of energy that is absorbed by the sample during melting. This indicates that the amount of crystalline fat may be higher in the butter made with the higher-fat cream since it requires more energy to melt the sample and results in a higher enthalpy. The enthalpy can also be an indicator of the structure of the crystal network. A tighter network requires more energy to break it down during heating. As mentioned before butter made with different creams had the same water content, and therefore same fat content, therefore it is likely that the correlation between fat content and enthalpy is due to the presence of a tighter and stronger crystalline network in butter made with high-fat creams. Fat content also has a positive correlation with delta. Delta is calculated from the ratio of \( G'' \) to \( G' \) for each cycle of deformation (Silva et al. 2013). A larger delta indicates that the ratio of liquid-like behavior to solid-like behavior is higher in the system. Understanding the enthalpy and the delta may suggest that the higher fat content cream will result in a higher ratio of liquid-like material with a highly dense crystalline network.

Water content is also positively correlated with fat content of the cream \( (P = 0.004, r = 0.948) \). From this correlation, we recognize that a higher amount of fat in the cream will result in a higher amount of water in the butter and therefore a lower amount of fat in the butter. The differences between the water content of the different samples are not significant, as previously mentioned, indicating that changes between samples are very small. The fat content was raised in each product by adding liquid AMF. Higher fat in the cream does not result in higher fat in the butter because the
amount of solids in the medium is not sufficient to stabilize the butter. If there are not enough solid particles that grain in the churning process, it cannot trap all the liquid fat in the butter matrix and that is why using a high amount of fat in the cream does not result in a butter with significantly higher fat content.

Water loss

As we can see in Figure 4-2b, there is a visible increase in water loss as the fat content of the cream increases. However, there is only a significant difference in these values between the butter made with the cream with the lowest and highest fat content. It seems that regardless of how much water was originally in the sample, there is a level of water that can be properly emulsified in conditions required for sheeting butter. The average amount of water retained among all the samples was $8.5 \pm 0.2\%$ water. With this as the optimal level of water, we can see a higher amount of fat incorporated in the butter, as in the case of butter made with low-fat content creams, which would help to drive the water content down and reduce water loss. In the correlation analysis, this concept is validated, indicating that there is a positive correlation between water loss and water content ($P = 0.001$, $r = 0.971$). This indicates that a higher water content will result in a higher amount of water loss. When the fat content of the cream drives a higher water content in the butter, we will also see an increasing amount of water lost in the lamination of butter.

We also see a significant correlation between water loss and a few other physical properties. A negative correlation with $G'$ ($P = 0.043$, $r = -0.826$) indicates that the higher elastic modulus follows a lower amount of water loss. Delta has a positive correlation
with the water loss \( (P = 0.001, r = 0.975) \). This shows that a higher delta (greater amount of liquid-like behavior compared to solid-like material) would result in a higher amount of water loss. Under pressure it seems that a more liquid behavior would allow for the water droplets in the system to coalesce and escape the butter emulsion. Both the correlation to \( G' \) and delta help to demonstrate an increase in elastic-like behavior as a key characteristic in reducing water loss. \( G' \) provides information about the solid-like behavior of the crystalline network which can be driven by the amount of solid material and by interactions at the molecular level. We would expect that if water loss was negatively correlated with \( G' \), then it will be also negatively correlated with melting enthalpy, since a fat network with a high elastic modulus could be a result of having more crystalline material and therefore a higher enthalpy. However, contrary to what was expected, a positive correlation between enthalpy and water loss \( (P = 0.004, r = 0.950) \) was observed. This suggests that water loss can be minimized by having a crystalline network with a high elastic modulus that indicates stronger molecular interactions, but with lower fat content or a less structured crystalline network measured by the enthalpy. This contradiction in physical properties is not completely understood. One potential explanation could be due to protein content. The protein content is known to assist in the emulsification of butter. AMF contains no protein as the protein is removed in the separation process. When adding AMF to cream in greater percentages, it is expected that the protein content would decrease. This decrease could affect the stability of emulsion and the overall water loss. However, changes in protein content are very small between samples. Another cause of this contradiction could be the structure and interactions of the crystals. It is our hypothesis that the less structured crystalline network, measured by the
enthalpy, allows for the fat to deform during the lamination, but the strong molecular interactions prevent the crystalline network from breaking and allow for water retention.

CONCLUSION

While we saw a significant difference in the fat content of the cream at each level, many of the physical properties did not display significant differences between the samples including water loss. However, strong correlations were identified with both the fat content and the water loss with a few physical properties. Strong correlations were identified between cream fat content and water content, enthalpy, delta and water loss. Similarly, strong correlations were identified between water loss and fat content of the cream, water content, enthalpy, $G'$ and delta. Understanding how the fat content affects all physical properties gives greater insight into the handling of cream before churning. These results also identify which specific properties play a role in water loss and how they are affected by cream fat content. Overall, we have found that there are correlations between water loss increase and increased fat content of the cream, a higher water content in the butter, a higher melting enthalpy, a higher delta and a decreased $G'$. Changing the fat content of the cream had strong correlations with the overall water loss. However, the changes in total water loss were comparatively small with only a significant difference between the highest and lowest levels. These findings show that to reduce the amount of water lost during extrusion during lamination processes, a cream with low-fat content and butter with low water content should be used. In addition, butter with lower melting enthalpies, but high elasticity and high shift phase angle will also result in low
water loss. Future research could look into changing the composition of the fat by adjusting the fatty acid/triacylglycerol balance to evaluate how fat composition affects water loss.

ACKNOWLEDGEMENTS

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REFERENCES


URL https://www.ecfr.gov/current/title-7/subtitle-B/chapter-I/subchapter-C/part-


CHAPTER 5

EFFECT OF ANHYDROUS MILK FAT FRACTION ADDITION IN BUTTER ON WATER LOSS REDUCTION

ABSTRACT

The objective of this chapter is to incorporate anhydrous milk fat (AMF) and fractions of AMF (AMF_f) to evaluate their effect on water loss and physical properties of the butter obtained. Two methods were used to incorporate AMF and AMF_f in butter production to increase the amount of fat in the cream. The first method incorporated AMF and AMF_f in the cream before churning and will be referred to as the “pre-churn method”. The second method incorporated AMF and AMF_f in the working step at the end of churning process and will be referred to as the “post-churn method”. In both of these methods four different AMF_f were incorporated: control (non-fractionated AMF), 20°C fraction (AMF20), 25°C fraction (AMF25), and 30°C (AMF30). The two methods had opposite effects on water loss. Butters made with the pre-churn method had a trend toward a lower water loss when formulated with higher melting point AMF_f. However, butters made with the post-churn method showed a decrease in water loss when lower melting AMF_f were used. The water loss for butters made with the pre-churn method had a significant positive correlation with water content and a significant negative
correlation with hardness, rheological crossover point, solid fat content at 5°C- 20°C, enthalpy, and amount of palmitic and oleic fatty acids. These characteristics suggest that a greater amount of crystalline fat in the butter results in lower amount of water loss. The water loss of butters obtained with the post-churn method had a significant positive correlation with hardness, solid fat content at 5-20°C, and myristic fatty acids. These results suggest that a high-melting fraction of AMF can be incorporated in a high-fat cream to reduce water loss. In a commercial application the pre-churn method can be used with a high melting fraction to increase the hardness and structure of the butter to allow for a reduction in water loss.

INTRODUCTION

Milk fat is very complex because it contains a wide variety of fatty acids (FA) and triacylglycerols (TAGs) in varying amounts based on the diet of the animal and the breed of cow where it comes from. These FA arranged in TAGs can affect the properties of the cream and the resultant butter. Individual FA and TAGs have very different melting points ranging from -50 to 80°C (Rønholt et al., 2013).

The melting profile of fat is highly dependent on the FA and TAG composition of the butter. The most prevalent FA in milk fat include palmitic acid (22-35%), stearic acid (9-14%), myristic acid (8-14%), and oleic acid (20-30%) while many others are present in smaller quantities (Buldo and Wilking, 2016). The proportion of these fatty acids helps to account for the physical properties of the fat. According to Kaylegian and Lindsay
decreases in long chain saturated fats and increases in short chain and long chain unsaturated fats leads to a lower melting point and thus a lower solid fat content.

Fractionation is a method to select a target melting profile by isolating TAGs in a certain melting point range. Anhydrous milk fat (AMF) can be fractionated because it has no additives and because the water has been removed leaving a 99.8% milk fat product (FAO and WHO, 2019; Mortensen, 2011). Three main fractions that have been identified in milk fat with melting points between -40 and 40°C. The low-melting fraction consists of TAGs that melt below 10°C, the medium-melting fraction melts between 10-19°C, and the high-melting fraction consists of TAGs that melt at temperatures above 20°C (Deffense, 1993). The melting profile of butters can be manipulated by incorporating different AMF_f throughout the processing. Spreadable butters are often supplemented with a low or medium melting fraction to decrease the solid fat content at lower temperatures. Pastry applications often use the higher milkfat fraction to allow the butter to be layered and worked without melting before baking, while cakes require medium fractions to allow for air incorporation and volume (Kalegian and Lindsay, 1995c).

When butters are used in laminated pastries, a defect is often seen during the extrusion process. During this process, butter is sheeted from 50 lb. blocks to a thin ribbon of butter that can be incorporated in lamination for pastries like croissants or Danishes. In this process, water is often seen dripping from the equipment. This defect of excess water is known as leaking (Bradley and Smukowski, 2009). In Chapter 3 commercial butters were studied to identify physical properties that are correlated to water loss. It was identified that high water content and high water loss follow a similar trend. In Chapter 4 the fat content of cream was tested to see its effect on water loss. This
study identified that the fat content of the cream does not significantly affect the physical properties of butter. However, a positive correlation between water loss, fat content of the cream, enthalpy, and G’ and a negative correlation between water loss and delta were found. As identified previously, addition of AMF_f can change the physical properties of butters and perhaps their capacity to hold water during extrusion. The aim of this work is to understand the physical properties that are affected by addition of different AMF_f and how they are correlated to water loss. This understanding will help to identify how butter processing and composition can be manipulated to reduce water loss.

