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DAIRY WHIPPING CREAM: SYNERGISTIC ROLES OF FAT CRYSTALLISATION AND PARTIAL COALESCENCE



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

Master of Philosophy
in
Food Technology

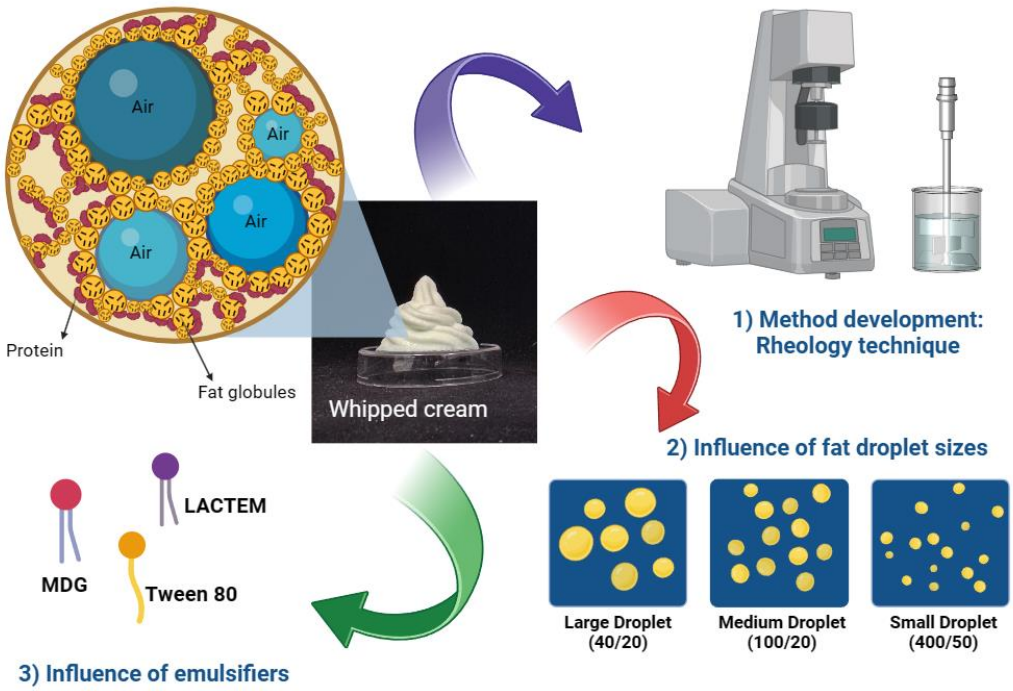
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For
My parents, who teach me.
Lara, Pat, and Emm who guide me.
Amy A.H, who inspires me.

Abstract



The study of fat crystallisation and shear-induced partial coalescence mechanisms has been crucial for the development of aerated colloidal food products like whipping cream, whipped toppings, and ice cream. This research examines the interdependent effects of these factors on the stability, foam structural properties, and underlying mechanisms of oil-in-water (O/W) emulsions in the recombined dairy whipping cream made using anhydrous milk and stabilised by sodium caseinate.

Partial coalescence in O/W emulsions can be influenced by factors such as droplet sizes, shear, air incorporation, and the addition of emulsifiers. This study investigates the impact of fat droplet sizes and emulsifiers on the development of stable whipped cream, with the aim of understanding the complex physico-chemical properties affected by these interrelated factors. The study is divided into three parts: 1) development of a novel technique using a rheometer to monitor the primary mechanism of whipping in the presence of air, 2) investigation of the influence of fat droplet sizes, and 3) examining the effect of different types and concentrations of emulsifiers on whipping properties.

In **Chapter 4**, a rheological setup and methodology were developed to systematically explore the influence of shear-induced partial coalescence in the presence of air in whipping cream systems. This methodology integrates rotational rheology with starch spindle to investigate

the kinetics of partial coalescence in the presence of air by examining the viscosity, particle size, and microstructure of foam and fat droplets in the analysed creams.

In **Chapter 5**, the study found that fat droplet size has a strong influence on emulsion stability and modulates the crystallisation of emulsified milk fat. Large droplets induce faster nucleation and crystal formation on the inner surface of the fat droplet interface, thus triggering nucleation events more quickly than smaller droplets. In contrast, smaller droplets have slower nucleation rates, potentially resulting in delayed or absent crystal formation within the droplets, hindering aggregation and subsequent fat crystallisation, and consequently, partial coalescence within the emulsion. The study also demonstrated that larger fat droplets have lower shear resistance than smaller fat droplets, resulting in shorter whipping times for aerated emulsions. Conversely, smaller fat droplets improve stability by hindering fat crystallisation and partial coalescence, thus extending the whipping time. When evaluating aerated emulsions, larger fat droplets were found to efficiently entrap air cells and encapsulate them with partially coalesced fats, resulting in maximum stability and overrun. Conversely, smaller fat droplets delay crystal nucleation within the droplets, resulting in a less cohesive and stable foam structure.

In **Chapter 6**, research also examined how different types and concentrations of emulsifiers affect homogenised milk creams and their whipping properties. The investigation focused on understanding the effects of low molecular weight (LMW) emulsifiers on fat crystallisation, polymorphic transitions, whipping properties, and foam structure in O/W emulsions. It was found that lipophilic emulsifiers such as MDG and LACTEM showed better adsorption and interfacial displacement compared to hydrophilic emulsifier (Tween 80), resulting in reduced protein adsorption at the O/W interface and mitigating phase separation.

In summary, the study highlights the crucial role of fat droplet size and emulsifiers in determining emulsion stability, crystallisation kinetics, and whipping properties. The insights gained provide valuable information for refining dairy product formulations and processing techniques and contribute to a deeper understanding of fat crystallisation and partial coalescence in emulsified systems. The successful development of this methodology has highlighted its potential for reliable and real-time use of this technique in the future. These results provide an interesting starting point for further work on controlling the partial coalescence of fat droplets and fat crystallisation behavior by selecting appropriate emulsifier formulas according to their different functionality to achieve betrer stability in the aerated emulsions of dairy and non-dairy products.

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List of symbols and abbreviations

α	Capture efficiency
γ	Interfacial tension
ΔH_m	Melting enthalpy
$\Delta\mu$	Difference in chemical potential
ΔP	Laplace pressure
ΔG	Gibbs free energy
η	Viscosity
θ_W	Contact angle measured in aqueous phase
γ_{AW}	Air-in-water interfacial tension
γ_{OW}	Oil-in-water interfacial tension
γ_{WS}	Water-in-solid interfacial tension
d	Distance
D	Fat droplet diameter
$d_{3,2}$	Sauter diameter
$d_{4,3}$	Volume-weighted average
T-t	Time-temperature program
Tm	Melting temperature
μm	Micrometre
α	Alpha
β	Beta
β'	Beta prime
A/O	Oil-in-air
A/W	Air-in-water
AFM	Atomic force microscopy
AMF	Anhydrous milk fat
C	Concentration
CB	Cocoa butter
CFR	Code of federal regulations
CLSM	Confocal laser scanning microscope
Cryo-SEM	Cryo-scanning electron micrographs
Cs	Saturation concentration
DAG	Diglycerides
DIC	Differential interference contrast
DSC	Differential scanning calorimetry
FA	Fatty acid

FC	Fresh cream
FDA	U.S. Food and drug administration
FFA	Free fatty acid
GDT	Geometric dimensioning and tolerancing
GMO	Glyceryl monooleate
HLB	Hydrophilic and lipophilic balances
HMF	High melting point fractions
HPH	High pressure homogeniser
HPLC	High-performance liquid chromatography
HTST	High temperature short-time processing
LACTEM	Lactic acid esters of MDG
LD	Large droplet
LMF	Low melting point fractions
LWM	Low molecular weight
MAG	Monoglycerides
MD	Medium droplet
MDG	Distilled mono and diglycerides
MFG	Milk fat droplets
MFGM	Milk fat droplet membrane
MMF	Medium melting point fractions
ND	Not detected
NMR	Nuclear magnetic resonance
NZMP	New Zealand Milk Products
O/W	Oil-in-water
PGFEs	Polyglycerol fatty acid esters
PKO	Palm kernel oil
PL	Phospholipid
PLM	Polarised light microscope
PMF	Palm mid fraction
PO	Palm oil
Polysorbate 20	Polyoxyethylene sorbitan monolaurate
PS	Palm stearin
PSD	Particle size distribution
RDC	Recombined dairy cream
RO	Reverse osmosis
RSM-CCD	Response surface method - central composite approach
SD	Small droplet

SDS	Sodium dodecyl sulphate
SE	Sucrose ester
SEM	Scanning electron microscopy
SNF	Solids-non-fat
SSL	Sodium stearoyl lactylate
SSS	Tristearin
TAG	Triacylglycerol
TEM	Transmission electron micrographs
TGMS	Triglycerol monostearate
Tween 80	Polysorbate 80
UHT WC	Ultra-high temperature whipping cream
UHT	Ultra-high temperature
WC	Whipping cream
WP	Whey protein
WPH	Whey protein hydrolysates
WPI	Whey protein isolate
WPs	Whey proteins
XRD	X-ray diffraction

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Chapter 1: Introduction

This research is a collaborative effort between the School of Food and Advanced Technology, Massey University of New Zealand and Synlait Milk Limited. The aim of this study was to investigate the mechanisms of fat crystallisation and shear-induced partial coalescence that influence the functionality and stability of recombined dairy whipping creams. This study contributes significantly to supporting sustainable whipping cream production by providing in-depth insights and a comprehensive scientific understanding of the complex interdependent effects of these factors on the stability, foam structural properties and underlying mechanisms of oil-in-water (O/W) emulsions in the recombined dairy whipping cream system.

Dairy cream is a fat-rich milk product characterised by a high fat content and, from a microstructural point of view, is considered an oil-in-water (O/W) emulsion. Whipping cream generally consists of 30 - 36% milk fat (CFR, 2023) and includes native, reconstituted, and recombined whipping cream. A notable feature of whipping cream is its ability to be whipped into rigid triphasic foam. This foam can be defined as an aerated emulsion system that exhibits partial destabilisation of the O/W emulsion, with stabilisation assisted by partially coalesced fat droplets at the air-in-water (A/W) interface (Fredrick et al., 2010a; Li et al., 2020; Xu et al., 2022).

The whipping mechanism is shown in *Figure 1* and can be summarised as follows: (i) agitation introduces large air bubbles into the cream, causing proteins to be rapidly adsorbed at the A/W interface; (ii) the air bubbles are fragmented into smaller bubbles, displacing some proteins from the bubble interface due to the presence of fat droplets; and (iii) mechanical damage to the fat droplets and the presence of large lipid crystals lead to partial coalescence of the fat droplets. Protruding crystals may pierce the film/membrane between approaching fat droplets and cause the cream to become stiff; and (iv) a network of coalesced fat droplets ultimately surrounds and stabilises the air bubbles, preventing the whipped cream from collapsing (Tamime, 2009).

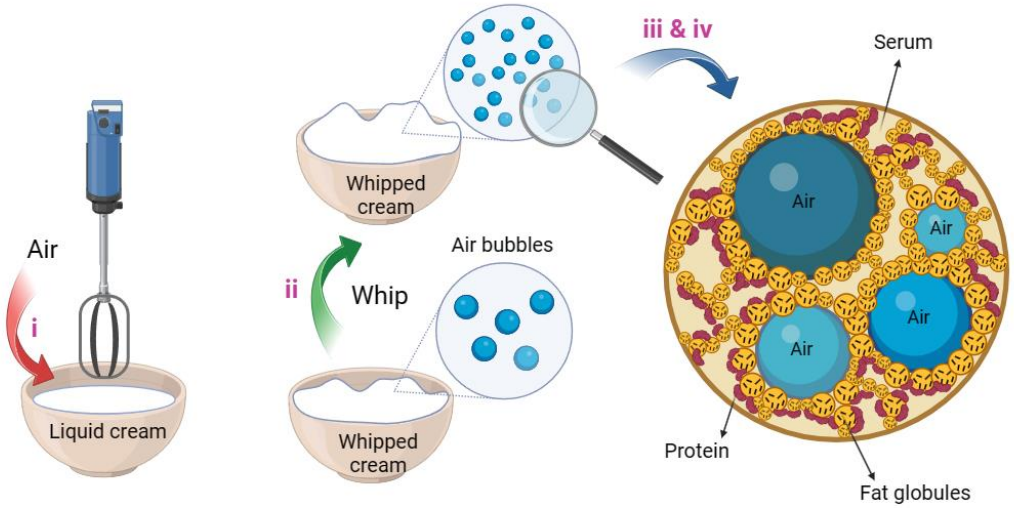


***Figure 1**: Mechanism of whipping.*

Recombined dairy cream (RDC) is an alternative to the native dairy cream. It is an O/W emulsion produced by high-pressure homogenisation (HPH) of a mixture of anhydrous milk fat (AMF) and an aqueous phase. RDCs are typically stabilised with dairy and non-dairy proteins, as well as other stabilisers (Li et al., 2020). The use of RDC offers advantages in terms of transport, storage and possibility of standardising and adjusting the composition and properties of cream, thus minimising the effects of production seasonality. However, to achieve the desired properties of creams like RDC, precise technical preparation is required.

Ideally, whipping creams should be easy to whip and high emulsion stability during storage and transport. It is important to find a balance between stability and destabilisation (Liu et al., 2022). The whippability of dairy cream is typically quantified by monitoring overrun (%), whipping time (*t*), chilled stability, rheological properties, and minimal serum drainage (syneresis). In general, whipped cream with shorter whipping time, relatively higher overrun, and minimum serum drainage is the ideal whipped cream often preferred by consumers (Dhungana et al., 2020).

The partial coalescence of O/W emulsions can be triggered by various factors, including temperature fluctuations, shear, air inclusion, and the addition of emulsifiers. Therefore, this study focuses on the development of a stable recombined dairy whipping cream by understanding the complex physicochemical properties influenced by these interrelated factors. To achieve this, several contributing factors are investigated, including emulsion droplet and fat droplet sizes, interface interactions, and key properties that determine fat crystal distribution at the interface or in the continuous phase, such as the use of low molecular weight (LMW) emulsifiers associated with fat crystallisation and partial coalescence. This

study analyses how these factors influence the stability, whippability, rheology, and microstructural properties of whipped cream.

This work consists of seven chapters and the outline of the thesis with research questions and project objectives, is presented in *Figure 2.*

In brief:

Chapter 1 describes the background and outlines the objectives and main framework of the research project.

Chapter 2 is a literature review of the fundamental knowledge on fat crystallisation and partial coalescence in dairy whipping cream. In addition, important parameters, and methods for studying the underlying mechanisms of fat crystallisation and partial coalescence are discussed.

Chapter 3 highlights the materials and methods used in the research project.

Chapter 4 discusses method development for monitoring the whipping cream using a rheological technique.

Chapter 5 describes the influence of the fat droplet size of homogenised dairy cream on fat crystallisation, partial coalescence, and subsequent whipping properties.

Chapter 6 examines the influence of types and concentrations of emulsifiers of homogenised dairy cream on fat crystallisation, partial coalescence, and subsequent whipping properties.

Chapter 7 summarises all research findings and provides recommendations and directions for future work.

1.1 Research directions and objectives

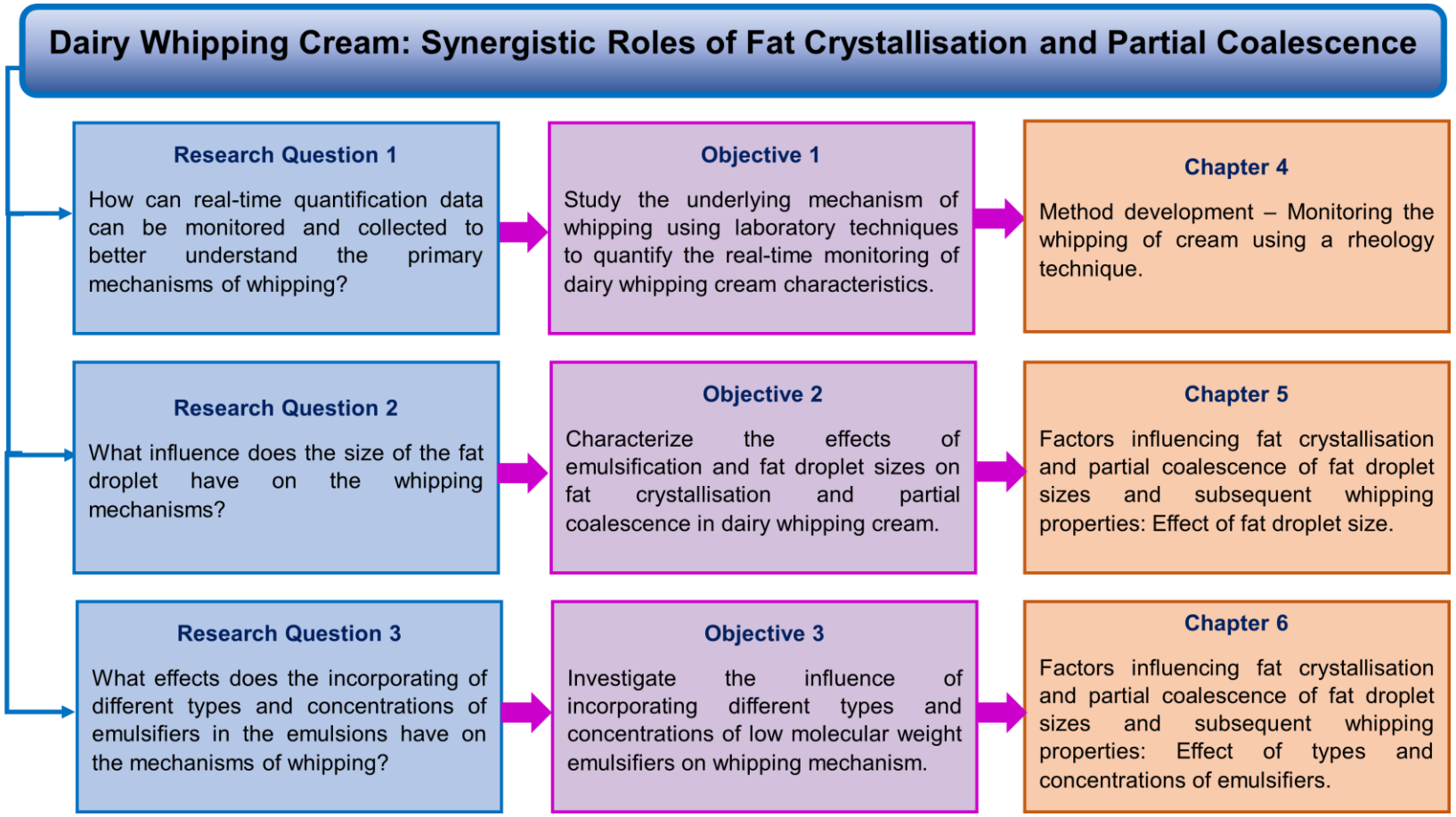


***Figure 2**: An overview of the thesis structure with research questions and project objectives.*

1.2 Rationale and expected benefits

This research is conducted to provide answers and offer pragmatic solutions through scientific explanations. Scientific communities and food manufacturers are the two target groups that could benefit most from the outcomes of this study.

For scientific communities, understanding the complexity of an aerated colloidal food system can provide valuable insight into the underlying mechanisms of partial coalescence and fat crystallisation in an oil-in-water emulsion. The study provides a systematic approach to identify key factors affecting the processes and to develop methods to control and improve the stability of the whipping cream systems.

The study also explores new ideas, disseminate new information and challenges existing theories about the interaction of fat droplets and the interfacial behaviors of protein molecules, whipping phases, and temperature in relation to the rate and extent of partial coalescence. The findings bring existing knowledge to a new level that will help validate and improve research protocols in the aerated whipping cream study.

For food companies, the new and emerging knowledge, techniques, and applications from this study can help optimise commercial interest and high-throughput production. By understanding the conditions under which fat crystallisation and partial coalescence occur and the factors that affect them, companies can produce a consistent, high-quality product that meets consumer expectations. This can help increase customer satisfaction and brand loyalty, ultimately increasing company efficiency and profitability.

Chapter 2: Literature review

2.1 Physicochemical properties of milkfat, natural and recombined creams

Milk fat is one of the most complex natural fats with a distinct combination of triglycerides, that include both long and short chain lengths, which distinguishes it from vegetable oils. In the food industry, dairy fat is valued for its excellent taste, excellent mouthfeel, and distinctive functional properties, particularly in whipped cream applications. It is noteworthy that milk fat contains over 400 fatty acids making it more diverse than vegetable and other animal fats (Huppertz and Kelly, 2006; Jensen et al., 1990). *Table 1* lists the major fatty acids in milk fat triacylglycerols.

***Table 1**: Principal fatty acids in milk fat. Adapted from Bylund, G. (2003).*

Faty acid	Percent of total fatty acid content	Melting point (°C)
Saturated		
Butyric acid ($C_4H_8O_2$)	3.0 – 4.5	-7.9
Caproic acid ($C_6H_{12}O_2$)	1.3 – 2.2	-1.5
Caprylic acid ($C_8H_{16}O_2$)	0.8 – 2.5	16.5
Capric acid ($C_{10}H_{20}O_2$)	1.8 – 3.8	31.4
Lauric acid ($C_{12}H_{24}O_2$)	2.0 – 5.0	43.6
Myristic acid ($C_{14}H_{28}O_2$)	7.0 – 11.0	53.8
Palmitic acid ($C_{16}H_{32}O_2$)	25.0 – 29.0	62.6
Stearic acid ($C_{18}H_{36}O_2$)	7.0 – 3.0	69.3
Unsaturated		
Oleic acid ($C_{18}H_{34}O_2$)	30.0 – 40.0	14.0
Linoleic acid ($C_{18}H_{32}O_2$)	2.0 – 3.0	-5.0
Linolenic acid ($C_{18}H_{30}O_2$)	Up to 1.0	-5.0
Arachidonic acid ($C_{20}H_{32}O_2$)	Up to 1.0	-49.5

Anhydrous milk fat (AMF) is a concentrated product obtained from pasteurised dairy cream. This cream is further processed using phase inversion and vacuum drying to produce a product that contains 99.8% milk fat, 0.1% non-fat solids and 0.1% water (Tamime, 2009). The production process for AMF is shown in *Figure 3* and the compositional differences between milk, cream, and AMF are presented in *Table 2*.

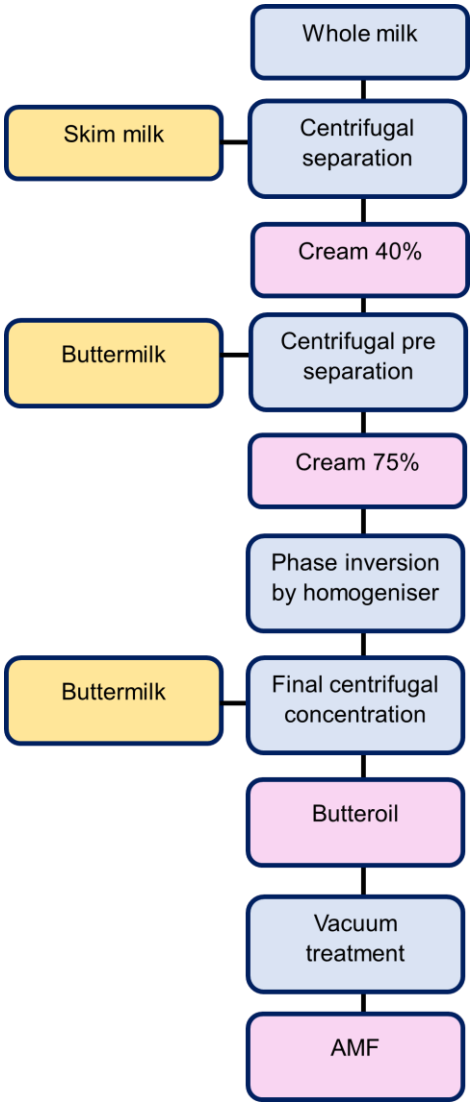


***Figure 3**: Process flow diagram of AMF production from milk. Adapted from Walstra et al. (2005).*

AMF consists of the inner core of milk fat droplets due to the lack of membrane components. Milk fat droplets consist of a triacylglycerol (TAG) core surrounded by a complex membrane known as milk fat droplet membrane (MFGM). MFGM constitutes 2-6% of the total mass of the fat droplet and contains a diverse range of components such as polar lipids, protein, glycoproteins, cholesterol, enzyme, and other bioactive compounds (Keenan and Mather, 2006; Dewettinck et al., 2008).

However, during processing the membrane is damaged and removed. Removal of MFGM during AMF production results in compositional differences between AMF and other dairy products. For instance, AMF lacks the polar lipids and proteins present in MFGM, which have various physiological functions, including acting as emulsifiers, stabilising agents, and bioactive compounds (Dewettinck et al., 2008).

***Table 2**: Typical composition of dairy products in New Zealand (Carr et al., 2003).*

Composition	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%

Dairy cream is a fat-rich fluid milk product derived from the physical separation of milk into skimmed milk and cream. From a microstructural perspective, dairy cream can be characterised as an O/W emulsion consisting of two or more immiscible liquids such as oil and water, with one liquid dispersed in the other (Allen et al., 2008; McCarthy, 2017). Therefore, natural cream derived from fresh milk contains milk lipids consists of these dispersed milk fat droplets within a continuous aqueous skimmed milk phase (Walstra, 2005). It is possible to classify cream according to its fat content, which depending on the level of standardisation (*Table 3*).

***Table 3:** Different types of cream (US Code of Federal Regulations, 2024).*

Type	Milk Fat Content (%)	Uses
Half and half cream	12 (range 10.5 – 18)	Cream in coffee, cooking
Single cream	20	Cooking
Light cream	20 (range 18 – 30)	Coffee or table cream, cooking
Whipping cream	30 – 36	Toppings and fillings
Heavy whipping cream	>36	Whipping
Double cream	48	British whipping cream
Clotted cream	55 – 60	British served with scones

Dairy cream is used not only as an ingredient in the production of many food products but also for direct consumption. Over the last decade, creams recombined from milk constituents have become increasingly popular due to their significant industrial production advantages over fresh cream. The storage cost of raw materials for recombined cream is lower, and the composition and desired properties of the cream can be standardised and changed regardless of the milking season. Additionally, recombined dairy cream may be more cost-effective in regions where fresh milk is scarce or storage and transportation options are limited (Li et al., 2020; Zhou et al., 2017).

Although recombination milk products have been extensively studied (Li et al., 2020; Fredrick et al., 2013; Zhou et al., 2017; van Lent et al., 2008), the early dairy literature paid little attention to the properties of milk produced by recombination formed fat droplets. However,

unlike their natural counterparts, fat droplets are the components that have changed the most due to processing, resulting in different properties (Fredrick et al., 2013: Melsen J.P., 1987).

Over the years, several dairy products have been used in studies to emulsify anhydrous milk fat (AMF) into recombined cream. Most studies have focused on the effects of protein types, fat compositions and emulsifiers on the creaming stability, fat droplet size, rheological properties, microstructure, particle size, cream stability and interfacial tension of recombined cream on the protein surface (Fredrick et al., 2013).

Tomas et al. (1994) prepared O/W recombined cream by combining AMF and skim milk, which were homogenised using a laboratory-scale microfluidiser. They observed that aggregation increased with fat-to-protein mass ratio, while the size of the fat droplets remained unchanged. They found that surface area increased, and protein coverage increased by up to 85% due to large numbers of fat droplets, and that the amount of adsorbed protein decreased slightly with higher fat-to-protein mass ratios. Meanwhile Ihara et al. (2015) investigated the effects of various emulsifying substances on the stability of fat droplets and the properties of whipped cream. They concluded that the addition of a low molecular weight emulsifier increased the stability of the fat droplets and reduced shape retention, while the addition of protein had little effect.

Li et al. (2020) studied the stability of recombined dairy cream with triglycerol monostearate (TGMS) and showed that 0.5% or more TGMS improves stability and whipping properties and effectively displaces almost half of the adsorbed protein. Melsen (1987) examined the stability of the fat emulsion in recombined cream with different fat droplet sizes. Their results showed that recombined cream made from AMF and skimmed milk was significantly more stable to partial coalescence than natural cream. However, phospholipid-containing fat droplets were less resistant. The study also found that the outermost layer of fat droplets is mostly made up of milk proteins.

Whipping the cream creates a firm foam that is often used as a topping for desserts, coffee, pastries and ice cream. In this process, air is incorporated into the cream and the milk fat droplets are destabilised via a partial coalescence mechanism due to the presence of fat crystals and shear forces. Therefore, whipped cream consists of a network of partially coalesced fat droplets that immobilise the air bubbles and the cream-serum phase. Several variables affect the whipping properties of cream, as well as the texture and consistency of whipped cream. Since whipping cream actually represents a destabilisation of the emulsion with partial coalescence of the fat droplets, the interfacial layer should not be too strong. Proteins attach to the fat droplet membrane, forming a steric barrier and reinforcing the

membrane (Matsumura and Matsumiya, 2012). According to Wilde et al. (2007), emulsions become stable when proteins are adsorbed on the surface of fat droplets. Jiang et al., 2018 concluded that different molecular weight of emulsifiers has different interfacial properties that affect the texture of final whipped cream.

On the other hand, LMW surfactants enhance partial coalescence (Goff, 1997) and contribute to shorter whipping times (Allen, 2008). According to Hotrum et al. (2005), cream stabilised with whey protein isolate whips faster than cream stabilised with sodium caseinate. The dairy cream in the presence of stabilisers can affect the texture and stability of the whipped cream, as well as vary the whipping time and overrun (Gafour and Aly, 2020).

2.2 Fat crystallisation in bulk

Due to the wide range of triacylglycerols (TAGs) and other constituents, the crystallisation of milk fat is a complicated process. The network of fat crystals that forms during the processing and storage of milk fat rich dairy products, such as butter, whipped cream, and ice cream, has a significant impact on their physicochemical properties, microstructure and sensory properties (Campos et al., 2002).

Figure 4 provides an overview of the general processes involved in the formation of a fat crystal network in fat crystallisation, where TAG nucleation, growth, aggregation, and sintering contribute to the formation of a continuous, three-dimensional network with small crystals. The properties of the crystal network can be altered by recrystallisation during storage (Walstra et al., 2001; Foubert et al., 2006; Kloek, 1998).

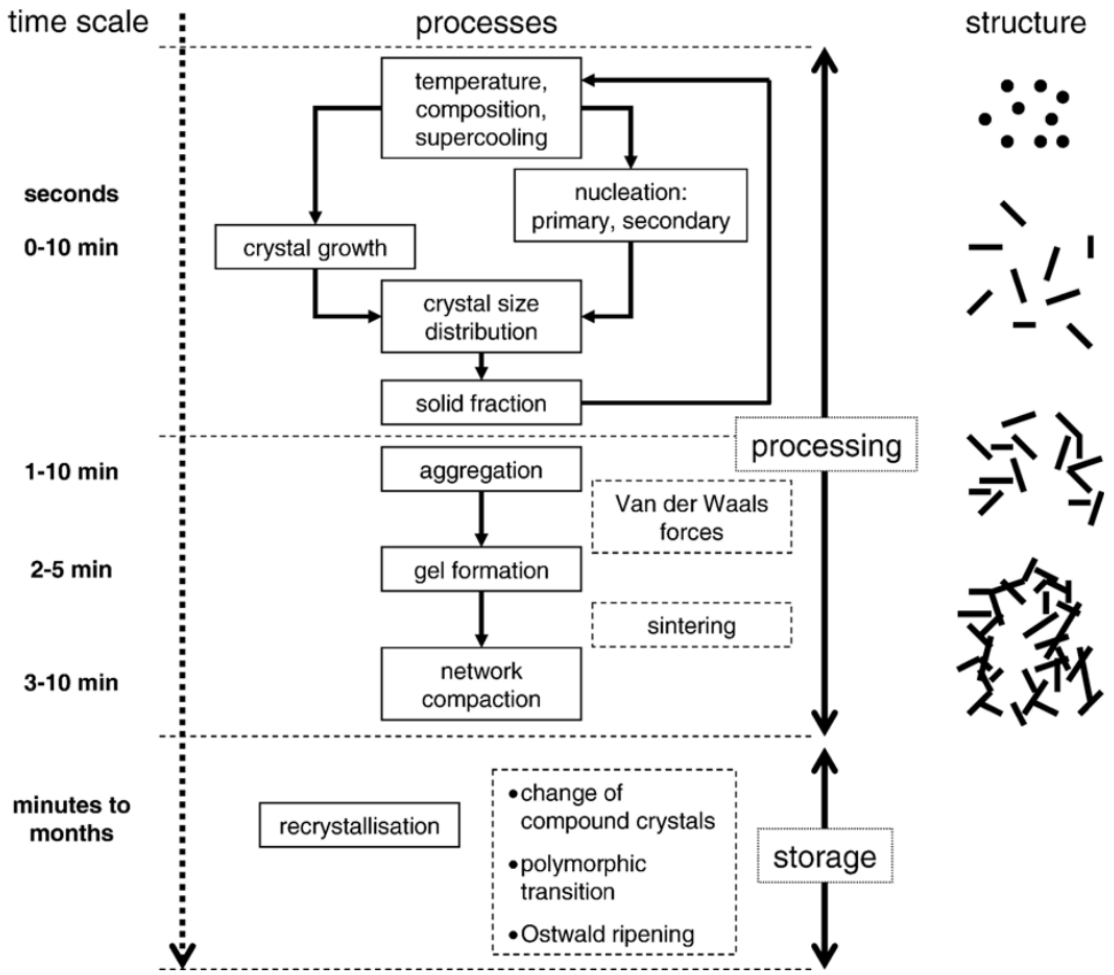


Figure 4: *Schematic illustration depicting the mechanisms involved in fat crystallisation (Walstra et al., 2001).*

2.2.1 The fundamental principle of crystallisation

Fat crystallisation is significant in food industry for optimisation and controlling the processing of products such as butter, ice-cream, and whipped cream as well as for the purification of fats and oils into fractions with specific properties and functionalities. Primary crystallisation involves generating sufficient thermodynamic driving force, nucleation, and crystal growth (Ashok and Dewettinck, 2015).

This section provides an overview of the principles and terminology related to fat crystallisation and a discussion of the process parameters that influence the rates of the various crystallisation processes. For detailed and comprehensive reviews on fat crystallisation, Wright et al. (2000), Sato (2001), Himawan et al. (2006), Ashok and Dewettinck (2015),

Mazzanti et al. (2005), Herrera and Hartel (2000), Coupland (2002), Ghosh and Rousseau (2011), Rousseau (2013) and Povey (2010) have written high-quality literatures.

In this context, the underlying mechanism of fat crystallisation is discussed. The driving force for all crystallisation processes is the difference in chemical potential ($J.mol^{-1}$) between the liquid and solid phase, which is also known as the partial molar Gibbs free energy. In the case of lipids, crystallisation is a process that happens from a solution, where lower melting triacylglycerols act as a solvent for the higher melting triacylglycerols, which are the solutes. Therefore, crystallisation can only take place when the system becomes supersaturated, which happens when the solute concentration (*C)* exceeds the saturation concentration (*Cs)*. The chemical potential difference at a given temperature (*T*) can be mathematically expressed as in **Equation 1**:

$$\Delta\mu = R_g \, \mathrm{T} \, \mathrm{In} \frac{C}{Cs} \qquad \textbf{Equation 1}$$

with R_g the universal gas constant (8.314 $J.K^{-1}.mol^{-1}$) and *T* the absolute temperature (K). Furthermore, the melt can also result in the crystallisation of fat. When the temperature of the molten fat is greater than the triacylglycerols with the highest melting point, the interaction between individual triacylglycerols is insufficient to counteract thermal Brownian motion.

However, upon cooling, thermal motion is diminished, and Van der Waals forces between triacylglycerols may exceed their kinetic energy. The thermodynamic driving force for this process is proportional to the difference between the actual temperature and the melting temperature (T_m). This difference in temperature is referred to as supercooling or undercooling. The chemical potential difference (Δμ) can be expressed as in **Equation 2**:

$$\Delta\mu = \Delta H_m \frac{T_m - T}{T_m} \qquad \textbf{Equation 2}$$

Fats can crystallise through nucleation from either solution or melt. The temperature difference between the actual and melting temperatures influences the thermodynamic driving force for nucleation from the melt. Through bimolecular interactions, nucleation can occur, resulting in the formation of ordered domains (Foubert, 2012).

Marangoni (2002) categorised nucleation into three types: primary homogeneous nucleation, primary heterogeneous nucleation, and secondary nucleation. Primary homogeneous nucleation occurs without external surface catalysis, but extreme supersaturation or

supercooling is required. Heterogeneous nucleation of primary phase, on the other hand, occurs on foreign surfaces that act as catalytic nucleation sites. These impurities lower the activation-free energy required for nucleation. In addition to dust particles, catalytic impurities include crystalliser walls, impeller blades, mono- and diacylglycerols, and other minor polar lipids or surfactants (Hartel, 1992; Ghotra and Narine, 2002).

In the presence of already formed crystals, secondary nucleation can occur after primary nucleation has occurred. Secondary nucleation is highly relevant to industrial applications and, depending on the location of nucleation, can be true, apparent, or contact secondary nucleation. It is the mechanism underlying crystal seeding, for instance. Kloek (1998) and Garside (1987) distinguished between true secondary nucleation, apparent secondary nucleation, and contact secondary nucleation. True secondary nucleation occurs when nucleation occurs in the vicinity of growing crystals, whereas apparent secondary nucleation occurs at fragments of growing crystals washed off by flow along the crystals and acting as nuclei. Contact secondary nucleation is the result of crystals colliding, impellers, crystalliser walls and others.

2.2.2 Nucleation

According to Rye et al., 2005, the formation of fat crystals is a multi-step process that dependents on two essential steps. First, it requires the diffusion of triacylglycerols from a bulk solution across a boundary layer, followed by their incorporation into the crystal lattice of an existing nucleus or crystal. However, triacylglycerols can detach from the crystal surface during the process, which can affect the growth rate of the crystals. According to Walstra (2003), the growth rate of fat crystals is determined by the combined attachment and detachment of triacylglycerols to the crystal surface.

As stated by Boistelle (1988) and Himawan et al. (2006), surface morphology of the crystals plays a crucial role in determining its growth rate. Based on their growth rate, crystals have traditionally been classified as flat, kinked or wavy surfaces. As reported by Ghotra and Narine (2002) and Himawan et al. (2006), research in recent years has focused primarily on smooth and rough surfaces. Rough surfaces have a diffuse structure in which the degree of crystallinity varies continuously across a few atomic levels across the solid-liquid interface, whereas smooth surfaces display an atomically immediate change in the degree of crystalline order across the solid-liquid boundary. Attachment to a rough surface requires contact with multiple molecules, while attachment to a smooth surface requires only one molecule. Due to the larger number of contact points, there is a greater likelihood of adhesion in the first scenario.

