Influence of Emulsifiers and Fat Type on The Shear-induced Destabilisation of Partially Crystalline Oil-In-Water Emulsions and Corresponding Aeration Properties

A thesis presented in partial fulfilment of the requirements for the degree of

Master

of

Food Technology

at Massey University, Manawatū, New Zealand.

Stella Simone Henderson

2022

Abstract

UHT whipping cream is an oil-in-water emulsion characteristically containing between 35-40% milk fat. A whipped cream is made up of three immiscible phases: air, fat and water. Air bubbles are stabilized by a network of partially coalesced fat globules and proteins, induced by mechanical forces (whipping). The percentage of solid fat and crystal morphology in dairy creams play a critical role in determining functional properties as it influences the degree of partial coalescence. The solid fat content of New Zealand milk fat can change depending on the stage of lactation, resulting in inconsistent functionality of creams. The purpose of this research was to investigate the influence of the oil-water interfacial film composition, emulsifiers and fat crystallization on the stability, structure and functionality of model partially crystalline oil-in-water emulsions for whipping cream applications.

A bench scale model whipping cream system was developed with the ability to destabilise under shear. The model system was used to investigate the influence of emulsifiers and the seasonal variation in SFC on whipping cream functionality. This research was spilt into two main sections. Firstly, the the impact of LACTEM, MDG and Tween 80 on the destabilization and crystallization of milk fat in the model system was studied. Secondly, the impact of seasonal variation of solid fat content and non-dairy fat/oil blends on whipping cream functionality was studied. Formulations were analyzed through emulsion characteristics (PSD, FGSD, composition, protein surface load), crystallization behaviour, microstructure and whipping properties (whipping time, overrun, foam firmness). A key method used throughout this research to determine mechanisms was the 'stop and return' DSC method. This method was able to identify small changes to the formulation which impacted polymorphic transition and functionality. The relative solid fat content was used to identify significant correlations between blends of fats/oil and functionality.

Results suggest that LACTEM significantly increased overrun but resulted in poor chilled foam stability probably due to the mixed layer of partially coalesced fat and proteins stabilizing air bubbles. MDG resulted in an emulsion that was stable against shear-induced aggregation, leading to long whipping times and weak foam structure. Tween80 resulted in fast destabilization of fat globules but could not efficiently incorporate air bubbles, leading to dense foams with minimal overrun. It was found that generally, a higher initial formation of α-crystals resulted in a more effective fat globule destabilization during whipping. It is recommended that the impact of emulsifier blends on the model system is investigated. Emulsifiers are rarely used singularly in whipping creams, and often require careful formulation to produce desired functionality through balancing the inhibition and promotion of destabilization.

Results suggest the SFC of an emulsion impacts functionality. A high SFC did not cause significant differences in functionality and may improve foam structure. A low SFC resulted in longer whipping times, lower overrun and softer foams with a less defined foam structure. It is recommended that extra caution is taken when

formulating with early season cream due to the low SFC. Potential solutions could include the addition of hard fraction AMF or adjusting emulsifier levels to account for the reduced SFC.

Hard fraction palm oil and canola oil were used in emulsions to determine if SFC is a key driving force in influencing functionality. Both provided some potential benefits to the functionality and manufacturing costs as both palm oil and canola oil are typically cheaper than AMF. Hard palm fraction may provide improved whipping properties at a lower SFC compared to pure AMF due to the amount of unsaturated fatty acids. Canola oil can potentially be a replacement for a percentage of milk fat in whipping creams if the SFC is adequate. It is recommended that the feasibility of including hard palm fraction and canola oil in whipping cream formulations is investigated as both provided some potential benefits to the functionality and manufacturing costs.

Acknowledgements

I am extremely grateful to my main supervisor Dr. Lara Matia-Merino for her expertise, advice and motivation throughout the project. I appreciate the trust and confidence you showed in me.

I would like to express my greatest appreciation to my co-supervisor Dr. Patrick Janssen for the continuous support throughout the project. For his patience, guidance and extensive knowledge.

I would like to recognise the invaluable support and encouragement from my industry supervisors Dr. Sam Rogers and Dr. Anna Williams. For their time and dedication to ensuring a successful and enjoyable research experience.

I would like to express my gratitude to all the staff members from the School of Food and Advance Technology for their support and assistance. Particularly Michelle Tamehana, Steve Glassgow, Gary Radford and Warwick Johnson who inducted me into labs an assisted with equipment training.

I would like to acknowledge the financial and other support provided by Synlait Milk Ltd and the help given by the Research and Development team. I have enjoyed working alongside everyone, the valuable insights and experience is deeply appreciated.

Table of Contents

List of Illustrations

List of Tables

Chapter 1: Thesis Introduction

The purpose of this research was to investigate the influence of the oil-water interfacial film composition, emulsifiers and fat crystallization on the stability, structure and functionality of model partially crystalline oil-in-water emulsions for whipping cream applications.

Fresh cream is produced from whole milk using centrifugal separators. Centrifugal force is used to separate whole milk into different phases (skim milk and cream) based on density (McCarthy, 2011). New Zealand cream is typically composed of 37% milk fat, 2% protein and 3% lactose (Southward & Creamer, 1997). The composition and structure of fresh cream can be modified to create a range of cream products designed for specific applications. Examples of modified creams include ultra heat treatment (UHT) whipping creams, aerosol creams, cooking creams and beverage creams. This research focuses on UHT whipping creams, a product commonly consumed after being whipped to a firm peak. Whipping creams can be formulated with fresh cream, Anhydrous milk fat (AMF), milk solids, emulsifiers and stabilizers. The additives in whipping creams help to increase overrun, reduce whipping time and improve stability compared to fresh cream. The required functional properties of UHT whipping creams are highly specific to the consumer applications. Applications of whipping creams include whipped toppings, baking, beverages and desserts. Desired characteristics of a whipping cream are the ability to form a stable foam with sufficient overrun, chilled stability and limited serum drainage. The un-whipped product should have reasonable shelf life and be stable against temperature cycling. A combination of short whipping time and large overrun is often preferred by consumers. Once whipped, the cream should be easily piped or spread, holding its shape with no cracks. Desserts made with whipped cream are often refrigerated overnight, requiring a firm foam to maintain its shape for at least 24 hours after whipping.

An emulsion is a mixture of two or more immiscible liquids such as oil and water, where one liquid is dispersed within the other (Allen, Murray, & Dickinson, 2008a). UHT Whipping cream is an oil-in-water emulsion, characteristically containing between 35 - 40% fat (Hoffmann, 2016). Emulsions are thermodynamically unstable and often require emulsifiers, stabilizers and thickeners to decrease the rate of phase separation. Primary causes of instability include creaming, flocculation and coalescence. Understanding mechanisms that occur between ingredients at the oil/water interface is critical for predicting and improving stability and functionality. The addition of emulsifiers and stabilizers can manipulate the stability of a whipping cream product by encouraging or suppressing a mechanism. For example, stability can be encouraged under conditions such as storage and thermal fluctuations, destabilization can be promoted under conditions such as whipping when it is desired. The mechanical force of whipping creates a three-phase system by incorporating air bubbles into the emulsion. The foam structure is stabilized by partially coalesced fat globules with the presence of solid fat crystals (Allen et al., 2008a). Partial coalescence refers to an irreversible state of

aggregation of partially crystalline fat droplets which are connected by a “neck” of liquid oil (Fuller, 2015). Partial coalescence can only occur when the dispersed liquid droplets contain both liquid and crystalline fat. The presence of crystalline fat prevents full coalescence. Partial coalescence is an unfavored phenomenon in liquid emulsions as it leads to instabilities, but a favored phenomenon in aerated creams as it contributes to the foam structure. The solid fat content (SFC) of milk fat in New Zealand is known to change depending on the stage of lactation and season (Li, Ye, & Singh, 2022). The amount of solid fat and crystal morphology in dairy creams play a critical role in determining functional properties as it influences the degree of partial coalescence.

The aim of this research was to develop a model cream system and explore characterising methods to evaluate formulation levers for UHT whipping creams by testing individual mechanisms. A model system was developed that allows the mechanisms to be studied in a simplified system. The model system includes a fat source, protein source and emulsifiers.

The objectives were to:

- Establish a bench scale model whipping cream system with the ability to destabilise under shear.
- Investigate the influence of emulsifiers on whipping cream functionality and their mechanism that impact fat crystallization.
- Investigate the impact of seasonal changes to solid fat content on whipping cream functionality and how it may be controlled by formulation.

Chapter 2: Literature Review

2.1. Introduction

UHT whipping cream is an oil-in-water emulsion, it is a complex food system that requires careful formulation to ensure optimal functionality and storage stability. Emulsions are not thermodynamically favored and often require the addition of emulsifiers, stabilizers and thickeners to decrease the rate of separation. Understanding the mechanisms that occur between ingredients at the oil/water interface is critical for predicting and improving stability and functionality. A whipped cream is made up of three immiscible phases: air, oil and water. Air bubbles are stabilized by a network of partially coalesced fat globules and proteins, induced by mechanical forces (whipping). It is expected that a whipping cream forms a stable foam with sufficient overrun, chilled stability and limited serum drainage. The selection of ingredients and fat type is critical for maximizing cream performance. Therefore, the interactions between fat type, fat structure, emulsifiers and stabilizers must be investigated to identify optimal formulation leavers.

This literature review investigates emulsion principles, ingredient interactions, aeration, composition, dairy fat alternatives and the methodologies used to study emulsions and the mechanisms driving emulsion stability.

2.2. Milk Fat

2.2.1. Overview

Milk fat is a unique blend of triglycerides, containing a range of long and short chain lengths compared to vegetable oils. In the food industry, dairy fat is used for its quality taste, mouthfeel and distinctive functionality in whipped cream. Over 400 fatty acids are present in milk fat, making it more diverse than vegetable and other animal fats (Jensen, Ferris, & Lammi-Keefe, 1991).

AMF is a concentrated product produced from pasteurized dairy cream. Concentrated cream is further processed using phase inversion and vacuum drying to produce a product containing 99.8% milk fat, 0.1% non-fat solids and 0.1% water (Southward & Creamer, 1997). Figure 1: Process flow diagram of AMF production from milk.Figure 1 illustrates the process flow diagram for AMF production and Table 1 shows the compositional differences between milk, cream and AMF. AMF is often the primary source of fat in recombined creams. The composition of AMF represents the inner core of milk fat globules as the membrane components are absent. The naturally occurring membrane of milk fat globules consists of phospholipids, proteins, acylglycerols, sterols and other compounds (Monteny, 2015). The membrane becomes damaged and removed during processing.

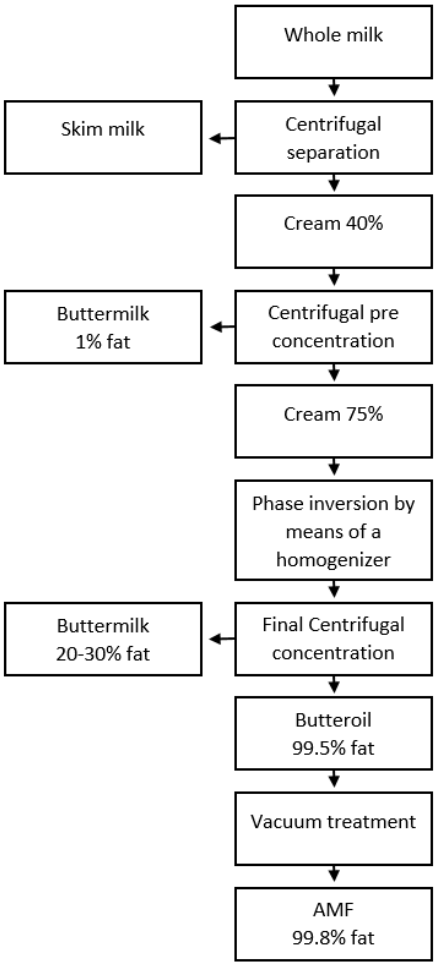


Figure 1: Process flow diagram of AMF production from milk.

Table 1: Typical composition of dairy products in New Zealand (C. R. Southward, 1997).

	Whole milk	**Cream**	**AMF**
Fat	4.0%	40.0%	99.8%
Protein	3.28%	2.0%	<0.01%
Lactose	4.7%	3.0%	<0.01%
Ash	0.71%	0.38%	<0.1%
Water	87.7%	54.9%	<0.1%

2.2.2. Seasonal Variation

The solid fat content of milk fat is known to change depending on the stage of milking and season (Li, Ye, & Singh, 2020; Norris, Gray, & Dolby, 1973). The amount of solid fat in a product at a given temperature plays a critical role in determining the functionality and stability for whipping cream.

In New Zealand, studies suggest that the early season (August – October) has the lowest solid fat content at ambient temperatures in comparison to the rest of the milking season (November – May), with the highest solid fat content occurring around December (Mid-season) (Li et al., 2022; Norris et al., 1973). The crystallization rate of summer milk fat increases in comparison the early season milk fat (Norris et al., 1973). Late season milk has highlighted some unique properties, including highest protein and fat levels, lowest lactose content and altered protein and salt compositions (Li et al., 2020).

The presence of solid fat in whipping cream is essential for partial coalesce to occur (Monteny, 2015). The percentage of solid fat in creams increases at lower temperatures, generating the recommended for cream to be whipped straight from the fridge (Ihara et al., 2010). However, the temperature at which customers whip a product cannot be controlled. Therefore, it is important to ensure that the product has sufficient levels of solid fat at ambient temperatures. The foam produced from products with low levels of solid fat are more likely to have poor foam stability as the structure is weaker and less rigid (Ihara et al., 2010). Results from Li et al. (2022) showed how the whipping properties of creams varied across the season. Early-season cream (lowest solid fat content) had the shortest whipping time, highest overrun and firmness. Late season cream (medium solid fat content) had the longest whipping time, lowest overrun and firmness.

2.2.2. Crystallization Characteristics

The crystallization characteristics of milk fat are responsible for the whipping properties of cream. When milk fat is cooled below melting point, triglycerides undergo a phase transition from liquid to crystalline. This occurs through a two-step process of nuclei formation and crystal growth (Smith, Bhaggan, Talbot, & Malssen, 2011). After nucleation, triglycerides arrange into one of three types of crystals: α, β and β'. Fats initially form into α or β' crystal polymorphs and may transform into β' and β crystals over time as they are more thermodynamically stable. This polymorphic transition is irreversible (unless heated above melting point) and depends on the temperature/time combination used during processing. The polymorphic form of crystals influences a products texture, appearance and functionality. β' crystals are often the preferred form as it provides the greatest functionality in terms of softness, aeration ability and creaming properties (Lopez et al., 2002).

The crystallization of fat droplets are affected by the type of emulsifier, droplet size distribution, temperature history and the presence of any impurities (Mutoh, Nakagawa, Noda, Shiinoki, & Matsumura, 2001). Smith et al. (2011) found that surfactants may influence fat crystal nucleation in bulk fat by either inhibiting or promoting crystal growth. The effects include a change in nucleation time or temperature and a change in the number and composition of nuclei (Smith et al., 2011).There are two rules that typically predict whether a surfactant will affect fat crystallization: 1) the similarity in triglyceride composition. 2) the affinity of the surfactant for the crystal matrix (Fuller, 2015). In general, the surface coverage of adsorbed proteins on an oil droplet is decreased by the transition from liquid to crystalline fat (S. R. Euston & Mayhill, 2001).

2.2.3. Partial Coalescence

Particle coalescence refers to an irreversible state of aggregation of partially crystalline fat droplets which are connected by a "neck" of liquid oil (Fuller, 2015). It occurs when the dispersed liquid droplets contain both liquid and crystalline fat. The mechanisms by which the "neck" of oil is formed occurs from surface crystals from one globule that rupture another. Once this contact occurs, liquid can flow between globules and form a junction if the contact time is sufficient (Walstra, 2003). The presence of crystalline fat prevents full coalescence due to the internal structure. Partial coalescence is an unfavored phenomenon in liquid emulsions as it leads to instabilities, but a favored phenomenon in aerated creams as it contributes to the foam structure.

Pathways that can lead to partial coalescence include shear, temperature fluctuations and the incorporation of air bubbles. In UHT creams the fat globule membrane has been removed and replaced with proteins. In order for partial coalescence to occur, the protein layer needs to be predominantly removed so that fat globules are able to approach each other (Walstra, 2003). Therefore, the surface roughness dictates the susceptibility to partial coalescence. The surface roughness can be controlled by the use of surfactants, crystal formation or altering the degree of droplet supercooling by increasing the number of nucleation sites (Fuller, 2015). To regulate partial coalescence, the protrusion of fat crystals should be restricted, and the thickened/strength of the interfacial film could be increased.

2.2.4. Cream and Recombined Creams

Natural cream is produced from the separation process of milk fat and skim milk by a centrifugal separator (Monteny, 2015). The resulting cream is an oil-in-water emulsion, physically characterized by its fat content (approximately 40%), particle size and viscosity (Fredrick et al., 2013).

Recombined dairy cream is generally produced by combining milk powder, AMF, water, emulsifiers, stabilizers and sometimes sugar. Recombined creams have gained popularity due to the accessibility of raw materials and the ability to alter the functionality. The raw materials for recombined cream do not require the same level of caution around the storage and transportation than fresh cream (Monteny, 2015).

2.2.5. Milk Fat and Vegetable Oil Blends

AMF can be separated into the different melting fractions and used in specific ratios to improve its functionality in products. Fractionation is a separation technique that divides the fat into different components depending on their melting point. First stage fractionation results in a high melting fraction (HMF) and a low melting fraction (LMF). The resulting HMF and LMF can be refine further through second and third stage fractionation (Deffense, 1995). To produce a dairy product with higher levels of solid fat, high melting fraction AMF can be added back into a formulation to improve the functionality and stability. The addition of hard fraction AMF into dairy products was studied by Monteny (2015), who investigated partial coalescence in recombined creams as influenced by fat composition. Monteny (2015) evaluated the fractionation process and the effect of AMF blends and AMF/vegetable oil blends. Monteny (2015) found that recombined creams containing a higher solid fat content resulted in faster formation of a stronger fat crystal network. The two promising formulations included a blend of AMF fractions (40% LMF/ 60% HMF) and a blend of AMF and vegetable oil (70% Rapeseed oil/ 30% HMF AMF).

The work by Martini, Herrera, and Hartel (2002) investigated the effect of sunflower oil addition on the crystallization behaviour of high melting fraction AMF. The results showed that the addition of sunflower oil resulted in a significant decrease of solid fat content due to dilution effect, but a relatively similar melting point. This suggests that sunflower oil addition to milk fat results in slower crystallization by an inhibition effect.

Palm oil is one of the most popular vegetable fats used throughout the food industry due to its cost, purity and high melting point. It is used in products such as spreads, cookies, peanut butter and instant noodles. However, the use of palm oil in products has sparked controversy due to health and environmental concerns (Monteny, 2015). The concept of increasing the solid fat content of AMF containing products with hard fraction palm oil is commonly adopted by the confectionary industry. The addition of hard palm oil stearin to AMF results in

improved functionality, melting profiles and solid fat contents at high temperatures (Hayati et al., 2000). Hayati et al. (2000) discovered that blends of AMF and soft palm oil stearin exhibited similar melting profiles to the HMF of milk fat at temperatures above 10˚C. Findings like this suggest that material cost of food products can be reduced by using palm oil/AMF blends.

Rapeseed oil is becoming the preferred alternative to palm oil in the food industry. It is commonly used as a replacement of AMF in products to reduce material costs. Several spread products in Europe have replaced up to 30% of the AMF in formulations with rapeseed oil (Monteny, 2015). Kaufmann, Andersen, and Wiking (2012) showed that the addition of rapeseed oil to AMF influenced the melting behaviour and cooling rate. Results by DSC showed that fast and slow cooling resulted in completely different crystallization behaviour due to the solubilisation effect of rapeseed oil. Kaufmann et al. (2012) concluded that the texture properties of the fat blends varied even when the solid fat content remained relatively constant. Slow and fast cooled blends with similar solid fat contents showed very different stress fracture results due to the different microstructure formed.

2.2.6. Milk Fat Alternatives

Due to current food trends, the demand for diary alternatives is increasing. The challenge product developers face is designing dairy free formulations that behave in the same unique way as dairy fat. Cream-like emulsions created with vegatable fats have been studied (Márquez & Wagner, 2012; Pérez, Tesei, Wagner, & Márquez, 2014; Shin, Heo, & Lee, 2019). When developing a cream-like emulsion, the objective is to have sufficent soild fat content for partial coalesece to occur.

The work by Pérez et al. (2014) investigated partial coalescence in cream-like emulsions prepared with low trans vegetable fats, bovine fat, partially hydrogenated soybean oil and sunflower oil. Temperature cycling and controlled stirring were used to compare results across formulations. Pérez et al. (2014) found that the low trans vegetable fat formulation was prone to partial coalescences when the process is desired (whipping or stirring) and more stable to the partial coalescences when it needs to be avoided (thermal fluctuations). This was concluded to be due to the lower solid fat content.

Márquez and Wagner (2012) investigated the cream-like emulsions prepared with soybean milk and low trans vegetable fat, focusing on rheology. The particle size and fat content of oil-in-water formulations were altered, and the effect was studied using viscoelastic behaviors and aggregation by shaking. Results suggested that partial coalescence can be reproduced in emulsions prepared with soybean milk and low trans vegetable oil. The combination of soybean milk and low trans vegetable oil could be a potential vegetable substitute for traditional dairy creams due to their similarity in rheological behavior.

2.3. Emulsions

2.3.1. Stability

Emulsion systems are not thermodynamically favored and possess minimal stability. Emulsion stability can be defined as the degree of phase separation over time. The primary causes of instability include creaming, flocculation and coalescence. Creaming is characterized by the movement of oil droplets under gravity to form a concentrated layer at the surface (Sjöblom, 2006). Creaming is reversable by agitation or gentle mixing depending on the degree of separation. Stokes law can be used to evaluate creaming. It shows that creaming can be controlled by particle size reduction, reduction of phase density differences and by increasing the viscosity of the continuous phase. Flocculation occurs when dispersed droplets form aggregates that have not entirely lost their identity. The two primary drivers of flocculation are Brownian motion and shear flow (Sjöblom, 2006). Flocculation can be prevented either by electrostatic stabilization or by steric stabilization. In electrostatic stabilization, droplets are kept apart by the repulsion of their charged surfaces. Steric stabilization is created by osmotic repulsion between macromolecular adsorbed layers. Surface active polyelectrolyte such as proteins can provide both electrostatic and steric stability (Sjöblom, 2006).

Coalescence refers to the joining of two or more droplets to form a single droplet. This results in greater volume and smaller interfacial area. Coalescence is energetically favored and irreversible. Coalescence occurs when droplets have been in proximity for a long period of time, such as a flocculated state. The two primary causes of coalescence are thinning and rupture of the oil-water interfacial film (Sjöblom, 2006). Film thinning is dependent on the colloidal forces acting on the film and the hydrodynamic aspects of film flow. Film rupture depends on the thickness of the film and the mechanical properties of the film.

The stability of an emulsion can be improved by controlling droplet size, increasing viscosity of the continuous phase, and controlling the membrane surface via addition of emulsifiers and stabilizers (Sjöblom, 2006). One method to evaluate the stability of an emulsion is by determining the particle size distribution. The particle size distribution of an emulsion should have a single peak under 1 micron. Signs of an unstable distribution are peaks larger than 1 micron and bimodal distribution.

Factors that may affect droplet size include the homogeniser, energy input, rheological properties of each phase, surfactant type and concentration, volume fraction, temperature and method of preparation (Sjöblom, 2006).

2.3.2 Emulsifiers

Emulsifiers are an essential ingredient in whipping cream formulations. A typical UHT whipping cream contains a carefully selected blend of emulsifiers to provide optimal functionality and stability. Common emulsifiers used in whipping cream formulations include polysorbates, mono and diglycerides (MDG), Lactic acid esters (LACTEM), sodium stearoyl lactylate (SSL) and sodium hexametaphosphate. Emulsifiers are amphiphilic molecules, possessing both hydrophobic and hydrophilic fragments. This structure allows the molecule to become solubilized in both phases, assembling at the interface. When selecting an emulsifier, it is important to understanding the molecular structure to achieve the desired functionality. Increasing the chain length of an emulsifier typically results in the molecule self-orientating into the liquid crystalline phase, particularly at low temperatures (Fuller, 2015). Surfactants can influence fat crystallization by inhibiting or promoting crystal growth (Álvarez Cerimedo, Cerdeira, Candal, & Herrera, 2008). The specific impact surfactants can have on crystal nucleation include a change in nucleation time, temperature, composition and number of nuclei.

Finding the ideal balance between emulsifiers in whipping cream is critical for producing a product with the desired functionality and stability. It is necessary to find a balance between emulsion stability and destabilization using emulsifiers. The emulsifiers selected in whipping cream products all play important roles by modifying the stability and inhibiting the full potential of other emulsifiers and proteins in the system. For example, MDG is known to increase the viscosity of an emulsion. This viscosity increase may be hindered by the addition of a more polar emulsifier such as lecithin or SSL (Moonen & Bas, 2014).

Mono and diglycerides (MDG) are a common food emulsifier created by the interesterification of edible fats or oils with glycerol. In dairy products, MDG's are primarily responsible for the partial destabilization of fat in an emulsion (Moonen & Bas, 2014). MDG's are known to reduce the oil/water interface tension more that milk proteins alone, creating a more effective homogenisation process by producing a narrower PSD (Moonen & Bas, 2014). Another primary role of MDG's in dairy products is the ability to assists with aeration. It does this by promoting the desorption of milk proteins from the oil-water interface, resulting in an interfacial layer with reduced strength (Moonen & Bas, 2014). This mechanism is required for controlled agglomeration of fat globules during aeration. The addition of MDG's can result in a stable crystalline structure by initiating robust fat crystallization (Moonen & Bas, 2014). The concentration of MDG's in whipping cream formulations is typically between 0.2-0.5%, depending on the fat content of a product (Xiao, 2020). MDG's are lipophilic emulsifiers and should be added into the fat phase before homogenisation.

Lactic acid esters (LACTEM) are based on the reaction between lactic acid and monoglycerides of fully hydrogenated vegetable or animal fats. Lactic acid esters contain between 15-35% esterified lactic acid, distributed over several isomeric compounds. The key benefits of using LACTEM in cream systems include improved aeration, foam stability, texture and volume (Gaupp & Adams, 2014). LACTEM is often used in

cream products in combination with saturated monoglycerides to produce a product that is stable in the crystalline form. LACTEM primarily stays in the α-crystal state and do not show polymorphous tendencies. This gives LACTEM the ability to stabilize the α-crystals of triglycerides in the system. The α-tending properties of LACTEM has been linked to improved whippability and overrun (Gaupp & Adams, 2014). The foam structure can become strengthened with α-tending emulsifiers as they promote agglomeration of fat globules (Gaupp & Adams, 2014).

Polysorbates are non-ionic hydrophilic surfactants created by a high-pressure reaction of sorbitan esters with ethylene oxide (Cottrell & Peij, 2014). The group of products consist of polysorbates 20, 60, 65 and 80, also commonly known as Tween. The inclusion of Tween emulsifiers in cream systems can potentially hinder partial coalescence by inhibiting liquid migration between fat globules. Long chain fatty acid tweens (40 ad 60) reportedly decreased the degree of supercooling required for crystallization (Fuller, 2015). Polysorbates are known to be highly effective at displacing proteins at the oil/water interface, allowing them to be used at low concentrations (Xiao, 2020). The typical dosage of polysorbates in dairy products is 0.04-0.07% (Dar & Light, 2014). Polysorbate 80 has be shown to have the greatest effect at low concentrations, which is important as the flavour of polysorbates can be easily recognised in products (Cottrell & Peij, 2014).

2.3.3 Protein Based Films

Oil-in-water emulsions stabilized by proteins are known to be resistant to partial coalescence (O'Regan & Mulvihill, 2010). The surface rheology of protein-stabilized emulsions is a significant factor influencing the coalescence kinetics. Protein layers resist coalescence as they have high surface elastic modules and can resist flow with high surface viscosity. Adsorbed proteins create thick layers and do not reduce surface tension the same way as other surfactants do. As a result, emulsion stability is achieved via the mechanical protection of the adsorbed protein rather than a reduction of interfacial tension (Fuller, 2015). It is recognized across literature that the transition from liquid oil to crystalline fat has been shown to decrease the surface coverage of adsorbed proteins at the oil-water interface (Euston & Mayhill, 2001).

Adsorbed protein at the oil-water interface can be partially displaced by low molecular weight surfactants due to competitive adsorption at the interface (Smith, Kakuda, & Goff, 2000). This is thought to occur through a three-stage mechanism: surfactant adsorption, expansion of the surfactant domains and the control of the interface by surfactants (Gunning et al., 2004). Gaps and weak areas in the protein film can be filled by surfactants. If the appropriate length of time is given and a high concentration of surfactants is present, the protein film can become fully displaced. This displacement of the protein film by surfactants increases the susceptibility of partially crystalline emulsions to partial coalescence (Fuller, 2015).

Whipping cream requires a highly specific form of stability. The product must be stable towards temperature cycling and extended shelf life, with the ability to destabilize under shear. The balance of attractive and repulsive forces acting on the fat globules must be careful managed to create this stability. The key force that needs to be controlled to achieve the required stability is depletion attraction. Depletion attraction illustrates that when two particles exist at a distance smaller that the diameter of another un-adsorbed polymer, the polymer can be excluded from the inter-globule space(Dickinson, Golding, & Povey, 1997). This promotes flocculation of fat globules due to the osmotic gradient. Xanthan gum is often used as a depletion agent. Vanapalli, Palanuwech, and Coupland (2002) found that xanthan gum at low levels resulted in depletion flocculation which increased the susceptibility to partial coalescence, whereas higher levels of xanthan gum resulted in increased viscosity which inherently reduce susceptibility to partial coalescence.

Low molecular weight surfactants affect competitive adsorption with proteins in various ways depending on the charge present. Surfactants can displace protein via one of two mechanisms: solubilization and replacement (Dickinson, 1999). The solubility mechanism describes how a surfactant binds to a protein molecule, resulting in an increase in solubility within the continuous phase. This leads to a more efficient desorption of protein-surfactant complex at the interface (Dickinson, 1999). The replacement mechanism occurs when the interfacial free energy becomes lowered to a greater extent than the protein alone, by the presence of a surfactant at the interface. This typically occurs when non-ionic surfactants are present (Fuller, 2015).

2.3.4 Aeration

Whipping cream creates a three-phase solid system by incorporating air bubbles into the system. The weak foam structure is stabilized by partially coalesced fat globules from the presence of solid fat crystals (Allen et al., 2008a). Monteny (2015) described the aeration process of cream in three stages. Firstly, the whisk introduces large air bubbles which eventually reduce in size and become stabilized by proteins. Fat globules then begin to adsorb at the new air interface once, releasing liquid oil which spreads onto the surface of the air bubble. This mechanism promotes surface-meditated partial coalescence, which occurs when the spreading oil promotes aggregation of fat globules and the flocculation of other fat globules at the interface. Finally, partial coalescence between fat globules occurs, resulting in a network that has encapsulated air bubbles. This system retains the serum phase in the foam structure and increases the stability of the whipped foam. A higher degree of partial coalescence in the final stage of whipping results in a firmer and stiffer foam (Monteny, 2015). Foams are a naturally unstable system. The destabilization of whipped cream systems are primarily due to liquid drainage, Ostwald ripening and air bubble coalescence (Fredrick, 2011). These phenomena can result in difficulty controlling whipped cream properties such as mouth feel, flavour and appearance.

The temperature creams are whipped at plays a significant role in the stability and functionality of the final foam. Ihara et al. (2010) studied the effect of whipping temperature (5 -15 degrees) on the whipping (time and

overrun) and rheological properties of whipped cream. The stability of the foams was studied by measuring the hardness, viscoelasticity, overrun, whipping time, aggregation ratio, serum viscosity, dynamic surface tension and the diameter and surface area of the air bubbles. Ihara et al. (2010) found that an increase in temperature promoted aggregation, reducing the time to reach a given hardness. It was recommended that a temperature between 7.5-12.5 degrees is used when whipping creams as it resulted in the optimal texture. It has been suggested that greater displacement of proteins by emulsifiers occur at lower temperatures due to greater emulsifier surface activity (Fuller, 2015).

2.4 Model Systems

A model system is a simplified version of a complete food system, it contains the major components in the system and aims to remove any variation or cofounding factors found in real food systems. Components are incorporated one at a time in order to understand the specific mechanisms and impact on the system without the influence of minor components. A cream model system typically consists of fat, water, protein and emulsifiers or stabilizers. Components that are excluded from a cream model system include fat globule membrane components, minerals, native proteins/micelles.

The paper by Dickinson (2019) highlights the uses of model systems in whipping cream research. Model systems have been used to study flocculation behavior of oil-in-water emulsions stabilized by sodium caseinate. This research demonstrates the importance of finding the optimal caseinate to oil ratio in model systems to avoid flocculation. Insufficient protein at the interface can lead to bridging flocculation which occurs when oil droplets share the proteinaceous material. Conversely, an excess of non-adsorbed protein in a system can lead to depletion flocculation. Bridging flocculation can only be disrupted by strong shear forces such as second stage homogenisation whereas depletion flocculation is reversible by gentle stirring or dilution (Dickinson, 2019). Figure 2 illustrates the difference between depletion and bridging flocculating as a result of polymer concentration.

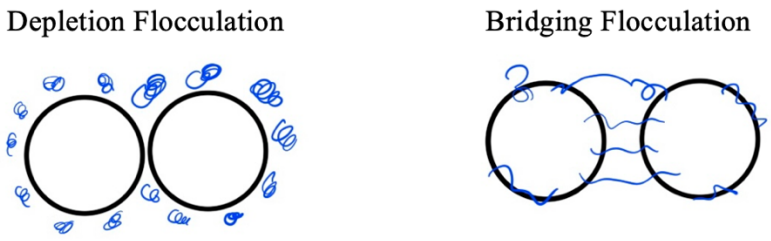


Figure 2: Schematic of depletion flocculation and bridging flocculation. Blue indicates polymers, black indicates colloids.

2.4.1 Emulsion Preparation

An emulsion needs to be prepared correctly and consistently to achieve statistically significant results. A stable emulsion can be identified by the particle size distribution. A stable emulsion will have a narrow distribution curve, with a peak around 1μm (Sjöblom, 2006). Signs of an unstable emulsion include double distribution peaks and large droplet sizes. To achieve homogeneous droplet distribution, the correct homogeniser pressure and number of passes through the homogeniser for the material should be used. The settings required for each homogeniser will vary slightly and preliminary experiments are required before trials to determine the optimal settings. A good starting point is setting a target droplet size and ensuring emulsions achieve this.

The emulsion preparation methodology in literature can vary depending on the type of homogeniser, temperature, pressure, passes and order of ingredients added. The order of ingredients added is dependent on the affinity of the molecule to either the water or oil phase. Generally, proteins and hydrophilic emulsifiers are dispersed in the water phase and lipophilic emulsifier are dispersed in the oil phase. Table 2 evaluates common emulsion preparation methods in literature. Table 2 shows that emulsions are typically pre-homogenised to form a coarse emulsion with either an Ultraturrax or Silverson mixer. The primary method of particle size reduction was a two-stage homogeniser, but similar results can also be achieved with ultrasonic processors and microfluidizers. Table 2 shows that the temperature samples are processed at is generally above melting point (above 40 ˚C) with the exception of (Michon et al., 2019) used a temperature of 18˚C.