**MATERIALS AND METHODS**

*Sample Preparation*

*Production of AMF*

Fractions of anhydrous milk fat (AMF) were obtained in a single step fractionation process as explained by Kalegian and Lindsay (1995b). AMF was obtained from butter made in our lab. The cream was stabilized between 15-18°C. Approximately 4 liters of cream were added to the butter churn (Armfield FT21 Butter Churn, Hampshire, England) and turned on to level three for 25 minutes. The speed was lowered to level two and run for two minutes. The speed was again lowered to level one and run for two minutes or until a significant amount of buttermilk had been collected and small grains of butter were observed. The buttermilk was drained and the butter was washed with distilled tap water. The churn was turned on low speed (setting 1) for 30 seconds and water was drained. Next, 1 L of distilled tap water and 1 L of ice water were then
added to the churn. Again, the butter and water were churned for one minute on low speed. Water was drained and replaced with 2 L ice water and churned for 1 minute. Water was drained, and the butter was scraped out of the churn and placed on multiple layers of cheesecloth. After wrapping the butter in cheesecloth, the butter was squeezed to remove any excess water. Butter was then stored in the fridge at 5°C until making AMF.

Making the butter into AMF followed a procedure similar to Shulka et al. (1994). Butter was removed from the refrigerator and placed in a beaker in the oven at 65°C until the butter was completely melted. Once melted, the butter was poured into a separatory funnel in the oven. The fat and water separated in the funnel and the water was drained from the bottom. Sodium sulfate was added to the fat fraction at a level of 1% to remove any remaining water. The salt was stirred and the mixture sat in the oven for 5 minutes at 65°C allowing for the salt to settle. The solution was stirred again and set in the oven for another 10 minutes. The solution was stirred one more time and filtered using vacuum filtration and a glass microfiber filter. The filtered fat was collected and stored at 5°C.

AMF Fractionation

The fractionation process is outlined in Figure 5-1. Anhydrous milk fat was melted and put in an oven at 65°C for 30 minutes to ensure all crystals were melted and crystal memory has been erased. Beakers filled with sample, about 600ml in a 1000ml beaker, were placed in a water bath at three different temperatures: 20°C, 25°C, and 30°C. These samples were left in the water bath at temperature for seven days. After seven days, samples were filtered using vacuum filtration and crystals were collected.
The long crystallization period allowed for large crystals to form to increase ability to filter. Fractions were stored at 5°C. Three fractions were obtained by this procedure. Crystals obtained after fractionating at 20°C will be referred to as the AMF20 fraction, crystals obtained after fractionating at 25°C will be referred to as the AMF25 fraction, and lastly, crystals obtained after fractionating at 30°C will be referred to as the AMF30 fraction.

**Figure 5-1:** Procedure of separation and fractionation of AMF from butter. Control and temperatures listed indicate the different crystallization setpoints for each of the different treatments.
Addition of AMF and AMF_f to cream:

Organic cream with no additives was purchased from a local grocery store. Cream was stored at 5°C for 24 hours before use. Cream fat content was measured using the Babcock method (described below). In Chapter 4, it was identified that the greatest amount of water loss was found in cream with 48% fat content. Therefore, a 48% level of fat in cream was used to make the butter. This allowed for testing on a product that is known to have high water loss and allows for optimizing reduction of water loss. To achieve a 48% fat content, the fat content of the cream was measured, and it was standardized by adding AMF or AMF_f (AMF20, AMF25, or AMF30). Proportions of AMF and AMF_f and cream that needed to be mixed to obtain 48% fat in the cream were determined using the following series of formulas:

\[ 100x + (\text{Cream fat content})y = \text{desired percent fat of cream} \]  
\[ x + y = 1 \]

In these formulas x and y represent the fraction of AMF (or AMF_f) and cream respectively that need to be mixed to obtain 48% fat cream (desired percent fat of cream). The cream used was 30.0 ± 0.3% fat. With this initial fat content (cream fat content), the cream would make up 74% of the total mixture. On average, about 342.27 ± 3.32g of AMF (or AMF_f) was added to 1088.67 ± 3.36g cream to obtain approximately 1.5 liters of 48% fat cream.

Butter manufacture

Two different methods were used to add the AMF and the AMF_f to the butter. The first method, designated as the “Pre-churn method” for the rest of the chapter,
followed the butter making method described in Chapter 3 and incorporated the AMF and the AMF_f in the cream before churning (Figure 5-2). During this process, the cream was heated to 15-18°C in a water bath and the melted AMF and AMF_f (30-32°C) were added by mixing with an immersion blender for 30 seconds. Samples of the mixture were taken and a Babcock test was performed to quantify fat content of the mixture. The cream/AMF or cream/AMF_f mixture was stabilized between 15-18°C. The cream/AMF (or cream/AMF_f) mixture was added to the butter churn (Armfield FT21 Butter churn, Hampshire, England) and churned on level 3 for four minutes. The speed was lowered to setting 2 for two minutes. Again, the speed was lowered to setting 1 for two minutes. The buttermilk was drained and collected. The fat content of the buttermilk was determined using the Babcock method. The buttermilk was weighed to determine how much was lost in processing. Distilled tap water (1L) was added to the butter in the churn to wash the butter. The churn was turned on low speed (setting 1) for 1 minute and water was drained. Half a liter of distilled tap water and half a liter of ice water was added to the churn. Again, the butter and water were churned for one minute on low speed. Water was drained and replaced with 1 L ice water and churned for 1 minute. Water was drained and the butter was worked in the churn on level 3 for one minute. Excess water was drained. The butter was then placed in a cheesecloth lined rectangle hand press. The butter was squeezed in the press to remove any excess water and was formed into blocks. Blocks were stored in the fridge at 5°C for at least 24 hours before testing. Three replicates were performed at each fractionation level content.
The second method, referred to as the “post-churn method” throughout the rest of the chapter, incorporated the AMF in the working step (Figure 5-3). The same equations above were used to calculate the amount of cream and AMF (or AMF_f) needed to have a 48% fat cream. The cream was heated to 15-18°C in a water bath. Once it had reached the appropriate temperature, the cream was added to the butter churn. Due to a lower fat content (~30%) the churn had to run at high speed (setting 3) for 25 minutes. The speed was reduced to setting 2 for two minutes and reduced again to setting 1 for two minutes. The buttermilk was drained and collected. The fat content of the buttermilk was determined using the Babcock method. The buttermilk was weighed to determine how much was lost in processing. Distilled tap water (1L) was added to the butter in the churn to wash the butter. The churn was turned on low speed (setting 1) for 1 minute and water was drained. Half a liter of distilled tap water and half a liter of ice water was added to the churn. Again, the butter and water were churned for one minute on low speed. Water was drained and replaced with 1 L ice water and churned for 1 minute. Water was
drained and the butter was worked in the churn on level 3 for one minute. Excess water was drained. The AMF (or the AMF_f) was heated to 30-32°C and was added slowly to the butter mixing on level 3 over the course of 2 minutes. The butter was churned for an additional minute. Ice water (1L) was added and churned for 1 minute at level 1. Water was drained and the butter was worked in the churn on level 3 for one minute. Excess water was drained. The butter was then placed in a cheesecloth lined rectangle hand press. The butter was squeezed in the press to remove any excess water and was formed into blocks. Blocks were stored in the fridge at 5°C for at least 24 hours before testing. Three replicates were performed at each fractionation level content.

**Figure 5-3:** Procedure for adding AMF in the post-churn method.
Fat Content of Cream:

The fat content of cream, cream/AMF mixture, and cream/AMF_f mixture was measured using the Babcock method (Lynch et al., 1996). In this method 9 grams of sample were placed in a cream Babcock bottle. Water was added to wash all sample to the bottom of the bottle and prevent burning the sample with the acid. Sulphuric acid (17.5 ml) was added to the bottle and the sample was mixed. Bottles were placed in the centrifuge for 10 minutes, filled with water to bring the solution up into the neck of the bottle, and centrifuged again for 5 minutes. Measurements were made by using the scale on the neck of the bottle. Measurements were done in triplicate for each sample.

Water Content:

Water content was determined by a rapid moisture analyzer (Sartorius MA 150 Moisture Analyzer, Sartorius, Weender Landstrasse, Goettingen, Germany) using the rapid moisture analyzer method. Using the “standard drying” setting at 110°C the sample was dried to remove all water. Approximately 5 g of sample was placed on a tared weighing pan (102 mm x 8 mm, 60 ml). Small amounts of butter sample were placed across the pan to ensure even melting and water reduction. The water content was then determined as the moisture analyzer heated the sample and recorded water loss. Samples were tested in triplicate.

Water Droplet Size:

Water droplet size was determined using Nuclear Magnetic Resonance (NMR) Spectroscopy (Minispec mq-20, Bruker Inc., Billerica, MA). A core of each butter
sample was taken using the back end of a pipette (9” disposable Pasteur pipette). Each pipette was put inside an NMR tube (10 mm in diameter and 180 mm in height) and stored at 5°C for 24 h. The NMR was set to 5°C using a water bath. The settings were such that a 90- and 180-degree pulse length was checked, and calculations were conducted at the end of the measurement. Results for D3_3 were reported. Each sample was tested in triplicate.

**Solid Fat Content:**

The solid fat content (SFC) of each sample was determined using Nuclear Magnetic Resonance (NMR) Spectroscopy (Minispec mq-20, Bruker Inc., Billerica, MA) following the AOCS Cd 16b-93 method (AOCS 2017a). Each sample was prepared by taking a core of the sample with the back end of a pipette (9” disposable Pasteur pipette). The end containing the sample was placed in an NMR tube (10 mm in diameter and 180 mm in height). All samples were placed in a water bath at 5°C for 1 hour and inserted into the NMR for reading. Results were recorded and the samples were then put in a water bath at 10°C for 1 hour. This process was repeated at 5°C intervals from 5°C-40°C. The SFC of the sample at each temperature was recorded to identify the melting trend and amount of solid fat at each temperature. Measurements were performed in triplicate. All results were adjusted for water content.

**Melting Behavior:**

Using a differential scanning calorimeter (Q20, TA Instruments, New Castle, DE), 9-12 mg of each sample was put into an aluminum hermetic pan. Each pan was
sealed and placed in the DSC set to 5°C. An empty pan was used as a reference. The pan was held at 5°C for one minute before temperature change. The DSC tracked melting behavior as the temperature increased from 5°C to 60°C at a ramp rate of 5°C per minute. The reported values include the change in enthalpy associated with the melting process, onset temperature, and peak temperature. Each of these parameters were determined using TA Universal Analysis software. Enthalpy measurements were adjusted for water content. Measurements were performed in triplicate.