As Marangoni (2005) points out, a higher viscosity results in slower dissipation of heat of crystallisation from the growing crystal. Therefore, viscosity and crystal growth rate are inversely proportional. Crystal growth in natural fats, containing a multicomponent system of supersaturated triacylglycerols completing for the same nucleation site is a time-consuming process. The slow growth is due to the large and flexible size of each TAG molecule, which requires a longer period of time to achieve the proper position and conformation for incorporation into the crystal lattice of a growing crystal. Due to the lower driving force required for crystal growth, nucleation dominates excessive growth in a highly supersaturated or supercooled liquid phase, resulting in the formation of multiple nuclei and subsequent crystals, as observed by Herrera and Hartel (2000).

Mazzanti et al. (2003) indicated that, in addition to orientation and phase transitions factors such as structure, shear, temperature, and liquid phase composition play a crucial role in fat crystal growth. Meanwhile, according to Walstra (2003), the composition and temperature of the liquid phase determine the degree of supersaturation or supercooling of triacylglycerols where a higher chemical potential is positively correlated with a faster crystal growth rate. In contrast, at lower temperatures, an increase in viscosity can inhibit the transport of triacylglycerols to the crystal surface, resulting in diffusion-limited crystal growth.

Tran and Rousseau (2016) summarised that shear greatly accelerates fat nucleation and polymorphic transformations. Below the critical shear rate, aggregation is promoted while above the mechanism is hindered. Shear also affected fat crystallisation by changing the orientation of fat crystals with the formation of a new spheroidal crystal microstructure.

2.2.3 Polymorphism

Polymorphism is the phenomenon by which molecules with mixed chemical composition can crystallise into different unit cell structures with more than one crystal structure (Hondoh and Ueno, 2016; Sato and Ueno, 2011). Nevertheless, the various polymorphic modifications have distinct physical properties, including melting point, solid fat content, rheology, microstructural, melting and crystallisation profiles (Metin and Hartel, 2005).

According to the classification of Larsson’s (1966), triacylglycerols have three fundamental polymorphs that are distinguished by their hydrocarbon subcell packing: α, β', and β. The hydrocarbon packing is characterised by the interchain distances of the subcell; these short spacings are chain-length independent and can be measured by wide-angle X-ray diffraction (d'Souza et al., 1990; Lopez et al., 2001). The degree of freedom and orientation of the fatty acid chains of the three basic polymorphs differ. The α polymorph features chains that are

perpendicular to the methyl end group plane, with high oscillatory and rotational freedom, whereas, in the β' and β crystals, the chains are tilted in relation to the methyl end group plane and oscillatory freedom is reduced. In the β' polymorph, adjacent zig-zag fatty acid chains are in different planes, while in the β polymorph, they are all in the same plane.

The melting point, melting enthalpy, stability, and density of α, β', and β polymorphs vary due to their distinct packing (*Figure 5*). The β polymorph is the most stable and has the lowest Gibbs free energy (denoted as ΔG) due to its more rigid packing; as a result, it has the highest melting point. In contrast, the intermediate behaviour is β' the polymorph and the α polymorph is the least stable because it possesses the lowest melting point due to the highest Gibbs free energy (Walstra, 1987).

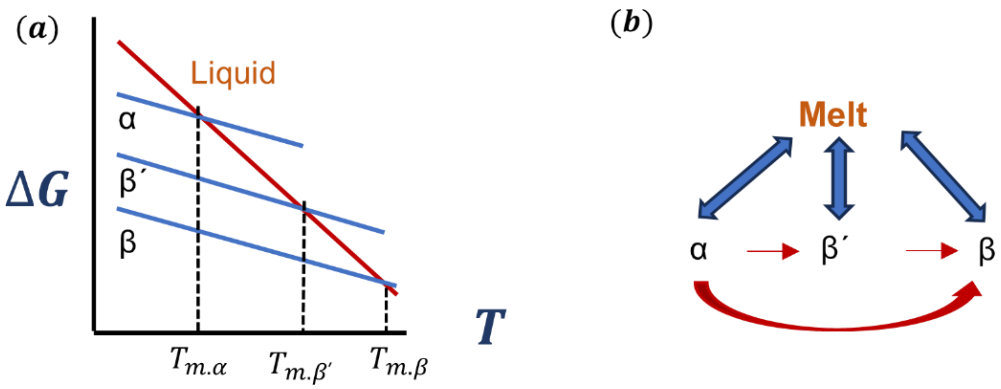


***Figure 5**: The relation between Gibbs free energy and temperature for the three main polymorphic forms of TAGs (monotropic polymorphism). (b) The polymorphic transformation pathways in fats involve liquid crystals. Adapted from Sato and Ueno. (2011).*

2.2.4 Factors affecting primary crystallisation

In a bulk fat system, the fat crystallisation mechanism and kinetics are largely determined by the applied time-temperature programme (*t*-*T*), which includes the actual temperature, storage time, cooling rate, and agitation.

It may be concluded from **Equations 1** and **2** that the actual temperature determines the degree of supersaturation and supercooling. Therefore, the driving force for nucleation is greater the lower the temperature. The driving force required for crystal growth is less than that required for nucleation. At higher supersaturation or supercooling, nucleation will therefore predominate, resulting in a high number of nuclei and, consequently, smaller

crystals. Crystal growth is encouraged at lower supercooling or supersaturation, resulting in fewer large crystals (Herrera and Hartel, 2000).

At a fast rate of cooling, nucleation must occur in a short period of time (Compos et al., 2002). Consequently, nucleation only occurs at extremely reduced induction durations (*i.e.*, an enhanced nucleation rate) and, consequently, with greater supercooling. As a result, the appropriate triacylglycerol conformation is easier to achieve, and compound crystals are favoured. As Herrera and Hartel have demonstrated, this entails a higher concentration of solid fat (2000).

In addition, the rate of cooling also determines the polymorphic transformation of the formed triacylglycerol crystal. At a high cooling rate, severe supercooling initiates crystallisation, resulting in many tiny compound crystals with metastable polymorphic modification. For low cooling rates, the inverse is true (*Figure 6*).

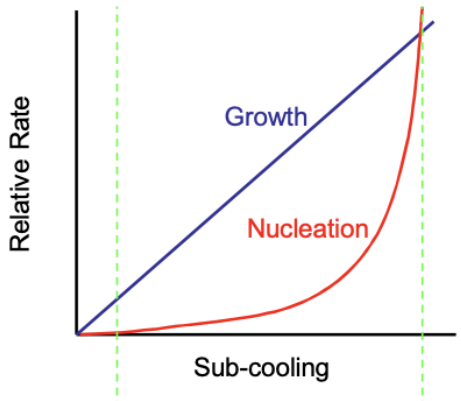


***Figure 6**: Effect of sub-cooling on growth and nucleation rate.*

Aggregation of fat crystals occur during storage, where recrystallisation involving changes in crystal forms, fat composition, and polymorphism can take place (Walstra et al., 2001). At low shear rates, agglomeration can occur, whereas, at greater shear rates, the formation of smaller crystals is possible. The impeller facilitates the nucleation and/or breaking of crystals and their aggregates, resulting in the formation of smaller crystals (Rafanan and Rousseau, 2019; Walstra et al., 2001). In addition, crystals may exhibit orientation in conditions of intense agitation (Mazzanti et al., 2003) and shear (Tran and Rousseau, 2016).

Fat crystallisation under shear has been studied by several researchers. Mazzanti et al. (2003) studied fat crystal orientation and polymorphic transitions for different types of fats under shear using a Couette shear cell coupled with X-ray diffraction. They concluded that under shear, the fat crystal aligns to the shear direction and polymorphic transition happen faster if higher

shear rates are applied. MacMillan and Roberts (2002) observed the same trends in cocoa butter, Mazzanti et al. (2009) in milk fat and Mazzanti et al. (2005) in palm stearin. Campos and Marangoni (2014) indicated that, cocoa butter (CB) crystallised under shear showed significant amount of mixed, small crystals with faster crystallisation rate. However, other authors showed that the crystal network was weaker after shearing (Acevedo and Marangoni, 2013) as corroborated by Patel and Dewettinck (2015) due to existence of critical shear rate threshold.

2.3 Fat crystallisation in oil-in-water (O/W) emulsions

2.3.1 Nucleation and crystal growth

Fat in O/W water emulsions is divided into small droplets, which results in the emergence of new phenomena that make fat crystallisation more complex than in bulk fat systems. The nucleation mechanism may vary, and Awad and Sato (2002) have identified three distinct types of heterogeneous nucleation that can be observed in O/W emulsions, as illustrated in *Figure 7*.

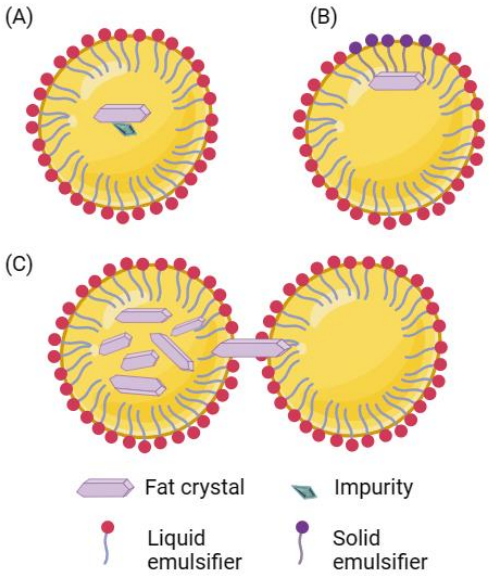


***Figure 7**: Schematic illustration of the different heterogeneous nucleation mechanisms in an O/W emulsion. (A) Volume heterogeneous nucleation, (B) Interfacial heterogeneous nucleation and (C) Interdroplet heterogeneous nucleation. Adapted from Awad and Sato (2002).*

Volume heterogeneous nucleation: Volume heterogeneous nucleation is often observed in bulk fat systems due to the presence of foreign surfaces or guest molecules (Walstra, 2003; Coupland, 2002; Povey, 2001). During emulsification, these catalytic impurities are partitioned like the fat, and depending on the droplet size, the number of impurities per droplet can range from a large number to approximately zero. As a result, an increase in supercooling is typically observed, and the kinetics are initially proportional to the volume of droplets and, therefore also to the cube of their diameter (Povey, 2001). However, when if the number of droplets exceeds the number of impurities or if the bulk mass is devoid of impurities, the fat crystallises through a homogeneous nucleation mechanism as elaborated by Skoda and Van de Tempel (1963); Walstra and van Beresteyn, (1975).

Interfacial heterogeneous nucleation: Emulsions generate a large interfacial area that can serve as a nucleation site if the hydrophobic portion of the surface-active components has a similar molecular structure to the crystallising components (Skoda and Van de Tempel, 1963; McClements et al., 1993). Therefore, the nucleation rate is proportional to the fat droplets' interfacial area and, consequently, to the square of their diameter (Coupland, 2002; Povey, 2001).

Interdroplet heterogeneous nucleation: Due to Brownian diffusion, droplets in emulsions move rapidly, resulting in frequent collisions. If a crystallised droplet collides with a supercooled liquid droplet and the crystal phase of the solid droplet encounters the supercooled liquid, it may induce nucleation in the liquid droplet (Walstra, 2003; Povey, 2001; Dickinson et al., 1993; McClements et al., 1994). It should be noted that this is a secondary nucleation process that occurs only if homogeneous or heterogeneous primary nucleation has already occurred (Skoda and Van De Tempel, 1963).

Like bulk fat systems, the nucleation rate and type of emulsions are affected by the complete temperature history and flow conditions. Moreover, the particle size distribution in emulsions has a significant impact as proposed by Lopez et al. (2002). These effects of dispersing oil into droplets can influence crystal growth and polymorphic evolutions during subsequent crystallisation, as well as the final quantity, size, morphology, and composition of fat crystals and their network in fat droplets.

2.3.2 Fat crystal network in fat droplets

The crystallisation behaviour of milk fat in bulk and natural cream has been extensively studied by Ollivon's research team over the past decade using X-ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) under both isothermal and non-isothermal conditions (Martínez-

Monteagudo et al., 2012; Fredrick et al., 2011; Foubert et al., 2008; Lopez et al., 2006; Lavigne and Ollivon, 1997; Lopez et al., 2001, Lopez and Ollivon, 2009; Lopez et al., 2002; Michalski et al., 2004; and Bugeat et al., 2011).

During cooling, the predominant crystals formed are α and β' crystals with 2L and/or 3L stackings. At low cooling rates and during isothermal conditioning at 4°C after quench cooling, only traces of the β polymorph are detected in bulk fat systems, indicating the pervasiveness of mixed crystals in milk fat. The precise positional distribution of the fatty acids on the glycerol backbone in milk fat explains the compatibility necessary for the formation of compound crystals within the wide variety of heterogeneous groups of triacylglycerols in milk fat (Marangoni and Lencki, 1998).

Natural cream produces unstable crystals and the delayed transition to more stable species, especially at low cooling rates of 0.15°C.min^{-1} as observed by Lopez et al. (2001). At higher supercooling temperatures milk fat in cream nucleates as 3L- polymorphs. Due to the lack of nucleation centres in most of the droplets, the cooling rate was insufficient to permit stable varieties to form in cream. In addition, through XRD analysis the Ollivon research team hypothesised that the crystals in fat droplets are more disordered and/or smaller due to the higher supercooling required to induce nucleation and the crystal packing constraints caused by the curved interface (Lopez et al., 2002).

The structural changes in natural cream were recorded separately for small droplets (1-3 μm) and large fat droplets (> 5 μm) (Michalski et al., 2004). Different degrees of supercooling resulted in the formation of crystals with a similar appearance. In addition, the long spacings varied slightly based on the size of the droplets, which was primarily attributed to the differences in fatty acids composition between the various dairy fat droplets (Briard et al., 2003).

Despite the triacylglycerol composition, the physical properties of fats can be affected by minor components such as LMW emulsifiers, which can be naturally present or purposefully added. LMW emulsifiers are amphiphilic molecules made up of hydrophobic and hydrophilic characteristics. Earlier research demonstrated that emulsifiers were initially used to slow down polymorphic transitions; however, tristearin (SSS) experiments demonstrated that emulsifiers can also speed up the transition. Liquid emulsifiers can speed up the polymorphic transition in SSS crystals by increasing their mobility, whereas solid emulsifiers can either retard or speed up the transition depending on their ability to form hydrogen bonds with adjacent triacylglycerols (Garti and Yano, 2001; Aronhime et al., 1987).

Cheng et al. (2020) investigated how emulsifiers like glyceryl monooleate (GMO), and sucrose ester (SE) affected the competitive adsorption and fat crystallisation behaviour of emulsified fat of AMF and coconut oil (CO). The outcomes showed that both GMO and SE had an impact on crystallisation behaviour, altering the crystal growth, morphology, and firmness of whipped cream and ice cream, respectively. In emulsion systems with various interactions between the fat component and emulsifier, a distinct mechanism was implicated in the mechanism of partial coalescence. According to Sakamoto et al. (2004), the presence of polyglycerol fatty acid esters (PGFEs) resulted in the heterogeneous nucleation of palm mid fraction in O/W emulsions. They observed that the temperature required for crystallisation rose with the increase in PGFE content, which was attributed to a higher hydrophobicity level.

Sato's team also conducted a comprehensive investigation into the effect of hydrophobic sucrose and polyglycerol esters on O/W emulsions. The emulsions were made from palm oil (PO), palm mid fraction (PMF), palm stearin (PS), or palm kernel oil (PKO). Their findings observed that the presence of these emulsifiers accelerated nucleation in the emulsions. For example, in the presence of sucrose esters, PKO crystallisation initiated to a more stable polymorph. Sato’s team concluded that the acceleration effect of sucrose and polyglycerol esters on nucleation rate is proportional to the chain length of saturated FAs and the degree of fatty acid esterification on the hydrophilic part of the emulsifiers.

It is suggested that the higher nucleation rate is attributed to interfacial heterogeneous nucleation rather than volume heterogeneous nucleation, according to the hypothesis. It has been discovered that hydrophobic sucrose esters induce interfacial heterogeneous nucleation by orienting the fatty acid chains of triacylglycerol molecules through hydrophobic interactions. In the presence of sucrose esters, the lamellar planes of fat crystals were found to be parallel to the oil-water interface, whereas a rather random orientation was observed in the absence of sucrose esters, as demonstrated using a microbeam small-angle X-ray diffraction technique.

Wang et al. (2021) investigate the stability of aerated milk fat emulsions employing multiple emulsifier types (mono- and diglycerides, Tween 80, LACTEM and sodium stearyl lactylate). The texture of the final aerated emulsion products was impacted by the interfacial characteristics and fat crystallisation behaviour of the various emulsifier behaviours, according to the results. The aerated emulsions' foam firmness and stability were improved by the lipophilic emulsifier, which also accelerated milk fat crystallisation and increases the solid fat content. The α-tending emulsifiers lactic acid esters of mono- and diglycerides (LACTEM) were shown to competitively replace protein from the O/W interface and produce shear

instability during aeration, which may, in some cases, cause fat droplet flocculation or coalescence (Munk et al., 2013 and 2014).

2.4 Destabilisation mechanisms in emulsions

Thermodynamically, emulsions are inherently unstable systems with low stability. The kinetic stability with no signs of fat droplets aggregation can be achieved by modifying certain parameters. *Figure 8* illustrates the types of emulsion instability, and the mechanisms are explored briefly as follows (Dickinson, 2019; Petrut et al., 2016; Moens et al., 2016; Fredrick et al., 2010; Walstra et al., 2003; Sjöblom, 2006).

Ostwald ripening **-** Due to the difference in Laplace pressure, molecules of the dispersed phase move from small to large droplets by diffusion. The Laplace pressure in the small droplets is greater, increasing the solubility of the smaller fat droplets in the continuous phase, diffusing towards the large ones. This means that small droplets disappear while large ones grow. The rate of transport is roughly proportional to how well the molecules of the dispersed phase dissolve in the molecules of the continuous phase.

Creaming - Creaming is a reversible instability by agitation or mixing depending on the degree of phase separation. It results from the disparity in mass density between the continuous water phase and the dispersed oil phase. The difference is almost always negative in O/W emulsions, which causes the droplets to rise. *Stokes' Law* can be used to evaluate the rate of creaming. The droplet size is the main factor contributing to this phase separation.

By controlling droplet size, increasing the viscosity of the continuous phase, and manipulating the membrane surface using emulsifiers and stabilisers, the stability of an emulsion can be generally enhanced. Determining the particle size distribution is one way to assess emulsion's stability. The particle size distribution of an emulsion should have a single peak under 1 µm - if creaming is to be avoided. Bimodal distributions and peaks greater than 1 µm are likely to undergo creaming. The type of homogeniser, energy input, rheological properties of each phase, surfactant types and concentration, volume fraction, temperature, and the method of preparation all can influence the droplet size.

Flocculation - Flocculation is a common occurrence that has an impact on the creaming and appearance of protein-stabilised O/W emulsions. Droplet flocculation can be reversible or permanent, depending on the kind and intensity of the molecular and colloidal interactions involved that are difficult to reverse. Other instability phenomena such coalescence, phase separation, and Ostwald ripening are affected by the flocculation state. However, it is most

clear that the presence of flocs is associated with noticeable alterations in emulsion appearance or storage stability. Observations of increased creaming are typically connected with flocculation in a diluted O/W emulsion. This can be explained by the fact that flocs experience stronger buoyant forces than individual drops. On the other hand, in a more concentrated O/W emulsion, the existence of a dense network of flocs can prevent creaming since the emulsion system as a whole form an interconnected gel-like structure (Dickinson, 2019).

Coalescence - Coalescence is the fusion of two or more fat droplets into a single droplet and is an irreversible process. This increases the fat droplet volume and decreases the interfacial area. Coalescence easily occurs in the presence of very weak interfacial layers when the fat droplets come into proximity. This may be under static conditions or shear force over longer periods of time in an O/W emulsion such as cream. Coalescence occurs in two stages, first is the thinning of the liquid film of the continuous phase between the two fat droplets, then, the film may spontaneously break due to temperature fluctuations and/or mechanical agitations. The type and concentration of the emulsifier or stabiliser used, and the degree of fat crystallisation will impact the mechanism of coalescence in the system.

Partial coalescence - Partial coalescence is also known as clumping when referring specifically to milk fat. Only if fat droplets in milk contain some solid particles, primarily fat crystals, this phenomenon observes. The fat crystals can protrude and pierce the adsorbed layer of a fat droplet into the continuous water phase. Due to collision with another fat droplet, the fat crystal can pierce the adsorbed layer of that fat droplet, allowing fat droplets interaction (Fredrick et al., 2010). The mechanisms and factors that influence partial coalescence will be examined in great depth in the following section.

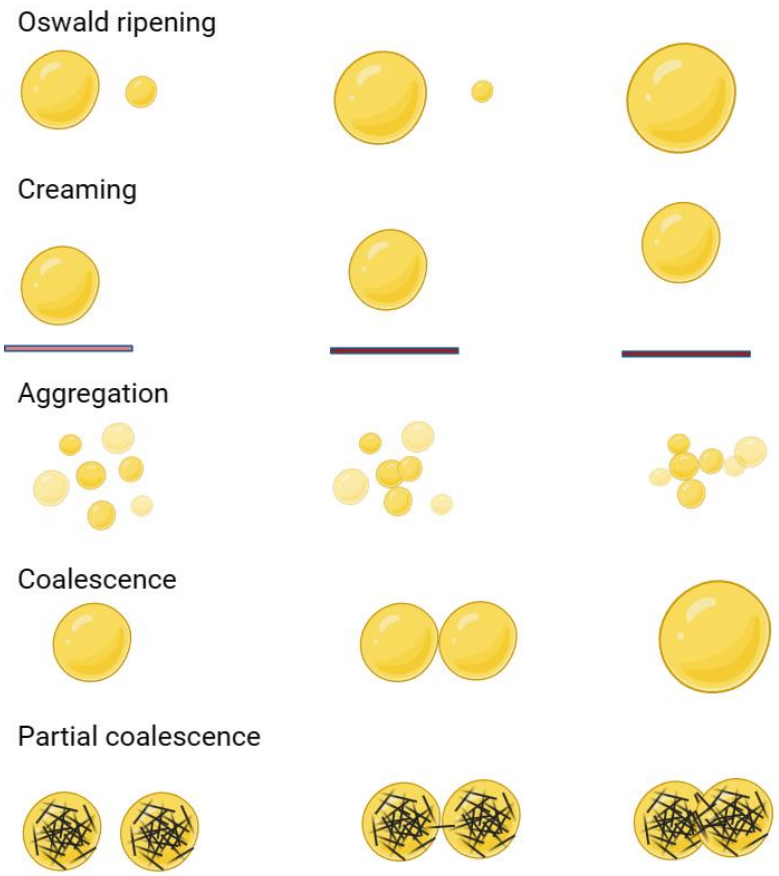


***Figure 8**: Schematic illustration of potential instability mechanisms in oil-in-water (O/W) emulsions. Adapted from Walstra (2003).*

2.4.1 Partial coalescence under static conditions

The occurrence of partial coalescence in semi-crystalline emulsions because of temperature cycling is well-documented. Interestingly, this process can take place in the absence of mechanical agitation or shear. According to Boode et al. (1991), the mechanism of partial coalescence generated by temperature cycling is like the mechanism of shear-induced partial coalescence, in which is suggested that fat crystals rupture the interface. It was also observed that reheating semi-crystalline emulsions increases their susceptibility to partial coalescence, potentially because of an increase in fat crystal size and interface protrusion distance after temperature cycling. Temperature cycling plays a vital role in causing partial coalescence in semi-crystalline emulsions, and reheating these emulsions can amplify this effect.

As indicated by Thivilliers et al. (2008), partial melting of crystals can result in their migration to the interface, where they serve as nucleation sites during recrystallisation, forming larger crystals. The rate of partial coalescence induced by temperature cycling is dependent on the

particle size and emulsifier-to-protein molar ratio. These variables affect the distance that the fat crystals protrude from droplets and the contact time between droplets during close approach. Larger fat droplets and substantial protein displacement facilitate partial coalescence, according to the authors, whereas small fat droplets may encounter jamming due to the interaction between their rough surfaces without considerable partial coalescence.

Moens et al. (2016) highlighted that tempering increased the susceptibility to partial coalescence caused by shear, which due to larger fat crystals have higher chance to close to the interface of the fat droplets. Rojas (2008) has postulated an alternative mechanism for temperature cycling-induced partial coalescence, involving the formation of liquid oil bridges between fat droplets as opposed to the rupture of the interface by fat crystals. The author observed the formation of 'beads' of liquid oil at the interface between fat droplets and water when the droplets were kept at temperatures where the fat was predominantly molten. The liquid oil droplets at the interface partially solidified upon re-cooling, indicating the existence of fat fractions with a medium to high melting point in the exuded fat. Boode and Walstra (1993) discovered fat crystals protruding from fat droplets, which do not exactly agree with Rojas (2008). The mechanism of partial coalescence generated by temperature cycling, may require both the protrusion of fat crystals and the development of liquid oil bridges, and the specific mechanism may depend on the experimental settings and properties of the semi-crystalline emulsions.

It is noteworthy that there are very few reports of other researchers confirming the protrusion of fat crystals from partially crystalline fat droplets using microscopy. This could be attributed to the use of SDS as a surfactant in these studies, and the observation of only large droplets with a diameter of several microns or greater. Boode and Walstra (1993) proposed that SDS may accelerate the migration of fat crystals into the aqueous phase via the Lanza effect, in which the crystals migrate because they are better wetted by the aqueous phase than by the oil phase. Therefore, the choice of surfactant and the size of droplets can affect the visualisation of the protrusion of fat crystals, and caution should be exercised in interpreting microscopy data regarding this phenomenon.

2.4.2 Shear-induced partial coalescence

Mechanical shear forces (flow conditions) and the time-temperature (*t*-*T*) processing programme, including solid fat content and fat crystal are the primary parameters that influence the occurrence and rate of partial coalescence in O/W emulsions.

Temperature

The susceptibility of O/W emulsions to partial coalescence is determined in large part by the physical state of the fat droplets, which is influenced by the type or composition of fats, the actual temperature, and the temperature history. When the fat droplets are subjected to a degree of supercooling, they may crystallise, leading to the nucleation and growth of crystals. The solid fat content, as well as the morphology, size, and polymorphism of the crystals, will have a substantial effect on the partial coalescence stability of the fat droplets (Ashok and Dewettinck, 2015; Allen et al., 2001; Van Aken, 2001).

Flow conditions

Both Brownian motion and creaming behaviour influence the frequency of interactions and movement of fat droplets in a quiescent O/W emulsion. Taylor vortices in turbulent flow or shear rates in simple shear flow are two examples of hydrodynamic mechanical forces that can raise the encounter frequency and, as a result, the partial coalescence rate (Walstra, 2003). This type of fat droplet clustering is known as shear-induced partial coalescence (Hotrum et al., 2005).

During processing, the velocity gradient developed can also increase the capture efficiency (α), which is the probability that two fat droplets will adhere after close proximity (Darling, 1982; Van Boekel and Walstra, 1981). During flow (*Figure 9*), there are two causes for the increased capture efficiency. Initially, the fat droplets will roll around one another, causing fat crystals to protrude from the interface between two approaching fat droplets. Secondly, the fat droplets are pushed closer together, resulting in a shorter interdroplet distance with the elimination of the repulsion barrier. This can result in the second fat droplet being pierced by the protruding crystal, thus increasing the capture efficiency.

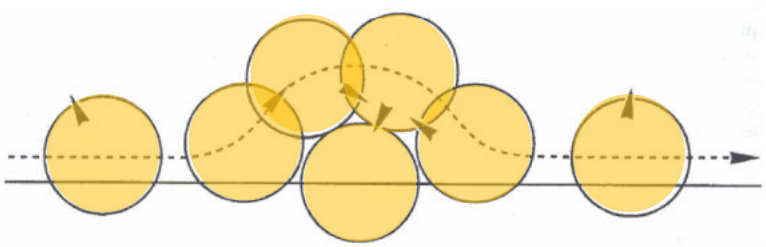


***Figure 9**: Movement of fat droplets relative to one another when shear flow is applied. Adapted from Van Boekel and Walstra (1981).*

Before the partial coalescence rate rises with the applied shear rate in emulsions, a shear rate threshold value may be observed (Hinrichs and Kessler, 1997). The fat droplets either do not clump or clump extremely slowly below this threshold value, probably because the distance between fat droplets is greater than the protrusion distance of the crystals.

Solid fat content

For fat droplets to undergo partial coalescence, Boode (1992 and 1993) demonstrated that a fat crystal network is required inside the droplet. Therefore, the rate of partial coalescence is closely related to the solid fat content of the fat droplets. Consequently, stability against partial coalescence can be maintained below a particular solid fat content threshold value. However, if the fat crystals do not form a space-spanning network that approximates the size of the droplets, they cannot extend extremely far and, upon collision, may be pushed into the fat droplets, resulting in "true" coalescence (McClements et al., 1994). Using the theory of fractal aggregation of fat crystals, which defines a critical diameter of a fractal aggregate required to form a continuous network, it is possible to determine the minimum solid fat content required to establish a crystal network throughout the droplet. This diameter should be smaller than the fat droplet's diameter to facilitate the formation of a network of fat crystals throughout the fat droplet. Therefore, this condition must be met (Thiel et al., 2016; Fredrick et al., 2012; Boode, 1993; Boode and Walstra, 1993).

Fat crystal arrangement

In the context of fat droplets, their movement to an equilibrium position in the O/W emulsion can influence the contact angle of the crystal in both the oil and aqueous phases. Three interfacial tensions determine the contact angle, as shown in *Figure 10*. It is important to note that the type and concentration of emulsifiers in the O/W or W/O phase can significantly change the interfacial tensions and contact angles of the fat crystal arrangement as stated by Van Boekel (1980).

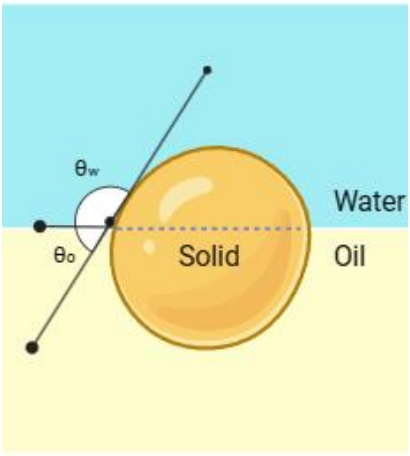


***Figure 10**: Schematic illustration of the contact angle of a spherical fat crystal at the O/W interface.*

In most O/W emulsions, the contact angle of a crystal in the aqueous phase ranges from 120° to 160°. This indicates that the emulsion interfacial free energy is minimised when crystals are positioned at the interface and exhibit a preference for being wetted by the liquid oil phase. This effect is more pronounced for smaller values of oil phase because of a greater increase in free energy relative to the state of complete oil wetting. *Figure 10* demonstrates that the orientation of free-moving crystals is tangential regardless of the wetting conditions represented by the contact angle, as noted by Boode and Walstra (1993). The attractive Van der Waals forces between fat crystals prevent them from diffusing freely to the O/W interface (Boode and Walstra, 1993; Xu, Nikolov, and Wasan, 2005). The interface is only accessible to fat crystals that are not connected to the internal crystal network.

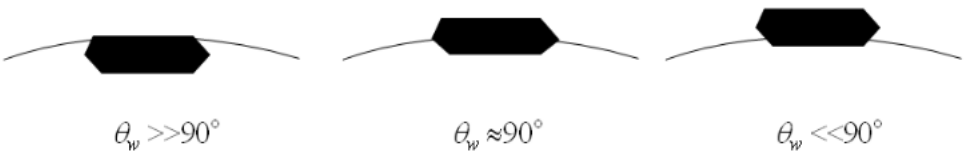


***Figure 11**: Orientation of free movable fat crystals at the O/W* interface*. Adapted from Boode (1992).*

Figure 11 highlighted the capture efficiency of layered (L-) type droplets is dependent on the crystals' contact angle. When θ_w is significantly greater than 90°, the crystals are preferentially wetted and positioned in the oil phase, while their edges protrude by a few tens of nanometres due to the curved interface. The capture efficiency for partial coalescence in this instance is

on the order of 10^{-6}. When θ_w is approximately 90° and the crystals are not forced into either phase, a similar situation occurs. When θ_w is significantly less than 90°, however, the crystals are primarily wetted by the aqueous phase and are located outside the O/W interface. This may result in the aggregation of fat droplets via bridging, but it is unlikely that partial coalescence will occur frequently.

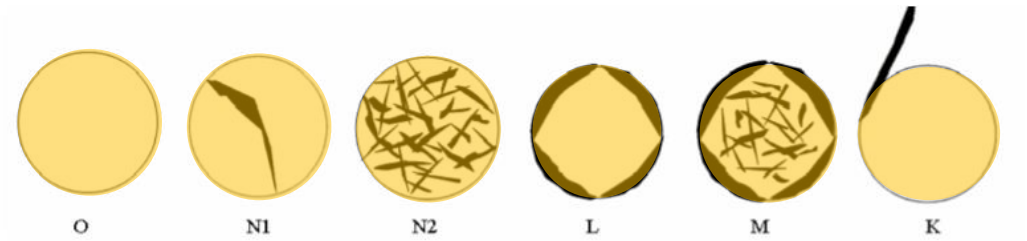


***Figure 12**: Schematic presentation of types of fat droplets as observed with the microscope. Adapted from Boode (1992).*

Boode and Walstra (1993) observed that fat crystals in N-type droplets exhibit a radial orientation, and the extent of crystal protrusion from the droplet is dependent on several factors, such as the angle of contact, the shape and size of crystals, and the contraction of the liquid perimeter due to fat crystallisation (*Figure 12*). N-type fat droplets are more susceptible to partial coalescence, and the capture efficiency (α) is dependent on the number of fat crystals present at the interface. The capture efficiency of N1-type fat droplets with few protruding crystals is approximately 10^{-6}, whereas the capture efficiency of N2-type fat droplets with numerous protruding fat crystals is $10^{-6} < \alpha < 1$. K-type droplets have thick fat crystals that grow from the fat droplet when the θ_w is 90° and are highly susceptible to partial coalescence in the presence of water-soluble low molecular emulsifiers. The type of fat droplet formed is influenced by fat composition, temperature, and temperature fluctuations (Fuller et al., 2015; Boode and Walstra, 1993; Xu, Nikolov, and Wasan, 2005).

2.4.3 Factors determining partial coalescence rate

Variations in O/W emulsions susceptibility to partial coalescence can result in differences in their final state. When droplets undergo rapid partial coalescence, they aggregate into a continuous network, causing the O/W emulsion to invert into a W/O emulsion. Subsequent melting of this emulsion causes phase separation, with an oil layer forming on top and a water layer forming on the bottom. Slow partial coalescence, on the other hand, can result in irregular clumps that are more susceptible to creaming than the original singlets.

In the cream layer, the clumps become denser, potentially increasing the rate of partial coalescence locally, whereas, in the bottom layer, the rate will be even lower than in the original emulsion. Different susceptibilities to partial coalescence indicate that several types of partial coalescence are possible.

Four types of partial coalescence have been empirically identified in the literature, distinguished by changes in fat droplet size distribution and the presence or absence of a fat membrane on the emulsion's surface following heating and creaming of the large clumps (Boode, 1992). Changes in particle size distribution and the type of partial coalescence are primarily determined by differences in coalescence efficiencies between clumps and single fat droplet of various sizes (Boode, 1992).

Type A: All fat droplets participate and form irregular clumps. Melting produces an emulsion with a unimodal particle size distribution and a larger mean droplet size.

Type B: Involves the formation of large clumps that cream upon melting, and the resulting fat droplets are larger than the initial ones.

Type C: Rapid partial coalescence between a sum of emulsion droplets results in the formation of large clumps that rapidly aggregate and coagulate upon heating. The remaining fat droplets have a particle size distribution that has not changed.

Type D: Large clumps form, which rapidly cream upon heating, and the remaining emulsion has a smaller fat droplet size distribution than the original.

The factors that significantly determine the rate of partial coalescence are assumed to influence the physical properties of the O/W emulsion. The rate of partial coalescence is the product of the encounter frequency and the capture efficiency, denoted by α. The capture efficiency is calculated as the ratio of the observed partially coalesced fat droplet aggregates to the theoretically calculated aggregates, if each collision results in the formation of an aggregate (Walstra and Dickinson, 2003).

2.4.4 Role of proteins and emulsifiers

A complex procedure is required to evaluate the stability of an emulsion with a modified interfacial composition. Changing the quantity and type of surface-active surfactants prior to emulsification can affect not only the composition, but also the particle size distribution,

surface load, thickness, viscoelasticity of the O/W interfacial layer, and the fat crystallisation behaviour. These factors' variability makes it difficult to compare experimental outcomes.

Colloidal repulsion is a typically stabilising force against partial coalescence, especially in quiescent emulsions, as it determines the minimum distance between approaching fat droplets and, consequently, the capture efficiency. The composition of the interfacial layer surrounding the fat droplets largely determines the magnitude and type of colloidal repulsion. O/W emulsions stabilised by proteins and certain LMW emulsifiers may exhibit significant steric and electrostatic repulsion.

Proteins typically exhibit greater steric repulsion than LMW emulsifiers, resulting in a lower partial coalescence rate for fat droplets stabilised by proteins (Van Boekel and Walstra, 1981; Dickinson, 2001). In addition, proteins tend to form a thicker and frequently more viscoelastic membrane at the O/W interface. This reduces conformational flexibility at the interface, preventing fat crystals from entering the aqueous phase or rupturing the membrane of an approaching fat droplet (Boode, 1992; Van Boekel and Walstra, 1981; Boode, 1991; Xu, Nikolov and Wasan, 2005).

Proteins

Proteins and LMW emulsifiers are active surfactants that can adsorb at the interface to reduce the interfacial free energy, thereby promoting emulsion formation. Each protein is a high molecular weight molecule consisting of different types of amino acids (Pugnaloni et al., 2004).

