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UNDERSTANDING THE STRUCTURE AND FUNCTION IN REDUCED FAT CHEESE USING DOUBLE EMULSION TECHNOLOGY

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DECLARATION

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and this work has not been submitted for any other degree or professional qualification except as specified.

ABSTRACT

Reduced fat cheese often suffers from inferior sensory and functional qualities compared to its full fat counterpart, which limits consumer acceptance despite the increasing demand for reduced fat products. Double emulsions, such as water-in-oil-in-water (W/O/W) are a novel technology, which use synthetic emulsifiers such as polyglycerol polyrincoleate (PGPR), can be utilised to improve the sensory and functional properties of reduced fat cheeses. However, challenges persist in identifying alternative natural lipophilic surfactants and minimise the use of synthetic emulsifiers.

In this study, the development of stable, small droplet (3 – 4 μm) double emulsions was explored for their application in reduced fat cheese production. The process involved creating primary emulsions, forming double emulsions and subsequently incorporating them into cheese formulations. Various analyses including nutritional, functional and sensory evaluations were conducted.

Initial attempts using polyphenol crystals curcumin and quercetin, as lipophilic surfactants were unsuccessful in achieving the desired small droplet sizes. However, sunflower lecithin proved effective, stabilising droplets at approximately 12 μm in sunflower oil. Transitioning from sunflower oil to milk fat with sunflower lecithin alone presented production challenges and resulted in larger droplet sizes. Nevertheless, partially replacing PGPR with sunflower lecithin in ratio of P1.5:L0.5 and P1:L1 produced stable droplets of around 3.6 μm. Further method development for skimmed milk-based double emulsions allowed for the successful encapsulation of reduced PGPR with sunflower lecithin, maintaining stable double emulsions for two hours under optimised conditions (35:65 W_1/O:W_2, 6000 rpm for 10 minutes), resulting in droplet size of 14 to 17 μm suitable for reduced fat cheese production.

These double emulsions, when incorporated into reduced fat cheese, enhanced texture and meltability. Sensory evaluations indicated positive outcomes, with similar aroma and flavour profiles across samples, though the mouth feel remained akin to that of reduced fat control cheese. This study demonstrates the potential of double emulsions with reduced synthetic emulsifiers to improve the functionality and sensory properties of reduced fat cheese.

ABBREVIATIONS

AMF – Anhydrous Milk Fat

ANOVA – Analysis of variance

CHD – Coronary heart disease

$D_{3,2}$ – Sauter mean

$D_{4,3}$ – Volume-weighted mean

DE – Double emulsion

DLP – Delactosed Permeate

FF – Full Fat

GPA – General Procrustes Analysis

HLB – Hydrophile-Lipophile Balance

HPH – High pressure homogenisation

LAB – Lactic Acid Bacteria

MFG – Milk Fat Globule

O/W – Oil-in-water

PANOVA – Procrustes analysis of variance

PC - Phosphatidycholine

PDI – Polydispersity Index

PE – Phosphatidylethanolamine

PGPR – Polyglycerol Polyricinoleate

PIT – Phase inversion temperature

RF – Reduced Fat

RPM – Revolutions per minute

SI – Serum Index

SL / L – Sunflower Lecithin / Lecithin

UK – United Kingdom

W/O – Water-in-oil

$W_1/O/W_2$ – Water-in-oil-in-water

WPI – Whey Protein Isolate

WPPC – Whey Protein Phospholipid Concentrate

CHAPTER ONE – INTRODUCTION

1.1 Background

Obesity has become a significant public health challenge in the United Kingdom (UK), with rates steadily increasing over the past few decades. Around 64 % of adults are estimated to be overweight or obese in the UK (Mintel, 2024). This rising trend is closely linked to dietary patterns, particularly the high consumption of foods rich in saturated fats such as some dairy products. While dairy can be part of a balanced diet, excessive intake of high saturated fat in products like cheese, butter and cream can contribute significantly to calorific intake and the accumulation of body fat.

The health consequences of obesity exacerbated by high fat diets, are severe, potentially leading to a range of chronic conditions, including type 2 diabetes, cardiovascular diseases and certain cancers. Diets high in saturated fat have been associated with health-related problems, such as coronary heart disease (CHD), stroke and high cholesterol levels. A meta-analysis, reviewing papers investigating the relationship between dairy fat consumption and potential health risks, found 16 papers which all referred to association of high dairy consumption and high cholesterol levels and greater risk for further health implications (Guo *et al.*, 2017). A further meta-analysis documented the detrimental effect of cheese on blood pressure, however, a direct relationship between cheese and CHD has not been proven (Zhang *et al.*, 2023a). Another study described the overall impact of cheese consumption and although cheese provides many beneficial nutrients, in excess it can cause a negative impact leading to higher risk of CHD (Beresford, 2023). As 100 g of Cheddar cheese equates to 50 % of an adults recommended daily intake of total fats, there is concern that diets high in fat can create a greater risk for health implications.

To combat this issue public health initiatives in the UK have focussed on promoting healthier eating habits, including the reduction of saturated fats in the diet and the encouragement of low fat or reduced fat alternatives. The reduction of fat content in foods results in a loss of sensory and functional attributes, therefore when formulating reduced fat and low fat products the use of fat mimetics, fat replacers or other novel technologies such as double emulsions require careful formulation to balance the fat reduction and sensory characteristics. This research thesis will explore the possibility of improving the sensory and functionality of reduced fat cheese using double emulsion technology.

1.2 Thesis Structure

The structure of the thesis is shown in Figure 1.1 and has a literature review (Chapter 2) encompassing background information on cheese and emulsion science, followed by the general materials and methods used in experiments (Chapter 3). Chapter 4 is a research chapter, which undertakes the exploration of potential natural surfactants. Objective 1 will be covered in this chapter. Following on from this, the next chapter will investigate the movement of sunflower oil to milk fat and the partial replacement of synthetic surfactants, where objective 2 will be covered (Chapter 5). These research chapters' results will enable the development of double emulsions for further application and answer objective 3 (Chapter 6). Finally, Chapter 7 will encompass the final piece of the puzzle by using the emulsions in reduced fat cheese application, summarised by objective 4. Finally, the thesis will be discussed and summarised in Chapter 8.

The detailed objectives are as follows:

Objective 1: Evaluating the efficacy of natural surfactants including polyphenol crystals and sunflower lecithin in stabilising water-in-sunflower-oil emulsions.

Objective 2: Exploring the potential of sunflower lecithin as a lipophilic surfactant in water-in-milk fat emulsions and assessing the feasibility of partially replacing synthetic surfactants with sunflower lecithin.

Objective 3: Evaluating the use of the designed primary emulsions from Chapter 5 into skimmed milk double emulsions and optimising production methods for further application.

Objective 4: Investigating the developed double emulsions from Chapter 6 in reduced fat Cheddar production and evaluating the functional and sensory characteristics double emulsion technology has on improving reduced fat cheeses (Chapter 7)

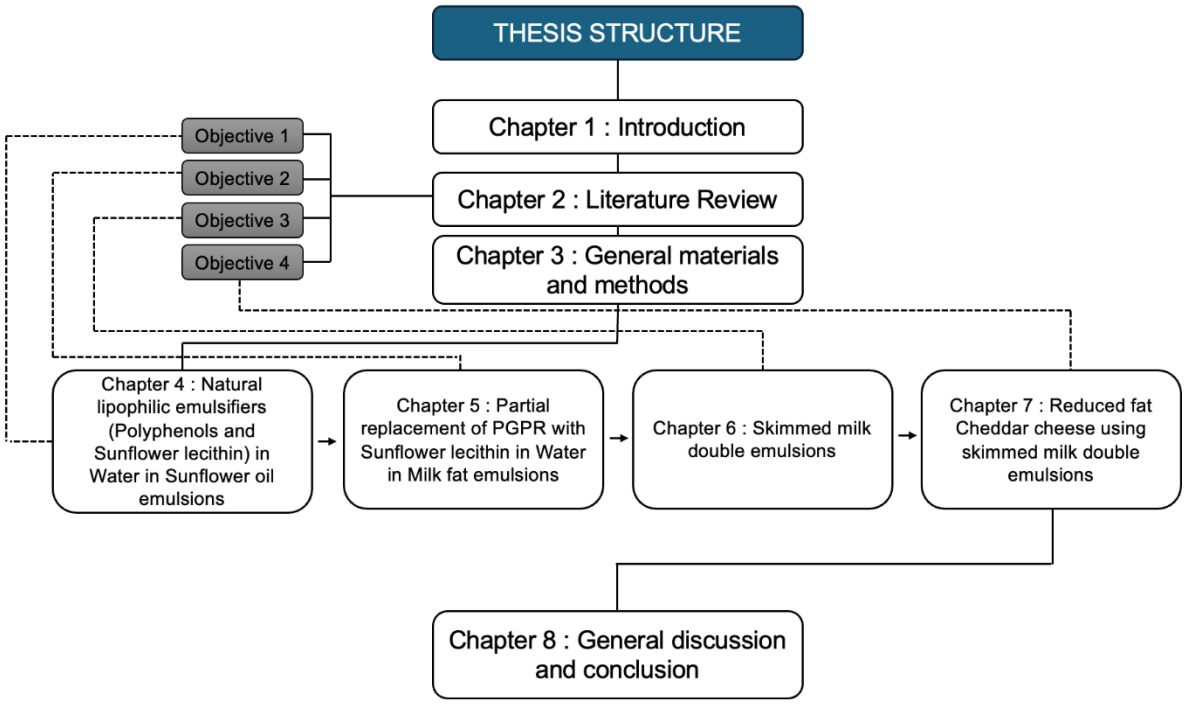


Figure 1.1 Thesis structure

For publication after thesis submission, Chapters 4 and 5 will be combined into a publication to be submitted to the Food Hydrocolloids journal to discuss the production of W/O emulsions with a reduction of synthetic lipophilic emulsifiers with natural alternatives. Currently there are limited studies addressing this issue and in milk fat systems. Chapter 7 will be submitted as a publication to the International Journal of Dairy Technology. This will provide an insight into double emulsions in Cheddar cheese and sensory evaluation of consumers.

CHAPTER TWO – LITERATURE REVIEW

2.1 The UK cheese market and health consequences

In the United Kingdom (UK) Cheddar cheese remains the nations favourite cheese, with 71 % of adults purchasing Cheddar (Mintel, 2023). According to Mintel (2019), 43 % of consumers were concerned about the health aspects of cheese consumption. In 2021, it was reported that the Cathedral City brand launched a new lower calorie '82' cheese, but it failed within the first twelve months, suggesting that it could be a sensory challenge to introduce healthier variants to the market, which could be speculated to be due to the poor sensory characteristics and consumer expectations of reduced fat cheese, where consumers weigh up the benefit of having the low calorie cheese compared to taste, is it worth it to them? Causing manufacturers to consider the impacts of low fat on the sensory aspects of cheese. Mintel (2021) reports the consumer focus on 'clean label' products and the conscious reduction in synthetic material usage. The 'clean label' definition is very subjective and can be interpreted in different ways. Generally, the term is referring to a food product which has no or a lack of artificial preservatives or ingredients. Consumers are looking for familiar ingredients and shorter ingredients lists which include the reduction of synthetic or highly processed ingredients in their foods (Cassiday, 2017; Maruyama, Streletskaya and Lim, 2021).

Cheddar cheese must meet specific characteristic parameters, and modified fat contents are restricted, such as external (non-dairy) fat or protein added to cheese (Dairy UK, 2018). In the UK, cheese can only be labelled as Cheddar if it also meets these parameters, as outlined in Table 2.1, and any cheese with fat values lower than the half fat values are not permitted to be labelled as Cheddar.

Table 2.1 – Compositional characteristics of Cheddar cheese in the United Kingdom.

Cheddar Type	Average Fat (%)	Minimum Fat (%)	Maximum Fat (%)	Moisture (%)
Standard	34.9	29.0	N/A	39.0
"Lighter" reduced fat (30 %)	22.1	17.5	24.4	44.5
Half fat (50 %)	15.8	13.5	17.5	50.0

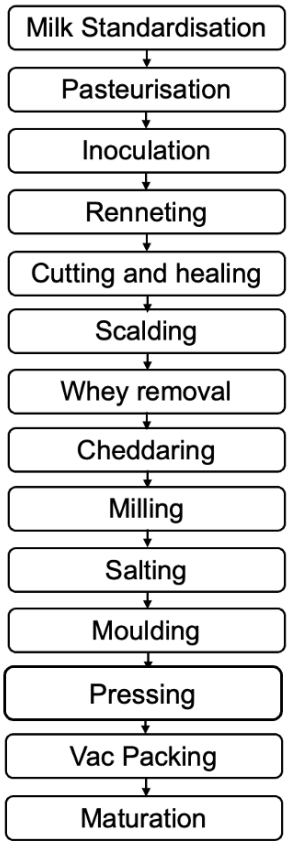
(Source adapted from Dairy UK (2018))

The term “cheese analogue” is used when sources other than milk, such as fat or protein, are added to cheese. These additions are often engineered for specific nutritional benefits, such as replacing milk fat with vegetable fat to create low fat cheese or catering to consumer trends like plant-based alternatives for vegan cheeses (Bachmann, 2001). According to the FAO Codex Alimentarius a low fat product must contain 3 g of fat per 100 g or less, and reduced fat product must have 30 % less fat than the standard.

2.2 Cheese

2.2.1 Cheddar Cheese Manufacture

The fundamental science of cheese making is consistent across different cheese types; however, variation in method, the addition of specific starter cultures, or the inclusion of moulds result in the diversity of cheese produced. Cheddar cheese is a traditional British Territorial cheese with a defined method of production. Figure 2.1 illustrates the manufacturing process of Cheddar cheese, adapted from Murtaza (2016) and Clark and Agarwal (2007).



(Source adapted from; (Murtaza, 2016) and (Clark and Agarwal, 2007)

Figure 2.1 – Flow diagram of the Cheddar Cheese Process

In large-scale cheese production, milk is typically standardised to control the casein-to-fat ratio. Pasteurisation, which precedes cheese manufacture, is commonly applied in industrial settings to eliminate pathogenic microorganisms. However, it is not universally required – many artisanal cheeses are made using raw milk. After pasteurisation, milk is cooled to approximately 30^{o}C for inoculation with starter cultures.

The starter cultures used in Cheddar production are primarily homofermentative lactic acid bacteria, meaning they metabolise lactose almost exclusively into lactic acid, with minimal production of gas or other by-products (Fox *et al.*, 2017). These cultures are mesophilic, thriving in moderate temperatures (30-40^{o}C), and typically include *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. The inoculated milk is left for approximately 50 minutes to allow for acidification, which is monitored throughout the process.

Rennet, containing the enzyme chymosin, is then added to initiate coagulation. Chymosin, traditionally sourced from the abomasum of milk-fed calves but now often produced microbially, cleaves the kappa-casein “hairy” layer from the casein micelle. This exposes the hydrophobic sites, leading to flocculation via calcium phosphate bridging, forming three-dimensional curd matrix that traps moisture, fat and micronutrients (Everett and Auty, 2017; Fox *et al.*, 2017). Coagulation typically takes about 45 minutes.

Once set, the curd is cut using cheese knives equipped with vertical and horizontal wires to increase surface area and promote whey expulsion. A resting period follows to allow a skin to form on the curd, making it more robust during subsequent handling. The scalding stage heats the curd to 38-40^{o}C, encouraging further syneresis (the contraction of protein and expulsion of whey), curd shrinkage, and slowing of starter culture activity.

After whey is drained, the cheddaring process begins, involving repeated flipping and stacking of curd blocks. This step enhances moisture loss, ensures uniform acidity, and contributes to Cheddar’s distinctive texture. As the pH drops progressively – typically from 5.4 to around 5.0 – the curd transitions from rubbery to a firm, plastic consistency characteristic of matured Cheddar (Clark and Agarwal, 2007).

The curds are then milled, a step that increases porosity and facilitates further whey removal. This also prepares the curds for salting, where salt is distributed evenly to inhibit microbial growth, influence texture and enhance flavour. Curds are then transferred to moulds and pressed at approximately 15 psi for 16 – 18 hours. The pressed cheese is vacuum-packed and matured for periods ranging from 3 to 18 months, depending on the desired final product characteristics.

2.2.2 Structure and function of cheese

Cheese is a complex food made from milk with the addition of starter cultures and a coagulant. It has a macrostructure consisting of curd particles, joins and structural elements visible to the naked eye, and a microstructure, visible through microscopy, consisting of compositional elements. Cheese consists of a protein (casein) matrix, joined by calcium phosphate bridges, which strengthen the cross linkage between proteins (Metzger, Barbano and Kindstedt, 2001; Zisu and Shah, 2007; Everett and Auty, 2017; Fox *et al.*, 2017). The casein matrix entraps fat during coagulation, which acts as an "inert filler" and impacts the rheology, functional, and textural properties (Everett and Auty, 2017; Ramel and Marangoni, 2017; Mattice and Marangoni, 2018; Sharma Khanal *et al.*, 2019).

There is some debate as to whether fat is truly an inert filler or interacts with the casein matrix. Some studies suggest that when the fat globules are homogenised and casein acts as the hydrophilic surfactant to stabilise the droplets, this impacts the interaction between casein molecules (Everett and Auty, 2017). Fat globules can range in size from 2 μm to 10 μm, and even up to 50 μm, with larger droplets often being aggregated globules (Everett and Auty, 2017). The temperature of the cheese and ultimately the milk fat, affects the structure, for example, solid fat can limit deformation, which refers to the cheese's ability to change shape under stress – such as stretching, cutting or flowing due to its viscoelastic nature (Everett and Auty, 2017; Farkye and Guinee, 2017). This is also linked to cheese melting, where fat globules coalesce and lubricate the layers of casein, enabling them to slide over one another (Guinee, 2016). As temperature increases, the casein matrix softens and loosens while melting fat acts as plasticiser, enhancing flow and stretchability. This is important in consumer application, such as on a pizza or cheese on toast. Fat is fundamental to taste and mouthfeel; as fat melts, it lubricates the mouth, contributing to perceived creaminess and smooth attributes (Tekin, Sahin and Sumnu, 2017; Mattice and Marangoni, 2018; Metha, 2018; Sharma Khanal *et al.*, 2019; Giha, Ordoñez and Villamil, 2021).

Milk fat has a wide variety of fatty acids (Espert *et al.*, 2020). During maturation, fat is broken down by lipolysis – a process in which lipase enzymes hydrolyse triglycerides, releasing free fatty acids. Lipase enzymes originate from native milk enzymes, starter cultures or are added (Collins, McSweeney and Wilkinson, 2003). These free fatty acids can be further converted into volatile compounds such as

aldehydes, ketones, and alcohols which contribute to the characteristic aroma and flavour profiles of ripened cheeses (Fenelon and Guinee, 2000; Mistry, 2001).

In addition to fat and protein, moisture content plays a central role in shaping the texture, flavour development, and microbial stability of cheese. Water in cheese exists in different forms: bound water, which is tightly associated with casein and minerals, and entrapped water, which is physically held within the curd matrix (McMahon and Brym, 2015). During early cheesemaking, water activity supports the metabolic functions of starter cultures and promotes enzymatic action during coagulation. However, as cheese matures, moisture content typically decreases, especially in hard cheeses like Cheddar, due to evaporation and syneresis, resulting in a firmer texture over time (Lamichhane, Kelly and Sheehan, 2018). While higher moisture can accelerate certain ripening reactions, excessive moisture may lead to textural defects, microbial spoilage, reduced shelf life, and unbalanced flavour development, all of which compromise cheese quality.

The pH of cheese also affects its structure and function. Specifically, a drop in pH promotes calcium solubilisation, which impacts the interaction between casein proteins, weakening their connection and resulting in a soft cheese (Pastorino, Hansen and McMahon, 2003). Additionally, salt influences the structure of cheese by affecting syneresis and helping with preservation through the control of microbial growth (Guinee and Fox, 2017).