**Texture/Hardness:**

Texture was analyzed by determining the hardness with a texture profile analyzer (TA-XT Plus texture analyzer, Texture Technologies Corp., Hamilton, MA) using a cylinder probe TA-4 (Texture Technologies Corp.). The sample was prepared by taking a core out of the butter with a plastic culture tube (12x75 mm, 5.0 ml). The end of the culture tube was cut off to allow for extrusion of the sample. Sample was refrigerated overnight at 5°C in the culture tube. Pushing the sample out of the tube, a 1 cm cylinder was cut and placed on the stage, resting on the flat side. A compression method was used to determine the hardness of the sample. The probe was calibrated to a starting position of 20 mm. The probe was lowered until the normal force just became positive, indicating the probe was barely touching the sample. At this level, the displacement was zeroed out and the test was initiated. The test was set to compress down 6 mm at a rate of 60 mm/s. After this step, the probe returned to the zeroed-out position and the 6 mm compression was repeated. Measurements were performed in triplicate.
Viscoelasticity:

The viscoelastic behavior of the samples was determined using a rheometer (AR-G2, TA instruments New Castle, DE) with an 8 mm Plate SST Smart Swap geometry (TA Instruments). The instrument was set to 5°C with a frequency of 1 Hz and a strain between $8.0 \times 10^{-4}$ to 10%. The sample was prepared by taking a core of the butter sample with a culture tube (12x75mm, 5.0 ml). The sample was refrigerated for 24 hours at 5°C. Similar to the hardness measurement, the end of the culture tube was cut off to allow for the sample to be pushed out of the tube. The butter was pushed out of the tube and a 1-2 mm cylinder was cut from the sample. The cylinder was placed, flat side down, on the stage. The geometry was lowered until it the normal force was just barely positive with a gap of 1000-1600µm. The test began and the $G'$, $G''$, and delta were recorded. The twelfth measurement was recorded because it falls in the middle of the linear viscoelastic region on the measurements at a 0.01% strain. The crossover point was calculated from this data as the strain values where $G' = G''$. For each experimental replicate, rheological data was collected in triplicate.

Water Loss:

A method to measure the amount of water lost during the lamination process was developed in-house. The goal of measuring water loss was to mimic the water loss observed in industry caused by the extrusion of the butter into a thin ribbon. Five grams of sample were placed in the center of a rectangular, grade 1 filter paper (11 cm x 27 cm) and covered with a similar piece of filter paper. The sample and filter paper were rolled through a pasta roller (Atlas 150 Macchina per pasta, Campodarsego, Italy) at level 0
once. This rolling was repeated at each consecutive level once up to level 6 (1.2 mm).
The purpose of this process is to absorb the water lost during the extrusion in the filter paper. After the rolling process, the sample and filter paper were cooled at 5°C for 30 min to mimic a resting step. This resting step also allowed the fat to harden after being handled in an effort to reduce fat loss. After 30 min the filter papers were gently pulled apart. Butter was scraped off the surface of each filter paper using a flat sided spatula. All butter scraped off was placed on a tared pan (102 mm x 8 mm, 60 ml) in the moisture analyzer (Sartorius MA 150 Moisture Analyzer) to measure the water content. The sample was evenly distributed across the surface of the pan. The moisture analyzer was set on a “standard drying” setting at 110°C and the analysis was started. Water loss was calculated by determining the difference between the original water content and the water content of the sample after rolling it out. Measurements were performed in triplicate. A two-way ANOVA was used to determine significant differences between the rolled sample and the control (water content) sample. A one-way ANOVA was also used to determine significant differences between the amount of water lost between samples. This test was performed in triplicate.

**Fatty Acid Composition:**

Fatty acid composition was quantified using an Agilent 6890 N Gas Chromatography system (Agilent, Santa Clara, CA, USA) with an FID detector. The AOAC official method 996.01 was used to analyze the sample (Satchithanandam 2001). 1µl of sample was injected into the column with a split ratio of 10:1(Supelco P/N 24056 - SP 2560, 100 m x 250 µm ID, 0.2 µm film) with an inlet temperature of 250°C. Helium
was used as a carrier gas used with a flow rate of 1.2mL/ minute. The oven temperature was isothermal at 100°C for 5 minutes before increasing to 240°C at a rate of 3.5°C/minute. The sample was held at 240°C for 15 minutes.

**Triacylglycerol Composition:**

Liquid Chromatography Mass Spectrometry (LC-MS) using a Bruker Elute Ultra-High-Performance Liquid Chromatography (UHPLC) (Billerica, Massachusetts, USA) with a high resolution and mass accurate Bruker Impact II Q-TOF detector (expect 5 PPM or better accuracy and >20k resolution). An Agilent Zorbax StableBond C18 column (5 μm, 80 Å, 250 × 4.6 mm, Santa Clara, CA, USA) was used at a controlled temperature of 40 °C. For each sample (dissolved in 1/1, v/v, chloroform/isopropyl alcohol, at about 1 mg/mL), an aliquot of 10 μL was injected and eluted at a flow rate of 1.0 mL min⁻¹. The mobile phase consisted of methanol with 8% water (A), and isopropyl alcohol with 8% water (B). The samples were eluted using a linear gradient from 58% (v/v) A and 42% (v/v) B to 18% A (v/v) and 82% (v/v) B in 130 min. The detector was sampled at a frequency of 1 Hz with a scan range from 200 to 950 m/z.

**Melting Points Determination:**

The melting point of the AMF and the AMF_f were determined using a differential scanning calorimeter (Q20, TA Instruments, New Castle, DE) using a method similar to Knothe and Dunn and AOCS method Cj 1-94 (Knothe and Dunn 2009, AOCS 2017b). Each sample (approximately 15 mg) were put into an aluminum hermetic pan that was sealed and placed in the DSC set to 5°C. An empty pan was used as a reference.
The pan was held at 5°C for one minute before temperature change. The DSC tracked melting behavior as the temperature increased from 5°C to 60°C at a ramp rate of 5°C per minute. The sample was held isothermal at 60°C for 15 minutes to ensure all sample had melted. The temperature was then decreased from 60°C to -20°C at a ramp rate of 5°C per minute. It was again held isothermal for 90 minutes to allow for all sample to crystalize. Lastly, the sample was heated to 60°C at a ramp rate of 5°C per minute. The melting temperature was determined by an integration of the peaks from the last heating step from -20°C to 60°C using TA Universal Analysis software. The peak temperature of this integration was reported as the melting point.

Statistical Analysis:

Data was analyzed using Prism 9.0 (GraphPad Software, San Diego, CA). One-way ANOVA was used with Tukey’s multiple comparison to analyze results of moisture analysis, water droplet size distribution, SFC, melting behavior, texture/hardness, and elasticity. A two-way ANOVA with Bonferroni multiple comparisons test was used for water loss. Correlation of all characteristics was determined in relation to water loss. A $\alpha = 0.05$ level of significance was used in statistical evaluation.

RESULTS AND DISCUSSION

Physical Properties:

The physical properties of the butters made by adding AMF and AMF_f using the two procedures described above (pre-churn and post-churn) were measured and
correlations between physical properties and water loss were determined. Throughout the rest of the paper butter including fractions will be referred to as control, AMF20, AMF25, and AMF30.

**Fatty Acid and TAG Composition AMF and Its Fractions**

Milk fat is composed of over 400 different FA, only 14 of which account for 1% or more of the total FA composition (Buldo and Wiking, 2016). The most abundant fatty acids observed in the AMF obtained for this study were capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1), and linolenic acid (C18:2) (Table 5-1). As expected, the amount of saturated fatty acids increased with fractionation temperature. The greatest increase was found in palmitic and stearic acid. It is expected that these fatty acids would be high in high melting fractions because the melting point for both palmitic (63°C) and stearic (71.2°C) acids are much higher than the temperatures used to fractionate the AMF (20, 25, and 30°C) (Rønholt et al. 2013). Interestingly, the content of capric acid was lower in the high-melting point fractions. Even though it is a saturated acid, it is not surprising for it to be lower because it has a melting point of 31.5°C. The fatty acid compositions in the AMF and AMF_f are in line with the TAG analysis results. Higher melting fractions of AMF had higher levels of saturated TAGs containing two or more palmitic or steric fatty acids (Table 5-2). The opposite is also true, TAGs with oleic, linoleic or linolenic acids, or with short chain FAs were in greater proportions in the low-melting fractions of AMF. The FA and TAG composition described in Table 5-1 and 5-2 resulted in different
melting points for the AMF and the AMF f. AMF30 had the highest melting point, followed by AMF_25, followed by AM_20, and by AMF.
**Table 5-1:** Fatty acid composition of anhydrous milk fat (AMF) and anhydrous milk fat fractions (AMF20, AMF25, AMF30) before addition to the butter. AMF20 means AMF fractionated at 20 °C, AMF25 means AMF fractionated at 25°C, AMF30 means AMF fractionated at 30°C. SFA means saturated fatty acids, UFA means unsaturated fatty acids. Values in the same row with the same letter (a-d) are not significantly different (α=0.05).

<table>
<thead>
<tr>
<th></th>
<th>AMF</th>
<th>AMF20</th>
<th>AMF25</th>
<th>AMF30</th>
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<tr>
<td>C4:0</td>
<td>1.95 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.54 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.60 ± 0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.41 ± 0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>C8:0</td>
<td>1.22 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.02 ± 0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.93 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>C10:0</td>
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<td>2.80 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>2.35 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C10:1</td>
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<td>0.20 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>3.29 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.81 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C15:0</td>
<td>1.11 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:2</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>33.87 ± 0.46&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34.97 ± 0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.89 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.44 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1 cis</td>
<td>1.86 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.55 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:0</td>
<td>11.43 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.02 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.29 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1 cis</td>
<td>24.43 ± 0.64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.29 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.89 ± 0.34&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2n6 trans</td>
<td>0.26 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2n6 cis</td>
<td>3.36 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.05 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.69 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.47 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.04 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>0.07 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>0.41 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C21:0</td>
<td>0.38 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total SFA</td>
<td>68.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>69.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total UFA</td>
<td>31.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>33.55</td>
<td>36.77</td>
<td>39.71</td>
<td>43.05</td>
</tr>
</tbody>
</table>
Table 5-2: Triacylglycerol composition of anhydrous milk fat (AMF) and anhydrous milk fat fractions (AMF20, AMF25, AMF30) before addition to the butter. Values in the same row with the same letter (a-d) are not significantly different (α=0.05). Triacylglycerols under 0.3 are not reported.