In an aqueous solution, protein molecules tend to adopt a coil-like structure to expose the most hydrophilic groups to the water while hiding the most hydrophobic groups in the coil's centre. When a protein molecule in an A/W or O/W interface, it partially unfolds, with its hydrophobic groups oriented towards the non-aqueous like fat phase. Proteins diffuse and adsorb significantly more slowly than LMW emulsifiers and typically do not reduce the interfacial tension as effectively. However, due to their amphiphilic structures, proteins can form a viscoelastic protective membrane at the oil droplets interface, providing long-term stability via steric and electrostatic stabilisation mechanisms for foams and emulsions (Jiang et al., 2018; Han et al., 2018; Wilde et al., 2004).

Due to the positive correlation between surface load and colloidal repulsion, thickness, and viscoelasticity of the interfacial layer, the partial coalescence rate of emulsions decreases with increasing protein surface load until the maximum load is reached and all adsorption sites at

the interface are occupied (Goff, 1997). In general, casein-stabilised emulsions are more resistant to partial coalescence than whey protein stabilised emulsions (Vanapalli et al., 2002; McClements, 1993; Thanasukarn et al., 2004 and Pelan et al., 1997).

Heat treatment prior to and after emulsification may have a significant effect on the stability of emulsions because it induces changes in protein hydrophobicity and flexibility, which affect both aggregation properties and protein behaviour at the O/W interface (Peng et al., 2016; Jiang et al., 2018; Moro et al., 2013; Das and Kinsella, 1990). Emulsions stabilised with caseins are less sensitive to heat than those stabilised with globular whey proteins (WPs) because caseins are heat-stable proteins and globular WPs denature easily (Jiang et al., 2018; Kinsella and Morr, 1984). Preheating WPs makes butter oil emulsions more susceptible to partial coalescence, whereas post-heated emulsions remain stable (Segall and Goff, 1999). Pre-treated WP-stabilised emulsions are the least stable, according to Kiokias and Bot (2005; 2006) and Kiokias et al. (2004), whereas post-treated whey-protein, skim milk powder, and caseinate-stabilised emulsions are significantly more stable.

Low-molecular-weight (LMW) surfactants (labelled as “emulsifiers” in the food industry)

Compared to proteins, LMW emulsifiers have superior surface-active properties, enabling them to efficiently pack at the O/W interface. In the presence of both emulsifiers and proteins, the phenomenon of competitive adsorption may take place. This has been the subject of extensive research over the past decade, especially in relation to emulsions using natural milk proteins (Li et al., 2020; Munk et al., 2014; Singh and Sarkar, 2011; Dickinson, 2001; Cornec et al., 1998; Dickinson and Hong, 1994; Dickinson, Iveson and Tanai, 1992; Dickinson et al., 1993; Courthaudon, Dickinson and Dalgleish, 1991; Chen and Dickinson, 1993).

In emulsions, the presence of LMW emulsifiers and their competitive adsorption with proteins can affect the O/W interface stability (Li et al., 2023; Munk et al., 2014; Ihara et al., 2015; Cornec et al., 1998; Dickinson et al., 1993; Chen and Dickinson, 1993, 1998). LMW emulsifiers can nucleate at interface, compress the protein network, and result in protein release into the aqueous phase (Mackie et al., 2000). This displacement can also impact the interface's viscoelasticity, repulsive interaction, and partial coalescence stability (Morris and Gunning, 2008; Palanuwech and Coupland, 2003; Wilde et al., 2004; Dickinson, 2001). It should be noted here that according to Dickinson (2001), this displacement of proteins from the interface can influence the overall stability of emulsions by altering the viscoelasticity and repulsive

interaction of the interface, decreasing its thickness, and lowering the partial coalescence stability.

Prior to cooling and/or whipping, ageing a protein-stabilised ice cream mix or dairy cream in the presence of LMW emulsifiers has been shown to improve the physicochemical properties of the whipped products (Lui et al., 2022; Jiang et al., 2018; Leal-Calderon, Schmitt and Bibette, 2007; Ihara et al., 2015; Tual et al., 2005 and 2006; Barfod et al., 1991). In addition, LMW emulsifiers can alter the contact angle of fat crystals, thereby potentially increasing the crystals' wettability and protrusion distance. In turn, this can increase the probability of partial coalescence (Boode and Walstra, 1993; Darling, 1982; Van Boekel and Walstra, 1981).

The addition of LMW emulsifiers to oil can influence the crystallisation behaviour in fat droplets by affecting nucleation, crystal growth, and polymorphic transitions, which in turn can influence crystal size, morphology, sintering, crystal arrangement in the droplet, fat crystal network, and solid fat content (Cheng et al., 2020; Goibier et al., 2017; Munk et al., 2014; Ghosh and Rousseau, 2011; Awad and Sato, 2001 and 2002; Hodate et al., 1997; Awad, 2004; Miura et al., 2006; Tual et al., 2005 and 2006; Douaire et al., 2014). According to the literature, the partial coalescence stability of emulsions is affected by the concentration and type of emulsifiers used (Cheng et al., 2020; Munk et al., 2014; Arima et al., 2009; Miura et al., 2006; Buchheim, Barfod and Krog, 1985; Harada and Yokomizo, 2000).

Jiang et al. (2018) investigated the interaction between three LMW emulsifiers (sodium stearoyl lactylate (SSL), phospholipid (PL), and sucrose ester (SE)) and caseinate to comprehend the mechanism of formation and stability of whipped creams. The combination of surfactants and caseinate enhanced the stability of emulsions. The adsorption of water-soluble emulsifiers, sucrose ester onto fat droplets was greater than that of oil-soluble emulsifiers, resulting in a high foaming activity. In sucrose ester creams, notable protrusions of fat crystals and the aggregation of fat droplets into a colloidal matrix with significant hardness and viscosity were observed. Phospholipid on the other hand, exhibited shear thinning followed by thickening, whereas sodium stearoyl lactylate was incapable of producing stable foam due to the formation of α gel at the interface. The study concluded that the cooperative actions of casein molecules and hydrophilic surfactants played a significant role in the production of aerated emulsions with enhanced stability and a tendency to coalesce.

2.5 Aerated food emulsion

2.5.1 Mechanisms of air-water (A/W) interface stabilisation

Due to the amphipathic nature of proteins, their capacity to generate adsorbed layers at the A/W and O/W interfaces is commonly used to control the features of food emulsions. This behaviour is a direct consequence of the protein's foaming properties. Particularly in the presence of emulsifiers, the composition of the interface may vary dramatically from that of the aqueous phase due to the selective adsorption of complex protein combinations (Brooker, Anderson, and Andrews, 1986).

Electron microscopy reveals protein at A/W interfaces as dense layers whose thickness (varying from 2 to 6 nm) depends on several variables, including the nature and concentration of protein. The proteins at A/W interface are found in food foams like meringue, beer, and sponge cake. Nonetheless, the competitive adsorption of other food components, such as lipids, may affect the stability of a protein at the A/W interface and, consequently, the properties of the bulk foam (Munk et al., 2013 and 2014).

Fats have a significant impact on foam properties despite being a relatively minor chemical component of the food system. In the numerous examples where fat is a major component, its interaction with the interfacial protein has a greater impact on the foam's bulk properties (Liu et al., 2022; Wang et al., 2021; Fillery-Travis et al., 2000; Wilde et al., 2003; Damodaran, 2005).

Dairy foams

In general, dairy foam is produced by incorporating air into creams that contains 36 to 40% fat. Air bubbles in the of whipped cream, are primarily stabilised by the surface adsorption of fat droplets. However, there is no fat involved at the beginning of the whipping process. It has been shown by Murray (2007) that during whipping, air bubbles are initially stabilised by the adsorption of soluble milk proteins, including casein and whey proteins, at the A/W interface, and that the adsorption of fat droplets at A/W interface is a secondary process (Fredrick et al., 2013). The fat droplets are the predominant components of the A/W interface. Nonetheless, remnants of the original proteins at the interface are always visible between the fat droplets at the surface of the air bubbles.

The stabilisation of air in a variety of aerated O/W food emulsions resembles what is described for whipped cream, although the number of fat droplets adsorbed is variable and dependent

on the fat content. Following the elucidation of the mechanism of air bubble stabilisation in whipped cream, structural studies have been conducted to identify the interfacial changes that occur in whipped creams that collapse after whipping or cream that fail to whip. The instability of whipped creams is attributed to fat droplet aggregation caused by mechanical damage to the cream during separation (Anderson and Brooker, 1988). It has since been suggested that freeze fracturing with Transmission Electron Microscopy (TEM) are required to obtain a sufficient spatial resolution for observing the subtle differences in interfacial structure between defective and normal foams (Brooker, 1990).

Small crystals of triacylglycerols fat are typically observed adsorbing to the protein at A/W interface of normal whipped cream, as demonstrated by the same studies. These crystals originate from the droplets of free fat that are produced when the fat droplets are mechanically damaged during processing and consequently, they likely present in small quantities creams, because of the rupture of some milk fat droplets and the release of free fat during the separation process.

In contrast to this, the defective whipping creams contain conspicuous large bodies of free fat dispersed throughout the aqueous phase, which are most likely the result of improper processing and handling of the cream, as discussed previously. When these creams are whipped, numerous and extremely large fat crystals are found adsorbing to the A/W interface or bubbles and consequently these crystals can inhibit the normal absorption of fat droplets during the whipping process. In addition to the destabilising effect of free liquid fat on air bubble formation, the inhibition of fat droplet adsorption and the loss of fat droplet crosslinking between air bubbles are responsible for the poor whipping properties of these creams (Dickinson, 1989; Brooker, 1993).

Given that the adsorbed triacylglycerols crystal are derived from free fat bodies, the mechanism by which they reach the A/W interface and adsorb to it is unknown, but there are two possibilities:

i) During whipping, the crystals are sheared from the surface of the free fat lumps and adhere to the protein at the A/W interface.

ii) The surface forces holding a mass of free fat are proportional to its radius, while the shear forces are proportional to the radius squared. *Figure 13* is a confocal micrograph of defective whipped cream demonstrating the ability of masses of free

fat containing fat crystals, to attach and remain to air bubbles until the end of whipping.

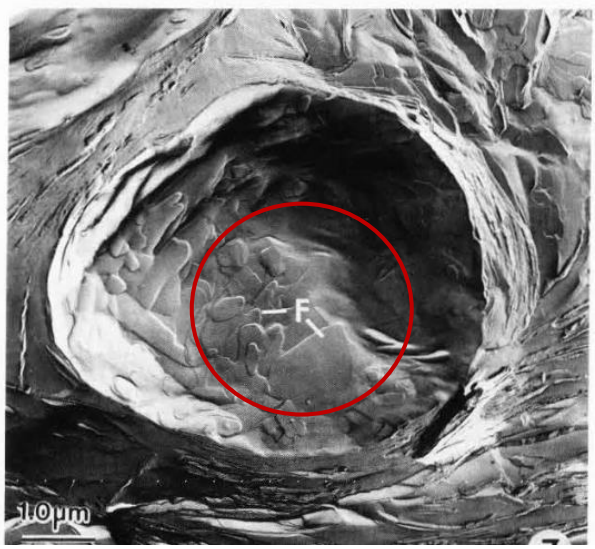


***Figure 13**: Confocal laser scanning micrograph of the defective whipped cream treated with Nile Blue and showing bodies of free fat (F) attached (at arrows) to air bubbles (A). Numerous small fat droplets are visible in the background. Adapted from Brooker et al. (1993)*

According to the known behaviours of lipid dispersions in protein-containing aqueous solutions, bodies of free fat in cream are predicted to be covered by an interfacial layer of adsorbed milk proteins. As a result, the interaction of this fat with the air bubbles, whether by route (i) or (ii) as discussed above, must involve an interfacial protein-protein interaction of the type previously discussed as part of the mechanism for normal fat droplet adsorption.

The mechanism of air stabilisation involves the adsorption of particulate lipids at the A/W interface, which requires an appropriate balance of interfacial free energies so that fat crystals are preferentially wetted by the continuous phase. Thus, for foams containing an aqueous and lipid phase, the ability of the lipid to stabilise (or destabilise) the foam is determined by the contact angle between the three interfaces, O/W, O/A, and A/W. Increasing the interfacial tension between O/W or between A/W interfaces strengthens the attachment of lipids to air bubbles and increases their stability. In practice, this can be accomplished either by manipulating the nature of the interfacial protein or by employing suitable emulsifiers.

2.5.2 Whipped cream

2.5.2.1 Definition and physical properties

Food emulsions such as mayonnaise, salad dressings and milk are two-phase systems of immiscible liquids. These phases are known as dispersed and continuous phases. Typically, in the food industry, the two phases are water and oil and most of the emulsified products are formulated to achieve long-term stability; however, it should be noted that, three phases aerated emulsions, such as whipped cream, ice cream, and whipped toppings are deliberately destabilised to allow fat droplets to partially coalesce and form at the A/W interface (Zeng et al., 2022; Jiang et al., 2018; Nguyen et al., 2015). In fact, the destabilisation of fat is essential for achieving the desired textural, rheological, microstructural and sensorial properties which physically measured the by desired firmness, high stability, high overrun and good whippability (Moens et al., 2016).

The fat droplets stabilise the foam structure by forming a three-dimensional network around the air bubbles that extends throughout the O/W phase. The dispersed air bubbles in the serum phase of whipped cream are primarily immobilised by partially coalesced fat droplets, which form a semi-continuous network in the system. Nevertheless, a protein layer stabilises part of the air bubble surface (Jiang et al., 2018; Liu et al., 2021; Zhou et al., 2020; Brooker, Anderson and Andrews, 1986). Several microscopic techniques (Du et al., 2021; Han et al., 2018; Pawar et al., 2012; Goff, 1997; Anderson, Brooker and Needs; 1987) demonstrate the development of this microstructure. *Figure 14* to *Figure 17* show Confocal Laser Scanning Microscopy (CLSM), Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM) images, of dairy products and recombined whipped cream, respectively.

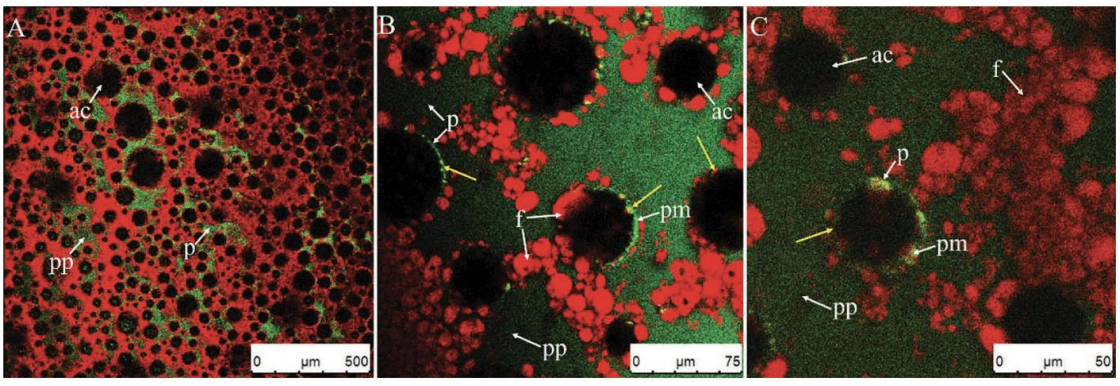


***Figure 14**: CLSM micrographs of cream whipped for 30 sec at low speed (950 rpm) and then for 130 sec at high speed (1100 rpm) (A-C). Red and green in plasma represent fat (f) and protein (p), black or green circled areas are the centre or surface of air bubbles, green circles and area signals indicate protein membrane respectively. Adapted from Han et al., (2018).*

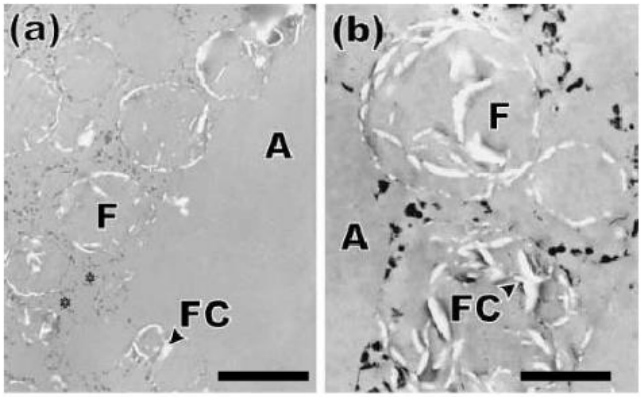


***Figure 15**: Transmission electron micrographs (TEM) of freeze-substituted and low-temperature embedded whipped cream at different magnifications: (a) length of scale bar is 4 µm and (b) length of scale bar is 1µm. Both images show the air-bubble (A) surface fat (F) containing fat crystals (FC), which appear brighter. Adapted from Smith, Goff and Sun (2004).*

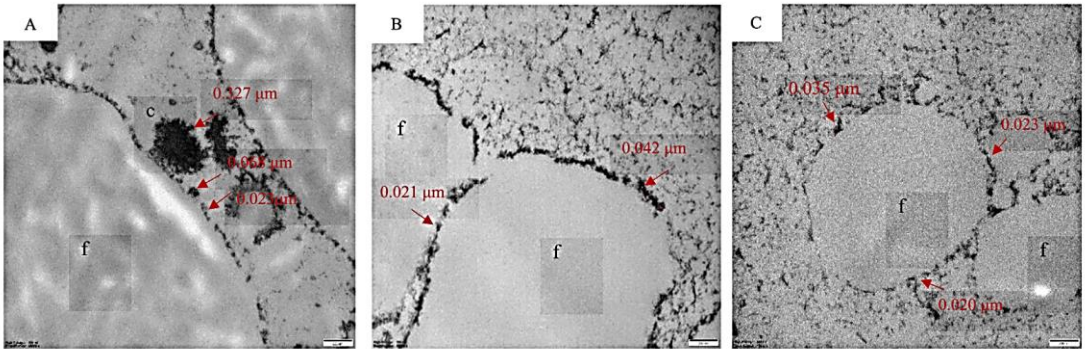


***Figure 16**: TEM images of the recombined dairy creams. f: fat droplets, c: casein aggregates. Adapted from Li et al., (2020).*

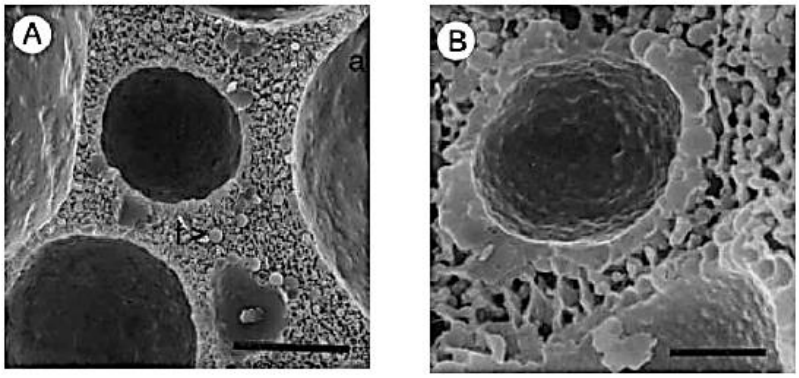


***Figure 17**: Cryo-scanning electron micrographs (cryo-SEM) of whipped cream at different magnifications: (a) Overview showing the relative size and prevalence of air bubbles and fat droplets (length of scale bar is 30 µm) and (b) the internal structure of the air bubble, showing the layer of* partially coalesced *fat stabilising the bubble. Adapted Goff (1997).*

During homogenisation, the formation of O/W emulsions is often accompanied by the rapid adsorption of proteins to the surface of newly formed fat droplets (Raikos, 2010). Proteins and LMW emulsifiers are responsible for fat stabilisation capacity and aeration potential of whipped cream. The cream emulsion must be stable prior to whipping, but can easily be destabilised by shearing to form a fat network that traps air bubbles. The formation of whipped cream is caused by the partial coalescence of oil droplets, creating a viscoelastic A/W interface. (Jiang et al., 2018; Nguyen et al., 2015; Goff, 1997; Brooker, Anderson, and Andrews, 1986; Smith, Goff, and Kakuda, 2000).

By forming an interfacial layer via steric and electrostatic stabilisation mechanisms, caseins prevent coalescence and flocculation of fat droplets. Prior to whipping, it is crucial that the whipping cream remain liquid during transport, storage, and handling. Since the formation of a network between fat droplets increases the viscosity of emulsions, it is important that fat droplet destabilisation does not occur prior to whipping the cream (Munk et al., 2014). Ultra-high temperature (UHT) treatment has extended the shelf life of whipped cream from a few days to over six months, eliminating the need for cold chains in storage and transportation (Li et al., 2020; Xu et al., 2022). The composition of conventional dairy whipping cream is detailed in *Table 4*.

***Table 4**: Gross composition properties of natural cream. Adapted from Walstra et al., (2005).*

Content (%)	Whipping cream
Fat	35
Casein	1.7
Other protein	1.15
Lactose	3.1
Salts	0.57
Water	58.4
pH	6.7
Density ($kg.m^{-3}$)	990
$d_{3.2}$ (µm)	4.0

Several studies have examined the effects of various fats (Liu et al., 2021; Moens, Tavernier and Dewettinck, 2018), proteins (Cao et al., 2020; Long et al., 2016), and LMW emulsifiers (Cheng et al., 2020) of whipped cream. In addition to traditional dairy whipped cream, the market also offers recombined creams and aerosol creams. Recombined cream is a dairy-free cream that typically consists of vegetable fats such as solidified lauric acids, such as palm kernel oil or coconut oil. Hardened lauric fats are suitable for recombined creams because they contain short and medium chain glycerides similar to those in butterfat and contribute to the proper mouthfeel and melting properties (Fauzi et al., 2013).

Compared to dairy whipped cream, the air bubbles in recombined creams are stabilised by a monolayer of individual fat droplets rather than a network of partially coalesced fat droplets (Krog, Barfod, and Buchheim, 1987), which negatively affects their storage stability after whipping. Aerosol cream is whipped dairy or vegetable cream dispensed from an aerosol or spray can. The overrun of these products is quite high (200 to 300%), and the firmness of aerosol-whipped cream is primarily due to the dense packing of numerous bubbles (Walstra et al., 2005). Additionally, aerosol whipped cream is extremely unstable. During the extremely rapid formation and expansion of bubbles, the absorption and clumping of fat droplets are limited (Walstra et al., 2005). Therefore, thickeners are often required to improve stability.

In general, the initial whipping properties of cream are evaluated by measuring its overrun, air cell/air bubble distribution, and firmness. The overrun is a measurement of the amount of air introduced into the whipped cream and, consequently, the increase in volume. The ideal whipping ratio for traditional dairy whipped cream is between 100 and 120% (Graf and Muller, 1965). The overrun offers no information about the size distribution of the air cells. Typically, microscopic techniques such as light microscopy, CLSM, TEM, SEM are used to determine this analysis.

The size of air bubbles in whipped cream is between 20 and 60 μm (Van Aken, 2001; Chang and Hartel, 2002). The firmness of whipped cream is determined primarily by a puncture probe attached to a texture analyser (Van Aken, 2001; Van Camp et al., 1996; Shamsi et al., 2002; Smith, 1999). The resultant firmness is highly dependent on the penetration depth and velocity, the probe geometry, and the sample dimensions. Therefore, there is no general target value for the ideal firmness of whipped cream. In recent studies, dynamic oscillatory rheology has been used to investigate the viscoelastic behaviour of whipped cream as a measure of structural rigidity (Drelon et al., 2006; Riaublanc et al., 2005; Gravier et al., 2006).

Like emulsions, foams are thermodynamically unstable systems and are therefore subject to multiple instability mechanisms (Langevin, 2019; Dickinson, 2015; Dalgleish at al., 2006; Prins, 1987). Serum leakage, loss, and drainage (syneresis) is a gravitationally unstable mechanism that simultaneously causes the downward movement of the heavy aqueous phase around the air cells and their ascent. Ostwald ripening is the development of larger air cells at the expense of smaller ones. The rate of disproportion depends extremely sensitively on the solubility of the gas phase in the aqueous phase and thus on the composition and temperature of both phases. For example, the high solubility of nitrogen oxide (N_2O) in water causes to rapid destabilisation of aerosol creams. The coalescence of two adjacent air cells results in the loss of two smaller cells and the formation of one larger cell (Dutta et al., 2004).

2.5.2.2 Factors governing the whipping properties

During whipping, cream transforms from a two-phase liquid system (O/W emulsion) into a three-phase solid system in which air is incorporated and enclosed by a network of fat droplets (Wang et al., 2021). This structure formation is based on two types of partial coalescence: shear-induced partial coalescence and A/W interface-mediated partial coalescence (Zeng et al., 2022; Moen et al., 2016 and 2018; Thivilliers et al., 2010; Hotrum et al., 2005). Shear-induced partial coalescence is the orthokinetic destabilisation of fat droplets in the serum phase, while A/W interface-mediated partial coalescence occurs at the surface of an air bubble, as depicted in *Figure 18*.

Fat droplets enter the surface of the air bubble, followed by the distribution of oil from the fat crystal network. When this occurs between two adjacent fat droplets on the surface of the bubble, the fat droplets easily form a connection and partial coalescence occurs (Moen et al., 2016 and 2018; Hotrum et al., 2005). Consequently, the capture efficiency for partial coalescence in the presence of air is enhanced by concentrating and immobilising fat droplets at the air bubble surface, as well as the enhancement of connection formation caused by oil spreading at the surface.

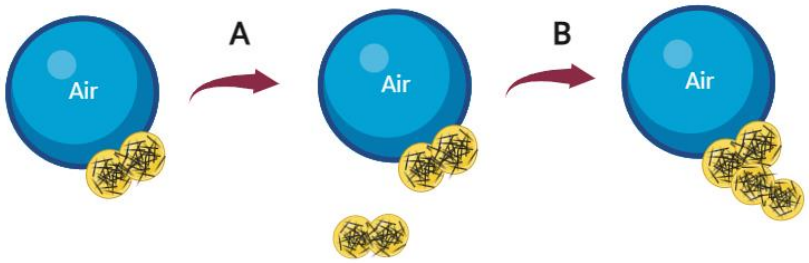


***Figure 18**: Schematic presentation of (A) air-water (A/W) interface-mediated partial coalescence combined with (B) shear-induced partial coalescence in whipped cream. Adapted from Hotrum et al., (2005).*

Adding an emulsifier to the cream improves whipping behaviour and foam stability. The adsorption of partially coalesced fat at the A/W interface is promoted by the addition of LMW emulsifiers by lowering the interfacial tension (Wang et al., 2011; Smith, Goff, and Kakuda 2000; Anderson and Brooker, 1988; Paquin and Dickinson, 1991). The addition of large molecular polysaccharide stabilisers causes an increase in the viscosity of the aqueous phase. Carrageenan has been shown to interact with casein micelles to form α-casein

glycomacropeptide complexes, which provide cohesion between membranes and serum while providing structural integrity to the foam.

According to Wang et al. (2011), hydrophobic emulsifiers accelerated fat crystallisation to increase whipping overrun and enhance foam stability. The foam had uniformly formed air bubbles surrounded by a network of fat crystals consisting of evenly distributed fat droplets. Douaire et al. (2014) analysed the arrangement of emulsifiers adsorbed at the O/W or O/A interface and their potential to impact partial coalescence due to the influence of fat crystallisation.

Partial coalescence is a complex phenomenon influenced by various factors. This research aims to elucidate the role of the fat crystal network within droplets. Our hypothesis posits that altering the arrangement and size of fat crystals can modify the susceptibility of fat droplets to shear-induced partial coalescence, thereby affecting the quality of whipped cream. *Figure 2* illustrates how different chapters contribute to the scope of this research.

The literature review in Chapter 1 focuses on fat crystallisation, particularly in oil-in-water (O/W) emulsions and explores partial coalescence with an emphasis on the fat crystal network and whippability of emulsions. It highlights the lack of knowledge regarding real-time quantification of the primary whipping mechanisms, the correlation between fat droplet size, and the incorporation of various types and concentrations of emulsifiers into the fat crystal network concerning partial coalescence. Additionally, the review identifies different strategies to modify the characteristics of this network.

Chapter 3: Materials and methods

3.1 Material

The materials used in this study were 99 wt.% premium grade of anhydrous milkfat (AMF) and sodium caseinate containing 92.5 wt.% protein, 0.8 wt.% fat, 4.66 wt.% moisture and 3.4 wt.% ash purchased from New Zealand Milk Products (NZMP) Fonterra New Zealand. Food grade Polysorbate 80 (Tween 80) and food grade Lactic Acid Esters of MDG (LACTEM) were kindly donated by Palsgaard, Hawkins Watts (Auckland, New Zealand). Food grade Distilled Mono and Diglycerides (MDG) were purchased from Danisco (St. Louis, MO, USA). Reverse osmosis (RO) water was used in all emulsions.

The commercial brands of Ultra-High Temperature whipping cream and fresh cream (referred to as UHT WC and FC) are sourced from local suppliers in the South and North Islands of New Zealand, respectively. Prior to use, emulsifiers, commercial creams, and AMF were stored at 4°C. All chemicals used were either analytical or high-performance liquid chromatography (HPLC) grade.

The detailed composition information for UHT WC, FC and AMF, as provided by the manufacturers, is shown in *Table 5*.

***Table 5**: Compositional information in average quantity per 100 mL for UHT WC, FC and AMF.*

Composition	UHT WC	FC	AMF
Fat	35.7 g	37.4 g	99.0
Saturated fat	24.3 g	24.7 g	N/A
Carbohydrate	3.0 g	3.0 g	N/A
Protein	2.3 g	2.4 g	N/A
Salt	35 mg	25 mg	N/A

** Abbreviations: UHT WC = UHT whipping cream, FC = Fresh cream, AMF = Anhydrous milk fat, N/A = Non-detectable*

3.2 Methods

3.2.1 Emulsion preparation

AMF was heated to 80°C before dissolving the lipophilic emulsifiers (MDG or LACTEM, if added) in the liquid AMF to form the oil phase. The prepared oil phase, sodium caseinate solution (1 wt.%) with hydrophilic emulsifier (Tween 80, if added) were subsequently mixed using high shear mixing (Silverson L5M-A, Silverson Machines Ltd., England) at 10,000 rpm

for 1 min. After the initial mixing, the emulsion was further homogenised in a two-stage high-pressure homogeniser (Rannie, Copenhagen, Denmark). The homogeniser previously preheated with water at about 40°C, carried out the homogenisation process at selected pressures in the ranges of 20 to 400 bar (see Chapter 5 for details) to produce different fat droplet sizes of emulsions after three passes.

The final emulsion consisted of 36 wt.% AMF stabilised by 1 wt.% sodium caseinate, as a control emulsion, with added emulsifier at two different concentrations (0.5% to 1.0%) as described in Chapter 6. The emulsions contained 0.01% sodium azide (NaN_3) as an antimicrobial agent. After homogenisation, the emulsions were immediately cooled to 4°C and aged at this temperature for 24 h before subjected to measurement and analysis, except for the average oil droplet diameter and protein surface load of the emulsions, which were measured immediately after homogenisation. This is intended to ensure that the original sizes and properties of the emulsions are preserved against aging and other influencing factors. Emulsions were prepared in triplicate for each experimental parameter examined.

3.2.2 Emulsion characterisation

3.2.2.1 Mean size and particle size distribution

The emulsion size distribution and fat droplet mean size were determined using a Malvern Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK). This laser light scattering analyser uses Mie theory and takes into account the refractive indices of milk fat (1.462) and water (1.330) as well as the assumption of particle sphericity to calculate the size distribution. In this study, before the particle size distribution analysis, the emulsions were diluted with a 1% sodium dodecyl sulphate (SDS) solution to exclude flocculated fat droplets and prevent the formation of reversible aggregates, representing true coalescence in the emulsions. The volume-weighted mean diameter $d_{4,3}$ was used to interpret the particle size data.

3.2.2.2 Stability and microstructure of emulsions

The physical stability of the emulsions was visually assessed to observe phase separation or creaming against gravity after 24 h and one week of storage at 4°C. The glass tube containing 20 mL of emulsion was levelled and photographed for observation. Each sample was measured in triplicate.

A microscopic examination was carried out to observe the aggregation state and size of fat droplets in the emulsion. This visualisation was performed under Polarised Light Microscope (Eclipse Ci POL, Nikon, Japan) and an Olympus BX53 Differential Interference Contrast (DIC) microscope (Olympus, Tokyo, Japan). The emulsion was first diluted in demineralised water

(10% v/v). A drop was then transferred to a microscope slide and covered with a cover slip. The microscopic images were taken at different magnifications at a constant room temperature. The microscopic images were taken in triplicate.

3.2.2.3 Protein surface load

The concentration of adsorbed protein was determined using the depletion method described by Segall and Goff (1999). The emulsions were stored at 4°C for 24 h before centrifugation. The samples were heated to 20°C in a water bath and centrifuged at 27,000 × g for 1 H to separate the fat droplets from the serum phase (Sorvall RC 6+, Thermo Scientific, MA, USA). The emulsions were. Serum was collected with a syringe and filtered through a 0.2 µm syringe filter. Protein content of the serum phase was determined with a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark) using a recalibrated sodium caseinate setting. The total mean surface area (S_1) was calculated using **Equation 3** (Berton, Genot and Ropers, 2011):

$$S_1 = S_{globule} \times \frac{m_{oil}}{V_{globule} \times p} \qquad \textbf{Equation 3}$$

Where, $S_{globule}$ is the surface area (m2) of a fat droplet and P is the fat density (g/m3). The $S_{globule}$ and $V_{globule}$ were calculated from $d_{3.2}$, the mean surface weight diameter. A density value of 0.915 g/m^3 was used for milk fat at 20ºC (Watson and Tittsler, 1961). Protein surface load was calculated as 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. The emulsions were measured in triplicate.

3.2.3 Fat crystallisation in emulsion

3.2.3.1 Refined stop-and-return DSC method

Isothermal crystallisation kinetics were analysed using the stop-and-return method described by Foubert et al. (2008) with modifications. Crystallisation curves were generated using a MDSC Q-2000 instrument (TA Instruments, New Castle, DE, USA). A hermetic aluminium pan (Tzero, TA Instruments USA) containing (<10 mg) emulsion was weighed with a microbalance, micro pipetted and sealed with a crimper press. An empty pan was used as a reference and nitrogen was used to flush the system. The time-temperature program (*t*-*T*) was used as follows: Hold at 65°C for 10 minutes to ensure a completely liquid state. Allow to cool to the isothermal crystallisation temperature at 25°C min^{-1} to 5°C, hold for the required crystallisation time, and then heat at 20°C min^{-1} to 65°C. The crystallisation time before remelting was varied for each cycle and ranged from 30 sec to 120 min. Melting curves were integrated using a

sloped straight line passing through the points at 45°C and 55°C, assuming that sensible heat is constant over the temperature range. The emulsions were measured in triplicate.

3.2.3.2 Nucleation behaviour of emulsion using Polarised Light Microscope (PLM)

Microscopic examination using a Polarised Light Microscope, PLM (Eclipse Ci POL, Nikon, Japan) equipped with a temperature control stage (Linkam Scientific Instrument, UK) was performed at a cooling temperature from 40°C to 4°C and a cooling rate of 5°C min^{-1} and holding each temperature for 30 min to observe the nucleation of the fat structure and correlate it with the results of the heating cycles derived from the refined stop-and-return DSC method.

3.2.4 Evaluation of aerated emulsion

3.2.4.1 Whippability using a rheometer

The rheological analysis was performed using a stress-controlled rotational rheometer (Physica MCR 302, Anton-Paar GmbH, Graz, Austria) equipped with a concentric cylindrical geometry (d_o = 27 mm, d_i = 25 mm, h = 40 mm) and a starch spindle (ST24-20/2V/2V-30) consists of a stirrer with three blades of 24 mm (length) and 8 mm (width). This spindle was chosen for its similarity to a mixer and its ability to introduce air from the surroundings to facilitate the incorporation of air into the emulsion during the whipping process. The cell was controlled at 8°C by a Peltier plate system.

Before pipetting into the geometry and cooling to 8°C for 5 min, the emulsion was equilibrated for 30 min at room temperature (approximately between 10 to 15°C). According to Jiang et al. (2019), storage time of the emulsion before aeration is generally required and therefore the quiescent stability of the emulsion is an important parameter to consider. During whipping, the rotational speed (rpm) of the stirrer was recorded until butter grains formed. The whipping time (*t*) was defined as the time required to reach the maximum apparent viscosity (η), which corresponds to the maximum foam firmness before the foam begins to collapse. Each sample was analysed in triplicate using RheoPlus/32 V3.62 for instrument control and rheological data analysis.

3.2.4.2 Foam structure using Confocal Laser Scanning Microscope (CLSM)

CLSM was used to observe the microstructure of aerated emulsions. Immediately before whipping, 0.02 wt.% Nile Red (Sigma-Aldrich, MA, USA) and Fast Green (Sigma-Aldrich, MA, USA) were added to the emulsion to stain the fat and protein, respectively. Stained emulsions were whipped at 8°C under conditioned parameters using a rheometer. The emulsions were

whipped until a firm peak formed. After whipping, the stained whipped emulsions were immediately placed on a microscope slide for observation. A high-resolution Zeiss LSM 900 Airyscan 2 system (Oberkochen, Germany) with a temperature control stage (Linkam Scientific Instrument, UK) was used to acquire the images. The excitation wavelength for Nile Red and Fast Green was 568 nm and 633 nm, and the emitted fluorescent light was detected at 569-640 nm and 650-700 nm, respectively.

3.2.4.3 Overrun

Liquid or whipped emulsion was poured into a 25 mL aluminium dish (40 mm diameter and 22 mm depth) until the container was full and levelled to the rim. When filling the container, air pockets were avoided, and the surface was smoothed with a scraper. The weight of the samples was measured, and the overrun of the foam was calculated according to the **Equation 4** below. The overrun of the foam was defined as the gas-to-liquid weight ratio expressed as a percentage (Allen et al., 2008a).

$$Overrun\ (\%) = \frac{m_1 - m_2}{m_2} \times 100\% \qquad \textbf{Equation 4}$$

m_1: the weight of the liquid cream (g)
m_2: the weight of the whipped cream (g)

3.2.4.4 Firmness

Deformation puncture measurements were performed at 8°C using a TA.XTPlusC Texture Analyzer (Stable Micro Systems, USA) equipped with a cylindrical acrylic probe (diameter 10 mm, height 35 mm). Penetration tests were performed on the surface of the aerated emulsions at a rate of 1 mm/s over 5 mm. The trigger threshold for starting of the measurement was set at 0.01 N (Fredrick et al., 2013a). The force required to reach this depth (5 mm) was defined as the firmness of the aerated emulsions. The aerated emulsions were gently poured into a 120 mL pottle until filled and levelled with a flat stainless-steel spatula. Each sample was measured in triplicate.