Starter cultures, typically comprising lactic acid-producing bacteria (LAB), primarily function to break down lactose into lactic acid. This acidification lowers the pH, creating an environment that inhibits the growth of spoilage and pathogenic bacteria, thus contributing to food preservation. In addition to acid production, LAB also contribute to flavour development during maturation through enzymatic activity, particularly the breakdown of proteins into peptides and amino acids, which serve as flavour precursors (Chandan and Kapoor, 2011).

2.3 Low fat cheese – What's the problem?

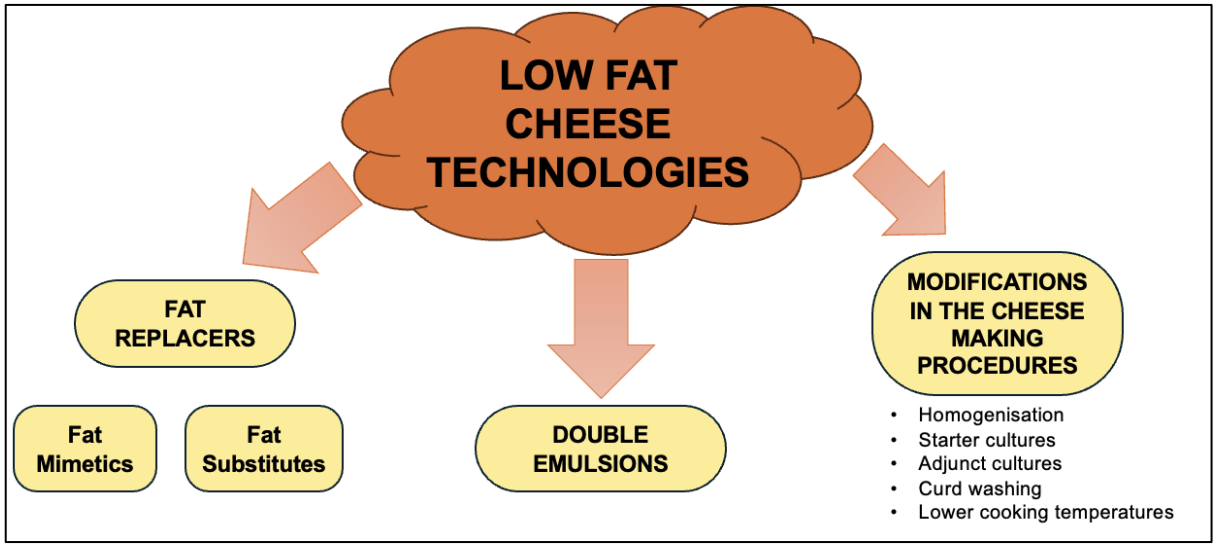
The cheese industry suffers loss in quality with low fat cheese manufacture, due to the technological changes within cheese that are crucial for sensory and functional attributes (Bourouis, Pang and Liu, 2023). As previously discussed in section 2.2, fat significantly contributes to mouthfeel. Generally, low fat cheese results in a higher protein-to-fat ratio, leading to a firmer texture often described as hard and rubbery due to the absence of fat globules dispersed within the casein matrix (Drake, Miracle and McMahon, 2010; Esen and Güzeler, 2023).

Low fat cheeses are also associated with poor sensory and flavour attributes. A study by Guan *et al.* (2021) involved adding lipase nanotubes to low fat cheese to counteract the low lipid methods that result in poor flavour. The addition of these lipid nanotubes was shown to increase the amount of free fatty acids during maturation. The authors recorded the free fatty acid content after 7 days of ripening and found that the sample with the lipase nanotubes had double the amount compared to the low fat control, even though the samples started with the same milk fat content. However, sensory evaluation was not conducted, so the claim that these nanotubes would improve the sensory of low fat cheese cannot be fully confirmed.

Drake, Miracle and McMahon (2010) investigated the flavour and flavour chemistry of fat reduction in Cheddar cheese. After two weeks maturation, no significant differences were found between the reduced fat and control cheeses. However, after three months, a bitter taste was evident within the reduced fat and low fat cheeses, which could be attributed to the higher moisture content and proteolysis, causing bitter flavour compounds from certain amino acid breakdowns (Drake, Miracle and McMahon, 2010).

2.4 Methods of improving low fat cheese

Numerous technologies have been utilised within the cheese industry and in research to improve the functional, textural and sensory properties of low fat cheese. Figure 2.2 outlines the main technologies and processes used to improve the characteristics of low fat cheese. Some are novel and not widely used in industry but are employed in research. Examples include cheese manufacturing methods, discussed further in section 2.4.1, fat replacers such as fat mimetics and fat substitutes which are discussed in more detail in 2.4.2 and novel technologies which are mentioned in section 2.4.3 and explained in further detail from section 2.5 onwards. Double emulsions in cheese specifically are discussed in section 2.7.



(Source: Authors Own)

Figure 2.2 – Technologies for improving low fat cheese.

When choosing a technology or method to improve a formulated low fat product, in this case cheese, it is important for businesses, researchers and cheesemakers to consider the following before embarking on production:

- Food safety aspects: the safety and approval of additional ingredients added, whether they are generally accepted by the Food Safety Standards within the UK.
- Functional properties
- Organoleptic/sensory properties
- Achieving “less fat” and lower calories
- “Green processing”

(Lim, Inglett and Lee, 2010)

Green processing is a term promoting the development of environmentally friendly products (Lim, Inglett and Lee, 2010). This aligns with the consumer focus highlighted in the Mintel (2023) report which notes a trend towards reducing ultra-processed foods and an increased prevalence of natural products in the market. When adding ingredients to low fat products such as fat mimetics, substitutes or surfactants within double emulsions, it is important to ensure they align with the ideals of being natural and environmentally friendly in their sourcing and production methods.

2.4.1 Cheese manufacturing methods

The modification of manufacturing methods has been found to enhance the textural, sensory and functional properties of low fat cheese. These modifications include homogenising the cheese milk, washing of cheese curds, adjusting cooking temperatures and times during cheesemaking, pre-acidification and using specific starter and adjunct cultures (Farkye and Guinee, 2017).

Homogenisation balances droplet break up and recoalescence to prevent two liquids from reverting to their original state (Leong *et al.*, 2009). In milk, homogenisation creates smaller fat droplets, which need to be stabilised to prevent coalescence. Stabilisation is achieved by the adsorption of surface-active ingredients in milk, such as casein, to the surface of the newly formed droplets (Everett and Auty, 2017). This process in cheese milk creates a denser casein matrix, as there are no larger fat droplets to disrupt the protein matrix. Smaller droplets have also been found to increase the amount of light scattered, creating a whiter cheese (Pastorino *et al.*, 2002; Van Hekken *et al.*, 2007; Everett and Auty, 2017).

Homogenisation has been used to improve low fat cheese by reducing the size of fat globules, leading to more fat being entrapped within the casein matrix. Thereby reducing loss of whey and increasing moisture, this increase in moisture aids the hydration of casein. As some of the casein is used to adsorb to the newly created droplet during homogenisation, this leaves less casein available to create strong cross linkages, thus reducing strength in matrix making the cheese softer. A study on Turkish white cheese found that homogenisation significantly affected hardness and flavour, with higher sensory scores for cheese made from homogenised milk. Although specific sensory descriptors were not provided, the authors noted that no off flavours, such as rancidity occurred, which can be a risk if lipase is excreted when fat is homogenised. The study also found that the increased dispersion of fat droplets within the matrix provided a creamier texture (Karaman and Akalın, 2013).

In cheese, pH influences softness and meltability (Muthukumarappan and Swamy, 2023). Pre-acidification is the addition of food-grade acid in cheese milk prior to the starter culture (Swaminathan *et al.*, 2025). This process lowers the pH in cheese milk, encouraging the removal of calcium from casein and converting it into an insoluble colloidal form (Smith, Metzger and Drake, 2016; Farkye and Guinee, 2017). Reducing soluble calcium prevents numerous strong protein cross-linkages, thereby producing a softer cheese (Zisu and Shah, 2007; Smith, Metzger and Drake, 2016). A study by Smith, Metzger and Drake (2016) found that pre-acidification of low fat Mozzarella decreased calcium content but adversely affected yield. The authors suggest that this method improves texture and functionality, but

manufacturers must balance lower yield against these benefits. However, the study lacked textural analysis, so it is difficult to conclude the impact of pre-acidification alone on cheese milk. Swaminathan *et al.* (2025) also found that pre-acidification of high casein milk in mozzarella production resulted in cheese that had a lower hardness. Conversely, Zisu and Shah (2007) found that pre-acidification alone did not influence moisture or texture.

Starter cultures, primarily LAB, are added to cheese for various reasons (Parente, Cogan and Powell, 2017). LAB break down lactose into lactic acid, lowering pH and creating an undesirable environment for spoilage bacteria. LAB also contributes to flavour development during ripening by producing volatile flavour compounds and carbon dioxide, which affects the cheese structure (Parente, Cogan and Powell, 2017; Ardö *et al.*, 2017). Secondary and adjunct cultures are added to improve organoleptic properties or serve specific functions such as white mould for brie or blue moulds for blue cheeses (Irlinger, Helinck and Jany, 2017; Parente, Cogan and Powell, 2017). Several studies have investigated the use of certain starter cultures to improve low fat cheeses by increasing moisture to aid softness and flavour. Some starter cultures produce exopolysaccharides (EPS) which enhance viscosity and water-binding properties (Broadbent *et al.*, 2001). EPS increases cheese moisture by binding water and retarding whey expulsion (Zisu and Shah, 2007). Zisu and Shah (2007) used both pre-acidification and an EPS-producing starter culture to improve the texture of low fat Mozzarella and found they significantly reduced hardness. Other studies also support that EPS starters increase moisture and positively affect sensory properties (Lynch *et al.*, 2014). Wang *et al.* (2019) found that Cheddar cheese with EPS-producing *Lactobacillus plantarum* JLK0142 had lower hardness like full fat cheese, with no significant difference in overall sensory acceptability. Adjunct cultures, such as those used by Lynch *et al.* (2014), can also improve sensory aspects. Ahmed *et al.* (2021) found that probiotic adjunct cultures enhanced the sensory attributes of low fat Feta cheese and maintained probiotic viability at the end of 14 days of storage.

Washing curds can influence moisture and calcium content by removing some soluble calcium and lactic acid, reducing strong protein cross-linkages and improving texture (Farkye and Guinee, 2017). However, a lack of soluble calcium can hinder meltability and functionality (Everett and Auty, 2017). Lowering cooking temperatures during cheesemaking can reduce syneresis and whey expulsion, leading to higher moisture content. Konuklar *et al.* (2004) investigated both fat mimetics (Nutrim, a β-glucan hydrocolloid suspension) and lower cooking temperatures to improve low fat Cheddar cheese and found that the fat mimetic increased moisture and disrupted the protein structure, improving the structure of low fat cheese.

2.4.2 Fat replacers

The term “fat replacers” is a generic term that can be divided into multiple sub-groups: *fat substitutes, fat mimetics* and *low calorie/ modified* fats (Roller and Jones, 1996). Generally, these are used to mimic some of the physicochemical, functional and sensory properties of fat (Zhao *et al.*, 2023). However, when choosing a fat replacer it is important to understand the physical properties of fat, as discussed previously, as well as the benefits and limitations of the non-fat ingredient being added and how it can impact the behaviour of the product, particularly in cheese, butter and fat crystallisation (Espert *et al.*, 2020).

A fat substitute is defined as the replacement of fat with an alternative on a weight-by-weight basis (Roller and Jones, 1996; O'Sullivan, 2016), such as replacing milk fat with vegetable oil. Lobato-Calleros *et al.* (2002) substituted milk fat with canola oil and emulsifiers in Manchego cheese and found the combination of emulsifiers and vegetable oil resulted in a reduction in hardness due to the emulsifier’s interaction with lactoglobulin. Shabani *et al.* (2016) substituted milk fat with sunflower oil in ultra-filtrated, white-brined cheese and found that an increase in sunflower oil content resulted in lower firmness in the low fat cheese. Myhan *et al.* (2020) investigated the substitution of milk fat with palm oil in Edam cheese and found no significant impact on the rheological properties at the initial stage of cheese made with palm oil. However, sensory properties were not assessed, and the difference in fatty acid composition between palm oil and milk fat could affect sensory attributes. Additionally, considerations need to be made regarding the type of oil used, as consumer demand for ethically and environmentally friendly products could impact the use of palm oil.

In comparison to fat substitutes, fat mimetics are typically protein- or carbohydrate-based (Sikorski and Kołakowska, 2010). Fat mimetics require a higher water content as they generally have a higher water-binding activity (Roller and Jones, 1996; Farkye and Guinee, 2017). Their role is to act as an ‘inert filler’, reducing the continuity of the casein matrix within cheese (Anvari and Joyner, 2019). Fat mimetics can also hinder syneresis, thereby increasing the moisture content with curd (Akin and Kirmaci, 2015; Stankey *et al.*, 2017). Stankey *et al.* (2017) found that syneresis was hindered because the micro-particulate whey protein blocked the pores, preventing water movement and thus hindering whey expulsion. Despite the benefits of increased moisture and improved textural characteristics, it is important to note that the increase in water can reduce the shelf life of the product, posing a food safety risk due to alterations in pH and the creation of favourable conditions for growth of spoilage bacteria (Konuklar *et al.*, 2004).

Esen and Güzeler (2023) used whey protein as a fat replacer to improve the microstructure of Boru-type Künefe cheese and found that cheese made with 1.5 % fat milk and 0.5 % whey protein was the closest in the textural results to full fat. Bourouis, Pang and Liu (2023) describe how protein can provide similar textural and sensory properties to fat, making it a viable option as a fat mimetic. Protein and fat particles act in a similar way to fat by fitting in the gap between the tongue and the palate, providing perceived creaminess and smoothness to the product but with a lower calorific value (Nourmohammadi, Austin and Chen, 2023).

Table 2.2 identifies and highlights the key findings of various fat replacers, both carbohydrate- and protein- based, that have been researched and used in low fat cheeses to improve functional, rheological, textural and sensorial properties. The majority of papers reviewed report positive effects of fat replacers on the improvement of low fat cheeses, primarily due to their moisture retention and syneresis hinderance. However, a small number of papers note the production of undesirable bitter and off-flavours, which can be attributed to the high moisture content and proteolytic breakdown of casein into bitter peptides. In full fat cheeses these bitter peptides can be diluted by the fat and are less likely to be detected by consumers. In contrast, in low fat cheeses the absence of fat makes these bitter peptides more noticeable.

642 **Table 2.2 – Fat replacers used in low fat cheese**

	Fat Mimetic	Cheese Type	Key Findings	Citation
Carbohydrate based	Maize Starch	Cheese (in *vitro*)	• Modified starch was found to be the better fat replacer as it disrupted the protein matrix and had good water binding properties	(Diamantino *et al.*, 2019)
	Konjac Glucomannan (KGM)	Mozzarella	• Addition of KGM resulted in lower firmness, similar to that of full fat cheese. • Meltability, overall functional and textural properties of KGM Mozzarella was comparable to full fat and proved to be a good replacer for fat and improving the qualities of low fat Mozzarella	(Dai *et al.*, 2019)
	β-glucan (Nutrim)	Cheddar	• Increased moisture, higher than the control. • Cheeses with higher concentration of Nutrim resulted in a smoother microstructure, with smaller voids being only 7 μm, whereas the low fat comparison had voids as large as 43 μm.	(Konuklar *et al.*, 2004)
	β-glucan and Inulin	Labneh cheese	• Addition of fat replacers affected the sensory, and caused a difference in texture, with low fat and reduced fat cheeses being harder than control. More research required to understand the full impact and amendment of quantities of fat replacers to improve the functional, sensorial and textural properties.	(Aydinol and Ozcan, 2018)
	Avicel plus ® (Cellulose), Nutrim (β-glucan), and	Kashar Cheese	• Cheese with fat replacers had higher moisture due to water binding activity (Avicel plus® the highest with 59.74 %). • Fat replacers improved the textural properties, Avicel plus® and Simplesse® had structures closest to their full fat counterparts and lower hardness that low-fat cheese (without fat replacers)	(Sahan *et al.*, 2008)

Protein Based	Simplesse® (Whey Protein),		• β-glucan cheeses had the worst scores for sensory due to starchy taste, but had better meltability scores • Appearance showed a significant difference, the fat replacers produced a 'green tint" compared to the full fat.	
	Microparticulate Whey Protein (MWP)	Cheddar	• Increase in moisture with the concentration of MWP, due to water binding properties. • No significant differences in sensory at the beginning of storage, by the end slight bitter flavours were observed with an increase in MWP concentration. • 0.5 % MWP chosen as preferred concentration, as this improved yield, created a softer cheese although less meltable but no distinctive sensory defects	(Stankey *et al.*, 2017)
	Whey Protein (Simplesse®, Dairy Lo and ProLo)	Edam	• Fat replacer cheese resulted in higher moisture and lower firmness than the control. • Positive flavour scores in the sensory evaluation • At the end of storage, a bitter taste with Simplesse® and ProLo was detected	(El-Aide, 2019)
	Whey Protein Isolate	Boru-type Küefe Cheese	• Low fat cheese had the highest results in firmness. • Overall sensory was rarely affected, sensory was similar to control. • 0.5 % WPI and 1.5 % Fat milk resulted in the cheese closest to full fat.	(Esen and Güzeler, 2023)
	Simplesse® (Whey Protein) Maltrin®	Beyaz pickled Cheese	• Higher moisture to protein ratio due to water binding activities of fat replacers. • Low fat cheeses were the hardest and the fat replacer cheeses were softer in comparison to low fat but still firmer than the full fat. • Sensory was deemed acceptable to participants and fat replacer cheeses had similar scores to that of the full fat.	(Akin and Kirmaci, 2015)

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2.4.3 Novel fat mimetic technologies

For years, fat mimetics have been classified as carbohydrates and proteins used to replicate the structure and function of fat in food products, achieving notable success. Recently, novel technologies such as colloidal systems, including emulsions and double emulsions, have emerged. These systems enhance low fat products by encapsulating water droplets, thereby improving texture and functionality to mimic the properties of full fat versions. These are discussed further in the next sections and their application in food products and potential for fortification of water-soluble vitamins.

2.5 Emulsions

Emulsions are defined as a dispersion of immiscible droplets in a continuous phase stabilised by emulsifiers (Rousseau, 2000). The liquid being dispersed is often spherical in shape (McClements, 2016) .The substance that makes up the droplets is known as the dispersed, discontinuous, or internal phase, compared to that of the substance that is surrounding the droplets, which is known as the continuous or external phase (McClements, 2016). Some foods, like milk, are emulsions that are found naturally. There are two main types of emulsions: oil-in-water (O/W) emulsions such as milk, where the milk fat globules are suspended in an aqueous phase; and water-in-oil (W/O) emulsions, such as margarine and butter. Emulsion systems are widely used in the cosmetic and pharmaceutical industries, and used extensively in the food industry, where they are used for several functions, such as flavour, delivery of micronutrients and lowering fat contents in products such as salad dressings (McClements, 2016).

An explanation of the different types of emulsions including advantages and limitations are stated in Table 2.3.