<table>
<thead>
<tr>
<th>Triacylglycerol composition</th>
<th>Control</th>
<th>AMF20</th>
<th>AMF25</th>
<th>AMF30</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC, CyCLa</td>
<td>0.78 ± 0.03a</td>
<td>0.72 ± 0.04a</td>
<td>0.56 ± 0.02a</td>
<td>0.47 ± 0.03a</td>
</tr>
<tr>
<td>CCLa, CaLaM, CyLaLa, BuMM</td>
<td>1.95 ± 0.07a</td>
<td>1.77 ± 0.11a</td>
<td>1.38 ± 0.05b</td>
<td>1.16 ± 0.07b</td>
</tr>
<tr>
<td>BuLaO, CaCO</td>
<td>0.92 ± 0.06a</td>
<td>0.84 ± 0.03a</td>
<td>0.67 ± 0.03a</td>
<td>0.54 ± 0.02b</td>
</tr>
<tr>
<td>BuMP</td>
<td>0.61 ± 0.06a</td>
<td>0.58 ± 0.05a</td>
<td>0.49 ± 0.09a</td>
<td>0.38 ± 0.06a</td>
</tr>
<tr>
<td>CyMLa, CLaLa (CCLn)</td>
<td>4.13 ± 0.29a</td>
<td>3.73 ± 0.20b</td>
<td>2.93 ± 0.18b</td>
<td>2.48 ± 0.10c</td>
</tr>
<tr>
<td>BuMO, CaLaO, BuPL, CaML, CCL (LLBu)</td>
<td>2.30 ± 0.14a</td>
<td>2.29 ± 0.46a</td>
<td>1.65 ± 0.02b</td>
<td>1.41 ± 0.09b</td>
</tr>
<tr>
<td>BuPL, CaML, CCL, CyLaL (BuPaP)</td>
<td>1.14 ± 0.13a</td>
<td>0.94 ± 0.18a</td>
<td>0.80 ± 0.05a</td>
<td>0.67 ± 0.03b</td>
</tr>
<tr>
<td>CaPLn, CyMLn, CLaLn, BuLO (BuPaO)</td>
<td>2.19 ± 0.11a</td>
<td>1.93 ± 0.21ab</td>
<td>1.62 ± 0.04b</td>
<td>1.33 ± 0.07c</td>
</tr>
<tr>
<td>BuPP</td>
<td>7.58 ± 0.49a</td>
<td>6.93 ± 0.46b</td>
<td>5.49 ± 0.32c</td>
<td>4.60 ± 0.19d</td>
</tr>
<tr>
<td>BuPO</td>
<td>5.22 ± 0.41a</td>
<td>5.23 ± 0.21a</td>
<td>3.81 ± 0.42b</td>
<td>3.48 ± 0.14b</td>
</tr>
<tr>
<td>CyML, CLaL</td>
<td>1.84 ± 0.39a</td>
<td>1.22 ± 0.05b</td>
<td>1.24 ± 0.32d</td>
<td>0.80 ± 0.06c</td>
</tr>
<tr>
<td>BuOO, CLaL</td>
<td>1.46 ± 0.78a</td>
<td>0.69 ± 0.07b</td>
<td>0.55 ± 0.06b</td>
<td>0.44 ± 0.05b</td>
</tr>
<tr>
<td>LaLaM</td>
<td>1.66 ± 0.95a</td>
<td>0.74 ± 0.07b</td>
<td>0.64 ± 0.04b</td>
<td>0.46 ± 0.04b</td>
</tr>
<tr>
<td>CMM</td>
<td>2.76 ± 0.29a</td>
<td>2.41 ± 0.05a</td>
<td>1.94 ± 0.11b</td>
<td>1.63 ± 0.06b</td>
</tr>
<tr>
<td>CLaO, CyMO, CaPO</td>
<td>4.02 ± 0.91a</td>
<td>4.36 ± 0.12a</td>
<td>2.87 ± 0.71b</td>
<td>1.89 ± 0.14c</td>
</tr>
<tr>
<td>CML, LaLaL</td>
<td>1.58 ± 0.85ab</td>
<td>2.08 ± 0.12a</td>
<td>2.24 ± 0.64a</td>
<td>2.36 ± 0.11a</td>
</tr>
<tr>
<td>CMP, LaLaP, LaMM (CyLL, CyLnO)</td>
<td>1.75 ± 0.06a</td>
<td>1.74 ± 0.03a</td>
<td>1.61 ± 0.05b</td>
<td>1.24 ± 0.04b</td>
</tr>
<tr>
<td>CLaS, CyPS</td>
<td>1.25 ± 0.10ab</td>
<td>1.14 ± 0.12a</td>
<td>0.94 ± 0.06b</td>
<td>0.75 ± 0.06b</td>
</tr>
<tr>
<td>CMO, LaLaO</td>
<td>1.66 ± 0.08b</td>
<td>2.29 ± 0.13a</td>
<td>1.29 ± 0.07b</td>
<td>1.06 ± 0.06c</td>
</tr>
<tr>
<td>COL, CSLn, LaPLn (PaLaPo, PaCO)</td>
<td>0.75 ± 0.12a</td>
<td>0.00 ± 0.00b</td>
<td>0.67 ± 0.15a</td>
<td>0.51 ± 0.06a</td>
</tr>
<tr>
<td>CaMS, CPP, LaLaS, LaMP, MMM</td>
<td>4.39 ± 0.17a</td>
<td>4.49 ± 0.12a</td>
<td>4.50 ± 0.16a</td>
<td>3.61 ± 0.13b</td>
</tr>
<tr>
<td>CPO, LaMO, MML, LaPL (CPaS, LaPaP, MpaM)</td>
<td>0.92 ± 0.19a</td>
<td>0.89 ± 0.16a</td>
<td>0.81 ± 0.09a</td>
<td>0.64 ± 0.07a</td>
</tr>
<tr>
<td>CPS, LaMS, LaPP, MMP</td>
<td>2.12 ± 0.10a</td>
<td>2.80 ± 0.19b</td>
<td>3.73 ± 0.12a</td>
<td>3.40 ± 0.09a</td>
</tr>
<tr>
<td>LaPO, MMO</td>
<td>2.37 ± 0.10a</td>
<td>2.37 ± 0.06a</td>
<td>2.20 ± 0.10b</td>
<td>1.83 ± 0.07a</td>
</tr>
<tr>
<td>MPL, LaOO</td>
<td>1.29 ± 0.15a</td>
<td>1.29 ± 0.11a</td>
<td>1.23 ± 0.07a</td>
<td>1.10 ± 0.07a</td>
</tr>
<tr>
<td>MOL, LaPLn, MSLn (MPaO)</td>
<td>0.57 ± 0.11a</td>
<td>0.55 ± 0.07a</td>
<td>0.51 ± 0.08a</td>
<td>0.42 ± 0.06a</td>
</tr>
<tr>
<td>MPP</td>
<td>2.49 ± 0.15c</td>
<td>3.35 ± 0.12c</td>
<td>5.13 ± 0.27b</td>
<td>5.70 ± 0.27a</td>
</tr>
<tr>
<td>MPO, LaSO</td>
<td>4.50 ± 0.29a</td>
<td>4.49 ± 0.07a</td>
<td>4.54 ± 0.15a</td>
<td>3.85 ± 0.19b</td>
</tr>
<tr>
<td>MSL, MOO (PaPP, PaSM)</td>
<td>3.07 ± 0.19a</td>
<td>2.98 ± 0.19a</td>
<td>2.80 ± 0.16b</td>
<td>2.49 ± 0.17b</td>
</tr>
<tr>
<td>PLO, PLnS</td>
<td>1.62 ± 0.15a</td>
<td>1.63 ± 0.16a</td>
<td>1.56 ± 0.19a</td>
<td>1.39 ± 0.17a</td>
</tr>
<tr>
<td>PPP</td>
<td>3.34 ± 0.35a</td>
<td>4.46 ± 0.26b</td>
<td>6.64 ± 0.36b</td>
<td>8.31 ± 0.47a</td>
</tr>
<tr>
<td>PPO</td>
<td>7.07 ± 0.43c</td>
<td>7.58 ± 0.28b</td>
<td>8.31 ± 0.21b</td>
<td>7.98 ± 0.27a</td>
</tr>
<tr>
<td>POO, PSL</td>
<td>6.75 ± 0.19a</td>
<td>6.57 ± 0.25a</td>
<td>6.08 ± 0.27b</td>
<td>5.83 ± 0.38b</td>
</tr>
<tr>
<td>PPS</td>
<td>2.50 ± 0.15a</td>
<td>3.71 ± 0.26b</td>
<td>5.74 ± 0.84b</td>
<td>10.36 ± 0.69a</td>
</tr>
<tr>
<td>PSO</td>
<td>5.46 ± 1.27c</td>
<td>5.50 ± 0.19c</td>
<td>6.18 ± 0.94b</td>
<td>6.66 ± 0.26b</td>
</tr>
<tr>
<td>OOS, SSL</td>
<td>1.98 ± 0.50a</td>
<td>1.79 ± 0.17a</td>
<td>1.66 ± 0.38a</td>
<td>1.99 ± 0.28a</td>
</tr>
<tr>
<td>PSS</td>
<td>0.77 ± 0.20c</td>
<td>0.95 ± 0.06c</td>
<td>2.01 ± 0.09b</td>
<td>3.73 ± 0.27a</td>
</tr>
<tr>
<td>SSO</td>
<td>0.55 ± 0.15a</td>
<td>0.53 ± 0.04a</td>
<td>0.71 ± 0.08a</td>
<td>0.79 ± 0.09a</td>
</tr>
<tr>
<td>SSS</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>0.21 ± 0.03b</td>
<td>0.54 ± 0.04a</td>
</tr>
</tbody>
</table>