3.2.5 Data analysis

One-way analysis of variance (ANOVA) and student's t-tests were performed using Minitab (version 14, Minitab Ltd., Coventry, UK) to determine differences in whipping time between samples ($P < 0.05$).

Chapter 4: Method development - Monitoring the whipping of cream using a rheological technique

4.1 Introduction

Determination of shear-induced partial coalescence is a crucial factor affecting the whipping properties of oil-in-water (O/W) dairy emulsions. To develop stable whipped cream and accurately predict properties such as whipping time, overrun, firmness and stability, evaluating these factors using analytical techniques such as rotational viscosity, particle size distribution and microscopic imaging is essential (Du et al., 2021; Fuller et al., 2015; Han et al., 2023; Pawar et al., 2012; Boode and Walstra, 1993; Van Boekel and Walstra, 1981).

Rotational viscosity analyses are commonly performed at constant or varying frequency speed or stresses without introducing air into the emulsion (Davies et al., 2000; Fuller et al., 2015; Liu et al., 2019; Thivilliers et al., 2006.; Thivilliers-Arvis et al., 2010; Wang et al., 2021). Microscopic imaging has confirmed the presence of fat clumps (Du et al., 2021; Fuller et al., 2015; Han et al., 2018; Pawar et al., 2012), while changes in particle size during shear have been used to estimate the shear-induced partial coalescence rate in other studies (Boode and Walstra, 1993; Fuller et al., 2015; Van Boekel and Walstra, 1981).

However, there is a notable gap in examining the relationship between the shear-induced partial coalescence rate, measured by viscosity analyses and the connection between the whipping properties, a gap was only addressed by Fredrick et al. (2010) in connection with dairy creams. Therefore, the objective of this study is to utilise a newly developed rheological technique and investigate the complicated phenomena of shear-induced partial coalescence in the presence of air in emulsified oil-in-water (O/W) cream. This study also presents an advanced technique to provide a better perspective on the phenomenon of shear-induced partial coalescence combined with Laser Diffraction and Confocal Laser Scanning Microscopy (CLSM) to analyse sizes and shapes of aggregates formed during whipping. The insights gained from this study aims to develop a prediction tool using a rheometer, optimise processing conditions, provide real-time monitoring of the whipping process, and develop simple testing techniques.

In this chapter, a stress-controlled rotational rheometer with concentric cylindrical cup geometry and a starch spindle is used to qualify real-time monitoring of shear-induced partial coalescence kinetics in the presence of air (*Figure 19*). This spindle was chosen for its ability to introduce air during mechanical whipping similar to a conventional mixer, it rotates rapidly

to create a low-pressure area that draws air into the mixture, resulting in whipped cream with a lighter texture and greater volume, a similar mechanism as a conventional mixer.



***Figure 19**: Concentric cylinder geometry with Starch spindle.*

To elucidate the behaviour of fat droplets during the shearing process, the viscosity analysis is interrupted intermittently until the cream reaches its maximum viscosity or maximum whippability. Beyond this point, the emulsion is inverted or churned. In addition, particle size distribution and microscopic analyses were performed to gain deeper insights into the shear-induced partial coalescence process during whipping (*Figure 20*).

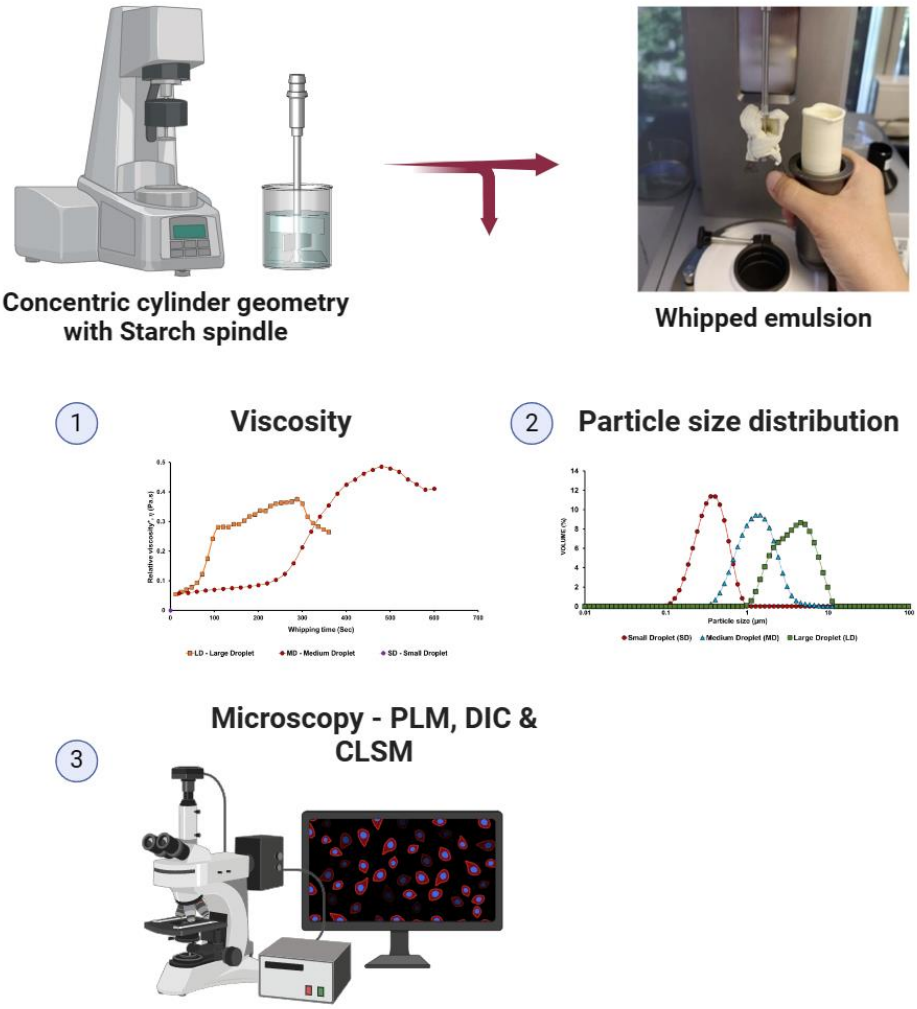


***Figure 20**: Analytical techniques used to monitor the whippability of cream sheared in the rheometer under conditioned parameters (volume: 10* mL*, speed: 1800 rpm, temperature 8 °C) in the presence of air. Abbreviations: PLM: polarised light microscope, DIC: Differential Interference contrast, CLSM: confocal laser scanning microscope.*

The potential of the rheometer as a prediction tool for whipping properties was evaluated by selecting three variables: rotational speed (rpm), temperature (°C) and sample volume (mL). The research examined the effects of these variables on the overrun (%) and whipping time (*t*).

4.2 Materials and methods

4.2.1 Materials

Please refer to section 3.1

4.2.2 Methods

4.2.2.1 Particle size distribution

Please refer to section 3.2.2.1

4.2.2.2 Stability and microstructure of emulsion

Please refer to section 3.2.2.2

4.2.2.3 Whippability using a rheometer

Please refer to section 3.2.4.1

4.2.2.4 Foam structure using confocal laser scanning microscope (CLSM)

Please refer to section 3.2.4.2

4.2.2.5 Data analysis

Please refer to section 3.2.5

4.3 Results and discussion

4.3.1 Characterisation of dairy creams

All experiments were carried out with commercial creams: Ultra-high temperature whipping cream (UHT WC) with a volume-weighted average droplet size $d_{4,3}$ of 1.88 µm and natural fresh cream (FC) with an average droplet size of 4.37 µm. The particle size distribution and microstructure of these creams were visually presented in *Figure 21* and *22*, respectively. The microstructure was captured under both differential interference contrast (DIC) and polarised light microscopy (PLM) at room temperature.

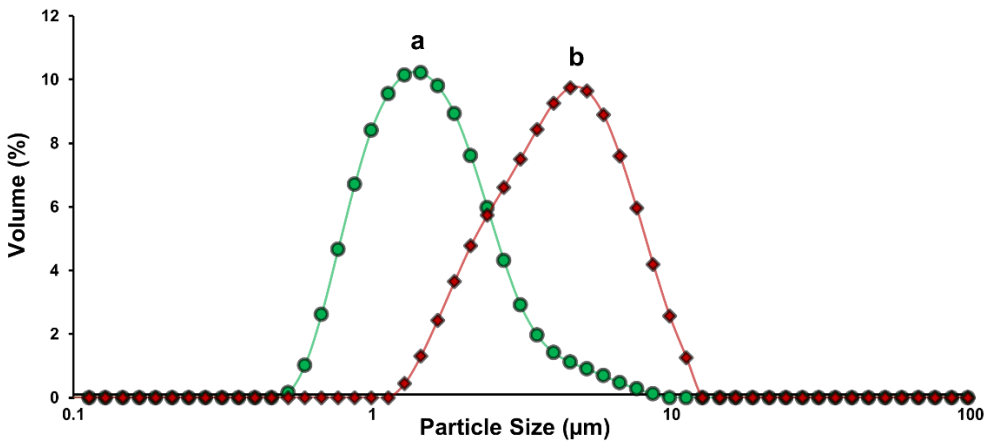


***Figure 21**: Particle size distributions of creams (a: UHT WC; b: FC). Abbreviations, UHT WC: Ultra-high temperature whipping cream, FC: Fresh cream.*

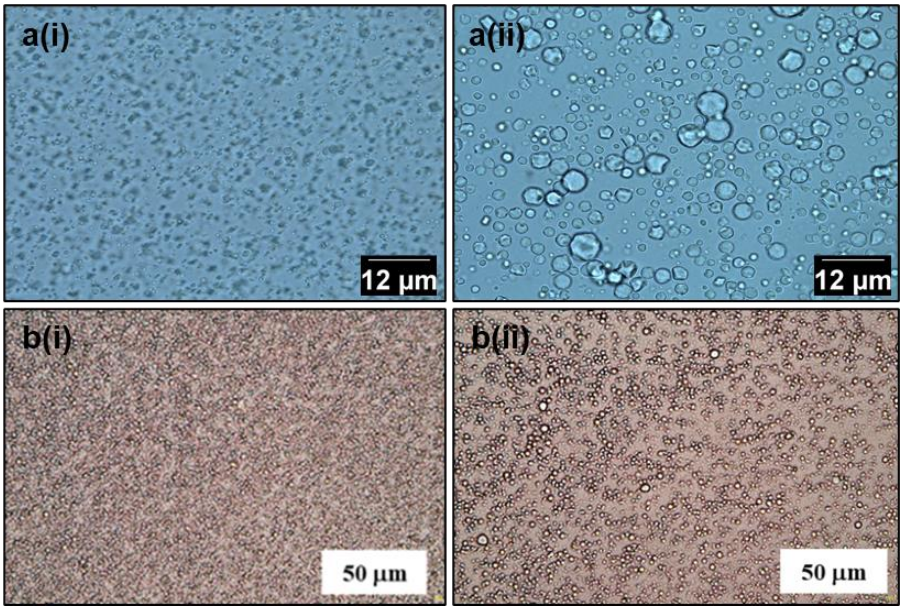


***Figure 22**: Differential Interference Contrast (DIC) and Polarised Light Microscope (PLM) micrographs of creams at different magnifications taken at room temperature: a(i) DIC of UHT WC, a(ii) DIC of FC; b(i) PLM of UHT WC and b(ii) PLM of FC.*

Fresh cream (FC) is a fat fraction of milk obtained by separating it from the non-fat portion of milk, called skimmed milk, either by centrifugal or gravitational forces (Early, 1998). The stability of fresh cream is attributed to the milk fat globule membrane (MFGM), a lipid bilayer membrane composed primarily of proteins and phospholipids, that acts as an emulsifier to stabilise the cream and prevent individual fat droplets from coalescing (Cano-Ruiz and Richter, 1997).

In the case of the UHT WC on the other hand, emulsifiers and stabilisers were added to the cream to improve its properties. The emulsifiers are surface-active substances introduced during processing leading to finer and well dispersed fat droplets. In addition, the homogenisation process produces narrower and smaller fat droplets with a more uniform size distribution, thereby reducing the rate of phase separation. This reduction in particle size distribution can be seen from the results shown in *Figure 21* and *22* and was later confirmed using the rheometer technique (see Chapter 4: 4.3.2, *Figure 23*). Without homogenisation, the particle size distribution of FC was wider compared to UHT WC, and the creaming rate is expected to be higher. This suggests that fat droplets are less uniform, and aggregation occurs more easily. This observation is supported by micrographs of FC taken with Differential Interference Contrast (DIC) and Polarised Light Microscope (PLM) at room temperature, as highlighted in *Figure 22*.

4.3.2 Rheometer technique

4.3.2.1 Experimental design

In the present study, a statistical experimental design with 18 experimental runs was designed using the Response Surface Method based on the Central Composite Approach, RSM-CCD (Design Expert 8.0.4, 2010). This experimental framework is used to determine conditioned parameters to evaluate the real-time whipping process in the presence of air. Three key variables were selected: rotational speed (X_1 :1600 – 2000 rpm), temperature (X_2 :4 – 12°C) and sample volume (X_3 :10 – 20 mL). The whipping (shear) time (300 sec) and overrun (approximately 50%) were selected as responses.

These responses were chosen due to the need to achieve a well-defined firmness/firm peak characterised by a visually stiff foam. With this technique, an acceptable overrun of approximately 50.00% is possible within a whipping time of 300 sec. It is noteworthy that the acceptable overrun for commercial whipped UHT WC and FC in our study is relatively low compared to the acceptable overrun reported in the literature using conventional mixer, which typically exceeding 80 - 90% (Jakubczyk and Niranjan, 2006). This deviation is due to the small sample volume used in the concentric cylindrical geometry. The optimal parameters used in this experimental design are presented in ***Appendix 1***.

Figure 23 shows the whipping progress of the UHT WC in the rheometer and depicts the apparent viscosity (η) versus time (t) at different rotational speeds (X_1) up to 300 sec with the observations of whipping phases as under-whipped (UW), ideally-whipped (IW) and over-whipped (OW). The decision to limit the whipping time in the rheometer was made to ensure that the whipping time does not exceed 5 minutes, as the typical whipping time for both UHT WC and FC with a conventional mixer is between 3 and 4 minutes to achieve the acceptable overrun.

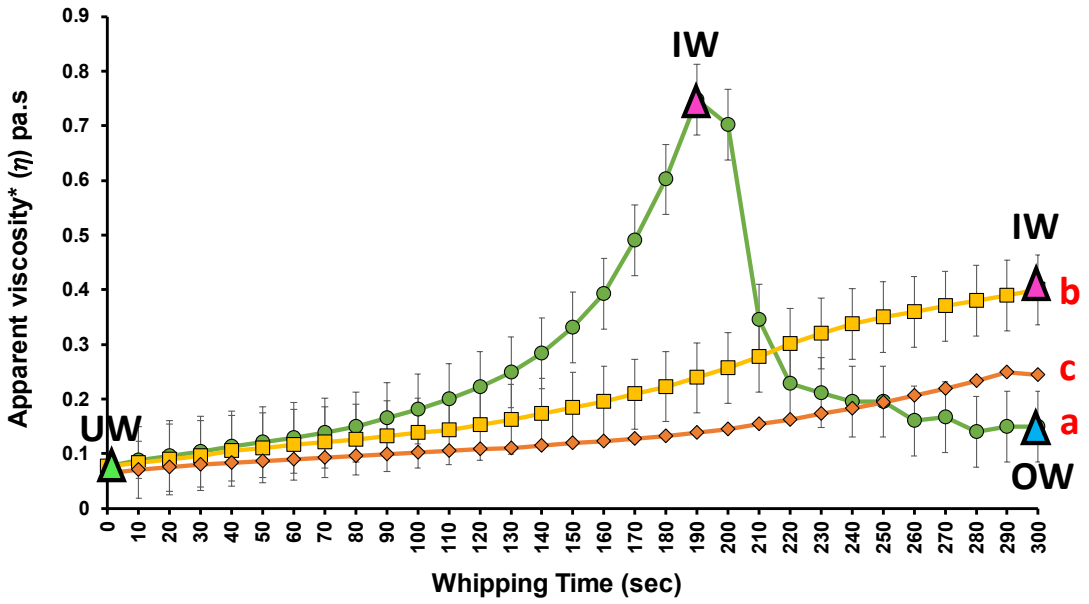


***Figure 23**: Apparent viscosity profile (η) versus whipping time, 300 sec (t) of 10 mL UHT WC at various rotational frequency speeds (rpm), where a = 2000 rpm (OW, over whipped), b = 1800 rpm (IW, ideally whipped), c = 1500 rpm (UW, under whipped) at temperature of 8°C.*

As highlighted in *Figure 23*, the apparent viscosity curves (η) showed an initial increase above 0 sec. of whipping time, indicating the under-whipped (UW) stage, where the fat droplet sizes remained unchanged until partial coalescence was initiated. Notably, the apparent viscosity (η) profile of UHT WC whipped at different rotation speeds (2000, 1800 and 1500 rpm) showed three phases of whipping, which were identified as under-whipped (UW), ideally-whipped (IW) and over-whipped (OW). The viscosity exhibited a progressive increase until reaching an initial peak, regardless of speed. This phenomenon is likely attributed to the irregular fat aggregation, which leads to a gradual increase in viscosity until a continuous network forms, thus increasing the volume. This continuous network gives the emulsion solid-like properties and immobilises other particles such as air bubbles, which characterises the ideally-whipped (IW) cream. According to Graf and Muller (1965), an ideal whipped cream can be defined as the foam that has a rigid structure and high overrun and is stable against deformation.

At 2000 rpm, the viscosity of the cream subsequently decreased with advancing shear and time due to partial coalescence, causing the UHT WC to transition to an over-whipped (OW) state, which is a phase inversion to a water-in-oil (W/O) emulsion.

As shown in *Figure 23*, phase inversion for UHT WC was only observed at the rotational frequency speed of 2000 rpm. According to Fredrick et al. (2010b), phase inversion from O/W to W/O emulsions occurs when fat droplets undergo rapid partial coalescence and aggregate into a continuous network. Evaluation of apparent viscosity profile (η) versus whipping time (t)

for UHT WC based on rotational speed (X_1 :1600 – 2000 rpm) and other 2 key variables temperature (X_2 :4 – 12°C) and sample volume (X_3 :10 – 20 mL) showed that a rotational speed of 1800 rpm produced a satisfactory firm peak of whipped cream within 300 sec of whipping at maximum overrun (*Figure 24*).

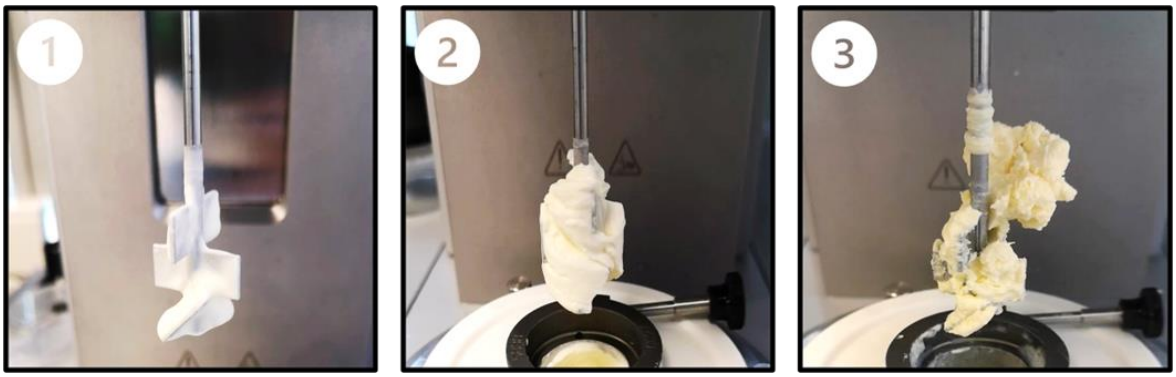


***Figure 24**: (1) Under whipped (UW), (2) Ideally whipped (IW) (3) Over whipped (OW) of UHT WC under conditioned parameters (volume 10 mL, temperature 8°C, and rotation speed, 1800 rpm).*

Additionally, method development runs demonstrated that a firm peak could be achieved with a 10 mL sample of whipping cream, suggesting that efficient whipping is possible on a relatively small scale. Various whipping temperatures were tested (ranging from 4 to 12°C) and 8°C was identified as the optimal temperature at which fat droplets partially crystallised and the ability to form a firm peak and high overrun. Consistent with previous studies by Buchheim et al. (1987) and Walstra et al. (2005), who found that a chilled temperature is required for whipping cream, because lower temperatures are associated with increased foam overrun and reduced foam density.

Furthermore, whipping the cream at a lower temperature (between 4 and 10°C) resulted in an increase in the cream viscosity, allowing the incorporation of air bubbles and the formation of a stable whipped foam. This effect is attributed to the reduced mobility of the fat droplets in the cream at lower temperatures, which hinders the melting of the fat crystals and promotes partial coalescence (Fredrick et al., 2010b). Overall, selecting an appropriate whipping temperature, sample volume and rotation speed for the rheometer is crucial to achieving the high-quality whipped cream.

The formation of a stable foam structure in whipped cream depends on the interaction between the fat droplets themselves, and between fat droplets and air bubbles. *Figure 25* shows the rosettes of the whipped UHT WC and FC immediately after whipping, whipped under the

conditioned parameters listed in *Table 6*. Both creams exhibited firm peaks and maximum air bubble entrapment. After 2 h of production, no syneresis was observed.

Based on the results obtained, the conditioned parameters were a rotational speed (X_1) of 1800 rpm, a temperature (X_2) of 8°C, a sample volume (X_3) of 10 mL with a shear time of 300s and an overrun of 54.10% for UHT WC and 56.10% for FC. These findings highlight the importance of a carefully selected experimental design to elucidate the relationship between variables and optimise the desired foam properties.

***Table 6**: Conditioned parameters for whipping with the rheological technique. Abbreviations: UHT WC: Ultra-high temperature whipping cream, FC: Fresh cream.*

Commercial cream	Rotational frequency speed (rpm)	Sample (mL)	Temperature (°C)	Time (Sec)	Overrun (%)
UHT WC	1800	10	8	300	54 ± 1.50^a
FC	1800	10	8	300	56 ± 1.21^a

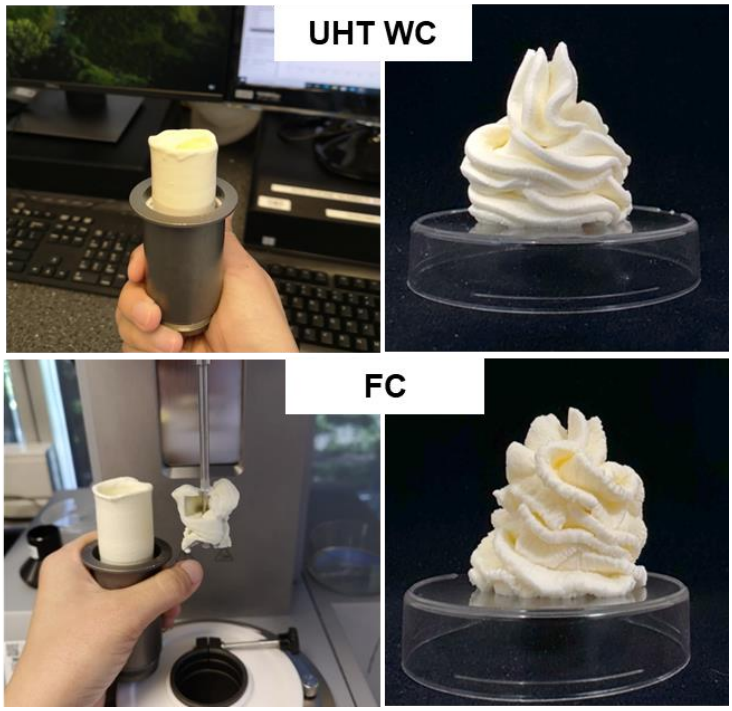


***Figure 25**: UHT WC and FC after being whipped following conditioned parameters and piped into a rosette. Abbreviations: UHT WC - Ultra-high temperature whipping cream, FC - Fresh cream.*

4.3.2.2 Apparent viscosity profile

The viscosity profiles of UHT WC and FC, subjected to the conditioned parameters of constant rotational speed at 1800 rpm for 300 sec at 8°C, are shown in *Figure 26*. The commercial whipping creams examined showed different apparent viscosity profiles, particularly with UHT WC having a higher viscosity than FC. The viscosity graph of UHT WC showed a gradually increasing curve until reaching a maximum viscosity, which was identified as a firm peak at 240 sec (IW), followed by a continuously decreasing apparent viscosity until 360 sec, indicating the entry into the churning phase. Conversely, FC exhibited a different trend, quickly reaching an ideally-whipped (IW) phase at 120 sec, with maximum viscosity evident through complete churning and subsequently leading to a noticeable cream separation with visible fat and serum phases at 180 sec.

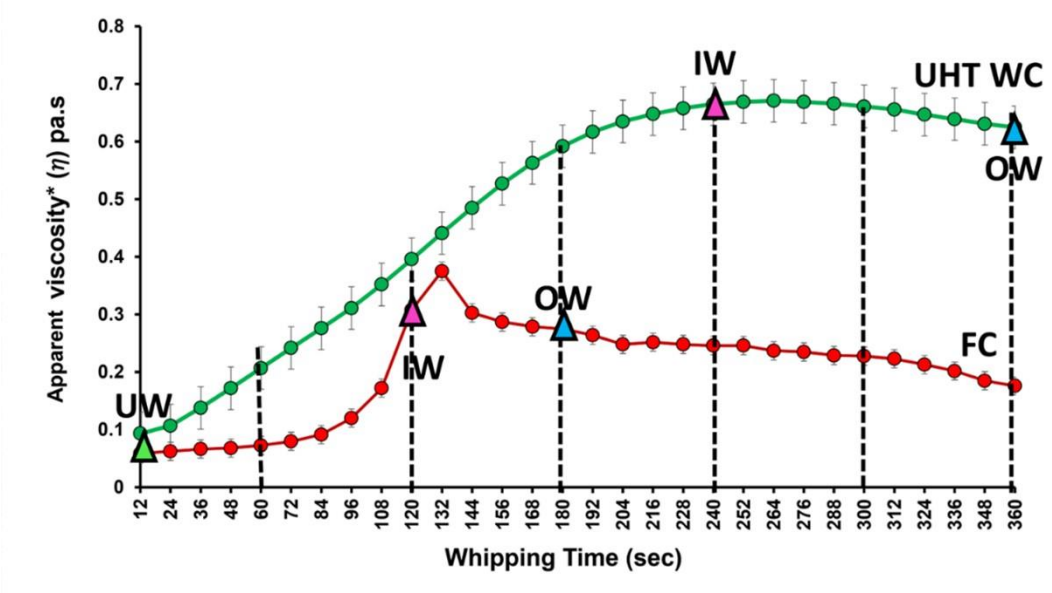


***Figure 26**: Viscosity profiles of UHT WC and FC for a duration of 360 sec at 8°C and at a rotational frequency speed of 1800 rpm. Where UW = under whipped, IW = ideally whipped and OW = over whipped.*

The results showed that the whipping time to reach the ideally-whipped phase (IW) of FC was significantly shorter compared to UHT WC, which was accompanied by an increase in the mean fat droplet size (*Figure 21*). This finding indicated that smaller fat droplets are relatively more shear resistant than larger fat droplets, which accounts for the longer whipping time in our study. Good agreement was found when comparing results from this work with published data by Panchal et al. (2017) and Truong et al. (2014), highlighting the size range of bovine milk (0.5 to 20 µm). Furthermore, the churning time of cream showed a significant increase

from 132 (FC) to 360 min (UHT WC) as the average fat droplet sizes decreased from 4.97 to 1.87 µm.

Particular attention was paid to UHT WC, whose higher viscosity and longer whipping time are due to the homogenisation effect, which reduces the particle size and increases the surface area. The addition of stabilisers in this recipe, also increases the viscosity of the aqueous phase. This provides cohesion between the dispersed phase and the serum and gives the overall structural integrity to the foam. This reduced size of the fat droplets affected their coalescence and stabilisation during whipping.

Additionally, surface-active substances and stabilisers are commonly added to UHT cream to create a finer foam, which requires a longer whipping time to break up the fat droplet interface and incorporate air bubbles into the stable emulsion. These observations were also supported by findings from Bruhn and Bruhn (1987) and Dhungana et al. (2019). In contrast, according to Moens et al. (2018), larger particles > 4.00 µm have more favourable properties when whipping. Their lower surface area-to-volume ratio allows for better stabilisation, because the lower number of interfaces allows for more effective air bubble entrapment. Additionally, the larger particles promote a more controlled fat crystallisation process and contribute to the formation of a stable partially coalesced crystalline network essential to the foam structure.

The initial delay in stabilisation of the air bubble may be an indication of a more stable interface in the UHT WC. A fine balance must be achieved for proper structure development and integrity; fat droplet membrane must be destabilised to support partial coalescence, MFGM must interact with proteins in the air serum during whipping and the resulting foam structure must resist collapse (Brooker, 1990).

4.3.2.3 Mean size and particle size distribution (PSD)

Understanding the intricate relationship between particle size and shear-induced partial coalescence in the presence of air using this rheometer technique provides valuable insights into optimising the whipping process to produce a desired foam characteristic. Adjusting the particle size within the suitable range can be a critical parameter to achieve the desired balance between stability and textural properties in whipped products such as whipped cream.

In emulsions, the crystallisation behaviour can be affected primarily by the different fat droplet sizes. When investigating behaviour of the cream under shear in the presence of air in the rheometer, the viscosity analysis was strategically interrupted at multiple intervals, 0, 60, 120, 180, 240, 300 and 360 sec. The particle size distribution (PSD) of the fat droplets and air

bubbles was analysed using Laser Diffraction analysis, concurrently with CLSM to visualise the microstructure of whipped cream samples and obtain better information about the aggregation process by imaging single focal plane in a sample of arbitrary thickness.

Before analysing the particle size distribution, the creams were diluted with a 1% sodium dodecyl sulphate (SDS) solution to exclude flocculated droplets and prevent the formation of reversible aggregates. Therefore, the growth of sizes measured represents true partial coalescence in the emulsion. *Figure 27* and *28* show the PSD curves from 0 to 360 sec and from 0 to 180 sec for the UHT WC and FC respectively, obtained during whipping with a rheometer.

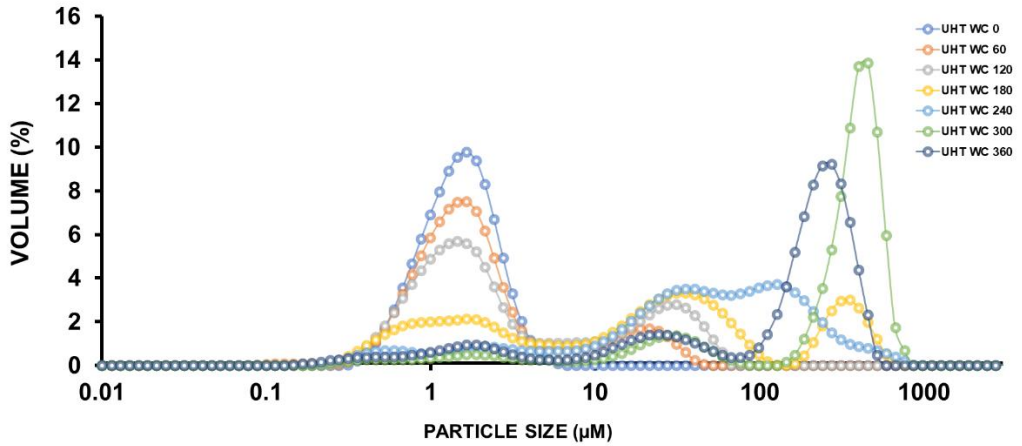


***Figure 27**: Particle size distribution of UHT WC whipped in the rheometer, from 0 to 360 sec (T = 8°C, rotation speed = 1800 rpm, volume = 10 mL).*

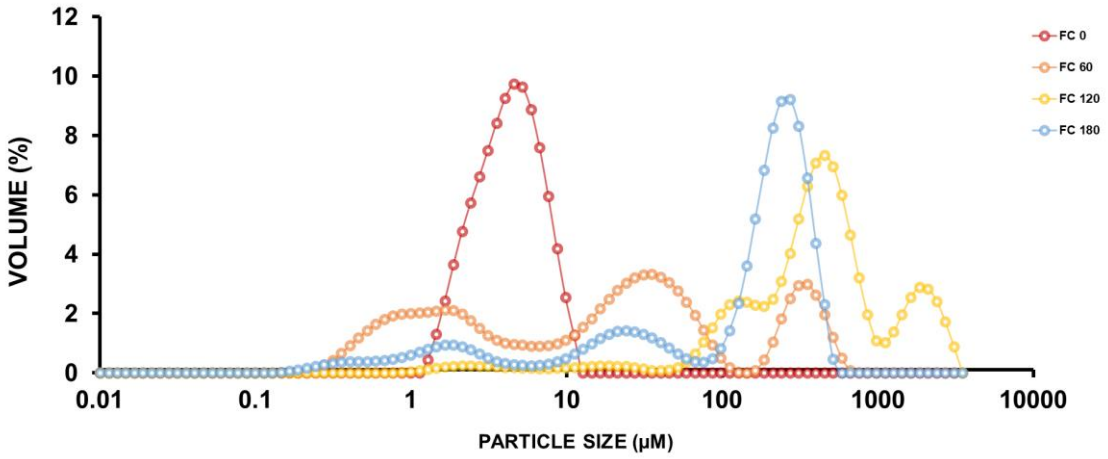


***Figure 28:** Particle size distribution of FC whipped in the rheometer, from 0 to 180 sec (T = 8°C, rotation speed = 1800 rpm, volume = 10 mL).*

The initial peak at about 2 µm, (*Figure 27*) and 5 µm (*Figure 28*) of the unwhipped UHT WC and FC, represents the original average particle size of the samples. As long as partial coalescence was not initiated, the droplet size remained unaltered and hence encounter of frequency (*i.e.,* number of collisions) was constant (Moens et al., 2018b).

As whipping progressed, the initial peak almost completely disappeared for both UHT WC and FC, indicating that all fat droplets are equally susceptible to shear-induced partial coalescence. The second stage of ideally-whipped phases for UHT WC (240 sec) and FC (120 sec), represents the formation of aggregates due to shear as peaks at larger sizes. These important peaks showed broadening to larger sizes, indicating enlargement of aggregates and incorporation of air bubbles, presumably between 10 and 100 µm (Hotrum et al., 2004, 2005). The greater the extent of partial coalescence, which increases with shear and time, the greater the width of the curve.

These multimodal peaks suggested that partial coalescence occurred due to the frequency of collision between semi crystalline fat droplets. At this phase, the emulsion was ideally whipped (IW), which was visually distinguished by the air bubbles stabilised by partially coalesced fats droplets, as shown in the subsequent section. In this phase, whipping is mainly influenced by the interfacial tension and the microstructure of the foam, which was formed by the spreading of fat droplets on the air bubble surface, forming a continuous network of fat droplets throughout the aqueous phase. The network formed provided the necessary mechanical strength required to give the whipped cream structure good stability (Goff and Hartel, 2013; Moens et al., 2018b).

The extent of partial coalescence increased with shear and time, causing the emulsion to transition to an over-whipped (OW) state. The third monomodal peaks at the larger sizes > 100 µm were observed for both creams (UHT WC > 300 – 360 sec; FC > 180 sec – 300 sec) and represent the over-whipped phase in which all fat droplets were particularly larger than the original droplets due to partial coalescence and churning, also known as phase inversion, along with a reduction in air bubbles sizes, indicating that the foams were partially collapsing. At this stage, a decrease in viscosity suggested phase inversion and a weaker network of coalesced fat droplets.

The shear-induced partial coalescence was faster for FC than for UHT WC, as we noticed that the viscosity of FC increased soon after shear was initiated, and at the same time the overall time to reach phase inversion was shorter. From this observation, we concluded that FC is significantly less stable to partial coalescence than UHT WC, which contributes to a larger particle size. In contrast, UHT WC allows for slower stabilisation due to the smaller particle

size and lower surface area to volume ratio, thus extending the whipping time until air bubbles are effectively trapped in the foam structure. The modified fat structure, significantly smaller than native fat droplets, resulting in a larger surface area, promoting greater contact with surrounding air bubbles, resulting in increased air-water (A/W) interfaces and making foam more difficult to stabilise.

4.3.2.4 Crystal morphology using Confocal Laser Scanning Microscopy

During whipping, cream undergoes a transformation from a two-phase liquid system, O/W emulsion to a three-phase solid system, incorporating air and trapping it in a crystalline network of fat droplets (Du et al., 2021). The mechanism of partial coalescence focusing on microstructural changes after whipping with a rheometer was further monitored using CLSM.

Confocal images of cream whipped in the rheometer at 8°C, are shown in *Figure 29*, and depict the whipping phases of UHT WC and FC. This image provides the real-time visualisation of the microstructure of fat droplets, proteins and air bubbles of UHT WC and FC whipped following conditioned parameters in the rheometer.

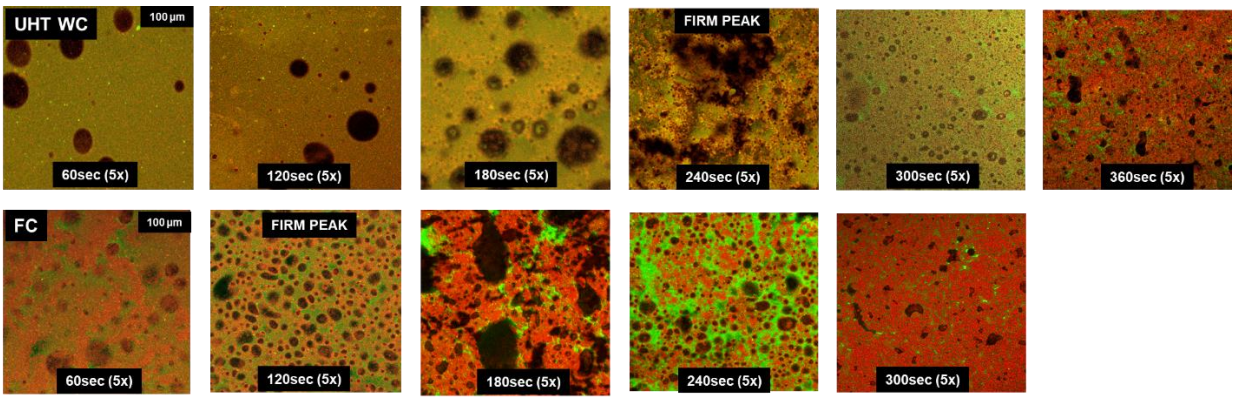


***Figure 29**: The confocal laser scanning microscopy (CLSM) micrographs of UHT WC and FC whipped under conditioned parameters in the rheometer taken at 8°C. In red the fat phase, in green the protein and in black the air bubbles.*

In the early stage of whipping (UHT WC: 60 sec – 180 sec; FC: 60 sec), large air bubbles were introduced, which were initially uncovered and incorporated into the cream after substantial bubble size reduction. The apparent viscosity remained low at this phase, allowing shear-induced partial coalescence and some adsorption of fat droplet at the surface of the air bubbles.