Table 2: Comparison of emulsion preparation methods.

Literature	Equipment	Settings	Temperature	Length of storage required
(Michon et al., 2019)	Rotor-stator homogeniser (Polytron PT3100D, Switzerland) Ultrasonic Processor 20 kHz, 130 W	5 minutes Rotational speed varying between 5,000-10,000 rpm	18 ˚C	
(Haddadian, Bremer, Eyres, Carne, & Everett, 2016)	Rotor-stator homogeniser (Polytron PT 2100, Kinematica, Luzern, Switzerland) M-110S Microfluidizer (Microfluidics Corporation, Newton, MA, USA)	30 seconds 4,000 rpm 56 MPa	50 ˚C	14 days
(Shin et al., 2019)	Silverson mixer Microfluidics processor	2 min 5,000 rpm Two passes at 3000 psi	40 ˚C	
(Márquez & Wagner, 2012)	Ultra-turrax T-25 Rotor–stator homogeniser	1 min at 24,000 rpm 100 bar in first valve 10 bar in second valve	60 ˚C	1 day at 4 degrees
(Mutoh et al., 2001)	Two-stage homogeniser	5 min at 10,000 rpm 10MPa in first stage 2MPa in second stage	60 ˚C	
(Jiang et al., 2018)	Two stage homogeniser	150 bar, 30 bar	60 ˚C	24 hours at 4 degrees

2.5 Methodologies

2.5.1 Differential Scanning Calorimetry

DSC can be used to produce thermograms which show the effect of both temperature cycles and crystallization of the lipid phase (Pérez et al., 2014). DSC is primarily used to study the melting behaviour of fats by subjecting the sample to a series of temperature cycles using pre-determined heating/cooling rates. Enthalpy changes are measured as a result of changes to the physical and chemical properties of the sample as a function of temperature and time (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011). The results provide an understanding of crystallization behaviour, crystal polymorphs and melting point range.

Wang, Hartel, and Zhang (2021) and Monteny (2015) utilised DSC methods uses to study the crystallization and melting behavior of AMF blends. Both methods involved heating samples above melting temperature, cooling to below zero at a rate of 5 degrees/minute and holding before heating back to melting temperature at 5 degrees/minute. From this, they were able to identify final melting points, initial crystallization and the different fat fractions in AMF.

2.5.2 Rheology

Shear-induced Aggregation

Food rheology is the study of the deformation and flow of food under specific applied forces (O'Sullivan, 2017). Fat globule aggregation can be evaluated using shear inducing methods across a range of temperatures. Many researchers utilized the cone and plate geometry to study shear-induced aggregation. However, there is a range of approaches used across literature. These include constant shear, oscillatory testing and stress ramps. Methods involving constant shear rates allow for the aggregation time to be determined, as the viscosity and shear stress remain relatively constant right before the onset of aggregation. Orthokinetic aggregation produces a viscosity versus time graph and helps to identify overcoming an energy barrier, identified by a sudden increase of viscosity caused by aggregation of the dispersed phase. The induction time can be used to determine the repulsive energy barrier between droplets. Oscillatory testing is useful when studying shear-induced aggregation for unstable emulsions with large droplet sizes. Stress ramp experiments can determine the critical stress value which induces aggregation.

When an emulsion is subjected to shear, particles inevitably encounter each other, collide and coalesce. The probability that two particles will interact is dependent on collision frequency and efficiency. Encounter frequency can be improved by increasing the shear rate, volume fraction and droplet size (Fuller, 2015). Equation 1 from Walstra (2003) describes the capture efficiency:

$$\alpha = 0.6\left(\frac{A}{\eta\,\psi\,d^3}\right)^{0.18} \quad (1)$$

Where α = capture efficiency, A = Hamaker constant, η = viscosity, ψ = velocity gradient and d = droplet diameter. This equation is only valid under laminar flow conditions, which are produced under low shear rates as the fluid moves in layers. Secondary flow is produced at higher shear rates due to the increased centrifugal and inertial forces over the viscous forces. The difference between laminar and secondary flow can be described by the deviation from smooth flow into vortices (Fuller, 2015). Turbulent flow can be produced by increasing the shear rate further to create random and chaotic behavior.

Márquez and Wagner (2012) studied shear-induced aggregation by magnetic stirring. Samples were exposed to a constant stir of 12,000 rpm until the rod had stopped as a result of thickness increase in the system. The time taken for the rod to become stationary was recorded. Monteny (2015) studied shear-induced aggregation using a starch pasting cell at a constant shear rate, to evaluate the change in viscosity over time.

Temperature Ramp

Temperature ramp rheology can be used to evaluate changes in fat crystallization as a function of temperature. Decreasing temperature ramps allow the onset of crystallization growth of different fat mediums to be studied. Lupi et al. (2012) investigated the effect of organogelator and fat source on the rheological properties of olive oil-based organogels. A decreasing temperature ramp from 70˚C to 20 ˚C was utilized to estimate the temperature at which the onset of crystallization occurred. A strong increase of the complex modulus G' was used to identify the onset of crystallization.

Foam Evaluation

Dynamic oscillatory testing is used to evaluate foam structure as it does not cause large deformation during the measurement. Tests can be performed on fresh and aged foams to understand differences in structural changes. Stress sweep is used to measure strain hardness, defined as the stress measured when the whipped cream strain reaches 10 % (Ihara et al., 2010). Values can be used to compare hardness across samples to understand any structural differences. Typically, samples are taken from the top of the foam or the side of the bowl after whipping.

2.5.3 Zetasizer

Zetasizer is an instrument that is used to measure particle size in the nano range using dynamic light scattering, particle mobility and charge (zeta potential) using electrophoretic light scattering and the molecular weight of particles using static light scattering ("Zetasizer Range," 2021). Zeta potential is often used as a measurement of emulsion stability as it can determine particle susceptibility to flocculation (Bhatt, Prasad, Singh, & Panpalia, 2010). There is an energy barrier resulting from the repulsive force that prevents two particles approaching one another. The size of this potential barrier can be indicated by the zeta potential, a measurement of the potential at the slipping plane between particles and the associated double layer (Duffy, Larsson, & Hill, 2012). The measurement is dependent on particle size, distance between particles and the strength of the interfacial film (Bhatt et al., 2010). A stronger interfacial film results in a lower zeta potential decay rate and a more stable emulsion. A zeta potential of ±30 mV is considered stable as the particles tend to repel each other (Duffy et al., 2012). However, a low zeta potential value can indicate insignificant repulsion to keep particles separated, resulting in flocculation.

2.5.4 Surface Tension

Any substance (protein, emulsifier, surfactant etc.) on the surface of a fat globule influences the surface tension. Typically, the presence of a substance results in a lower surface tension. The degree of adsorption at the interface increases with the surface age and the decrease in surface tension (Ihara et al., 2010). The relationship between surface tension and bubble age can be used to evaluate adsorption speed. Surface tension can be evaluated using force based or optical methods. One method primarily used in literature is the pendant drop tensiometer (Ihara et al., 2010; Wang et al., 2021). This method is an optical based, pendant drop shape analysis. A needle produces a hanging drop of one liquid within another. The balance of forces between the liquids is determines the shape of the droplet. The surface tension of two immiscible liquids with known densities can be determined by the droplet shape.

2.5.5 Whipping Properties

Two of the most common whipping properties evaluated are whipping time and overrun. Whipping time refers to the time to reach a predetermined stability. This parameter is assessed visually and has raised concerns over its subjectivity. The visual determination generally is assessed when the foam forms a firm peak and/or when there a change in motor frequency is heard. Overrun describes the percentage increase in volume due to the inclusion of gas. Generally, optimal whipping properties are an overrun between 100-150% and a whipping

time around 2-3 minutes (Walstra, Wouters, & Geurts, 2006). The stability of foams are typically assessed by serum loss and foam hardness (Monteny, 2015). The foam stability can also be evaluated by piping the cream into shapes such as rosettes and measuring the chilled stability of the rosette overnight (appearance based). Figure 3 shows an example of a good and poor whipped cream when piped and how visual tools can be used to assess this. A simplified method to study shear-induced aggregation of emulations was used by Pérez et al. (2014) which involved stirring an emulsion with a magnetic stirrer at 150 rpm at room temperature. The time was recorded for the stirring rod to become immobilized as a result of thickness increase in the system.

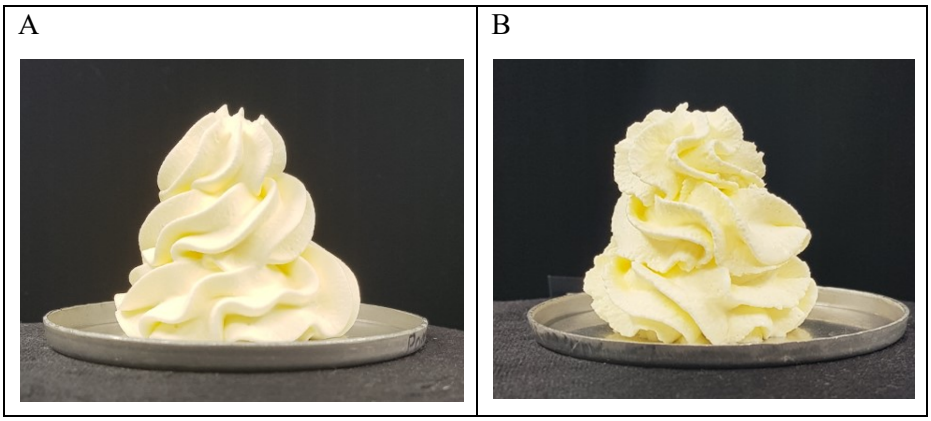


Figure 3: Images of whipped cream piped into rosettes, showing a good cream versus a bad cream.

2.6 Summary

In summary, a substantial amount of work has been carried out to understand the stability of oil-in-water emulsions and the primary factors and mechanisms that affect this. However, the influence of solid fat content due to seasonal variation on emulsion stability and how it can be controlled through formulation has not been well researched. This work will involve studying the stability of a model emulsion formulated with different interfacial compositions and solid fat contents. The mechanisms as which emulsifiers influence the fat structuring and overall stability will be investigated.

Critical analysis of literature has identified several key research questions to be defined:

- What influence do emulsifiers have on the fat structuring of oil-in-water emulsions and how can the ratios be adjusted to control stability?
- How does the seasonal variation in solid fat content affect the stability and functionality of UHT whipping cream?

Chapter 3: Methodology

A range of methodologies were used to analyse properties of the emulsions. A fatty acid analysis of the emulsifiers was completed, and multiple emulsion preparation methods explored to determine any influence on the model system. Emulsions characteristics and foam characteristics of model systems were applied throughout this study.

3.1. Fatty Acid Analysis of Ingredients

Fatty acid profiles of the emulsifiers were determined by the Massey University Nutrition Laboratory, using the gas chromatography method as described by Sukhija and Palmquist (1988), Table 3.

Table 3: Fatty acid profile of the used emulsifiers.

Fatty Acid Profile (g/100g)	**Tween80**	**MDG**	**LACTEM**
C6:0	0.03	0.02	0.02
C8:0	-	0.01	0.01
C10:0	-	0.01	0.01
C12:0	0.03	0.16	0.13
C13:0	< 0.01	< 0.01	< 0.01
C14:0	0.06	0.94	0.82
C16:0	0.79	48.00	42.85
C17:0	0.04	0.14	0.12
C18:0	0.45	33.20	27.96
C18:1n9c	17.89	0.04	0.10
C18:1n7c	0.12	-	-
C18:2n6c	0.03	-	0.02
C20:0	0.02	0.37	0.31
C21:0	0.02	-	-
C22:0	-	0.06	0.04
C23:0	-	0.01	0.01
C24:0	-	0.06	0.05

3.2. Emulsion Preparation

Three emulsion preparation methods were investigated throughout this study (Chapter 5) to determine the influence on the model system. The method selected was chosen based on experimental objectives and the required arrangement of fat globule membrane being replicated. Figure 4 illustrate the process flow diagram for the three emulsion preparation methods.

- Method 1 involved creating a stock emulsion from AMF and sodium caseinate initially, then introducing an emulsifier solution to the protein stabilized emulsion after homogenisation.
- Method 2 involved adding the emulsifiers to either the fat or water phase depending on their hydrophobicity prior to homogenisation, then forming the final emulsion.
- Method 3 involved adding the fat-soluble emulsifiers into the fat phase before homogenisation, followed by a Tween80 solution 24 hours after homogenisation

To ensure homogeneous melting fractions throughout experiments using bulk AMF, the AMF was melted to 70°C and mixed well before pouring into individual containers.

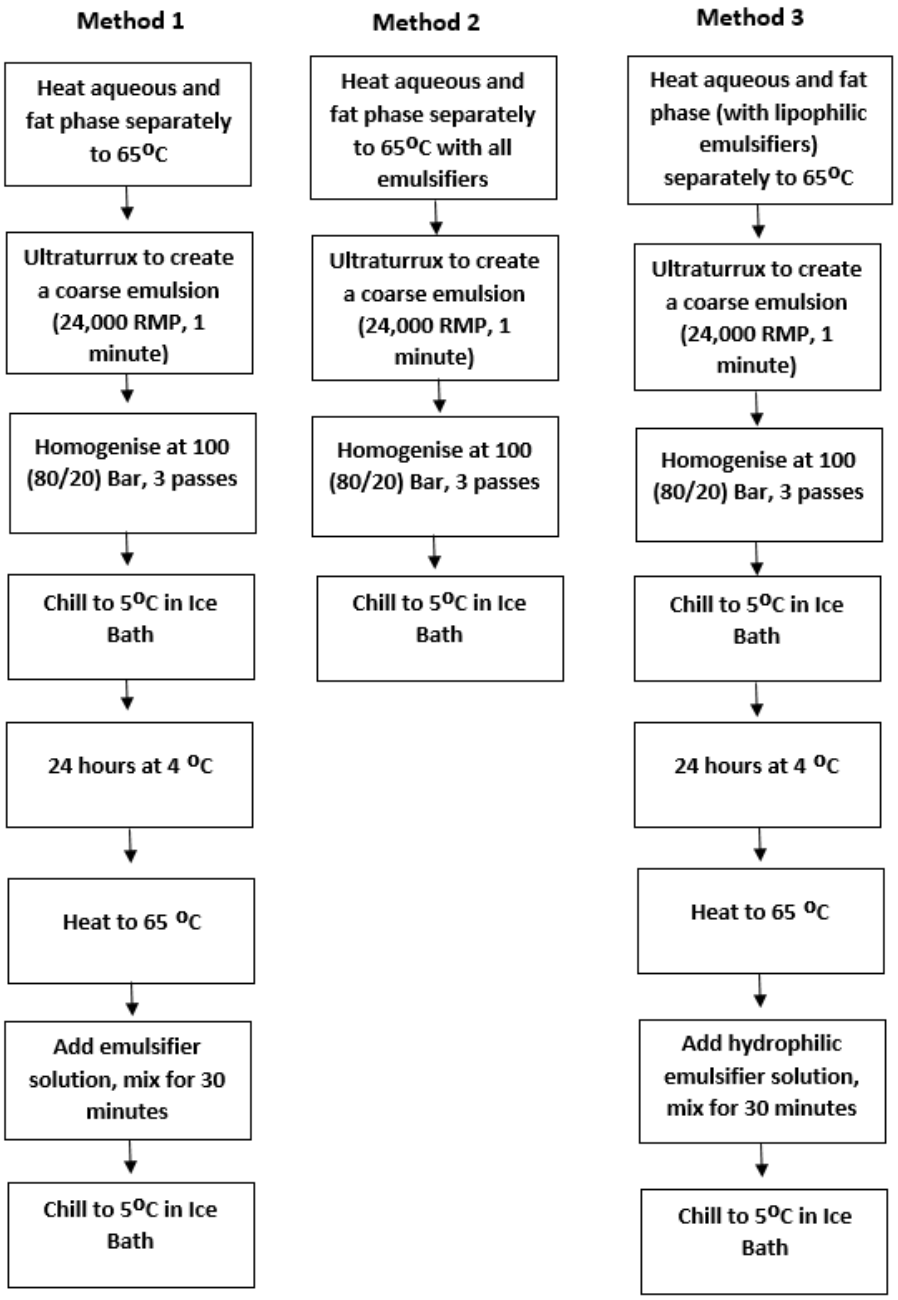


Figure 4: Process flow diagram for the three emulsion preparation methods.

3.2.1. Method 1

A stock, protein stabilized oil-in-water emulsion was prepared initially. To do this, sodium caseinate was hydrated by gently dispersing in water overnight at room temperature. The fat phase and sodium caseinate solution were heated separately in a water bath to 70°C before combining. A coarse emulsion was prepared by high shear mixing (Ultra-Turrax, IKA, Germany) for 1 minute at 24,000 rpm. Samples were heated to 70°C in a water bath before homogenisation. Samples were homogenised using a preheated two-stage homogeniser (APV Rannie, Copenhagen, Denmark) at 100 bar (80/20) and 3 passes (unless specified otherwise) and stored at 4°C for 24 hours before further use. The typical composition of a stock emulsion was 42 wt% fat and 2 wt% sodium caseinate (unless specified otherwise).

Working emulsions were created by diluting the stock emulsion with and emulsifier solution. Emulsifier solutions were prepared by dispersing emulsifiers in water (type and concentration defined in individual sections). Emulsifier solutions were heated to 65°C and stirred with a stir bar before being left to mix overnight at room temperature. Stock emulsions were heated to 65°C for 30 minutes in a water bath before diluting with emulsifier solution to 36 wt% fat. After mixing, samples were cooled to 5°C in an ice bath before storage. All emulsions were stored at 4°C for 6 days before testing to allow for the SFC ad interfacial film composition to reach equilibrium. A typical working emulsion contained 36 wt% fat, 2 wt% sodium caseinate (unless specified otherwise). All emulsion formulations studied were prepared and tested in at least duplicate or greater batches.

3.2.2. Method 2

Sodium caseinate was hydrated by gently dispersing in water overnight at room temperature. Emulsifiers were weighed out and dispersed in individual mediums, hydrophilic emulsifiers (Tween80) in the water phase and lipophilic emulsifiers (MDG, LACTEM) in the fat phase. The fat phase and sodium caseinate solutions containing emulsifiers were heated separately in a water bath to 70°C and well mixed before combining. A coarse emulsion was prepared by high shear mixing (Ultra-Turrax, IKA, Germany) for 1 minute at 24,000 rpm. Samples were heated to 70°C in a water bath before homogenisation. Samples were homogenised using a preheated two-stage homogeniser (APV Rannie, Copenhagen, Denmark) at 100 bar (80/20) and 3 passes (unless specified otherwise). Samples were cooled to 5°C in an ice bath before storage. All emulsions were stored at 4°C for 6 days before testing to allow for the SFC ad interfacial film composition to reach equilibrium. A typical emulsion contained 36 wt% fat, 2 wt% sodium caseinate and 0.3% emulsifier (unless specified otherwise). All emulsion formulations studied were prepared and tested in at least duplicate or greater batches.

3.2.3. Method 3

Sodium caseinate was hydrated by gently dispersing in water overnight at room temperature. Lipophilic emulsifiers (MDG, LACTEM) were weighed out and dispersed in the fat phase. The fat phase and sodium caseinate solutions were heated separately in a water bath to 70°C and well mixed before combining. A coarse emulsion was prepared by high shear mixing (Ultra-Turrax, IKA, Germany) for 1 minute at 24,000 rpm. Samples were heated to 70°C in a water bath before homogenisation. Samples were homogenised using a preheated two-stage homogeniser (APV Rannie, Copenhagen, Denmark) at 100 bar (80/20) and 3 passes (unless specified otherwise).

Emulsions containing Tween80 were included 24 hours after homogenisation. Tween80 solutions were prepared by dispersing emulsifiers in water (concentration defined in individual sections), heating to 65°C with a stir bar before being left to mix overnight at room temperature. Emulsions were heated to 65°C for 30 minutes in a water bath before diluting with Tween80 solution to 36 wt% fat. After mixing, samples were cooled to 5°C in an ice bath before storage. All emulsions were stored at 4°C for 6 days before testing to allow for the SFC ad interfacial film composition to reach equilibrium. A typical emulsion contained 36 wt% fat, 2 wt% sodium caseinate and 0.3% emulsifier (unless specified otherwise). All emulsion formulations studied were prepared and tested in at least duplicate or greater batches.

3.3. Emulsion Characteristics

3.3.1. Particle Size Analysis

The particle size distribution (PSD) and fat globule size distribution (FGSD) of emulsions were measured at room temperature by laser diffraction particle size analyzer, Malvern Mastersizer 3000 (Malvern Instruments Ltd. Worcester, UK). Volume weighted mean particle diameter ($D_{4,3}$), volume/surface mean ($D_{3,2}$) and number mean (d_{10}, d_{50} d_{90}) measurements were recorded. Prior to testing, 1 part emulsion was diluted with 4 parts RO water. Of this dilution, 1-part diluted sample to 9-parts 2% SDS solution to isolate the fat globule. SDS helps to dissociate weak interactions between fat globules that can potentially impact the particle size results. SDS does not separate agglomerated fat globules. A refractive index for milk fat of 1.45 was used. Each emulsion was measured in triplicate.

3.3.2. Composition

Emulsion composition was measured using a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark). 70ml of sample was taken after homogenisationfor measurement. Fat, protein, total solids and solids non-fat were measured.

3.3.3. Protein Surface Load

Adsorbed protein concentration was determined by depletion method described by Segall and Goff (1999). Emulsions were stored for 24 hours at 5ºC prior to centrifugation. Samples were heated to 20ºC in a water bath then transferred into the centrifuge (Sorvall RC 6+, Thermo Scientific, MA, USA). Emulsions were spun at 27,000xg for 1 hour to separate the fat globules from the serum phase. The serum was collected by a syringe and filtered through a 0.2µm syringe filter. The protein content of the serum phase was determined using a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark) using a pre-calibrated sodium caseinate setting (method development detailed in Section 4.2). The total mean surface area (s_1) was calculated using equation 2 (Berton, Genot, & Ropers, 2011):

$$S_1 = s_{globule} \times \frac{m_{oil}}{V_{globule} \times \rho} \qquad (2)$$

Where $s_{globule}$ is the surface area (m^2) of one fat globule and P is the density of fat (g/m^3). The $s_{globule}$ and $V_{globule}$ were calculated from d_{32}, the surface weight mean diameter. A density value of 0.915 g/m^3 for milk fat at 20°C was used (Watson & Tittsler, 1961).

Protein surface load was calculated from the difference between the initial protein concentration in the emulsion and the protein concentration in the serum phase after centrifugation, divided by the total mean surface interfacial area.

3.3.4. Light Microscopy

Microstructure of samples were analyzed using light microscopy (Lecia DM1000, Leica Microsystems, Wetzlar, Germany). Samples were evaluated after 7 days of storage at 4°C. One drop of emulsion was placed on a microscope slide and covered with a cover slip. X10 and X100 magnification were applied for the images.

3.3.5. Differential Scanning Calorimetry

Stop-and-Return Method

Isothermal crystallization kinetics were analyzed using the stop-and-return method described by Foubert, Fredrick, Vereecken, Sichien, and Dewettinck (2008). Crystallization curves were obtained using a MDSC Q-2000 instrument (TA Instruments; New Castle, DE, USA). A hermetic aluminum pan (Tzero, TA Instruments, USA) filled with (5-15mg) of sample was sealed using a press. An empty pan was used as a reference and nitrogen was used to purge the system.

The applied time-temperature program was as follows: Holding at 65°C for 10 minutes to ensure a completely liquid state. Cooling at 25 °C min^{-1} to the isothermal crystallization temperature (5 °C), holding for the required crystallization time, and then heating at 20°C min^{-1} to 65°C. The crystallization time before remelting was varied for each cycle, ranging between 30-seconds and 120-minutes. Figure 5 demonstrates the heat flow versus temperature graph produced using this method.

Melting curves were integrated using a horizontal baseline of 55 °C, assuming the sensible heat is constant over the temperature range. Emulsions were measured in duplicate.

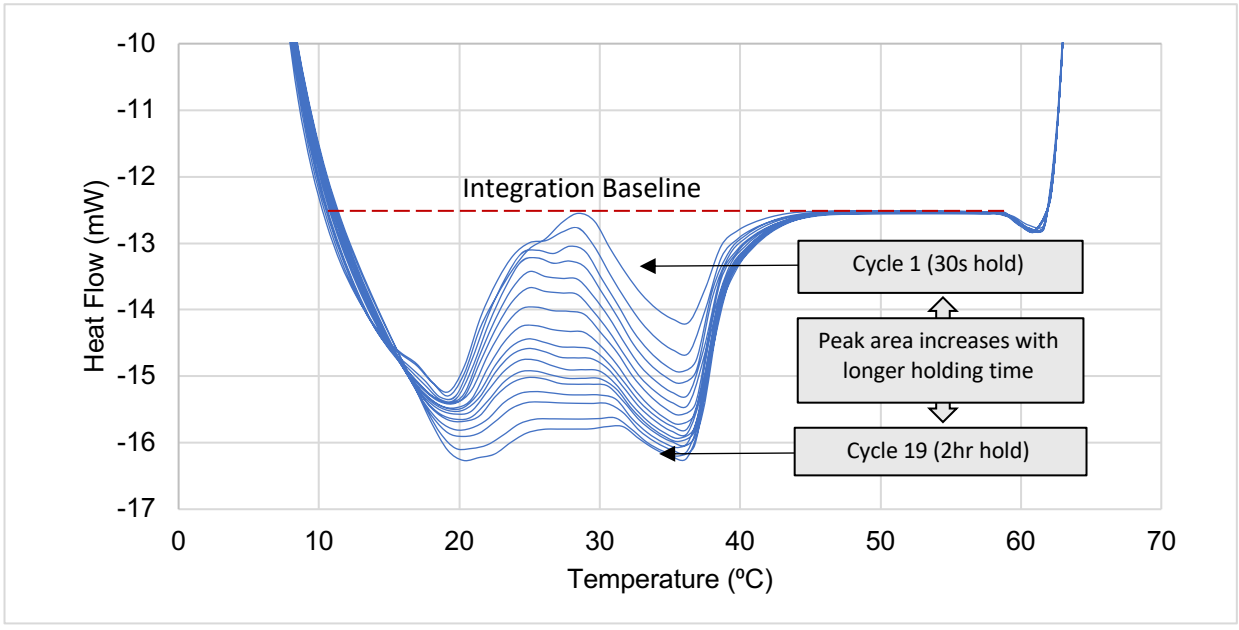


Figure 5: Example heat flow versus temperature graph for a 10mg cream sample after 19 heating and cooling cycles with increasing crystallization time.

Integrated melting heat values can be plotted against holding time to produce an isothermal crystallization curve. Figure 6 illustrates an example melting heat as a function of holding time graph. This graph can indicate α and β'crystal growth by the first and second step, often identified by an inflex point. The final point is a measurement of solid fat content, relative to other samples.

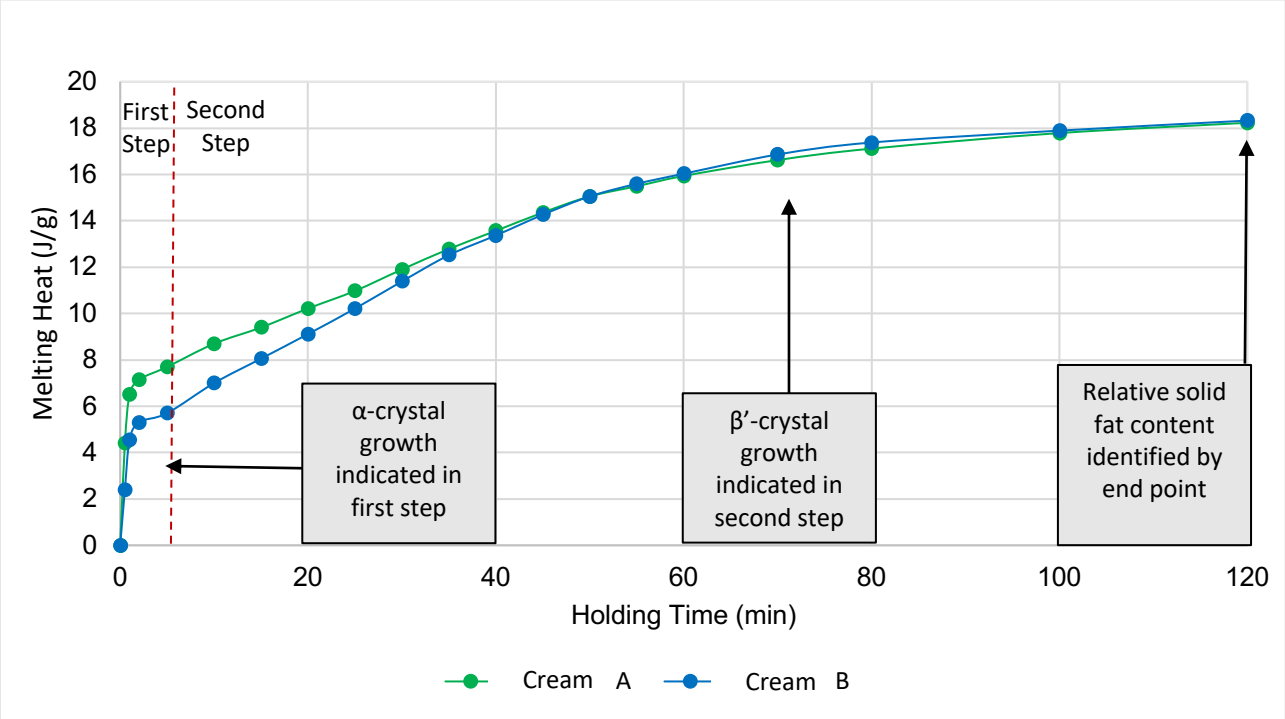


Figure 6: Example melting heat as a function of time for isothermal crystallization at 5 ℃ for a 10mg cream sample.

Calculation of Mass Fraction of Crystals in the Filter Cake

To assess the effectiveness of the fat fractionation, DSC thermograms were utilized to estimate the concentration of solid crystals in the hard fraction cake after filtration. The aim was to allow the fat to equilibrate the fractionation temperature of 22°C with purely β'-crystals. Crystallization curves were obtained using a MDSC Q-2000 instrument (TA Instruments; New Castle, DE, USA). A hermetic aluminum pan (Tzero, TA Instruments, USA) filled with (5-15mg) of sample was sealed using a press. An empty pan was used as a reference and nitrogen was used to purge the system.

Samples were heated to 65°C and equilibrated for 5 minutes before cooling to 5°C at a rate of 25°C /minute. Samples were then held at 5°C for 60 minutes, heated to 22°C at a rate of 20°C/minute and held for a further 60 minutes before heating back to 65°C at a rate of 20°C/minute. Each sample was measured in at least duplicate. The data was recorded and analysed using a software Universal Analysis 2000 (TA Instrument, USA).

The melting curve between 22-65°C was integrated using a linear baseline of 55°C. The total melting heat (J/g) was divided by the heat of crystallization of β'-crystals to get an estimate of the mass fraction of crystals in the filter cake. Heat of crystallization value for AMF used was 122 J/g.

3.4. Foam Characteristics

3.4.1. Whipping Time

Emulsions were whipped at 4ºC using a KitchenAid KSM160 Artisan Stand Mixer (MI, USA) set on speed 8. Bowls were chilled to 4°C beforehand to maintain temperature during whipping. Emulsions were stored for 7 days at 4°C before whipping. Emulsions were whipped until a firm peak formed. Whipping time was determined visually after reaching maximum overrun and a firm peak. The whipped emulsion should not stick to the sides of the bowl or around the whisk (Monteny, 2015).

3.4.2. Overrun

The overrun was determined at 4ºC for each aerated emulsion. Overrun refers to the amount of air introduced in the emulsion relative to the sample volume (Smith et al., 2000). Overrun was calculated using equation 3:

$$overrun\ (\%) = \frac{m_{liquid\ sample} - m_{whipped\ sample}}{m_{whipped\ sample}} \times 100 \qquad (3)$$

3.4.3. Foam Firmness

Deformation puncture measurements were evaluated at 4ºC using a TA.XT*Plus*C texture analyzer (Stable Micro Systems, USA) equipped with an acrylic cylindrical flat probe (Diameter 10mm) Puncture tests were performed at a velocity of 1mm/s over a distance of 5mm. Aerated emulsions were piped into a 120ml pottle until completely full and leveled with a spatula. The peak force at a depth of 5mm was defined as the firmness of the aerated emulsions. Each sample was measured in triplicate.

3.4.4. Shear-Induced Aggregation

Stability under shear was investigated over time at a constant shear rate based on methods described by Fuller (2015) using a Malvern Kinexus PRO rheometer (Malvern Instruments Ltd., Worcestershire, UK) equipped with cone and plate geometry (50mm diameter, 2^{o} angle, 70um gap). The plate temperature was controlled at 5^{o}C by a Peltier plate system. The cone and plate were pre-chilled before use to maintain and emulsion temperature of 5^{o}C. A constant shear rate of 500s^{-1} was applied over 300s. Viscosity versus time was measured.

3.4.5. Confocal Scanning Light Microscopy

Emulsions were stored for 7 days at 4°C prior to testing. 100g of sample was poured into the mixing bowl and 1mL of 0.02 wt% Nile Red (Sigma-Aldrich, MA, USA) and Fast Green (Sigma-Aldrich, MA, USA) were added to stain the fats and protein, respectively. Dyed emulsions were whipped at 4ºC using a KitchenAid KSM160 Artisan Stand Mixer (MI, USA) set on speed 8. Bowls were chilled to 4°C beforehand to maintain temperature during whipping. Emulsions were whipped until a firm peak formed. After whipping, the stained samples were immediately placed on a slide of observation.

A Zeiss LSM 900 Airyscan 2 super-resolution system (Oberkochen, Germany) equipped with a 10x objective and a 100x oil-immersion objective was applied to obtain the images. The excitation wavelength for Nile Red and Fast green were 568 nm and 633 nm, respectively. The fluorescence light emitted by the Nile Red and Fast green were detected at 569-640 nm and 650-700 nm, respectively.

Chapter 4: Method Development

4.1. Shear-Induced Aggregation

Stability under shear was investigated over time at a constant shear rate based on methods described by Fuller (2015). Shear rates between 100-2000 s^{-1} were investigated using a Malvern Kinexus PRO rheometer (Malvern Instruments Ltd., Worcestershire, UK) equipped with cone and plate geometry (50mm diameter, 2º angle, 70um gap). The plate temperature was controlled at 4ºC by a Peltier plate system. The cone and plate were pre-chilled before use to maintain an emulsion temperature of 4ºC.

Cone-and-plate geometry was selected in order for primary laminar flow conditions to be generated under shear. From the viscosity versus time curve, the aggregation time was determined as the period of rapid viscosity increase following Guery et al. (2006). This point of aggregation typically occurs right before the jamming transition. Jamming transition is described as when a fluid emulsion changes into a semisolid or paste due to the overcrowding of aggregates as they grow in size, entrapping a portion of the continuous phase (Fuller, 2015). On the viscosity versus time graph (example: Figure 7), the point of initial viscosity increase represents the onset of aggregation, whereas the maximum peak represents the jamming transition.