667 **Table 2.3 – Comparison of emulsion types used in the food industry**

	Description	**Advantages/ Limitations**	**Average Droplet size**	**Citations**
Macroemulsions (Conventional Emulsions)	-Water in oil (W/O) or oil in water (O/W). -Utilised for lipid delivery and encapsulation of fatty acids.	Advantages – low cost and easy to produce. Limitations – prone to physical instability when exposed to different environmental conditions	0.1 μm - 100 μm	(McClements, 2007; Tadros, 2016; McClements, 2016)
Multilayer Emulsions	-Numerous layers of different emulsifiers -Undergo several homogenisation steps	Advantages- these emulsions have improved physical stability and are useful delivery systems. Limitations – costly and time consuming.	-	(Guzey and McClements, 2006; McClements, 2007; McClements, Decker and Weiss, 2007)
Multiple Emulsions (Double Emulsions)	- Oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) -Undergo a two-step process.	Advantages - Allow a controlled release of bioactives and offers some protection from degradation during storage and consumption.	Vary in size	(McClements, 2016; McClements, 2007; McClements, Decker and Weiss, 2007)

	- Used as delivery systems or to lower the fat content of products (without altering textural and sensory properties)	Limitations – these are novel mechanisms and not widely used in the food manufacture.		
Nano-emulsions	-Have very small droplet diameters -Often produced using high-energy techniques.	Advantages – small droplet size benefits stability and efficient delivery of bioactives. Limitations - require specialist equipment to produce.	20 – 200 nm	(Tadros *et al.*, 2004; Anton and Vandamme, 2011; Tadros, 2016)
Filled Hydrogel Particles	-Referred to as confining oil in a solid like aqueous network that prevents droplet movement.	Advantages – They are efficient in drug delivery in the pharmaceutical industry. Limitations - uses in the food industry requires better production methods as these are novel.	-	(Farjami and Madadlou, 2019)

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2.5.1 Emulsion formation and production

The production method varies based on the desired emulsion and its intended use. As emulsions are unstable (Rousseau, 2000), the use of emulsifiers and/or thickeners are required to help stabilise the droplet in the continuous phase. To incorporate these ingredients the emulsifiers or thickeners are either mixed, dissolved or dispersed into their relative phases. Often the mixing of emulsifiers in the external or internal phases require high-speed stirring to prevent them from being unstable (McClements, 2016) and some may require heat. For example, thickeners such as polysaccharides require a warm temperature to allow gelatinisation to form the required viscosity. These steps occur prior to the production of an emulsion.

According to McClements (2016) the process of creating an emulsion is known as *homogenisation.* Oil and water are two immiscible liquids, which when placed together position themselves to limit the surface area between the two interfaces, leaving two layers. The homogenisation process balances droplet break up and droplet recoalescence to prevent the liquids reverting to their original state (Leong *et al.*, 2009). A mechanical energy or shear force is required to break the dispersed phase into droplets, which exceeds the Laplace pressure (refer to section 2.5.3.4), allowing droplets to be formed (Walstra, 1993). A surfactant, by adsorbing to the surface, plays a role in lowering the interfacial tension of the droplet, causing a reduction in the resistance to droplet deformation (Walstra, 1993; Walstra and Smulders, 1998; Leong *et al.*, 2009; McClements, 2016). The surfactant also prevents recoalescence by generating repulsive forces in the interfacial layer, thus leading to stabilisation (Walstra, 1993; Binks, 1998; Leong *et al.*, 2009).

Homogenisation can be achieved using a range of machines and methods, for example high shear blenders, ultrasonic homogenisers and high pressure homogenisers (HPH). The homogenisers can vary in speed, size and efficiency and processing parameters depend on the final properties that are desired. The aim of homogenisation is to distribute the internal phase evenly and in similar sized droplets within the external phase. There can be a two-step homogenisation process, the first known as primary homogenisation, which creates a coarse emulsion with droplets of different sizes that tend to be more unstable compared to those which have undergone secondary homogenisation. Secondary homogenisation is where the primary homogenised solution is homogenised further to produce much smaller droplets, which benefit stability. However, through more recent developments and improvements to equipment, some homogenisers can create droplets at the required diameter without secondary homogenisation (McClements, 2016).

Homogenisation can have a strong influence on emulsion characteristics (Binks, 1998; Camino and Pilosof, 2011). Qamar, Bhandari and Prakash (2019) found that homogenisation in dairy-based beverages affected the functional behaviour of the emulsion. The creation of smaller droplets improves kinetic stability in an emulsion (Leong *et al.*, 2018). Different methods of emulsification are critiqued in Table 2.4.

Table 2.4 – Comparison of homogenisation methods.

	Method of homogenisation	**Advantages**	**Disadvantages**	**Citation**
High shear – rotor-stator	Referred to as 'rotor-stator' having a rotating and a static disk in which the emulsion is forced between to create the droplets. Creates a coarse emulsion and can be used to premix emulsions before using the other methods.	Conventional emulsion technique, easily used in industry and can be used in the production line.	Both droplet size and distribution are not easily controlled.	(Spyropoulosa, Hancocks and Norton, 2011; McClements, 2016)
High Pressure homogenisers	Requires pre-mixed emulsion first. The emulsion is pulled through a narrow valve and into a chamber. Emulsion experiences a combination of disruptive forces causing the larger droplets to be broken into smaller ones.	Fine emulsions can be created rapidly	Suited to small scale production only.	(Dickinson, 1994; Schultz *et al.*, 2004; McClements, 2016)

	Method of homogenisation	**Advantages**	**Disadvantages**	**Citation**
Ultrasonic homogenisation	Requires pre-mixed emulsion first. Droplets are created due to the mechanism of oscillation of the liquid due to the action of sound, causing severe stress, breaking the droplets into smaller ones.	Efficient method to produce a fine dispersion of droplets.	Suited to small scale production only.	(Behrend, Ax and Schubert, 2000; Schultz *et al.*, 2004; McClements, 2016)
Membrane emulsification	Requires a pre-mixed emulsion. The emulsion is forced through a membrane pore, creating a droplet the size of the pore.	Aims to create a monodisperse emulsion	A slow method of emulsification	(Dickinson, 1994; Yuan, Williams and Biggs, 2009)

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High shear homogenisers are rotor-stator machines, whereby one element rotates while the other remains stationary (McClements, 2016). According to Tadros (2016) operating rotor-stator mixers can significantly reduce the processing time. One brand of high-shear mixer is the Silverson, which is a batch radial discharge mixer, whereby the emulsion is created in batches (Tadros, 2016). The machine works via the four blade rotor which pumps the fluid through a stationary stator that is perforated with small holes (Tadros, 2013). An example of this can be seen in Figure 2.3 (b). The rotor generates a turbulent flow, causing the droplets to be broken up by shear and inertial stress in a turbulent regime (Urban *et al.*, 2006; Ashar *et al.*, 2018). The flow is displayed in Figure 2.3 (a).

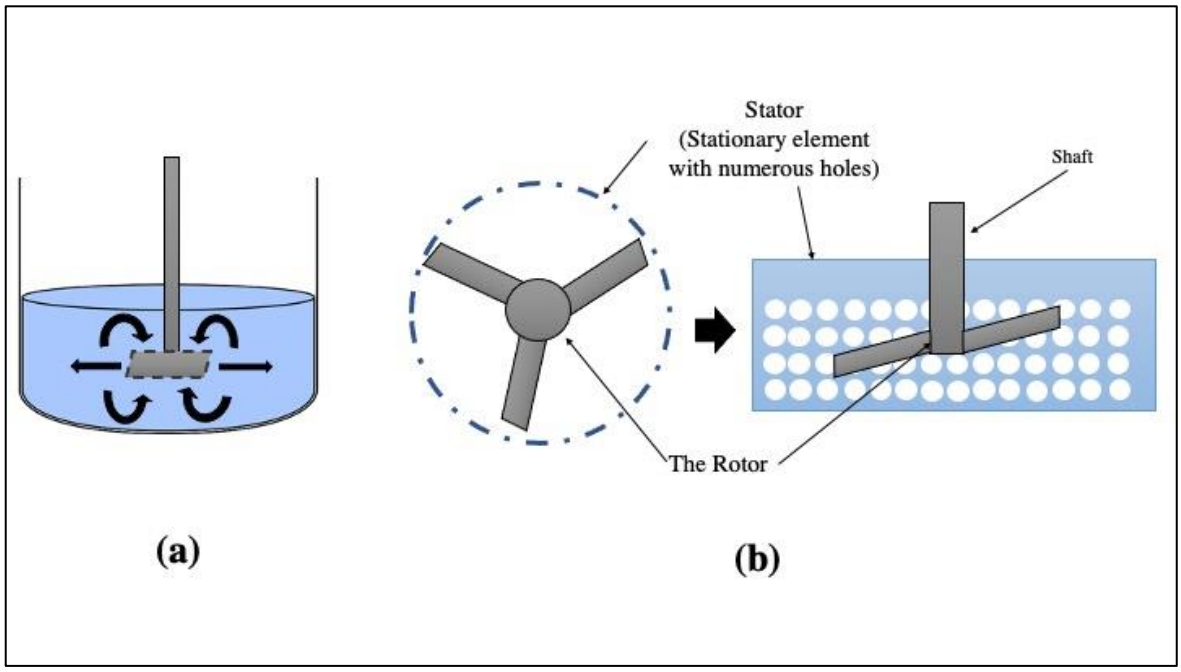
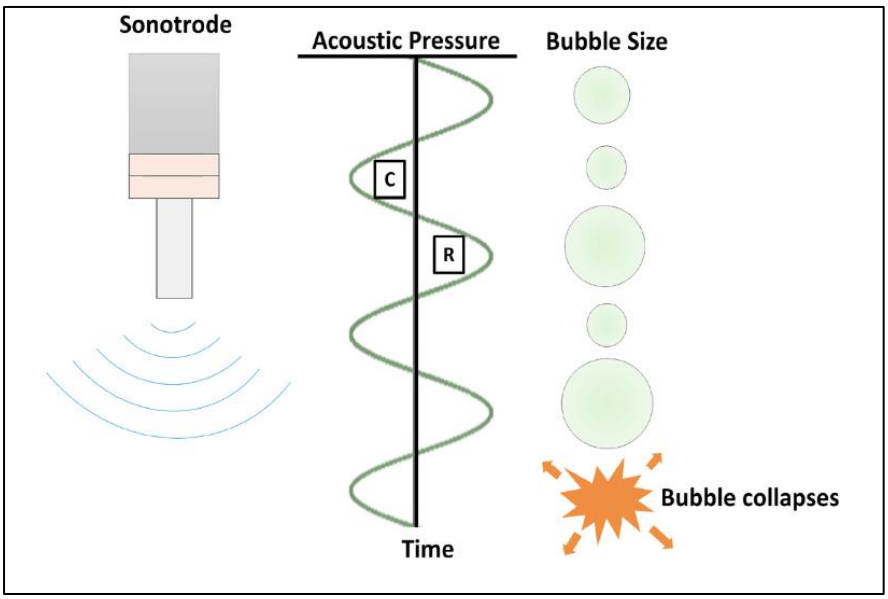


Figure 2.3 – Diagram representing a Rotor-Stator mixer – (a) showing the flow of fluid through the mixer during emulsification, creating a turbulent flow. (b) showing a diagram of the rotor and the stator with holes as seen in a Silverson mixer.

Ultrasonic homogenisation is where acoustic waves in the range of 20 to 30 kHz are capable of breaking droplets (Truong *et al.*, 2016; Berk, 2018). The droplet break up is by cavitation of the fluids, where bubble sizes change quickly, collapse, and then implode (Urban *et al.*, 2006; Bermúdez-Aguirre, Mawson and Barbosa-Cánovas, 2008). This cavitation is caused by the acoustic waves (Truong *et al.*, 2016; Berk, 2018; Pollet and Ashokkumar, 2019). A visual representation of ultrasonic emulsification can be seen in Figure 2.4, highlighting how the droplets (bubbles) change size, implode and form smaller droplets. O'Sullivan *et al.* (2015) found that ultrasonic emulsification could form submicron emulsions with efficient formation achieved at higher amplitudes and lower processing volumes

Bermúdez-Aguirre, Mawson and Barbosa-Cánovas (2008) found that ultrasonic homogenisation of the milk fat globule to 0.5 to 0.7 μm was achieved at 400 to 450 W.



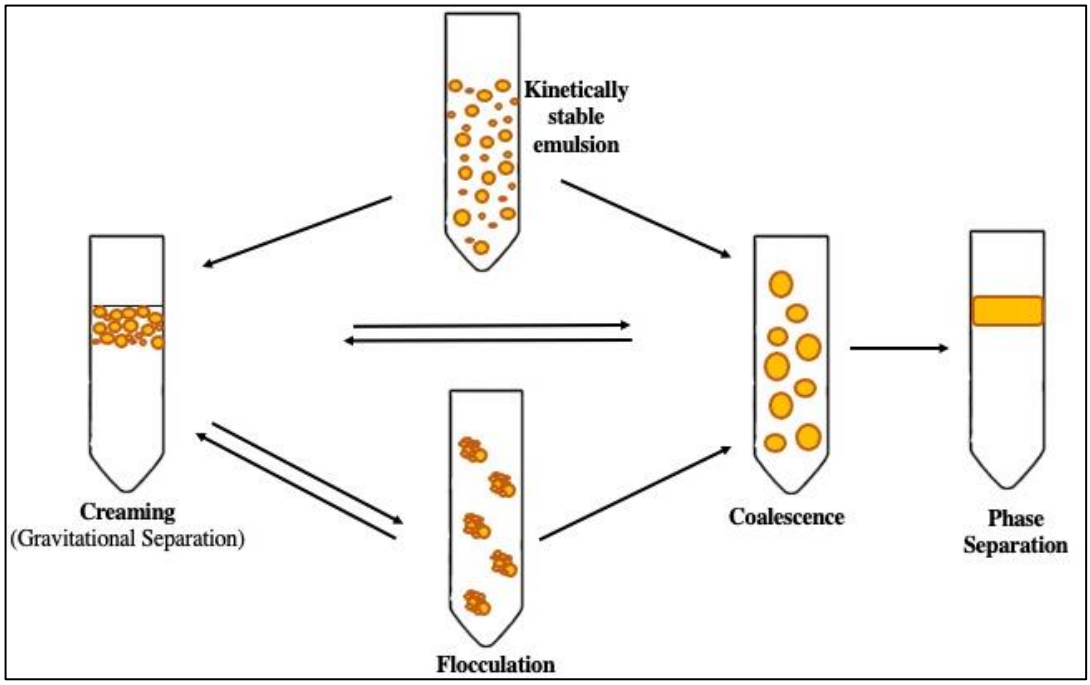
(Source Taha *et al.* (2020))

Figure 2.4 – Diagram depicting ultrasonic emulsification with acoustic waves and the visual representation of the changing droplet sizes until they collapse into smaller droplets (C = compression and R = refraction).

High pressure homogenisation (HPH) has been used in several studies where increasing the pressure decreases the droplet size (Floury, Desrumaux and Lardières, 2000; Leong *et al.*, 2009) but there are some exceptions when certain emulsifiers are used (Qian and McClements, 2011). Leong *et al.* (2018), studying the formation of double emulsions comparing HPH and ultrasonic techniques, found that when 150 bar for the HPH and 6 W for the sonication was used to create emulsions, the size distribution was statistically indistinguishable. However, the reported P value in the study was 0.69, suggesting no statistically significant difference between the two methods. While this may imply comparable particle sizes, the original study did not report a formal power analysis, and the sample size appears limited. This raises the possibility that the study may have lacked sufficient statistical power to detect a true difference, if one exists. In a different study investigating the development of oil-in-water emulsions, comparing both HPH and ultrasonic found that the ultrasonic produced smaller particle sizes compared to HPH. The ultrasonic-produced emulsion also demonstrated signs of being monodisperse whereas the HPH emulsion exhibited signs of larger droplets and aggregation (Li and Xiang, 2019). In another study by McCarthy *et al.* (2016) ultrasonic homogenisation produced small sized particles, however, they did tend to floc together.

2.5.2 Emulsion destabilisation mechanisms

When generating a colloidal state (or emulsion) of two immiscible components energy is expended in the production. Systems adopt the lowest free energy therefore the colloidal state reverses, meaning emulsions are thermodynamically unstable (Rousseau, 2000; Guzey and McClements, 2006; Yuan, Williams and Biggs, 2009; McClements, 2016; McClements and Jafari, 2018). According to McClements (2016) and Camino and Pilosof (2011) the term emulsion stability is used to describe the ability to resist changes in its properties. There are several types of destabilisation mechanisms that can occur in an emulsion. The destabilisation mechanism of an emulsion is dependent on the emulsion type and the components or ingredients used. In addition, the rate at which an emulsion breaks down is influenced by pH, temperature, and other environmental conditions (Rousseau, 2000; Dalgleish, 2001; McClements, 2016). Figure 2.5 outlines some of the physical types of destabilisation mechanisms which can occur in emulsions.



(Source adapted from McClements, 2016)

Figure 2.5 - Forms of emulsion instability

2.5.2.1 Gravitational separation

Gravitational separation (also referred to as creaming or sedimentation) describes the movement of droplets in an emulsion. Creaming involves the upward movement of droplets compared to sedimentation where the droplets collate at the bottom (Fredrick, Walstra and Dewettinck, 2010; McClements, 2016). Creaming occurs because the droplets have a lower density than the outer phase so can ‘cream’ at the top whereas sedimentation is the

opposite, and the droplets have a higher density than that of the outer phase so gather at the bottom (Fredrick, Walstra and Dewettinck, 2010; McClements, 2016; Tadros, 2016). Figure 2.5 displays a diagram of the 'creaming' mechanism in an emulsion. Creaming can be calculated by the creaming velocity or also known as Stokes' Law, equating the forces moving the droplet upwards and the drag force of the opposite direction. The equation for Stokes' Law is:

Equation 1:

$$v_{Stokes} = \frac{2gr^2(\rho_1 - \rho_2)}{9\eta_1}$$

(Chanamai and McClements, 2000; McClements, 2016).

Where v_{Stokes} is the creaming velocity using the Stokes' Law, η is the shear viscosity, *r* is the particle radius, *g* is the gravitational acceleration and r is the density (subscripts 1 and 2 refer to the continuous and dispersed phases respectively) (Chanamai and McClements, 2000; McClements, 2016). Stokes' Law assumes that there are no interaction forces between droplets and assumes that the movement of one droplet does not influence its neighbour. In practice, there are other factors which can impact the creaming velocity, as food emulsion droplets are not always perfectly spherical and rigid nor are they always within an ideal liquid. Stokes' Law ignores the effect Brownian Motion has on the emulsion droplets. There are numerous mathematical deviations to the equation depending on what the emulsion is experiencing for example, temperature variations causing change in viscosity or electrostatic or Van de Waals forces.

2.5.2.2 Flocculation

Flocculation is the aggregation of droplets to form larger units known as flocs as seen in Figure 2.5. The droplets come together due to the Brownian motion (which is explained further in section 2.5.3.2) (Fredrick, Walstra and Dewettinck, 2010), as a result of insufficient repulsion to keep droplets apart, where the Van de Waals forces of attraction are weak (Tadros, 2013; McClements, 2016). In addition, the electrostatic forces of the droplets cause an attractive depletion force, encouraging droplets to floc together without rupturing the membrane (Binks, 1998; Fredrick, Walstra and Dewettinck, 2010).

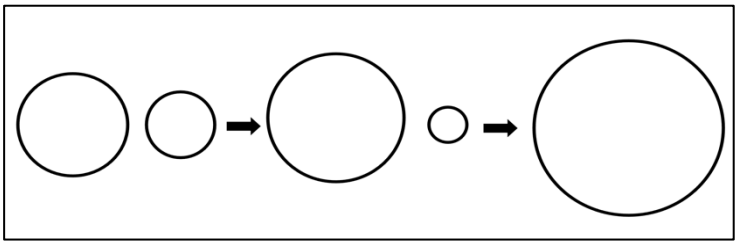
2.5.2.3 Coalescence

Coalescence is a process relating to the thinning or disruption of the liquid film between droplets resulting in the fusion of two or more droplets into a larger one (Binks, 1998; Fredrick, Walstra and Dewettinck, 2010; McClements, 2016; Tadros, 2016). Refer to Figure

2.5 for a diagram of coalescence compared to the kinetically stable emulsion. This often occurs after droplets have come together, when the liquid film may spontaneously break or if the film is thin. Flocculation and creaming enhance and aid coalescence because if droplets are together for long enough to interact with constantly changing dipoles this can cause spontaneous film breakage.