Structure indicated by FA composition (e.g., OOO for triolein) using the following abbreviations: Bu, butyric acid (C4:0); Ca, caproic acid (C6:0); Cy, caprylic acid (C8:0); C, capric acid (C10:0); La, lauric acid (C12:0); M, myristic acid (C14:0); Pa, n-pentadecanoic acid (C15:0); P, palmitic acid (C16:0); Po, palmitoleic acid (C16:1); S, stearic acid (C18:0); O, oleic acid (C18:1); L, linoleic acid (C18:2); Ln, linolenic acid (C18:3).
**Water Content**

The procedures used to incorporate the AMF and the AMF_f to the butter had different effects on the water content. When AMF or AMF_f were added using the pre-churn procedure, water content was lower in the butters made with the high-melting fraction (p<0.05, Figure 5-4A). Water content ranged from 7.7 to 12.7% in the pre-churn procedure. In high AMF_f the fatty acids that have high melting points may have a greater amount of crystalline material. The increase in crystalline material may increase water release in churning and working stages. Contrary to the pre-churn method, the type of AMF_f incorporated using post-churn method did not affect the water content of the butters (p> 0.05). The water content of the butters processed using the post-churn method had an average of 11.50 ± 0.43%. The similar water content in all the butters of the post-churn procedure may be because all the butters started with cream churned with the same procedure and properties. The formation of the water-in-oil emulsion occurs during the churning process resulting in the separation and draining of the majority of the water. The working step was consistent for each sample, making any change in water content in this step similar for each sample. Once the AMF or AMF_f was added no additional water was lost from the sample. To ensure that fat and water were accounted for in all steps of buttermaking the buttermilk was analyzed for fat content and the total weight. No significant differences were found in the total weight of buttermilk or the fat content (p>0.05).
**Figure 5-4:** Analysis of physical properties related to water. Results are separated by procedure. pre-churn indicates procedure adding AMF to cream, post-churn indicates procedure adding AMF in working step. A) Water content B) Water droplet C) Water loss. Error bars indicate standard error of the mean. Results sharing the same letter (a-c) are not significantly different between procedures. Results sharing the same letter (A-C) are not significantly different within procedures (α=0.05).
Water Droplet size

The water droplet sizes in the butters processed with the pre-churn and post-churn methods increased with the addition of high-temperature AMF_f (p<0.05; Figure 5-4B). The largest water droplet size, 25.7µm, was identified in the butter made with AMF30 added in the cream step (pre-churn method). The smallest water droplet size, 5.6 µm, was found in the butter with AMF20 added in the working step. When semi-crystalline emulsions encounter shear working, water droplets can deform causing partial coalescence and increasing the size of water droplets (Estevez et al., 2013). The higher amount of crystalline material from the palmitic and stearic fatty acids in the high melting fractions may increase partial coalescence in the working stage resulting in larger water droplets. The ideal water droplet size in commercial butters ranges between 1 and 5µm to reduce microbiological activity (Mortensen, 2011). However, other studies have reported water droplet values similar to those achieved in this study with 2.6-10.6µm for commercial products and 28-35µm for small scale manufacturing (van Lent et al., 2009; van Dalen, 2002; Rønholt et al., 2013). Typically, a large water droplet size is associated with a less stable butter (Juriaanse and Hertje, 1988). However, in a study by Estevez et al. it was determined that the overall SFC was a major component for hardness and stability of the crystalline structure (2013). Their study identified water droplet size to highly influence early processing of butter but after hardening at 4°C the water droplet size was no longer a significant factor in the textural properties (Estevez et al., 2013). While water droplet can play a role in the structural integrity of the butter, the change in amount of crystalline material, due to different compositions of fatty acids, may overshadow the effects of water droplet size.
Water Loss

There was a significant amount of water loss in all the samples (Figure 5-4C). The pre-churn procedure and post-churn procedure had opposite effects on water loss. The pre-churn procedure had a pattern of decreasing water loss for butters formulated with the high-melting fraction of AMF. While Shukla et al. did not measure water loss, the results from their study indicated that higher melting fractions resulted in a reduced water activity and moisture migration, with the potential to reduce leakiness (1994). Similarly, this study identified a reduced leakiness in butters incorporating high melting fraction. However, when butters were processed using the post-churn procedure the water loss increased in butters formulated with high-melting fractions. In both procedures significant differences were only seen between the control and the AMF25 and AMF30.

Hardness

The hardness of the butters increased almost linearly with the use of high-melting fractions of AMF for both procedures (Figure 5-5). A much larger difference in in the pre-churn method hardness values than the post-churn method values can be observed. It has been determined that small crystal size, high amount of butter grains, strength of the crystal-crystal bonds, amount of crystalline material in the sample, low water content, and small water droplet size can all be contributing factors to increasing the overall hardness (Rønholt et al., 2014; Lee and Martini, 2018, Rønholt et al. 2013, Rønholt et al. 2012). With a higher-melting fraction, one would expect to see more crystalline material in the butter resulting in a higher hardness. Our findings support the assumption that a higher melting fraction results in an increased hardness. The high amounts of stearic and
palmitic acid and the high melting point contribute to the higher amount of crystalline material. Marangoni et al. (2022) identified a correlation with increased palmitic acid and increased hardness. Inversely, Queirós et al. (2016) identified a decrease in hardness and solid fat content with increased olein. Both of these studies support the increase in hardness with the higher fraction additions. The water content will also play a role in decreasing the liquid portion of the solid to liquid ratio, increasing hardness. The increased size in water droplets is typically a factor in lower hardness. However, the low water content increases the stability of the fat network which may allow for increased stability of larger water droplets.

Figure 5-5: Hardness of butter samples from both methods. Results sharing the same letter (a-c) are not significantly different between procedures. Results sharing the same letter (A-C) are not significantly different within procedures (α=0.05).
Rheological Properties

The pre-churn and post-churn procedures were both analyzed for rheological properties to understand the effect of AMF_f addition to the butter. In all measurements it was apparent that the pre-churn procedure had a more distinct trend than the post-churn procedure (Figure 3). This is especially exaggerated in the delta measurements, where a clear trend is visible of lower delta value in butters made with higher-melting fractions of AMF in the pre-churn procedure and no significant difference in the post-churn procedure (α=0.05).

Figure 5-6: Rheological values from both methods. A) G’, B) G”, C) Delta, and D) Cross over point. Results sharing the same letter (a-c) are not significantly different between procedures. Results sharing the same letter (A-C) are not significantly different within procedures (α=0.05).
Both procedures indicated that the use of a high-melting fraction of AMF increased the G’ and G” (Figure 5-6a, 5-6b). Shukla et al. (1994) found similar results with a higher G’ and G” in their higher melting butter. A high G’ is associated with more elastic-like behavior and a high G” is associated with a higher viscous-like behavior. When both parameters (G’ and G”) are steadily increasing it can be difficult to recognize if the substance is more viscous or elastic. The delta or phase angle gives a ratio of G” to G’ helping to indicate which behavior is dominant. In the pre-churn method our decrease in delta indicates that the butters have a more elastic behavior as high-melting fractions of AMF are used (Figure 5-6c). This indicates more solid like material. With a higher amount of crystalline material in the high melting fraction, it is not surprising that that the G’ and delta indicate an increasing amount of elastic like behavior as the fraction temperature increases. However, in the post-churn procedure there is not a significant change in the ration of viscous to elastic behavior.

The crossover point is the point when the viscous like behavior and the elastic like behavior are equal. The deformation over time from the rheometer will slowly decrease the elastic like behavior and increase the viscous like behavior. Both procedures had a steady decreasing crossover point when higher-melting point fractions were used (Figure 5-6d). These results are in line with expectations because a softer product, like the control, may have a higher crossover point because it can withstand more deformation without breaking the structure. The harder butters like the ones obtained with AMF25 and the AMF30 would have a lower cross over point because the structure has a higher amount of crystalline material that can be disrupted more easily.
Melting Profile

According to Vanhoutte et al., three melting fractions can be identified in AMF such as low-melting fraction (<10°C), medium-melting fraction (10-21°C), and high-melting fraction (>21°C). Our medium melting fraction peak, at 15°C, is seen in the control and in a less noticeable peak in the AMF20 (Figure 5-7). This peak is visible but is not the largest peak represented in any of the samples. The peak at around 22°C, in the medium-melting fraction range, is visible in all of the melting profiles. This is the peak where the onset temperature is taken from in all our results. However, this peak is only the largest in the butter made with AMF (control) and with AMF20. Lastly, the peak at around 36°C, a high-melting fraction peak, is seen the strongest in the butters made with AMF25 and AMF30 but a small peak can be seen in the AMF (control) and in the AMF20. This is the largest peak in the in the highest two fractions These different peaks are similar for butters obtained with the two procedures (pre-churn and post-churn) and are similar to those found in other studies of milk fat fractionation (Vanhoutte et al., 2002; Małkowska et al., 2021).
Figure 5-7: Melting profile of butter samples from both procedures A) Pre-churn method B) Post-churn method measured by differential scanning calorimeter.

The analysis of the melting profile includes enthalpy, onset temperature, and peak temperature measurements. Enthalpy in the pre-churn method showed a larger enthalpy in the AMF25 and AMF30 than in the control or AMF20 (Figure 5-8A). In the post-churn method the AMF25 has the highest enthalpy but is the only fraction that is significantly different from the control. The higher enthalpy in these higher fractions follows the
results found in hardness and $G'$. The higher melting fractions should have a greater amount of crystalline material resulting in a greater hardness, $G'$, and Enthalpy

**Figure 5-8:** Melting parameters obtained from the melting profiles of butter samples from both procedures. Results sharing the same letter (a-c) are not significantly different between procedures. Results sharing the same letter (A-C) are not significantly different within procedures ($\alpha=0.05$). A) Enthalpy B) Onset Temperature C) Peak Temperature.
The onset temperature had no significant difference in any of the procedures or fractions (p>0.05; Figure 5-8B). While the addition of the fractions to the cream affected the melting profile, the cream base in all the butters should have a similar melting profile. The cream made up for about 70% of the total, making it a major component to the butter. It is likely that the cream had an influence on the consistent onset temperature at 18.76 ± 0.53°C which correspond to the melting of TAGs with intermediate melting points. In the peak temperature there are two groupings, the control and AMF20 and the AMF25 and AMF30 (Figure 5-8C). We would expect to see a higher peak temperature in AMF25 and AMF30 because the fractions used have high melting points. As seen in Figure 5-7, The amount of material melting in the AMF 25 and AMF30 is greater in the 36°C peak than in the 21°C peak. These fractions also have a higher amount of palmitic and steric acid, which may account for the larger peak. This pattern in the peak temperature is present in both the pre-churn and post-churn methods.