Samples were exposed to shear for a period of 300s. The challenge was determining the corresponding shear rate for a sample that allowed it to destabilize within the given time period. When a low shear rate was applied, the viscosity remained constant throughout the measurement and did not aggregate, conversely when the shear rate was too high, samples destabilized almost immediately and were unable to produced practical data. Figure 7 illustrates the expected viscosity vs time curve as well as what happens when the shear rate is too high or low. The viscosity of the sample was another determining factor influencing destabilization. If a sample had a considerably low viscosity, it could not be measured at the predetermined shear rate as it would not remain within the geometry. Samples were expelled due to insufficient surface tension to keep the sample between the cone and plate.

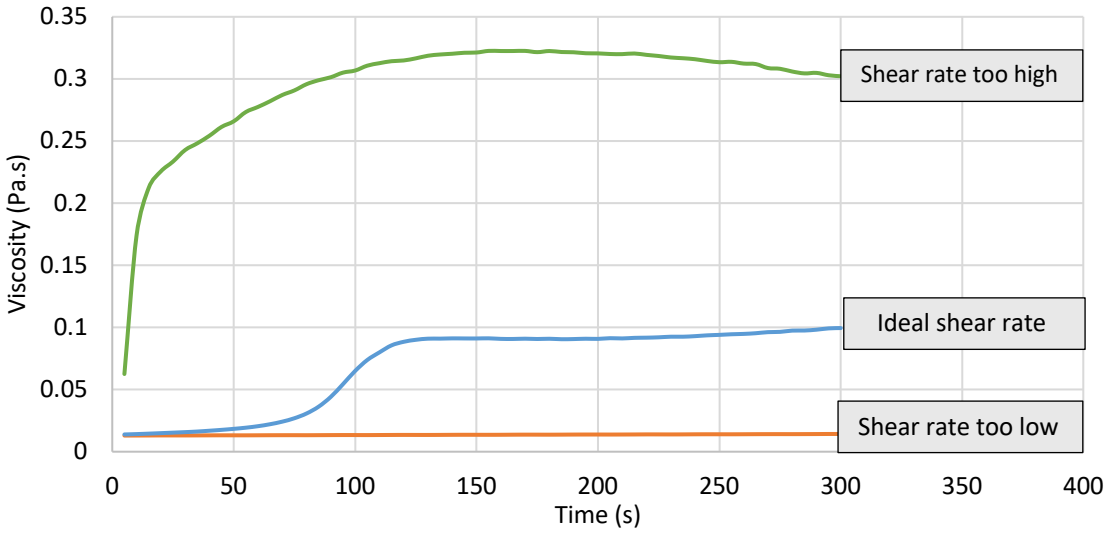


Figure 7: Viscosity versus time graph showing viscosity results when the shear rate is ideal and not adjusted for the formulation.

The ideal shear rate was depending on the characteristics of the emulsion. Factors that effected the rate of aggregation and the amount of shear required included emulsifier presence, emulsifier concentration, fat globule size and protein concentration.

It was discovered that only emulsions containing Tween80 had the ability to destabilize under the given conditions (Figure 41, Appendix). Samples that contained another emulsifier or a combination of Tween80 and another emulsifier such as MDG and LACTEM did not destabilize as they were either very stable or too low in viscosity to aggregate.

The FGSD was another factor that influenced the shear-induced aggregation method. The particle size influenced the onset of aggregation and the shear rate required to destabilize the emulsion. Figure 8 illustrated that samples containing larger fat globules required a lower shear rate and less time to aggregate in comparison to samples with smaller fat globules. This was expected, as larger droplets are less stable and more susceptible to instability mechanisms such as creaming and partial coalescence.

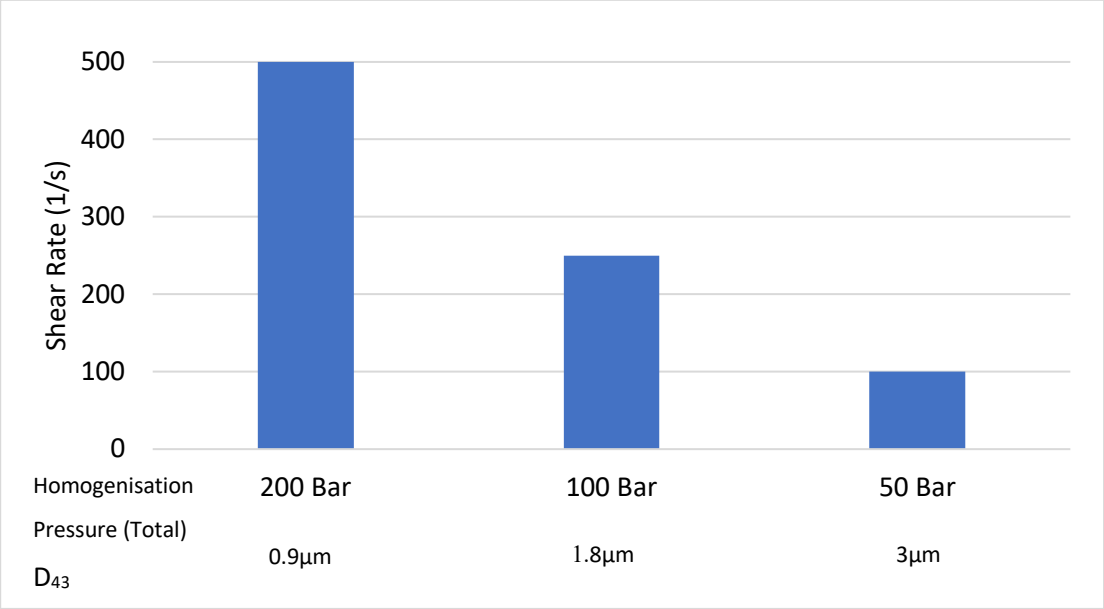


Figure 8: Shear rate required to destabilize emulsions containing 0.4% Tween80 between 100-300s of constant shear.

The shear-induced aggregation rheometer method can be an important tool to identify factors influencing emulsion destabilization. Method development experiments for these formulations determined that results can only be comparative if the PSD, shear rate and viscosity of the samples are consistent. Tween80 must also be present in the formulation for the emulsion to destabilize. Key results from the viscosity versus time graph that can be used to make assumptions about emulsions are the time of sudden viscosity increase and the maximum viscosity reached. The point of initial viscosity increase can determine a formulations susceptibility to destabilization under shear and potentially correlate with whipping time.

4.2. Protein Surface Load

Adsorbed protein concentration was determined by depletion method (Segall & Goff, 1999). It was recommended by Fuller (2015) for samples to be centrifuged at 27,500g for 60 minutes at 40ºC to minimize solid fat and inhibit partial coalescence that may occur due to centrifugation. Partial coalescences would impact the fat globule interfacial area, leading to an inaccurate protein surface load measurement. Experiments using this method resulted in damaged centrifuge tubes (Oak Ridge Centrifuge Tube, PPCO, Nalge Nunc International, NY, USA) as they could not withstand the temperature/force combination, despite claimed capabilities of the product. A significant number of tubes removed from the centrifuge had warped and some had cracked. A lower temperature of 20ºC was trialed which resulted in the tubes staying intact without warping. It was decided to continue with a centrifugation temperature 20ºC for the tube to last the duration of the test.

The impact of centrifuging at a lower temperature on the fat globule interfacial area should be considered when analyzing protein surface load results. It is important to ensure that all samples within an experiment are centrifuged at the same temperature to compare results and draw conclusions.

4.2.1. MilkoScan Calibration

A calibration curve was developed using a sodium caseinate solution (Sodium caseinate, RO water) on a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark). Calibration samples were prepared containing a protein concentration between 0 - 3%, in 0.5% increments. Calibration samples were run through the MilkoScan, the curve was adjusted based on calculated protein concentration. Samples were performed in triplicate to ensure the MilkoScan results were reading consistently.

The calibration samples were analyzed for protein content by the Massey University Nutrition Laboratory to confirm and adjust the accuracy of the calibration curve. Genuine protein serum samples from centrifuged emulsions were also analyzed for protein concentration using both the MilkoScan and the laboratory to validate the accuracy of the method. The Nutrition Laboratory protein results were equivalent to the estimated readings from the MilkoScan (Table 4), concluding the method as an acceptable technique to determine the amount of un-adsorbed protein in the serum for future experiments. The calibration was maintained by performing a new set of calibration data points every 8 weeks.

Table 4: Results comparing protein concentration results from the Nutrition Laboratory and the MilkoScan method developed.

Sample	**Expected Protein Concentration (%)**	**MilkoScan Result (%)**	**Laboratory Result (%)**
Calibration (low)	0.51	0.5	0.4
Calibration (high)	2.99	2.99	2.9
Protein serum	-	0.68	0.7
Protein Serum	-	1.68	1.7
Protein Serum	-	2.37	2.3

Chapter 5: Optimizing the Model System

5.1. Introduction

A model food system is a simplified version of a complete food system. Components are introduced one by one in order to understand specific mechanisms and their impact on the system, without the influence of minor components. The cream model system being investigated consists of a fat source, water, milk protein and emulsifiers. Components that are excluded from the model system include fat globule membrane components, minerals, native proteins/micelles, sugars and stabilizers.

AMF and sodium caseinate were selected as the primary ingredients in the model system as they are standardized forms of dairy fat and protein. AMF and sodium caseinate provide predictable and consistent stabilizing mechanisms. Reliable fat and protein components are essential to confidently identify mechanisms caused by intentionally changed factors such as processing conditions, emulsifier type and concentration.

AMF was selected as the fat source for the model system as it is a standardized version of dairy fat. The use of a fat/oil source allows emulsions to be formed in experimental conditions. Fresh cream can have a highly variable membrane surface composition, dependent on season, breed of cow, feed etc. Variations in membrane composition can significantly impact the functionality and stability of whipped cream products (Wang et al., 2021). AMF is a concentrated milk fat product, derived from pasteurized cream. Concentrated cream is processed using phase inversion and vacuum drying to produce AMF (Rajah, 1991). When the fat globule membrane is removed, the resulting AMF has a composition of 99.8% milk fat, 0.1% non-fat solids and 0.1% water (Rajah, 1991). AMF is expected to have consistent and predictable properties in the model system, compared to a cream system formulated with fresh cream.

The protein present in whipping creams made from fresh ingredients include both whey and casein. The protein selected for the model system was sodium caseinate as it is standardized and will have a predictable effect on the system. Sodium caseinate is produced from extracting casein precipitates from skim milk. Enzymes or acidic compounds are added to skim milk to produce casein curds that are separated from the whey. The curds are treated with sodium hydroxide and dried into a powder (Felix da Silva, 2018). When in an emulsion, sodium caseinate stabilized films are composed of a mixture of casein proteins which are stronger than β-casein and slightly weaker than κ-casein films (Fuller, 2015).

The aim of these experiments was to optimize the model system in regard to ingredients, formulation and preparation methods.

The objectives were to:

- Develop a method in preparing the model emulsions
- Investigate different fat and protein sources and how they impact the model system
- Optimize ingredient concentrations

5.2. Materials and Methods

5.2.1. Materials

Sodium caseinate (92.5 wt% protein, 0.8 wt% fat, 4.66 wt% moisture and 3.4 wt% ash) was purchased from Fonterra Co-Operative Group Ltd (Auckland, New Zealand). Beta Serum powder (30.8 wt% protein, 15.2 wt% fat (5.8 wt% phospholipids), 3.6 wt% moisture 47.2% lactose and 6.5 wt% ash) was purchased from The Tatua Co-Operative Dairy Company Ltd (Morrinsville, New Zealand). AMF (99.8 wt% fat) was provided by Synlait Milk Ltd (Christchurch, New Zealand). Food grade Polysorbate 80 (Tween80) was purchased from Invita (Auckland, New Zealand). Food grade LACTEM was purchased from Hawkins Watts (Auckland, New Zealand). Food grade HP60 Mono- and di-glycerides (MDG) was purchased from Hawkins Watts (Auckland, New Zealand). Canary Clarified Butter (Ghee) (Waikato, New Zealand) was purchased from a local food service supplier. RO water was used in all emulsions.

AMF was the preferred choice of fat source for all experiments, however procurement delays meant that an alternative fat source needed to be used to start experiments. Ghee was selected as the alternative fat source. No significant differences ($P > 0.05$) to the model system were observed when the fat source was changed from Ghee to AMF.

5.2.2. Selection of Homogeniser, Pressure and Passes

Two homogenisers were chosen to compare FGSD and stability results for stock emulsions across a range of pressures. Emulsions were composed of 42 wt% Ghee and 2 wt% Sodium Caseinate. Homogeniser 1 (APV Rannie, Copenhagen, Denmark), homogeniser 2: bench-top (Homolab 2, FBF Italia, Italy). Samples were processed through homogeniser 1 using a two-stage homogenisation and three passes. The total pressures applied were 50 bar (30/20), 100 bar (80/20), 150 bar (100/50) and 200 bar (150/50). Samples were processed through homogeniser 2 using single stage homogenisation at 50 bar, 100 bar, 150 bar and 200 bar with 1-3 passes and two stage homogenisation at 150/50 with 1-3 passes. The FGSD of each emulsion was measured according to Section 3.3.1. The FGSD and visual separation were used to determine the acceptability of the homogeniser and pressure combinations. Results were used to determine the optimal pressure and pass combination to move forward with experiments.

5.2.3. Optimising Protein Concentration

The target fat globule D_{43} particle size of 1.3-1.7µm in the emulsion is larger than other well studied model cream systems. It is the target fat globule size for this study as this closely represented the fat globule size of commercial UHT whipping creams. A review of literature found that a combination of D_{32} 0.4µm particle size and 2% sodium caseinate was a commonly used formulation (Fuller, 2015). The model system used in this study will have less interfacial surface area due to the larger fat globule particle size. Therefore, it is thought that a reduced amount of sodium caseinate is required to cover the surface of fat globules. Excess protein in the aqueous phase may cause unwanted flocculation, potentially influencing experimental results.

Four stock emulsions containing 42 wt% Ghee and sodium Caseinate (0.5 wt%, 1 wt%, 1.5 wt% and 2 wt%) were prepared according to Section 3.2.1, using 100 bar total pressure (80/20). The adsorbed protein content was measured according to Section 3.3.3 after 24 hours of storage at 4°C. The FGSD was measured according to Section 3.3.1 after 24 hours of storage at 4°C. Light microscopy was used according to Section 3.3.4, after 24 hours of storage at 4°C to identify instabilities. Emulsions were made and tested in duplicate.

5.2.4. Impact of Emulsion Preparation Method on the Model System

Three emulsion preparation methods were proposed for creating the model system. The first method involved creating a stock solution with a higher fat percentage (42 wt% fat) and diluting with an emulsifier solution to 36 wt% fat as described in Section 3.2.1. The second method involved creating a 36 wt% fat emulsion by adding the emulsifiers to either the fat or water phase depending on their hydrophobicity, prior to homogenisationas described in Section 3.2.2. The third method involved creating a 36 wt% fat emulsion by adding fat soluble emulsifiers into the fat before homogenisationand Tween 80 24 hours after homogenisationas described in Section 3.2.3. A sodium caseinate level of 1 wt% was selected across formulations.

A set of emulsifiers (0.2% LACTEM, 0.1% MDG, 0.1% Tween80) were added either before or after homogenisation, depending on the emulsion preparation method followed. The FGSD was measured immediately after homogenisationand after 6 days or storage at 4ºC, according to Section 3.3.1. Protein surface load was measured according to Section 3.3.3 after 24 hours of storage at 4ºC. The protein load was measured both before and after emulsifier addition to understand the degree of protein displacement taking place at the interface. Whipping properties were measured according to Section 3.4.1 and 3.4.2, after 7 days of storage at 4ºC. Emulsions were studied using light microscopy, according to Section 3.3.4 after 7 days of storage at 4ºC. Emulsions were made and tested in duplicate.

5.2.5. Influence of Beta-Serum Powder on the Model System

To bring the model system closer to an emulsion made with fresh cream, the addition of Beta Serum powder (BSP) was investigated. BSP is a concentrated source of milk phospholipids, extracted from milk fat globule membranes. Milk phospholipids provide functional benefits in food systems as they are effective emulsifiers, surfactants and foaming agents (Huang et al., 2020). The hypothesis was that by including BSP in the formulation, it would replace the milk fat globule membrane that is lost in the manufacturing process of AMF, bringing the model system closer to an emulsion made with fresh cream.

Three formulations were prepared according to Table 5 to investigate correlations between model emulsions prepared with AMF versus AMF and BSP versus fresh cream.

Previous protein surface load results from AMF emulsions without emulsifiers were used to determine the amount of protein adsorbed on the fat globule surface in the model system. Results showed that 33% of sodium caseinate added became adsorbed at the interface. This value was used as an expected amount of membrane protein that would potentially be present in a fresh cream system with a similar particle size. Therefore, 0.66% sodium caseinate and 0.33% BSP was used when preparing the emulsion.

The control and BSP samples were prepared according to Section 3.2.1, using 100 bar total pressure (80/20). The fresh cream sample was diluted to 36% fat and combined with emulsifiers. The particle size distribution was measured immediately after homogenisationand after 6 days of storage at 4ºC, according to Section 3.3.1. The adsorbed protein content was measured according to Section 3.3.3 after 24 hours of storage at 5ºC. Whipping properties were measured according to Section 3.4.1 and 3.4.2 after 7 days of storage at 4ºC. Emulsions were made and tested in duplicate.

Table 5: Formulations for beta serum powder experiments.

Control	**Beta Serum Powder**	**Fresh Cream**
36% AMF	36% AMF	36% fat (950g fresh
1% Sodium caseinate	0.66% Sodium caseinate	cream, 115g water)
0.2% LACTEM	0.33% BSP	0.2% LACTEM
0.1% MDG	0.2% LACTEM	0.1% MDG
0.1% T80	0.1% MDG	0.1% T80
	0.1% T80	

5.3. Results and Discussion

5.3.1. Selection of Homogeniser, Pressure and Passes

Two homogenisers were selected as potential pieces of equipment to use in for experimental work. Homogeniser 1 had a volume range between 1L-20L compared to homogeniser 2 which had a range of between 300ml-2L. It was expected that that both pieces of equipment could be used interchangeably throughout the project, depending on the batch size required for an experiment. The hypothesis was that emulsions made using the same pressure and passes would result in the same FGSD and stability for both homogenisers.

Figure 9 below shows the FGSD from the two homogenisers at 150/50 bar and three passes, Taken immediately after homogenisation. Figure 9 illustrated that the two homogenisers did not produce the same FGSD when the same pressure and number of passes were applied. Homogeniser 1 had a narrow peak between 0.314-2.24µm in comparison to homogeniser 2 that displayed a wide peak between 0.276 – 4.03µm.

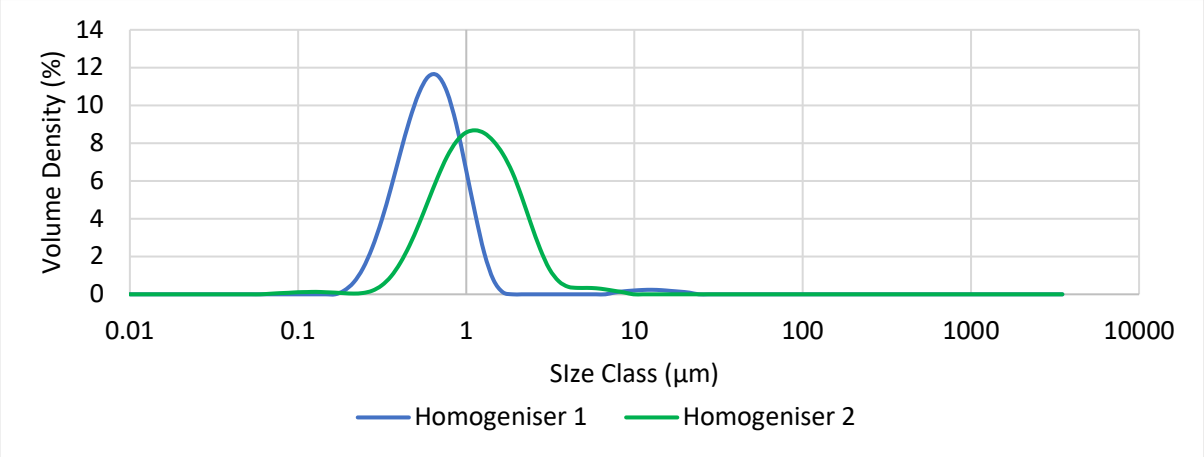


Figure 9: Comparison of FGSD from homogeniser 1 and 2 at 150/50 bar, 3 passes, immediately after homogenisation.

The FGSD results from homogeniser 2 (Figure 10) showed that emulsions were often produced with distributions ranging from 0.5 - 10µm. A consistent peak was only achieved using three passes, double peaks were observed when a single or double pass was used. All emulsions made in homogeniser 2 displayed visual separation after 24 hours of storage at 4ºC, regardless of the pressure and number of passes, demonstrating that emulsions produced from homogeniser 2 contained oversized particles.

The FGSD and overnight stability results concluded that homogeniser 1 should be selected to continue experiments as it provided more stable emulsion characteristics.

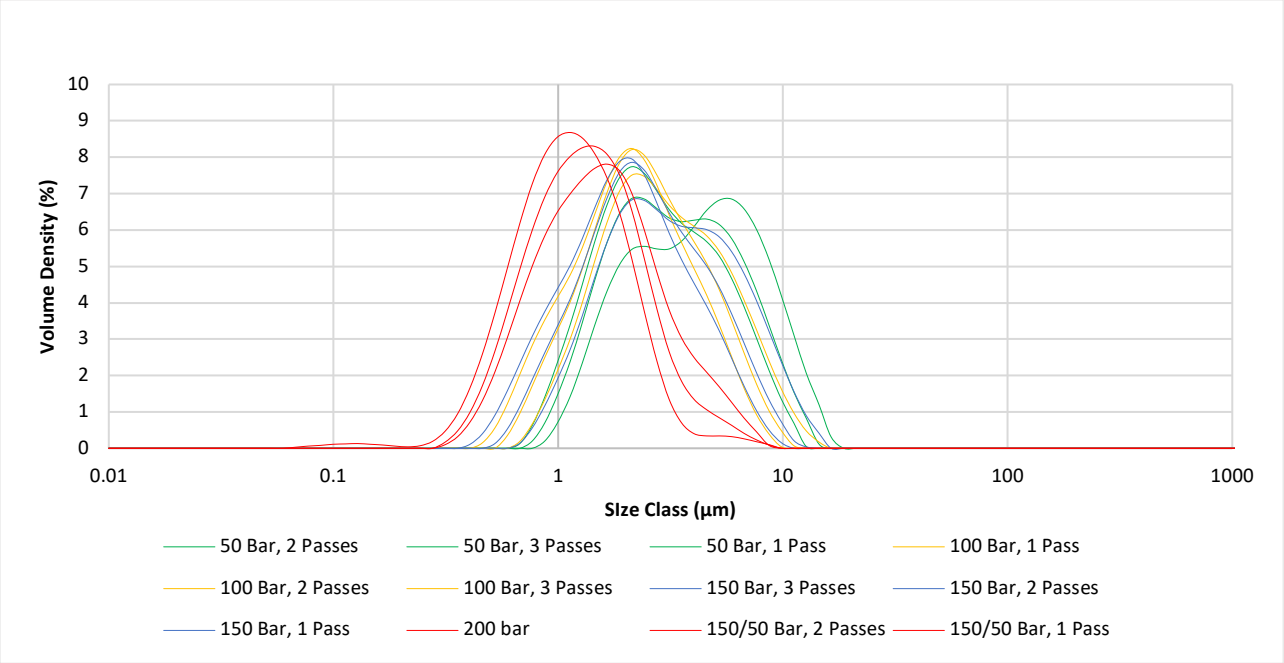


Figure 10: FGSD results from homogeniser 2, immediately after homogenisation.

The target FGSD for the model system was set between D_{43} values of 1.3-1.7µm. Figure 11 illustrates the FGSD produced across 4 different pressures using homogeniser 1. The D_{43} of the samples were 0.9, 1.1, 1.8 and 3µm. From these results, it was concluded that a pressure and pass combination of 100 Bar (80/20) and three passes should be used for future experiments.

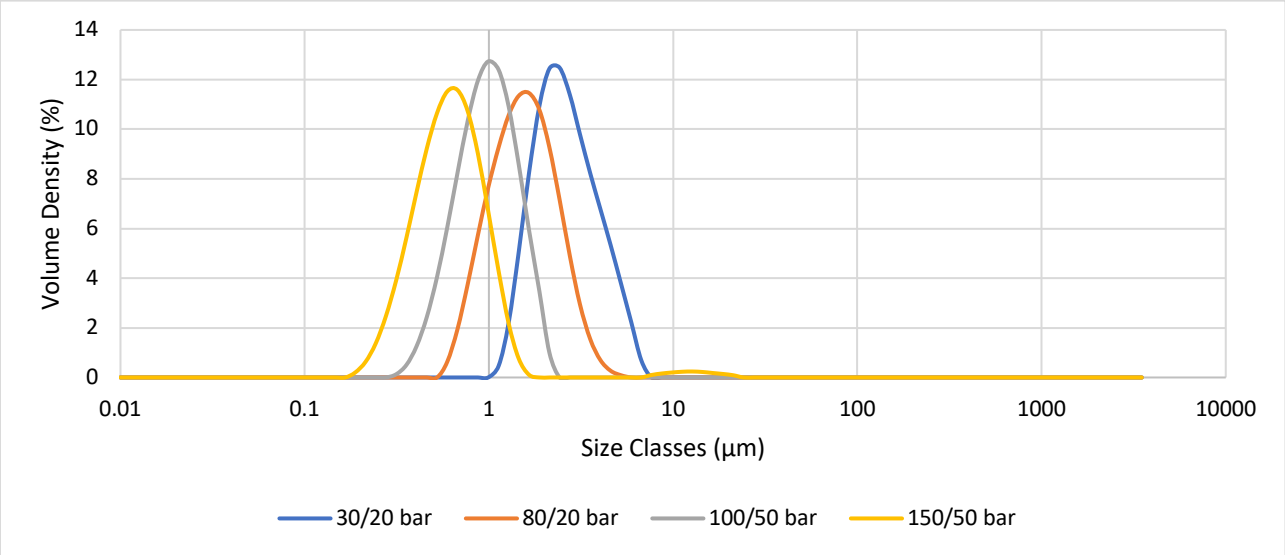


Figure 11: FGSD of emulsions produced on homogeniser 1, using a range of pressures and 3 passes.

5.3.2. Optimising Protein Concentration

The four formulations displayed varying degrees of stability and physical characteristics, dependent on protein concentration. Figure 42 (Appendix) illustrated the separation that occurred after 24 hours of storage at 4°C. There was a direct link between protein concentration and separation. Fat globule aggregation is based on interactions between adsorbed proteins at the droplet interface and non-adsorbed proteins in the aqueous phase (Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014). The sample with the lowest protein concentration had the least amount of visual separation across samples, the degree of separation increased with the increase in protein content suggesting protein concentration was responsible for the separation. Figure 12 showed that the 0.5% NaCas sample displayed a slightly smaller than expected FGSD, whereas the 2% NaCas sample displayed a slightly higher FGSD. This change in FGSD was likely due to the thickness of the protein layer at the oil-water interface as a result of protein concentration.

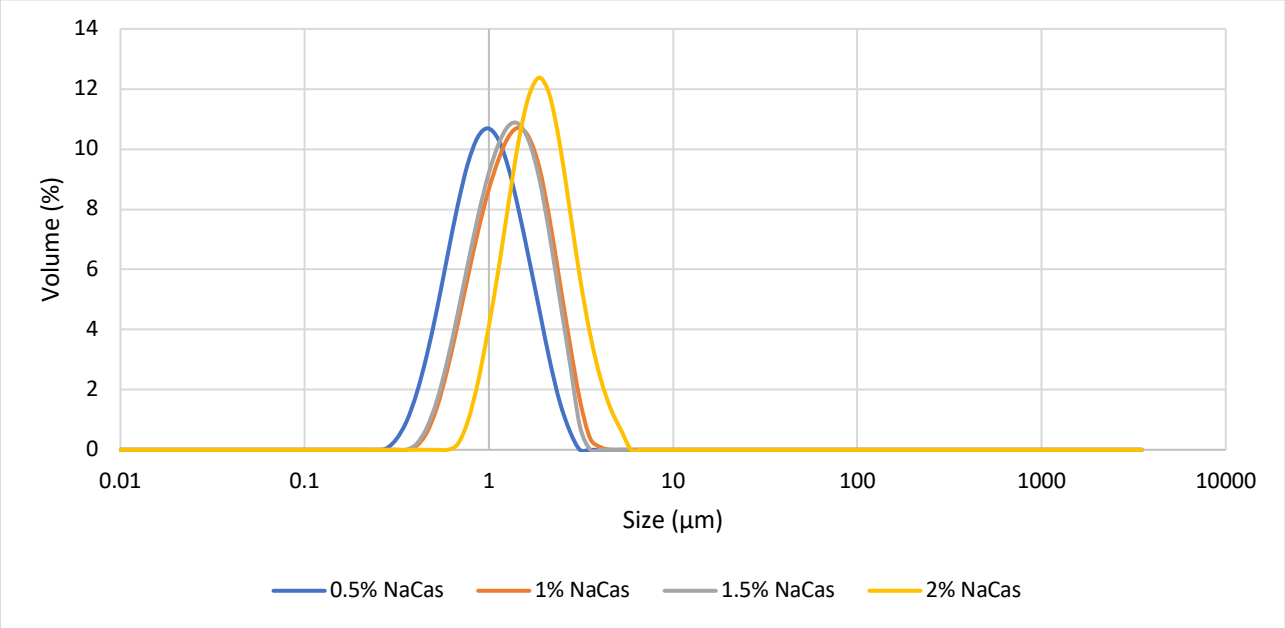


Figure 12: FGSD for samples prepared with varying levels of sodium caseinate, measured immediately after homogenisation.

Figure 13 showed there was a direct correlation between protein in the system and the adsorbed protein surface load. The surface load increased with the amount of protein in the system. It is important that the protein load does not get too high as increasing the protein load can prevent partial coalescence entirely (Pelan, Watts, Campbell, & Lips, 1997). Increased caseinate surface coverage of fat globules can result in an increased viscosity of an emulsion (Munk et al., 2014).

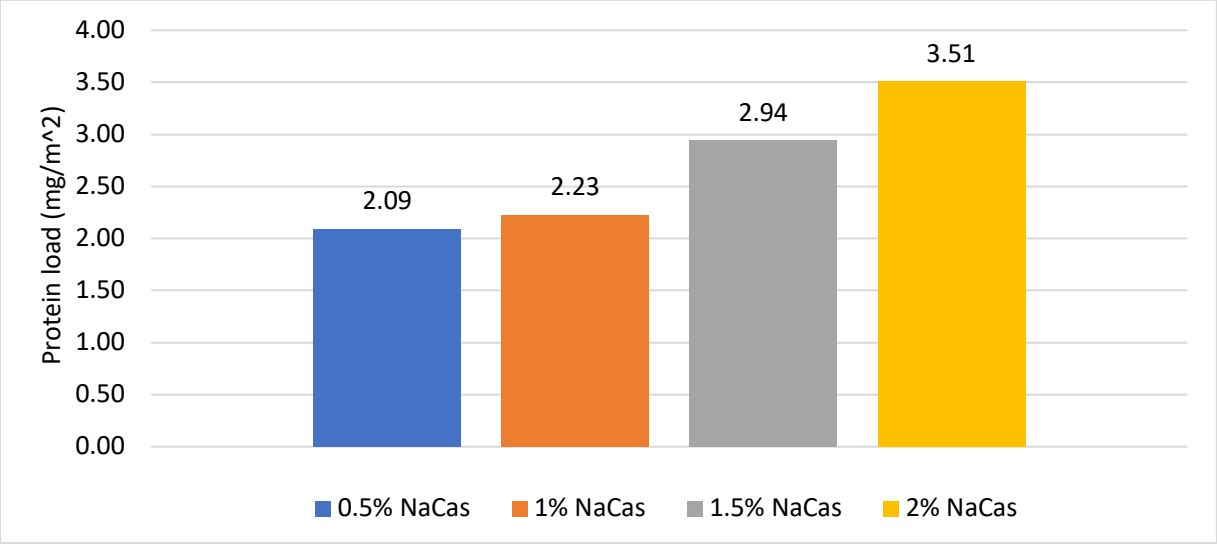


Figure 13: Adsorbed protein surface load results for samples prepared with varying levels of sodium caseinate.

Figure 14 of the light microscopy images of the four formulations illustrates the behaviour of fat globules in the emulsion. Samples containing 0.5%, 1.5% and 2% NaCas exhibited signs of flocculation, 1% NaCas formulation was the only sample that did not show signs of flocculation. Flocculation refers to the aggregation of droplets in an emulsion, the forces can be weak (reversable) or strong (difficult to reverse) (Dickinson, 2019). The light microscopy images and protein surface load results suggest there are two types of flocculation occurring that resulted in separation. The sample containing 0.5% NaCas most likely has bridging flocculation occurring while the 1.5% and 2% NaCas samples have depletion flocculation occurring.

Depletion flocculation is defined as the presence of excess polymer in the aqueous continuous phase (Dickinson, 2019). Non-adsorbed polymers promote droplet flocculation through an osmotic pressure difference (Chanamai & McClements, 2001). Bridging flocculation refers to the direct bridging of polymers between different droplet surfaces, or the crosslinking of polymers through physical and chemical bonds (Dickinson, 2019). Clusters held together by protein bridges can only be disrupted by strong shear forces, whereas flocs of droplets caused by depletion can be redispersed by gentle stirring (Dickinson, 2019).

It is important that the protein concentration in the system does not create instabilities as methods will be used to evaluate the impact of other ingredients on emulsion destabilisation. The combine results of the FGSD, protein surface load and microscopy suggest the model system should be prepared with 1% NaCas. This concentration of protein in the system does not cause any significant flocculation that could potentially impact future results.