2.5.2.4 Ostwald Ripening

Ostwald ripening is the transport by diffusion of molecules in the internal phase to the external phase. This ripening effect is due to the Laplace pressure, which is the pressure gradient between the convex and the concave side of a cured interface and to deform the droplet a large stress must be applied on the pressure gradient (Walstra, 1993; Binks, 1998). During the Ostwald ripening smaller particles shrink and larger particles swell and increase in size, as seen in Figure 2.6. However, Ostwald ripening is generally not seen in O/W food emulsions.



(Source adapted from Fredrick, Walstra and Dewettinck, 2010)

Figure 2.6 - Diagram of the droplets changing size during the Ostwald ripening.

2.5.2.5 Phase separation and phase inversion

Phase separation is the separation of two immiscible liquids back to their original state after being homogenised (McClements, 2016). However, there is another phenomenon where phase inversion occurs. For example, in a O/W emulsion the continuous phase (aqueous) could begin to be emulsified into the oil droplets forming a temporary $W_1/O/W_2$ emulsion and if the phenomenon continues it could ultimately form a W/O where the water is dispersed in the oil phase (Tadros, 2016). This can be catastrophic, as the process may alter the intended function of the emulsion. The phase inversion temperature (PIT) is the temperature at which an emulsion inverts, where a W/O becomes a O/W or vice versa (Rosen, 2004; Holmberg, Lindman and Kronberg, 2014).

2.5.3 Emulsion characteristics

2.5.3.1 Droplet Concentration and Size

The concentration of droplets is referred to as the *dispersed volume fraction* (ϕ) and is calculated by the *volume of emulsion droplets* (V_D) divided by the total volume of the emulsion (V_E):

Equation 2:

$$\phi = \frac{V_D}{V_E}$$

(McClements, 2016)

Both the concentration of droplets and droplet size impact the numerous characteristics of emulsions, for example, texture, appearance, shelf life and release characteristics for any fortified ingredients (vitamins or minerals) (Dickinson, 2001; Rodriguez Patino and Pilosof, 2011; McClements, 2016). When characterising an emulsion, the droplet size data and particle distribution is investigated to evaluate the stability. Droplets that have all the same diameter are classed as *monodisperse* whereas a range of droplet sizes is referred to as *polydisperse* (McClements, 2016). A monodisperse emulsion is preferred as this produces a more stable emulsion (Zhang *et al.*, 2021) and it is assumed that the emulsion is stable due to the balanced electrostatic repulsion between droplets (Souilem *et al.*, 2014). As larger droplets adsorb a larger proportion of emulsifiers, this causes a difference in attractive forces between droplets, which could cause the aggregation of droplets. A polydispersity index (PDI) can be calculated using the following equation:

Equation 3:

$$PDI = \frac{D_{90} - D_{10}}{D_{50}}$$

(Paximada, Howarth and Dubey, 2021)

where D_{90}, D_{10} and D_{50} is used, which are determined as the cumulative size distribution (Moghadam, Zakeri and Samimi, 2019). PDI shows the distribution of particles, the larger difference between the mean values the greater the polydispersity (McClements, 2016). If the PDI number is more than 1 is gives rise to a polydisperse emulsion.

2.5.3.2 Brownian Motion

Brownian motion is the random stochastic movement of particles within a fluid induced by random collision of droplets (Metcalfe *et al.*, 2012). A less viscous fluid with smaller particle

sizes and a higher temperature can witness a stronger and random displacement of particles. Brownian motion can be regarded as a diffusion process and can be related to the diffusion coefficient. The diffusion coefficient is inversely proportional to viscosity in the continuous phase, meaning that increasing viscosity can aid the retarding of Brownian motion (Hao, 2005). Brownian motion can also play a role in creaming behaviour, as discussed in relation to the creaming velocity in section 2.5.2.1.

2.5.3.3 Droplet interactions

There are many different types of interactions between droplets within food emulsions, which can affect the sensory and physical properties of the emulsions (McClements, 2016). Being able to understand how the droplets interact can help to identify the stability of an emulsion.

Van der Waals forces influence the droplet interactions in three ways: (i) dispersion forces, whereby the interaction of an instantaneous dipole caused by the movement of negative electrons around the positive nucleus creating a dipole which then induces a dipole in a neighbouring droplet, (ii) induction forces, where a permanent dipole induces a dipole in a neighbour; and (iii) orientation forces which are permanent dipoles that are continuously rotating (McClements, 2016; Tadros, 2016).

Electrostatic interactions are important as often droplets have an electrical charge, the sign of which depends on the emulsifier and environmental conditions such as pH and temperature (McClements, 2016; Tadros, 2016). Often droplets in an emulsion are coated with the same emulsifier, giving the droplets the same charge, which causes the droplets to repel from each other, benefiting stability of the emulsion (McClements, 2016; Tadros, 2016). Steric interactions occur when two droplets approach each other within a close proximity and the interfacial layers overlap or compress, which can cause the droplets to repel (McClements, 2016; Tadros, 2016).

There are some general characteristics and features of the interactions described which are outlined in Table 2.5. All are influenced by the composition of the interfacial layer and tend to increase in strength as droplet size increases.

907 **Table 2.5 - Comparing the general features of each of the droplet interactions.**

Van der Waals	Electrostatic Interactions	Steric Interactions
• Interaction between droplets is always attractive • Strength of interaction decreases with droplet separation • Strength of interaction increases with droplet size • Strength of interaction depends on thickness and composition of the interfacial layer • Strength of interaction depends on the environmental conditions	• Interaction between droplets can be either repulsive or attractive • Strength of interaction decreases with droplet separation • Strength of interaction increases with droplet size. • Dependent on the interfacial layer composition.	• Always repulsive at short separations but can be attractive or repulsive at intermediate separations. • Strength of the interaction increases with droplet size. • Dependent on the interfacial layer composition.

908 (Source adapted from McClements, 2016 and Tadros, 2016)

909

910 **2.5.3.4 The Laplace Pressure**

911 Curved interfaces cause a phenomenon which can affect the emulsion properties. Across a
912 curved liquid interface there is a pressure differential which is known as the *Laplace*
913 *Pressure*. Interfacial tension causes the droplet to compress, increasing the internal
914 pressure, which contributes to stabilising the emulsion. This pressure difference between the
915 inside and the outside of the droplet is known as the Laplace pressure gradient. Once
916 equilibrium is reached, where the inward stress is balanced to the outward stress from
917 compressing the bonds in the droplet (Tadros, 2009; Cheng and Wang, 2013; McClements,
918 2016), the resulting pressure stabilises a spherical droplet. This pressure must be achieved
919 for effective homogenisation. It can be identified using Young-Laplace equation (Equation 4):

Equation 4:
$$\triangle p = \frac{2\gamma}{r}$$

γ is the energy per unit area (mJm^{-2}) which is equivalent to force per unit length (mNm^{-1}) and can be used to define surface or interfacial tension (Tadros, 2009), while *r* is the radius of the droplets.

2.5.3.5 Interfacial Tension and the Interfacial Layer

In an emulsion the interface is the area between the two phases (oil and water). *Interfacial* or *surface* pressure or tension is the contracting force at the surface of the droplet, whereby the droplet contracts to reduce the surface area (Schramm, 2014). This *interfacial tension* is lowered by the adsorption of a surface-active compound, which are often of low-molecular weight, to the droplet interface (Lucassen-Reynders, 1993; Shui, Berg and Eijkel, 2009; Schramm, 2014). During emulsification the stress on the fluid must overcome the Laplace Pressure to create the droplets, which generally decreases as interfacial tension decreases, meaning that there is less free energy required to break up droplets and allow the formation of an emulsion (Lucassen-Reynders, 1993; Norde, 2011; Schramm, 2014).

The interfacial layer is created depending on the number or type of surfactants within the emulsion. These surfactants will form layers around the droplet (McClements, 2016). The composition and thickness of the interfacial layer influences production, processing and stability of emulsions (McClements, 2016; Tadros, 2016; Ravera *et al.*, 2021). The electrical and steric interactions between layers can impact the destabilisation mechanisms, for example the adsorption of some emulsifiers can lower the interfacial tension and stabilise droplets against coalescence (Ravera *et al.*, 2021).

2.5.3.6 Flow profiles in droplet formation

Flow profiles of the fluid in an emulsion are responsible for droplet formation and droplet distribution (Walstra, 1993; Walstra and Smulders, 1998; McClements, 2016; Tadros, 2016). The flow depends on the balancing of viscous and inertial forces acting on the fluid characterised by Reynolds Number (*Re)* (Equation 5):

Equation 5
$$Re = \frac{vl\rho}{\eta}$$

(Walstra, 1993; Walstra and Smulders, 1998; Dalmazzone, 2005; McClements, 2016; Tadros, 2016)

where v is the linear liquid velocity, *l* is the length scale flow cavity radius of the cylindrical tube within the fluid will pass, ρ is the density and η is the viscosity.

There are three types of flows:

- *Laminar Flow*: Fluid flow tends to be smooth and well defined (McClements, 2016), meaning the flow is fairly low, resulting in a *Re* number <1000. These can be rotational, simple shear or extensional (McClements, 2016; Tadros, 2016).
- *Turbulent Flow:* Fluid tends to be irregular and chaotic (McClements, 2016), resulting in a *Re* number >2000 (McClements, 2016; Tadros, 2016), seen in a high shear mixer.
- *Cavitational Flow:* Occurs when there are highly fluctuating pressure variations in a fluid and small cavities form which can violently implode and generate shock waves (McClements, 2016), seen during ultrasonication.

2.5.3.7 Droplet break-up

Droplet break-up or deformation is when the ratio of the external stress overcomes the Laplace pressure, characterised by the *Weber Number (We).* For laminar flow the *We* can be calculated by equation 6:

Equation 6
$$We = \frac{Shear\ Forces}{Interfacial\ Forces} = \frac{G\eta_{cd}}{2\gamma}$$

For Turbulent flow the *We* can be calculated by equation 7:

Equation 7
$$We = \frac{Turbulent\ Forces}{Interfacial\ Forces}$$

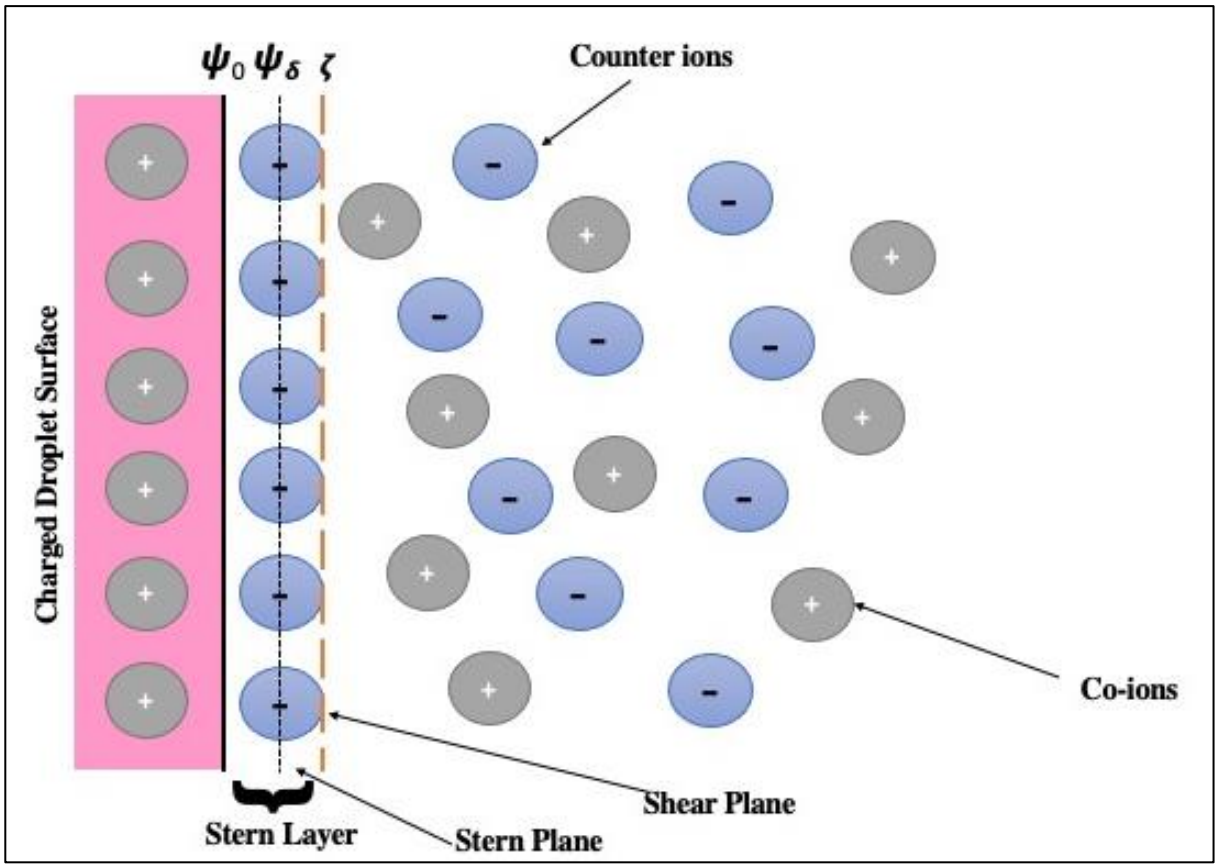
(Walstra and Smulders, 1998; McClements, 2016; Tadros, 2016)

where *G* is the shear force, η_{cd} the viscosity of the continuous phase and λ previously defined, above the *We* critical value (*We*$_{CR}$) then the droplet will burst into smaller droplets, hence the term droplet break-up (Walstra, 1993; Walstra and Smulders, 1998; Dalmazzone, 2005). The *We*$_{CR}$ is dependent on two parameters, the velocity and viscosity ratio. As viscosity of the oil is important during the breakup of droplets, the higher the viscosity the longer it takes to deform a droplet (Walstra, 1993). In addition, a surfactant lowers the interfacial tension therefore benefits the break-up of droplets as the lowering of the interfacial tension means there is less external stress required to overcome the Laplace pressure (Walstra and Smulders, 1998; Dalmazzone, 2005).

2.5.3.8 Droplet charge

Droplets can have an electrical surface charge due to the adsorption of charged materials, such as an emulsifier (McClements, 2016; Tadros, 2016; Ravera *et al.*, 2021). The charge of a droplet can influence the stability of an emulsion, rheology, colour, texture and flavour but also the way in which it interacts (Cano-Sarmiento *et al.*, 2018). The charge can be characterised in three different ways: (i) surface charge density (σ) which is the amount of charge per unit surface area (McClements, 2016); (ii) electrical surface potential (φ_0) which is the amount of free energy required to increase the charge from zero to σ (McClements, 2016); and (iii) zeta potential (ζ), which is the difference between the charge on the layer and bulk phase in which the droplet is dispersed within. It gives a net charge providing the zeta-potential charge which is important in characterising the electrical interactions (Li and Tian, 2007; Cano-Sarmiento *et al.*, 2018). A charged surface attracts the opposite charge, these ions are known as counterions, as seen in Figure 2.7, the positive ions on the charged surface attract negative counterions. This results in a strong layer of counterions and forms a neutralising layer. The other ions further away from the surface are within the electrical double layer and contain, co-ions. The closest layer, of the neutralising counterions is defined as the *stern plane* and is the boundary between the inner (*Stern* Layer) and the outer layer. The Debye *Screening length* is the measure of the thickness of the electrical double layer consisting of a mixture of counterions and co-ions which are not closely bound to the droplet surface. The *shear plane* is positioned and determined by the size of the

counterions attracted to the charged surface, this is also known as the ζ-potential and determined by electrokinetic techniques.



(Source adapted from McClements, 2016)

Figure 2.7 – Visual representation of droplet charge.

The zeta potential is commonly used in laboratories to identify the charge of a droplet when analysing emulsions.

2.5.4 Emulsifiers used for emulsion stabilisation

A surfactant can be defined as a substance that reduces the surface tension by adsorbing onto the surface of the droplet (Krog, Larsson and Fridberg, 1990; Norn, 2015; McClements, 2016). This surfactant stabilises the droplet, but a thickener or gelling agent can also be used to hinder droplet movement and aid stabilisation. Food emulsifiers can be a range of molecules, including phospholipids and amphiphilic macromolecules (McClements, 2016). Table 2.6 highlights some molecules which are used as emulsifiers in the food industry

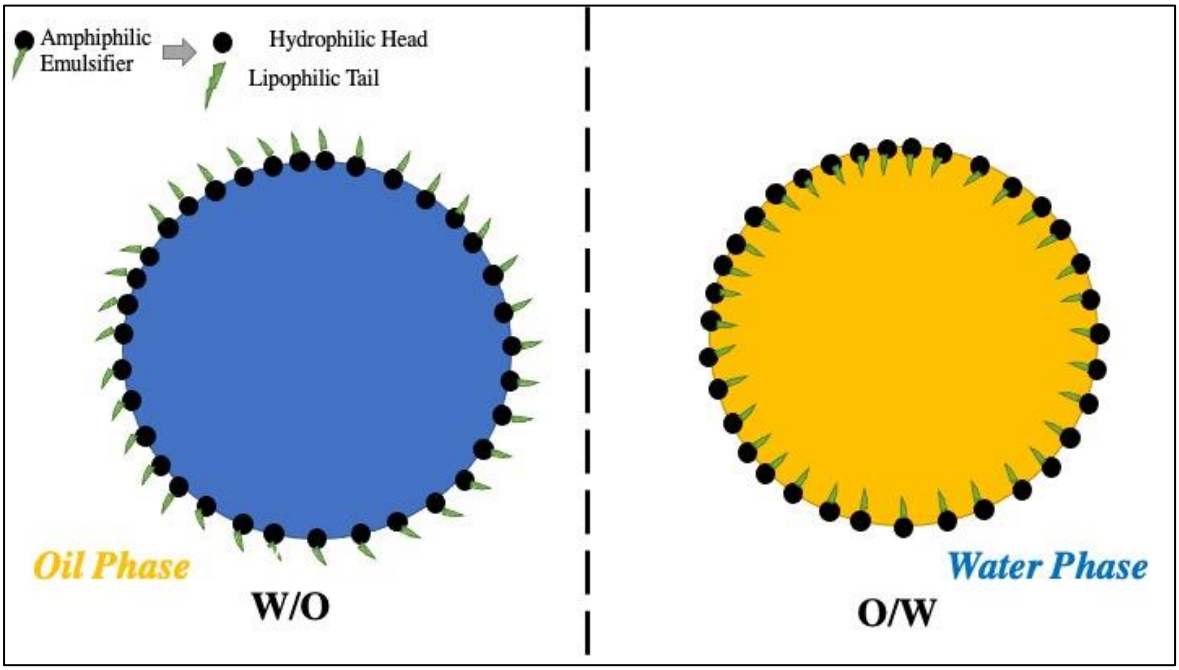
1010 **Table 2.6 - Examples of emulsifiers commonly used in the food industry.**

Amphiphilic macromolecules	Milk proteins • Whey Proteins • Casein Animal Proteins • Gelatine • Myosin • Actin • Actomyosin Egg Proteins Plant Proteins • Legumes (e.g., Pea) • Soy	Proteins are mainly surface active. Amphiphilic molecules Relatively small molecules that adsorb to the surface of the droplet	(McClements, 2016; Ozturk and McClements, 2016)
Phospholipids (Lecithins)	Naturally occurring in membranes	Naturally amphiphilic molecules that are surface active	(Ozturk and McClements, 2016)
Saponins	Natural sugars from the bark of a tree.	Hydrophilic sugar	Ozturk and McClements, 2016)
Organic Acid Esters	Long chains of hydrocarbons for example - Polyglycerol fatty acid esters (PGFEs)	Synthesised chemically or enzymatically. PGFEs are non-ionic surfactants which have several properties including thickening.	(Peng *et al.*, 2018)
Stearoyl Lactylate Salts	Manufactured by reacting stearic acid and lactic acid in presence of Sodium.	Anionic surfactant	(Flores *et al.*, 2007)

1011

2.5.4.1 Surfactants

A group of emulsifiers which are classed as surface-active or also known as surfactants are comprised of amphiphilic molecules (McClements, 2016). This means that they have two parts, one which is hydrophilic, and the other is hydrophobic, also known as lipophilic (Msagati, 2012; Norn, 2015; McClements, 2016; McClements and Jafari, 2018). The amphiphilic molecules position themselves between the two phases to stabilise the droplets (Msagati, 2012; Norn, 2015). The positioning of these emulsifiers is shown in Figure 2.8, on whether it is stabilising a W/O emulsion or a O/W emulsion and forming a protective barrier(Msagati, 2012).