During the ideally-whipped (IW) phase (UHT WC: 240 sec), the continuous rupture of the air bubble led to an expansion in the bubble surface area and a dynamic change in surface tension. Adsorption of fat droplets occurred, resulting in dense packing of the bubbles and a shift to laminar flow due to increased viscosity. Bubble coalescence occurred to a lesser extent than disruption, and firmness increased gradually with a significant decrease in serum loss *(Figure 29 firm peak).*

Meanwhile FC reached the IW phase faster (120 sec) than UHT WC (240 sec) suggesting that the kinetics of partial coalescence, influenced by fat droplet susceptibility and collision frequency, exhibited greater rates in FC than at UHT WC, which is supported by viscosity observation and particle size analyses. Networks of shear-induced partially coalesced fat droplets contributed to high foam stability, as indicated by well-dispersed spherical-shaped air bubbles immobilised in a rigid network of fat droplets. This observation is also confirmed by Moens et al. (2019).

In the OW phase, intense red areas can be observed for both UHT WC (360 sec) and FC (180 sec), referred to as fat phase. This phase resulting in a lack of air bubble retention and lower overrun. The deformation of the air bubbles (black areas), whose irregular air bubble structure indicated poor foam stability, which was related to a reduction in overrun, as large fat aggregates ruptured the lamellae enclosing the air bubbles and then coalesced along with the air loss (Wang et al., 2021b).

4.4 Conclusions

In this study, we elucidated the significant impact of droplet size of two types of commercial creams, UHT WC and FC. The rheological setup and methodology developed provided a means to systematically explore the influence of shear-induced partial coalescence in the presence of air in whipping cream systems. By integrating rotational rheology with starch spindle analysis, we comprehensively assessed the kinetics of partial coalescence in the presence of air, examining the viscosity, particle size, and microstructure of air bubbles and fat droplets in the analysed creams. The successful development of this methodology has highlighted its potential for reliable and real-time use of this technique in the future and contributed to a more comprehensive understanding of partial coalescence in the presence of air in cream systems.

Chapter 5: Factors influencing fat crystallisation and partial coalescence of fat droplets and subsequent whipping properties: i: Effect of fat droplet size

5.1 Introduction

The aim of this chapter was to explain the influence of fat droplet size in homogenised milk creams and their whipping properties. To achieve this, a two-stage high pressure homogeniser (HPH) was used to produce milk creams with different fat droplet sizes. The size of the emulsion droplets is known to influence the crystallisation properties of the dispersed fat phase (Lopez et al., 2005a; Lopez, 2020; Montenegro et al., 2003).

In a dispersed system, each droplet requires a nucleus or impurity to initiate nucleation which requires the need for extensive supercooling compared to bulk fat (Fredrick et al., 2010a; Walstra et al., 2005). Variations in the droplet size of the emulsion can alter the crystallisation, structural behaviour and stability of the foam, as well as and partial coalescence of emulsified fats (Dhungana et al., 2020; Truong et al., 2019). Studies have shown that submicron droplets have a lower crystallisation temperature than micron droplets in *n*-hexadecane O/W emulsions (Dickinson et al., 1991).

Although recent research has reported the crystallisation properties of milk fat blended with various oil sources; i) palm oil (Nguyen et al., 2020), ii) palm stearin and palm oil (Mao et al., 2022), iii) unsaturated fatty acids (Bugeat et al., 2011), iv) sunflower oil blends (Martini et al., 2002), and the use of various emulsifiers (Fredrick et al., 2013a; Jiang et al., 2019a; Wang et al., 2021), the crystallisation behaviour of milk fat is still far from being fully understood. The main reason is the very high diversity and complexity of triacylglycerols (TAGs), with more than 200 species accounting for > 98% of the total milk fat composition. There is limited information about the crystallisation behaviour of milk fat emulsions due to their TAG's complexity (Jensen et al., 1990).

In addition to this complexity, the composition of milk fat TAGs can vary greatly due to many factors such as milking seasonality (Li et al., 2022), animal breed (Tzompa-Sosa and Arita-Merino, 2022), and lactations duration (Pacheco-Pappenheim et al., 2022), feed (Palmquist et al., 1993), and regional variability (Shi et al., 2001). Therefore, a better understanding of the droplet size-dependent crystallisation properties of milk fat emulsions are required to evaluate the mechanism of partial coalescence and subsequent whipping properties of dairy emulsions (Cerdeira et al., 2005; Cheng et al., 2020; Dickinson, 2019; Fredrick et al., 2010; Jiang et al., 2019b; Liu et al., 2022; Rizzo et al., 2015; Warren and Hartel, 2018).

This chapter addresses the behaviour of fat crystallisation in an emulsified fat, by manipulating the key variable, micron scale fat droplet size, as illustrated in *Figure 30*.

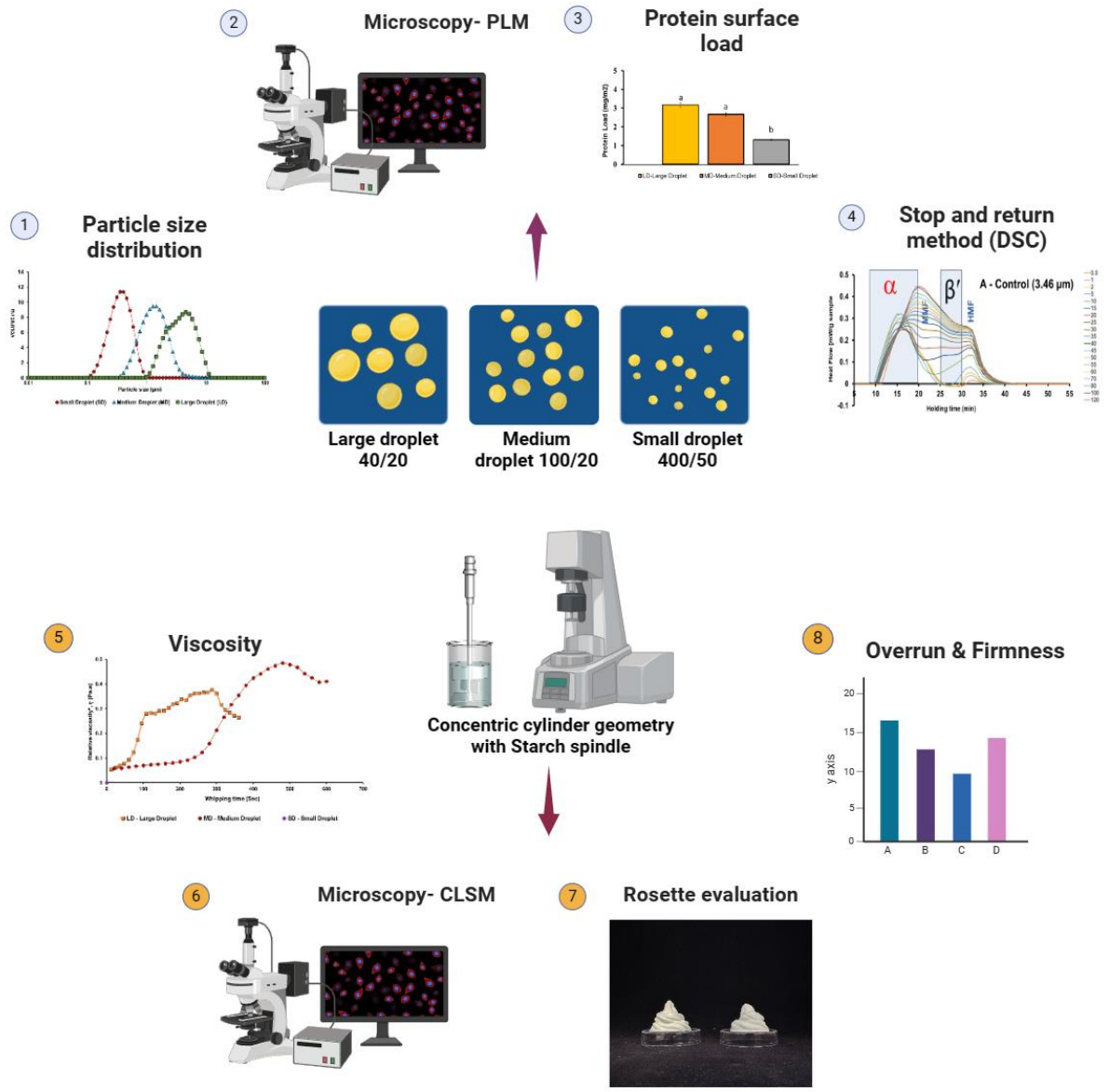


***Figure 30**: The analytical techniques such as viscosity, differential scanning calorimetry (DSC) properties, particle size distribution, protein surface load and microscopy will provide the notable strength to fill the crucial knowledge gaps. These techniques provide a new perspective and deeper understanding of how fat droplet size influences emulsion crystallisation.*

The insights gained in this chapter provide a better understanding of how variations in fat droplet size modulate the crystallisation of emulsified milk fat. In addition, it offers valuable information for better control of this key process, that determines the structure and functionality

of whipping cream. Furthermore, the use of the novel of the novel technique for evaluating the whippability of cream using the rheometer, presented in Chapter 4, and further applied in this section, enhances the comprehensiveness of our study.

The hypothesis is that the following factors can have a very important effect on crystallisation: particle size distribution, protein surface load at the O/W interface, and surface nucleation. It is suggested that different nucleation mechanisms and crystallisation kinetics due to the emulsion droplet size could influence the rate of partial coalescence and, consequently, affect the whipping properties of the recombined cream model system.

5.2 Materials and methods

5.2.1 Materials

Please refer to section 3.1

5.2.2 Methods

5.2.2.1 Emulsion preparation

Please refer to section 3.2.1

5.2.2.2 Particle size distribution

Please refer to section 3.2.2.1

5.2.2.3 Stability and microstructure of emulsion

Please refer to section 3.2.2.2

5.2.2.4 Protein surface load

Please refer to section 3.2.2.3

5.2.2.5 Refined stop-and-return DSC method

Please refer to section 3.2.3.1

5.2.2.6 Whippability using a rheometer

Please refer to section 3.2.4.1

5.2.2.7 Foam structure using confocal laser scanning microscope (CLSM)

Please refer to section 3.2.4.2

5.2.2.8 Overrun

Please refer to section 3.2.4.3

5.2.2.9 Firmness

Please refer to section 3.2.4.4

5.2.2.10 Data analysis

Please refer to section 3.2.5

5.3 Results and discussion

5.3.1 Emulsion characterisation

5.3.1.1 Mean size and particle size distribution

Milk is an O/W emulsion consisting of fat droplets dispersed in a continuous serum phase. To improve emulsion stability, homogenisation is generally used in the dairy industry. Homogenisation breaks down the oil phase into small particles increasing the surface area of the oil droplets. The mean droplet size and the droplet size distribution are important characteristics of emulsions. High-pressure homogenisation (HPH) mechanically reduces the size of the fat droplets to less than 1 µm, thereby producing emulsions characterised by uniform fat droplet size and higher stability, as highlighted by Lee et al. (2009).

The effect on particle size using the volume-weighted mean diameter $d_{4,3}$ at three different homogenisation pressures; 400/50 bar produces small droplets (SD), 100/20 bar produces medium droplets (MD) and 40/20 bar produces large droplets (LD) measured immediately after homogenisation, as shown in *Figure 31*. The emulsions exhibited statistically significant differences (P>0.05) in particle size distribution and mean values across samples.

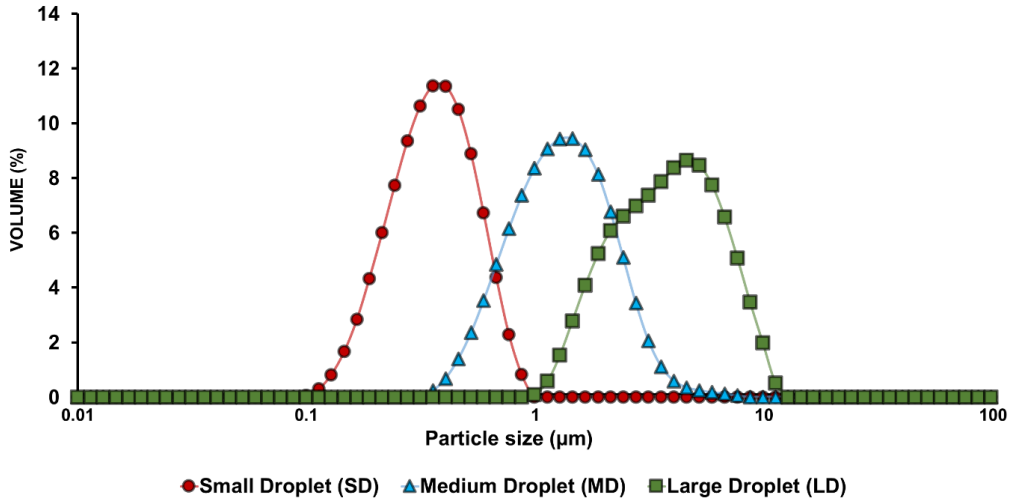


***Figure 31**: Particle size distributions of emulsions at three homogenisation pressures. Small droplet (SD) with homogenisation pressure of 400/50, medium droplet (MD) with 100/20 and large droplet (LD) with 40/20.*

For the large droplet emulsion (LD), the particle size was between 1 and 10 µm, with a volume-weighted mean diameter, $d_{4,3}$, of 4.52 µm. The medium droplet emulsion (MD) had a size

distribution between 0.5 and 7 μm, with a $d_{4,3}$ of 1.57 μm. In contrast, the small droplet emulsion (SD) had sizes between 0.1 and 1 μm with an average $d_{4,3}$, of 0.41 μm.

In the absence of surfactants, all three emulsions exhibited particle size $d_{4,3}$ values that were in the micron range, with a monomodal distribution, indicating that the fat droplets are homogeneous and well-protected, without significant fat aggregation or tendency to cream. Notably, the distribution size decreased with increasing pressure.

This observation is consistent with the fundamental principles of HPH, in which larger fat droplets in the emulsion are broken down into smaller, more uniform droplets. The increased pressure and high shear forces generated by the turbulent motion facilitate the fragmentation of these fat droplets, resulting in a reduction in their size to the submicron level. The mechanical forces exerted during HPH serve to disperse, homogenise, and refine the emulsion, ultimately leading to the observed inverse relationship between applied pressure and droplet size. This finding is consistent with established literature, which has demonstrated that HPH effectively produces emulsions with smaller droplet sizes, contributing to improved stability compositional uniformity (Lee et al., 2009; Tadros et al., 2004).

5.3.1.2 Stability and microstructure of emulsions

Figure 32 highlighted the stability of emulsions after 24 storage at 4°C, along with the microstructure visualised under polarised light microscope (PLM). Particle size measurements and visual observations demonstrated that all emulsions remained stable, maintaining consistent particle size distributions over storage. No visible aggregation or creaming of fat droplets was observed even after a week of storage at chilled temperature (4°C), and the droplet size distribution was found to remain constant during storage (picture and data not shown). Sodium caseinate has good solubility and excellent emulsifying properties as previously observed by Dickinson (1999). Walstra et al. (2005) also reported that casein molecules and aggregates become rapidly associated with the surface of newly formed oil droplets generated during emulsification using HPH. In addition, the steric stabilising casein

layer protects fine fat droplets against immediate recoalescence, and it confers high stability during subsequent processing and storage against droplet aggregation.

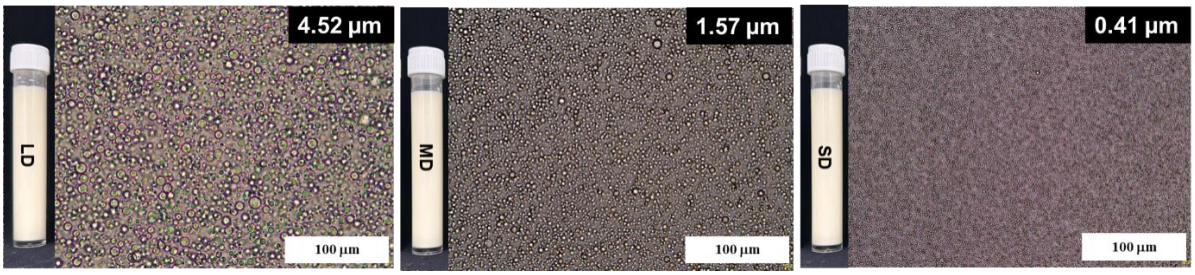


***Figure 32**: Stability of emulsions after 24 h stored at 4°C and microstructure of emulsions visualised under Polarised Light Microscope, PLM (Scale bar: 100 µm with oil immersion). Small droplet (SD) with homogenisation pressure of 400/50, medium droplet (MD) with 100/20 and large droplet (LD) with 40/20.*

Fat crystallisation in fat droplet can increase the instability of the emulsion. To monitor the effect of fat droplet size on crystallisation, the emulsions (large droplet, LD, medium droplet, MD, and small droplet, SD) were examined under PLM using a temperature-controlled stage cooling from 40°C to 4°C at 5°C/min. Nevertheless, the results showed that it is difficult to study the crystal formation in an emulsion due to its size. According to Lopez et al. (2000), the emulsion must be larger than 5 µm to monitor the crystal morphology.

5.3.1.3 Protein surface load

Stabilisation of the emulsion against droplet-droplet aggregation is predominantly governed by proteins at the interface. This protein presence leads to the formation of a densely adsorbed layer on the fat droplet surfaces, which is crucial for effective steric stabilisation as well as electrostatic stabilisation above or below the protein. The protein surface loading of these emulsions is a key factor in evaluating their interfacial membranes stability, as discussed in detail by Dickinson in both 2001 and 2013. The effect of fat droplet size on the degree of protein surface load of emulsions with similar protein content is shown in *Figure 33*.

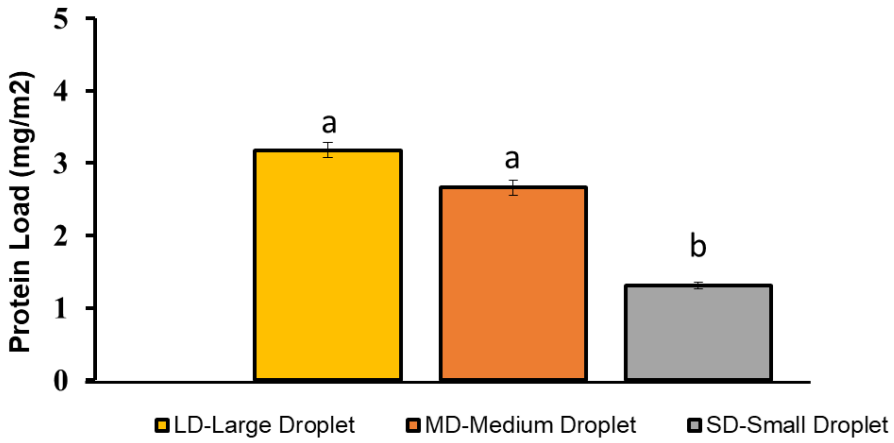


***Figure 33**: Impact of fat droplet sizes on the degree of protein surface load in emulsions. Emulsions were dispersed with 36 wt.% of anhydrous milkfat and stabilised with 1 wt.% of sodium caseinate. Fat droplet size of LD – 4.52 µm, MD – 1.57 µm and SD – 0.41 µm.*

Homogenisation of emulsions results in a reduction in fat droplet size with an increase in surface area that needs to be covered and stabilised by the adsorption of protein molecules such as sodium caseinate at the O/W interface (Floury et al., 2000; Truong et al., 2014).

The results showed that the emulsion with large fat droplet sizes (LD – 4.52 µm) had a surface load of approximately 3.18 mg/m^2, which corresponds to the largest amount of protein distributed at the O/W interface. A reduction in fat droplet size was observed, which correlated with a significant decrease in protein load concentration. Given this, the emulsion with small droplet size (SD – 0.41 µm) produced a protein load of ~1.3 mg/m^2, significantly smaller than the other two. This agrees well with the literature, as with average values below 0.5 µm, the casein protein load is between 0.5 and 3 mg/m^2 depending on the total amount of protein added (Dickinson and Golding, 1997).

This observation can be attributed to the properties of smaller fat droplets, which facilitate the formation of a more densely packed adsorbed protein layer at the interface due to their increased surface area relative to volume. In summary, smaller fat droplets can optimise the formation of a compact and effective protein layer at the O/W interface. Consequently, this newly formed compact protein layer resulted in lower protein load because the available surface area on smaller droplets is efficiently covered by a smaller amount of protein.

It is noteworthy that the surface protein concentration also decreases with increasing homogenising pressure in the range of 20–50 MPa, as elucidated by Long et al. (2016). This finding supports the connection between the homogenisation conditions and the resulting protein distribution at the emulsion interface. Protein load is important because if it becomes

too high, partial coalescence can be completely prevented, affecting whippability (Pelan et al., 1997).

5.3.2 Fat crystallisation in emulsion

5.3.2.1 Refined stop-and-return DSC method

The polymorphic transition and crystallisation within fat droplets have significant effects, influencing not only the texture of butter and the physical stability of whipped cream but also the rheological and sensory properties of various dairy products. Therefore, it is of interest to elucidate the mechanisms leading to partial coalescence, particularly the kinetics of fat crystallisation at isothermal temperatures to improve product quality and cost efficiency. Fat crystallisation in O/W emulsions is a complex phenomenon due to several factors (i) it occurs in the confined volume of small droplets, (ii) natural fats have a diverse and complex TAGs composition along with various minor components, and (iii) fat crystallisation can occur differently in each fat droplet, which poses challenges for data interpretation (Moens et al., 2018a).

Various techniques, including freeze fracture electron microscopy (Buchheim et al., 1985), polarised light microscopy (Walstra et al., 2005), confocal laser scanning microscopy (Du et al., 2021; Han et al., 2018; Liang et al., 2014), ultrasonic velocity (McClements et al., 1993), small deformation rheology, nuclear magnetic resonance and x-ray scattering (Buldo et al., 2013) have been used to monitor the crystallisation of O/W emulsions. Although the previous studies provided insights into the microscopic aspects of emulsion crystallisation, they offer limited information for monitoring the influence of the kinetics of crystallisation of emulsified AMF stabilised by of sodium caseinate, as it is the case in the present study. In particular, there is a gap in understanding how the time-temperature (*t*-*T*) history influences primary crystallisation processes such as nucleation, crystal growth and polymorphism, which serves as the initial stage in the formation of a crystalline network within the droplets.

The refined stop-and-return method using Differential Scanning Calorimetry (DSC) represents a promising technique for addressing this existing knowledge gap on the crystallisation mechanism in fat droplets. This technique involves halting the crystallisation kinetics at various intervals during isothermal crystallisation and then heating the sample to achieve the melting profile of the fat crystals formed during specific isothermal durations. This technique is particularly valuable because it allows researcher to study the *in-situ* mechanism of fat crystallisation, gain insight that different polymorphic forms and fractions have different melting temperatures, and postulate the effects of droplet size on crystallisation mechanisms.

The crystallisation kinetics in the control emulsion with fat droplet sizes of 4.52 µm, which was subjected to a specific time-temperature program (*t,T*) to obtain a melting profile. The diagram showed four different regions of interest, as depicted in *Figure 34*. These regions were observed over hold times of 0.5 to 120 min at 5°C to ensure initial crystallisation in all droplets and to show polymorphic transformations over hold times of 120 min. Disruption of isothermal crystallisation results in a shift of the endothermic peaks to higher temperatures, indicating a transition to a more stable polymorph.

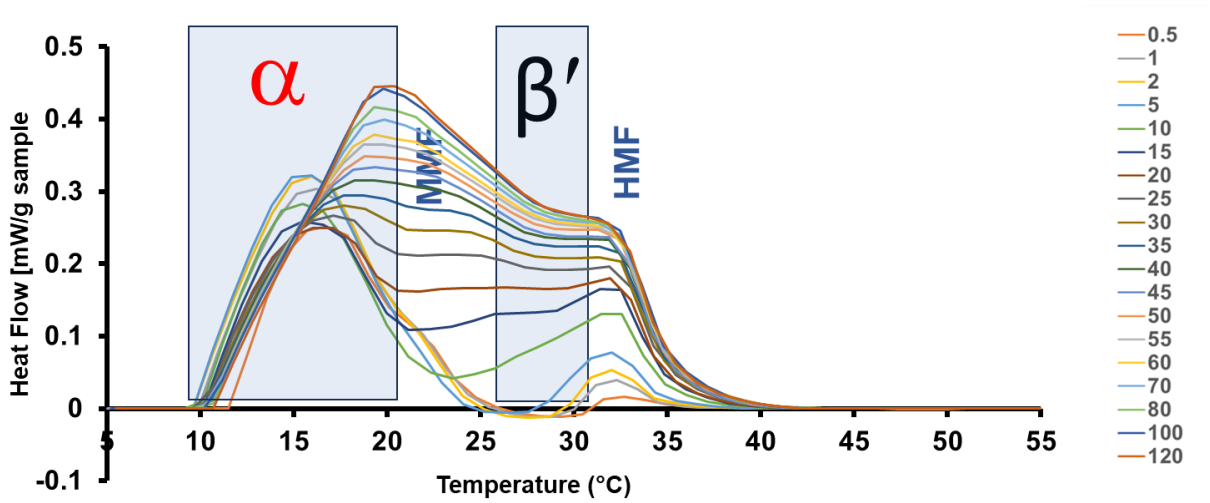


***Figure 34**: Kinetic of isothermal crystallisation for the control emulsion recorded as function of heat flow against temperature at isothermal crystallisation at 5°C, with the holding time between 0.5 s to 120 min by stop-and-return method using DSC. The control is made of 36 wt.% AMF stabilised by 1 wt.% of sodium caseinate ($d_{4.3}$ = 4.52 µm).*

The appearance of a single strong characteristic peak at the far left of the graph corresponds to the metastable polymorphic form, likely α polymorph crystals (hexagonal), developing from the melt. This phenomenon suggests fast crystallisation kinetics, exclusive to anhydrous milk fat (AMF) at temperatures below ~20°C. This finding is consistent with previous studies by Grotenhuis et al. (1999) and Mazzanti et al. (2003), who also reported similar observations. In addition, previous studies have reported that the crystalline packing structures of milk fat formed during fast cooling are extremely unstable and less densely packed and have a relatively short lifetime (Cisneros et al., 2006; Lopez et al., 2005b).

The peaks that form more gradually at around 20°C represent compositional changes within the α to β′ polymorphs of the MMF, while the slow forming shoulder peaks on the far right caused a shift of the endothermic peaks to higher temperatures, presumably a transition the compositional to a more stable polymorph means the β′ form. At around 25 - 30°C, a downward

dip below the baseline of the DSC curve indicated polymorphic transitions from α crystals to predominantly β′ crystals (orthorhombic). The estimated energy of approximately 40 kJ/mol due to the exothermic polymorphic transformation. Due to the heat released by the nucleation and initial growth of β′ in the medium melting fraction (MMF) from phase α to β′. The observed different heats of crystallisation between α and β′ crystals, approximately 80 and 120 kJ/mol, respectively, are in complete agreement with Ten Grotenhuis et al. (1999) and Mazzanti et al. (2003), concluded that crystallisation into the α form does not occur above 20°C, but that only β′ crystals form above this temperature.

Manipulation of emulsions through the different fat droplet sizes, has revealed significant differences in crystallisation events and polymorphic changes within the emulsions. The relationship between fat droplet size in emulsions and the influence on fat crystallisation behavior is summarised in *Figure 35* after an isothermal period of 120 min at 5°C.

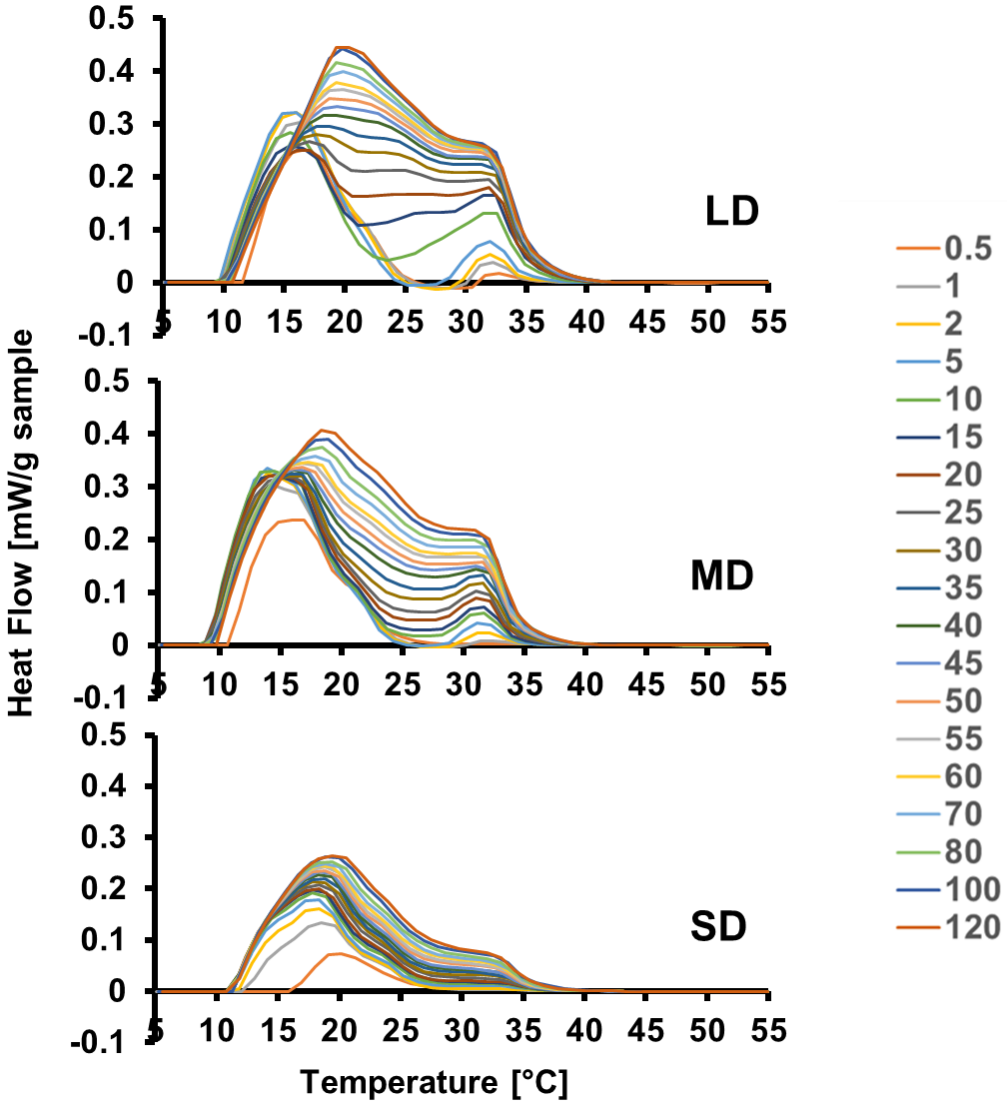


***Figure 35**: Effects of droplet sizes on the crystallisation events of fat droplets in 36 wt.% AMF emulsions stabilised with 1 wt.% of sodium caseinate, where fat droplet sizes vary as: LD; 4.52 µm, MD: 1.57 µm and SD: 0.41 µm.*

It is widely accepted that reducing fat droplet size induces several changes: an increase in interfacial surface area, interfacial curvature, Laplace pressure within the fat droplets and modifies the quantity and conformation of the protein that stabilised the interface (Truong et al., 2014). However, the exact influence of fat droplet size on the crystalline structures formed remains unclear.

To achieve complete crystallisation, nucleation must take place in each individual droplet. Large droplets (LD) exhibited the formation of β′ polymorphs within both the MMF and HMF regions, with near-complete crystallisation of β′ HMF crystals occurring after approximately 50 min. In contrast, a medium droplet (MD) with a particle size of 1.57 µm displayed an on-going crystallisation for both the β′ polymorph of HMF and MMF crystals, suggesting that there was

no stable state at the end of the experiment was achieved. Another hypothesis derived from the observations postulated a lower nucleation rate of polymorphic transitions of α to β′ HMF crystals observed in MD, which was due to a delay in the nucleation events required in each droplet. This delay may also be due to a lack of α nucleation on the inner surface of the fat droplet interface, which triggers the nucleation event.

The time required for the formation of the first nucleus is inversely proportional to the droplet volume, meaning that a finer/smaller droplet requires a lower temperature, because more catalytic impurities are needed to achieve the same crystallisation rate (Walstra et al., 2005). This observation is consistent with previous studies (Bugeat et al., 2011; Montenegro et al., 2003; Truong et al., 2014), which suggest that the crystallisation process is delayed in smaller droplets due to the higher number of catalytic impurities required for the crystallisation process to occur.

This delay was particularly evident for small droplets (SD – 0.41 µm), where emulsions showed the slowest crystallisation despite having the largest interfacial area, suggesting that no nucleation occurs at the droplet interface when an emulsifier has no affinity to oil (sodium caseinate) is used to prepare emulsions. The investigation of the observed peaks in the heat flow as a function of time also provides interesting insights. The small droplets (SD) emulsion exhibited approximately half the height of the large droplets (LD) emulsion, suggesting that smaller droplets nucleating more slowly, and the emulsion is further from reaching equilibrium.

5.3.3 Evaluation of aerated emulsion

5.3.3.1 Whippability using a rheometer

The current literature addressing the effect of fat droplet size on the whipping properties of native and/or homogenised cream is notably limited. Edén et al. (2016) reported that reducing the average milk fat droplet size of native cream resulted in longer whipping times and decreased overrun. However, it is noteworthy that their investigation included droplet sizes ranging from 4.0 to 4.9 µm. As the study did not encompass smaller fat droplets (<4 µm), which constitute a significant proportion of the total fat droplets present in milk, further evidence is needed to investigate the size effect.

Figure 36 depicts the relationship between apparent viscosity (η) and whipping time (*t*) across different fat droplet sizes, demonstrating the increase in viscosity over the course of whipping under conditioned parameters using a rheometer with constant rotational speed (1800 rpm),

volume (10 mL), and temperature (8ºC). This graphical representation provides insight into the progress of whippability, as indicated by the phases of under-whipped (UW), ideally-whipped (IW) and over-whipped (OW) emulsions. These phases were used to quantitatively assess the effects of changes in the fat crystal network of droplets on the shear-induced partial coalescence. The three phases are showed in *Figure 36*.

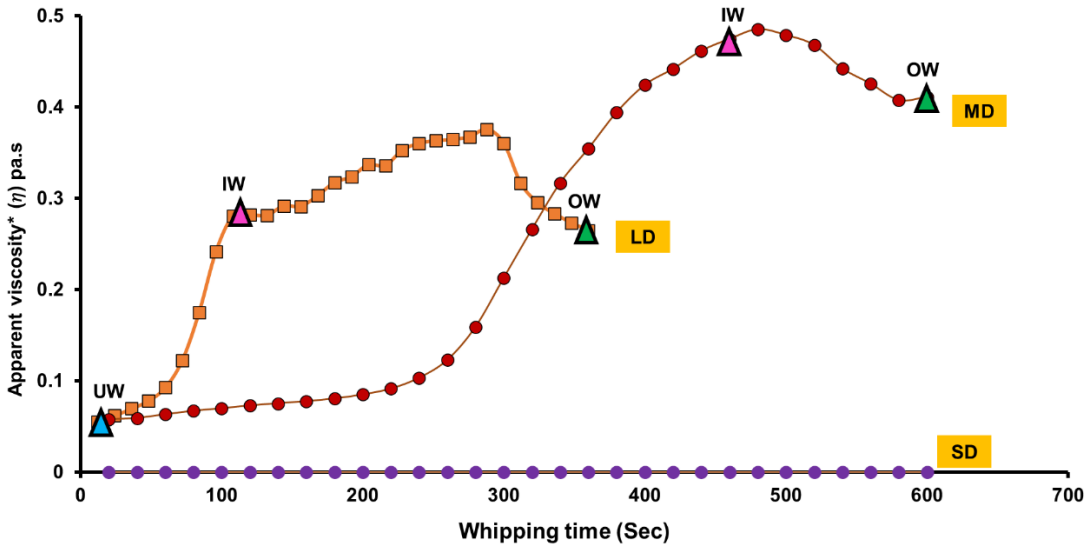


***Figure 36**: Apparent viscosity profile of 10 mL of 36 wt.% AMF emulsions stabilised with 1 wt.% of sodium caseinate sheared at constant rotational speed of 1800 rpm and temperature of 8ºC. Abbreviations: LD – large droplets, MD – Medium-droplet, SD – Small-droplets, UW – Under-whipped, IW – Ideally-whipped and OW – Over-whipped.*

The formation of stable foam structures during whipping is influenced by the adsorption behavior of individual fat particles and milk proteins at the air-water interface (Liu et al., 2022b). As whipping progress, a partially crystalline O/W emulsion transforms into a foam, with air bubbles are stabilised by a network of surface-mediated partially coalesced fat droplets. Furthermore, these fat droplets form a network throughout the aqueous phase and provide the foam with sufficient solidity and stability (Moens et al., 2018b; Zeng et al., 2022).

As shown in *Figure 36*, the shape of the viscosity increases differed depending on the droplet size. In both cases of LD and MD, peak viscosity was achieved because of an enhanced fat crystal network was formed as a result of the partial coalescence process, which stabilised and held the aggregated fat droplets. Additionally, fat crystals can adsorb at the A/W and O/W interfaces and act as Pickering particles, reducing interfacial and surface tensions (Rousseau, 2000). This phenomenon is likely attributed to increasing fat aggregation which allows air entrapment and result into a gradual increase in viscosity until a continuous network form

throughout the volume. This continuous network gives the aerated system its solid-like properties. Where the immobilisation of air bubbles is possible, signifying the ideally-whipped (IW).