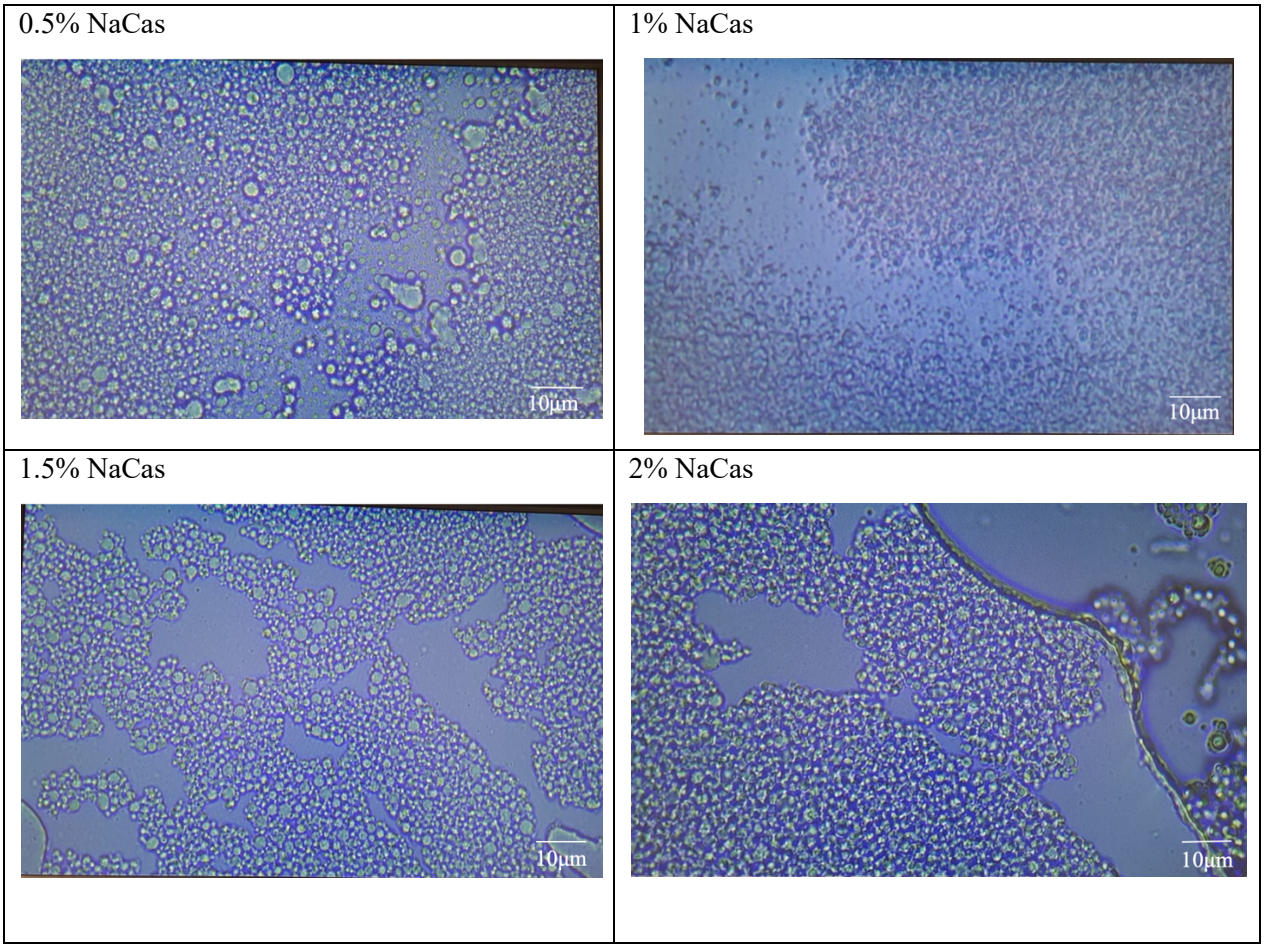


Figure 14: Light microscopy results at x100 magnification for four emulsions with varying protein levels, showing signs of phase separation through bridging flocculation (0.5% NaCas) and depletion flocculation (1.5%, 2% NaCas).

5.3.3. Impact of Emulsion Preparation Method on the Model System

Figure 15 shows the FGSD of emulsions prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation) and After (all emulsifiers added after homogenisation). All samples were homogenised at 100 (80/20) Bar but showed variation in size distribution. The smallest FGSD was observed in emulsions that had emulsifiers added before homogenisation, while a mixture of emulsifier addition had the largest FGSD. This was expected as lipophilic emulsifiers are known to reduce FGSD during homogenisation. MDG reduces the particle size as it decreases the interfacial tension more than milk proteins, resulting in an effective homogenisation process. A narrow FGSD and a well-defined surface area is produced (Moonen & Bas, 2014).

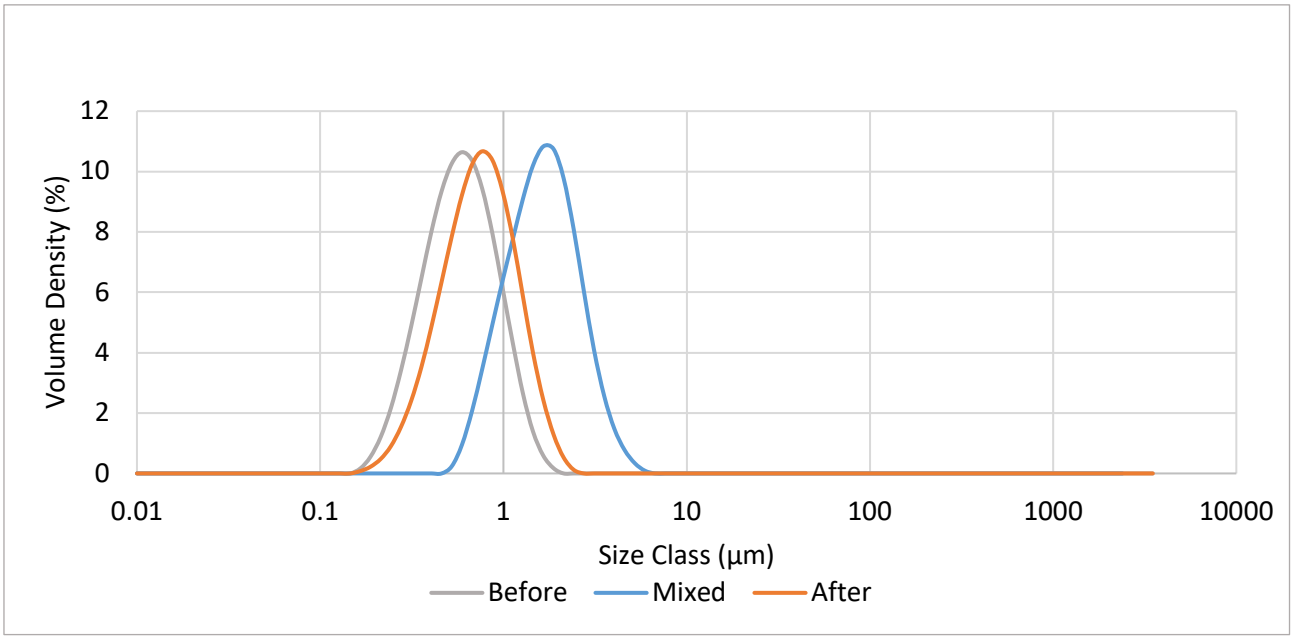


Figure 15: FGSD of emulsion immediately after homogenisation, prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation.

Figure 16 illustrated how the emulsion preparation method impacted the particle size after one week of storage at 4°C. Samples prepared with emulsifiers added after homogenisation showed the most significant change in FGSD. Numerous peaks were observed between 4 - 310µm, indicating signs of instabilities such as partial coalescence.

The FGSD was measured using SDS, a dissociating agent that solubilizes aggregated protein and disperses flocs via competitive displacement (Allen, Murray, & Dickinson, 2008b). Indicating that the formation of peaks above 10µm was likely due to partial coalescence. Peaks below the primary population indicated there may be unabsorbed emulsifiers in the serum phase interacting and potentially forming micelles. The FGSD results suggested that adding emulsifiers after homogenisation was not a viable option due to the instabilities it caused.

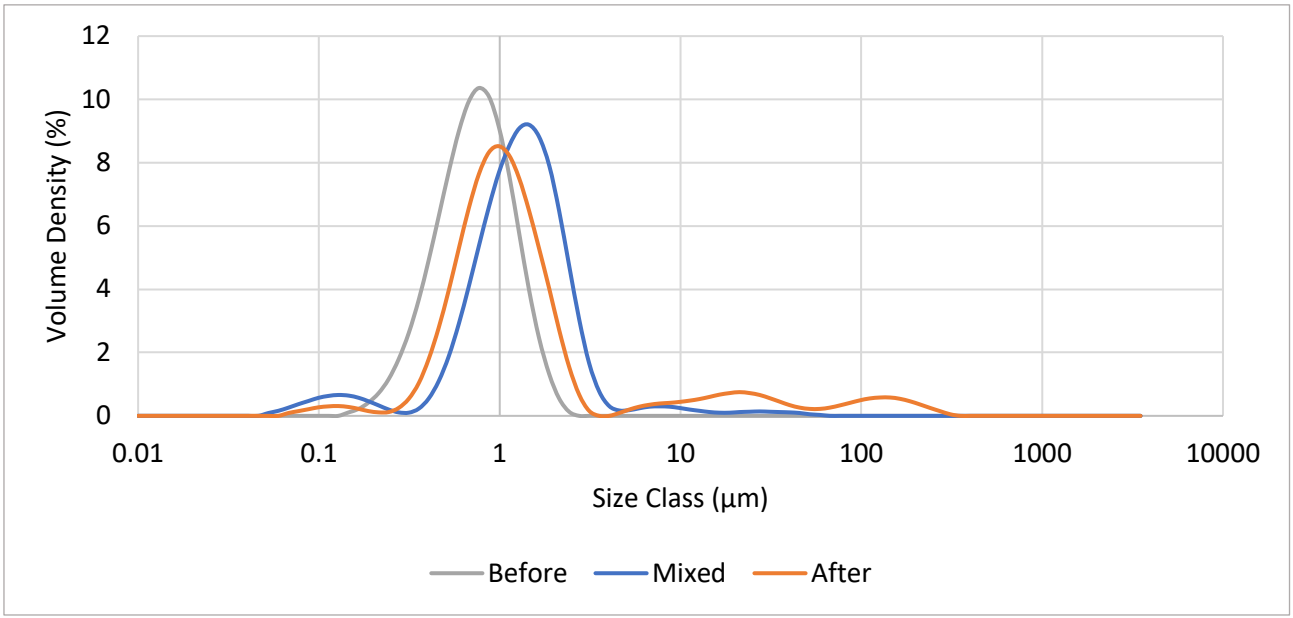


Figure 16: FGSD of emulsion 7 days after homogenisation, prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation.

The light microscopy results illustrated that a smaller particle size and homogeneous emulsion is produced when emulsifiers are added before homogenisation (Sample A). This is also supported by the stable FGSD results observed in Figure 17. Adding emulsifiers before homogenisation typically results in in a more effective homogenisation process due to decreased surface tension (Moonen & Bas, 2014).

When the emulsifiers were added after homogenisation (Sample B), irregular fat globules were observed. This was likely due to the emulsifiers competing to displace protein at the interface, leading to an un-homogeneous interfacial layer. Excess emulsifiers in the aqueous phase can lead to interactions between emulsifiers.

In Sample C, the fat-soluble emulsifiers were added before homogenisation and Tween80 was added after. This led to the similar reduced particle size as observed in sample A and the small molecule surfactant interactions as seen in sample B. When the Tween80 was not included before homogenisation, it allowed a protein layer to form around the fat globule which subsequently can become displaced. The Tween80 was more effective at displacing protein at the interface when added after homogenisation as indicated in the surface load results (Figure 18).

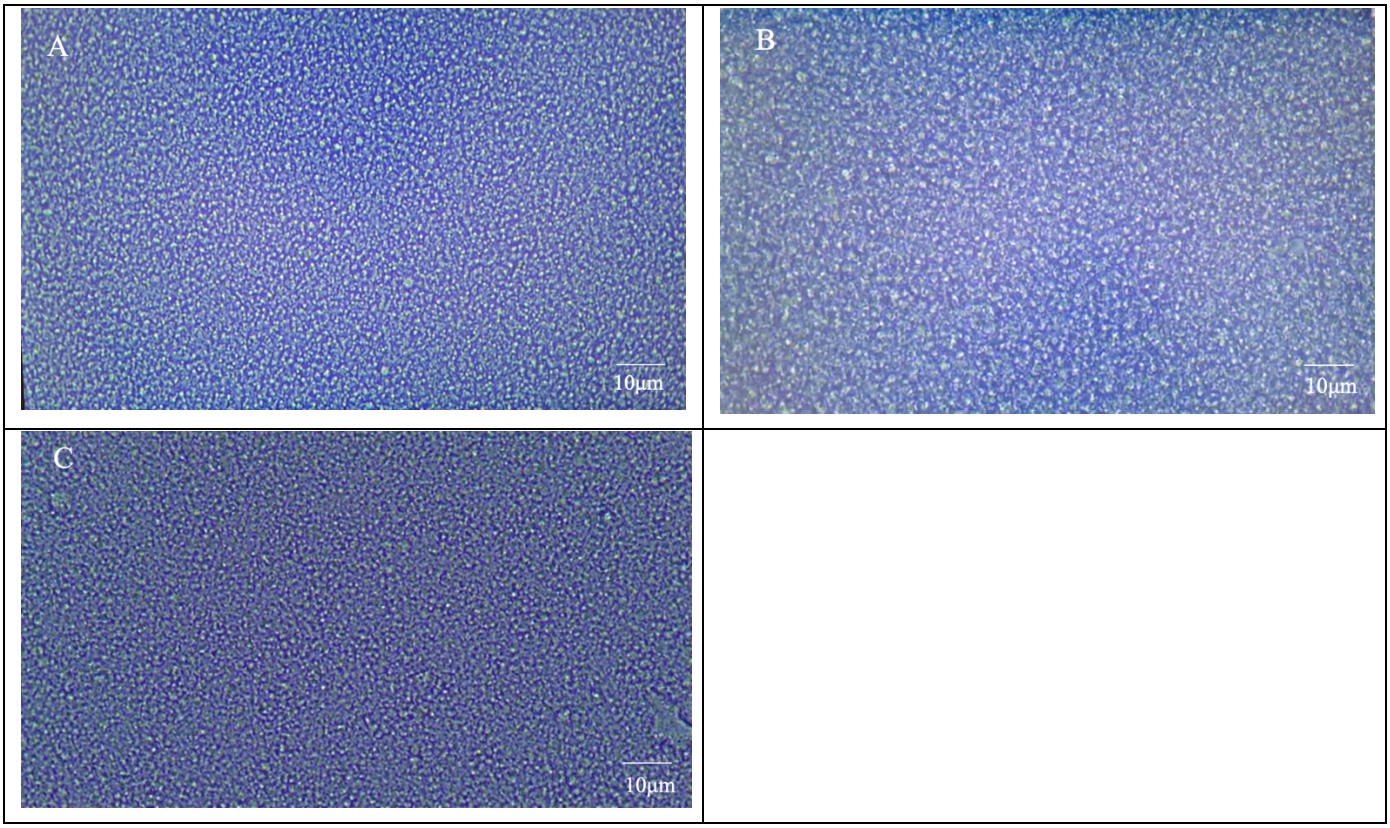


Figure 17: Light microscopy results at 100x magnification for emulsions with emulsifiers before homogenisation (A), emulsifiers added after homogenisation (B) and a mixture: fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation (C).

The adsorbed protein load results showed that there was an increased amount of protein in the serum phase of emulsions when emulsifiers were added, suggesting the emulsifiers do adsorb at the interface by competitive displacing proteins. Competitive adsorption occurs when there are multiple surface-active components in a system. Emulsifiers compete at the interface, the most hydrophobic emulsifier typically has the strongest affinity for the interface (Hartel & Hasenhuettl, 2019).

The mixed preparation method had the highest degree of protein displacement. Tween 80 was able to displace protein after a mixed layer of caseinate and lipophilic emulsifiers was formed initially. Oil droplets stabilized by emulsifiers are less stable than protein stabilized emulsions (Wilde, Mackie, Husband, Gunning, & Morris, 2004). This can lead to improved whipping properties when destabilization is favored.

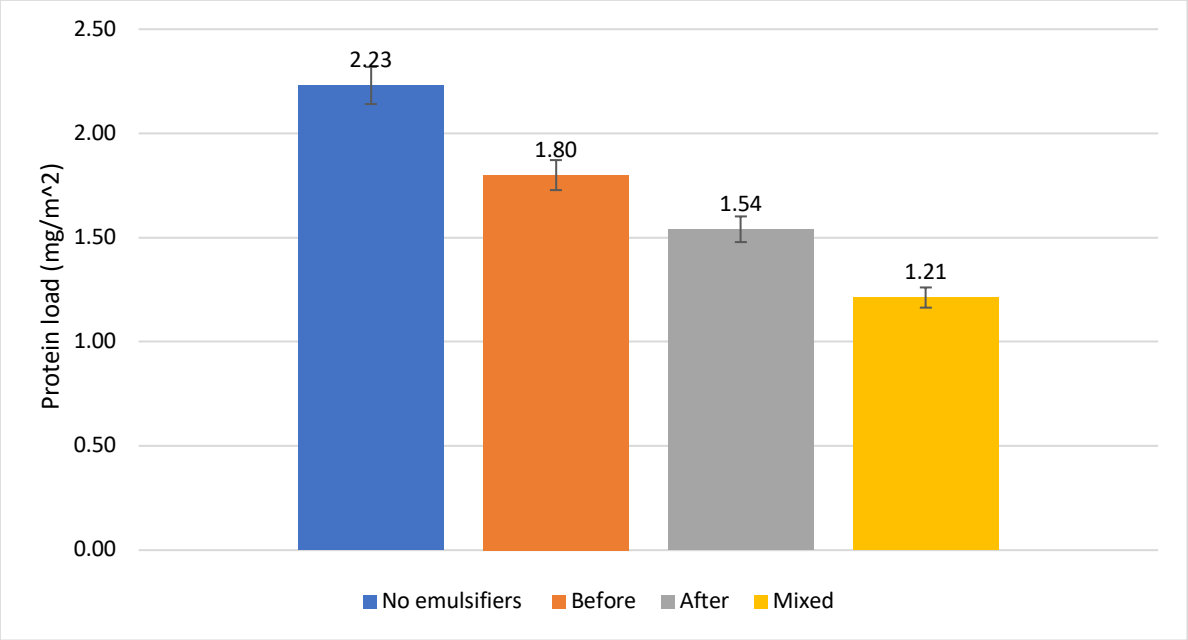


Figure 18: Adsorbed protein surface load result for emulsion prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation) and After (all emulsifiers homogenisation).

Results suggest there was no significant difference in whipping time and piped rosettes across samples ($P > 0.05$) (Figure 19 and Figure 20). Adding the emulsifiers after homogenisation resulted in higher overrun compared to other preparation methods. This may be due to a higher concentration of emulsifiers in the aqueous phase compared to the oil/water interface, leading to increased emulsifier adsorption at the air bubble interface.

Before	After	Mixed
		

Figure 19: Whipped rosettes for emulsion prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation) and After (all emulsifiers added after homogenisation).

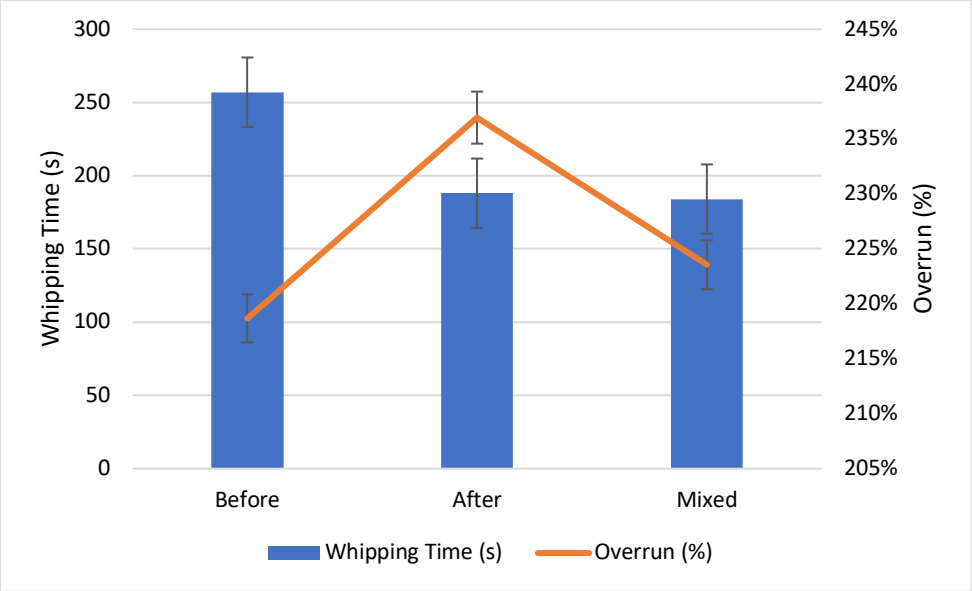


Figure 20: Whipping time (s) and overrun (%) result for emulsion prepared in three ways: Before (all emulsifiers added before homogenisation), Mixed (fat soluble emulsifiers added before homogenisation, Tween80 added after homogenisation) and After (all emulsifiers added after homogenisation).

Results from the FGSD, light microscopy, protein surface load and whipping properties suggested that the favored preparation method was adding fat soluble emulsifiers before homogenisation (LACTEM and MDG) add Tween80 after homogenisation. This preparation method was selected as it provided a stable and homogeneous emulsion over storage and sufficient whipping properties.

5.3.4. Influence of Beta-Serum Powder on the Model System

Figure 21 below illustrates the FGSD for emulsions prepare with NaCas, BSP and fresh cream. There was no significant difference FGSD between the NaCas and BSP samples, however the fresh cream sample had a considerably larger FGSD (D_{43} of 4.2µm compared to 0.7µm). This was due to the fresh cream sample being unhomogenised. It was expected that the fresh cream sample would have a shorter whipping time due to the larger fat globule size which may decrease stability.

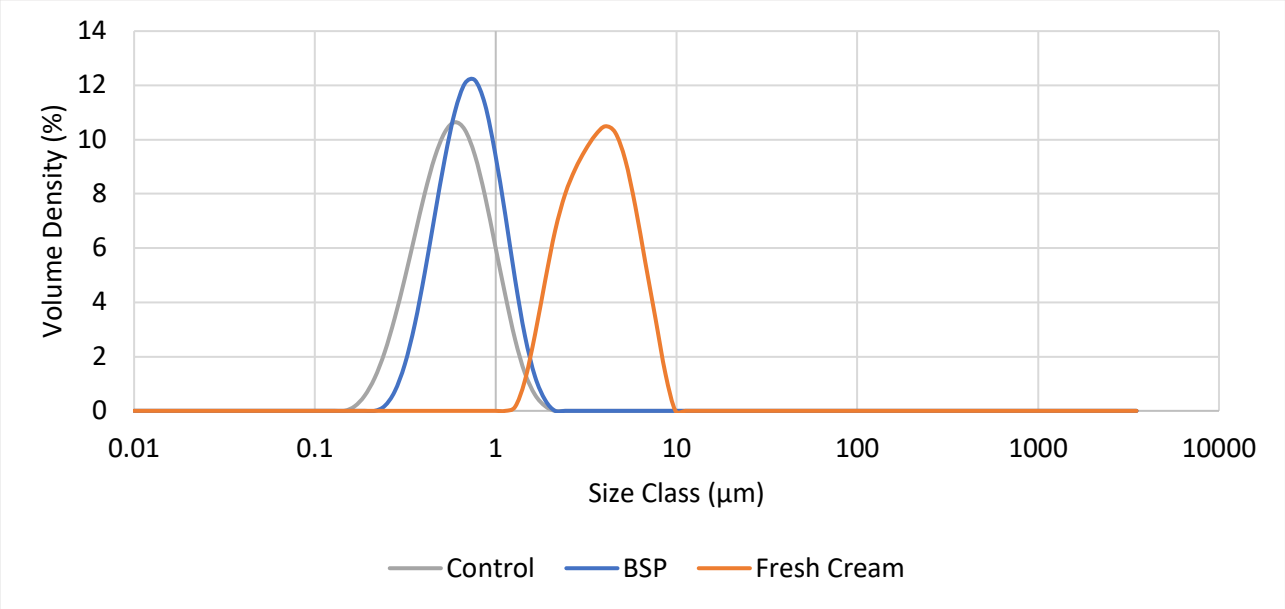


Figure 21: FGSD for samples prepared with NaCas (Control), BSP + NaCas and unhomogenised fresh cream.

Protein surface load results (Figure 22) showed a higher amount of adsorbed protein at the interface when BSP was included in the system. Similar surface load results were observed by Phan, Le, Van der Meeren, and Dewettinck (2014) who suggest that emulsions prepared with buttermilk powder had a higher surface load than commercial emulsions made with whey protein hydrolysate. Samples with low protein surface loads have higher free protein in the serum which reduces partial coalescence, leading to longer whipping times (van Lent, Le, Vanlerberghe, & Van der Meeren, 2008)

Although there was an increase in protein surface load between samples, there are limitations with the method that need to be considered when drawing conclusions. The method used to determine the protein content in the serum phase was developed using NaCas calibrations. The Milkoscan data set was not optimised to measure protein content in serums that contained other sources of protein such as those in BSP. This is expected to create a higher level of uncertainty towards the accuracy of measurements from BSP samples.

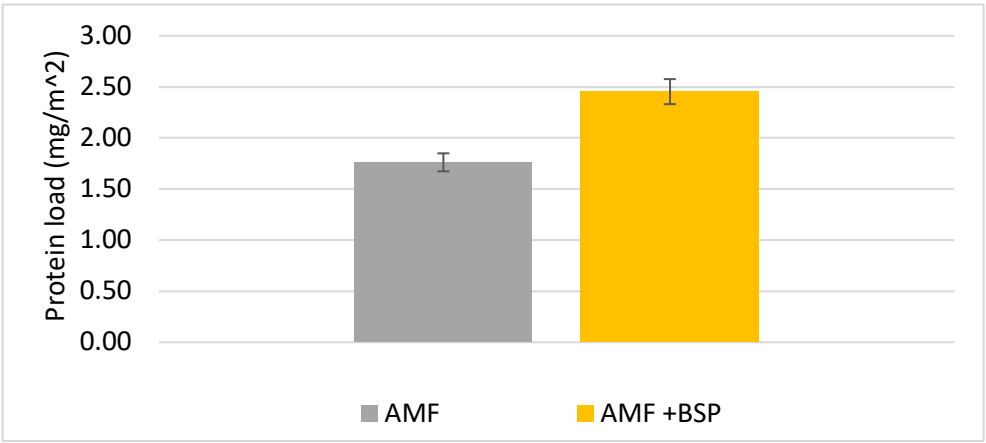


Figure 22: Comparison of adsorbed protein content for emulsions prepared with NaCas (Control) and BSP + NaCas.

Figure 23 shows that both whipping time and overrun were significantly improved when BSP was added to the system by shortening whipping time and increaseing overrun. Having the BSP in the formulation provided whipping property results closer to a sample prepared with fresh cream. BSP improves emulsification by limiting protein adsorption at the interface during homogenisation. This produces increased the non-adsorbed protein in the aqueous phase post-homogenisation, which can improve whipping properties (Hartel & Hasenhuettl, 2019).

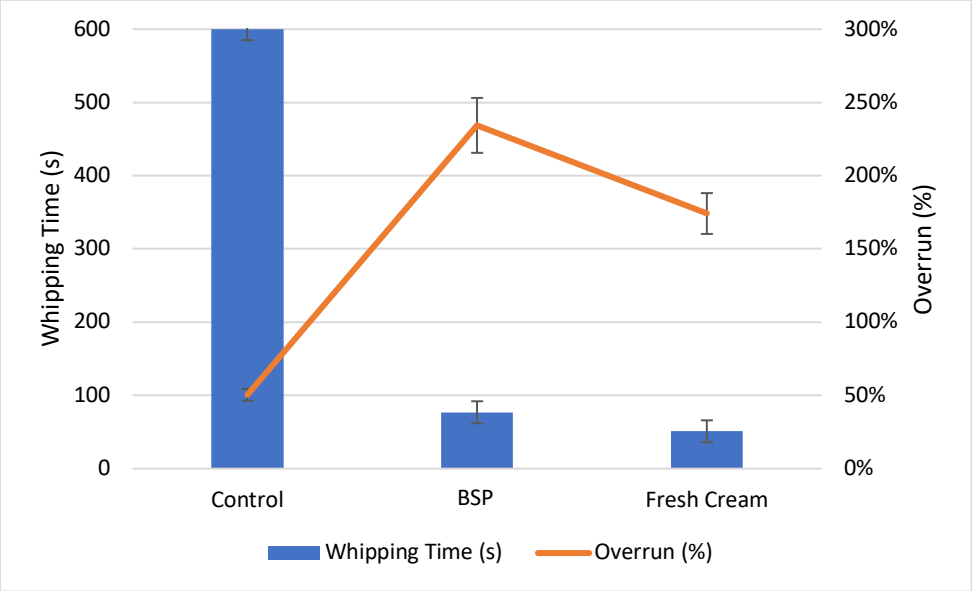


Figure 23: Whipping time (s) and Overrun (%) measurements for emulsions prepared with NaCas (Control) and BSP + NaCas.

It was concluded that adding BSP to the model significantly improved the functionality in terms of whipping time, overrun due to the emulsifying effects of the fat globule membrane components. The whipping properties of samples prepared with BSP were similar to the fresh cream sample, indicating that the addition of BSP did in fact bring the model system closer to a complete cream system. However, BSP introduced numerous phospholipids and membrane components, making the model too complex to identify specific mechanisms causing changes in functionality. It was decided that BSP would not be included in the final model system but offered as a recommendation for future work as it provided noteworthy benefits to whipping properties.

5.3. Conclusion

The following conditions were selected as the optimal processing, preparation, and formulation for the model system. These conditions produced a stable and reproducible emulsion that was able to destabilize under shear. A clear understanding of how the model system performs across storage and testing methods was established, allowing specific changes to the formulation to be studied in confidence to help identify responsible mechanisms. The optimal conditions included:

- Processing on homogeniser 1 (APV Rannie, Copenhagen, Denmark).
- Homogenisation pressure of 100 (80/20) Bar to produce a D_{43} between 1.3-1.7μm.
- Protein level of 1 wt% sodium caseinate and a fat level of 36 wt% AMF.
- Emulsion preparation method 3– fat soluble emulsifiers before homogenisation in the fat phase and tween80 24 hours after homogenisation.

Emulsion preparation method 3 was selected as it provided a stable and homogeneous emulsion during storage and displayed the ability to destabilize during whipping. This method produced partially protein-stabilized fat globules, the protein at the oil-water interface could be effectively displaced by Tween80 when added after homogenisation. Beta serum powder was concluded to make the model system too complex to identify mechanisms but provided an interesting opportunity for future study due to the significant improvement in whipping properties. No significant differences to the model system we observed when the fat source was changed from Ghee to AMF.

Chapter 6: Influence of Emulsifiers on Shear-induced Destabilization and Corresponding Aeration Properties

6.1. Introduction

UHT whipping creams are characteristically formulated with a blend of emulsifiers, selected to optimize stability and functionality. Emulsifiers are amphiphilic molecules that possess both hydrophobic and hydrophilic fragments. The structure of emulsifiers allows them to arrange at water-oil interfaces and become solubilized in both phases. Emulsifiers influence the interaction between fat globules by complexing and competitive adsorbing with proteins at the interface (Bos, Nylander, Arnebrant, & Clark, 1997). Emulsifiers in a system can alter the mechanical properties of the interfacial film by arranging in either a liquid, liquid-crystalline or crystalline state (Krog, 1997). Fat crystallization near the interface may also be altered by emulsifiers as they can act as nucleation sites for fat crystals via interfacial heterogeneous nucleation (Fuller, 2015).

In this chapter, the effect of LACTEM, MDG and Tween80 on the destabilization and crystallization behavior of partially crystalline oil-in-water emulsions was examined. LACTEM is a lactic ester of mono and diglycerides, containing a mixture of esters formed by lactic acid and fatty acids of edible food fats with glycerol (Gaupp & Adams, 2014). LACTEM is a lipophilic emulsifier and is added to whipping creams to improve aeration, foam stability, texture and volume (Gaupp & Adams, 2014). Mono and diglycerides (MDG) are lipophilic emulsifiers and are primarily responsible for the partial destabilization of fat in an emulsion during whipping (Moonen & Bas, 2014). MDG's are known to reduce the oil/water interface tension more that milk proteins alone, creating a more effective homogenisation process by producing a narrower FGSD (Moonen & Bas, 2014). Tween80 is a non-ionic hydrophilic surfactant, known to be highly effective at displacing proteins at the oil/water interface (Xiao, 2020). Tween80 destabilizes emulsions by either displacing protein from the oil/water interface or modifying the fat crystal properties (Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt, 2008). Protein displacement at the oil/water interface reduces surface tension, resulting in the membrane becoming more suspectable to destabilization (Goff, 1997). Emulsifier type and levels are selected to either inhibit or promote destabilization in a cream system.

The aim of this study was to determine how LACTEM, MDG and Tween 80 impact the destabilization and crystallization of milk fat in the model system.

The objectives were to:

- Determine how individual emulsifiers affect milk fat crystallization and polymorphs.
- Determine how individual emulsifiers impact the destabilization of the model system by investigating stability and whipping properties.

6.2. Materials and Methods

6.2.1. Materials

Sodium caseinate (92.5 wt% protein, 0.8 wt% fat, 4.66 wt% moisture and 3.4 wt% ash) was purchased from Fonterra Co-Operative Group Ltd (Auckland, New Zealand). AMF (99.8 wt% fat) was provided by Synlait Milk Ltd (Christchurch, New Zealand). Food grade Polysorbate 80 (Tween80) was purchased from Invita (Auckland, New Zealand). Food grade LACTEM was purchased from Hawkins Watts (Auckland, New Zealand). Food grade HP60 Mono- and di-glycerides (MDG)was purchased from Hawkins Watts (Auckland, New Zealand). RO water was used in all emulsions.

6.2.2. Methods

Four formulations containing either LACTEM, MDG, Tween80 or no emulsifier (control) were prepared according to Section 3.2.3. Samples were homogenised at 65°C using a two-stage homogeniser at 100 Bar total (80/20) and three passes. Each emulsion contained 0.3 wt% emulsifier (if added), 1 wt% sodium caseinate and 36 wt% AMF. The FGSD was measured immediately after homogenisationand after 6 days of storage at 4°C, according to Section 3.3.1. The composition was measured according to Section 3.3.2 immediately after homogenisation. Protein surface load was measured according to Section 3.3.3, after 24 hours of storage at 4°C. Whipping properties (whipping time and overrun) were measured according to Section 3.4.1 and 3.4.2 after 7 days of storage at 4°C. Whipped foams were piped into rosettes and images taken to assess performance. Foam structure was studied using CSLM, as described in Section 3.4.5, immediately after whipping. Crystallization behavior was studied using DSC according to Section 3.3.5 after 6 days of storage at 4°C.

Emulsions were prepared in duplicate across several independent trials in a randomized order. Statistical analysis was performed using a one-way analysis of variance (ANOVA) to determine statistically significant differences between samples. The analysis was carried out using Minitab 19 with a probability level for statistical significance of $P < 0.05$.

6.3. Results

6.3.1. Emulsion Characteristics

Emulsion characteristics were determined by measuring the particle size distribution (PSD), fat globule size distribution (FGSD), composition, and protein surface load. Findings were used to determine any significant difference between formulations caused by the emulsifiers. There were no significant differences ($P > 0.05$) in composition (Table 7, Appendix), PSD and FGSD across samples, indicating successful replication of emulsions during preparation. Figure 24 illustrates the FGSD measured immediately after homogenisation. The average D_{43} of samples were 1.5µm, all within the target values of 1.3-1.7µm. It was expected that the presence of an emulsifier would result in a narrower peak due to the decrease in surface tension (Moonen & Bas, 2014). Results suggest emulsifier addition did not affect the PSD and FGSD. The MDG samples exhibited a small peak around 0.13µm, indicating potential un-dissolved or non-adsorbed emulsifiers in the system. This could be due to insufficient heating of the fat phase before homogenisationor excess of surfactants resulting in micelles/bilayers. This may have impacted results and was taken into consideration when discussing conclusions. There was no difference in FGSD after one week of storage at 4°C (Figure 43, Appendix), indicating no emulsion instabilities such as partial coalescence or aggregation occurring.