(Source adapted from Chung and McClements, 2014)

Figure 2.8 – Diagram showing the droplet (internal phase) stabilised by a surface-active emulsifier.

Surfactants are often molecules with a low molecular weight (Lucassen-Reynders, 1993; Holmberg, Lindman and Kronberg, 2014; Schramm, 2014). These radically alter the surface and interfacial tension and reducing the Laplace pressure (Norde, 2011; Schramm, 2014), aiding emulsification and stabilisation, as previously discussed. In addition to this, the surfactants hold an electric charge which can generate repulsive forces between droplets, thus reducing aggregation and coalescence (Tadros, 2013; Silva, Cerqueira and Vicente, 2015).

Table 2.7 outlines the type of surfactants in relation to the ionic charge, which can impact the types of emulsion they stabilise and their ability to interact with other surfactants.

Table 2.7 Types of surfactants in relation to their ionic charge

Type	Description	Key points
Anionic	A portion of the molecule bears a negative charge	Largest class of surfactants. Has limited compatibility with cationic surfactants.
Cationic	A portion of the molecule bears a positive charge	Third largest class of surfactants. Limited combability with anionic surfactants. Adsorb strongly to most surfaces.
Zwitterionic	The molecule has both a positive and negative charge.	Smallest class of surfactants. Compatible with all surfactants. Stable in acids and bases.
Non-ionic	The molecule has no apparent ionic charge.	Second largest class of surfactants. Compatible with all surfactants. Solubility could be affected by temperature.

(Source adapted from Rosen 2004 and Holmberg, Lindman and Kronberg, 2014)

When choosing a surfactant for use in a food emulsion there are properties that must be considered, for example food emulsifiers must meet food additive standards which are accepted by the Food Standards Agency in the UK (Karsa, 2006). Their emulsification ability, adsorption, viscosity modification ability and stability in acid and alkaline media are also to be considered (Karsa, 2006). The geometry of a surfactant is also important (Figure 2.6), as the positioning and the way in which the surfactants fit along the interface is crucial in the stabilisation of a droplet and helping to reduce coalescence (Holmberg, Lindman and Kronberg, 2014).

The Hydrophile-Lipophile balance (HLB) is a number given to surfactants depending on their molecular properties and gives an indication of its affinity to the oil and aqueous phases (Msagati, 2012; Schramm, 2014; Norn, 2015; McClements, 2016). Surfactants are classified by their HLB number which indicates their emulsifying characteristics (Msagati, 2012; Schramm, 2014; Norn, 2015; McClements, 2016). HLB numbers range from 0 to 18 (Table 2.8), where a high HLB number has a high ratio hydrophilic to lipophilic groups, meaning it

has a hydrophilic tendency, whereas a low HLB number has a lower ratio of hydrophilic to lipophilic groups meaning it has a hydrophobic (or lipophilic) character (Msagati, 2012; Norn, 2015; McClements, 2016).

Table 2.8 – Hydrophile-Lipophile balance number classification and key properties

HLB Number	Classification/Properties
0 - 3	Anti-foaming properties
4 - 6	Stabilises water-in-oil (W/O) emulsions
7 – 9	Wetting agent
10 - 18	Stabilises oil-in-water (O/W) emulsions

(Msagati, 2012; Norn, 2015; McClements, 2016)

The HLB can be related to the PIT, whereby the higher the HLB value the greater the PIT (Rosen, 2004; Holmberg, Lindman and Kronberg, 2014). A change in environmental conditions such as pH or temperature can alter the HLB value of the surfactant (Rosen, 2004; Schramm, 2014).

2.5.4.2 Thickeners

Thickening agents are used to enhance the stability of emulsions by increasing viscosity and acting as a physical barrier and helps to reduce movement of droplets (McClements, 2016; Ozturk and McClements, 2016; Mao *et al.*, 2017). The increase in viscosity of the continuous phase reduces the diffusion co-efficient of the droplets (D), meaning that as D reduces, the frequency of collisions between droplets is reduced too, resulting in a lower rate of coalescence (Rosen, 2004).

The diffusion co-efficient (D) is calculated using the Stokes-Einstein Equation (Equation 8):

Equation 8:

$$D = \frac{kT}{6\pi\eta R}$$

(Rosen, 2004; Shire, 2015)

where *k* is the Boltzmann constant. This figure provides a measure of the amount of energy corresponding to the random thermal motions of the particles (Pitre *et al.*, 2011). *T* is temperature, η is viscosity and *R* is the radius of the particle.

Thickeners can also benefit stability by steric interactions, whereby a coated droplet is surrounded by the opposite charge in the continuous phase (McClements, 2016; Paximada, Howarth and Dubey, 2021).

2.5.5 Pickering emulsions

Although Pickering emulsions can be produced in the same way as other emulsions described previously they differ as they are stabilised by solid particles (Jafari *et al.*, 2020). According to Norton, Fryer and Norton (2013) the colloidal particles attached to the interface provide a steric barrier to coalescence.

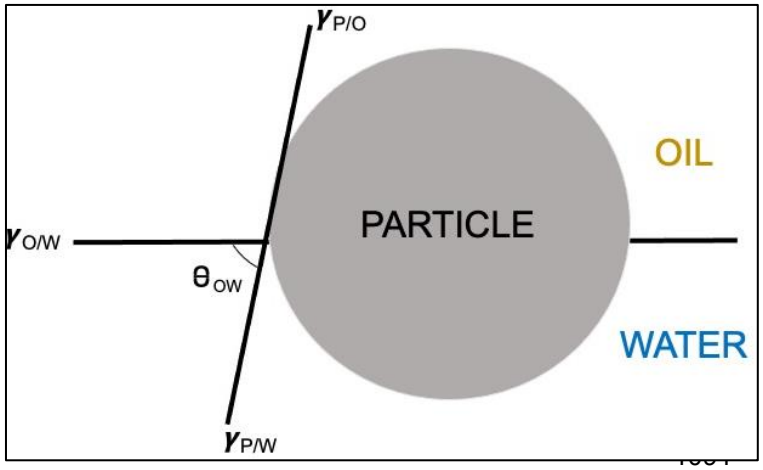
For a Pickering emulsion, the wettability of a particle determines whether it will be able to be immersed in a certain phase. Young's equation for determining the contact angle (θ) is (Equation 9):

Equation 9:

$$\cos\theta = \frac{(\gamma_{p/o} - \gamma_{p/w})}{\gamma_{o/w}}$$

(Linke and Drusch, 2018)

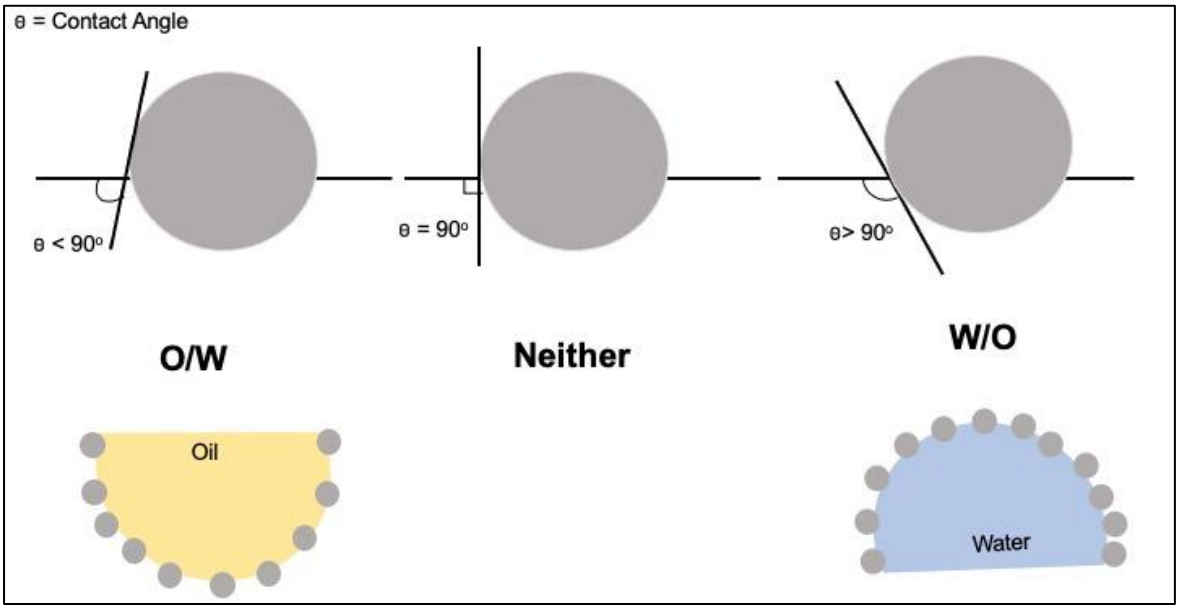
where $g_{p/o}$ is the interfacial tension between the particle and oil phase, as demonstrated in Figure 2.7. $g_{p/w}$ is the interfacial tension between the particle and water phase and finally $g_{o/w}$ is the interfacial tension between the oil and water.



(Source adapted from Linke and. Drusch, 2018)

Figure 2.9 – Particle contact surfaces in a Pickering emulsion.

The contact angle is important in wettability as previously mentioned, as the angle determines the type of emulsion the particle will stabilise. As seen in Figure 2.9 and Figure 2.10, for a O/W emulsion an angle of less than 90° is required, whereas if the angle is exactly 90° then these particles will not stabilise either emulsion (Norton, Fryer and Norton, 2013).



(Source adapted from Norton, Fryer and Norton, 2013)

Figure 2.10 – Visual representation of contact angles of Pickering emulsions at the interface.

Once the particle has adsorbed to the surface, it has been mentioned that it is irreversible, this is because there is a large amount of energy required to detach the particles from the surface. The energy required is known as the detachment energy or the free energy of desorption ΔGd (Linke and Drusch, 2018; Sarkar and Dickinson, 2020). As a particle is not always the same size, the equation differs depending on the shape. For a sphere the ΔGd equation is shown in (Equation 10):

Equation 10:

$$\Delta Gd_{sphere} = \gamma_{\alpha\beta}\pi r^2(1 - \cos\theta)^2$$

(Sarkar and Dickinson, 2020)

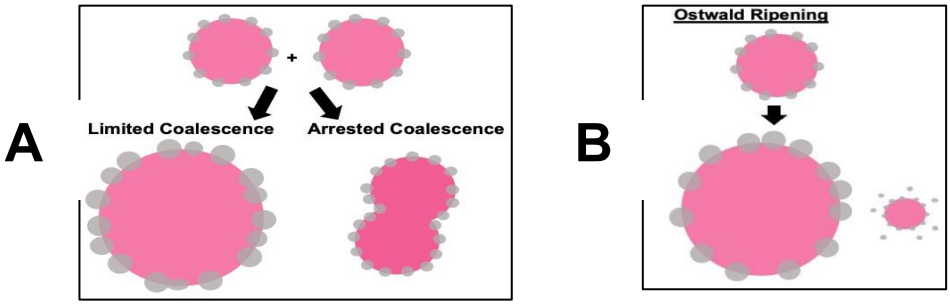
where g_{ab} is the fluid-fluid interfacial tension, r is the radius of the spherical particle and θ is the contact angle.

Numerous types of particles have been used to stabilise Pickering emulsions. These can consist of, starches, polyphenol crystals, citrus pectin, chitosan, cellulose, fat crystals,

proteins such as whey, zein, soy and peanut (Norton, Fryer and Norton, 2013; Xiao, Li and Huang, 2016; Chen *et al.*, 2020; Jiang, Sheng and Ngai, 2020; Cen *et al.*, 2023). The emulsions in which they stabilise depends on the contact angle as discussed previously.

2.5.5.1 Destabilisation mechanisms of Pickering emulsions

A Pickering emulsion has a physical barrier surrounding the droplet and the detachment energy required to remove the particle is high, and therefore unlikely to detach. This benefits stability by altering the interfacial tension meaning it is not easily destabilised (Xia, Xue and Wei, 2021). However, destabilisation is possible, Figure 2.11 is a visual representation of the types of destabilisations that can occur. Coalescence can occur but due to the strong attachment of particles to the interface, it can cause limited coalescence (A) or arrested coalescence where the particles do not fully combine causing this unusually shaped particle as seen in (B) (Whitby and Wanless, 2016).



(Source adapted from Whitby and Wanless, 2016)

Figure 2.11 Destabilisation of Pickering emulsions (A) coalescence and (B) Ostwald ripening.

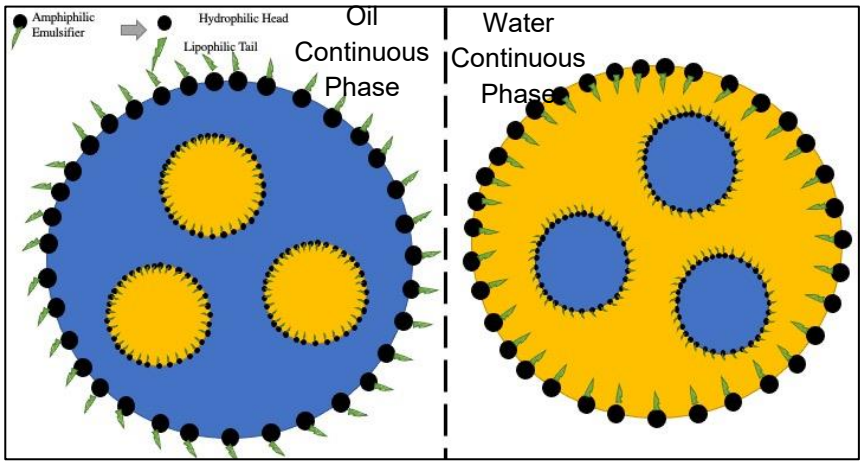
Another factor which affects stability of Pickering emulsions is the shape of a particle as it influences the number and packing ability of particles along the interface, thus impacting the interfacial tension (Xia, Xue and Wei, 2021). In addition, the size of the solid particle should be smaller than the desired droplet size, as this will impact the stability of the emulsion.

2.6 Double emulsions

Double emulsions consist of water-in-oil-in-water ($W_1/O/W_2$) or oil-in-water-in-oil ($O_1/W/O_2$) (Chung and McClements, 2014; McClements, 2016). These systems have numerous uses, including cosmetics, drug delivery in the pharmaceutical industry and recent developments for use in the food industry (Dickinson, 2011; Chung and McClements, 2014; McClements, 2016). Double emulsions have been used in the food industry for multiple strategies, which include using these systems to reduce the fat content but still providing a similar perceived mouth feel (Ding *et al.*, 2018). A study using double emulsions to reduce the fat content in

mayonnaise found that the amended low fat recipe did not affect the sensory characteristics compared to the full fat counterpart (Yildirim, Sumnu and Sahin, 2016). Likewise, the use of double emulsions to reduce the fat content in meat products has also been found to have a positive influence on the texture (Serdaroğlu, Öztürk and Urgu, 2016). Double emulsions are not only used in lowering fat content but also have been used to facilitate the controlled release of active agents and encapsulation of substances (Yildirim, Sumnu and Sahin, 2016). For example, Li *et al.* (2012) used double emulsions stabilised with whey protein and polysaccharide complexes to control the release of Vitamin E and Vitamin B_2. The study concluded that the protein-polysaccharide complexes served as selective barriers to protect and control release of the vitamins.

Figure 2.12 shows a diagram of a double emulsion, left is a $O_1/W/O_2$ and right is a $W_1/O/W_2$ and highlights the positioning of the amphiphilic surfactants to stabilise the droplet in the respective phases. The most common double emulsion used in the food industry is a $W_1/O/W_2$, and to stabilise these droplets two types of surfactants are required. A lipophilic emulsifier, with a HLB number lower than 6 is advised for the primary W_1/O emulsion (Ding *et al.*, 2018).



(Source adapted from Chung and McClements, 2014)

Figure 2.12 – Diagram of double emulsions stabilised by a surfactant.

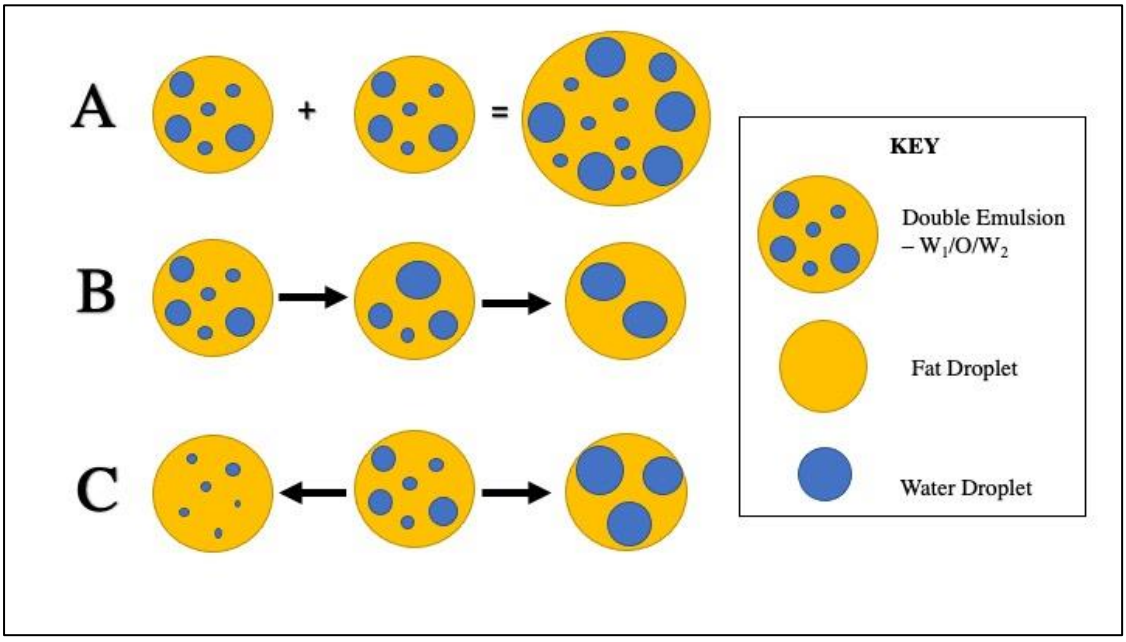
As seen in Figure 2.10 the amphiphilic emulsifier is positioned so the lipophilic tails are positioned toward the oil phase whereas the hydrophilic head is to the surface edge of the water droplet. For the secondary $W_1/O/W_2$ emulsion, a hydrophilic emulsifier with a HLB number higher than 8 is advised (Ding *et al.*, 2018). As seen in the diagram the hydrophilic head is on the surface of the oil droplet covering the droplet and creating an interfacial layer between the oil droplet and water continuous phase.