**Solid Fat Content**

The solid fat content (SFC) of the butter followed the expected results. As the temperature increased the solid fat content decreased indicating the melting of the fat (Figure 5-9). Butters formulated with AMF25 and AMF30 had a consistently significant higher solid fat content at each temperature, except for 40°C, than the butters made with the AMF (control) and with the AMF20 fraction in both procedures (p<0.05). In the pre-churn method, the significantly highest solid fat content was reported at all temperature levels for butters made with the AMF30 (α=0.05). With this understanding, it can be concluded that the pre-churn method with the AMF30 yields the highest solid fat content
from temperatures 5°C-40°C. It is very likely that this is because of the greater melting point and the greater amounts of palmitic and stearic fatty acids that make up the composition of the AMF30. This increase in saturated fatty acids will increase the total amount of crystalline material, therefore increasing solid fat content. A similar increase in saturated fatty acid and overall SFC in of high melting fraction has been observed on other studies (Reddy, 2010; Deffense, 1987; Kalegian, 1995e). The total crystalline material is a characteristic that ties SFC to hardness, rheology, and enthalpy. This assumption is validated by the significant correlation between SFC 15°C and hardness (p=0.005, r=0.99), delta (p=0.037, r=-0.96), and enthalpy (p=0.017, r=0.98). Similar correlations were observed for the SFC at other temperatures. In the post-churn method, there is only a significant difference between the SFC of butters made with AMF25 and AMF30 at 10, 30, and 35°C (p<0.05). Butters made with AMF25 and AMF30 had significantly higher SFC than butter made with AMF (control) and AMF20 (p<0.05). There is also a positive correlation between SFC at 20°C of the post-churn method with hardness (p=0.007, r=0.99) and G’ (p=0.023, r=0.98) indicating that the crystalline material of both procedures is high in the high melting fractions.
Figure 5-9: Solid fat content of butter samples from both procedures at temperatures ranging from 5°C-40°C. A) Pre-churn method B) Post-churn method

Correlations:
Understanding the effect of addition of different AMF fractions and the procedure of incorporation on the physical properties of butter allows for manipulation of these physical properties to fit specific needs. Reduction of water loss in the butter is one of the specific needs in this study. This correlation analysis will help to understand the physical properties and their effect on water loss.
Pre-Churn Method

A correlation analysis of the physical properties and the water loss in the pre-churn method was performed (Table 5-3). Physical properties from this procedure were identified to be significantly correlated with water loss including water content, hardness, rheological crossover point, solid fat content, enthalpy, and fatty acid composition ($\alpha=0.05$).
Table 5-3: Correlation factors of physical properties to water loss of butters made with various fractions of anhydrous milk fat added to the cream (pre-churn) or during the working step (post-churn). P-value significance $\alpha < 0.05$

<table>
<thead>
<tr>
<th></th>
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<th>Post-Churn Correlation</th>
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<tr>
<td>G'</td>
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<tr>
<td>G&quot;</td>
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<tr>
<td>Delta</td>
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<tr>
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<td>0.033</td>
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<tr>
<td>C18:1 cis</td>
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</table>

Water content has been consistently correlated with water loss, as seen in Chapters 3 and 4. In this study there is a positive correlation with water loss ($p=0.047$, $r=0.95$). This indicated that an increase in water content will result in an increase in water loss. This is expected because an increase in water requires incorporation of more water
droplets or larger water droplets in the fat phase of the butter. Rønholt et al. (2013) identified that an increase in water content can create a softer product which can reduce water droplet stability (Wright and Marangoni, 2006). To reduce water loss, the emulsion of water droplets in the oil matrix must be strong enough to hold up under pressure of extrusion. For this reason, it is not surprising that there is a negative correlation with water loss and hardness \( (p=0.021, r=-0.98) \) indicating that a butter with higher hardness will result in less water loss.

The crossover point was positively correlated with the water loss of the butter \( (p=0.033, r=0.97) \). This indicates that a butter with a smaller crossover point is likely to have a lower amount of water loss. As described earlier, the crossover point is the strain where the viscous like behavior and the elastic like behavior are the same. This correlation indicates that a product that cannot sustain a high amount of deformation before becoming more viscous-like will result in less water loss. High proportions of crystalline material are more prone to breaking upon deformation because there are more crystals available. This correlation may be another indication of the key role crystalline material plays in reducing water loss in the pre-churn method.

The solid fat content of the butter was significantly correlated at lower temperatures \( (5-20^\circ\text{C}) \). While there are significant differences in the SFC of butters made from the control and AMF20 with AMF25 and AMF30 at all temperatures it is only the lower temperatures that show a significant correlation with water loss \( (\alpha=0.05) \). SFC between \( 5^\circ\text{C} \) and \( 20^\circ\text{C} \) were negatively correlated with water loss, meaning that the higher SFC resulted in a lower amount of water loss. This is in line with hardness. The greater amount of solid fat content would increase the overall hardness of the butter. Both
of these factors point toward a higher temperature fraction for a lower amount of water loss.

The enthalpy is negatively correlated to water loss, demonstrating a high enthalpy will result in a low amount of water loss. Enthalpy in the pre-churn method was split into two significantly different groups, control and AMF20 significantly different from AMF25 and AMF30. AMF25 and AMF30 had the highest measured enthalpy. This correlation shows that AMF25 and AMF 30 are likely to have less water loss. As was demonstrated with SFC, it appears a higher amount of solid material follows a trend of lower water loss.

Lastly, water loss showed a positive correlation with the amount of oleic acid (18:1 cis) and a negative correlation with the amount palmitic acid (16:0). While the correlations of these two are opposite the effect is the same. Oleic acid is an unsaturated fatty acid which has a melting point at 15.40°C, making it liquid at room temperature (Knothe and Dunn, 2009). Palmitic acid has a melting point of 62.20°C making it solid at temperatures much higher than room temperature (Knothe and Dunn, 2009). The correlations indicate that a higher amount of saturated fats, specifically palmitic acid, and a lower amount of unsaturated fats, specifically oleic acid, will decrease the water loss. This correlation is in line with the solid fat and enthalpy correlations, in that palmitic acid will increase the solid fat content while at temperatures above 15.4°C oleic acid will not remain solid.

In summary, when incorporating AMF_f using the pre-churn method, water loss can be reduced by incorporating AMF_f with high melting points (higher contents of
palmitic acid and lower content of oleic acid) that will result in harder butters, more elastic, with lower water content, higher enthalpy, lower crossover point, and higher SFC.

*Post-Churn Method*

The post-churn method was also analyzed to identify correlations with the butter’s physical properties and water loss. This procedure yielded fewer significant correlations than the pre-churn method. The physical properties identified to have a significant correlation were hardness, solid fat content, and myristic fatty acid. All these characteristics had a positive correlation with water loss. That is, higher water loss was observed in butters with higher content of myristic acid that were harder, and had higher SFC values. The trend of water loss in the post-churn butter was the opposite of the pre-churn method (Figure 5-4C). While there is the least amount of water loss in the highest melting fraction of AMF using the pre-churn method, the post-churn method found the highest amount of water loss in butters made with AMF25 and AMF30.

With the opposite trend in water loss, it is expected to see opposite correlations as well. An increase in hardness is correlated with an increase in water loss. With incorporation of the AMF and the AMF_f in the last step, the AMF and the AMF_f was added to butter that was already washed with cold water. The difference in temperature from the semi-solid butter to the melted fat made the AMF crystalize quickly and didn’t blend completely with the butter. The AMF and AMF_f were not subjected to as much agitation as the churning provided. This left visible AMF crystals in the butter matrix. The large AMF crystals from the higher temperature fractions would help to increase hardness just as the high melting fraction increased hardness in the pre-churn method.
However, the fact that it did not blend completely may contribute to emulsion instability. Large crystals like this could not provide the stabilizing factor as coalesced butter fat does to the water droplets. Under pressure harder butters may be more brittle and breakage could lead to a higher amount of water loss. This same principle would be present in solid fat content. Solid fat content at 5°C-20°C had a positive correlation with water loss. This indicates that a higher SFC follows the trend of a higher amount of water loss.

Lastly myristic acid content was found to have a strong positive correlation with water content (p=0.013, r=0.99). Myristic acid is a saturated fatty acid with a melting point of 53.47°C (Knothe and Dunn, 2009). This is another factor that would play into the hardness and solid fat content of the butter. With a high melting point it is expected that this would be solid at temperatures above room temperature. All of the strongest correlations seem to deal with the structural components of the butter. While the results do not follow what was expected, it seems the difference in the structure of the butter is a key characteristic in the water loss. The constant water content may also be a factor in correlations found. With no significant difference in water content of the post-churn samples, the significance of other physical properties functionality may be more prominent.

As a whole, AMF_f addition in the working step can help to reduce water loss when low-melting AMF f are incorporated. These butters are expected to have a softer texture with a lower SFC and a lower amount of myristic acid.
CONCLUSION

This study indicates that the pre-churn method results in a decrease in water loss when high-melting fractions of AMF were used to formulate butter; while lower-melting point AFM fractions are needed in the post-churn procedure to decrease water loss. The pre-churn method also had more correlating physical properties related to water loss. These correlations allow for greater determination of how to manipulate physical properties to reduce water loss. Water loss can be decreased in butter by adding AMF fractions to the cream. When high-melting fractions are added, harder butters with higher SFC, higher ratio of elastic like behavior are obtained which result in less water loss. However, if the AMF fractions are added during the working process low melting fractions are recommended. When lower melting fractions are incorporated, lower solid fat content, decreased hardness, and a smaller amount of myristic acid contribute to a reduction in water loss. For all these reasons, it is recommended that a high fraction AMF is incorporated in a high fat cream to reduce water loss or low fraction is added to the working stage of the butter. While a reduction in water loss was achieved in both methods the post-churn method is not recommended for commercial application due to large crystals that could decrease consumer acceptance. AMF addition in the pre-churn method could be applied in an industrial setting by adding the AMF after pasteurization when the cream is hot. This will result in harder butter and the capacity of equipment will need to be assessed to see if it can handle the increased strain.
ACKNOWLEDGEMENT

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CHAPTER 6

CONCLUSION

Physical properties of butter play a significant role in the loss of water. In testing commercial and lab produced samples, trends and correlations were identified in physical characteristics that impacted the total water loss in the product. Compositional characteristics played a large role in the magnitude of the impact of physical characteristics on water loss. High water content was a consistent indicator of water loss in all studies. In commercial products the fat content, and therefore total water content, of the product played a role in significance of correlations. The separation based on fat content highlighted the need for smaller water droplet size and lower hardness in water loss reduction for products with a lower fat content (around 80%). Conversely, higher water loss in high fat products (83-85% fat) correlated with an increase in solid fat content at low temperatures and a decrease in liquid and solid like behavior.