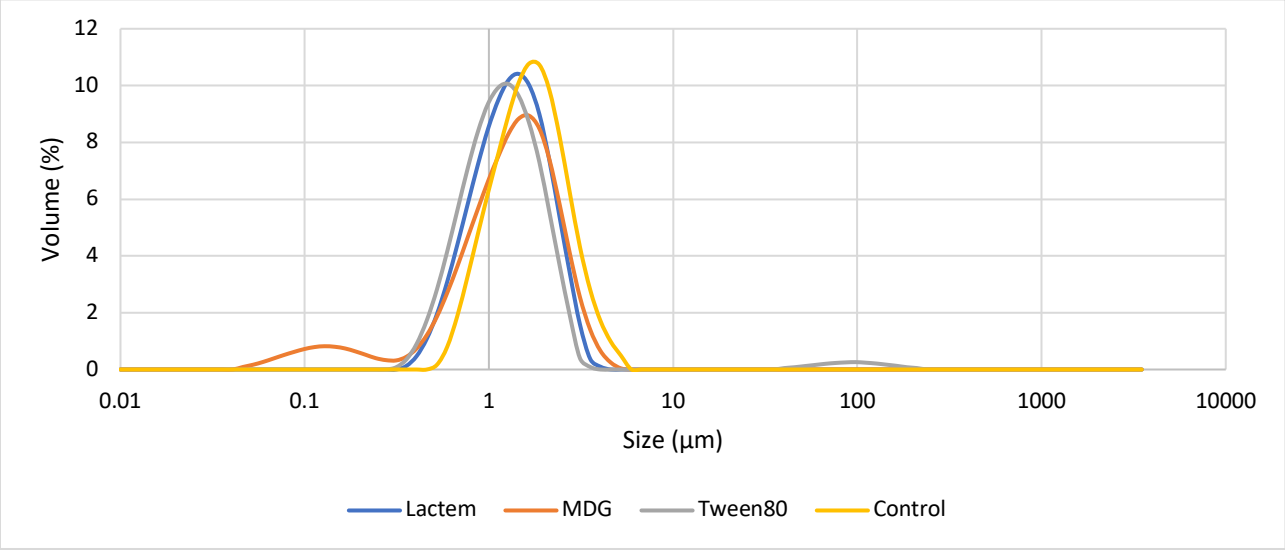


Figure 24: FGSD for emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, measured immediately after homogenisation.

The protein surface load results (Figure 25) illustrated that all three of the emulsifiers reduced the protein load, indicating the displacement of protein from the fat globule surface and the adsorption of surfactants at the interface. There were no significant differences ($P > 0.05$) in the degree of protein desorption between LACTEM and Tween80. MDG resulted in a lower protein surface load than Tween80, indicating that MDG was more effective at displacing proteins from the oil/water interface than Tween80.

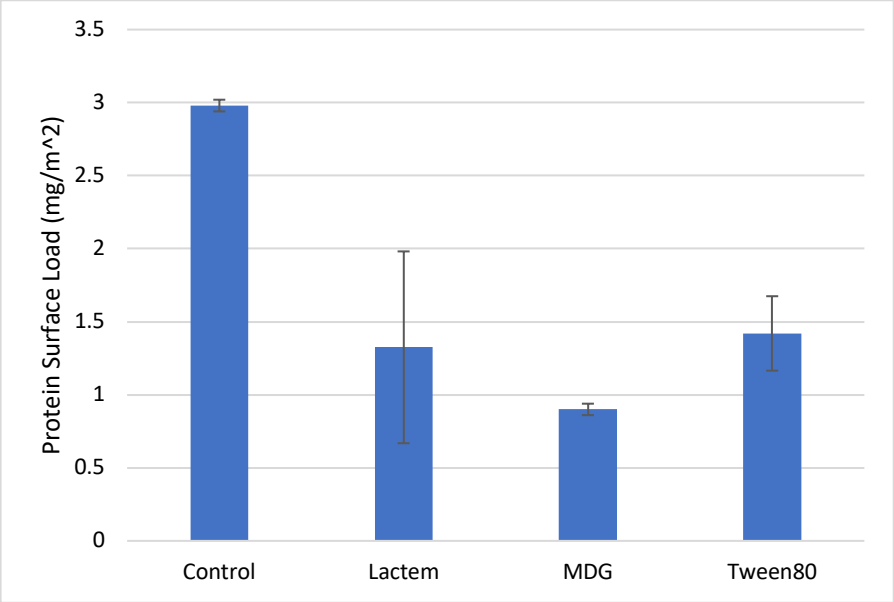


Figure 25: Protein surface load results for emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, measured 24 hours after homogenisation.

6.3.2. Milk Fat Crystallization Characteristics

The milk fat crystallization characteristics of emulsions were measured using the stop and return DSC method (Figure 44 - Figure 47, Appendix). Melting peaks were integrated to produce a melting heat as a function of time graph, Figure 26. Results suggest differences in the initial formation of α-crystals across samples. The amount of initial α-crystals are indicated by the first step, occurring at the 2minute hold point. Tween80 resulted in the highest quantity of initial α-crystals, followed by LACTEM, control and MDG. MDG resulted in the lowest quantity of initial α-crystals, potentially indicating crystallization directly into β'-crystals.

LACTEM primarily stays in the α-crystal state and do not show polymorphous tendencies, giving it the ability to stabilize the α-crystal modification of triglycerides in the system (Gaupp & Adams, 2014). The α-tending properties of LACTEM may improve whippability and overrun (Gaupp & Adams, 2014). α-tending emulsifiers strengthen foam structure as they promote agglomeration of fat globules (Gaupp & Adams, 2014).

MDG may have resulted in more fat crystals forming directly into the β' state. MDG can induce heterogeneous nucleation and accelerate crystal polymorph changes from α to β' to β, leading to less protruding surface fat crystals and reduced partial coalescence (Euston & Goff, 2019).The addition of MDG's can result in a stable crystalline structure by initiating robust fat crystallization (Moonen & Bas, 2014).

Tween80 does not undergo temperature dependent phase transitions like LACTEM and MDG (Gülseren & Coupland, 2008). Tween80 can act as a nucleation site for fat crystals through heterogeneous nucleation and this may enhance parallel orientation of fat crystal lamella at the oil-water interface (Arima, Ueno, Ogawa, & Sato, 2009). Fuller (2015) found that Tween80 did not influence the crystallization rate or crystallization onset of the milk fat.

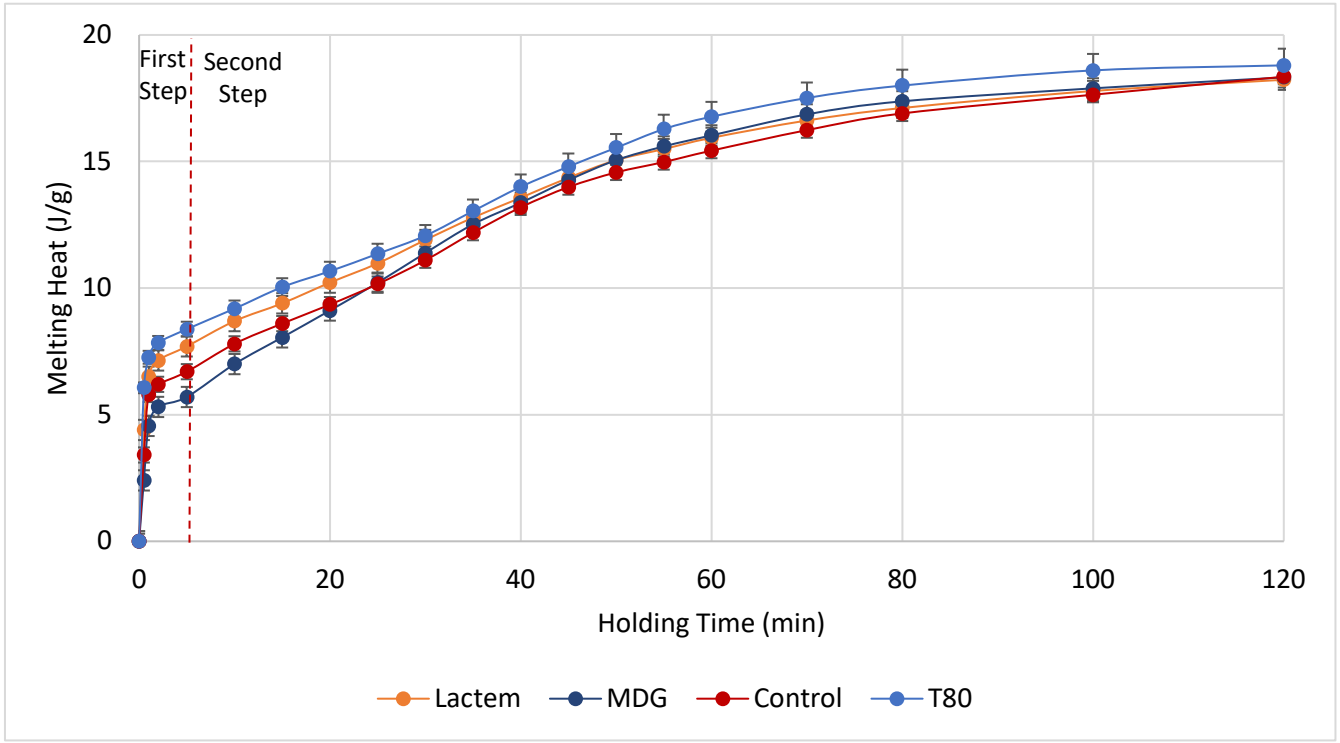


Figure 26: Melting heat as a function of time for isothermal crystallization at 5℃ of emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, measured after 6 days of storage at 4℃.

6.3.3. Whipping Properties

The four formulations had significant differences in whipping properties, illustrated in Figure 27. LACTEM and Tween80 resulted in a faster whipping time compared to the control, whereas MDG resulted in an increased whipping time as the sample did not reach a firm peak within the 10minute limit. LACTEM was the only emulsifier that resulted in increased overrun compared to the control ($P < 0.05$). Figure 28 illustrates the piped rosettes of the whipped emulsions immediately after whipping. The control formulation had rough edges and a dense foam. LACTEM resulted in smoother edges and a light, fluffy foam. MDG resulted in an emulsion that did not reach a firm peak, the piped foam was slumped and showed no foam structure, consistent with a stabilized emulsion that could not partially coalesce. Tween80 resulted in a minimal air incorporated and showed smoother edges.

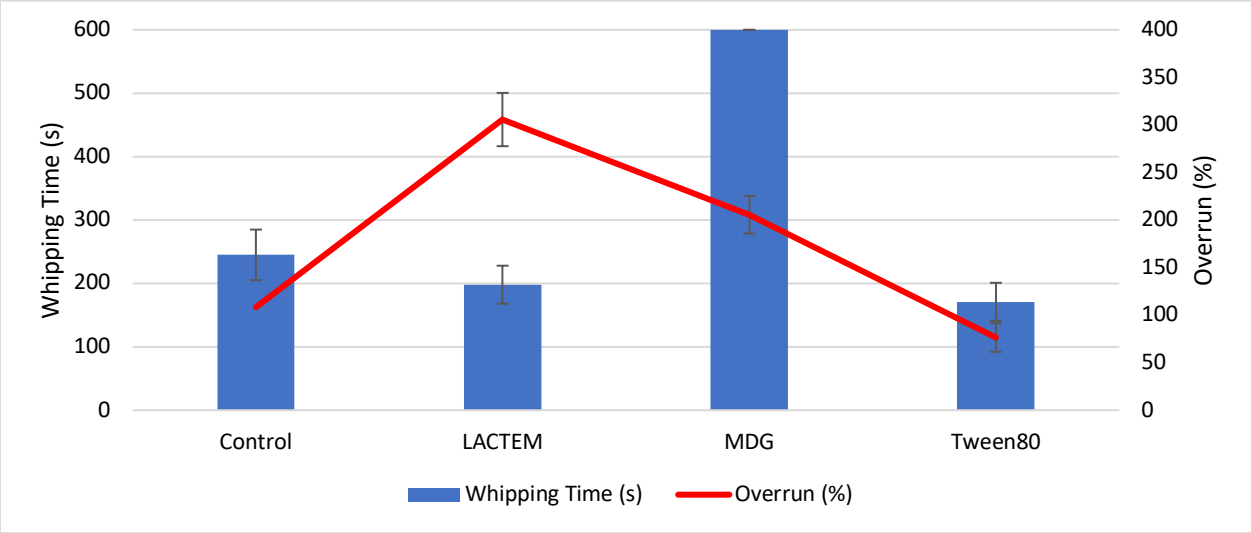


Figure 27: Whipping properties (whipping time and overrun) for emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, measured after 7 days of storage at 4 ℃.

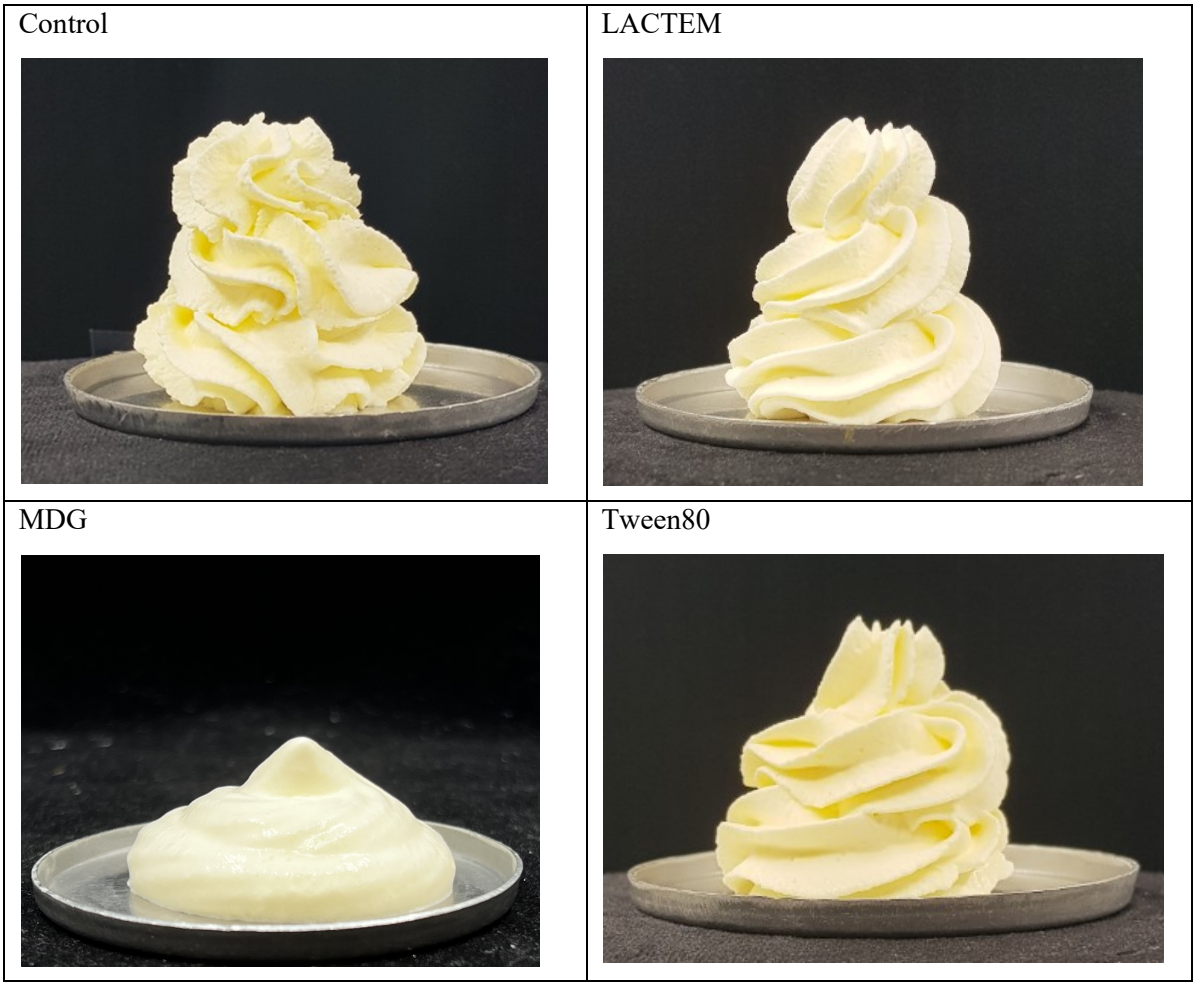


Figure 28: Piped rosettes for emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, photographed immediately after whipping.

6.3.4. Confocal Scanning Light microscopy

CLSM images (Figure 29 and Figure 30) of the whipped emulsions showed how the foam structures varied due to emulsifiers addition. Red and green signals represent fat and protein phases, respectively. The black signals represent air bubbles. The control formulation (no emulsifiers) had irregular shaped air bubble, stabilized by a thick layer of partially coalesced fat globules. Only small amounts of protein were part of the fat structure supporting the air bubbles. The air bubbles are likely irregularly shaped due to compression of the foam under the microscope. Samples prepared with LACTEM resulted in the stabilization of larger air bubbles and higher overall overrun. The layer of partially coalesced fat globules supporting the air bubbles was thinner in comparison to the control formulation. This air bubble interfacial layer was also not homogeneous, indicating emulsifiers or proteins were also present at the interface, contributing to the stabilization and foam structure. Samples prepared with MDG did not reach a firm peak during whipping, this was shown by the minimal partial coalescence of fat globules. Fat globules remained dispersed and did not show signs of partial coalescence or stabilization of air bubbles. MDG likely resulted in increased emulsion stability against shear

(Moens, De Clercq, Verstringe, & Dewettinck, 2015). Samples prepared with Tween80 displayed the highest degree of partial coalescence and fat globule aggregation. The air bubble interface appears to be primarily coated with fat globules. Majority of the fat globules are either aggregated or partially coalesced, helping to stabilizer air bubbles. The minimal protein signals indicated poor protein foaming during the first stage of whipping (Han et al., 2018).

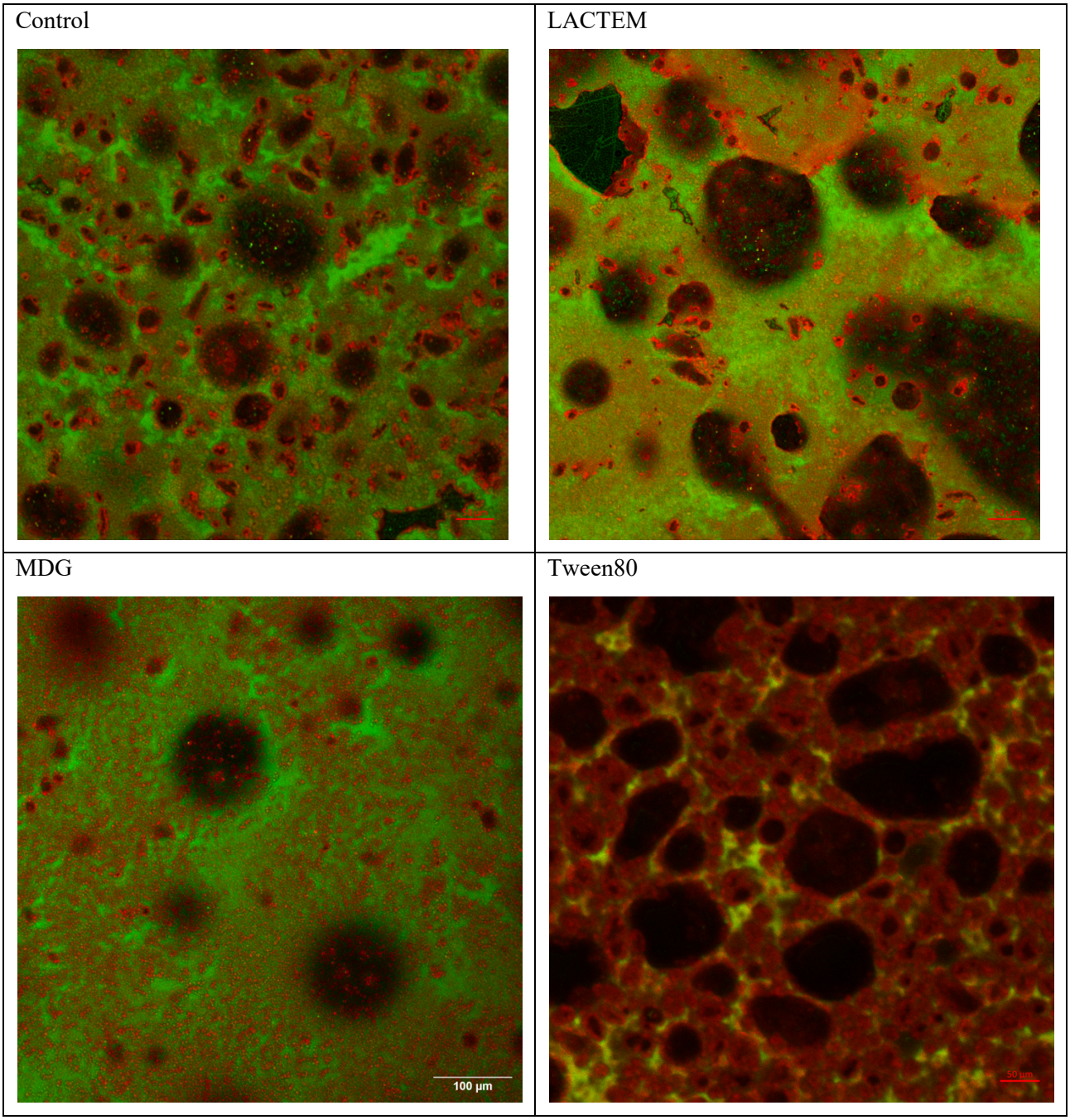


Figure 29: Confocal scanning light microscopy images of whipped emulsions, dyed with Nile Red and Fast Green. Emulsions formulated with LACTEM, MDG, Tween80 or no emulsifiers, taken immediately after whipping. Red and green signals represent fat and protein phase, respectively.

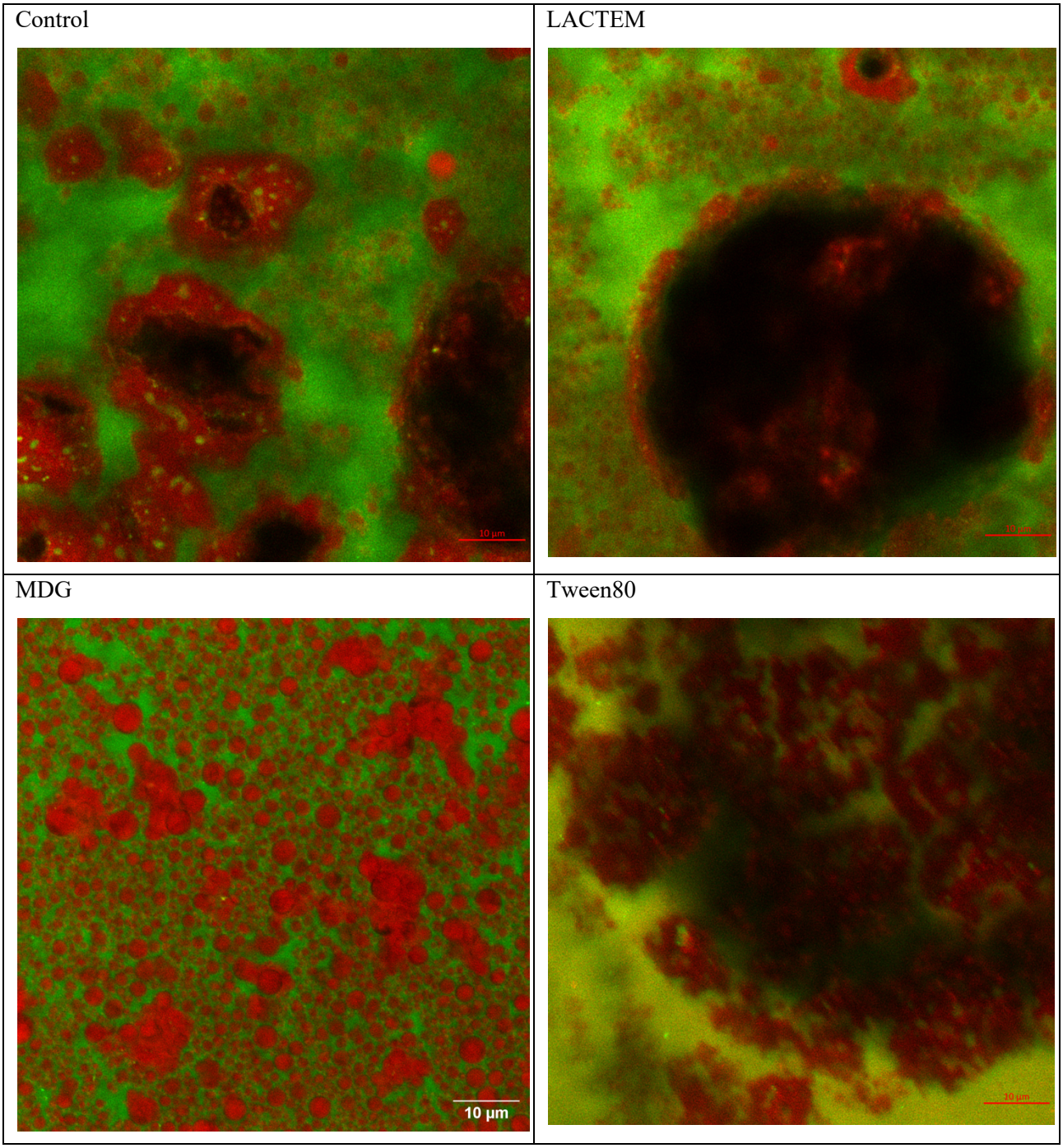


Figure 30: Confocal scanning light microscopy images of whipped emulsions, dyed with Nile Red and Fast Green. Emulsions formulated with LACTEM, MDG, Tween80 or no emulsifiers, taken immediately after whipping. Red and green signals represent fat and protein phase, respectively.

6.4. Discussion

In this chapter, the effect of LACTEM, MDG and Tween80 on the destabilization and crystallization behavior on partially crystalline oil-in-water emulsions was investigated. Throughout this study, it was generally found that a higher initial formation of α-crystals resulted in a more effective fat globule destabilization during whipping. Samples containing Tween80 displayed the highest initial formation of α-crystals, compared to samples containing MDG which resulted in the lowest (Figure 26). Microscopy images of the whipped emulsions showed that Tween80 samples had the highest degree of fat globule destabilization through partial coalescence, whereas samples containing MDG showed minimal fat destabilization (Figure 29 and Figure 30). This was identified by the degree of fat globules taking part in the foam structure. Tween80 may have resulted in higher initial α-crystals by acting as nucleation sites for fat crystals through heterogeneous nucleation at the oil-water interface (Arima et al., 2009). Both LACTEM and Tween80 resulted in a higher initial α-crystals compared to the control but displayed different whipping properties. The samples had similar whipping times, indicating the same degree of fat stabilization. However, the overrun was significantly higher for the LACTEM sample. This indicated that LACTEM was able to stabilize air bubbles more efficiently than Tween80. It is suggested that higher initial formation of α-crystals may result in β'-crystals which promote fat globule destabilization.

Emulsions formulated with LACTEM showed improved functionality through shortened whipping times, increased overrun and improved rosette appearance. It was found that samples containing LACTEM had a high initial formation of α-crystals compared to the control sample. The α-tending properties of LACTEM may have improve whippability and overrun by strengthening foam structure and promoting agglomeration of fat globules (Gaupp & Adams, 2014). It has been suggested that an α-crystalline gel forms at the oil-water interface, linking fat globules together and promoting partial coalescence (Munk et al., 2014). Microscopy images of whipped LACTEM containing emulsions showed a mixed layer of partially coalesced fat, protein and potentially emulsifiers stabilizing air bubbles. This allowed larger air bubbles to be stabilized and had more effective protein foaming during the first stage of whipping, leading to an increase in overrun and improved rosette appearance (Han et al., 2018). Although the overrun was significantly increased, the mixed layer may result in poor whipped and chilled stability due to reduced fat globules at the air bubble interface. Partially coalesced fat globules are more effective at stabilizing air bubbles than proteins (Smith et al., 2000). These results agree with the study by Munk et al. (2014), who found that emulsions made with LACTEM were sensitive to shear and led to high levels of caseinate in the serum phase. Resulting in a fat globule network with low strength and weak foam structure. Protein surface load results (Figure 25) suggest that LACTEM also displaced proteins from the interface, reducing the interfacial tension and increasing the membranes susceptibility to destabilization (Goff, 1997).

Emulsions prepared with MDG did not reach a firm peak within the 10-minute whipping time frame. Resulting in soft foams, long whipping times and poor rosettes. Microscopy images of whipped MDG emulsions showed minimal partial coalescence or flocculation and did not stabilize air bubbles. This indicates that MDG caused greater emulsion stability against whipping and shear forces. These results agree with Munk et al. (2014), who reported that interactions between caseinates and MDG at the oil-water interface increased interfacial viscoelasticity and reduced fat globule coalescence. FGSD results showed a small peak around 0.13µm, indicating potential non-adsorbed MDG in the serum phase. This may have increased interactions with caseinate, affecting fat globule coalescence. The displacement of proteins and adsorption of nanocrystals of MDG's at the oil-water interface may stabilize emulsions through a Pickering mechanism (Munk et al., 2014). The caseinate-MDG interactions, Pickering stabilization and polymorph changes previously described may be responsible for the observed increased emulsion stability against shear forces. Figure 26 shows that MDG may have resulted in fat crystals forming directly into the β' state. Crystallizing directly into β'-crystals may slow fat crystallization compared to crystals that form into the α state first (Janssen & MacGibbon, 2007). The slower crystallization rate may have resulted in the formation of purer β-crystals which are more stable against shear, resulting in minimal partial coalescence of fat globules (Moens et al., 2015). The β-crystal form does not allow the incorporation of significant amounts of air (Heymans, Tavernier, Dewettinck, & Van der Meeren, 2017).

Emulsions prepared with Tween80 displayed shortened whipping times and minimal overrun. Tween80 likely resulted in an increased emulsion viscosity which can be due to increased shear-induced aggregation (Fuller, 2015). Microscopy images of whipped Tween80 emulsions showed that the foam structure was formed primarily from partially coalesced fat globules. There was minimal protein foaming during the first stage of whipping, resulting in reduced incorporation of air bubbles. Although there was minimal overrun, the large amount of partially coalesced fat globules at the air bubble interface may produce excellent whipped and chilled foam stability due to the strong structure. Air bubbles stabilized by partially coalesced fat globules show a more effective stabilization than bubble coated by protein or emulsifier films (Smith et al., 2000). Protein displacement at the oil-water interface by Tween80 is likely the mechanism inducing the high degree of partial coalescence. Water-soluble surfactants are effective at displacing proteins from the oil-water interface as they are loosely anchored to the outer droplet surface and can be easily swept off the droplet with proteins during agitation (Davies, Dickinson, & Bee, 2000). As proteins become displaced, the membrane becomes susceptible to destabilization due to a reduction interfacial tension (Goff, 1997).

6.5. Conclusion

LACTEM, MDG and Tween80 impacted the destabilization and crystallization behavior of partially crystalline oil-in-water emulsions. LACTEM significantly increased overrun but may have poor chilled foam stability due to the mixed layer of partially coalesced fat and proteins stabilizing air bubbles. MDG resulted in an emulsion that was stable against shear-induced aggregation, leading to long whipping times and weak foam structure. Tween80 resulted in fast destabilization of fat globules but could not efficiently incorporate air bubbles, leading to dense foams with minimal overrun. It was found that generally, a higher initial formation of α-crystals resulted in a more effective fat globule destabilization during whipping. Tween80 resulted in the highest quantity of initial α-crystals, followed by LACTEM, no emulsifiers (control) and MDG.

Each emulsifier displayed advantages and disadvantages for whipping cream characteristics. It is recommended that emulsifiers are used in a formulated blend to produce desired functionality by balancing the inhibition and promotion of destabilization mechanisms. Further work is required to understand the impact of emulsifier blends on the model system.

Chapter 7: Influence of Solid Fat Content on Shear-induced Destabilization and Corresponding Aeration Properties

7.1. Introduction

The solid fat content (SFC) of milk fat plays a critical role in determining the functionality of whipping creams. The SFC is the percentage of crystalline fat at a certain temperature, it is required in creams for partial coalescence to take place (Monteny, 2015). Partial coalescence occurs when droplets contain both liquid and crystalline fat, the presence of crystalline fat prevents full coalescence (Walstra, 2003). Low levels of solid fat can produce foams that have poor stability as the structure is weaker and less ridged (Ihara et al., 2010).

The SFC of milk fat is known to change depending on the stage of milking and season (Li et al., 2020; Norris et al., 1973). In New Zealand, studies suggest that the early season (August – October) has the lowest solid fat content at ambient temperatures in comparison to the rest of the milking season (November – May), with the highest solid fat content occurring around December (Mid-season) (Li et al., 2022; Norris et al., 1973). Results from Li et al. (2022) found that the whipping properties of creams varied across the season. Early-season cream (lowest solid fat content) had the shortest whipping time, highest overrun and highest firmness. Late season cream (medium solid fat content) had the longest whipping time, lowest overrun and lowest firmness.

Milk fat is a mixture of triglycerides which exhibit different physical properties such as melting point. Purified AMF can be separated into the different fractions based on melting point through a separation technique called fractionation. First stage fractionation results in a high melting fraction (HMF) and a low melting fraction (LMF). The resulting HMF and LMF can be refine further through second and third stage fractionation (Deffense, 1995). HMF can be added back into product to improve functionality and stability by increasing the SFC.

Milk fat can be blended with vegetable oils to obtain specific functionality and properties in recombined creams. Monteny (2015) found that recombined creams containing a higher solid fat content than typical dairy creams resulted in the faster formation of a stronger fat crystal network. Palm oil is one of the most popular vegetable fats used throughout the food industry due to its cost, purity and high melting point. The SFC of AMF containing products can be increased with hard fraction palm oil, this approach is commonly utilized by the confectionary industry. The addition of hard palm oil stearin to AMF can result in improved functionality in food systems, melting profiles and solid fat contents at high temperatures (Hayati et al., 2000). Hayati et al. (2000) reported that blends of AMF and palm oil stearin exhibited similar melting profiles to the HMF of milk fat at temperatures above 10˚C.

The aim of this study was to investigate the impact of simulated seasonal variation of solid fat content and non-dairy fat/oil blends on whipping cream functionality.

The objectives were to:

- Fractionate AMF into high and low melting fractions.
- Create blends of AMF fractions that replicate the seasonal extremes of SFC.
- Investigate blends of AMF and non-dairy fat and oil sources to determine if SFC is a key driving force in influencing functionality.

7.2. Materials and Methods

7.2.1. Materials

Sodium caseinate (92.5 wt% protein, 0.8 wt% fat, 4.66 wt% moisture and 3.4 wt% ash) was purchased from Fonterra Co-Operative Group Ltd (Auckland, New Zealand). AMF (99.8 wt% fat) was provided by Synlait Milk Ltd (Christchurch, New Zealand). Food grade Polysorbate 80 (Tween80) was purchased from Invita (Auckland, New Zealand). Food grade LACTEM was purchased from Hawkins Watts (Auckland, New Zealand). Food grade HP60 Mono- and di-glycerides (MDG) was purchased from Hawkins Watts (Auckland, New Zealand). Canola oil was purchased from local supermarkets (Sunfield Oils, Auckland New Zealand). Palm oil was purchased from Pure Nature (Auckland, New Zealand). RO water was used in all emulsions.