2.6.1 Double emulsion destabilisation mechanisms

Like single emulsions, double emulsions are unstable and require the use of emulsifiers to make them kinetically stable. However, stabilisation can be a challenge as at certain conditions or times the double emulsion may need to be destabilised to release encapsulated ingredients (Leister and Karbstein, 2020).

The principles of instability are the same for primary and secondary emulsions. However, as shown in Figure 2.13 double emulsions can undergo different destabilisation mechanisms, for example coalescence can be of the outer droplets or the inner droplets. Internal droplets can experience shrinkage or swelling, due to the osmotic pressure gradient between W_1 and W_2, whereby a higher Laplace pressure causes the small W_1 droplets to migrate to the outer phase. It has been found that the addition of solutes to the inner water phase can cause the osmotic gradient to counteract the Laplace pressure and improve stability (Mezzenga, Folmer and Hughes, 2004; Sapei, Naqvi and Rousseau, 2012; Ding *et al.*, 2018).

When double emulsions are used as fat replacers it is important that W_1 droplets remain in place. If W_1 droplets shrink, this reduces the performance of the reduced fat emulsion and decreases viscosity. In comparison, if the W_1 droplets increase in size, this causes oil droplet growth, thus increasing viscosity, and is known as *Osmotic Swelling* (Oppermann *et al.*, 2015; Leister and Karbstein, 2020).



(Source adapted from Dickinson, 2011)

Figure 2.13 – Double emulsion destabilisation mechanisms; (A) Coalescence of the outer droplets, (B) Coalescence of the inner droplets, (C) Shrinkage or swelling of inner droplets.

2.6.2 Double emulsion use in the food industry for fat reduction

Double emulsions have been utilised in the food industry to lower the fat content of various food products. Research into their applications spans a wide range of food types including dairy, meat, sauces and bakery items. Tekin, Sahin and Sumnu (2017) investigated the use of double emulsions to reduce the fat content in ice cream. Their study found that using a Polyglycerol Polyrincoleate (PGPR) – Lecithin blend and Guar Gum double emulsion could reduce the ice cream's fat content without adversely affecting its sensory characteristics or functionality. Although regular ice cream had the highest intensity scores for mouth feel, which can be attributed to the fat being the main contributor of mouthfeel and flavour richness, the double emulsion ice cream did score higher than the lower fat ice cream without double emulsion. While the rheology of the ice cream was investigated the study did not examine the emulsion droplet size or how emulsifiers impacted the ice cream's structure both of which could also influence the ice cream.

Rakshit and Srivastav (2022) used double emulsions to reduce fat in short dough biscuits. They found that samples with a 40 % fat reduction had better perceived sensory attributes than the control. Another application of this mechanism is in replacing animal fat in meat emulsions. Choi *et al.* (2009) successfully used double emulsions for this purpose, although the change did alter the rheological composition, with a difference in texture between meat batter with vegetable oil compared to the control. Overall, there has been promising research on successful use of double emulsions in improving the structure and function of low fat food products.

2.6.3 Double emulsion use in the food industry – Fortification

The use of double emulsions as a method of fortifying a food product has also been an area of interest for food manufactures because the food acts as a vehicle for the active ingredient, protecting it during digestion and providing a controlled release. As Ye *et al.* (2009) mention, dairy products such as cheese are good vehicles for fortification as they can protect the bioactive ingredients due to their high pH, solid consistency and fat content.

Herzi and Essafi (2020) found that a double emulsion system proved beneficial in carrying magnesium to fortify yoghurt. In comparison, El Kadri *et al.* (2018) encapsulated a probiotic, *Lactobacillus paracasei,* in a double emulsion without impacting major characteristics such as texture. However, there was no sensory evaluation undertaken, and to clearly state that the incorporation of the probiotic did not affect all characteristics, then a consumer panel would be required to compare the control to the fortified yogurt to identify any difference. Another study by Jamshidi *et al.* (2019) which involved the fortification of natural yogurt with fish oil microcapsules containing vitamin B_{12}, these were successfully encapsulated and

found that there were no significant differences between the control and the fortified yogurt. However, the sensory evaluation proved otherwise, the fortified yogurt had notable fish flavours. Contributing to this, another study successfully encapsulated fish oils in processed cheese, but the sensory of these fortified cheeses developed undesirable fishy flavours (Ye *et al.*, 2009).

Double emulsions provide suitable novel technological opportunities for fortifying foods with vitamins and minerals and have been proven successful in several studies. However, further investigation and research could still be undertaken to improve the fortification of certain food stuffs with double emulsions and sensitive ingredients such as polyphenols, vitamins or minerals.

2.6.4 Surfactants in double emulsions

When stabilising double emulsions two surfactants are required. To achieve a stable W_1/O a lipophilic surfactant or emulsifier is required to stabilise the inner water droplets. This ingredient must have a low HLB value to stabilise the droplets within the oil phase, whereas the O/W_2 requires a hydrophilic surfactant which has a higher HLB value. There has already been extensive research into the use of natural surfactants to stabilise a O/W, which has been utilised in double emulsion production, such as milk proteins (casein and whey). Table 2.9 compares the different surfactants used in stabilising $W_1/O/W_2$ emulsion.

Polyglycerol polyricinoleate (PGPR) is a common lipophilic surfactant used and accepted within the food industry to stabilise emulsions (as discussed in section 2.6.2). The use of this ingredient, although synthetic, has proven beneficial in stabilising both W/O and O/W emulsions, depending on the concentration (Eisinaite *et al.*, 2018). The polyglycerol polar head interacts with water through hydrogen bonds, whereas the polyrincoleate part binds to the non-polar fatty acids of the oil phase through Van der Waals forces (Su, De Meulenaer and Van der Meeren, 2023). PGPR is commonly used in chocolate production to reduce viscosity during production to enable smooth enrobing and dipping. This synthetic emulsifier has been utilised in numerous studies with double emulsions for low fat or fortification purposes. A comprehensive range of studies are presented in Table 2.9, showing a wide usage of PGPR as the lipophilic emulsifier in food products. Despite the use of PGPR in food production the European Food Safety Authority has suggested a limit of 25 mg/kg body weight per day as an acceptable daily intake of PGPR (Younes *et al.*, 2022). This has led to recent research in the reduction or replacement of PGPR in food emulsions for a clean label and sustainable product.

1264 **Table 2.9 – Summary of literature using different surfactants in double emulsions**

Research	Oil Phase	Lipophilic Surfactant	Hydrophilic Surfactant	Citation
Double emulsions in skimmed milk	Sunflower oil	PGPR Sunflower Oil	Skimmed milk	(Leong *et al.*, 2018)
Double emulsions fortified with casein-whey proteins	Oil (not specified)	PGPR	Panodan	(Silva *et al.*, 2018)
Investigating crystallisable double emulsions.	Anhydrous milk fat	PGPR	Sodium Caseinate	(Herzi and Essafi, 2020)
Encapsulation of sweet whey using double emulsions	Canola oil	PGPR	Panodan	(Pimentel-González *et al.*, 2009)
Investigating beef fat replacers	Extra virgin olive oil	PGPR	Sodium Caseinate	(Serdaroğlu, Öztürk and Urgu, 2016)
Delivery of Lactobacillus paracasei in yogurt	Sunflower oil	PGPR	Skimmed milk	(El Kadri *et al.*, 2018)
Pickering W/O/W emulsions	Sunflower oil	PGPR	Tween 20	(Spyropoulos *et al.*, 2019)

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Research	Oil Phase	Lipophilic Surfactant	Hydrophilic Surfactant	Citation
W/O/W with Carragean	Canola oil	PGPR Sunflower Lecithin	Skimmed milk	(Klojdová, Troshchynska and Štětina, 2018)
Finding a clean emulsifier with a low HLB value in W/O/W	Medium chain triglyceride oil Long chain triglyceride oil	Soy Lecithin Sunflower Lecithin PGPR	WPI Sodium caseinate	(Balcaen *et al.*, 2021)
Encapsulation of grapeseed oil	Sunflower oil	PGPR	Sodium caseinate	(Estévez *et al.*, 2019)
Looking at the effect of the outer phase on W/O/W	Sunflower oil	PGPR	Tween 20 Sodium caseinate WPI	(Oppermann *et al.*, 2018)
Encapsulating tea polyphenols	Coconut oil	PGPR	Soy lecithin	(Tian, Xiang and Li, 2021)
Using W/O/W to reduce sugar	Olive oil	PGPR	Sodium caseinate	(Ilyasoglu Buyukkestelli and El, 2019)

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Research	Oil Phase	Lipophilic Surfactant	Hydrophilic Surfactant	Citation
Natural W/O/W double emulsions	Soybean oil	Soy lecithin	Gliadin colloid particle	(Zhang *et al.*, 2023b)
Encapsulation of “Pitanga” leaf hydroethanolic extract in films	Soybean oil	PGPR	Tween 80 Sodium caseinate	(Tessaro *et al.*, 2021)
Investigating different fats in double emulsions	Soybean oil	PGPR	Skimmed milk	(Pérez, Wagner and Márquez, 2017)
Fortification of calcium	Sunflower oil	PGPR	Soy milk	(MÁRquez and Wagner, 2010)

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2.6.4.1 Lipophilic - Phospholipids

Phospholipids, being amphiphilic molecules, can undergo further processing and purification to serve as surface-active ingredient for stabilising emulsions (Ozturk and McClements, 2016). They operate by both reducing surface tension (Kjellin and Johansson, 2010) and creating a protective barrier around droplets to hinder coalescence (Wang *et al.*, 2021). Lecithin, containing a blend of phospholipids, can be derived from either plant or animal sources (Bueschelberger, 2004; Zhu and Damodaran, 2013). These phospholipids encompass Phosphatidycholine (PC), Phosphatidylethanolamine (PE), Phosphatidylinositol (PI) and Lysophosphatidylcholine (LPC), along with minor components like phosphatidic acid (PA) and Sphingomyelin (Bueschelberger, 2004; Kjellin and Johansson, 2010; Zhu and Damodaran, 2013). Lecithin is a commercially viable food additive (E322) (Bueschelberger, 2004; Kjellin and Johansson, 2010; Zhu and Damodaran, 2013) but vegetable-derived lecithin is more readily accessible commercially, mainly due to the challenges associated with extracting it from animal sources (Bueschelberger, 2004).

The geometry of phospholipid impacts the emulsification properties including stability and formation, with PC usually forming a lamella layer. These phospholipids position themselves at the interface to fit together to protect the droplet, which links to the HLB value. According to an historic paper, van Nieuwenhuyzen and Szuhaj (1998) outlined the different fractions of lecithin, where standard lecithin has a HLB value of 4.0 and PI-3F fraction had a lower HLB of 2.0, due to the higher ratio of the PE, favouring a W/O emulsion and resulting in a lower HLB. Contrary to this, the PC fraction had a higher HLB value of around 7.0, favouring an O/W emulsion.

Phospholipids sourced from milk and by-products, such as cheese whey, are primarily derived from the milk fat globule membrane (MFGM) (Zhu and Damodaran, 2013) which has a thickness of approximately 8 – 10 nm, comprising of multiple layers of phospholipids and proteins, thereby stabilising in the aqueous phase (Michalski *et al.*, 2002; Singh and Gallier, 2017). As per Mulder and Walstra (1974), composition of MFGM, includes 900 mg/100 g (41 % w/w) proteins and 600 mg/100 g (27 % w/w) phospholipids. The surface tension of a milk fat globule (MFG) was noted by Michalski *et al.* (2002) as -13.5 ± 0.9 mV, which is influenced by the combination of proteins and phospholipids that surround it, resulting in its negative surface tension. Due to the layered structure of the MFGM, the phospholipids and proteins possess varying HLB values to safeguard the fat globule, with phospholipids of low HLB values being situated closest to the triglyceride centre (Michalski *et al.,* 2002). Dewettinck *et al.* (2008) and Contarini and Povolo (2013) suggest that components of the MFGM, such as phospholipids, hold promise for emulsion stabilisation applications.

In their study, Michalski *et al.* (2002) conducted a comparison between milk phospholipids and soybean lecithin, revealing that the stability of O/W emulsions was notably lower when employing milk phospholipids. This inferior stability was attributed to the higher proportion of PE relative to PC in milk phospholipids, which favours the W/O rather than the O/W. Another investigation, substituting PGPR with lecithin, demonstrated that lecithin alone might not suffice for stabilisation purposes. Hence, the supplementation of whey protein isolate (WPI) or sodium caseinate was implemented to enhance stabilisation (Wang *et al.*, 2021). The outcomes indicated that emulsions fortified with WPI exhibited enhanced protection and formed a protective layer around the phospholipids. Over time, the efficacy of WPI improved, resulting in greater stability even at day 70 compared to that of sodium caseinate.

Both van Nieuwenhuyzen and Szuhaj (1998) and Wang *et al.* (2021) have highlighted a concern regarding phospholipids as surfactants, specifically regarding the sensitivity of PE to calcium and magnesium in milk. This discrepancy may be attributed to the multiple layers of phospholipids present, as suggested by Michalski *et al.* (2002), wherein phospholipids with low HLB values are located nearer to the triglyceride centre and shielded by other phospholipids and proteins with higher HLB values.

Research has investigated the utilisation of phospholipids with elevated HLB values for the stabilisation of O/W emulsions. Horn *et al.* (2011) explored the oxidative stability of fish oil emulsions in water, employing sodium caseinate, WPI, soy lecithin and milk phospholipids. Their investigation revealed that emulsions based on phospholipids exhibited superior oxidative stability compared to those based on protein.

Furthermore, investigations into the use of phospholipids and concentrates derived from dairy products have been conducted to some extent. Surh, Ward and McClements (2006) assessed the efficacy of modified whey protein concentrate (MWPC) and conventional whey protein concentrate (CWPC) in emulsion stabilisation. While MWPC boasted a higher phospholipids content at 4 % compared to CWPC's < 1 %, CWPC possessed a greater protein content of 76 %. Results indicated that MWPC yielded smaller mean emulsion droplet diameters with fewer larger droplets than CWPC. The authors proposed that phospholipids rapidly adsorbed to the surface, interacting with proteins to form a layer around the droplets, like the MFGM, thereby enhancing emulsion stability.

Levin, Burrington and Hartel (2016) investigated the replacement of synthetic emulsifiers, such as PGPR in ice cream, sweetened condensed milk in caramel and egg in cake, using Whey Protein Phospholipid Concentrate (WPPC) from three different suppliers and delactosed permeate (DLP). WPPC and DLP were intended to substitute synthetic emulsifiers in ice cream, with partial and full replacement explored. While ice cream made

with WPPC exhibited slight discrepancies in viscosity, flow index and yield stress compared to synthetic emulsifiers, the study concluded that full replacement was not feasible. Nevertheless, there was potential for producing clean label ice cream using natural emulsifiers. However, the claim of achieving a fully “clean” label product seems unsubstantiated as the difference between synthetic and natural emulsifiers may have been negligible. A sensory evaluation was not conducted which could have provided insights into whether the observed differences in physical properties impacted sensory perception.

While the substitution of WPPC for PGPR in ice cream constitutes as an oil-in-air-in-water emulsion, various studies have explored the potential of milk phospholipids in O/W and W/O. There is a potential for milk phospholipids to stabilise W/O emulsions, provided that the phospholipids composition exhibits a higher proportion of PE than PC, given PE’s presumed lower HLB value. Knoth, Scherze and Muschiolik (2005) successfully employed PC-depleted lecithin with a ratio of 0.16 [PC/(PI,PE) to stabilise W/O emulsions. However, they noted that the type of oil influenced the stability of emulsions stabilised with this surfactant.

2.6.4.2 Lipophilic - Polyphenols

Polyphenols can be classed as flavonoids, tannins and phenolic acid compounds (Williamson, 2017) and can be obtained from plants. Curcumin, for example, is derived from the *Curcuma longa* plant, and has hydrophobic tendencies. Similarly, quercetin is a flavonoid found in the skin of fruit and vegetables (Li *et al.*, 2016). Polyphenols have numerous health benefits, for example, both curcumin and quercetin have antioxidant, antimicrobial and anti-inflammatory properties and have been utilised in the food industry as a fortification element. Other polyphenol containing ingredients, such as green tea have similar health benefits and have been used to successfully fortify Cheddar cheese using double emulsions (Giroux *et al.*, 2013). Polyphenols have been utilised in stabilising emulsions by the Pickering mechanism. Luo *et al.* (2019) used tea polyphenol palimitate (Tp-P) to stabilise W/O emulsions as a fat replacer. Their findings revealed that with increasing Tp-P concentration enhanced stability was achieved, resulting in smaller droplets measuring 3.07 ± 0.22 mm and a low polydispersity index of 0.42, leading to a monodisperse emulsion. This combination highlights the success of Tp-P as a stabiliser in W/O. Wang, Bai and Shao (2020) similarly utilised tea polyphenols alongside gelatine and chitosan to stabilise emulsions. By varying the ratios of different ingredients, they identified the optimal ratio as gelatine:tea polyphenols at a 1:2, yielding droplets averaging 880 nm. The authors attributed this concentration as optimal due to the structural compatibility of polyphenols with gelatine, facilitating the formation of robust interfaces between oil and water, therefore impeding aggregation (Wang, Bai and Shao, 2020). In contrast, Tong *et al.* (2021)also used green tea polyphenols in an high internal phase O/W emulsions but achieved much larger droplets in

the range of 27.68 μm ($D_{4,3}$). Shi *et al.* (2024) used modified epigallocatechin gallate derivatives to stabilise W/O at a 20:80 ratio. The study showed potential, with droplets reaching 2.45 μm to 7.18 μm ($D_{4,3}$), which contributed to stability of the emulsion, with creaming indexes of 0 % and 1.45 % for epigallocatechin gallate stearate and epigallocatechin gallate palmitate, respectively. The combined results from the study identified that there was opportunity for epigallocatechin gallate derivatives to be a natural alternative surfactant for W/O. However, due to the low crystallisation temperature of these surfactants, this could limit their application in all in all food stuffs. For example, it would not be suited for products which undergo pasteurisation such as some dairy products.

In their investigation, Aditya, Hamilton and Norton (2017) utilised Curcumin particles to stabilise nano-emulsions within an O/W system. While the study successfully achieved stable emulsion with curcumin, it relied on WPI coating the curcumin particles to facilitate emulsion stabilisation. Consequently, curcumin alone proved insufficient for stabilising this O/W emulsion. This limitation may stem from curcumin's oil solubility and a wettability contact angle exceeding 90°, suggesting an inclination towards stabilising W/O rather than a O/W system. Another study by Ghirro *et al.* (2022) explored the use of curcumin alongside k-carrageenan as a carrier in vegan low fat mayonnaise-like products, with parameters closely resembling traditional and light mayonnaise, except minor differences in colour.

Notable research by Zembyla, Murray and Sarkar (2018) employed polyphenol crystals (curcumin and quercetin) alongside whey protein to stabilise W/O emulsions via Pickering mechanism. Their investigation revealed that a concentration of 0.14 % wt. curcumin, the smallest droplet size was achieved, ensuring effective coating the droplets. Higher water to oil ratios (10:90) resulted in larger droplet sizes ranging from 20 to 24 μm, whereas lower ratios (5:95) yielded smaller droplets measuring 7 to 9 μm. Curcumin exhibited a contact angle of 175.3° at pH 3 and 115.7° at a pH 7, indicating its potential suitability for stability W/O emulsions. The study highlighted the efficacy of utilising polyphenol crystals to attach to the oil-water interface, thereby stabilising the emulsions. Additionally, the incorporation of 0.5 % WPI contributed to enhanced stability through the interaction between proteins and polyphenols (Zembyla, Murray and Sarkar, 2018). To summarise, this leads to the potential of these polyphenol crystals as natural W/O emulsifiers to be investigated further.