The rate of shear-induced partial coalescence is greatly influenced by the size of fat droplet and the spindle rotation speed, as discussed in detail the method development overview in Chapter 4. Large fat droplets were more reactive and at the same time induced a faster aggregation rate, due to the lesser protrusion distance of the crystalline droplets evident as early as 40 sec in the process. In contrast, medium droplets size (MD) displayed a slightly delayed and more gradual increase in viscosity, starting around 80 sec and increasing logarithmically only after 260 sec. It is noteworthy that no aggregation was observed for small fat droplets (SD) as the viscosity remained constant throughout. This observation suggests that crystal formation within the droplets may be absent or delayed, hindering fat crystallisation and partial coalescence in the emulsion (see section 5.3.2.1 for detailed discussion). In summary, the average whipping times to reach the ideally-whipped phase for LD (~4.52 µm) and MD (~1.57 µm) were 2.02 and 7.50 min, respectively, with a non-detectable (ND) time for small droplet sizes of ~0.41 (SD). Previous findings by Edén et al. (2016), who reported a 47 ± 7% reduction in whipping time as the average fat droplet size of native cream increased from 4.0 to 4.9 µm.

Our study found that smaller fat droplets had higher shear resistance than larger ones, resulting in longer whipping time for aerated emulsions. The presence of smaller fat droplets helps improve stability and prevents fat crystallisation and partial coalescence, confirming the findings of Goff (1997) and consequently increasing the whipping time. It is obvious that droplets with an average size of less than 1 µm size could not entrap air cells, because no viscosity was developed.

To better understand the mechanisms behind this whipping phases in the context of the shear-induced partial coalescence, particle size distribution analysis and *in-situ* microscopic studies were performed at different intervals along the viscosity profile as shown in *Figure 37A* and *Figure 37B.*

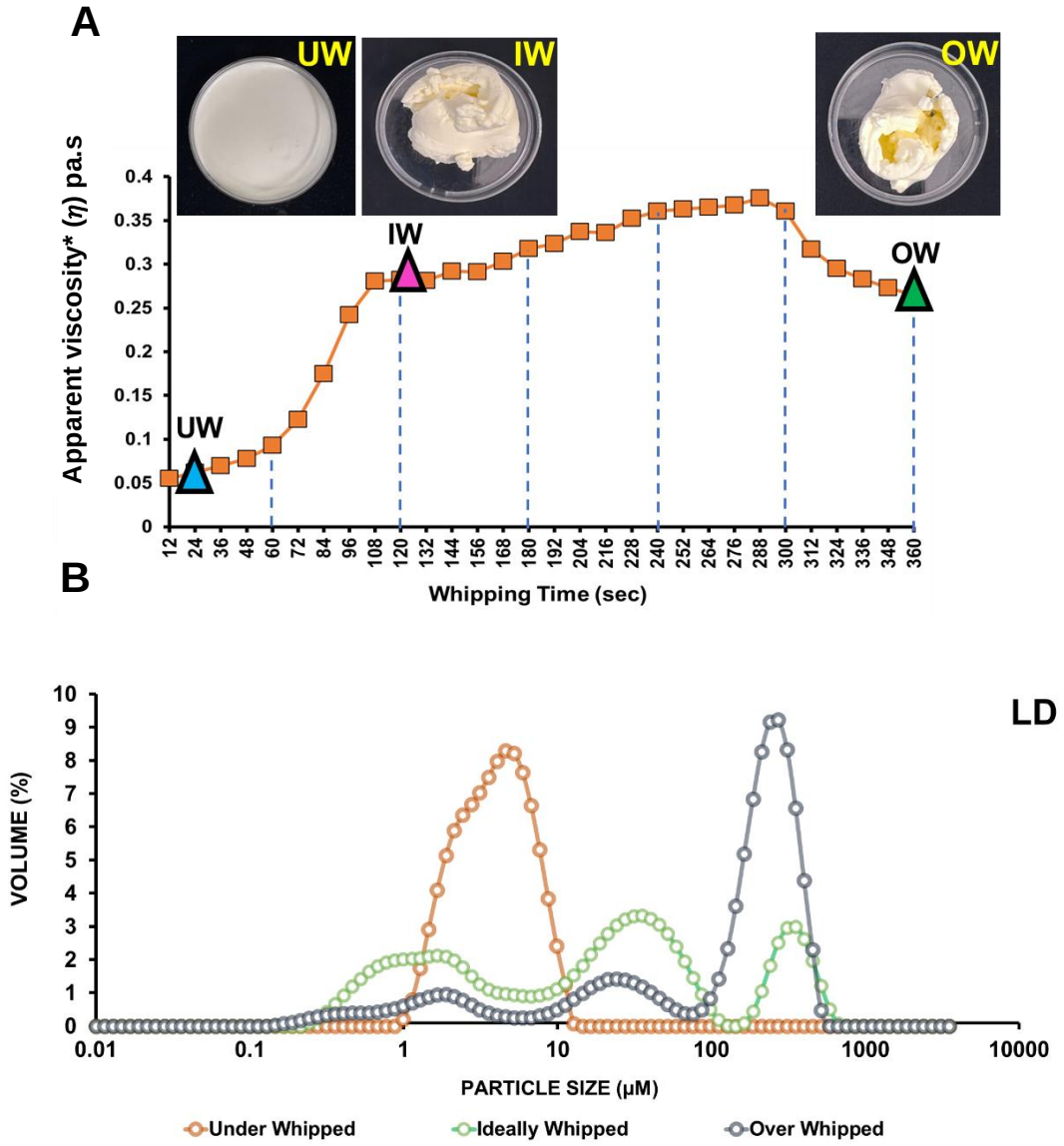


Figure 37: A) *Graph showing apparent viscosity versus whipping time of 10 mL large droplets, LD emulsion (36 wt.% AMF emulsions, stabilised with 1 wt.% of sodium caseinate) at constant rotational frequency of 1800 rpm and 8ºC. The measurements were interrupted at 3 whipping phases; UW (under whipped), IW (ideally whipped) and OW (over whipped) for further analysis* ***B)*** *particle size distribution of UW, IW and OW using laser light scattering.*

The apparent viscosity analysis during shear in the rheometer shown in *Figure 37A* was strategically interrupted based on three phases of whippability—under-whipped (UW), ideally-whipped (IW), and over-whipped (OW). Before analysing the particle size distribution (PSD) using laser diffraction, the whipped emulsion had to be diluted with a 1% SDS solution to prevent the detection of flocculated fat droplets. The SDS surfactant solution breaks up any flocs leaving only semi-coalesced fat or individual fat droplets for measurement. *Figure 37B* shows the PSD curve for large fat droplet size (LD), which captures the distribution of

individual milk fat droplets and partially coalesced fat as well as the air bubbles present in the emulsion. Notably, the initial curve showed a peak between 0 and 30 sec. of whipping time, indicating the under-whipped (UW) phase where the fat droplet sizes correspond to those in the original emulsion, mainly between 1 and 10 µm size. As long as partial coalescence was not initiated, the droplet size remained unchanged and therefore the encounter of frequency (*i.e.,* number of collisions) was constant (Moens et al., 2018b).

As whipping progressed, the size distribution showed three peaks at t = 120 sec, indicating the incorporation of air bubbles, presumably between 10 and 100 µm. This resulted in an escalation in the initial rate of shear-induced partial coalescence, as small individual fat droplets were aggregated into larger clusters, resulting in irreversible aggregated fat droplets over 100 µm. At this phase, the emulsion was characterised as ideally whipped (IW), visually indicated by the production of the best rosette and greatest overrun, as shown in the following section. In this phase, whipping is mainly influenced by the spreading of fat droplets on the air bubble surface to form a continuous network of fat droplets throughout the aqueous phase (Moens et al., 2018b).

The extent of shear-induced partial coalescence increased with shear and time, causing the emulsion to transition to an over-whipped (OW) state. This transition was characterised by a larger third peak approximately 300 µm in size, along with a reduction in air bubbles between 10 and 100 µm, as well as a reduction in primary fat droplets, leaving a small portion of approximately 2 µm fat droplets. At this phase, a decrease in viscosity was also observed indicating a phase inversion and continuous break-up of the partially coalesced fat droplet network.

Similar observations were found for the medium droplet size (MD) emulsion, as shown in *Figure 38A* and *Figure 38B* albeit, as described above, significantly longer times are required to reach the IW state.

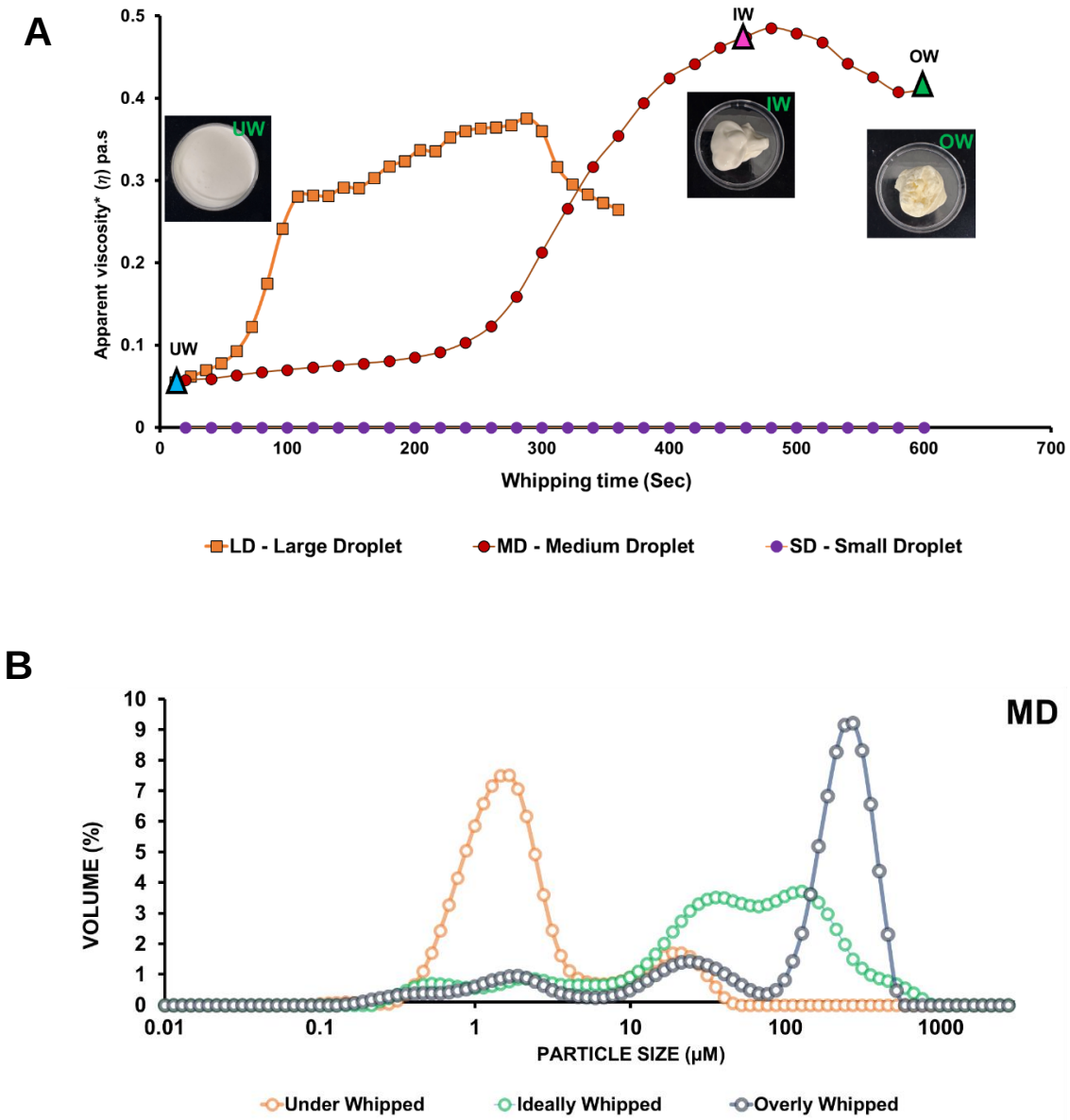


Figure 38: A) *Plot showing apparent viscosity versus whipping time of 10 mL medium droplets, MD emulsion (36 wt.% AMF emulsions, stabilised with 1 wt.% of sodium caseinate) at constant rotational frequency of 1800 rpm and 8ºC. The measurements were interrupted at 3 whipping phases; UW (under whipped), IW (ideally whipped) and OW (over whipped) for further analysis* ***B)*** *particle size distribution of UW, IW and OW using laser light scattering.*

The smaller fat droplets clearly exhibited higher shear resistance than larger counterparts, which increased the whipping time for aerated emulsions, by hindering of fat crystallisation and partial coalescence (Goff,1997), as described earlier in this section. However, the peak viscosity achieved by these MD emulsions was higher, presumably indicating that a stronger interfacial tension between O/W and A/W interfaces enhances the attachment of fat droplets to air bubbles and their stability and thus a greater extent of partial coalescence elevated, once the process was initiated. Furthermore, the size distribution of the IW emulsions shows

a higher proportion of large particles, which is probably related to a greater extent of partial coalescence, which would explain the increased viscosity.

5.3.3.2 Foam structure, overrun and firmness

The stability of aerated emulsions is closely related to the distribution of air bubbles and fat droplets, which formed a delicate balance crucial to the foam structure. The ideal whipped foam structure, showed in *Figure 39* visualised using Confocal Laser Scanning Microscopy (CLSM), reflects the relationship between the partial coalescence of fat droplets and the stabilised air bubbles (Goff, 1997; Jiang et al., 2019; Moens et al., 2018a; Wang et al., 2021c).

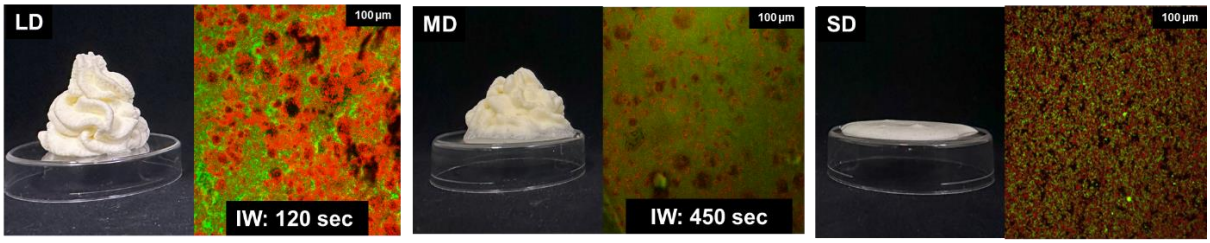


***Figure 39**: Confocal scanning laser microscopy (CSLM) micrographs depict ideally whipped (IW) rosettes of the 36 wt.% AMF aerated emulsions, stabilised with 1 wt.% of sodium caseinate, taken at 4°C. In the images, proteins are fluoresced in green, fat in red, and serum in black.*

The large fat droplet (LD) emulsion, stabilised solely by protein, has a distinctive feature in which semi-coalesced fat particles mainly coated the numerous small air cells (black regions) with a size between 10 and 100 µm. This covering of the air cell serves to prevent its collapse, which may account for the observed highest overrun of 130% and slightly less firm (~17 N) whipped cream compared to the aerated MD emulsion with an overrun 76% and firmness of ~20 N explained in *Table 7*.

In general, the smaller fat droplets could not adequately support the air cells, resulting in more irregular gas structures and poor foam stability. One can speculate that the partially coalesced network of fat droplet surrounding the air bubbles was inadequate and failed to provide the foam with the necessary cohesion and stability. Furthermore, the smaller fat droplet sizes, especially those <2 µm (MD), were less reactive and showed a slower crystallisation rate, increasing the whipping time to build a robust fat network over time, which did not even happen for droplets with mean averages around 0.4 µm (SD).

The whipped cream formed from MD emulsions appeared to trap significantly less air, with an approximate overrun of about ~76%. It was observed that the fat appeared to be less partially coalesced, as indicated by the small amount of aggregated fat in red in the microstructure

(*Figure 39*) and the particle size distribution obtained for the IW state (*Figure 38B*). In this case, the inefficient packing and stabilisation of the air cells was evident, characterised by very irregular and small air channels, with fewer dark areas indicating that scarcer air cells were present. This results in a slightly higher viscosity displayed with proteins in green dominating in the emulsion (*Figure 38A*) and a softer, less stable whipped rosette with some syneresis noted (*Figure 39*).

On the other hand, a small droplet (SD) emulsion with $d_{4,3}$, of 0.41 µm was very stable and high shear resistant and showed a slower crystallisation rate, as discussed in previous section, hindered partial coalescence and could not trap air.

***Table 7**: Effects of droplet sizes on the overrun and firmness of 36% AMF aerated emulsions, stabilised with 1% of sodium caseinate.*

Emulsion	Overrun (%)	Firmness (N)
LD – Large droplet	130.54 ±0.30a	17.11 ±0.65b
MD – Medium droplet	76.12 ±1.05b	20.10 ±0.52a
SD – Small droplet	ND	ND

**Abbreviation: ND: Not detected*

5.4 Conclusions

The aim of this chapter was to elucidate the effect of fat droplet size in homogenised dairy creams and their whipping properties. The results allow several important conclusions:

First, the size of fat droplet has a strong influence on the emulsion stability and in modulating the crystallisation of emulsified milk fat. Large droplets induce faster nucleation and polymorph formation on the inner surface of the fat droplet interface, triggering nucleation events more quickly compared to smaller droplets. Conversely, smaller droplets have slower nucleation rates, potentially resulting in delayed or absent crystal formation within the droplets, hindering aggregation and subsequent fat crystallisation and consequently partial coalescence within the emulsion.

Second, our study demonstrates that larger fat droplets have lower shear resistance than smaller fat droplets, resulting in shorter whipping times for aerated emulsions. On the contrary, smaller fat droplets contribute to enhanced stability by hindering fat crystallisation and partial coalescence, thus extending the whipping time.

Additionally, when evaluating aerated emulsions, we found that larger fat droplets efficiently entrap air cells and encapsulate them with partially coalesced fats, resulting in maximum

stability and overrun. Conversely, smaller fat droplets delay crystal nucleation within the droplets, resulting in a less cohesive and stable foam structure.

In summary, the rate of partial coalescence appears to have a close correlation with fat droplet size, with larger droplets allowing faster nucleation and promoting foam stabilisation. The presence of a crystal network fat droplets not only modulates their behaviour during shear-induced partial coalescence but also exerts remarkable influence on the whipping properties of the emulsion. Whether it is the effect of fat droplet size or the influence of fat crystal network, both factors exert a significant impact on shear-induced partial coalescence or other aspects of the whipping properties such as overrun, firmness, microstructure, apparent viscosity, and rosette evaluation.

In summary, these results highlight the critical role of fat droplet size in determining emulsion stability, crystallisation kinetics, and whipping properties. The insights gained in this chapter provide valuable guidance for refining dairy product formulations and processing techniques and contribute to a deeper understanding of fat crystallisation and partial coalescence in emulsified systems.

Chapter 6: Factors influencing fat crystallisation and partial coalescence of fat droplets and subsequent whipping properties: ii: Effect of types and concentrations of emulsifiers

6.1 Introduction

The incorporation of low molecular weight (LMW) emulsifiers, particularly mono- and diglycerides (MDG), lactic acid esters of MDG (LACTEM) and polysorbate 80 (Tween 80), improves the whippability of the cream, while stabilisers prevent cream from separating during storage and improve the firmness and stability of the whipped cream (Wang et al., 2023). Both protein and LMW emulsifiers can coexist as emulsifiers in the O/W emulsion. LMW emulsifiers such as MDG, LACTEM and Tween 80 are widely used in various types of emulsified products. MDG and LACTEM are oil-soluble, while Tween 80 is water-soluble, distinguishing them as different emulsifiers due to their hydrophilic-lipophilic (HLB) properties. Extensive research has been conducted to improve the whipping characteristics and stability of creams, primarily by optimising the selection of emulsifiers based on their surface-activity and interfacial tension (Cerdeira et al., 2005; Cheng et al., 2020; Dickinson, 2019; Fredrick et al., 2010; Jiang et al., 2019; Liu et al., 2022; Rizzo et al., 2015; Warren and Hartel, 2018).

As illustrated in *Figure 40,* this chapter discusses the use of advanced analytical techniques, including differential scanning calorimetry (DSC), rheometer and microscopy to examine factors influencing fat crystallisation and partial coalescence of fat droplets, and their subsequent effect on whipping properties. The particular interest of this chapter is to understand the relationships between the types and concentrations of LMW emulsifiers in O/W emulsions. Furthermore, whipping properties and foam structure are studied by manipulating the key variables – emulsifiers with different hydrophilic-lipophobic balance (HLB) and chain lengths as shown in *Figure 41*. These include lipophilic emulsifiers such as MDG and LACTEM, as well as the hydrophilic emulsifier, Tween 80, each in concentrations of 0.5 and 1.0 wt.%.

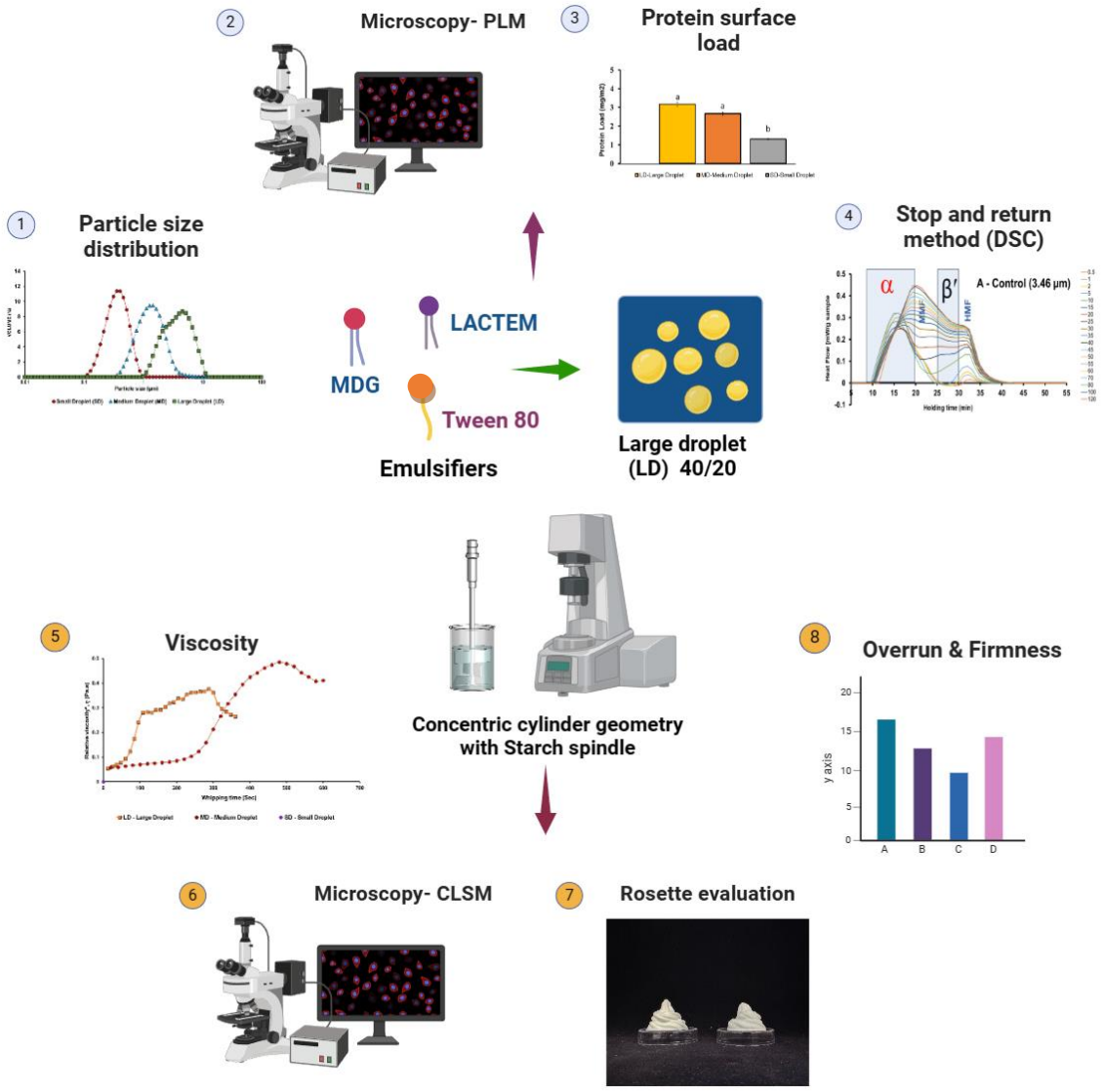


***Figure 40**: Schematic representation of the analytical techniques i.e., particle size distribution, protein surface load, stop and return method using DSC, viscosity, microscopy, overrun, rosette evaluation, and firmness to explain the effect of types (MDG, LACTEM and Tween 80) and concentrations (0.5 and 1.0 wt.%) of emulsifiers and subsequent whipping properties of large droplet (LD) emulsion homogenised at 40/20 bar.*

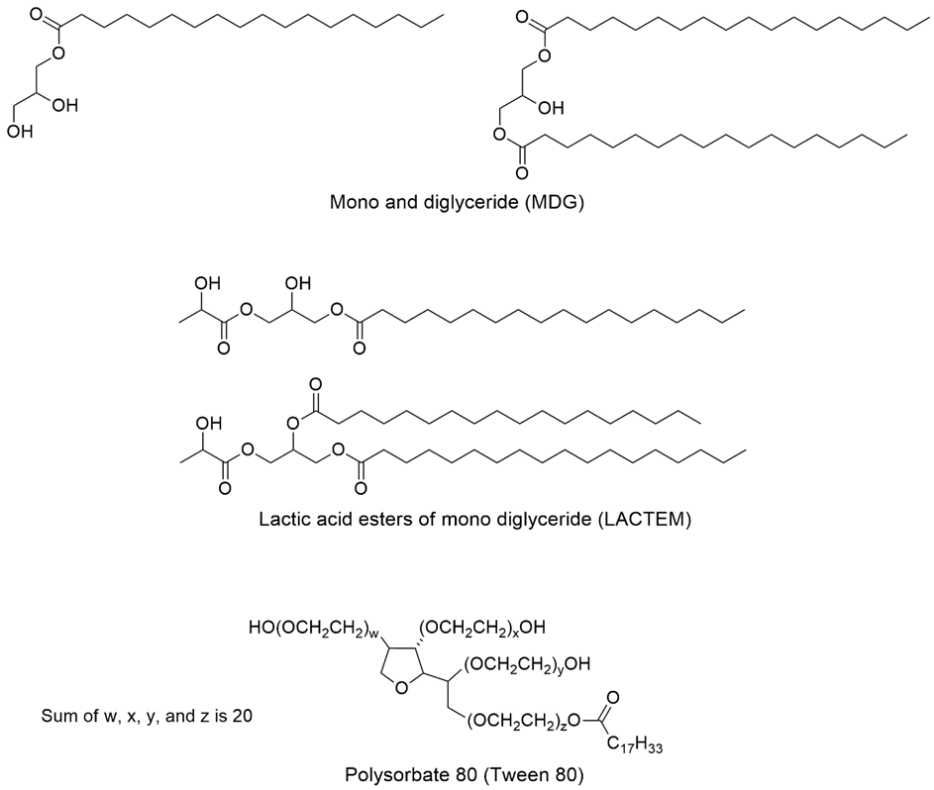


***Figure 41**: Molecular structures of emulsifiers.*

Two emulsifiers are commonly used in food industry, *i.e.,* Tween 80, and mono- and diglycerides (Goff and Hartel, 2013). These two emulsifiers differ in their molecular structure: Tween 80 is more hydrophilic and has a bent carbon chain due to large polyoxyethylated sorbitol head while MDGs have straight carbon chains and glycerol heads, making them more hydrophobic than Tween 80 (Euston, 2008).

6.2 Materials and methods

6.2.1 Materials

Please refer to section 3.1

6.2.2 Methods

6.2.2.1 Emulsion preparation

Please refer to section 3.2.1

6.2.2.2 Particle size distribution

Please refer to section 3.2.2.1

6.2.2.3 Stability and microstructure of emulsion

Please refer to section 3.2.2.2

6.2.2.4 Protein surface load

Please refer to section 3.2.2.3

6.2.2.5 Refined stop-and-return DSC method

Please refer to section 3.2.3.1

6.2.2.6 Nucleation behaviour of emulsion

Please refer to section 3.2.3.2

6.2.2.7 Whippability using a rheometer

Please refer to section 3.2.4.1

6.2.2.8 Foam structure using confocal laser scanning microscope (CLSM)

Please refer to section 3.2.4.2

6.2.2.9 Overrun

Please refer to section 3.2.4.3

6.2.2.10 Firmness

Please refer to section 3.2.4.4

6.2.2.11 Data analysis

Please refer to section 3.2.5

6.3 Results and discussion

6.3.1 Emulsion characterisation

6.3.1.1 Mean size and particle size distribution

The characterisation of emulsions was performed by assessing particle size distributions, stability, microstructure and protein surface load. The effect of homogenisation with and without incorporation of LMW emulsifiers on particle size using the volume-weighted mean diameter $d_{4,3}$ versus pressure at 40/20 bar which was measured immediately after homogenisation, is showed in *Figure 42* and *Table 8*.

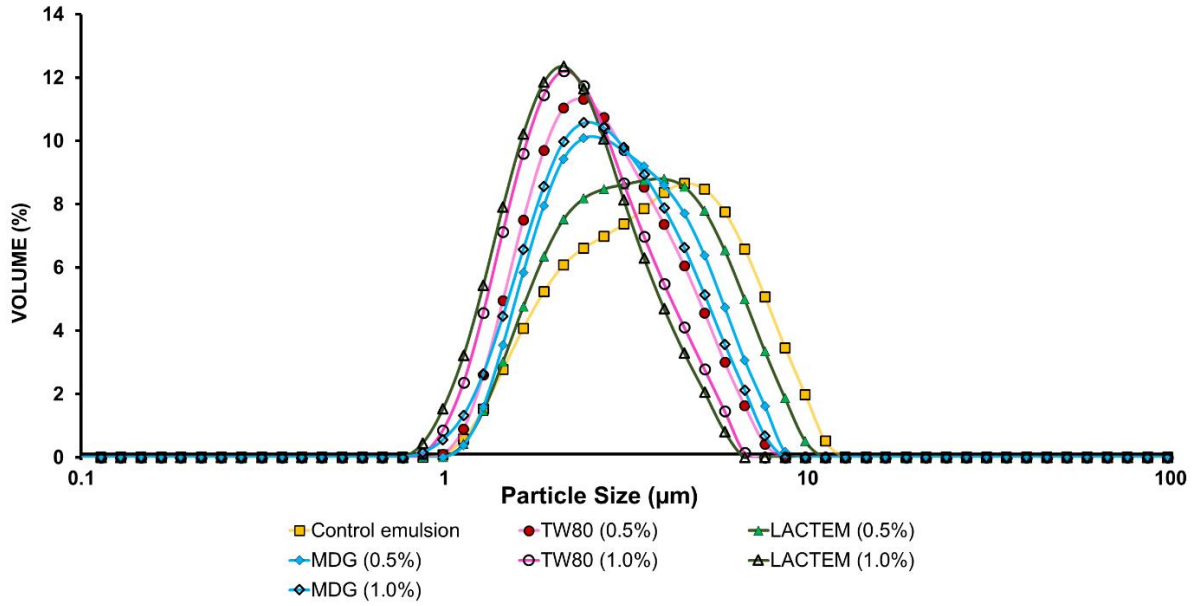


***Figure 42**: Particle size distributions of emulsions with different emulsifier types (MDG, LACTEM and Tween 80) and concentrations (0.5 to 1.0 wt.%) and without (control). Abbreviations: MDG (mono- and diglycerides), LACTEM (lactic acid esters of MDG) and Tween 80 (polysorbate 80).*

***Table 8**: Particle size volume weighted means ($d_{4,3}$) of emulsions.*

Emulsion	$d_{4,3}$ (µm)
A – Control	4.52[a]
B – MDG (0.5%)	3.55[b]
C – MDG (1.0%)	3.27[b]
D – LACTEM (0.5%)	4.03[a]
E – LACTEM (1.0%)	2.59[c]
F – Tween 80 (0.5%)	3.16[b]
G – Tween 80 (1.0%)	2.74[c]

The particle size distribution of each oil-in-water (O/W) emulsion prepared was evaluated using dynamic laser light scattering immediately post-homogenisation. The 36 wt.% anhydrous milk fat (AMF) emulsions stabilised by 1 wt.% of sodium caseinate, both with and without LMW emulsifiers, exhibited monomodal peaks after homogenisation. This suggested dispersion of the fat droplets rather than aggregation. This was further evidenced by the average of a single predominant peak in the particle size distribution of the emulsions and the fat droplets were relatively well distributed in the emulsions (*Figure 42*).

In contrast, in the absence of emulsifiers, the control emulsion showed the least narrow peak with a large volume-weighted mean diameter, $d_{4,3}$, of 4.52 µm with value of span greater than 1, indicating less uniform fat droplets and a greater tendency for creaming. According to McClements et al. (2011), milk protein as a macromolecular emulsifier diffuses and adsorbs significantly more slowly and is typically less effective in reducing interfacial tension compared to LMW emulsifiers.

The comparison with the control emulsion (A), stabilised by 1.0% protein (sodium caseinate), revealed that emulsions stabilised by low molecular weight (LMW) emulsifiers generally had lower $d_{4,3}$ values. The control and 0.5% LACTEM emulsions, were not significantly different, but were significantly different from all other emulsions, as shown in *Figure 42* and *Table 8*, as both emulsions showed no significant difference for $d_{4,3}$ values. The presence of three different types of LMW emulsifiers added to the O/W emulsions before homogenisation effectively reduced the size of fat droplets. This reduction was attributed to the ability of the emulsifiers to lower the interfacial tension, allowing for faster adsorption of the emulsifiers to the fat droplet surfaces during homogenisation. This finding is consistent with the observations of van Lent et al. (2008), who observed a similar effect with recombined dairy cream. In general, $d_{4,3}$ values decreased with higher concentration of LMW emulsifiers.

It is noteworthy that emulsions with 1.0% emulsifiers have significantly narrower and more uniform particle sizes than those with 0.5% emulsifiers, as visually confirmed in *Figure 42*. Among the emulsifiers evaluated, 1.0% LACTEM, a lipophilic emulsifier, demonstrated the most significant reduction in particle sizes, followed by Tween 80 (1.0%), a hydrophilic emulsifier. The results indicate that lipophilic emulsifiers with lower HLB, had slightly better adsorption and rapid interfacial displacement at the newly created O/W emulsion interface compared to hydrophilic emulsifier because the emulsifiers are very surface-active and tend to dominate the interface rather than caseinate, which as observed contributes to smaller droplets and therefore, allows less protein to adhere to the O/W interface. This partial displacement of the protein at the interface alters the membrane thickness, and thus affects the particle size.

The results also showed that the concentration of emulsifiers played a significant role in reducing the particle size of the fat droplets and that the type of emulsifiers was secondary. In summary, LMW emulsifiers can influence the properties of protein-stabilised emulsions in various ways. The much more rapid lowering of the interfacial tension than with protein alone, and the higher Gibbs elasticity of the film between pairs of approaching fat droplets during

emulsification, result in the formation of smaller fat droplets during homogenisation (Dickinson, 2001b, 2009).

6.3.1.2 Stability and microstructure of emulsion

An emulsion is a thermodynamically unstable system and droplets tend to aggregate due to attractive forces resulting in flocculation, creaming, coalescence, phase inversion and Ostwald ripening (McClements, 2012). In the context of aerated emulsions, where aeration is preceded by 24 h of storage, the quiescent stability of the emulsion becomes a critical consideration. Factors such as fat droplet size, viscosity and interfacial film properties significantly influence emulsion stability as highlighted by Jiang et al. (2019); Liu et al. (2022) and Zhao et al. (2019).

Creaming which has been identified as one of the instabilities in O/W emulsions (Tadros et al., 2004), was manifested by a high rate of separation, which resulted in the formation of a significant creaming layer. This phenomenon indicates low stability and is often accompanied by the visual separation of phases in emulsion (Wu and Ma, 2016; Zhou et al., 2017). In *Figure 43*, visual assessment of emulsion stability showed significant phase separation for emulsions containing 1.0% MDG, 0.5% Tween 80, and most clearly at 1.0% Tween 80.

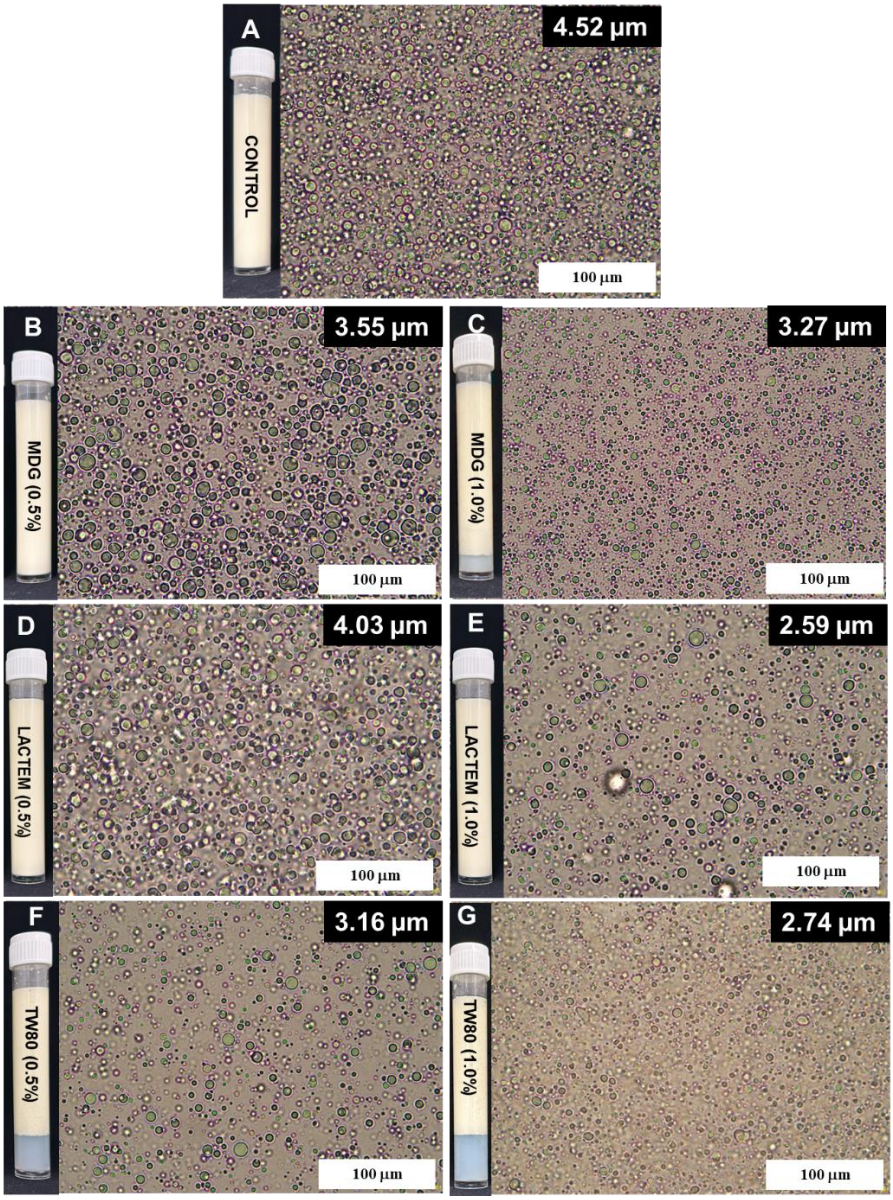


***Figure 43**: Stability of the emulsions after 24 h of storage at 4°C and microstructure of the emulsions visualised under the Polarised Light Microscope, PLM (scale bar: 100 µm with oil immersion). Large droplet (LD) emulsion with a homogenisation pressure of 40/20, stabilised with 1 wt.% sodium caseinate with and without incorporation of different concentrations of LMW emulsifiers.*

In contrast, emulsions A, B, D and E, which were the control, 0.5% MDG, 0.5% and 1.0% LACTEM, showed effective creaming and phase separation delay. Notably, LACTEM proves to be more effective than the other two emulsifiers in preventing creaming. It is worth noting that only a very minimal degree of phase separation was observed at a higher MDG concentration (1.0%) and no separation at a lower concentration of 0.5%.