Processing methods in lab-made butters were also identified to impact both the physical properties and water loss. In chapter 4, changing the amount of fat in cream used in churning did not change the physical properties in a significant way but did identify the need for higher elastic-like behavior with a lower total enthalpy and decreased water content to reduce water loss. The butter making step when AMF fractions were added also impacted the effect of the physical properties on water loss. This resulted in drastically different results identifying a need for an increase in crystalline material in
products made with AMF and its fractions in the cream and a decrease in crystalline material in butter made with AMF addition in the working step to decrease water loss.

In the end, some general trends were identified. In butters with a lower water content, crystalline structure and amount of solid fat was consistently negatively correlated with water loss. This was seen in high SFC at low temperatures, high G’ values, low delta values, high hardness, and high enthalpy. For best results in water loss reduction a lower fat cream (38-46%) with a high melting fraction (AMF25 or AMF30) incorporated in the cream before churning is recommended. Lower fat cream did have a significantly lower water loss than the 48% fat cream. The addition of AMF and AMF_f to cream created a more homogenous mix with more predictable outcomes. The added fractions incorporated a higher amount of crystalline material and a lower amount of water in the product resulting in a lower water loss.

In future studies it would be beneficial to understand physical properties in butter with a consistent amount of water. Water content was a determining factor in the results of all our studies. It would be interesting to see how physical properties are affected when water content is not a variable. Water droplet size was recognized as a contributing factor in the regression analysis performed in Chapter 3. However, in Chapters 4 and 5 water droplet sizes were much larger and did not have a significant correlation with water loss. Isolating water droplet size as a variable would allow for a greater understanding of its effect. The large water droplet size is often a result of small-scale manufacturing. Greater understanding is still necessary to identify if decreases in water droplet size from large scale manufacturing would affect the results discovered. Proteins within the butter are also known to be a part of the stability of emulsions. Manipulation of proteins in the
butter could lead to a stronger emulsion, reducing water loss. Lastly, combinations of milk fat fractions could lead to a butter with strong crystal interactions and reduction in brittleness which may bridge the gap in water loss between high and low water content butters. Lastly, these studies did not address the effect of spreadability on the butter and its water loss. While components reducing water loss have been identified, these characteristics need to be evaluated for the spreadability to allow them to be used in a pastry setting.
APPENDICES
Appendix A: Supplementary Tables and Figures from Chapter 3

Figure A-1 Melting profile obtained by differential scanning calorimetry for sample 6C. Slight variations are apparent in other curves. This curve is simply to demonstrate the two peaks observed in the melting profiles of all samples. Initial peak (peak 1) is gradual and small and second peak (peak 2) is more drastic and sharp.
Table A-1 SFC mean and standard deviation are recorded. Capital letters identify significant difference between temperatures of the same sample. Results sharing the same Capital letter (A-G) within the same row are not significantly different ($\alpha=0.05$). Lowercase letters identify a significant difference between samples at the same temperature. Results sharing the same lowercase letter (a-j) within the same column are not significantly different ($\alpha=0.05$)

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<td>38.4 ± 3.3Cghi</td>
<td>27.7 ± 3.1Dcdef</td>
<td>15.1 ± 1.2Eab</td>
<td>8.8 ± 0.9Fa</td>
<td>4.4 ± 0.5Gabc</td>
<td>3.6 ± 0.8Gabcd</td>
</tr>
<tr>
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<td>52.5 ± 1.2Acddef</td>
<td>48.0 ± 0.3Babcde</td>
<td>41.5 ± 0.2Cdefg</td>
<td>26.6 ± 0.9Ddef</td>
<td>13.8 ± 0.5Eab</td>
<td>8.0 ± 0.5Fa</td>
<td>2.1 ± 0.4Gc</td>
<td>1.5 ± 0.2Gabcd</td>
</tr>
<tr>
<td>6B</td>
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<td>44.4 ± 1.1Bghi</td>
<td>36.9 ± 1.2Cij</td>
<td>25.7 ± 0.7Df</td>
<td>13.8 ± 0.2Eab</td>
<td>8.4 ± 0.7Fa</td>
<td>5.4 ± 0.8Gabc</td>
<td>4.6 ± 0.1Gabc</td>
</tr>
<tr>
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<td>46.5 ± 1.5Bcdefg</td>
<td>38.8 ± 1.8Cghi</td>
<td>25.4 ± 0.3Def</td>
<td>14.3 ± 0.3Eab</td>
<td>8.0 ± 0.6Fa</td>
<td>6.2 ± 0.9Fab</td>
<td>5.3 ± 0.9Fa</td>
</tr>
<tr>
<td>7A</td>
<td>52.0 ± 1.1Adef</td>
<td>47.2 ± 0.4Babcde</td>
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<td>27.9 ± 0.8Dcdef</td>
<td>14.5 ± 0.6Eab</td>
<td>8.6 ± 0.6Fa</td>
<td>3.3 ± 0.3Gbc</td>
<td>1.4 ± 0.1Gabcd</td>
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<td>27.4 ± 0.6Dcdef</td>
<td>14.9 ± 0.5Eab</td>
<td>9.1 ± 1.0Fa</td>
<td>7.2 ± 0.4GFa</td>
<td>5.7 ± 1.4Ga</td>
</tr>
<tr>
<td>7C</td>
<td>51.3 ± 1.1Aefg</td>
<td>44.7 ± 1.3Befg</td>
<td>36.9 ± 1.0Cij</td>
<td>26.4 ± 0.1Ddef</td>
<td>14.0 ± 0.7Eab</td>
<td>7.7 ± 0.2Fa</td>
<td>5.5 ± 0.2Gfab</td>
<td>4.7 ± 0.7Gab</td>
</tr>
</tbody>
</table>
Table A-2: Correlation parameters for SFC and water loss of all commercial butter samples. No significant correlations are identified at with solid fat and water loss content any temperature. Correlation determined at a $\alpha = 0.05$ level of significance.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>p-value</th>
<th>Pearson r</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>0.977</td>
<td>-0.007</td>
</tr>
<tr>
<td>10°C</td>
<td>0.673</td>
<td>-0.098</td>
</tr>
<tr>
<td>15°C</td>
<td>0.534</td>
<td>-0.144</td>
</tr>
<tr>
<td>20°C</td>
<td>0.364</td>
<td>-0.209</td>
</tr>
<tr>
<td>25°C</td>
<td>0.502</td>
<td>-0.155</td>
</tr>
<tr>
<td>30°C</td>
<td>0.841</td>
<td>0.047</td>
</tr>
<tr>
<td>35°C</td>
<td>0.828</td>
<td>-0.050</td>
</tr>
<tr>
<td>40°C</td>
<td>0.987</td>
<td>0.004</td>
</tr>
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**Table A-3** Rheology values obtained for the butter samples. G’ is identified in Figure 3-7. G” indicates the viscous-like behavior of the sample. Delta represents the degree of viscoelasticity. Crossover point is the strain value (%) at which G’ = G”. Results sharing the same letter (a-b) within the same column are not significantly different (α=0.05)

<table>
<thead>
<tr>
<th>Sample</th>
<th>G” (MPa)</th>
<th>Delta (°)</th>
<th>Crossover Point (%)</th>
</tr>
</thead>
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<tr>
<td>1A</td>
<td>0.58 ± 0.14&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.9± 0.1&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.014 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1B</td>
<td>0.17 ± 0.05&lt;sup&gt;de&lt;/sup&gt;</td>
<td>10.1 ± 0.5&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.020 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1C</td>
<td>0.21 ± 0.09&lt;sup&gt;de&lt;/sup&gt;</td>
<td>9.8 ± 0.6&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.018 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2A</td>
<td>0.57 ± 0.14&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.7 ± 0.3&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.015 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2B</td>
<td>0.26 ± 0.21&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.7 ± 1.2&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.014 ± 0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2C</td>
<td>0.32 ± 0.29&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>11.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.023 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3A</td>
<td>0.68 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.3 ± 0.3&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.016 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3B</td>
<td>0.11 ± 0.12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.7 ± 1.0&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.019 ± 0.005&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3C</td>
<td>0.40 ± 0.26&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>11.2 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.014 ± 0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4A</td>
<td>0.25 ± 0.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.8 ± 1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.012 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4B</td>
<td>0.58 ± 0.04&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>9.8 ± 0.9&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.013 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>4C</td>
<td>0.30 ± 0.16&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>10.0 ± 0.5&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.011 ± 0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5A</td>
<td>0.67 ± 0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.7 ± 0.7&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.008 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5B</td>
<td>0.23 ± 0.11&lt;sup&gt;de&lt;/sup&gt;</td>
<td>10.1 ± 0.5&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.016 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5C</td>
<td>0.27 ± 0.13&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>10.5 ± 0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.013 ± 0.002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6A</td>
<td>0.37 ± 0.06&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>10.0 ± 0.8&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.019 ± 0.010&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>6B</td>
<td>0.16 ± 0.07&lt;sup&gt;de&lt;/sup&gt;</td>
<td>10.5 ± 1.6&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.015 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6C</td>
<td>0.17 ± 0.09&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.8 ± 1.5&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>0.016 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>7A</td>
<td>0.70 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0 ± 0.4&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.017 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>7B</td>
<td>0.26 ± 0.12&lt;sup&gt;de&lt;/sup&gt;</td>
<td>10.5 ± 0.6&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.022 ± 0.006&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>7C</td>
<td>0.27 ± 0.12&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>11.0 ± 3.5&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.016 ± 0.008&lt;sup&gt;ab&lt;/sup&gt;</td>
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Table A-4: Correlation values of all characteristics in relation to water loss. Correlation determined at a $\alpha = 0.05$ level of significance.