7.2.2. Methods

Seven formulations were prepared according to Table 6, following the emulsion preparation method described in Section 3.2.3. Samples were homogenised at 65°C using a two-stage homogeniser at 100 Bar total (80/20) and three passes. Each emulsion contained 0.2 wt% LACTEM, 0.1 wt% MDG, 0.1 wt% Tween80, 1 wt% sodium caseinate and 36 wt% fat/oil. The FGSD was measured immediately after homogenisationand after 6 days of storage at 4°C, according to Section 3.3.1. The composition was measured according to Section 3.3.2 immediately after homogenisation. Protein surface load was measured according to Section 3.3.3, after 24 hours of storage at 4°C. Whipping properties (whipping time and overrun) were measured according to Section 3.4.1 and 3.4.2 after 7 days of storage at 4°C. Whipped foams were piped into rosettes and images taken to assess performance. Foam structure was studied using CSLM for the control and low SFC samples, as described in Section 3.4.5, immediately after whipping. Foam firmness was measured immediately after whipping, according to Section 3.4.3. Crystallization behavior was studied using DSC according to Section 3.3.5 after 6 days of storage at 4°C.

Emulsions were prepared in duplicate across several independent trials in a randomized order. Statistical analysis was performed using a one-way analysis of variance (ANOVA) to determine statistically significant differences between samples. The analysis was carried out using Minitab 19 with a probability level for statistical significance of $P < 0.05$.

Table 6: Breakdown of fat/oil blends for each formulation, containing a total of 36 wt% fat per emulsion.

Formulation	AMF	High Melting Fraction	Low Melting Fraction	Hard Palm Oil Fraction	Canola Oil
1 – Control	100%				
2 – Low SFC	50%		50%		
3 – High SFC	50%	50%			
4 – Canola Oil/AMF	70%				30%
5 – Hard Palm Oil/AMF	70%			30%	
6 – Hard Palm Oil/ Canola/ AMF	33%			33%	33%
7 – Canola Oil/HMF		80%			20%

7.2.3. AMF and Palm Oil Fractionation

Procurement issues lead to in the inability to purchase hard fraction AMF or hard fraction palm oil. The alternative solution was to complete a first stage fractionation on the fats at a lab scale. The principle of first stage fractionation is based on slowly cooling melted fats to a temperature which produces a slurry of solid and liquid fat. The slurry is then filtered, producing a high and a low melting fraction. This procedure was not as efficient as commercially fractionated fats due to the limitations of lab scale equipment creating a less effective filtration step. The lower crystal content of the filter cake was considered when selecting formulations. It was expected that the hard fraction AMF would have a less sharp melting profile compared to commercial hard fraction AMF.

Fats were separately heated above melting point (50°C) using a water bath. Melted fats were poured into glass beakers and placed inside an incubator set at 22°C. AMF was left to slowly cool over 36 hours, palm oil was cooled over 12 hours. After the cooling time had elapsed, the resulting slurry was filtered using a Buchner funnel and flask. Mixtures were filtered for approximately 3 hours or until all the liquid fat was separated from the filter cake.

The quantity of crystals in the resulting hard fraction AMF was analyzed according to Section 3.3.5 to evaluate the purity. The resulting hard fraction palm oil was not evaluated for crystal purity. Results showed that the hard fraction AMF contained 38.3% crystals in the cake at 22°C. In comparison, commercial grade hard fraction AMF contains approximately 45-50% solid fat at 20°C (Hayati et al., 2000). These results were used to calculate mixtures of AMF fractions to achieve a SFC that represents seasonal extremes.

7.3. Results

7.3.1. Emulsion Characteristics

Emulsion characteristics were determined by measuring the particle size distribution (PSD), fat globule size distribution (FGSD), composition, and protein surface load. Findings were used to determine any significant difference between formulations as a result of the fat blends. There were no significant differences ($P > 0.05$) in composition (Table 8, Appendix) across samples, indicating successful replication of emulsions during preparation. Figure 31 illustrates the fat globule size distribution measured immediately after homogenisation. The average D_{43} of samples were 1.5μm, all within the target values of 1.3-1.7μm. There were two populations of FGSD. This was likely a result of a slight homogenisation pressure difference than anticipated on a single day of processing, the only emulsions affected were made all on the same day. There was no difference in FGSD after one week of storage at 4°C (Figure 48, Appendix), indicating no emulsion instabilities such as partial coalescence or aggregation occurring.

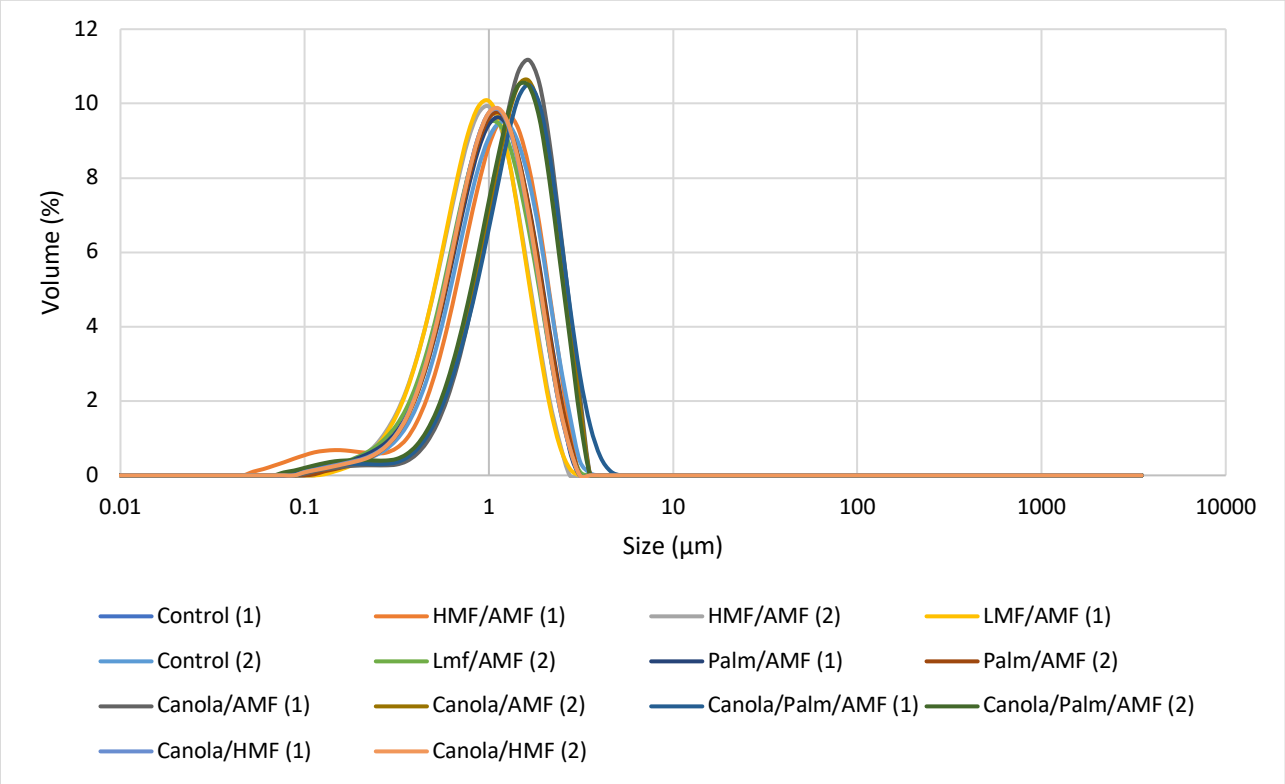


Figure 31: Fat globule size distribution for emulsions containing blends of AMF, canola oil or hard fraction palm oil, measured immediately after homogenisation.

The protein surface load results (Figure 32) illustrated that there were no significant differences ($P > 0.05$) in the level of protein desorption across all samples. This indicated that the fat source did not affect displacement of proteins or adsorption of emulsifiers at the oil-water interface. Protein displacement can therefore be ruled out as a mechanism responsible for any differences in functionality between samples.

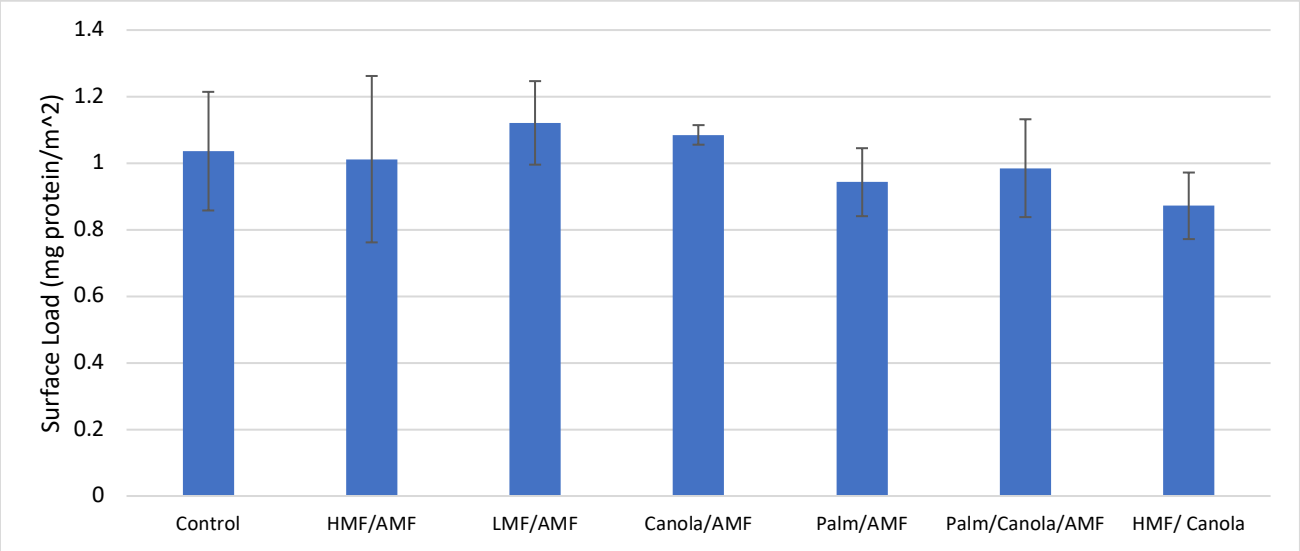


Figure 32: Protein surface load results for emulsions containing blends of AMF, canola oil or hard fraction palm oil, measured 24 hours after homogenisation.

7.3.2. Milk Fat Crystallization Characteristics

The fat crystallization characteristics of emulsions were measured using the stop and return DSC method (Figure 49 - Figure 55, Appendix). Melting peaks were integrated to produce a melting heat as a function of time graph, Figure 33. Mixing several types of fats and oils provides a mixture of triglycerides and chain length. In the raw data, fat blends of dairy and non-dairy resulted in numerous melting energy peaks which changed in number and intensity with each holding time, each representing a different crystal population. Due to the variation in crystallization peaks observed in blends of fats and oils, conclusions cannot be draw about polymorphic transitions between α - β' crystals, such as those previously described in Section 6.3.2. The melting heat values shown in Figure 33 are the total integrated heat released and therefore not a fair representation of individual crystal populations produced.

The final point at 120-minute holding time represents the solid fat content, relative to other samples. The relative SFC of each formulation is summarized in Figure 34. These values were used to determine significant correlations between SFC and whipping properties. The LMF/AMF and Palm/AMF samples appear to have not leveled off at the 120-minute holding time, indicating that these samples may have a higher relative SFC that measured.

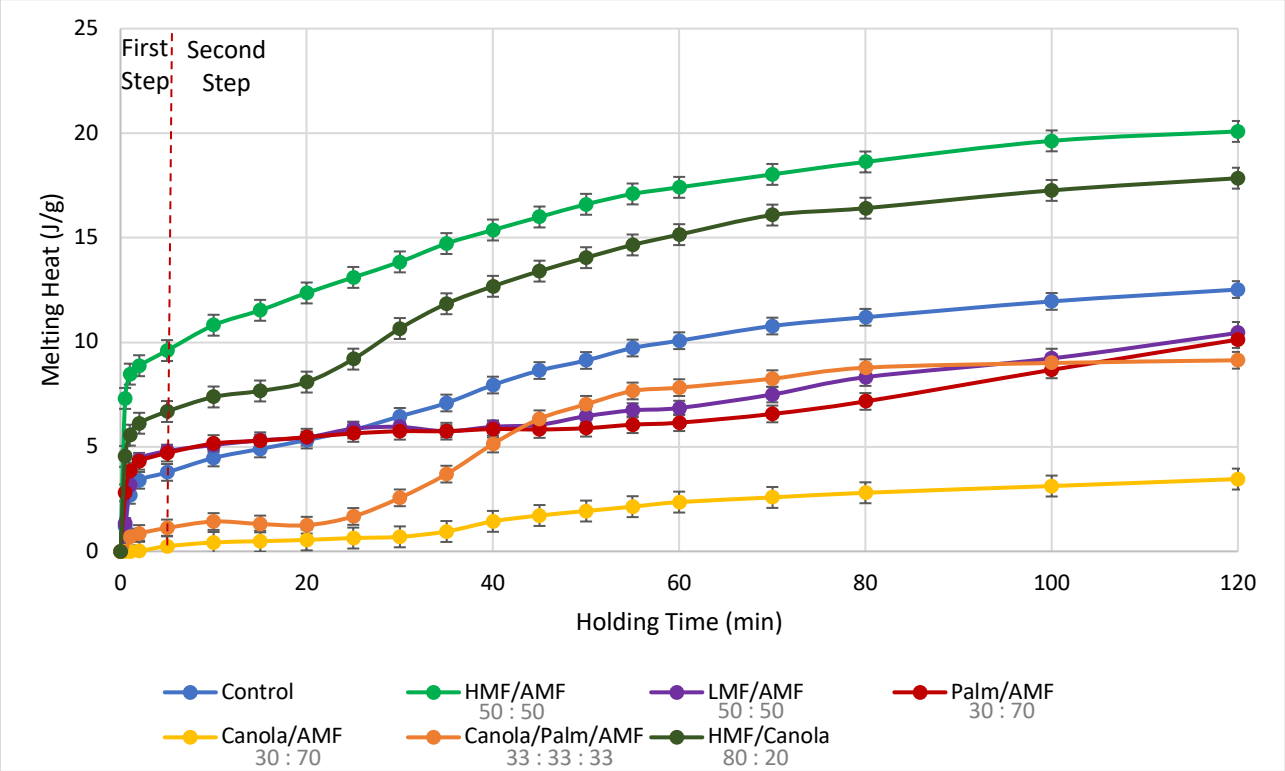


Figure 33: Melting heat as a function of time for isothermal crystallization at 5 ℃ of emulsions containing blends of AMF, canola oil or hard fraction palm oil, measured after 6 days of storage at 4 ℃.

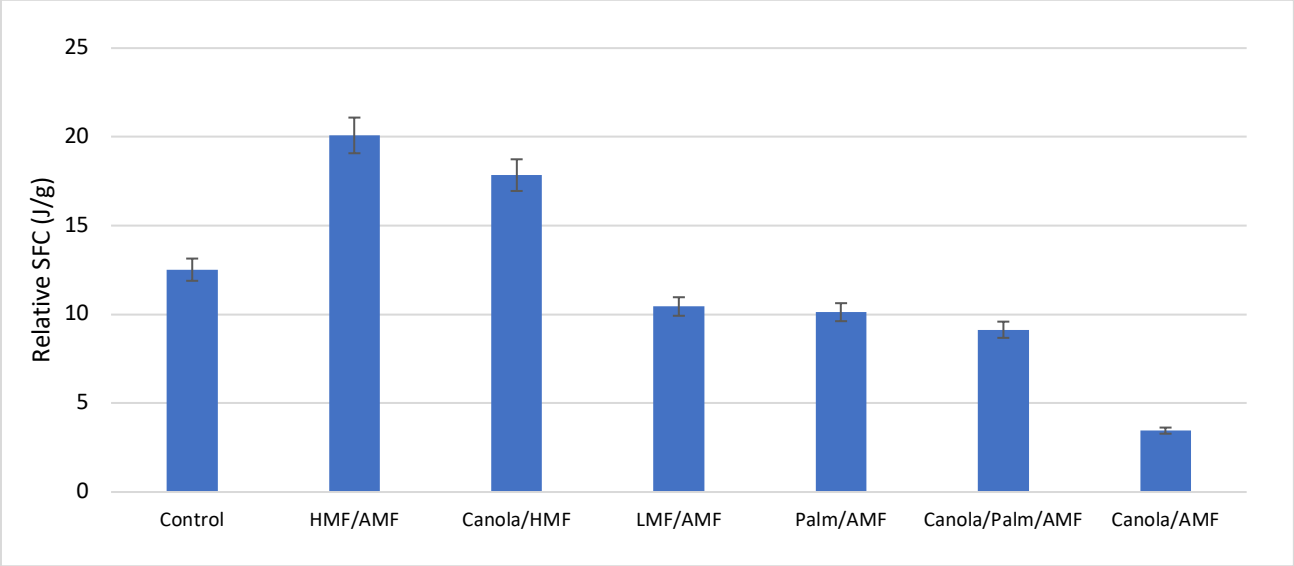


Figure 34: Relative solid fat content at 5℃ for emulsions containing blends of AMF, canola oil or hard fraction palm oil, measured after 6 days of storage at 4℃.

7.3.3. Whipping Properties

The seven formulations each displayed unique whipping properties, illustrated in Figure 35. The LMF/AMF, canola/AMF and canola/palm/AMF formulations all did not reach a firm peak within the 10-minute time frame. All three also resulted in a significantly lower overrun ($P < 0.05$) compared to the control, the overrun did not vary between the three formulations. Both the canola/AMF and canola/palm/AMF formulations resulted in a liquid after 10minutes (Figure 36), indicating insufficient SFC for partial coalescence to occur. Although the LMF/AMF formulation did not reach a firm peak with the 10-mintue time frame, the resulting soft foam was able to be piped into a rosette (Figure 36). The LMF/AMF rosettes were slumped and exhibited a less defined structure. The HMF/AMF and Palm/AMF formulations did not show any significant differences ($P > 0.05$) in both whipping time and overrun compared to the control. The HMF/AMF piped rosettes were similar to the control, the palm/AMF rosettes showed a less defined structure even though the whipping properties were similar (Figure 36). This indicated that the addition of hard palm fraction may have resulted in some changes to the microstructure. The canola/HMF formulation were the only samples that resulted in a faster whipping time ($P < 0.05$) compared to the control. This formulation also showed a less defined rosette structure when piped into rosettes (Figure 36).

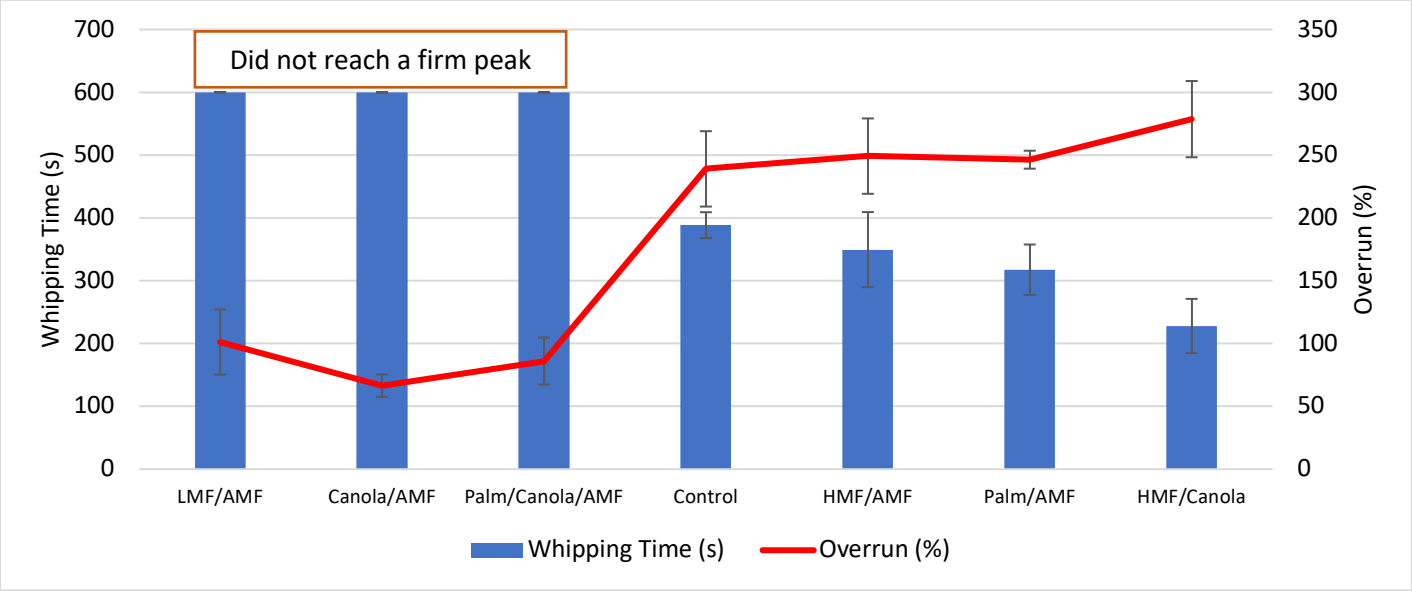


Figure 35: Whipping properties (whipping time and overrun) for emulsions blends of AMF, canola oil or hard fraction palm oil, measured after 7 days of storage at 4 ℃.

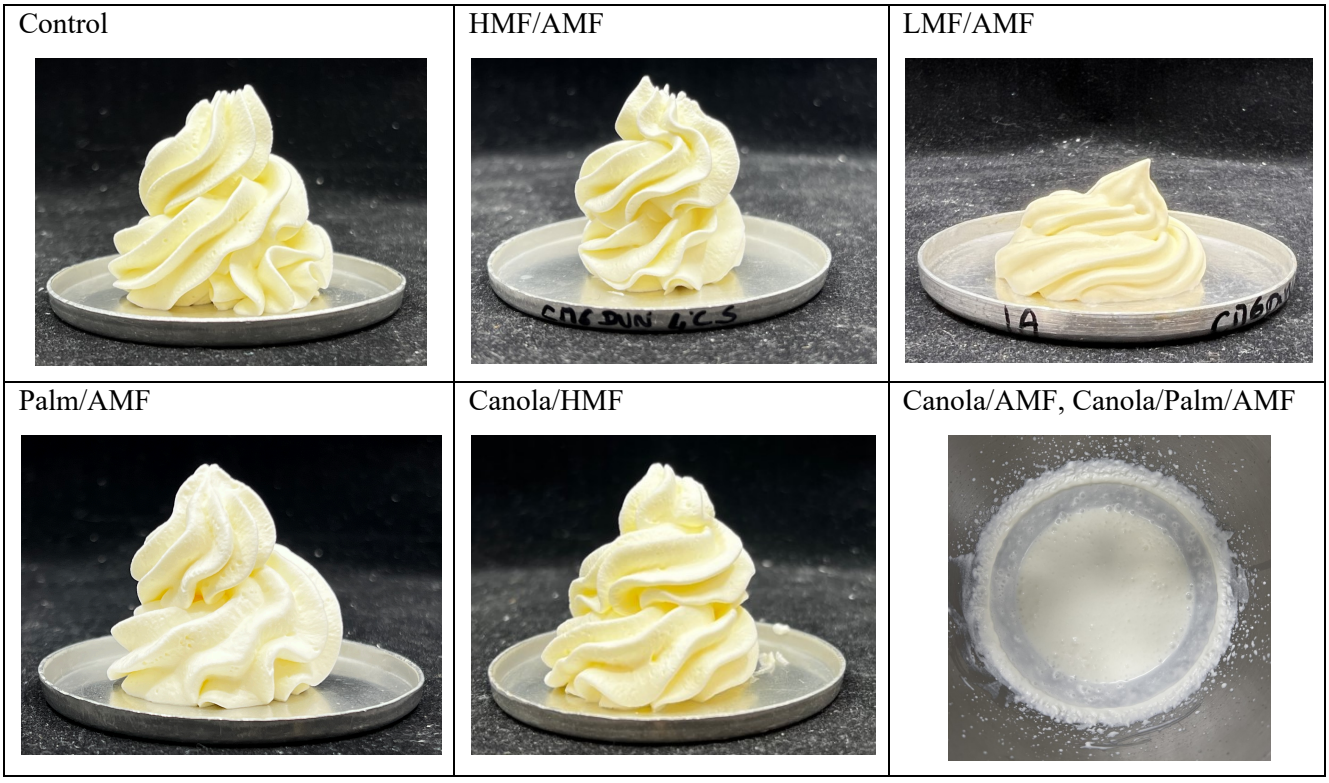


Figure 36: Piped rosettes for emulsions containing blends of AMF, canola oil or hard fraction palm oil, photographed immediately after whipping.

The foam firmness of whipped emulsions are illustrated in Figure 37. Results are consistent with rosette observations and show a correlation with the relative SFC (Figure 40). The HMF/AMF, canola/HMF, palm/AMF formulations did not exhibit any significant differences in foam firmness compared to the control ($P > 0.05$). the LMF/AMF, canola/palm/AMF and canola/AMF formulations all had a significantly lower foam firmness compared to the control ($P < 0.05$). This was anticipated as the three formulations did not reach a firm peak during the whipping process.

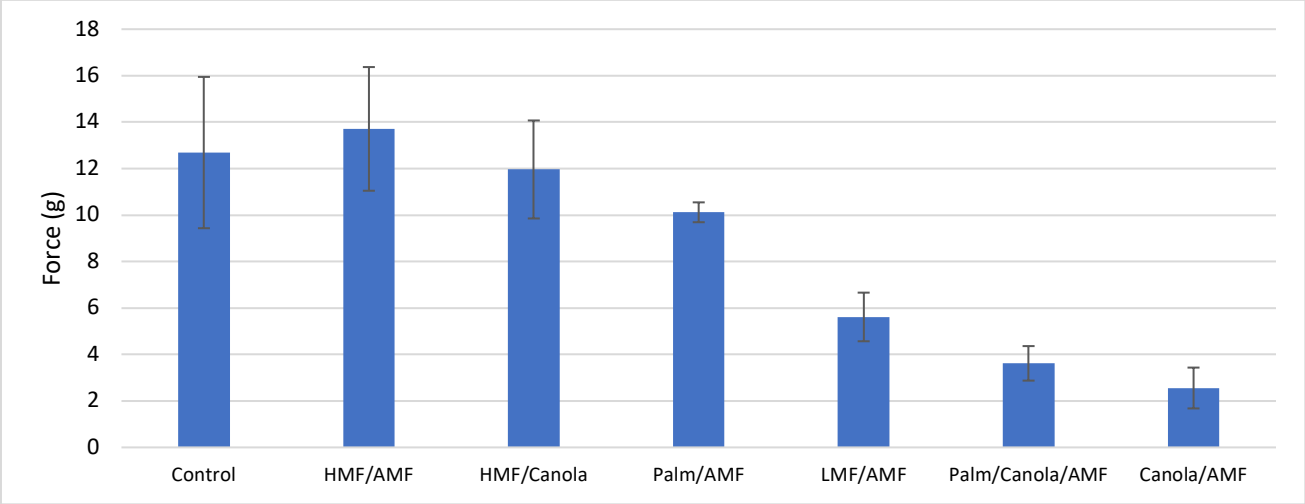


Figure 37: Foam firmness for whipped emulsions at 5 ℃, containing blends of AMF, canola oil or hard fraction palm oil, measured immediately after whipping.

7.3.4. Confocal Scanning Light Microscopy

CLSM images (Figure 38 and Figure 39) of the whipped emulsions showed how the foam structures varied for samples prepare with AMF to LMF/AMF. Red and green signals represent fat and protein phases, respectively. The black signals represent air bubbles. Both samples resulted in partially coalesced fat stabilizing air bubbles at the interface. AMF resulted in more uniform layer of fat globules at the air bubble interface compared to the LMF/AMF formulation which displayed thick and irregular fat globule structures. The structure of the AMF foam is predominately made up of destabilized fat with a small amount of protein within the partially coalesced fat globules at the air bubble interface. The LMF/AMF formulation resulted in smaller air bubbles being stabilized.

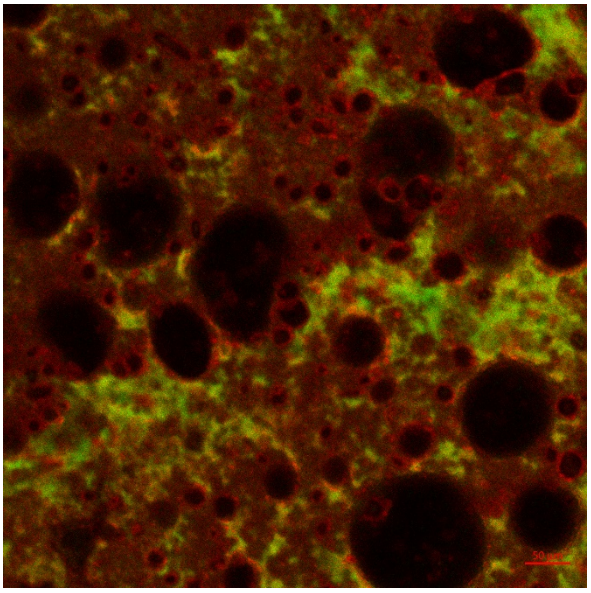
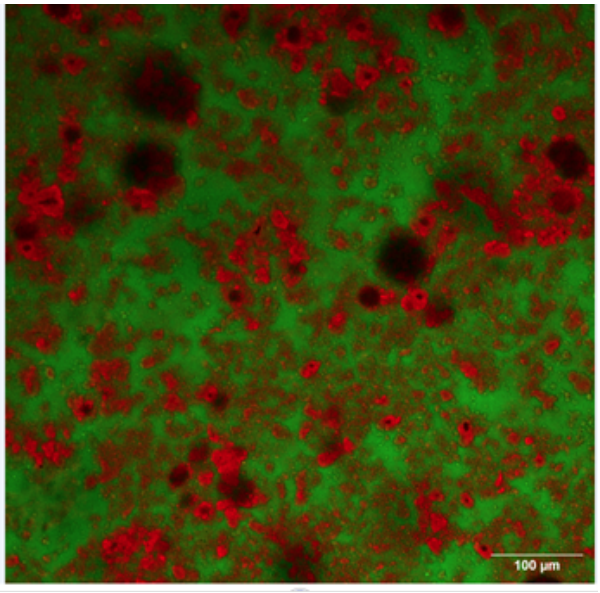
Control (AMF)	LMF/AMF
	

Figure 38: Confocal scanning light microscopy images of whipped emulsions, dyed with Nile Red and Fast Green. Emulsions formulated with AMF and LMF/AMF blend, taken immediately after whipping. Red and green signals represent fat and protein phase, respectively.

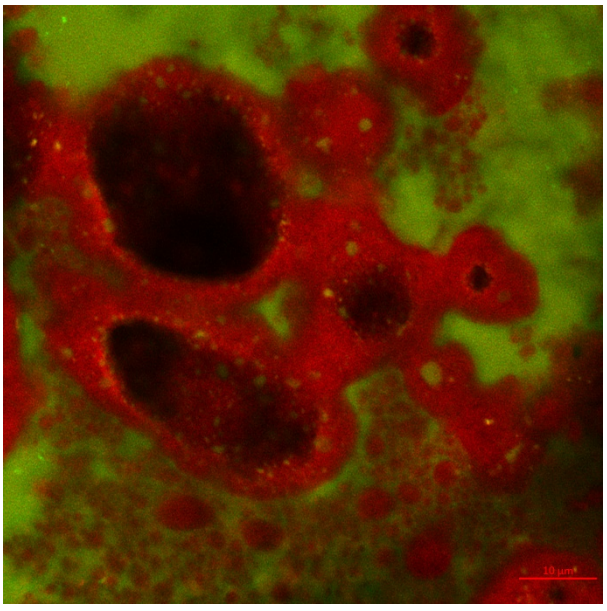
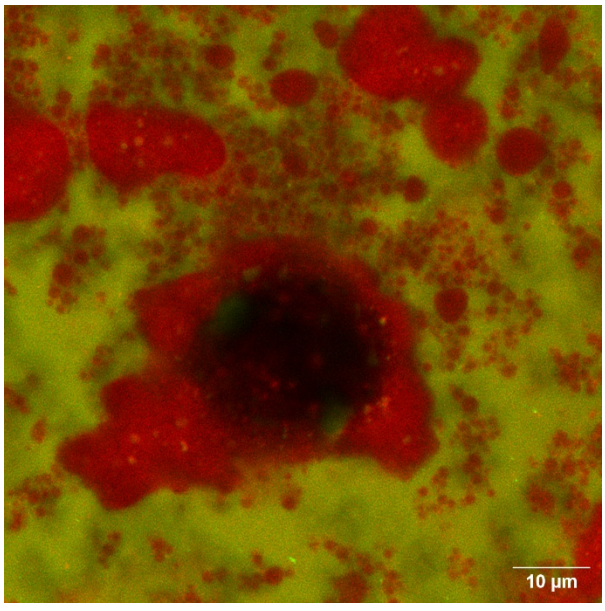
Control (AMF)	LMF/AMF
	

Figure 39: Confocal scanning light microscopy images of whipped emulsions, dyed with Nile Red and Fast Green. Emulsions formulated with AMF and LMF/AMF blend, taken immediately after whipping. Red and green signals represent fat and protein phase, respectively.

7.4. Discussion

In this chapter, the possible mechanisms of solid fat content impact on the functionality of partially crystalline oil-in-water emulsions were investigated. The pure AMF formulations were formulated to have a SFC level that replicated seasonal extremities. In New Zealand, the SFC of milk fat can vary between 18-28% at 20°C (Meagher, Holroyd, Illingworth, van de Ven, & Lane, 2007). In this study, the high SFC and control formulations both exhibited similar functionality in terms of whipping time, overrun, foam firmness and piped rosettes. This demonstrates that the periods producing high SFC i.e. typically summer, were not a cause for concern as it does not impact functionality and may provide slight benefits. The low SFC formulation however was unable to reach a firm peak and resulted in low foam firmness and poor rosettes. This indicated that a low SFC often associated with early season lactation may cause functionality issues for cream produced during this time. Hillbrick, Augustin, and Udabage (2006) reported that cream produced from early season milk had low SFC which resulted in longer whipping times and serum leakage. The study by Li et al. (2022) also reported similar results on un-homogenised pasteurized cream, early season cream displayed the lowest SFC and resulted in longer whipping times. However, Li et al. (2022) did not find a significant correlation between SFC and whipping properties (whipping time, overrun and foam firmness), suggesting that the seasonal variation in other parameters such as milk fat globules size and chain length may have caused differences. Due to the impact of seasonal variation on cream composition, it is recommended that extra caution is taken when formulating with early season cream. Potential solutions could include addition of hard fraction AMF or adjusting emulsifier levels to account for the reduced SFC.

The LMF/AMF and palm/AMF formulations exhibited similar crystallization curves and relative solid fat content. However, the LMF/AMF formulation did not reach a firm peak within the 10-minute time frame and the palm/AMF formulation did. These results suggest that the addition of hard palm fraction provided benefits for the functionality. These results agree with the study by Shamsi, Che Man, Yusoff, and Jinap (2002), who reported that whipping creams produced with palm oil resulted in a more stable foam than a dairy whipping cream. This was likely due to the amount of unsaturated fatty acids, specifically oleic acid which may have improved foam stability, identified by an increase in iodine value (Shamsi et al., 2002). Che Man, Shamsi, Yusoff, and Jinap (2003) also reported improved foam structure and appearance in whipping creams made with palm oil compared to dairy. This was likely due to the affinity of palm oil to crystalize in the β' state similarly to milk fat, which provides stability to many bubbles and a greater volume by promoting partial calescence.