The results suggest that lipophilic emulsifiers with lower HLB showed better adsorption and interfacial displacement compared to hydrophilic emulsifier due to weak competitive adsorption with protein, and thus less protein adhered to the O/W interface. This explains why there was no or less phase separation in emulsions prepared with lipophilic emulsifiers together with milk protein, with the exception of Emulsion C with 1.0% MDG. This discrepancy is attributed to the solid nature of MDG at room temperature, which requires at least 80°C to melt completely.

The incompatibility of the stereochemical structure of mono and diglycerides (MAGs and DAGs) in MDG, which contain a significant proportion of saturated fatty acids, compared to the mixed composition of medium-long chain lengths and unsaturated TAGs in anhydrous milk fat resulted in inhomogeneous fat droplets and the tendency to fat aggregation, as shown in *Figure 43*. According to Bus et al., (1990), the use of infrared spectroscopy showed that the hydroxyl groups in MDG can form hydrogen bond (H-bond) with the carbonyl moieties of fatty acids in oils, thereby shifting their adsorption/desorption equilibrium strongly toward desorption, resulting in coalescence of the emulsion.

Figure 42 shows that although Tween 80 successfully reduced the fat droplet size in the emulsion, we postulated that with prolonged presence in the emulsion Tween 80 changed further the composition of the interfacial layer. Despite Tween 80's ability to produce smaller fat droplets, evidence of fat aggregation led to creaming being observed. This ineffectiveness can be attributed to the molecular structure of Tween 80, which is characterised by a long unsaturated carbon chain with a large hydrophilic head (*Figure 41*), which may hinder effective coverage of the fat droplet surface, causing transient stabilisation and eventually resulting in phase separation, as highlighted in *Figure 43*.

This observation is also supported by Dickinson et al. (2003), who postulated that the presence of excess or unabsorbed protein in the form of small caseinate aggregates (sub-micelles) may lead to deterioration in creaming stability due to depletion flocculation. Meanwhile Cornec et al. (1996), found that competitive adsorption of emulsifiers such as Tween 80 and protein results in hindered surface diffusion of the emulsifier through adsorbed protein and disruption of protein-protein interactions in the adsorbed layer by the emulsifiers. They emphasized that these two mechanisms are incompatible with each other and often lead to instability when a mixed component of emulsifier and protein interface is formed. This inadequacy leads to poor stability followed by phase separation.

The increased concentration of Tween 80 to 1.0% implies an imbalance between Tween 80 and protein on the surface of oil droplets', resulting in poor combined emulsification effect. The negative synergism, reported by Dickinson (2019) further supports this observation. This may have affected the results and was taken into account when discussing the conclusions. Lower creaming rate and no phase separation, indicating better colloidal stability (Hong et al., 2018), were observed for the control and lipophilic emulsifiers – 0.5% and 1.0% LACTEM and 0.5% MDG emulsions, which maintained stability throughout the one-month experimental period.

6.3.1.3 Protein surface load

In O/W emulsions, stabilisation is primarily influenced by proteins, that form a dense adsorbed layer on the surface of fat droplets, with their surface load serving as an indicator of interfacial membrane integrity (Dickinson, 2001 and 2013). However, after homogenisation and aging, the interfacial properties of the fat droplets are mainly influenced by emulsifiers rather than proteins (Goff and Hartel, 2013). This is because emulsifiers tend to migrate from the serum phase to the interface, resulting in competitive adsorption between both proteins and emulsifiers (Bolliger et al., 2000; Jiang et al., 2019). However, we need to understand that both protein and emulsifiers were present during emulsification, so a mixed interfacial layer is expected to be formed in the first place, depending upon the surface activity of the emulsifiers determined by the structural properties. The effect of emulsifier types and concentrations on the protein surface load at the same protein content is shown in *Figure 44*.

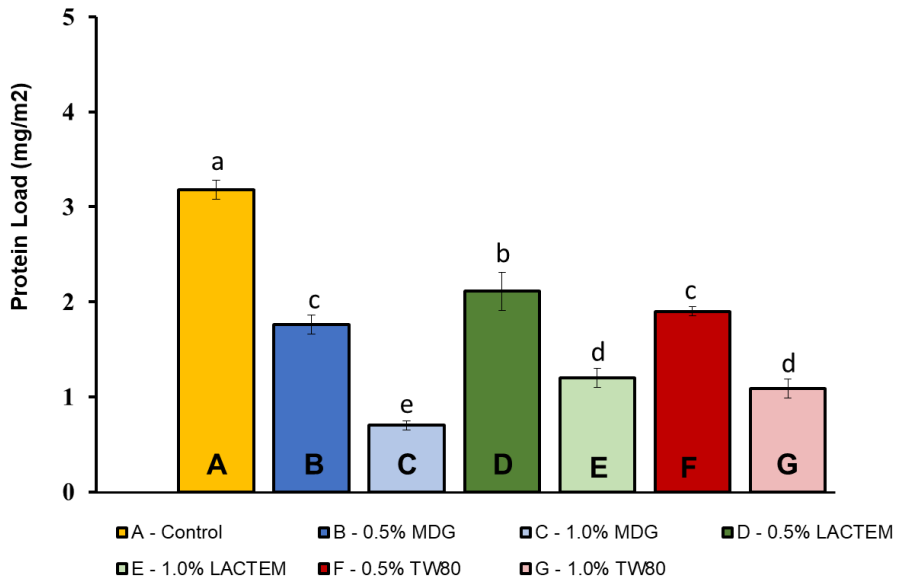


***Figure 44**: Effect of emulsifier types (MDG, LACTEM and Tween 80) and concentrations (0.5% and 1.0%) on the degree of protein surface load of emulsions.*

In the control emulsion stabilised solely by milk protein, approximately 3.18 mg/m^2 available protein was distributed at the interface. When used in combination with proteins, LMW emulsifiers can influence the stability of emulsions by competing with the proteins for adsorption at the interface, where the most surface-active and abundant emulsifiers dominate the interface and displacing proteins due to the high surface activity (Munk et al., 2014).

Figure 44 shows that the variation of protein surface load depended on the emulsifier concentration. At an emulsifier concentration of 0.5% (emulsions B, D and F), the protein load decreased significantly as the emulsifier concentration increased compared to the control emulsion (without emulsifier). With an emulsifier concentration of a 1.0%, a dramatic reduction in protein surface load was observed, dominated by lipophilic emulsifiers (MAG and LACTEM), followed by hydrophilic emulsifier, Tween 80. This observation suggested that MDG and LACTEM with a lower HLB value effectively reduced the interfacial protein due to the highly competitive adsorption of the lipophilic emulsifier on the surface of fat droplets compared to the hydrophilic emulsifier.

The observation for a 1.0% Tween 80 emulsion could be explained by the fact that the presence of excess or unabsorbed protein in the form of small caseinate aggregates such as sub-micelles may lead to deterioration in creaming stability due to depletion flocculation (Dickinson et al., 2003). The detailed mechanism behind the competitive adsorption of Tween 80 and protein on the surface of fat droplets was discussed in the previous section (6.3.1.2).

Emulsions with lipophilic emulsifiers showed higher displacement than hydrophilic emulsifiers through reduced interfacial tension at the protein interface, which could lead to more efficient packing within the interfacial film and facilitate the formation of a more densely packed adsorbed protein layer at the interface and showed a further reduction in protein surface load. This observation is consistent with the findings of Dickinson (2019), and further supports our results.

6.3.2. Fat crystallisation in emulsion

6.3.2.1 Refined stop-and-return DSC method

Manipulation of emulsions by incorporating different emulsifiers, both in type and concentration, has revealed significant differences in crystallisation events and polymorphic changes within the emulsions. The relationships between emulsifiers and fat droplet size also exert a noticeable influence on fat crystallisation behavior, which is supported by several

studies that emphasize the impact of emulsifier arrangement at the O/W interface on fat crystallisation in emulsions. With the displacement of milk protein by emulsifiers, the interfacial composition and behavior control the formation of fat crystal networks and/or the adsorption of fat droplets at the A/W interface (Awad and Sato, 2002; Douaire et al., 2014; Goibier et al., 2017a; Wang et al., 2021a; Murray, 2002).

As Garti and Yano (2001) have pointed out, emulsifiers can retard or accelerate nucleation, crystal growth and/or polymorphic transitions depending on the homogeneity between emulsifier and lipid in terms of chain length and degree of saturation. In the previous chapter, we examined in detail the fundamental mechanisms affecting polymorphic transformation and crystallisation in fat droplets in emulsions, as evidenced by stop-and-return DSC thermogram. The focus of this chapter is to examine the influence of emulsifier types and concentrations on the changes observed in the stop-and-return DSC thermogram as shown in *Figure 45*.

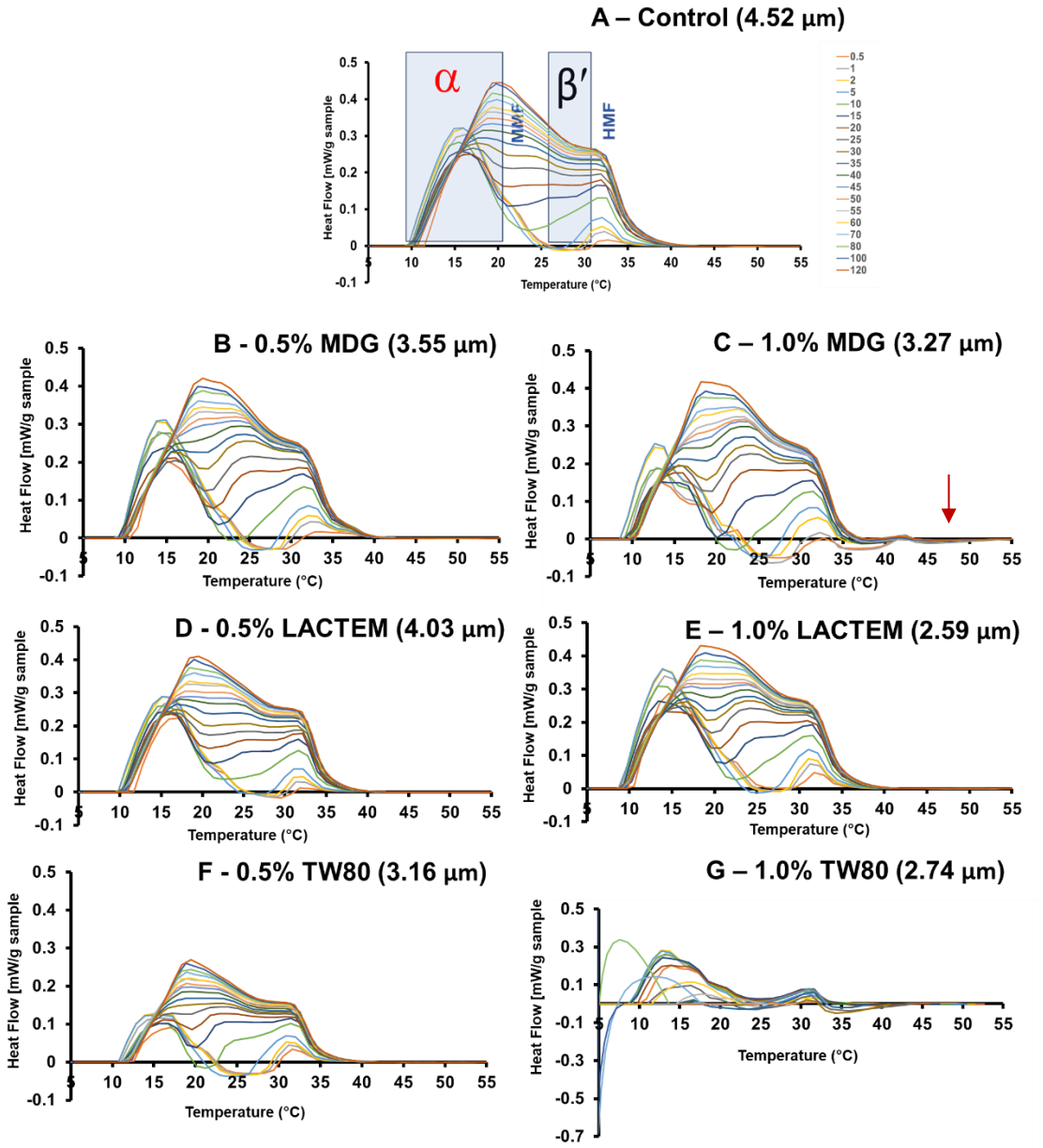


***Figure 45**: Effects of emulsifier types (MDG, LACTEM and Tween 80) and concentrations (0.5 and 1.0 wt.%) on the crystallisation events of fat droplets in emulsions stabilised with 1 wt.% sodium caseinate containing 36 wt.% AMF.*

In contrast to the control emulsion (A), emulsions containing lipophilic emulsifiers, in particular MDG at a concentration of 1.0%, showed clear changes characterised by deep dips, indicating a transition from the metastable α to the stable β′ polymorphic crystals. This observation indicates that the lipophilic emulsifier MDG accelerates nucleation and co-crystallises with fat on the surface of fat droplets, thereby promoting crystal growth and polymorphic transition. This effect is likely due to the structural homogeneity between the saturated carbon chain (particularly stearic acid) of anhydrous milk fat and MDG, which is mainly composed of stearic acid (C18:0) and is likely to favor fat crystallisation. Similar observations were found in

following analysis (see 6.3.2.2) highlighting the dependence of polymorphism transitions on emulsifier concentration, which is particularly evident for lipophilic emulsifiers such as MDG and LACTEM.

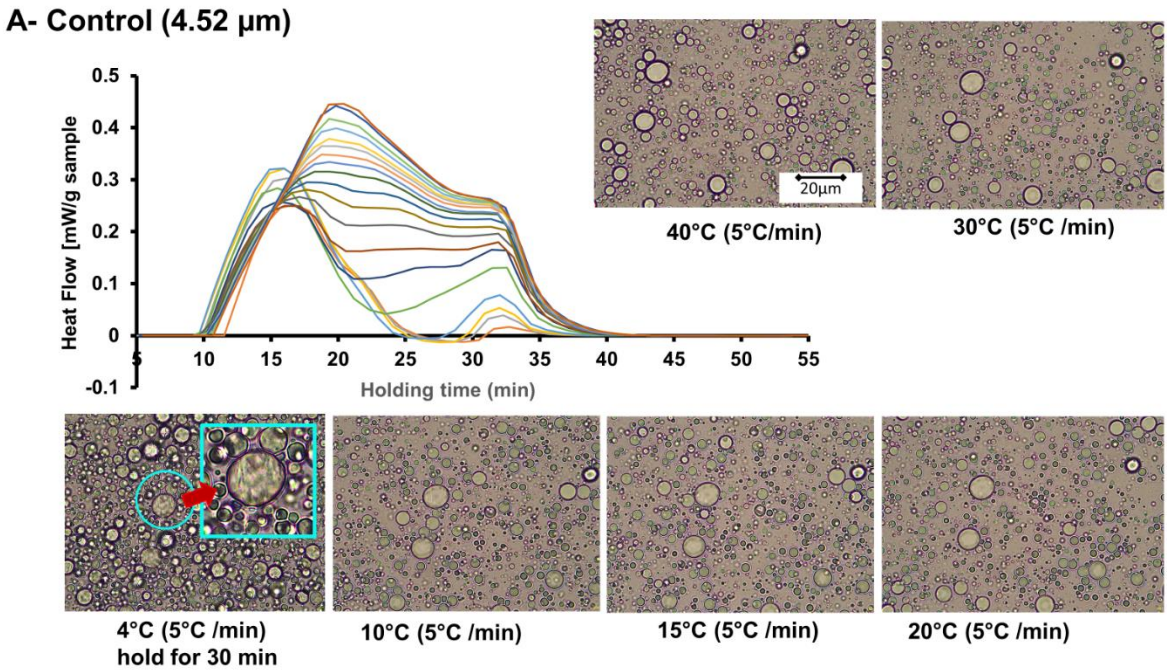
Emulsions containing 1.0% MDG exhibited an increased melting point to 50°C (marked by the red arrow in Emulsion C, *Figure 45*), attributed to the high concentration of highly saturated MDG, which promotes a densely packed crystal lattice and thereby a higher energy require for complete melting of the fat droplets in the emulsion. This observation is supported by previous studies indicating that crystallisation of MDG occurs simultaneously with anhydrous milk fat, which significantly increases the solid fat content and acts as nuclei for crystallisation, thereby increasing nucleation rates and heterogeneous interfacial nucleation due to their template effect (Arima et al., 2007; Coupland, 2002; Sato and Ueno, 2011).

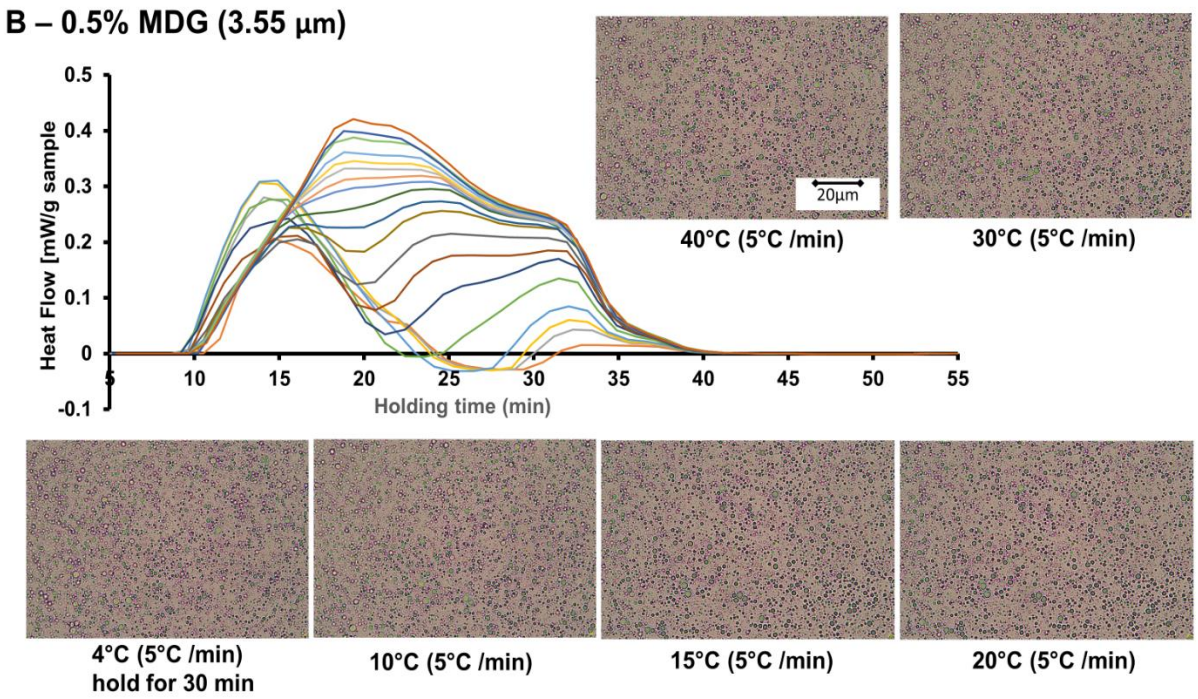
Notably, MDG demonstrated a more pronounced effect than LACTEM, which is likely due to the high melting mono- and diglycerides present in this MDG ingredient, which enables faster nucleation rates compared to LACTEM. The LACTEM-containing emulsion showed a similar trend to the control, but it is interesting to note that Emulsion E with 1.0% LACTEM showed the appearance of a single strong α polymorph peak on the leftmost side of the graph. This could be attributed to LACTEM as they are often referred to as α-tendency emulsifiers and have been shown to competitively replace protein from the O/W interface and induce shear instability during the aeration process, leading to some degree of flocculation or coalescence of fat droplets and achieving the characteristic foam structure (Allen et al., 2008; Munk et al., 2013; Munk and Andersen, 2015).

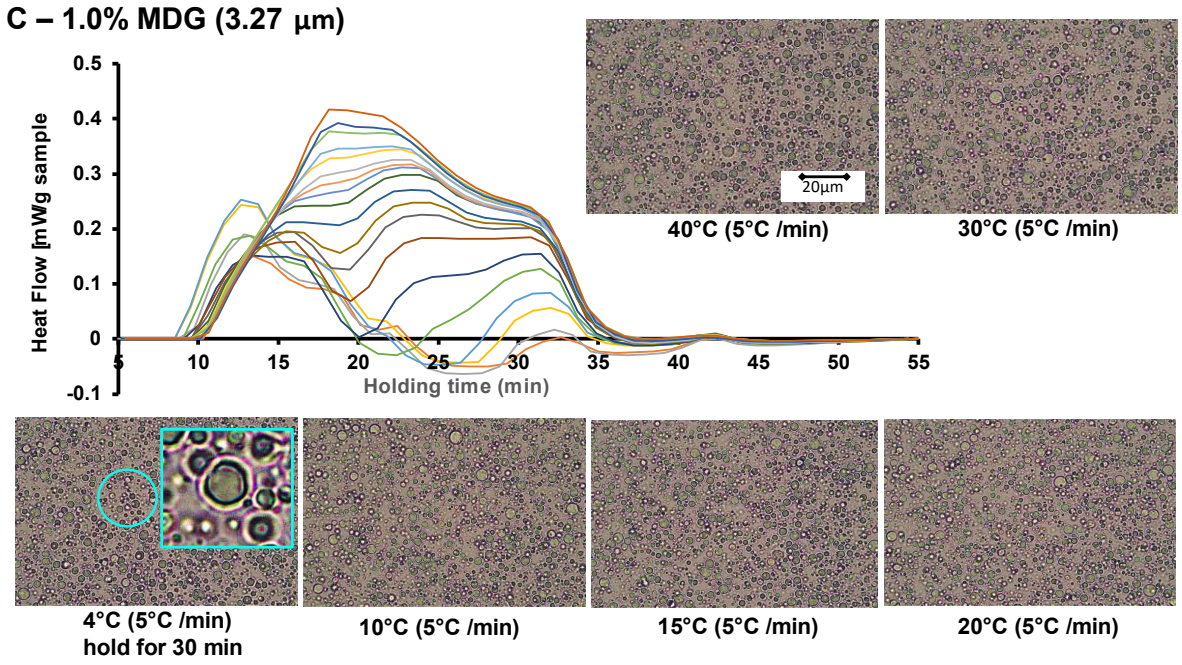
In contrast, emulsions containing Tween 80 demonstrated limited polymorphic transition and impaired with fat crystallisation due to the unsaturated carbon chain and large hydrophilic head. This effect was particularly pronounced at high concentrations, rendering Tween 80 ineffective as a template for fat crystals. Similar observations were reported by Rizzo et al. (2015). Furthermore, Gulseren and Coupland (2008) emphasized that Tween 80 is incapable of undergoing temperature-dependent phase transitions such as MDG and LACTEM. Fuller et al. (2015) also found that Tween 80 had no effect on the crystallisation rate or onset of crystallisation of milk fat. Besides, higher concentrations of Tween 80 showed a negative synergistic effect, as too large amounts did not trigger nucleation and hindered crystallisation, so it was likely to remain in the liquid state at lower temperatures.

6.3.2.2 Nucleation behaviour of emulsion

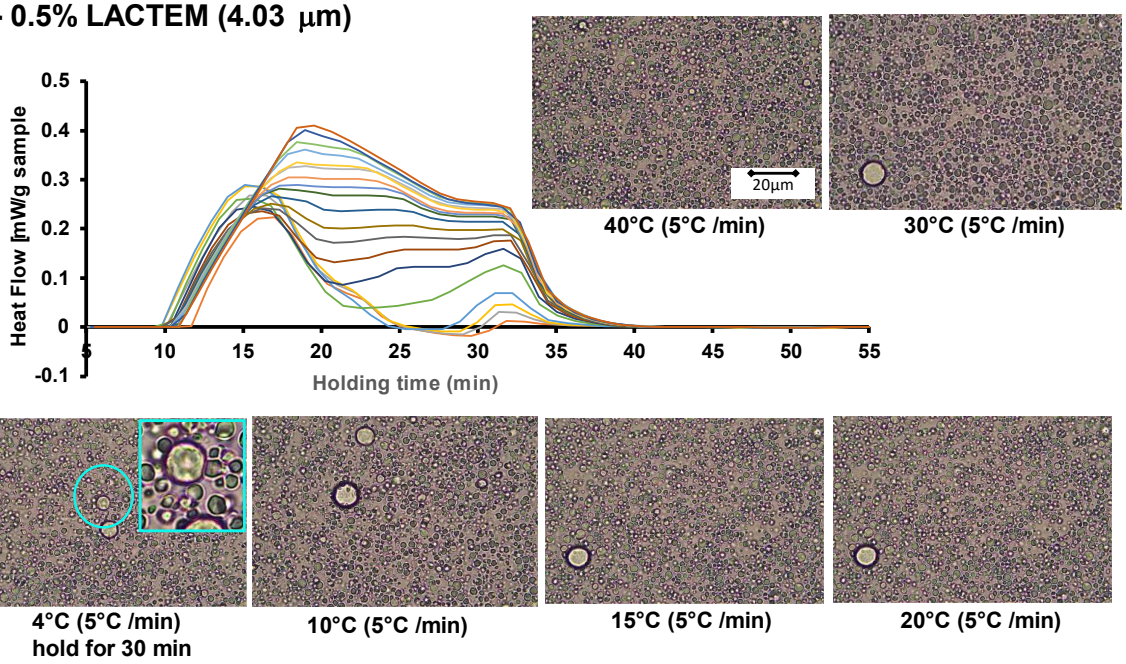
To obtain a more comprehensive understanding of nucleation kinetics and to identify partial coalescence of fat structure (*e.g.,* fat clustering, aggregates, and crystallisation) in O/W emulsions, we performed a microscopic study to attain correlations from the refined stop-and-return method using DSC. By studying crystallisation events derived from heating cycles, we aimed to gain insight into the nature of crystal nucleation. This review included observing polymorphic changes performed under a polarised light microscope (PLM), cooling from 40°C to 4°C at a cooling rate of 5°C/min and holding each temperature for 30 min, as shown in *Figure 46*. Our objective was to investigate the initial formation of interfacial nucleation and its interactions with crystal growth within the emulsion, as well as correlation with other properties such as particle size distribution and whipping properties.



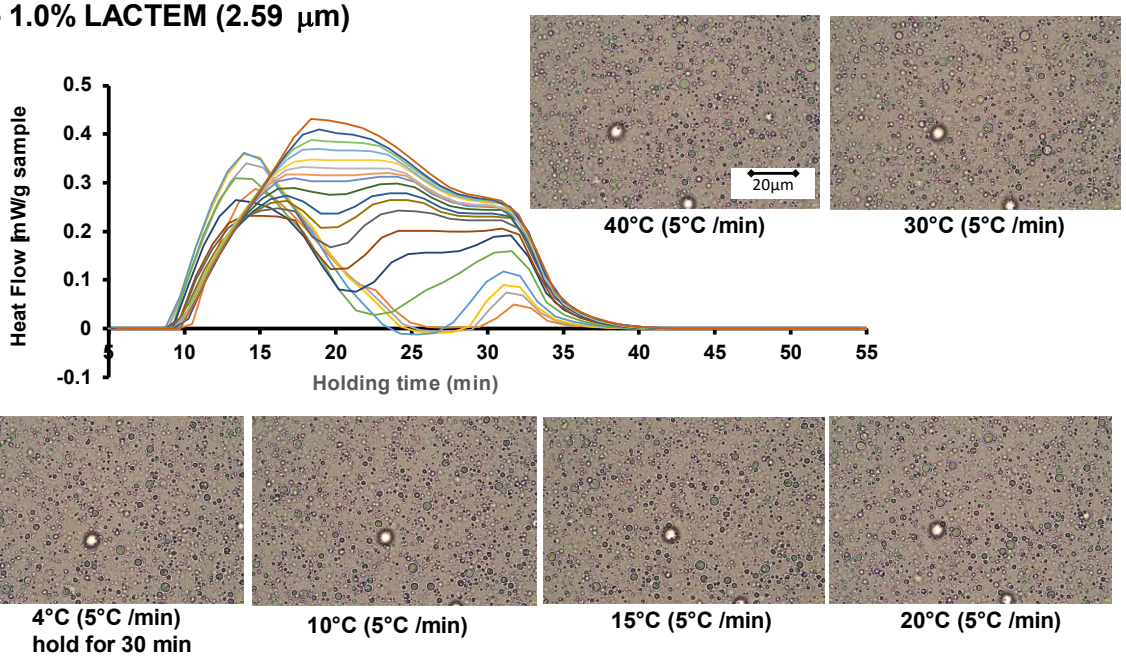

B – 0.5% MDG (3.55 µm)
Heat Flow [mW/g sample
0.5
0.4
0.3
0.2
0.1
0
-0.1
5
10
15
20
25
30
35
40
45
50
55
Holding time (min)
20µm
40°C (5°C /min)
30°C (5°C /min)
4°C (5°C /min)
hold for 30 min
10°C (5°C /min)
15°C (5°C /min)
20°C (5°C /min)

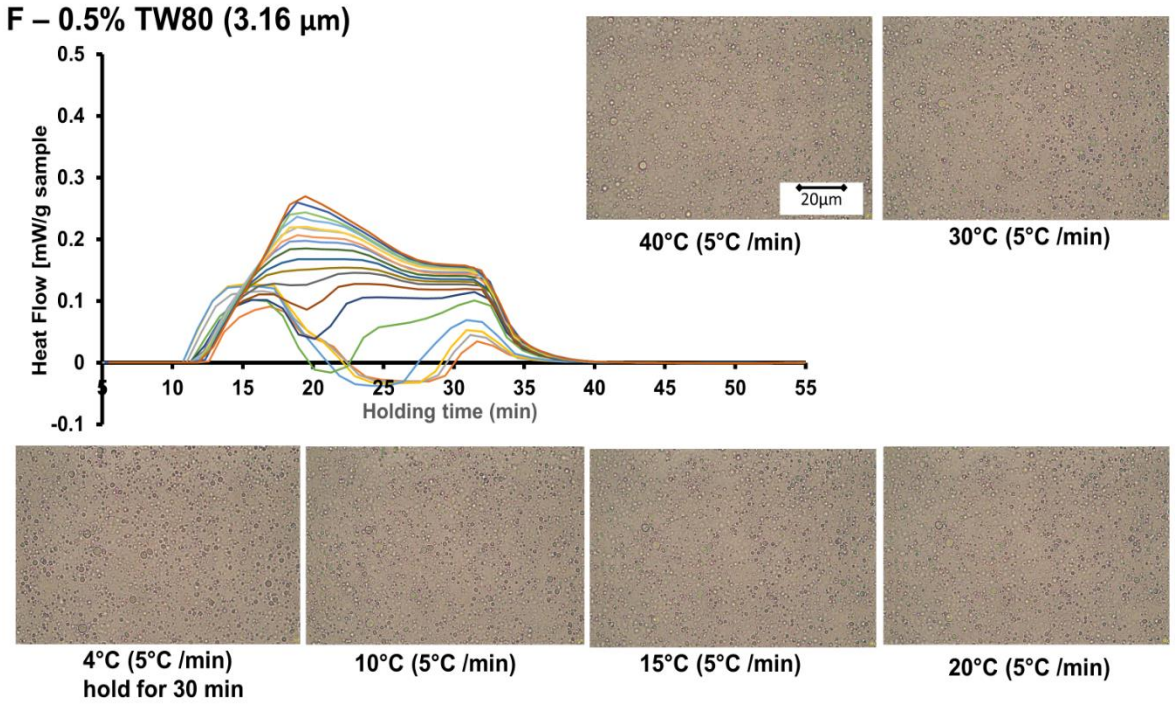

C – 1.0% MDG (3.27 µm)
Heat Flow [mWg sample
0.5
0.4
0.3
0.2
0.1
0
-0.1
5
10
15
20
25
30
35
40
45
50
55
Holding time (min)
20µm
40°C (5°C /min)
30°C (5°C /min)
4°C (5°C /min)
hold for 30 min
10°C (5°C /min)
15°C (5°C /min)
20°C (5°C /min)

D – 0.5% LACTEM (4.03 μm)



E – 1.0% LACTEM (2.59 μm)





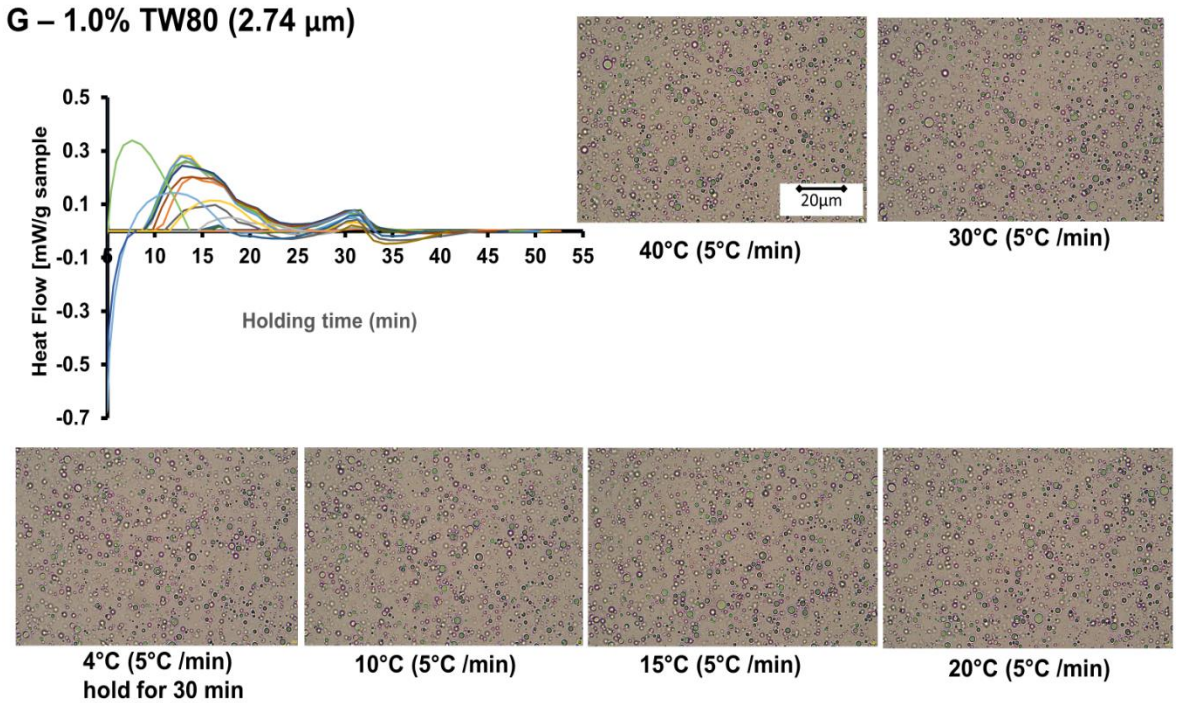


Figure 46: *The effects of different emulsifier types (MDG, LACTEM, and Tween 80) and concentrations (0.5 and 1.0 wt.%) on the crystallisation events of fat droplets in emulsions stabilised with 1 wt.% sodium caseinate and 36 wt.% AMF, in relation to the microstructure and nucleation behavior of O/W emulsions taken at 8°C with PLM.*

In emulsions containing lipophilic emulsifiers such as MDG (*Figure 46 C*) and LACTEM (*Figure 46 D*), their affinity for the oil phase and their ability to displace interfacial proteins contribute to the nucleation kinetics that occur predominantly at the surface of fat droplets. The micrograph shows that crystals form externally on the surface, in contrast to the control emulsion where the typical crystal formation occurs within the droplets as observed in *Figure 46 A.*

Displacement of interfacial proteins by lipophilic emulsifiers alters the conditions at the O/W interface and promotes an environment that favors nucleation on the outer interface of fat droplets. This surface nucleation phenomenon can be postulated due to the rearrangement of emulsifiers at the interface, which affects the interactions between fat molecules and water. The presence of lipophilic emulsifiers induces a distinct crystallisation pattern, that leads to the formation of external crystal instead of internal crystallisation within the fat droplets, which can be postulated to arise from the solidification of the emulsifiers at the interface, as observed in the control emulsion. In contrast, hydrophilic emulsifiers, exemplified by Tween 80 in *Figure 46 F and G*, exhibited limited solubility in oil, making them ineffective as templates for fat crystals.

*6.3.*3 Evaluation of aerated emulsion

6.3.3.1 Whippability using a rheometer

The arrangement of emulsifiers adsorbed at the O/W or O/A interface and the ability to influence partial coalescence of fat droplet are closely linked to fat crystallisation behavior. Partial coalescence is enhanced in the presence of liquid-like emulsifiers but is partially inhibited by high melting point emulsifiers. Bayard et al. (2022) demonstrated that emulsifiers with long saturated moieties, such as palmitic acid, can accelerate fat crystallisation while reducing the extent of partial coalescence (Goibier et al., 2017). Saturated monoglycerides have been found to prevent partial coalescence, whereas unsaturated monoglycerides, such as monoolein, facilitate partial coalescence due to morphological changes (Davies et al., 2000.; Fredrick et al., 2010, 2013).