2.7 Double emulsions in low fat cheese

Several studies have investigated the use of double emulsions to improve the functional and textural properties of reduced fat cheese, many of which have involved the fortification of proteins of vitamins for additional health trends. Most studies reported promising outcomes with double emulsions. Table 2.10 summarises several papers that applied double

emulsions in low fat cheese to either enhance functionality or as a method for vitamin or mineral fortification. In nearly all cases, synthetic lipophilic emulsifier was utilised, and the majority employed skimmed milk as a secondary emulsifier, leveraging its naturally occurring proteins (whey and casein). Interestingly, Sharma Khanal *et al.* (2019) explored single emulsions to improve the characteristics of low fat mozzarella cheese. While the emulsion did not enhance the textural properties, some improvement in colour was observed. Notably, this approach involved single rather than double emulsions.

Some interesting and notable papers have successfully used double emulsions to improve the function and structure of low fat cheese. Paximada, Howarth and Dubey (2021) found that the use of 25 % whey protein in the inner water phase along with PGPR and Span 80 was able to successfully create skimmed milk double emulsions. These were added to cheese milk and the structure and functional attributes were investigated. The results found that double emulsion cheeses had improved structure and texture compared to that of low fat control. However, this study lacked sensory evaluation of cheeses. Similarly, Leong *et al.* (2018) investigated the use of double and single emulsions, using canola oil to incorporate whey protein fortification in Cheddar cheese analogues using 2 % PGPR. The study found that double emulsions were successfully incorporated into cheese and were matured for 7 months, although these double emulsion cheeses did have a higher firmness and lower meltability compared to the control. Gamlath *et al.* (2023) also investigated the use of double emulsions with reduced amounts of surfactant, with a combination of PGPR and soy lecithin, and successfully incorporated these emulsions into cooked cheese curds, suggesting future research to investigating their sensory attributes.

Table 2.10 A summary of double emulsion technologies used in reduced and low fat cheeses.

Research	Cheese	Oil Type	Lipophilic Surfactant	Hydrophilic Surfactant	Outcome	Citation
Reducing fat using $W_1/O/W_2$	White fresh cheese	Canola oil	PGPR	Panodan	Hydrocolloid interactions caused a significant difference in the spatial arrangement of $W_1/O/W_2$ droplets Textural properties and the microstructure were altered when using DE. DE cheeses were harder but not significantly different from full fat	(Lobato-Calleros *et al.*, 2008)
Reducing fat using O/W and $W_1/O/W_2$	Cheddar	Canola oil	PGPR	Skimmed milk	Aroma of emulsion cheeses was notably different. DE cheese was the hardest but had highest water content entrapped in DE technology and not freely available to aid softening of the cheese. Single O/W emulsion was the softest	(Leong *et al.*, 2020)

Research	Cheese	Oil Type	Lipophilic Surfactant	Hydrophilic Surfactant	Outcome	Citation
Encapsulation of *Lactobacillus plantarum*	Oxaca	Canola oil	PGPR	Panodan, mesquite gum, maltodextrin and Gum Arabic	Successfully entrapped *Lactobacillus plantarum* in $W_1/O/W_2$ and made into Oxaca cheese	(Rodríguez-Huezo *et al.*, 2014)
Fortification of cheese with Vitamin B_{12}	Model cheese	Butter oil	PGPR	Skimmed milk	Successfully encapsulated Vitamin B_{12} to fortify model cheese.	(Giroux *et al.*, 2013)
Fortifying $W_1/O/W_2$ with protein in cheese	Cheese curds	Sunflower oil	PGPR Lecithin	Whey Protein Concentrate Skimmed Milk	Whey protein was successfully fortified in $W_1/O/W_2$ using reduced or minimal amounts of surfactants and were successfully incorporated into cheese.	(Gamlath *et al.*, 2023)
Fortification of cheese with antioxidants (polyphenols)	Chihuahua	Canola oil	PGPR	Panodan	Successfully encapsulated the polyphenols in cheese and protect the antioxidant through simulated gastro-intestinal action.	(Pimentel-González *et al.*, 2015)

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Research	Cheese	Oil Type	Lipophilic Surfactant	Hydrophilic Surfactant	Outcome	Citation
Reducing fat content and fortification with protein (Whey, Pea and Rice proteins)	Cheddar	Anhydrous milk fat	PGPR Span 80	Skimmed Milk	Successfully fortified $W_1/O/W_2$ with protein. DE cheeses improved the function and structure compared to that of the low-fat control.	(Paximada, Howarth and Dubey, 2021)

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2.8 Research gap and research aim

Extensive research has focussed on double emulsions and their application in low fat and reduced fat products, aiming to enhance functional and sensory attributes compared to traditional low fat alternatives. Several studies have explored the use of fat mimetics and replacers in cheese, with few employing double emulsion systems. Many of these studies have relied on synthetic lipophilic surfactants to stabilise W/O emulsions. Others have replaced milk fat with vegetable fats to increase unsaturated fatty acids. Notable contributions include the work of Leong *et al.* (2020) using canola oil and Paximada, Howarth and Dubey (2021), who used double emulsions in Cheddar cheese to improve the characteristics of low fat cheese which are often deemed as undesirable by consumers. Gamlath *et al.* (2023) used a combination of PGPR and soy lecithin to produce double emulsions and incorporate it into cooked cheese curds. These three notable studies found positive results with the use of double emulsion technology that are incorporated into cheese for dairy application. However, these studies did not include the sensory evaluation of the final product to determine the implications of this technology on the final product.

A significant gap exists in identifying natural lipophilic surfactants that can effectively stabilise W/O emulsions within double emulsion systems. As currently the research predominately relies on synthetic surfactants, with limited exploration into the potential of reducing synthetic surfactant use by partially replacing them with natural alternatives in a dairy application. Achieving small W/O droplets stabilised by natural surfactants presents a challenge, especially in creating droplets suitable for integration into a double emulsion system for cheese production. A partial replacement approach could address the consumer demand for cleaner labels and healthier products while maintaining the functionality of the emulsions. Furthermore, while there is some research on the use of double emulsion in Cheddar cheese, there is a gap in the use of double emulsions with native milk fat and critically evaluating the sensory characteristics of cheese which has incorporated the novel double emulsion technology.

To address these gaps and contribute to literature the overarching aim of this thesis is to advance the use of natural surfactants in skimmed milk double emulsions, focussing on improving the quality and consumer acceptability of reduced fat cheese using this designed novel technology. The objectives are:

Objective 1: Evaluating the efficacy of natural surfactants including polyphenol crystals and sunflower lecithin in stabilising water-in-sunflower oil emulsions (Chapter 4)

Objective 2: Exploring the potential of sunflower lecithin as a lipophilic surfactant in water-in-milk fat emulsions and assessing the feasibility of partially replacing synthetic surfactants with sunflower lecithin (Chapter 5)

Objective 3: Evaluating the use of the designed primary emulsions from Chapter 5 into skimmed milk double emulsions and optimising production methods for further application (Chapter 6)

Objective 4: Investigating the developed double emulsions from Chapter 6 in reduced fat Cheddar production and evaluating the functional and sensory characteristics double emulsion technology has on improving reduced fat cheeses (Chapter 7).

Overall thesis hypothesis:

Dairy based double emulsions made with natural or partially natural lipophilic surfactants will improve the sensory and functional characteristics of reduced fat cheese.

CHAPTER THREE – GENERAL METHODOLOGY

3.1 Materials

The following materials were used in this study; 100g of polyphenol crystals (curcumin and quercetin) donated from Direct Foods (Macclesfield, UK). Two kilograms of LeciTAs® 4437 sunflower lecithin, with a HLB value of 4.0 donated from Thew Arnott (Flintshire, UK). One kilogram of Palsgaard® Polyglycerol-polyricinoleate (PGPR) 4150 with a HLB value of 3.1 donated from Palsgaard (Denmark). Sunflower oil was purchased from a local supermarket, skimmed milk (0.1% fat) and whole milk (3.7% fat) were purchased from Wells Farm Dairy. Four kilograms of anhydrous milk fat (AMF; pure butter ghee) was donated by County Milks (UK) and additional was purchased from a local supermarket. Mesophilic cultures (MESO O and MESO O2) containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and vegetarian rennet were all purchased from Cheese and Yogurt Making (Kent, UK). All percentages for water and oil phases listed are weight/total weight of the emulsion and percentages of the components within the oil or water phases are given as a percentage weight of the total emulsion.

3.2 Methods

The following sections describe the methods used for the primary and double emulsions and their subsequent characterisation. Characterisation techniques were the same for primary and secondary emulsions, unless stated otherwise. The Cheddar cheese method was used for all cheese trials and characterisation of the cheeses was the same, unless stated otherwise.

3.2.1 Primary emulsion production

Primary emulsions were produced to a ratio of 60:40 oil/fat to water ratio. This ratio was chosen after preliminary experiments investigating the impact of ratio of the phases found that this resulted in the smaller droplet size and low serum index. The oil/fat phase components included varying concentrations and ratio of surfactants, Polyphenol crystals, sunflower lecithin and PGPR, as stated in Chapter 4, investigating the W/O surfactant in sunflower oil and Chapter 5 investigating W/O surfactant in milk fat.

AMF was heated to 50°C to ensure fat had completely melted before the addition of surfactant. The surfactant was added to sunflower oil/AMF at 50 °C and stirred using a magnetic hot plate stirrer at 450 rpm for one hour.

Emulsions were produced in 250 g samples, unless stated otherwise. Deionised water was added to the oil phase by pipette at 1,000 rpm on the Silverson High Shear Mixer

(Chesham, UK) and then increased to 4,000 rpm for 5 minutes until combined. Temperature was recorded using a temperature probe and sample was placed in a cool water bath (approx. 10°C) before undergoing ultrasonic homogenisation using a Branson Ultrasonic cell Disrupter (SFX550, 20kHz, max. 550W, Branson, UK) with a 12.7 mm horn at a 70% amplitude for 5 minutes (10-, 15- and 20-minute ultrasound times were used in some experiments). Joules (J) and Watt (W) results were recorded for each sample as well as temperature prior and post ultrasonication to monitor temperature increase.

3.2.2 Double Emulsion (DE) production ($W_1/O/W_2$)

The secondary phase (W_2) was skimmed milk for all double emulsions. Milk contains casein and whey proteins that act as the secondary hydrophilic emulsifier.

Double emulsions were created to a starting ratio of 20:80 ($W_1/O:W_2$), but differing ratios explored in Chapter 6, emulsions were created in 250 g samples for experiments in Chapter 6 whereas for Chapter 7 double emulsions were made in 1.5 L batches for cheese production. The primary emulsion was made two hours before double emulsification, investigated in Chapter 5.

3.2.3 Emulsion droplet size measurements and microstructure evaluation

Ten grams of emulsion were taken for microstructure analysis using optical light microscopy. Numerous images of each sample were obtained and processed further using Image J software (Java, NIH Image). A sample was placed on a 0.22 mm glass microscope slide with a 0.17 mm cover slip and observed under varying magnifications (x 4, x 10 and x 40) using a Zeiss Primostar 3 Optical Microscope (Germany) and a Motic Microscope Camera (Europe). For each repetition, the diameter of 600 droplets of each replicate was measured providing 1800 droplet measurements in total. Data were further processed in Excel (Microsoft) using the following equations to determine the Sauter mean (Equation 11) and volume weighted means (Equation 12), where n_i is the number of droplets of diameter d_i.

Equation 11 – Sauter mean ($D_{3,2}$)	Equation 12 – Volume weighted mean ($D_{4,3}$)
$d_{3,2} = \frac{\sum n_i d^3}{\sum n_i d^2}$	$d_{4,3} = \frac{\sum n_i d^4}{\sum n_i d^3}$

(Coupland and Julian McClements, 2001)

Calculation of droplet size distribution from the data was based on volume frequency by percentage based on the assumptions that the droplets are spherical, and the distribution is log normal.

3.2.4 Serum Index

Emulsion stability during storage was determined using a similar procedure as Paximada, Howarth and Dubey (2021), where samples were stored in the fridge (~ 4 °C) and the serum layers were measured using a ruler on day 1, 3, 5, 10, 15, 20 and 30 for W/O emulsions and measured at 30 min, 1 h, 2 h, day 1, 3, 5 and 7 for double emulsions. Serum Index (SI) was calculated using equation 13:

Equation 13 – Serum Index (SI)
$SI\ (\%) = \frac{H_s}{H_e}\ x\ 100$

(Paximada, Howarth and Dubey, 2021)

3.2.5 Polydispersity Index

Polydispersity index (PDI) was calculated from the droplet analysis following similar analysis as described by Paximada, Howarth and Dubey (2021) using equation 3:

Equation 3 – Polydispersity index (PDI)
$PDI = \frac{D_{90-}D_{10}}{D_{50}}$

(Paximada *et al.*, 2016)

D_{10}, D_{50}, and D_{90} are the percentile values which indicate the size of the particle below which 10% (D_{10}), 50% (D_{50}) and 90% (D_{90}) of all particles are found.

3.2.6 Confocal Fluorescence microscopy

Confocal Fluorescence microscopy (at the University of Warwick) was used to confirm the creation of double emulsion in milk emulsions and cheese. Nile red dye was used to stain the fat phase and was added to the fat phase prior to primary emulsification for the identification of the creation of double emulsion and Fast Green dye was diluted in distilled water to stain the aqueous phase. For identifying double emulsions in cheese, a similar method to that described in Leong *et al.* (2020) was used, which involved Nile red [NR] (NR; 1 mg mL^{-1} in dimethylsulphoxide) diluted 10 times with distilled water, and Fast Green FCF (0.1 mg mL^{-1} in distilled water). Cheese was cut into 2 mm cubes and placed in a petri dish. NR dye was added to the cheese and left for 5 minutes, excess dye was removed with a pipette, followed by the addition of Fast Green for 5 minutes, after which dye was removed. Samples were then placed on a microscope slide and the dyes were excited at 633 nm (Fast Green FCF) and 488 nm (NR) with emission filters set at 660 – 750 nm (Fast Green FCF) and 520 – 590 nm (NR). Images were then processed through Image J software.

3.2.9 Cheddar Cheese Production

3.2.9.1 Cheddar Production

Thirty litres of milk was heated to 32 °C in a 50 L Jongia (Solihull, UK) cheese vat. Double emulsion (1.5 L) was added and mixed into the milk and stirred by hand. A pH reading was taken, using a HANNA Cheese pH tester (Bedforshire, UK), this reading was taken prior to starter culture addition, as was a sample for titratable acidity (3.2.9.2). Mesophilic starter culture (Meso O and Meso O2, from Cheese and Yogurt Making, Kent, UK) was added at 0.05 g per 1 L, allowed to rehydrate on the surface for two minutes before gentle stirring by hand, and left for 50 minutes during which the milk was agitated gently to prevent the double emulsion from separating. Following starter culture addition, pH and titratable acidity values were recorded. Vegetarian rennet (Cheese and Yogurt Making, Kent, UK) was added at a dilution rate of 4:16 mL, rennet to water per 10 L and allowed to coagulate for 30 minutes. After, the curd was tested to ensure a gel-like coagulum had formed, then cut using a 1 cm square cheese wire knife. Curds were heated to 38 °C held for 10 minutes, stirring continuously, and whey was removed once a pH of 6.3 had been reached. The cheddaring process followed, with a series of cuts and turns of cheese curds, monitoring pH throughout. Once a pH of < 5.4 was reached, curds were broken by hand into ~ 1 cm cubes and salted at a 3 % concentration. Curds were placed into 1 kg Laude Gouda semi-permeated cheese moulds and placed in the pneumatic pressure press at 10 psi for an hour. Cheeses were turned and pressed for a further 19 hours at 40 psi. Following pressing, each cheese was removed from its mould, a pH reading was taken, each cheese wheel was vacuum packed using a Microprocessor Controller MCV-011, cheeses were placed in the maturing cabinet (Staginello) at 12 °C, 40 % relative humidity and turned every 3 days. Samples from the cheese wheels were taken at 4 weeks for analysis.

3.2.9.2 Acidity of Milk

Acidity was monitored during the cheese making process using a HANNA H199161 pH probe and titratable acidity was calculated by using 10 mL of milk with 1 mL (3 drops) of Phenolphthalein indicator and recording the amount of Sodium hydroxide (NaOH) used to turn the solution a pale pink. Titratable acidity was calculated using equation 14, and samples were recorded in triplicate.

Equation 14 – Titratable acidity
$TA\ (\%) = \frac{0.09\ x\ Amount\ of\ NaOH}{Amount\ of\ milk\ (mL)}\ x\ 100$

3.2.9.3 Cheese yield

Cheese yield was calculated using equation 15, adapted from Paximada, Howarth and Dubey (2021).

Equation 15 – Cheese yield
Cheese Yield (%) = $\frac{Weight\ of\ cheese\ (kg)}{Weight\ of\ milk\ (kg)}\ x\ 100$

3.2.10 Cheese Composition

Cheese composition which included fat by Gerber, protein, moisture, water activity and salt was conducted by ALS Laboratories (Chatteris, UK). Samples were sent to ALS in triplicate and results recorded in Chapter 7.

3.2.11 Melting Profile

Melting profile was performed with modification described by Paximada, Howarth and Dubey (2021) and Altan, Turhan and Gunasekaran (2005). Each sample was cut into 20 mm cubes, placed on a baking tray and measured using concentric circles in a 10 mm diameter increments. Samples were transferred to a preheated oven at 200 °C for 10 minutes and then left to cool to room temperature (20 °C) for 20 minutes. The diameter of the cheese was measured at three different points prior and post heat, and the percentage increase was calculated.

3.2.12 Oil Loss

Oil loss was measured as described by Ramel and Marangoni (2017) using a grade 4 Whatman filter paper and following equation 16, where W_f is the final weight of the paper after seven days of storage, W_i is the weight of the paper before adding cheese, W_{cf} is the final weight of the empty paper and W_{ci} is the initial weight of the empty paper.

Equation 16 – Oil loss
Oil Loss (%) = $\frac{(W_f - W_i) - (W_{cf} - W_{ci})}{W_i}$

3.2.13 Texture Analysis

Firmness and springiness of cheese were measured using TA. HD Plus Texture Analyser (Stable Micro Systems, UK), with a P/3 probe, a 3 mm cylinder and a 30 kg load cell. Cheese was cut into 3 cm cubes, with two repetitions on the same cube and for each wheel produced three cubes were taken. The texture analyser was set at a pre speed of 1 mm/s,

speed of 1 mm/s and post speed of 10 mm/s, with the probe held at 5 mm depth for 60 seconds. Firmness was measured by force (g) of the probe to enter the cheese and springiness was expressed as a percentage.

3.2.14 Microbiological Analysis

Microbiological analysis was carried out by ALS Laboratories (Shrewsbury, UK). Cheese samples were sent for microbiological analysis to ensure that they were within the legal limits of *Enterobacteriaceae,* Coagulase positive staphylococci, *Listeria monocytogenes, Escherichia coli, Bacillus cereus,* Sulphite reducing clostridia and *Salmonella*, before being used for sensory evaluation. Results for microbiological analysis (Appendix 1) and reference criteria (Appendix 2) were recorded.