The addition of canola oil reduced the SFC of formulations as it does not contain many high melting point triglycerides. This produced emulsions with increased whipping times, reduced overrun, soft foams and poor rosettes. These results were expected as the SFC of the canola/AMF and canola/palm/AMF formulations were low. A low SFC can result in a reduction in capture efficiency of colliding fat globules during shear and a

slower rate of partial coalescence (Monteny, 2015). To see the effect of canola oil on the model system with sufficient SFC, a formulation of canola oil and hard fraction HMF was investigated. Results from this formulation showed a decreased whipping time and increased overrun, while maintaining a similar SFC to the control. This potentially indicated that canola oil can be used to replace a percentage of the fat source in a whipping cream if the SFC remained consistent. However, there are other factors impacting functionality that canola oil may affect which are not explored here, such as protein interactions, fatty acid analysis and fat globule membrane components.

Throughout this study, it was found that the SFC and fat composition impacted the whipping properties. Statistical analysis revealed that emulsions which produced firmer foams resulted in improved rosettes. The improved foam structure typically associated with firmer foams likely resulted in a material that could be easily molded into the desired shape and remain in that structure without slumping. It was also found that longer whipping times were correlated with a reduced overrun ($P < 0.05$). Samples that produced the lowest overrun did not reach a firm peak within the 10-minute time frame: LMF/AMF, canola/AMF and canola/palm/AMF. The low overrun may have been a result of low solid fat content, reducing the rate of partial coalescence and/or potentially whipping past the point of maximum overrun for the emulsion. None of the three samples reached a firm enough peak to stop whipping and were therefore whipped until the time limit was reached. This may have resulted in the overrun decreasing due to excess whipping and/or an increase in temperature of the whipped sample, leading to the breakdown in foam structure due to less solid fat present. These results agree with Jakubczyk and Niranjan (2006) who reported that a whipped emulsion shows increasing overrun as whipping time proceeds until a maximum overrun is reached, whipping after this point resulted in a reduction of overrun. Maximum stability and foam firmness is observed at the point of maximum overrun as air bubbles are effectively encapsulated by partially coalesced fat globules. Ihara et al. (2010) observed that the temperature of emulsions can increase from 5 up to 15°C during the whipping process, showing that an increase in temperature during whipping can reduce the amount of solid fat and decrease functionality.

The SFC of the formulations is one of the key factors impacting the functionality of the emulsions. Solid fat is required in whipping cream emulsions for partial coalescence to occur. Partially coalesced fat globules stabilized air bubbles effectively at the air-liquid interface. Statistical analysis showed that the SFC was significantly correlated with whipping time, overrun and foam hardness ($P < 0.05$), Figure 40. A higher SFC resulted in a faster whipping time, higher overrun and firmer foam. It has been suggested that the rate of partial coalescence is strongly related to the SFC of the emulsion as solid fat is required to form a continuous crystal network (Fredrick, Walstra, & Dewettinck, 2010). An excessively high SFC can also cause issues as liquid fat is required for partial coalescence to occur. When the SFC exceeds the optimal range, there is not enough liquid fat to wet fat crystals, resulting in a permanent junction (Fredrick et al., 2010). A low SFC can result in a decrease in size and quantity of crystals in fat droplets, reducing capture efficiency of colliding fat globules during shear and a slower rate of partial coalescence (Monteny, 2015). As the rate of partial coalescence

impacts whipping time, overrun and foam firmness, it was expected that formulations containing the highest SFC displayed improved whipping properties in comparison to formulations containing low SFC, up to a point.

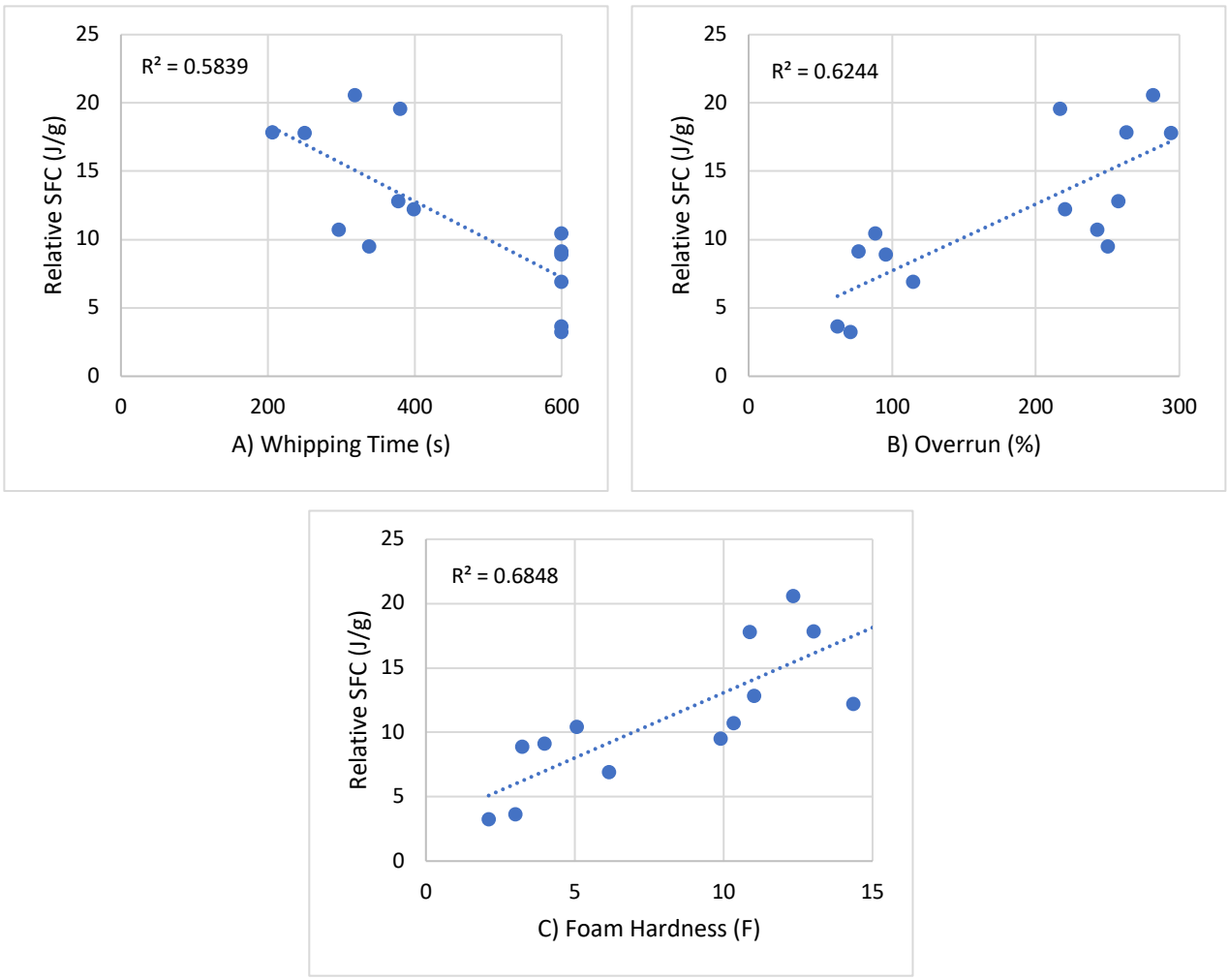


Figure 40: Significant correlations between relative SFC and a) whipping time, b) overrun and c) foam hardness.

7.5. Conclusion

The SFC and fat source of model emulsions had a significant impact on the whipping properties and functionality. Seasonal extremes of SFC were replicated using blends of AMF fractions, results suggest a high SFC did not impact functionality, but a low SFC did. A low SFC resulted in longer whipping times, lower overrun and softer foams with a less defined structure. It is recommended that extra caution is taken when formulating with early season cream (lowest SFC). Potential solutions could include the addition of hard fraction AMF or adjusting emulsifier levels to account for the reduced SFC.

Non-dairy fat and oil sources provided some potential benefits to the functionality and manufacturing costs as both palm oil and canola oil are typically cheaper than AMF. Hard palm fraction may provide improved whipping properties at a lower SFC compared to pure AMF, likely due to amount of unsaturated fatty acids. Canola oil can potentially be a replacement for a percentage of milk fat if the SFC is adequate. The addition of a non-dairy fat or oil source would affect labeling requirements as it can no longer be classed as a dairy cream, potentially impacting consumer perception. Further work on palm fraction and canola oil is recommended to determine feasibility such as sensory and shelf -life analysis.

Chapter 8: Summary

8.1. Conclusions

The aim of this research was to develop a model cream system and explore characterising methods to evaluate formulation levers for UHT whipping creams. This was completed through establishing a bench scale model whipping cream system with the ability to destabilise under shear. The model system was used to investigate the influence of emulsifiers and the seasonal variation in SFC on whipping cream functionality.

In Chapters 3 and 4, methods to evaluate whipping cream characteristics and functionality were explored. A key method that was used throughout this research was the stop and return DSC method. The stop and return method uses numerous heating and cooling cycles, the amount of energy released during a heating cycle is a measurement of the amount of crystallization and can be plotted against time. Integrated peaks are used to create a melting enthalpy curve that shows polymorphic forms and relative solid fat content. The stop and return method was able to identify that small changes to the formulation had impacted polymorphic transition and functionality. The relative solid fat content was a used to identify significant correlations between blends of fats/oil and functionality.

In Chapter 5, the ingredients, formulation and preparation method of the model system was optimized. The selected conditions resulted in a stable and reproducible emulsion that was able to destabilize under shear. The optimal conditions included:

- Homogenisation pressure of 100 (80/20) Bar to produce a D_{43} between 1.3-1.7μm.
- Protein level of 1 wt% sodium caseinate and a fat level of 36 wt% AMF.
- Emulsion preparation method 3– fat soluble emulsifiers before homogenisation in the fat phase and tween80 24 hours after homogenisation.

In Chapter 6, the impact of LACTEM, MDG and Tween 80 on the destabilization and crystallization of milk fat in the model system was studied. LACTEM significantly increased overrun but resulted in poor chilled foam stability probably due to the mixed layer of partially coalesced fat and proteins stabilizing air bubbles. MDG resulted in an emulsion that was stable against shear-induced aggregation, leading to long whipping times and weak foam structure. Tween80 resulted in fast destabilization of fat globules but could not efficiently incorporate air bubbles, leading to dense foams with minimal overrun. It was found that generally, a higher initial formation of α-crystals resulted in a more effective fat globule destabilization during whipping. α-tending emulsifiers produced a higher initial formation of α-crystals and improved whipping properties. It is expected that a blend of the three emulsifiers would provide better functionality.

In Chapter 7, the impact of simulated seasonal variation of solid fat content and non-dairy fat/oil blends on whipping cream functionality was studied. Blends of AMF fractions were created to replicate the seasonal

extremes of SFC. Results suggest a high SFC did not impact functionality. A low SFC resulted in a failure to whip to a firm peak, with long whipping times, low overrun and soft foams. Hard fraction palm oil and canola oil were used in emulsions to determine if SFC is a key driving force in influencing functionality. Both provided some potential benefits to the functionality and manufacturing costs as both palm oil and canola oil are typically cheaper than AMF. Hard palm fraction may provide improved whipping properties at a lower SFC compared to pure AMF, likely due to the amount of unsaturated fatty acids. Canola oil can potentially be a replacement for a percentage of milk fat in whipping creams if the SFC is adequate.

8.2. Recommendations for Future Work

Throughout this research, key recommendations for future work were identified:

- It is recommended that the use of beta-serum powder in whipping creams is explored due to the significant improvement in whipping properties observed.
- It is recommended that the impact of emulsifier blends on the model system is investigated. Emulsifiers are rarely used singularly in whipping creams, and often require careful formulation to produce desired functionality through balancing the inhibition and promotion of destabilization.
- It is recommended that extra caution is taken when formulating with early season cream due to the low SFC. Potential solutions could include the addition of hard fraction AMF or adjusting emulsifier levels to account for the reduced SFC.
- It is recommended that the feasibility of including hard palm fraction and canola oil in whipping cream formulations is investigated. Both provided some potential benefits to the functionality and manufacturing costs, however other factors such as sensory and shelf -life analysis should be explored.
- It is recommended that the model system is validated using UHT heat treatment and scaled up to confirm findings.

9. References

Allen, K. E., Murray, B. S., & Dickinson, E. (2008a). Development of a model whipped cream: Effects of emulsion droplet liquid/solid character and added hydrocolloid. *Food Hydrocolloids, 22*(4), 690-699. doi:https://doi.org/10.1016/j.foodhyd.2007.01.017

Allen, K. E., Murray, B. S., & Dickinson, E. (2008b). Whipped cream-like textured systems based on acidified caseinate-stabilized oil-in-water emulsions. *International Dairy Journal, 18*(10), 1011-1021. doi:https://doi.org/10.1016/j.idairyj.2008.04.003

Álvarez Cerimedo, M. S., Cerdeira, M., Candal, R. J., & Herrera, M. L. (2008). Microencapsulation of a Low-trans Fat in Trehalose as Affected by Emulsifier Type. *85*(9), 797-807. doi:https://doi.org/10.1007/s11746-008-1267-1

Arima, S., Ueno, S., Ogawa, A., & Sato, K. (2009). Scanning Microbeam Small-Angle X-ray Diffraction Study of Interfacial Heterogeneous Crystallization of Fat Crystals in Oil-in-Water Emulsion Droplets. *Langmuir, 25*(17), 9777-9784. doi:10.1021/la901115x

Berton, C., Genot, C., & Ropers, M.-H. (2011). Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. *Journal of Colloid and Interface Science, 354*(2), 739-748. doi:https://doi.org/10.1016/j.jcis.2010.11.055

Bhatt, N., Prasad, R. K., Singh, K., & Panpalia, G. M. (2010). Stability study of O/W emulsions using zeta potential *Journal of Chemical and Pharmaceutical Research 2*(1), 512-527.

Bos, M., Nylander, T., Arnebrant, T., & Clark, D. C. (1997). Protein/Emulsifier Interactions. In G. L. Hasenhuettl & R. W. Hartel (Eds.), *Food Emulsifiers and Their Applications* (pp. 95-146). Boston, MA: Springer US.

Chanamai, R., & McClements, D. J. (2001). Depletion flocculation of beverage emulsions by gum arabic and modified starch. *Journal of Food Science, 66*(3), 457-463. doi:10.1111/j.1365-2621.2001.tb16129.x

Chandrapala, J., Zisu, B., Palmer, M., Kentish, S., & Ashokkumar, M. (2011). Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. *Ultrasonics - Sonochemistry, 18*(5), 951-957. doi:10.1016/j.ultsonch.2010.12.016

Che Man, Y., Shamsi, K., Yusoff, M., & Jinap, S. (2003). A study on the crystal structure of palm oil-based whipping cream. *Journal of the American Oil Chemists' Society, 80*(5), 409-415.

Cottrell, T., & Peij, J. v. (2014). Sorbitan Esters and Polysorbates. In *Emulsifiers in Food Technology* (pp. 271-296).

Dar, Y. L., & Light, J. M. (2014). *Food texture design and optimization*: John Wiley & Sons.

Davies, E., Dickinson, E., & Bee, R. (2000). Shear stability of sodium caseinate emulsions containing monoglyceride and triglyceride crystals. *Food Hydrocolloids, 14*(2), 145-153. doi:https://doi.org/10.1016/S0268-005X(99)00060-0

Deffense, E. (1995). Dry multiple fractionation: Trends in products and applications. *Lipid Technology, 7*, 34-38.

Dickinson. (1999). Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and surfaces B: Biointerfaces, 15*(2), 161-176.

Dickinson. (2019). Strategies to control and inhibit the flocculation of protein-stabilized oil-in-water emulsions. *Food Hydrocolloids, 96*, 209-223. doi:https://doi.org/10.1016/j.foodhyd.2019.05.021

Dickinson, Golding, & Povey. (1997). Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. *Journal of colloid interface science, 185*(2), 515-529.

Duffy, J., Larsson, M., & Hill, A. (2012). Suspension Stability; Why Particle Size, Zeta Potential and Rheology are Important. *ANNUAL TRANSACTIONS OF THE NORDIC RHEOLOGY SOCIETY, 20*.

Euston, & Goff, H. (2019). Emulsifiers in Dairy Products and Dairy Substitutes. In *Dairy Fat Products and Functionality* (pp. 217-254).

Euston, S. R., & Mayhill, P. J. F. r. i. (2001). Time-and temperature-dependent changes in the adsorbed layer on caseinate-stabilized emulsion droplets. *34*(5), 369-376.

Felix da Silva, D. A., Lilia Ipsen, Richard Hougaard, Anni Bygvraa. (2018). Casein-Based Powders: Characteristics and Rehydration Properties. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 240-254. doi:https://doi.org/10.1111/1541-4337.12319

Foubert, I., Fredrick, E., Vereecken, J., Sichien, M., & Dewettinck, K. (2008). Stop-and-return DSC method to study fat crystallization. *Thermochimica Acta, 471*(1), 7-13. doi:https://doi.org/10.1016/j.tca.2008.02.005

Fredrick, E. (2011). *Fat crystallization and partial coalescence in dairy creams: Role of monoacylglycerols*: Ghent University.

Fredrick, E., Heyman, B., Moens, K., Fischer, S., Verwijlen, T., Moldenaers, P., . . . Dewettinck, K. (2013). Monoacylglycerols in dairy recombined cream: II. The effect on partial coalescence and whipping properties. *Food Research International, 51*(2), 936-945. doi:https://doi.org/10.1016/j.foodres.2013.02.006

Fredrick, E., Walstra, P., & Dewettinck, K. (2010). Factors governing partial coalescence in oil-in-water emulsions. *Advances in Colloid and Interface Science, 153*(1), 30-42. doi:https://doi.org/10.1016/j.cis.2009.10.003

Fuller, G. T. (2015). *Studies on the shear stability of partially crystalline oil-in-water emulsions : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology at Massey University, Palmerston North, New Zealand.* Massey University,

Gaupp, R., & Adams, W. (2014). Acid Esters of Mono- and Diglycerides. In *Emulsifiers in Food Technology* (pp. 93-120).

Goff, H. D. (1997). Instability and Partial Coalescence in Whippable Dairy Emulsions. *Journal of Dairy Science, 80*(10), 2620-2630. doi:10.3168/jds.S0022-0302(97)76219-2

Guery, J., Bertrand, E., Rouzeau, C., Levitz, P., Weitz, D. A., & Bibette, J. (2006). Irreversible Shear-Activated Aggregation in Non-Brownian Suspensions. *Physical review letters, 96*, 198301. doi:10.1103/PhysRevLett.96.198301

Gülseren, İ., & Coupland, J. N. (2008). Surface Melting in Alkane Emulsion Droplets as Affected by Surfactant Type. *Journal of the American Oil Chemists' Society, 85*(5), 413-419. doi:https://doi.org/10.1007/s11746-008-1216-z

Gunning, P. A., Mackie, A. R., Gunning, A. P., Woodward, N. C., Wilde, P. J., & Morris, V. J. J. B. (2004). Effect of surfactant type on surfactant− protein interactions at the air− water interface. *5*(3), 984-991.

Haddadian, Z., Bremer, P., Eyres, G. T., Carne, A., & Everett, D. W. (2016). The impact of cream churning conditions on xanthine oxidase activity and oxidation–reduction potential in model emulsion systems. *International Dairy Journal, 60*, 55-61. doi:https://doi.org/10.1016/j.idairyj.2016.01.017

Han, J., Zhou, X., Cao, J., Wang, Y., Sun, B., Li, Y., & Zhang, L. (2018). Microstructural evolution of whipped cream in whipping process observed by confocal laser scanning microscopy. *International Journal of Food Properties, 21*(1), 593-605. doi:10.1080/10942912.2018.1437630

Hartel, R. W., & Hasenhuettl, G. L. (2019). *Food emulsifiers and their applications* (Third edition ed.): Springer.

Hayati, I. N., Aminah, A., Mamot, S., Aini, I. N., Lida, H. N., & Sabariah, S. J. J. o. F. L. (2000). Melting characteristic and solid fat content of milk fat and palm stearin blends before and after enzymatic interesterification. *7*(3), 175-193.

Heymans, R., Tavernier, I., Dewettinck, K., & Van der Meeren, P. (2017). Crystal stabilization of edible oil foams. *Trends in Food Science & Technology, 69*, 13-24. doi:https://doi.org/10.1016/j.tifs.2017.08.015

Hillbrick, G., Augustin, M., & Udabage, P. (2006). Whipping properties of dairy creams. *Food Australia, 58*(4), 151-154.

Hoffmann, W. (2016). Cream: Manufacture. In *Reference Module in Food Science*: Elsevier.

Huang, Z., Zheng, H., Brennan, C. S., Mohan, M. S., Stipkovits, L., Li, L., & Kulasiri, D. (2020). Production of Milk Phospholipid-Enriched Dairy Ingredients. *Foods (Basel, Switzerland), 9*(3), 263. doi:10.3390/foods9030263

Ihara, K., Habara, K., Ozaki, Y., Nakamura, K., Ochi, H., Saito, H., . . . Iwatsuki, K. (2010). Influence of whipping temperature on the whipping properties and rheological characteristics of whipped cream. *Journal of Dairy Science, 93*(7), 2887-2895. doi:https://doi.org/10.3168/jds.2009-3012

Jakubczyk, E., & Niranjan, K. (2006). Transient development of whipped cream properties. *Journal of Food Engineering, 77*(1), 79-83. doi:https://doi.org/10.1016/j.jfoodeng.2005.06.046

Janssen, P. W. M., & MacGibbon, A. K. H. (2007). Non-Isothermal Crystallization of Bovine Milk Fat. *Journal of the American Oil Chemists' Society, 84*(9), 871-875. doi:10.1007/s11746-007-1105-x

Jensen, R. G., Ferris, A. M., & Lammi-Keefe, C. J. J. J. o. D. S. (1991). The composition of milk fat. *74*(9), 3228-3243.
Jiang, J., Jin, Y., Liang, X., Piatko, M., Campbell, S., Lo, S. K., & Liu, Y. (2018). Synergetic interfacial adsorption of protein and low-molecular-weight emulsifiers in aerated emulsions. *Food Hydrocolloids, 81*, 15-22. doi:https://doi.org/10.1016/j.foodhyd.2018.02.038
Kaufmann, N., Andersen, U., & Wiking, L. J. I. D. J. (2012). The effect of cooling rate and rapeseed oil addition on the melting behaviour, texture and microstructure of anhydrous milk fat. *25*(2), 73-79.
Krog, N. (1997). *Food emulsifiers and their chemical and physical properties*.
Li, S., Ye, A., & Singh, H. (2020). Effect of seasonal variations on the acid gelation of milk. *Journal of Dairy Science, 103*(6), 4965-4974. doi:https://doi.org/10.3168/jds.2019-17603
Li, S., Ye, A., & Singh, H. (2022). Seasonal variations in the characteristics of milk fat and the whipping properties of cream. *International Dairy Journal, 127*, 105288. doi:https://doi.org/10.1016/j.idairyj.2021.105288
Lopez, C., Bourgaux, C., Lesieur, P., Bernadou, S., Keller, G., & Ollivon, M. (2002). Thermal and Structural Behavior of Milk Fat: 3. Influence of Cooling Rate and Droplet Size on Cream Crystallization. *Journal of Colloid and Interface Science, 254*(1), 64-78. doi:https://doi.org/10.1006/jcis.2002.8548
Lupi, F. R., Gabriele, D., Facciolo, D., Baldino, N., Seta, L., & de Cindio, B. (2012). Effect of organogelator and fat source on rheological properties of olive oil-based organogels. *Food Research International, 46*(1), 177-184. doi:https://doi.org/10.1016/j.foodres.2011.11.029
Márquez, A. L., & Wagner, J. R. (2012). Rheology of Cream-like Emulsions Prepared with Soybean Milk and Low Trans Vegetable Fat. *89*(10), 1857-1865. doi:https://doi.org/10.1007/s11746-012-2093-z
Martini, S., Herrera, M., & Hartel, R. J. J. o. t. A. O. C. S. (2002). Effect of cooling rate on crystallization behavior of milk fat fraction/sunflower oil blends. *79*(11), 1055-1062.
McCarthy, O. J. (2011). Plant and Equipment | Centrifuges and Separators: Applications in the Dairy Industry. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences (Second Edition)* (pp. 175-183). San Diego: Academic Press.
Meagher, L. P., Holroyd, S. E., Illingworth, D., van de Ven, F., & Lane, S. (2007). At-Line Near-Infrared Spectroscopy for Prediction of the Solid Fat Content of Milk Fat from New Zealand Butter. *Journal of Agricultural and Food Chemistry, 55*(8), 2791-2796. doi:10.1021/jf063215m
Michon, B. M. G., Granda, P., Mat, D. J. L., Souchon, I., Le Feunteun, S., & Michon, C. (2019). Scale-down emulsion homogenisation: Conditions to mimic pilot homogenizer depending on the emulsifier. *Journal of Food Engineering, 261*, 117-124. doi:https://doi.org/10.1016/j.jfoodeng.2019.06.003
Moens, K., De Clercq, N., Verstringe, S., & Dewettinck, K. (2015). Revealing the Influence of Tempering on Polymorphism and Crystal Arrangement in Semicrystalline Oil-in-Water Emulsions. *Crystal Growth & Design, 15*(12), 5693-5704. doi:10.1021/acs.cgd.5b00665
Monteny, I. (2015). *Partial coalescence in recombined cream as influenced by fat composition.* Universiteit Gent
Moonen, H., & Bas, H. (2014). Mono- and Diglycerides. In *Emulsifiers in Food Technology* (pp. 73-92).
Munk, M. B., Larsen, F. H., van den Berg, F. W. J., Knudsen, J. C., & Andersen, M. L. (2014). Competitive Displacement of Sodium Caseinate by Low-Molecular-Weight Emulsifiers and the Effects on Emulsion Texture and Rheology. *Langmuir, 30*(29), 8687-8696. doi:10.1021/la5011743
Mutoh, T. A., Nakagawa, S., Noda, M., Shiinoki, Y., & Matsumura, Y. (2001). Relationship between characteristics of oil droplets and solidification of thermally treated creams. *JAOCS, Journal of the American Oil Chemists' Society, 78*(2), 177-182. doi:10.1007/s11746-001-0240-0
Norris, G. E., Gray, I. K., & Dolby, R. M. (1973). Seasonal variations in the composition and thermal properties of New Zealand milk fat: II. Thermal properties of milk fat and their relation to composition. *Journal of Dairy Research, 40*(3), 311-321. doi:10.1017/S0022029900014680
O'Sullivan, M. G. (2017). Instrumental Assessment of the Sensory Quality of Food and Beverage Products. In M. G. O'Sullivan (Ed.), *A Handbook for Sensory and Consumer-Driven New Product Development* (pp. 151-175): Woodhead Publishing.
O'Regan, J., & Mulvihill, D. M. (2010). Sodium caseinate–maltodextrin conjugate stabilized double emulsions: Encapsulation and stability. *Food Research International, 43*(1), 224-231. doi:https://doi.org/10.1016/j.foodres.2009.09.031
Pelan, B. M. C., Watts, K. M., Campbell, I. J., & Lips, A. (1997). The Stability of Aerated Milk Protein Emulsions in the Presence of Small Molecule Surfactants. *Journal of Dairy Science, 80*, 2631-2638.

Pérez, M. P., Tesei, M. F., Wagner, J. R., & Márquez, A. L. (2014). Partial coalescence in cream-like emulsions prepared with alternative fats: Effect of controlled stirring and temperature cycles. *Journal of Texture Studies, 45*(5), 396-407. doi:10.1111/jtxs.12090

Phan, T. T. Q., Le, T. T., Van der Meeren, P., & Dewettinck, K. (2014). Comparison of emulsifying properties of milk fat globule membrane materials isolated from different dairy by-products. *Journal of Dairy Science, 97*(8), 4799-4810. doi:https://doi.org/10.3168/jds.2014-8030

Rajah, K. (1991). *Anhydrous milk fat and fractionated products*.

Segall, K. I., & Goff, H. D. (1999). Determination of protein surface concentration for emulsions containing a partially crystalline dispersed phase. *Food Hydrocolloids, 13*(4), 291-297. doi:https://doi.org/10.1016/S0268-005X(99)00011-9

Shamsi, K., Che Man, Y., Yusoff, M., & Jinap, S. (2002). A comparative study of dairy whipping cream and palm oil-based whipping cream in terms of FA composition and foam stability. *Journal of the American Oil Chemists' Society, 79*(6), 583-588.

Shin, J.-A., Heo, Y.-J., & Lee, K.-T. (2019). Physicochemical characteristics of fat blend from hydrogenated coconut oil and acyl migrated palm mid-fraction. *Food Chemistry, 275*, 739-745. doi:https://doi.org/10.1016/j.foodchem.2018.09.131

Sjöblom, J. (2006). *Emulsions and emulsion stability* (2nd ed ed.): Taylor & Francis.

Smith, Bhaggan, Talbot, & Malssen, v. (2011). Crystallization of Fats: Influence of Minor Components and Additives. *Journal of the American Oil Chemists' Society, 88*(8), 1085-1101. doi:10.1007/s11746-011-1819-7

Smith, Kakuda, Y., & Goff, H. D. (2000). Changes in protein and fat structure in whipped cream caused by heat treatment and addition of stabilizer to the cream. *Food Research International, 33*(8), 697-706. doi:https://doi.org/10.1016/S0963-9969(00)00115-0

Southward, C. R., & Creamer, L. K. (1997). INTRODUCTION TO THE NEW ZEALAND DAIRY INDUSTRY. Retrieved from https://nzic.org.nz/app/uploads/2017/10/3A.pdf#:~:text=The%20major%20components%20in%20New%20Zealand%20cows%E2%84%A2%20milk,major%20contributor%20to%20the%20economy%20of%20this%20country.

Sukhija, P. S., & Palmquist, D. L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry, 36*(6), 1202-1206. doi:10.1021/jf00084a019

Thivilliers, F., Laurichesse, E., Saadaoui, H., Leal-Calderon, F., & Schmitt, V. (2008). Thermally Induced Gelling of Oil-in-Water Emulsions Comprising Partially Crystallized Droplets: The Impact of Interfacial Crystals. *Langmuir, 24*(23), 13364-13375. doi:10.1021/la802521f

van Lent, K., Le, C. T., Vanlerberghe, B., & Van der Meeren, P. (2008). Effect of formulation on the emulsion and whipping properties of recombined dairy cream. *International Dairy Journal, 18*(10), 1003-1010. doi:https://doi.org/10.1016/j.idairyj.2008.04.002

Vanapalli, S. A., Palanuwech, J., & Coupland, J. N. (2002). Influence of fat crystallization on the stability of flocculated emulsions. *Journal of Agricultural and Food Chemistry, 50*(18), 5224-5228.

Walstra. (2003). *Physical chemistry of foods.* . New York: Marcel Dekker.

Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). *Dairy Science and technology: Second Edition* CRC Press Taylor & Francis Group.

Wang, Y., Hartel, R. W., & Zhang, L. (2021). The stability of aerated emulsions: Effects of emulsifier synergy on partial coalescence and crystallization of milk fat. *Journal of Food Engineering, 291*, 110257. doi:https://doi.org/10.1016/j.jfoodeng.2020.110257

Watson, P. D., & Tittsler, R. P. (1961). The Density of Milk at Low Temperatures1. *Journal of Dairy Science, 44*(3), 416-424. doi:https://doi.org/10.3168/jds.S0022-0302(61)89758-0

Wilde, P., Mackie, A., Husband, F., Gunning, P., & Morris, V. (2004). Proteins and emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science, 108-109*, 63-71. doi:https://doi.org/10.1016/j.cis.2003.10.011

Xiao, Z. (2020). *The development of two types of emulsifier-free ice cream : a thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology at Massey University, Manawatū, New Zealand*.

Zetasizer Range. (2021). Retrieved from https://www.malvernpanalytical.com/en/products/product-range/zetasizer-range/

10. Appendix

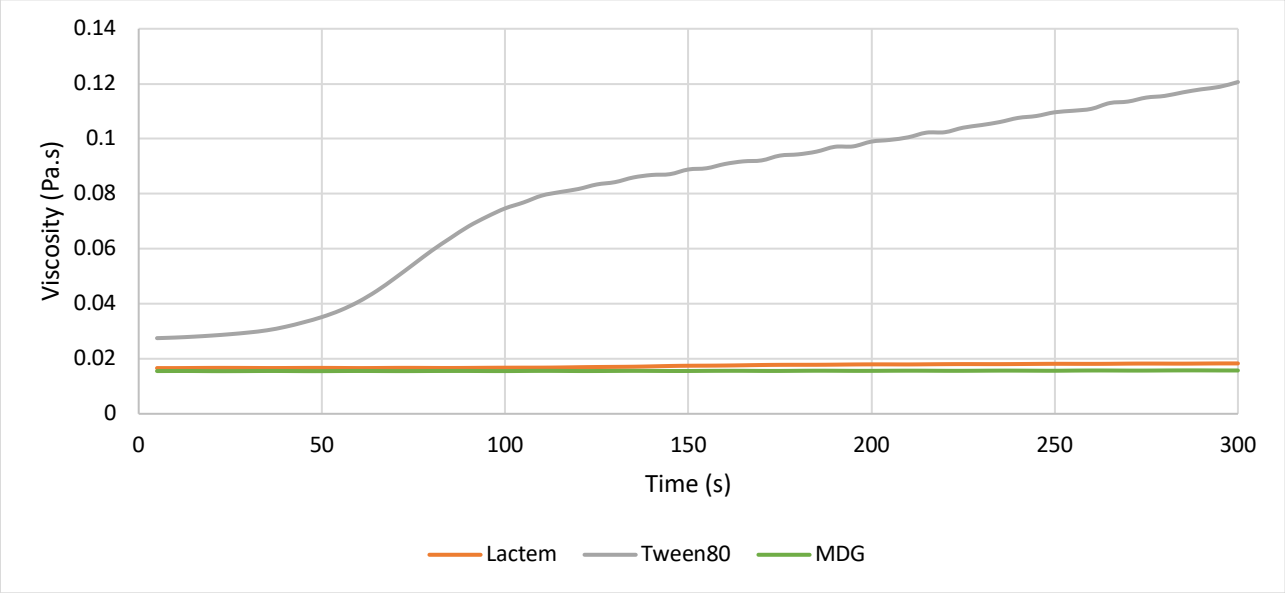


Figure 41: Viscosity versus time graph at 500s[1], showing only emulsions containing Tween80 destabilising under shear.