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<th>Regular Fat</th>
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<td>p-value Pearson r</td>
<td>p-value Pearson r</td>
<td>p-value Pearson r</td>
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<td>Water Content</td>
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</tr>
<tr>
<td>Enthalpy</td>
<td>0.009 0.519</td>
<td>0.03 0.71</td>
<td>0.58 0.18</td>
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<tr>
<td>Peak Temp (1st Peak)</td>
<td>0.437 -0.166</td>
<td>0.24 -0.43</td>
<td>0.31 0.32</td>
</tr>
<tr>
<td>Enthalpy (2nd Peak)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset Temp (2nd Peak)</td>
<td>0.613 -0.109</td>
<td>0.64 -0.18</td>
<td>0.69 0.13</td>
</tr>
<tr>
<td>Peak Temp (2nd Peak)</td>
<td>0.308 -0.217</td>
<td>0.29 -0.40</td>
<td>0.20 0.40</td>
</tr>
<tr>
<td>Enthalpy (Whole)</td>
<td>0.646 0.099</td>
<td>0.91 -0.05</td>
<td>0.59 0.17</td>
</tr>
<tr>
<td>G'</td>
<td>0.523 -0.137</td>
<td>0.01 -0.80</td>
<td>0.75 0.10</td>
</tr>
<tr>
<td>G''</td>
<td>0.513 -0.140</td>
<td>0.01 -0.79</td>
<td>0.71 0.12</td>
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<tr>
<td>Delta</td>
<td>0.296 -0.223</td>
<td>0.74 -0.13</td>
<td>0.87 -0.05</td>
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<tr>
<td>Crossover Point</td>
<td>0.863 0.037</td>
<td>0.67 -0.17</td>
<td>0.28 -0.34</td>
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<tr>
<td>Yield</td>
<td>0.887 0.031</td>
<td>0.38 0.33</td>
<td>0.13 -0.46</td>
</tr>
<tr>
<td>Hardness</td>
<td>0.950 0.014</td>
<td>0.72 -0.14</td>
<td>0.03 0.62</td>
</tr>
<tr>
<td>Water Droplet</td>
<td>0.842 0.043</td>
<td>0.26 0.42</td>
<td>0.04 0.59</td>
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<tr>
<td>SFC 5</td>
<td>0.772 -0.062</td>
<td>0.02 0.77</td>
<td>0.75 0.10</td>
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<tr>
<td>SFC 10</td>
<td>0.599 -0.113</td>
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<td>0.63 0.16</td>
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<tr>
<td>SFC 15</td>
<td>0.479 -0.152</td>
<td>0.04 0.70</td>
<td>0.38 0.28</td>
</tr>
<tr>
<td>SFC 20</td>
<td>0.285 -0.227</td>
<td>0.57 0.22</td>
<td>0.21 0.39</td>
</tr>
<tr>
<td>SFC 25</td>
<td>0.434 -0.168</td>
<td>0.07 0.63</td>
<td>0.12 0.47</td>
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<tr>
<td>SFC 30</td>
<td>0.934 0.018</td>
<td>0.37 0.34</td>
<td>0.01 0.73</td>
</tr>
<tr>
<td>SFC 35</td>
<td>0.546 -0.130</td>
<td>0.19 0.48</td>
<td>0.35 -0.30</td>
</tr>
<tr>
<td>SFC 40</td>
<td>0.689 -0.086</td>
<td>0.22 0.46</td>
<td>0.30 -0.33</td>
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</table>
Appendix B: Supplementary Tables and Figures from Chapter 4

**Figure B-1:** Water droplet size for butters obtained using cream with various fat contents. Results sharing the same letter are not significantly different ($\alpha=0.05$). Error bars represent standard error of the mean.
**Figure B-2:** Solid fat content (SFC) for butters obtained using creams with various fat contents. SFC measured at 5°C intervals from 5°C-35°C.
**Figure B-3:** Water content for butters obtained using creams with various fat contents.

Results sharing the same letter are not significantly different ($\alpha=0.05$). Error bars represent standard error of the mean.
Figure B-4: Melting properties for butters obtained using creams with various fat contents Enthalpy (A), onset temperature (B), and peak Temperature (C). Results sharing the same letter are not significantly different ($\alpha=0.05$). Error bars represent standard error of the mean.
Figure B-5: Melting profile of butter obtained using creams with various fat contents measured with differential scanning calorimeter.
**Figure B-6:** Hardness for butters obtained using creams with various fat contents.

Results sharing the same letter are not significantly different ($\alpha=0.05$). Error bars represent standard error of the mean.
**Figure B-7:** Rheological properties for butters obtained using creams with various fat contents including $G'$ (A), $G''$ (B), delta (C), crossover point (D), and yield stress (E). Results sharing the same letter are not significantly different ($\alpha=0.05$). Error bars represent standard error of the mean.
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ANNALISA JONES

Phone: (208)-881-6659
annalisajones12@gmail.com

Department of Nutrition, Dietetics and Food
Sciences Utah State University
Logan, UT 84322-8700

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<tbody>
<tr>
<td>R&amp;D INTERN</td>
<td></td>
</tr>
<tr>
<td>• Reclaim product going to waste to improve product yield and cost savings</td>
<td></td>
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<tr>
<td>• Understand and analyze issues due to crop year and develop tracking and improvement systems</td>
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<tr>
<td>• Understand and use equipment in the lab including Texture analyzer, Agtron, Moisture analyzer, RoTap, and RVA.</td>
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<table>
<thead>
<tr>
<th>UTAH STATE UNIVERSITY, LOGAN, UT</th>
<th>2020-2022</th>
</tr>
</thead>
<tbody>
<tr>
<td>STUDENT RESEARCH ASSISTANT</td>
<td></td>
</tr>
<tr>
<td>• Perform testing in the lab to identify characteristics necessary for quality pastry butter</td>
<td></td>
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<tr>
<td>• Record and report result to be reviewed for publishing</td>
<td></td>
</tr>
<tr>
<td>• Understand and use equipment in the lab including DSC, NMR, Rheometer, Texture analyzer, Polarized Light Microscope, and Moisture Analyzer</td>
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<table>
<thead>
<tr>
<th>LACTALIS AMERICAN GROUP INC., Nampa, Id</th>
<th>2020</th>
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</thead>
<tbody>
<tr>
<td>RAW SAVINGS MANAGEMENT INTERN</td>
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</tr>
<tr>
<td>• Identify areas and processes with high levels of product loss and create action plans to reduce loss in identified areas</td>
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<tr>
<td>• Direct changes made to processes working with supervisors, engineers, programmers, and floor workers.</td>
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<tr>
<td>• Put in place programs and plans to reduce loss by $30,000 monthly</td>
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<tr>
<th>BRIGHAM YOUNG UNIVERSITY-IDAHO, Rexburg, Id</th>
<th>2019-2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEACHING ASSISTANT</td>
<td></td>
</tr>
<tr>
<td>• Prepare students for upcoming assignments and evaluate progress</td>
<td></td>
</tr>
</tbody>
</table>
• Assist professor in developing lessons, teaching, and giving students opportunities to research

**BASIC AMERICAN FOODS CORP., Blackfoot, Id**

**QUALITY ASSURANCE INTERN**

• Calibrated an NIR Machine in order to advance accuracy and efficiency of quality assurance testing
• Collected data and tested products to evaluate safety, quality of products, and identify content characteristics.
• Developed a system to organize products in long term storage library to improve accessibility and easily store future additions
• Performed regular inspections of equipment to assure use of correct practices

**BRIGHAM YOUNG UNIVERSITY-IDAHO, Rexburg, Id**

**ONLINE COACHING ASSISTANT**

• Documented weekly performance of 60+ instructors addressing concerns in courses and helping elevate the student’s experience
• Designed new methods of data collection to increase productivity and accuracy of logging instructor performance

**SCHOLARSHIPS AND AWARDS**

**THIRD PLACE SMART SNACKS FOR KIDS PRODUCT DEVELOPMENT COMPETITION**

Product created for kids with health aspects in mind. Merengue and oat-based cookie that looked like a dinosaur egg was the final product

**DR. NIRANJAN R. GANDHI & MRS. JOSEPHINE N. GANDHI SCHOLARSHIP**

Utah State Scholarship based on need and academic achievement.

**FIRST PLACE OCEAN SPRAY PRODUCT DEVELOPMENT COMPETITION**

Product created with cranberry condiment or dip as the inspiration. Cranberry hot sauce was the finished product.

**DR. NIRANJAN R. GANDHI & MRS. JOSEPHINE N. GANDHI TUITION WAIVER**

Utah State Scholarship based on need and academic achievement.

**INTERMOUNTAIN INSTITUTE OF FOOD TECHNOLOGISTS SCHOLARSHIP**

Four students at Brigham Young University-Idaho were selected for their commitment to careers in the food science field in Idaho.

**BYU-IDAHO THOMAS E. RICKS AND OR TALENT GRANT**

This grant was awarded for academic excellence.

**IDAHO OPPORTUNITY SCHOLARSHIP**

Scholarship awarded to Idaho High school graduates to assist in secondary education
PUBLICATIONS


NATIONAL AND REGIONAL ABSTRACT PRESENTATIONS

- A. Jones & S. Martini. Fat Content of Cream Affects the Capacity of Butter to Hold Water. AOCs Annual Research Meeting, May 2022, Atlanta, GA, USA. (Poster)

LEADERSHIP

Utah State University
Food Science Club Committee Member, Logan, UT, 2020-2022

Brigham Young University-Idaho
Student Support Council -Involvement Representative, Rexburg, ID, 2018

COMMUNITY SERVICE

Workaway
International Volunteer, Bangkok, Thailand, 2019

The Church of Jesus Christ of Latter-day Saints
Full-Time Volunteer Representative, Carlsbad, Ca, 2016-2017
MEMBERSHIPS / AFFILIATIONS

- Institute of Food Technologists
- American Oil Chemists’ Society

TEACHING EXPERIENCE

- NDFS 5500-Food Analysis Lab Spring 2022
- NDFS 1010-Chocolate Lab Fall 2021