Figure 47 demonstrates the relationship between apparent viscosity (η) and whipping time (*t*) for emulsions with emulsifiers and shows the increase in viscosity over the course of whipping at constant rotational speed (1800 rpm), volume (10 mL), and temperature (8°C). For a comprehensive understanding of the apparent viscosity profile, please read the previous chapter (5.3.3.1), where we presented the graphical representation that provides insight into

the whippability progression, through the phases of under-whipped (UW), ideally-whipped (IW) and over-whipped (OW) emulsions. These phases were used to quantitatively evaluate the effects of shear-induced partial coalescence in the fat crystal network in droplets, which was influenced by the type and concentration of emulsifiers.

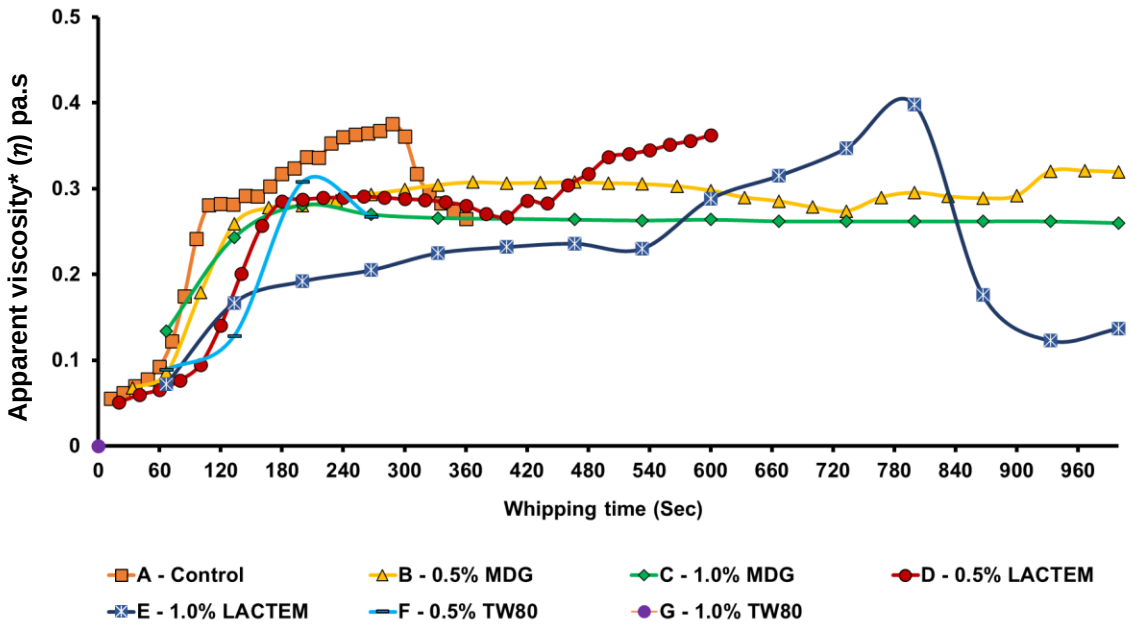


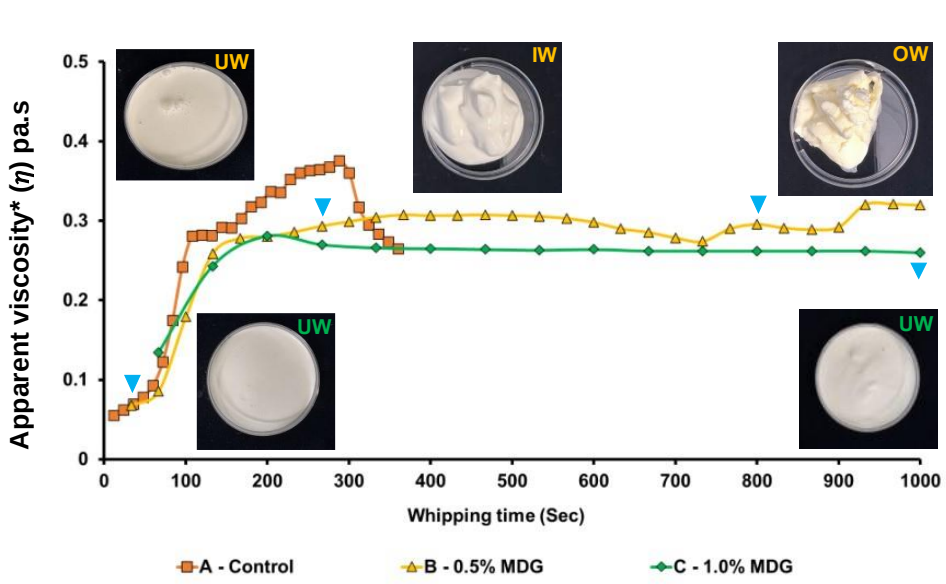
***Figure 47**: Apparent viscosity profile of 10 mL of emulsions incorporated with different types and concentrations of emulsifiers sheared at constant rotational speed of 1800 rpm and temperature of 8°C.*

Figure 47 depicts the relationship between apparent viscosity (η) and whipping time (*t*), marking the point at which viscosity increased as the whipping commenced. This graph elucidates the whippability of emulsions containing different emulsifier types and concentrations. The addition of emulsifiers uniformly extended the whipping time for all O/W emulsions, compared to the control except for the Tween 80 emulsions. In particular, higher concentrations of the lipophilic emulsifiers, MDG and LACTEM, resulted in longer whipping times to achieve a firm peak or ideally whipped emulsion. This observation demonstrated the role of emulsifiers in reducing fat droplet size, thereby improving shear stability and preventing partial coalescence, thereby expanding the whipping window and increasing overall whipping time.

While the incorporation of emulsifiers has been shown to weaken the interface and promote fat crystallisation as previously mentioned, this finding highlights that fat droplet size was found to be a major factor influencing whippability. The presence of a smaller fat droplet size helps

to improve stability by hindering fat crystallisation and partial coalescence, which is consistent with the findings of Goff (1997) and consequently extending the whipping time. In both LACTEM- and MDG-incorporated emulsions, the emulsifiers effectively prevented rapid partial coalescence during the whipping process, resulting in closer contact of the fat droplets without premature aggregation, in contrast to the control emulsion (A).

Figure 48 explains the course of the MDG-incorporated emulsion compared to the control emulsion (A) and shows three prominent shoulders. The first shoulder indicates an under-whipped state (UW) during the initial phase of incorporating air into the liquid emulsion. The second shoulder, which extends between 300 sec and 800 sec, signifies the onset of partial coalescence of fat droplets, leading to the formation of an ideally-whipped (IW) emulsion. During this phase, the viscosity progressively increases until it reaches an initial peak at the IW phase, indicating irregular aggregation and the gradual formation of a continuous network throughout the emulsion volume. This continuous network gives the emulsion solid properties, immobilises other particles such as air bubbles and thus characterises the emulsion as ideally-whipped (IW). The third shoulder marks the beginning of the built-up fat structure and the collapse of air bubbles, causing the emulsion to transition to an over-whipped (OW) state. However, this OW phase for a 1.0% MDG emulsion is not shown in the graph because a longer whipping time (> 1000 sec) is required to reach the OW phase.



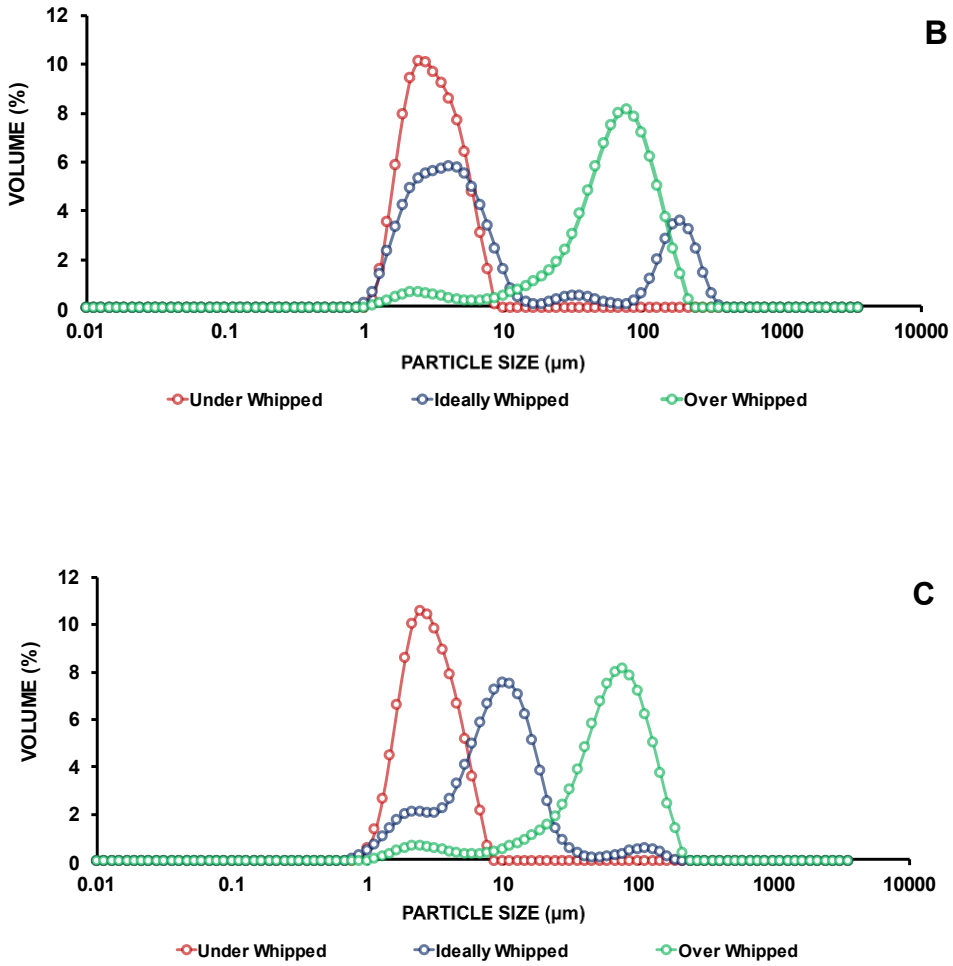


***Figure 48**: A) Apparent viscosity profile of 10 mL of MDG-incorporated emulsions compared to control, sheared at constant rotational speed of 1800 rpm and temperature of 8°C. B) Particle size distributions of the emulsion with 0.5% MDG. C) Particle size distributions of the emulsion with 1.0% MDG.*

The viscosity analysis during shear in the rheometer was strategically interrupted based on three phases of whippability: under-whipped (UW), ideally-whipped (IW), and over-whipped (OW). Prior to investigating the particle size distribution (PSD) analysis using laser diffraction, the whipped emulsion had to be diluted with a 1% SDS solution to prevent the analysis of flocculated fat droplets. The SDS surfactant solution breaks up all flocs leaving only semi-coalesced fat or individual fat droplets for measurement.

Figure 48 B and *C* illustrate the PSD curve for 0.5% and 1.0% MDG emulsions and capture the distributions of individual milk fat droplets and partially coalesced fat as well as air bubbles present in the emulsions. Notably, the viscosity curve exhibited a peak between 0 and 300 sec

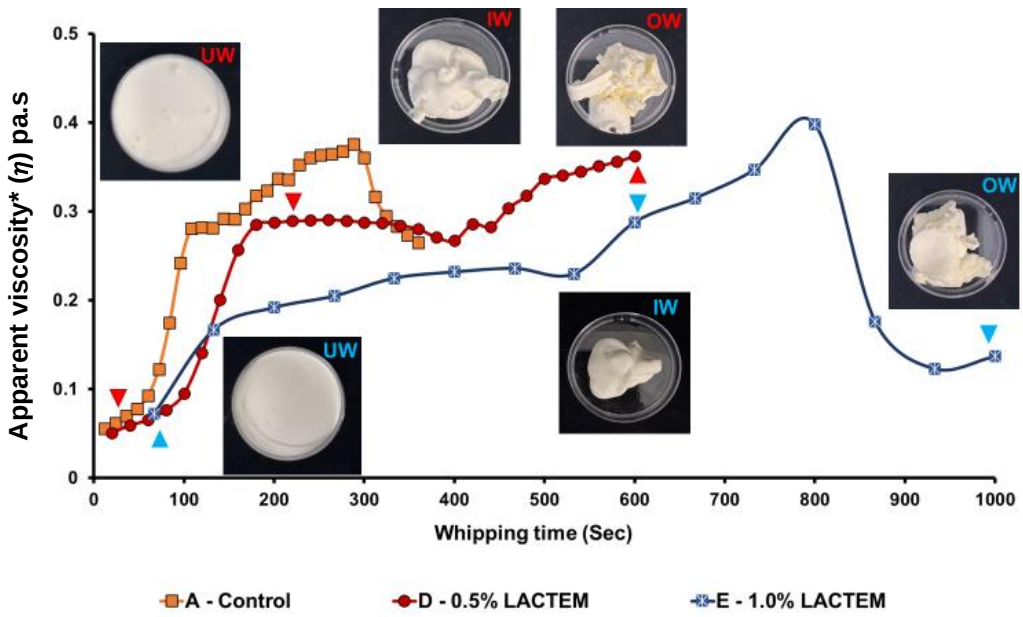
of whipping time, signifying the under-whipped (UW) phase where the fat droplet sizes correspond to those of the original emulsion and are mainly between 1 and 10 μm in size. As long as partial coalescence was not initiated, the droplet size remained unaltered and therefore the frequency of encounters (*i.e.,* the number of collisions) was constant (Moens et al., 2018b).

As whipping progressed, the size distribution showed three peaks for samples taken at t = 300 sec for 0.5% MDG and t = 1500 sec for 1.0% MDG (not shown in the graph), indicating the incorporation of air bubbles, presumably between 10 and 100 μm. This resulted in an escalation in the initial rate of shear-induced partial coalescence, as small individual fat droplets were aggregated into larger clusters, resulting in irreversible aggregated fat droplets over 100 μm. At this phase, the emulsion was characterised as ideally whipped, IW (not shown in the graph), visually marked by the production of the best rosette and greatest overrun, as shown in the following section. In this phase, whipping is mainly influenced by the spreading of fat droplets on the air bubble surface to form a continuous network of fat droplets throughout the aqueous phase (Moens et al., 2018b).

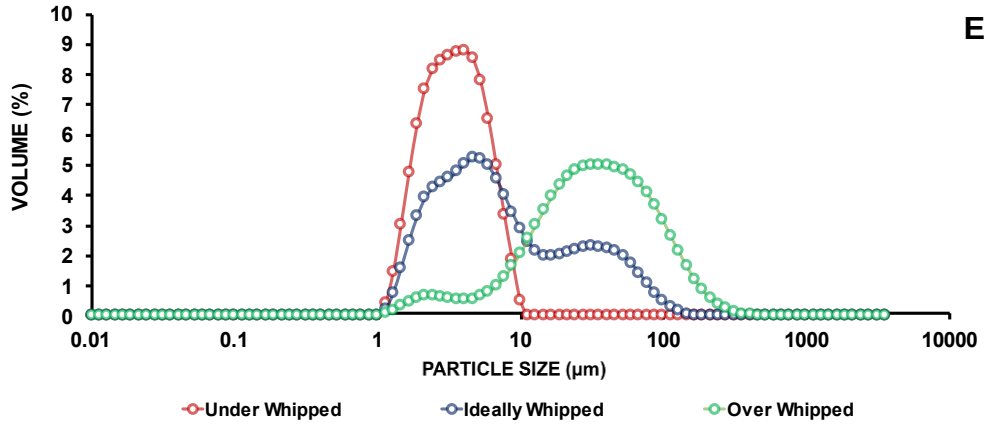
The extent of partial coalescence increased with shearing and time, causing the emulsions to transition to an over-whipped (OW) state. This transition was characterised by a single third peak measuring 100 μm in size, along with a reduction in air bubbles between 10 and 100 μm, as well as a reduction in primary fat droplets, leaving a small proportion of approximately 5 μm fat droplets. At this phase, a decrease in viscosity indicated phase inversion and weakening of the partially coalesced fat droplet network.

A parallel trend can be observed in for the LACTEM-incorporated emulsions (*Figure 49*), with the whipping process following a similar progression, but slightly faster than with MDG. This could be explained by the fact that the emulsion with 0.5% LACTEM produced slightly larger fat droplets than the 0.5% MDG. Large fat droplets showed lower stability and at the same time resulted in a faster aggregation rate due to the smaller protrusion distance of the crystalline droplets. Similar observations were found for the particle size distributions of the 0.5% and 1.0% LACTEM-incorporated emulsions as whipping progresses (*Figure 49E* and *F)* albeit significantly shorter times are required to reach the IW state.

D


Apparent viscosity* (η) pa.s
0.5
0.4
0.3
0.2
0.1
0
UW
IW
OW
OW
IW
UW
0
100
200
300
400
500
600
700
800
900
1000
Whipping time (Sec)
A - Control
D - 0.5% LACTEM
E - 1.0% LACTEM

E


VOLUME (%)
10
9
8
7
6
5
4
3
2
1
0
0.01
0.1
1
10
100
1000
10000
PARTICLE SIZE (µm)
Under Whipped
Ideally Whipped
Over Whipped

F

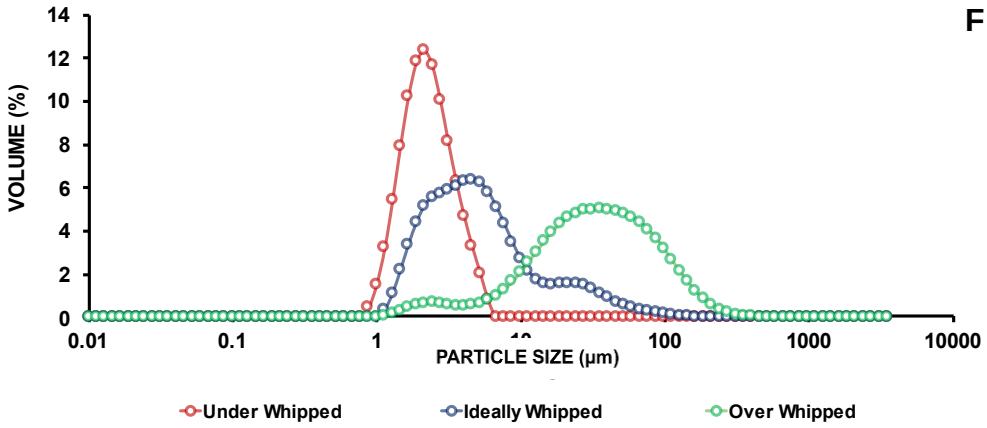

VOLUME (%)
14
12
10
8
6
4
2
0
0.01
0.1
1
100
1000
10000
PARTICLE SIZE (µm)
Under Whipped
Ideally Whipped
Over Whipped

***Figure 49**: D) Apparent viscosity profile of 10 mL of LACTEM-incorporated emulsions compared to control, sheared at constant rotational speed of 1800 rpm and temperature of 8°C. E) Particle size distributions of the emulsion with 0.5% MDG. F) Particle size distributions of the emulsion with 1.0% MDG.*

Conversely, emulsions containing the hydrophilic emulsifier Tween 80 failed to whip, as shown in *Figure 50*. It seems that a very fast aggregation takes place resulting in a fast semi coalescence at 1% Tween that does not allow for any air incorporation. The weakened interfacial layer in the presence of Tween 80, together with the fact that these emulsions are likely to be flocculated (given the phase separation observed) before whipping, this all contributes towards a fast emulsion destabilisation under shear. This is also observed at 0.5% Tween as the viscosity increases due to fat partial coalescence happening but fails to trap any air before the emulsion churns achieving the O/W state.

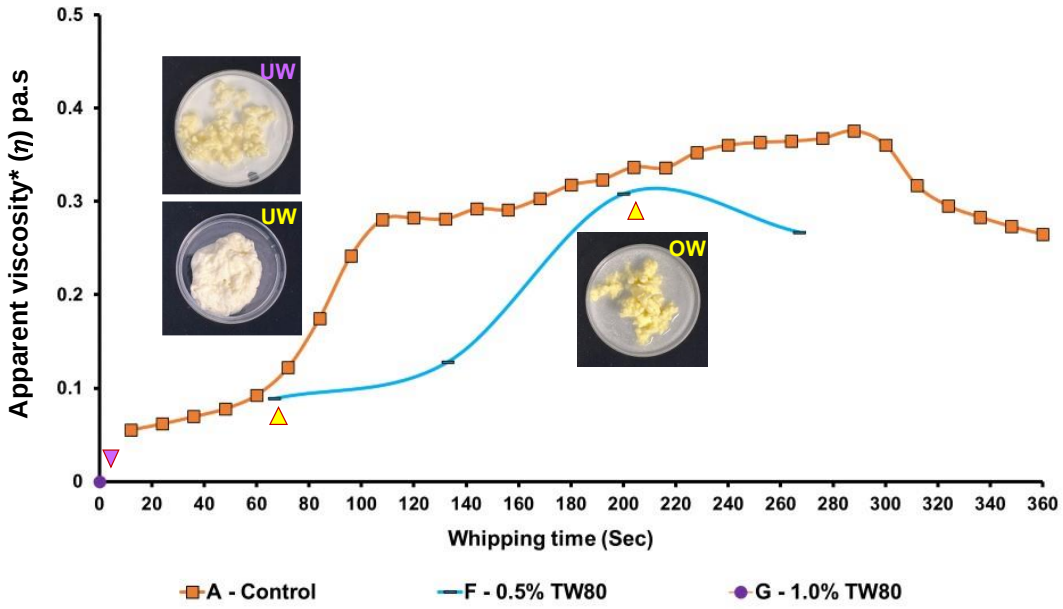


***Figure 50**: Apparent viscosity profile of 10 mL of Tween 80-incorporated emulsions compared to control, sheared at constant rotational speed of 1800 rpm and temperature of 8°C.*

In conclusion, our observations highlight the important role of particle size in whippability, with emulsifier effects being secondary. Smaller particle sizes contribute to enhanced stability, preventing fat crystallisation and partial coalescence, aligning with findings by Goff (1997) and subsequently extending the whipping time.

6.3.3.2 Foam structure, overrun and firmness

The stability of aerated emulsions is greatly influenced by the distribution of air bubbles and the dispersion of fat droplets (Goff et al., 1999; Jiang et al., 2018). The distinct foam structure formed through aeration reflects the equilibrium between the partial coalescence of fat droplets and air bubbles (Moens et al., 2016). The variations observed among the O/W emulsions in terms of foam microstructure as influenced by emulsifier types and concentrations are illustrated in *Figure 51*, captured through CLSM imaging.

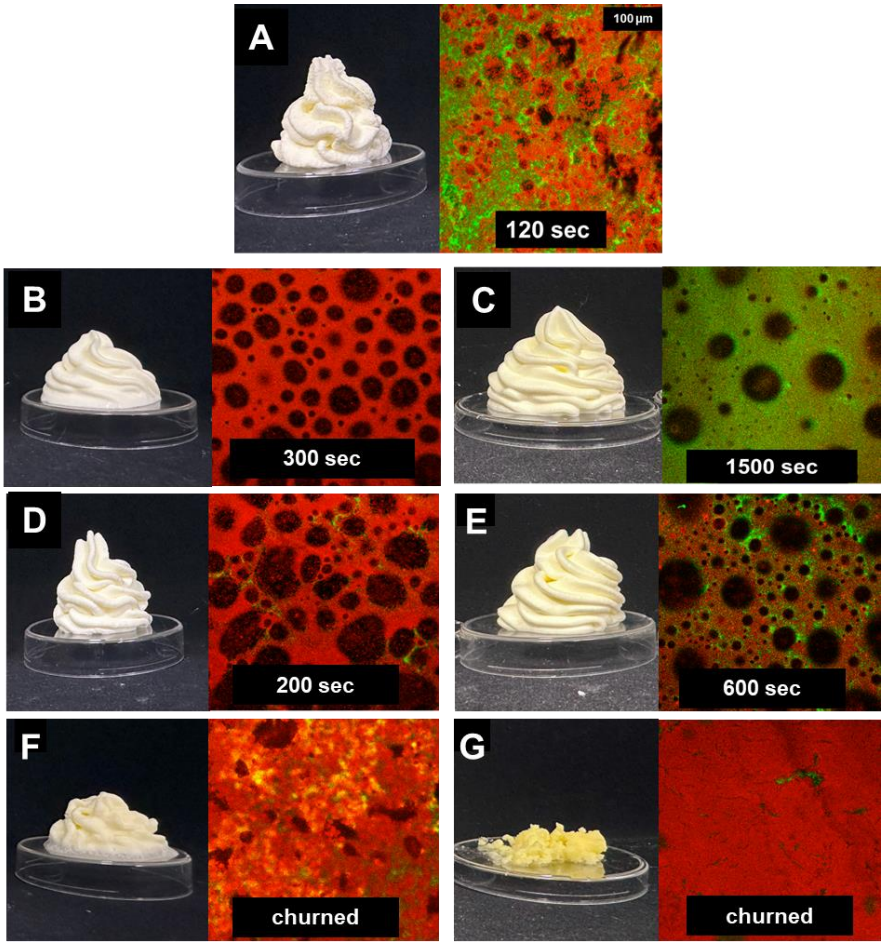


Figure 51: *The confocal laser scanning microscopy (CSLM) micrographs of the O/W emulsions incorporated with different types and concentration of emulsifiers. In the images, proteins are fluoresced in green and fat in red. Abbreviations: A: Control, B: 0.5% MDG, C: 1.0% MDG, D: 0.5% LACTEM, E: 1.0% LACTEM, F: 0.5% Tween 80 and G: 1.0% Tween 80.*

Figure 51 shows the different foam structures of the whipped emulsions, which vary due to the incorporation of emulsifiers. It is evident that the lipophilic emulsifiers (MDG and LACTEM) dominate the overrun and entrapment of air bubbles, surpassing the efficiency of hydrophilic emulsifiers such as Tween 80 (Emulsions F and G). In contrast, the control emulsion (A),

stabilised solely by protein, has fewer dispersed, irregularly shaped air bubbles (black areas), which are stabilised by a thick layer of partially coalesced fat droplets. The irregular shapes of the air bubbles may be due to inefficient packing and stabilisation of the air bubbles by a surface-mediated partial coalescence network, indicated by fewer red areas representing a network of coalesced fat.

A clear effect was observed when lipophilic emulsifiers were added. MDG (0.5% B and 1.0% C) and LACTEM (0.5% D and 1.0% E) produced a more evenly distributed, spherical network of air bubbles within the emulsified crystalline system, with well-dispersed fat droplets forming a stable network formed. This uniform air bubble distribution supports enhanced foam stability, and it is noteworthy that emulsions containing MDG (0.5% B and 1.0% C) had more homogeneously distributed and regularly shaped air bubbles. LACTEM, known for forming a protective α-crystalline membrane on fat droplets, stabilised both small and larger air bubbles, resulting in a higher overrun (*Table 9*). The layer of surface mediated-partially coalesced fat droplets supporting the air bubbles was thinner compared to the control formulation and showed a softer rosette as shown in the firmness results (*Table 10*). Although this air bubble interfacial layer is not homogeneous, it suggests the presence of emulsifiers and proteins at the interface that contribute to foam stabilisation and structure.

Emulsions prepared with MDG also resulted in satisfactory rosettes, indicating the stabilisation of air bubbles by surface mediated-partially coalesced fat. The MDG emulsifier resulted in faster nucleation compared to other emulsifiers, resulting in uniformly spherical air bubbles and a rigid structure connected by fat crystals. This makes it easier to form a protective layer around air bubbles during whipping, thus increasing foam firmness.

Table 9: *Effects of types and concentrations of emulsifiers on the overrun of O/W emulsions.*

Type of emulsifiers	**Overrun (%)**	
	Concentration 0.5%	**Concentration 1.0%**
Control	144.83 ±1.92*^{b}	144.83 ±1.92*^{a}
MDG	154.05 ±2.03^{c}	318.10 ±2.52^{c}
LACTEM	218.91 ±1.02^{d}	258.65 ±3.20^{b}
Tween 80	105.40 ±2.31^{a}	Churned

***Table 10**: Effects of types and concentrations of emulsifiers on the firmness of O/W emulsions.*

Type of emulsifiers	Firmness (*N*)	
	Concentration 0.5%	**Concentration 1.0%**
Control	20.11 ± 0.95*^{c}	20.11 ± 0.95^{*c}
MDG	10.82 ± 0.62^{ab}	6.76 ± 0.11^{a}
LACTEM	10.71 ± 0.65^{a}	9.62 ± 0.33^{b}
Tween 80	17.90 ± 1.54^{b}	2209.76 ± 11.46^{d}

Higher concentrations of lipophilic emulsifiers (1.0% MDG and LACTEM) result in smaller fat droplets, requiring longer whipping time to build a more robust fat network over time. This rigid network supports higher overrun, as indicated in *Table 9*, with a denser arrangement of fat droplets providing foam cohesion. The higher viscosity of these emulsions prevents incorporated air bubbles from escaping and thus additionally stabilises the foam.

6.4 Conclusion

In this chapter we examine how different types and concentrations of emulsifiers affect homogenised milk creams and their whipping properties. Our investigation focused on understanding the effects of low molecular weight (LMW) emulsifiers on fat crystallisation, polymorphic transitions, whipping properties, and foam structure in O/W emulsions. Through our research we have come to several important conclusions.

First, lipophilic emulsifiers such as MDG and LACTEM showed superior adsorption and interfacial displacement compared to hydrophilic emulsifiers (Tween 80), resulting in reduced protein adsorption at the O/W interface and mitigating phase separation. This improved stability was attributed to the ability of lipophilic emulsifiers to efficiently pack into the interfacial film, resulting in a reduced protein surface load. In addition, lipophilic emulsifiers, particularly MDG, have been found to accelerate fat nucleation and co-crystallisation, thereby promoting crystal growth and polymorphic transitions. The structural similarity between the saturated carbon chain of MDG and anhydrous milk fat (AMF) facilitated fat crystallisation and contributed to the formation of a stable fat network. In contrast, hydrophilic emulsifier such as Tween 80 showed limited polymorphic transition and interfered with fat crystallisation due to their unsaturated carbon chain and large hydrophilic head. Higher concentrations of Tween 80 did not serve as a template for fat crystals, resulting in compromised stability.

Additionally, the study highlighted the importance of particle size to shear-induced emulsion stability, with smaller fat droplets exhibiting increased stability by hindering fat crystallisation and partial coalescence. Emulsions containing the lipophilic emulsifiers MDG and LACTEM

demonstrated longer whipping times and improved foam stability, attributed to their ability to reduce fat droplet size and improve shear stability. Overall, our results highlight the critical role of emulsifiers in modulating the properties of O/W emulsions, with lipophilic emulsifiers offering superior stability and performance compared to hydrophilic counterparts. In summary, the importance of particle size in determining whipping ability is the primary factor of whippability, while emulsifier effects are secondary.

Smaller particle sizes contribute to improved stability, effectively preventing fat crystallisation and partial coalescence, thus improving the overall quality of the emulsion. These results provide an interesting starting point for further work on controlling the partial coalescence of fat droplets and fat crystallisation behavior by selecting appropriate emulsifier formulas according to their different functionality to achieve improved functionality in the aerated emulsions of dairy and non-dairy products.

Overall conclusions and recommendations

The results obtained from this study provide a clearer picture of the synergistic role of partial coalescence and fat crystallisation as well as the underlying mechanisms in whipping oil-in-water (O/W) emulsions using the rheometer technique. The following research questions were answered:

1. **Chapter 4**
   How can real-time quantification data be monitored and collected to better understand the primary mechanisms of whipping?

2. **Chapter 5**
   What influence does the size of the fat droplets have on the whipping mechanism?

3. **Chapter 6**
   What effects does the incorporating of different types and concentrations of emulsifiers in the oil-in-water (O/W) emulsions have on the mechanisms of whipping?

The rheological setup developed in Chapter 4 offered the opportunity to systematically investigate the influence of shear-induced partial coalescence in the presence of air in whipping cream systems. By integrating rotational rheology with starch spindle analysis, we were able to comprehensively assess the kinetics of partial coalescence in the presence of air, by examining the relationships between the viscosity, particle size, and microstructure of air bubbles and fat droplets in the analysed creams. The successful development of this methodology has demonstrated its potential for reliable and real-time use of this technique in the future and contributed to a more comprehensive understanding of the underlying mechanism of primary whipping such as fat crystallisation and partial coalescence in oil-in-water (O/W) emulsion systems.

The aim of Chapter 5 was to elucidate the effect of fat droplet size in homogenised dairy creams and their whipping properties. The results allow several important conclusions:

First, the size of the fat droplets has a strong influence on the emulsion stability and on the modulation of the crystallisation of oil-in-water emulsion. Large fat droplets induce faster nucleation and polymorph formation on the inner surface of the fat droplet interface, triggering nucleation events more quickly compared to smaller fat droplets. Conversely, smaller droplets have slower nucleation rates, potentially resulting in delayed or absent crystal formation within

the droplets, hindering aggregation and subsequent fat crystallisation and consequently partial coalescence within the emulsion.

Second, our study demonstrates that larger fat droplets have lower shear resistance than smaller fat droplets, resulting in shorter whipping times for aerated emulsions. On the contrary, smaller fat droplets contribute to enhanced stability by hindering fat crystallisation and partial coalescence, thus extending the whipping time. Additionally, when evaluating aerated emulsions, we found that larger fat droplets efficiently entrap air cells and encapsulate them with partially coalesced fat droplets, resulting in maximum stability and overrun. Conversely, smaller fat droplets delay crystal nucleation within the droplets, resulting in a less cohesive and stable foam structure.

In summary, the rate of shear-induced partial coalescence appears to have a close correlation with fat droplet size, with larger droplets allowing faster nucleation and promoting foam stabilisation. The presence of a crystal network of fat droplets not only modulates their behaviour during shear-induced partial coalescence but also exerts a notable influence on the whipping properties of the emulsion. Whether it is the effect of the fat droplet size or the influence of fat crystal network, both factors have a significant impact on shear-induced partial coalescence or other aspects of the whipping properties such as overrun, firmness, microstructure, apparent viscosity, and rosette evaluation.

In Chapter 6 we examine how different types and concentrations of emulsifiers affect homogenised oil-in-water emulsion and their whipping properties. Our investigation focused on understanding the effects of low molecular weight (LMW) emulsifiers on fat crystallisation, shear-induced partial coalescence, polymorphic transitions, whipping properties, and foam structure in O/W emulsions. Through our research we have come to several important conclusions.

First, lipophilic emulsifiers such as MDG and LACTEM showed better adsorption and interfacial displacement compared to hydrophilic emulsifiers (Tween 80), resulting in higher protein adsorption at the O/W interface. This improved stability was attributed to the ability of lipophilic emulsifiers to efficiently pack into the interfacial film, resulting in reduced protein surface load. In addition, lipophilic emulsifiers, particularly MDG, have been found to accelerate fat nucleation and co-crystallisation, promoting faster crystal growth and polymorphic transitions. The structural similarity between the saturated carbon chain of MDG and anhydrous milk fat facilitated fat crystallisation and contributed to the formation of a stable fat network. In contrast, hydrophilic emulsifiers such as Tween 80 showed limited polymorphic

transition and interfered with fat crystallisation due to their unsaturated carbon chain and large hydrophilic head. Higher concentrations of Tween 80 did not serve as a template for fat crystals, resulting in compromised stability.

Additionally, the study highlighted the importance of particle size to emulsion stability, with smaller fat droplets exhibiting increased stability by hindering fat crystallisation and partial coalescence. Emulsions containing the lipophilic emulsifiers MDG and LACTEM demonstrated longer whipping times and improved foam stability, attributed to their ability to reduce fat droplet size and improve shear stability. Smaller particle sizes contribute to improved stability, effectively preventing fat crystallisation and partial coalescence, thus improving the overall quality of the emulsion. In summary, the importance of particle size in determining whipping ability is the primary factor of whippability, while emulsifier effects are secondary.

These results provide an interesting starting point for further work on controlling the partial coalescence of fat droplets and fat crystallisation behaviour by selecting appropriate emulsifier formulas according to their different functionality to achieve improved functionality in the aerated emulsions of dairy and non-dairy products.

This research identified several key recommendations for future investigations:

1. It is recommended to examine the seasonality of the fat phase in recombined whipping cream. This study will provide insights into the influence of different fatty acids on the mechanisms of fat crystallisation and partial coalescence.

2. An evaluation of the mechanisms of combined hydrophilic and hydrophobic emulsifiers on fat crystallisation and partial coalescence is proposed. This study will enhance our understanding of their influence on subsequent whipping properties in recombined whipping cream.

3. The application of advanced techniques such as Transmission Electron Microscopy (TEM) and X-Ray Diffraction (XRD) is recommended for a detailed study of the imaging techniques and microstructure of crystal formation as well as air bubble morphology in aerated foods.

4. An investigation into the effect of tempering cycling on dairy whipping cream is proposed. This will help understand the impact of fat crystallisation, partial coalescence, and subsequent whipping properties.

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Appendixes

Appendix 1 supplementary information for Chapter 4

APPENDIX 1

Experimental Runs

Experiment	Whipping Time (s)	Volume (mL)	Temperature (°C)	Rotational frequency speed (RPM)	Overrun (%)	Observation
1	300	10	4	1600	55.63	Over-whipped
2	300	10	4	1800	46.88	Under-whipped
3	300	10	4	2000	50.63	Under-whipped
4	300	10	8	1600	54.38	Ideal-whipped
5	300	10	8	1800	49.38	Ideal-whipped
6	300	10	8	2000	54.38	Under-whipped
7	300	10	12	1600	54.38	Under-whipped
8	300	10	12	1800	56.88	Over-whipped
9	300	10	12	2000	55.63	Over-whipped
10	300	20	4	1600	21.02	Under-whipped
11	300	20	4	1800	26.70	Ideal-whipped
12	300	20	4	2000	25.00	Over-whipped
13	300	20	8	1600	18.75	Under-whipped
14	300	20	8	1800	25.00	Under-whipped
15	300	20	8	2000	22.73	Under-whipped
16	300	20	12	1600	27.27	Over-whipped
17	300	20	12	1800	29.55	Over-whipped
18	300	20	12	2000	27.84	Over-whipped