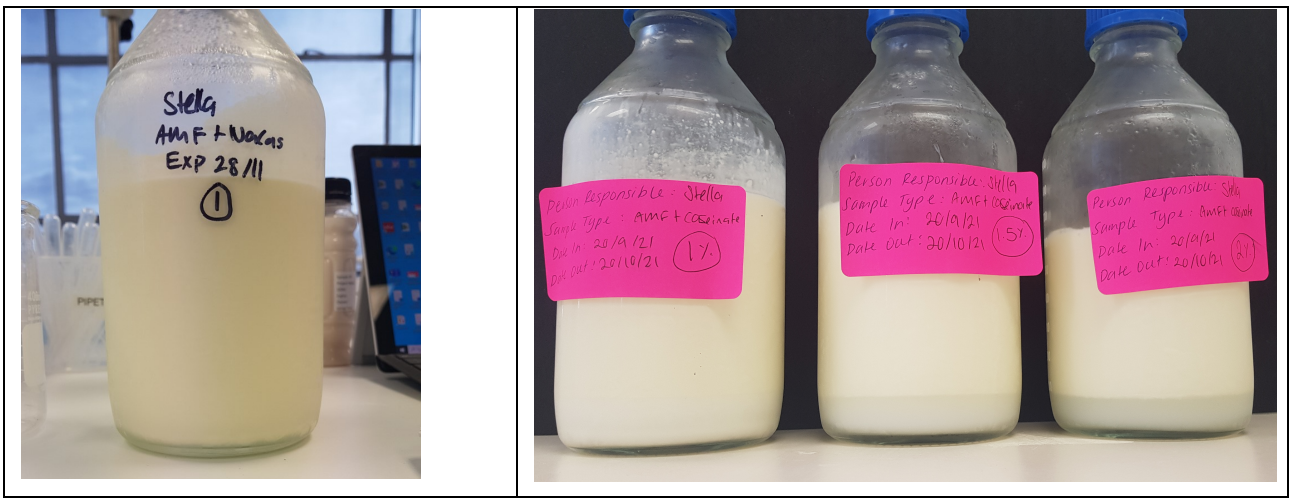


Figure 42: Emulsions prepared with 0.5%, 1%, 1.5% and 2% NaCas showing signs of bridging flocculation (0.5%) and depletion flocculation (1.5%, 2%) after 24 hours of storage at 4°C.

Table 7: Composition of emulsions from chapter 6, measured on the MilkoScan.

Sample	Fat (%)	Protein (%)	Total Solids (%)	Total Solids Non-Fat (%)
Control (1)	36.66	0.95	40.51	4.96
Control (2)	36.54	0.94	40.60	4.97
LACTEM (1)	36.31	0.92	40.88	4.96

LACTEM (2)	36.65	0.93	41.11	4.91
MDG (1)	37.29	0.94	40.37	4.99
MDG (2)	36.98	0.93	40.96	4.97
Tween80 (1)	36.53	0.92	40.98	4.91
Tween80 (2)	37.73	0.93	40.92	4.95

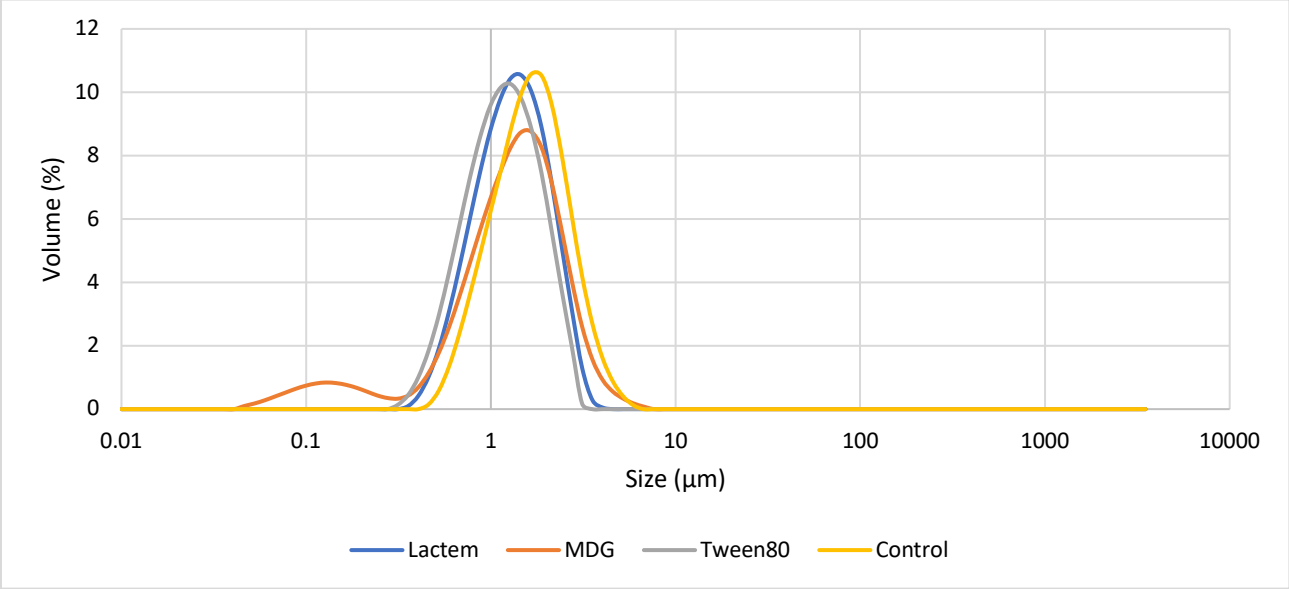


Figure 43: Fat globule size distribution for emulsions containing either LACTEM, MDG, Tween80 or no emulsifiers, measured 7 days after homogenisation.

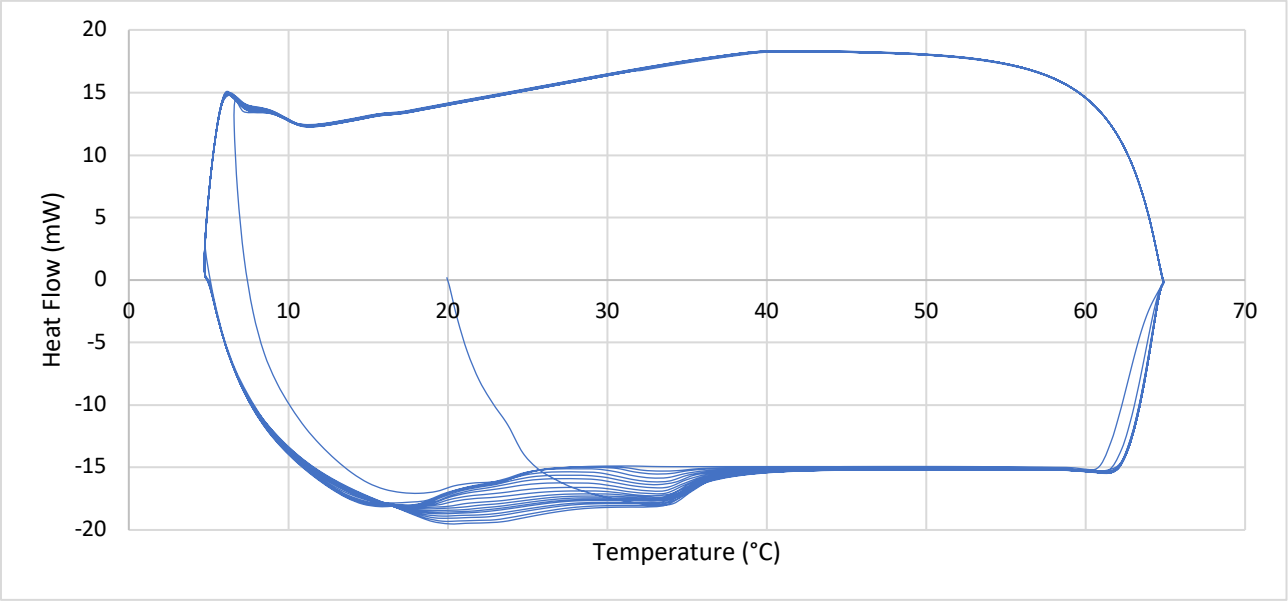


Figure 44: Heat flow versus temperature graph for the chapter 6 control formulation (no emulsifiers) after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

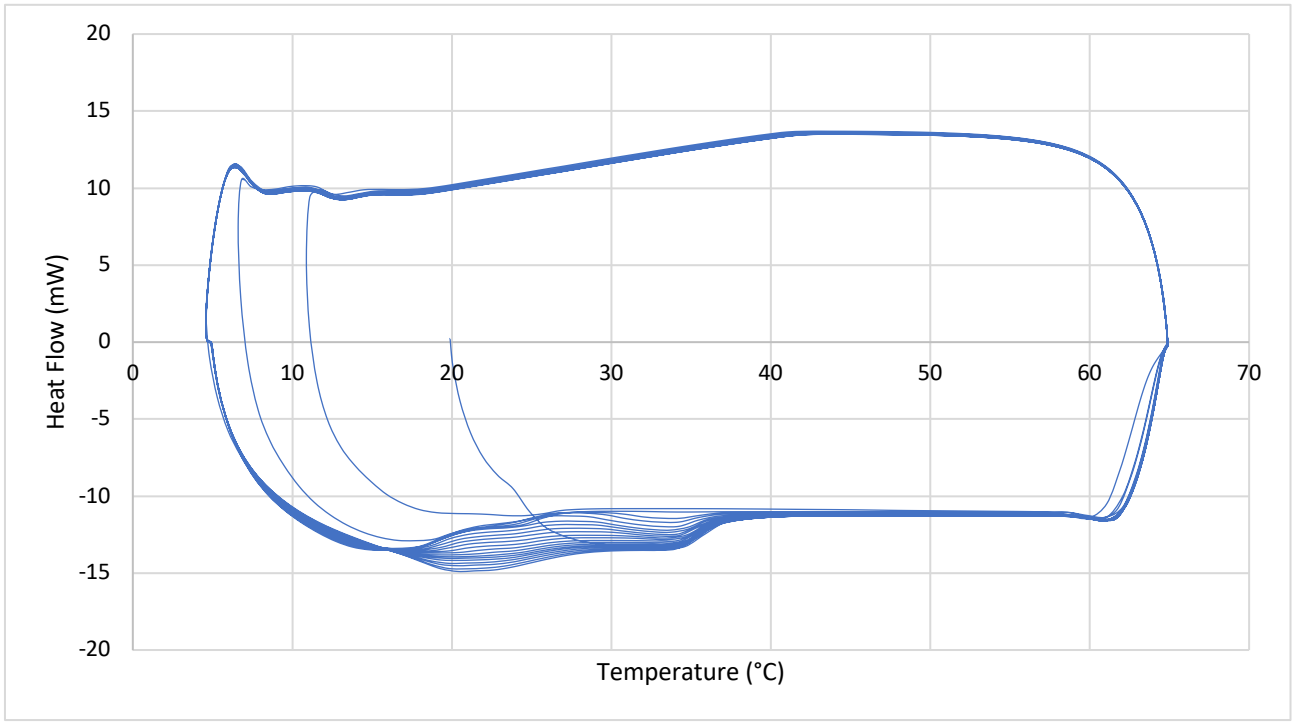


Figure 45: Heat flow versus temperature graph for the LACTEM formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

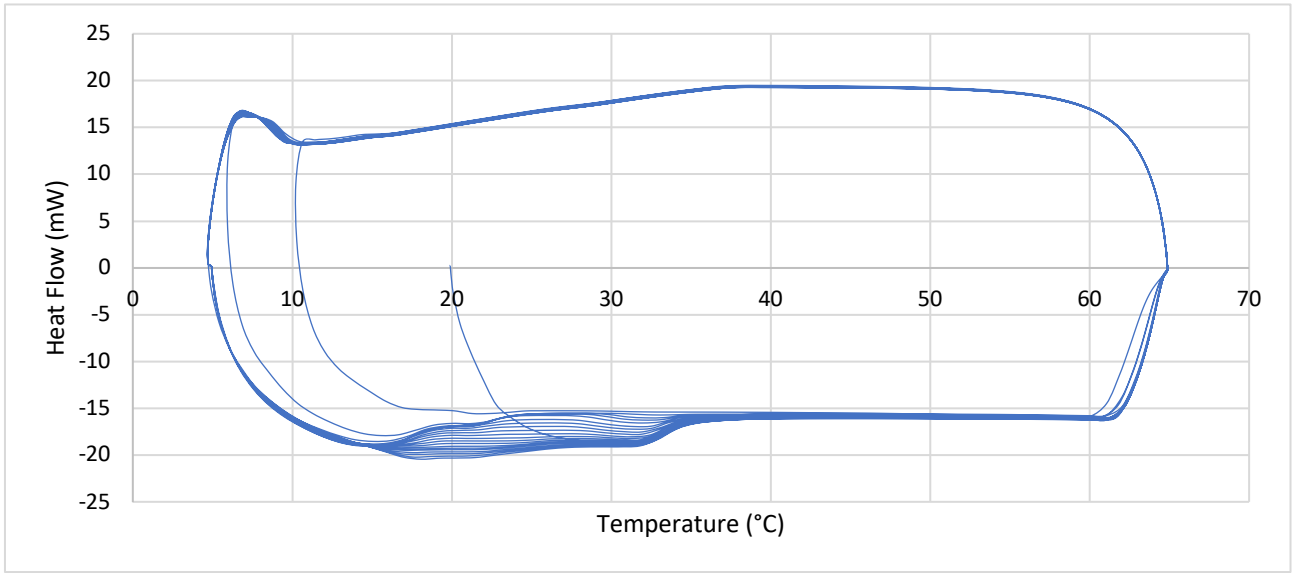


Figure 46: Heat flow versus temperature graph for the MDG formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

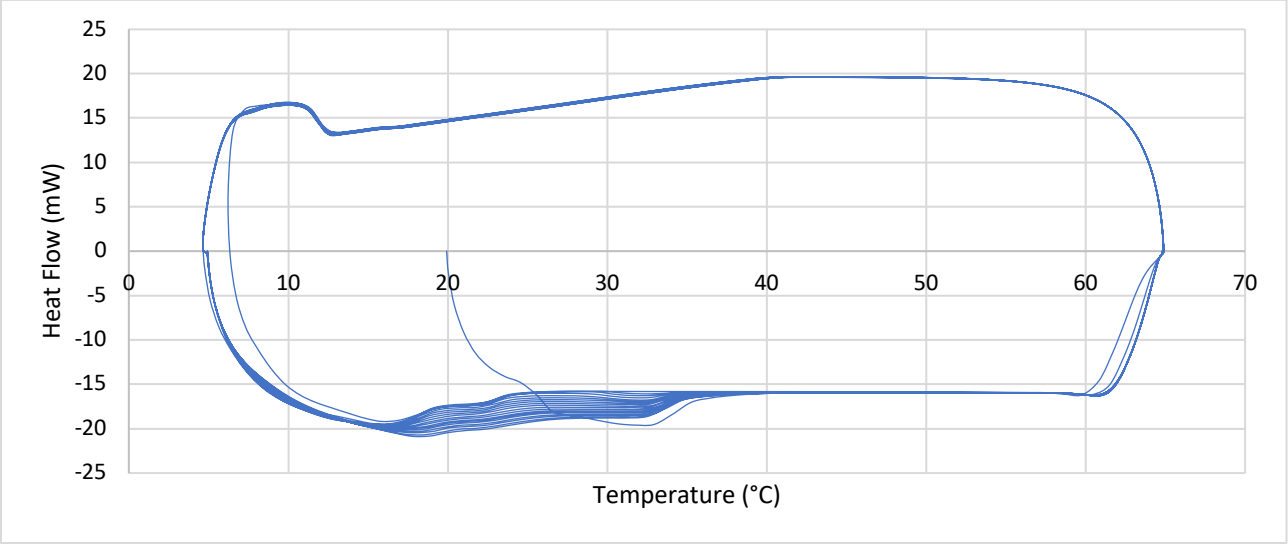


Figure 47: Heat flow versus temperature graph for the Tween80 formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an across from emulsion replicates.

Table 8: Composition of emulsions from chapter 7, measured on the MilkoScan.

Sample	**Fat (%)**	**Protein (%)**	**Total Solids (%)**	**Total Solids Non-Fat (%)**
Control (1)	36.6	0.91	40.5	4.96
Control (2)	36.71	0.94	40.96	4.97
HMF/AMF (1)	36.61	0.93	40.88	4.96
HMF/AMF (2)	36.92	0.91	41.11	4.91
LMF/AMF (1)	36.33	0.91	40.37	4.99
LMF/AMF (2)	36.32	0.94	40.48	5.01
Palm/AMF (1)	36.02	0.95	40.29	5.07
Palm/AMF (1)	35.67	0.96	39.02	5.27
Canola/AMF (1)	36.11	0.94	40.32	5.04
Canola/AMF (2)	35.43	0.96	38.76	5.29
Canola/Palm/AMF (1)	35.01	0.96	38.66	5.28
Canola/Palm/AMF (2)	35.21	0.96	38.88	5.28
Canola/HMF (1)	36.08	0.95	40.44	5.01
Canola/AMF (2)	36.11	0.96	40.35	5.04

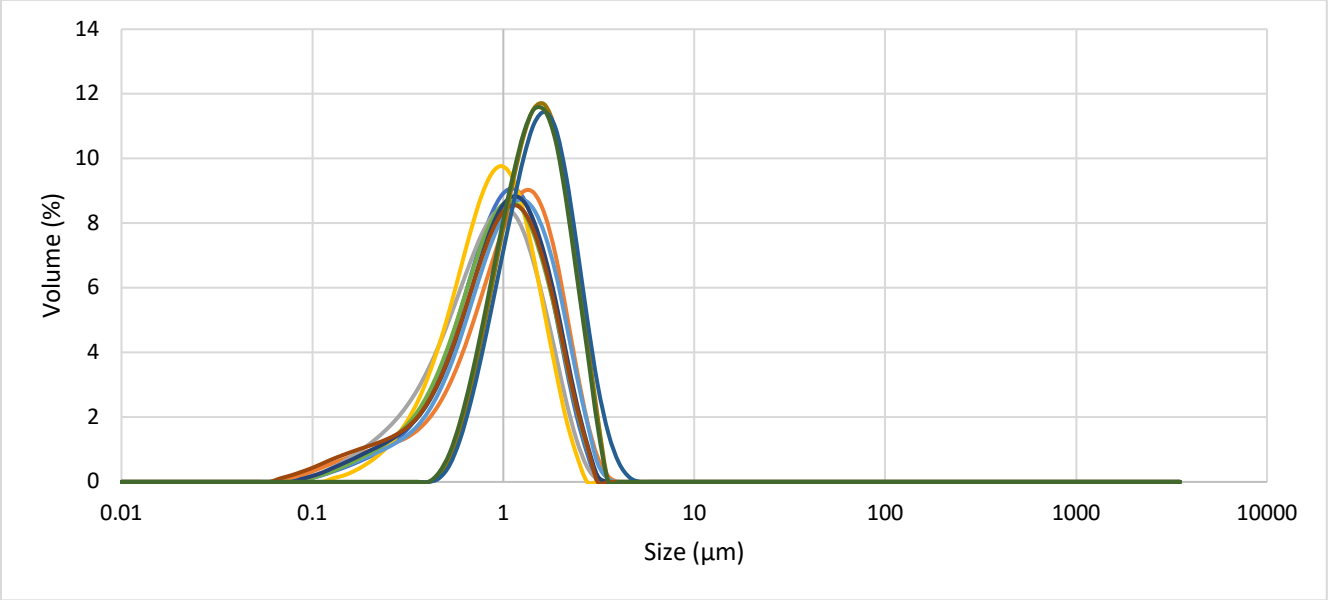


Figure 48: Fat globule size distribution for emulsions containing blends of AMF, canola oil or hard fraction palm oil, measured 7 days after homogenisation.

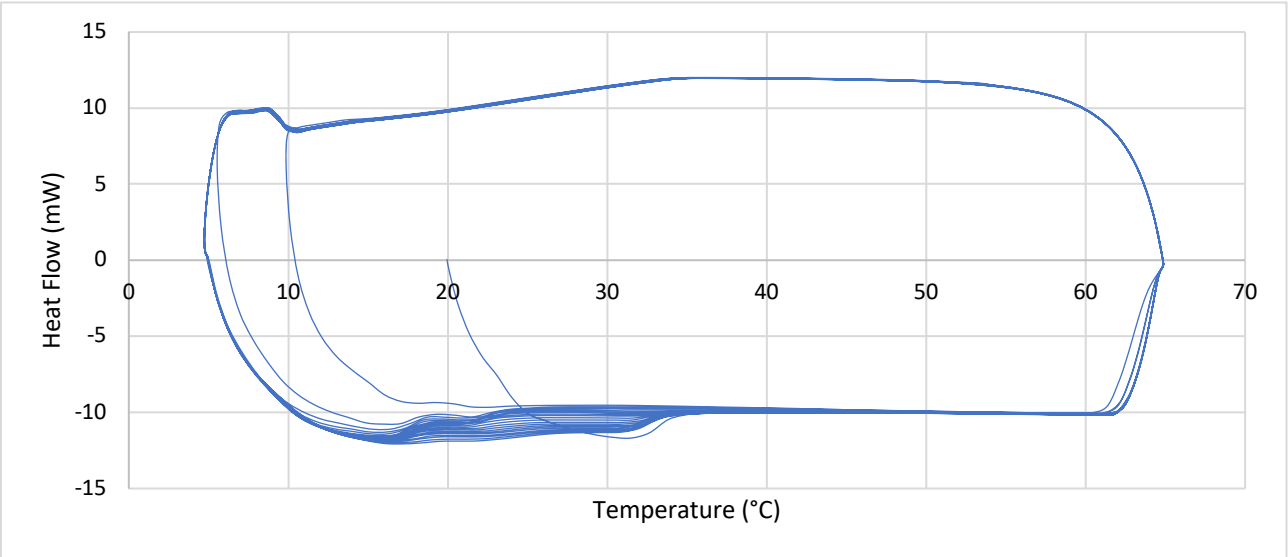


Figure 49: Heat flow versus temperature graph for the chapter 7 control formulation (AMF) after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

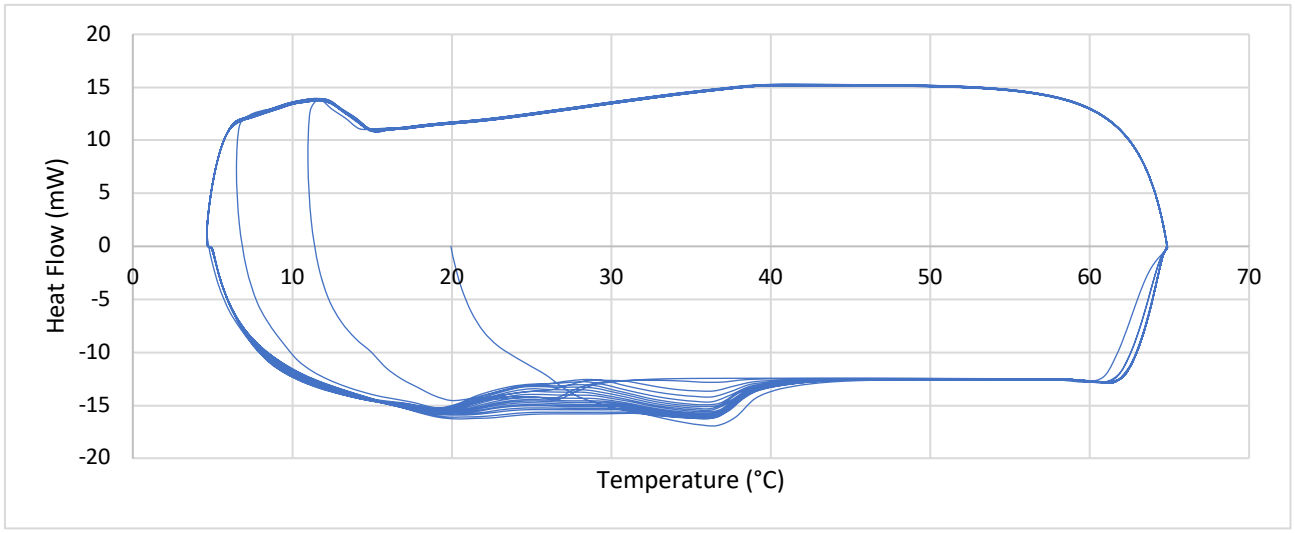


Figure 50: Heat flow versus temperature graph for the HMF/AMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

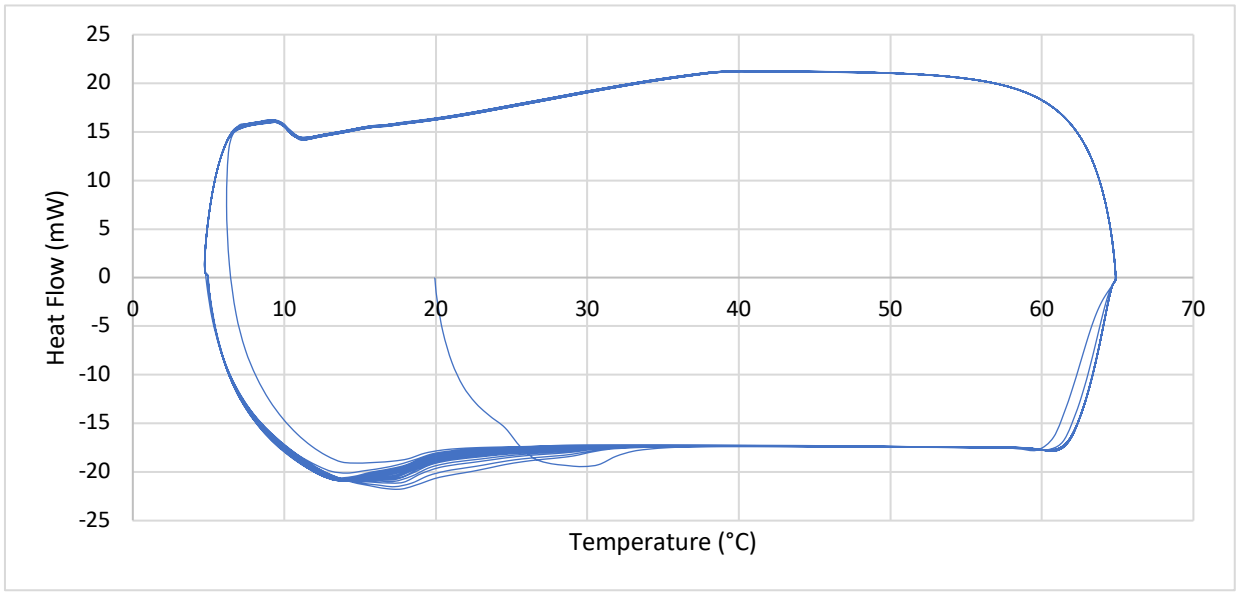


Figure 51: Heat flow versus temperature graph for the LMF/HMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

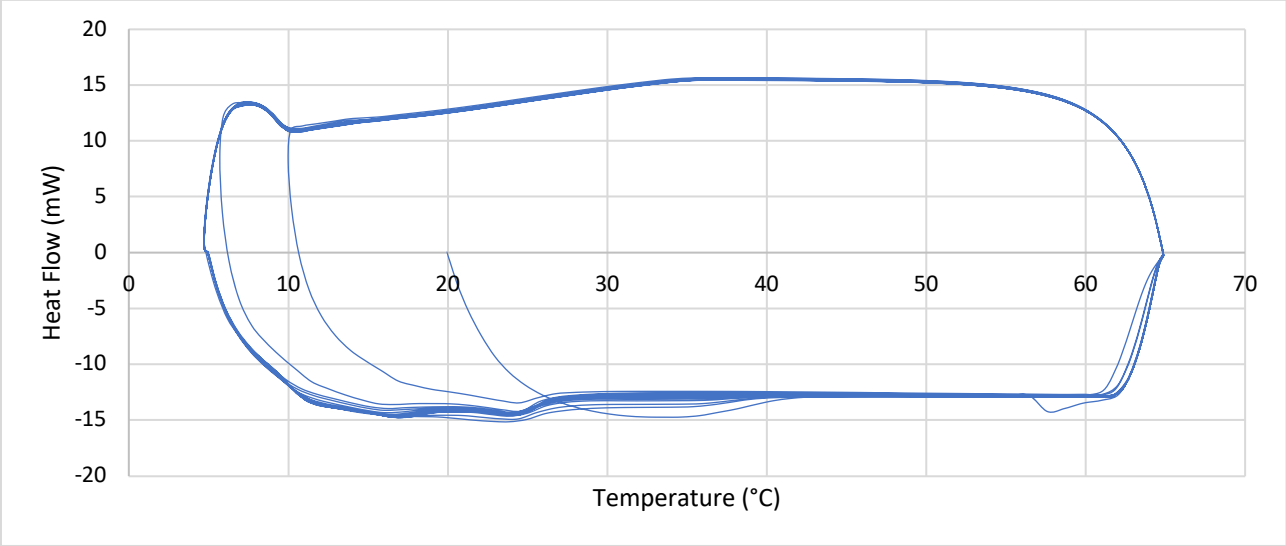


Figure 52: Heat flow versus temperature graph for the Palm/AMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

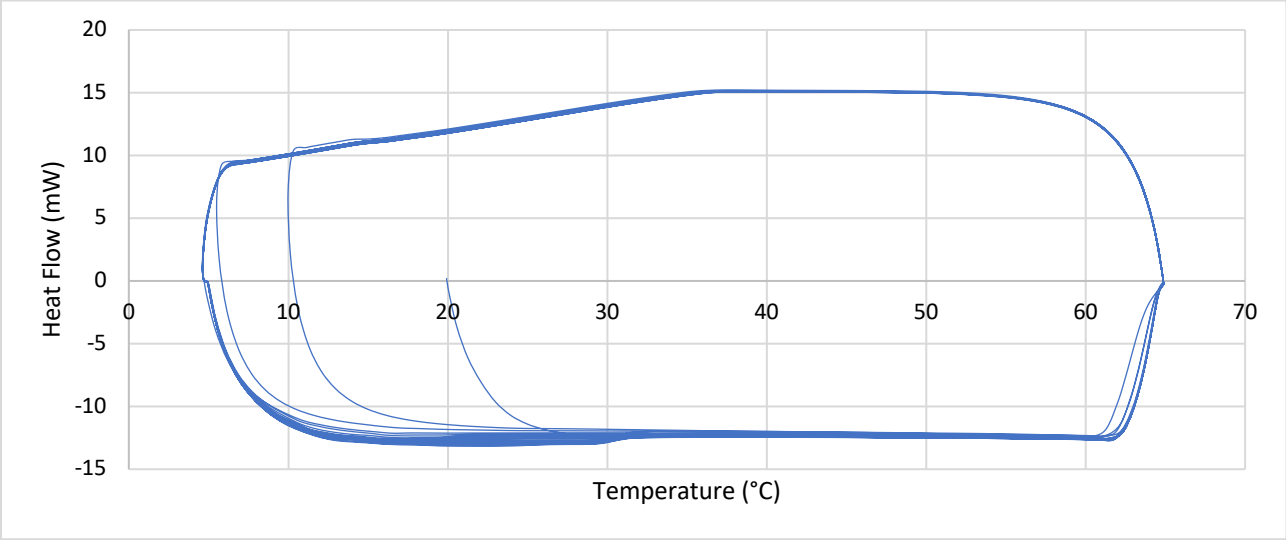


Figure 53: Heat flow versus temperature graph for the canola/AMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

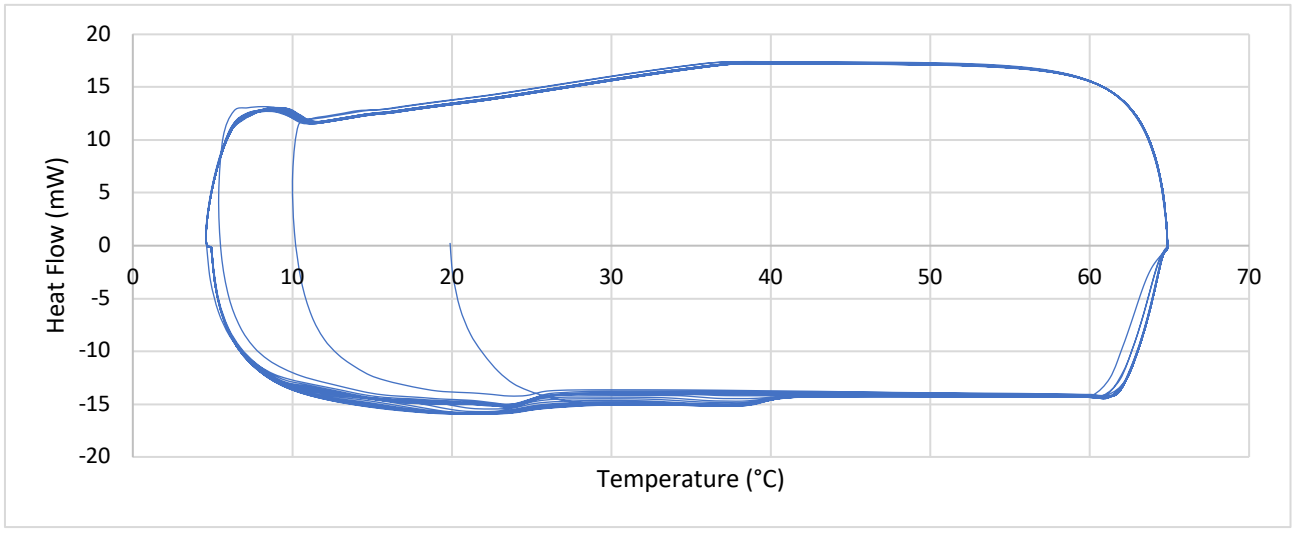


Figure 54: Heat flow versus temperature graph for the Canola/palm/AMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.

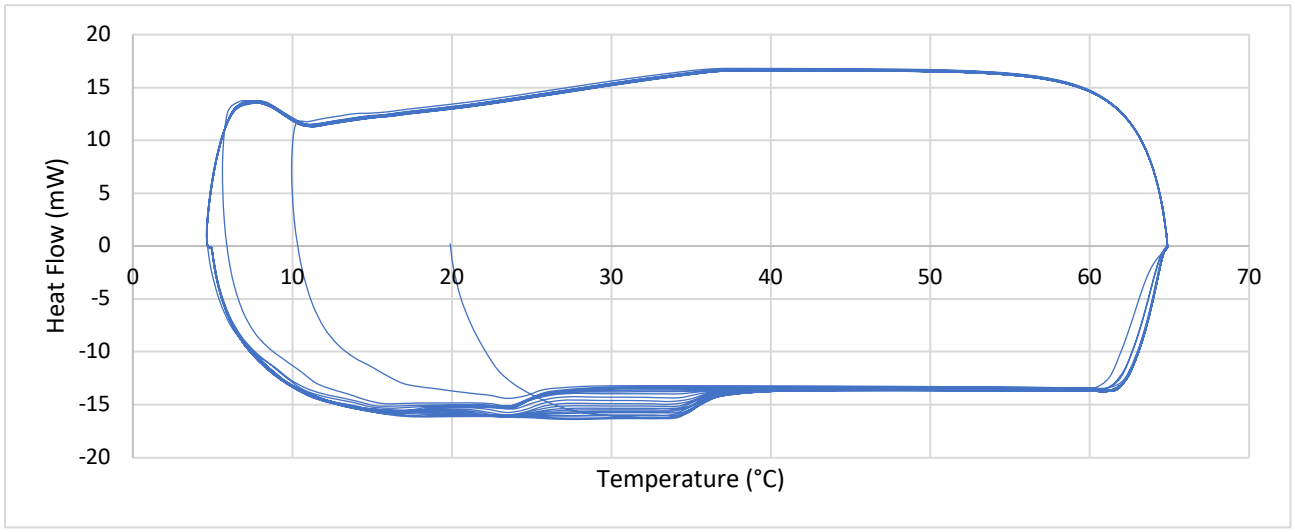


Figure 55: Heat flow versus temperature graph for the Canola/HMF formulation after 19 heating and cooling cycles with increasing crystallization times. Results expressed as an average across emulsion replicates.