3.2.15 Sensory Evaluation

Sensory evaluation was carried out using a rapid descriptive sensory method known as flash profile. This sensory testing method involves evaluation of the entire sample set simultaneously, based on a set of attributes generated by the panellists, and involves ranking each sample against one another. Samples were ranked 1 to 6, with 1 being least intense and 6 being intense for that characteristic. Flash profile utilises free vocabulary allowing the panellists to select attributes, which is particularly useful for exploratory studies and new products when sensory attributes are being extracted from the sample. This method enables researchers to investigate similarities and differences between samples in a 'rapid' manner utilising naïve consumers or skilled panellists to judge (Juemanee *et al.*, 2018; Liu *et al.*, 2018). Unlike conventional methods such as descriptive analysis, which often require extensive training, flash profile can be implemented more efficiently (Gkatzionis *et al.*, 2013; Petit, 2023) and with a smaller number of panellists. Heymann *et al.* (2012) suggested that a panel size of 8 to 10 is sufficient for exploratory profiling, which supports the validity of the sample size.

A total of 14 screened panellists were recruited. These panellists were in good health and regular consumers of cheese. They attended a 2-hour training session to develop the sensory characteristics perceived from the cheese range (low, medium, high fat cheeses as well as the experimental cheeses) and to familiarise with the flash profile method. This ensured a combined understanding of each characteristic and the evaluation procedures. This training session was a slight modification of the original flash profiling method to reduce repetitive sensory terms and to increase efficiency and reliability of the sensory profiling results (Juemanee *et al.*, 2018). During the session, perceived sensory characteristics describing similarities and differences among the cheese samples were listed by the order of perception and evaluation in separated categories of; smelling the sample; visually

observing the sample; texture by hand; texture in the mouth; followed by flavours and aftertaste. The chosen descriptors and explanations provided by the panellists are detailedin Tables 3.1 to 3.5. The descriptions of the sensory characteristics were defined through discussions during training and supplemented by a review of existing literature on cheese sensory evaluation (Fenelon *et al.*, 2000; Drake, 2007; Rogers *et al.*, 2009; Møller *et al.*, 2013).

Table 3.1 "Sniffing" sensory descriptors given to panellists

Sensory descriptor	**Description**
Yeasty	Bread-like, farmy or mild silage
Burnt caramelised smell	Burnt and sweet smell
Cooked milk	Milky, mixture of heated protein and sugar smell
Sour	Acidic, lactic, sharp
Fruity	Pineapple, butyric acid smell
Nutty	Mushroom, nutty, earthy tones
Cheddar	Smells like cheddar cheese
Packaging smell	Metallic, plastic or other chemical smells
Sweaty cheese	Warmed cheese, musty, oxidised cheese smell.

1672 **Table 3.2 “Visual” sensory descriptors given to panellists**

Sensory descriptor	Description
Glossiness	Shiny, light reflecting, oily look, a sheen to the surface
Dense appearance	Closed texture, no eye-pockets
Yellow	Colour ranging from white to cream to pale yellow
Sweaty appearance	Small droplets of liquid on the surface
Rubbery look	Chewy, springy, sticky look
Translucent	Cut the sample in two halves and see the light through
Smooth surface	On the cut surface, the colour and texture homogeneity.

1673

1674 **Table 3.3 “Texture by hand” sensory descriptors given to panellists**

Sensory descriptor	Description
Bounciness	Chewy, rubbery feel, jelly-like
Moist	Oily sensation on the fingers
Paste-like on fingers	Cheese turned to a paste upon squeezing
Hardness	Pressing a finger on the cube, resistance to pressing force, firmness.
Waxy texture	Like wax on pressing the cut cheese.

1675

Table 3.4 "Texture in the mouth" sensory descriptors given to panellists

Sensory descriptor	Description
Hardness	First bite firmness, resistance to the bite.
Grainy texture	Gritty, small hard particles while chewing
Creaminess	Rich moistness, thick fat-like, lubricating feel while chewing
Powdery	Dryness at the end of the chew.

Table 3.5 "Flavours and after taste" sensory descriptors given to panellists

Sensory descriptor	Description
Cooked milk	Tastes like warmed cheese or heated milk, slight oxidised flavour
Nutty	Nutty, mushroom or earthy tones
Sharp	Tangy, acidic, sour taste
Cheesy	Aged cheddar flavour
Burnt caramelised flavour	Mixture of heated sugar and burnt milk
Bitter aftertaste	Bitter taste left over, feeling dry in the throat after chewing.

Following training, panellists were asked to return on a separate day to assess the set of six cheeses (five experimental and one commercial cheese samples), during a 2-hour session that included a short break. The study comprised two replications, each using different 3-digit randomised sample codes to reduce order effects. During the session panellists were asked to rank their perceived intensity of the six samples, per descriptor at a time. If the panellists did not detect the presence of any descriptor on any sample, they were instructed to put in the ranked score of 1 (the least intense sample); it was also possible to rank more than one sample in the same ranking position. By enabling tied ranking of samples, key

sensory characteristics among the cheese samples would be more distinctive (Gkatzionis *et al.*, 2013). The Flash profile test ballot was designed, pre-tested and implemented using Compusense® software (Compusense, Inc., Guelph, ON, Canada).

Data analysis was conducted using a combination of methods. Generalised Procrustes Analysis (GPA) was used to visualise similarities and differences in sensory space between samples. Procrustes ANOVA (PANOVA) tested for panel consistency across replications. Where applicable, ANOVA was used to compare mean ranked intensities (standard deviations) across samples for individual descriptors. These statistical tools provided a robust framework to assess panellist agreement and interpret the multidimensional sensory space.

The test was conducted in the sensory science laboratory at Harper Adams University, using individual booths and Compusense® software (Compusense Inc., Guelph, Canada). Cheese samples were cut into 1.5cm cubes (3 per sample), served in coded lidded containers. Panellists were provided with palate cleansers (water, cucumber sticks and crackers) between samples (Till *et al.*, 2019). All procedures were approved by Harper Adams University Research Ethics Committee.

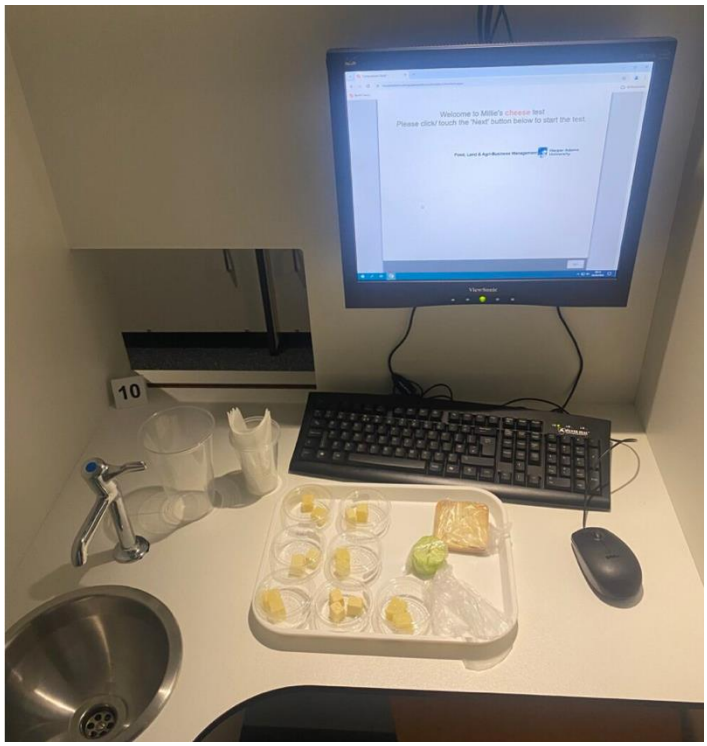


Figure 3.1 – Image of the sensory booths set up for panellists

3.2.16 Statistical analysis

All statistical analyses were conducted to ensure that appropriate methods were applied for reach dataset, based on distribution characteristics and research objectives. The significance level was set at $P < 0.05$. Analyses were performed using IBM SPSS Statistics (Version 29), XLSTAT (Microsoft, 2011) and RStudio.

When assessing if datasets were normally distributed, the Shapiro-Wilk test was applied. Where results indicated normality ($P > 0.05$), parametric tests were used:

- One-way Analysis of Variance (ANOVA) was employed to compare means between groups
- Tukey's Honestly Significant Difference (HSD) post hoc test was used to identify pairwise differences where ANOVA results were significant ($P < 0.05$)

For those which were non-normally distributed ($P < 0.05$), non-parametric alternatives were applied:

- Kruskal Wallis test was used to compare medians between groups
- If significance ($P < 0.05$) was observed, Dunn's post hoc test with Bonferroni correction was used to locate specific differences.

Sensory analysis data, as discussed in section 3.2.15, the data was obtained from flash profiling and analysed using multivariate and variance-based techniques:

- Generalised Procrustes Analysis (GPA) was conducted to visualise differences and similarities among cheese samples based on panellist sensory configurations
- Procrustes Analysis of Variance (PANOVA) was used to assess panellist consistency across replications and to test the robustness of the consensus space.

Additionally, where relevant, ANOVA was used to analyse mean ranked intensities of sensory descriptors, accompanied by standard deviation to reflect panellist variation.

To evaluate relationships between variables, the following correlation and trend analysis was used:

- Simple linear regression was performed to investigate key relationships (e.g. between emulsifier concentration and viscosity in Chapter 5). Where applicable, the coefficient of determination R^2 was reported to indicate the proportion of variance explained by the regression model.
- Pearson's correlation coefficient was used to determine the strength of association between fat content and cheese yield (Chapter 7)

CHAPTER FOUR: FINDING A NATURAL LIPOPHILIC EMULSIFIER FOR A WATER-IN-OIL EMULSION

4.1 Introduction

As outlined in Chapter 2, consumer demand for natural surfactants has driven food researchers to reduce the use of synthetic surfactants in food applications. Natural surfactants for stabilising O/W emulsions such as whey proteins, caseins and vegetable proteins have been successfully proven (Kralova, 2009). However, the key to addressing the research gap explained in Chapter 2 lies in finding a natural alternative to PGPR, a synthetic surfactant used for stabilising W/O emulsions. A surfactant with a Hydrophilic-Lipophilic balance (HLB) of below 4.0 is required. Research suggests that phospholipids, known as lecithin, obtained and modified from plants like sunflower, rapeseed and soy, could serve as a complete or partial replacement for PGPR. This chapter explores the potential of sunflower lecithin and polyphenols for stabilising W/O emulsions.

Polyphenols include flavonoids, tannins and phenolic acid compounds, which can be sourced from plants (Williamson, 2017). Examples include curcumin, derived from the *Curcuma longa* plant and quercetin, found in the skin of fruits and vegetables (Li and Tian, 2007). These compounds offer numerous health benefits, such as antioxidant, antimicrobial and anti-inflammatory properties, and have been used in food fortification. For instance, Giroux *et al.* (2013) successfully fortified Cheddar cheese with green tea using double emulsions.

Polyphenols have also shown promise in stabilising W/O emulsions. For example Luo *et al.* (2019) used tea polyphenol palmitate (Tp-P) to stabilise a W/O emulsion, achieving small droplets which enhanced stability by increasing Tp-P concentration. Wang, Bai and Shao (2020) also found that tea polyphenols combined with gelatine stabilised emulsions effectively. These studies create Pickering emulsions, which are emulsions that are stabilised by solid particles that form a thick protective film at the oil-water interface, preventing coalescence (Cheng and Wang, 2013; Duffas *et al.*, 2016; Jafari *et al.*, 2020; Sarkar and Dickinson, 2020). Various solid particles including chitosan, cellulose, fat crystals, proteins and polyphenol crystals have been used to stabilise emulsions (Cheng and Wang, 2013; Norton, Fryer and Norton, 2013; Xiao, Li and Huang, 2016; Jiang, Sheng and Ngai, 2020). A notable study by Zembyla *et al.* (2019) used polyphenol crystals (curcumin and quercetin) alongside whey protein to stabilise W/O emulsions via the Pickering mechanism. They achieved smaller droplet sizes with curcumin at a concentration of 0.14 % wt., demonstrating its potential for stabilising W/O emulsions.

Phospholipids, being amphiphilic molecules, can serve as a surfactant for stabilising emulsions. Lecithin, which can be derived from animal or plant sources, is referred to as a mixture of phospholipids, and has been utilised in the food industry as previously discussed in Chapter 2. As mentioned in section 2.6.4.1 a study by Levin, Burrington and Hartel (2016) looked at Whey Protein Phospholipid Concentrate (WPPC) and discussed the benefit this had on stabilising emulsions in ice cream. A preliminary investigation not discussed in this thesis, involved investigation into WPPC as a W/O, but issues arose when WPPC did not fully dissolve within the oil phase, due to the high protein content. Further purification of the powder could have been investigated to separate the phospholipids from the protein to see the efficacy. However, due to time and availability of resources at Harper Adams University, this was not explored further.

Despite this, research surrounding sunflower lecithin provided a more viable option for investigation, as it has a recognised E number and is used in the food industry. Utilised within margarine and fat spreads to aid anti-splattering during frying and used in chocolate to aid the flow index for enrobing products (van Nieuwenhuyzen and Szuhaj, 1998). The additional benefit of sunflower and rapeseed lecithin compared to that of soy, is that it is not genetically modified and is classed as hypoallergenic (van Nieuwenhuyzen and Szuhaj, 1998; Ying *et al.*, 2021).

In addition to surfactants, as discussed, the type of homogenisation method used when creating emulsions can influence the final droplet size, thus ultimately impacting emulsion stability. With ultrasonication, as previously discussed in Chapter 2, the alteration of ultrasonic parameters can influence the droplet size. O'Sullivan *et al.* (2015) found that an increase in production time, caused a reduction in emulsion droplet size, similarly so did Truong *et al.* (2016) with treatment temperature having an impact on emulsion production.

4.1.1 Aim and objectives

The aim of the current chapter was to find an alternative to PGPR and evaluate the use of the natural alternatives, such as polyphenol crystals and sunflower lecithin as the lipophilic emulsifier in W/O emulsions. The desired droplet size for the W/O emulsion was to be < 1 μm. The objectives include:

- Developing the parameters of curcumin and quercetin to produce W/O emulsions in sunflower oil.
- Evaluating the use of curcumin and quercetin in W/O emulsions in sunflower oil and their potential in further applications.

- Developing the parameters required to produce W/O emulsions using sunflower lecithin in sunflower oil.
- Evaluating the parameters used to create W/O emulsions using sunflower lecithin and how they can be progressed into further application.

4.2 Materials and Methods

Sunflower oil was used as the fat phase for this chapter because of its consistency and cost-effectiveness, allowing for predictable and stable initial formulation trials before transitioning to milk fat. Emulsion production and characterisation procedures were carried out as described in Chapter 3, unless stated otherwise.

4.2.1 Grinding of Polyphenol crystals

One gram of polyphenol powder was placed in a marble pestle and mortar, following a similar procedure as Renza-Diaz *et al.* (2021) with a goal to reduce polyphenol particle size. The sample was ground by hand for 10 minutes, with a 0.2 g sample removed after 2, 5 and 10 minutes, for particle size analysis under the optical microscope and image processing with Image J (NIH Image). Samples were repeated in duplicate.

4.2.2 Production of Polyphenol stabilised W/O

Preparation of polyphenols underwent some preliminary investigation. First, a method by Zembyla *et al.* (2019) was used, whereby 0.14 % of curcumin (or quercetin) was added to the oil phase and homogenised using Silverson high shear mixer (Chesham, UK) at 9,400 rpm for 5 minutes. However, the temperature of the emulsions at the end of homogenisation was beyond the crystal melting points of 120 °C and 142.7 °C for curcumin and quercetin, respectively (Donsí *et al.*, 2010; Srinivas *et al.*, 2010; Kharat, Zhang and McClements, 2018). An alternative method was used, which consisted of placing oil and 0.14 % polyphenol crystal on a magnetic stirrer at 450 rpm, set at 40 °C for two hours following methods from Duffas *et al.* (2016) and Kharat, Zhang and McClements (2018).

A 50 mL W/O emulsion was then prepared with a 40:60 ratio of water to oil, and a concentration of 0.14 % polyphenol. Deionised water was pipetted into the oil phase while stirring at 1,000 rpm on the Silverson high shear mixer, then increasing to 4,000 rpm for 2 minutes followed by sonication at 65 % amplitude for 1 minute 30 seconds, while the sample was kept within 40 to 50 °C using an ice bath. Ten grams were taken for microstructure analysis and 15 g for the stability analysis.

4.2.3 Sunflower lecithin stabilised W/O

For comparison, sunflower lecithin (SL) stabilised emulsions were created using varying concentration of SL, in sunflower oil and made as per section 3.2.1 in Chapter 3 in the general materials and methods.

4.3 Results and Discussion

4.3.1 Polyphenols

4.3.1.1 Particle size distribution of polyphenol crystals

Curcumin crystals are displayed in a polyhedral form, with a mean width of 6.76 μm ± 3.34 μm, compared to the more elongated, rod-like shape quercetin with a mean length of 7.22 μm ± 6.35 μm (Figure 4.1 (a) and Figure 4.1 (b)). Vasisht *et al.* (2016) reported quercetin crystals measuring 8.5 μm. In contrast, the preparation methods described by Zembyla *et al.* (2019) yielded smaller crystal sizes, measuring 0.2 μm and 5.9 μm for curcumin and quercetin, respectively. Duffas *et al.* (2016) observed that larger crystals had a slower adsorption rate to the oil-water interface, potentially hindering the production of small droplets as the crystals did not quickly adsorb to the interface to stabilise them. Thus, achieving a smaller crystal size is essential to support the production of smaller emulsion droplets.

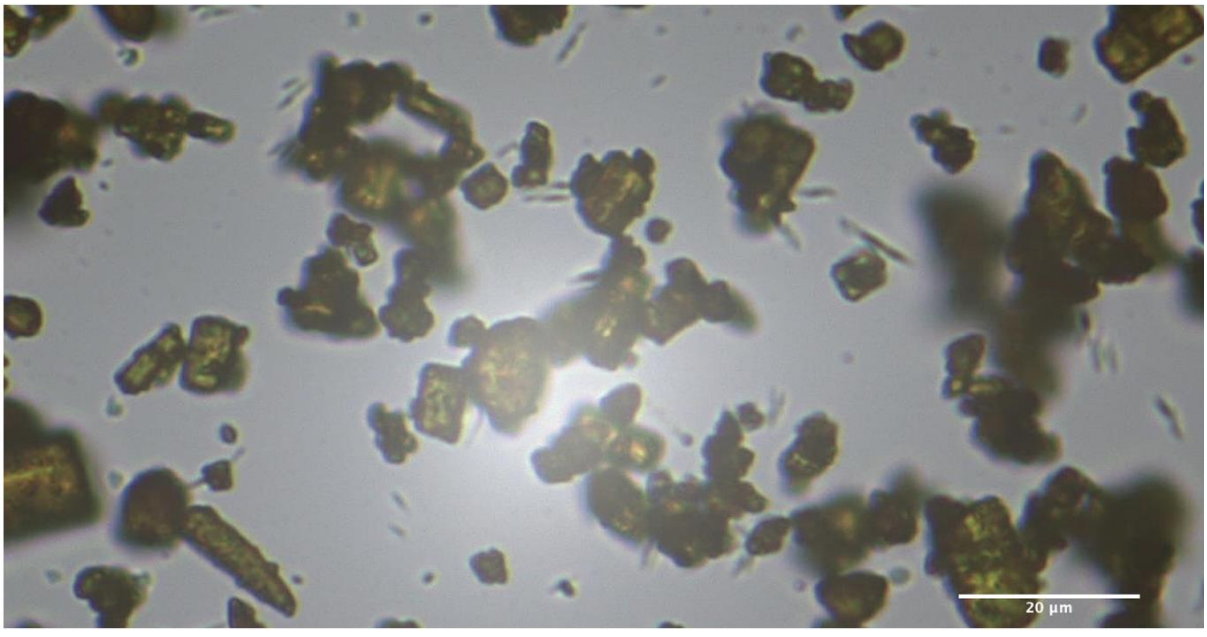


Figure 4.1 (a) Curcumin crystals under a 40 x magnification lens with a 20 μm scale.

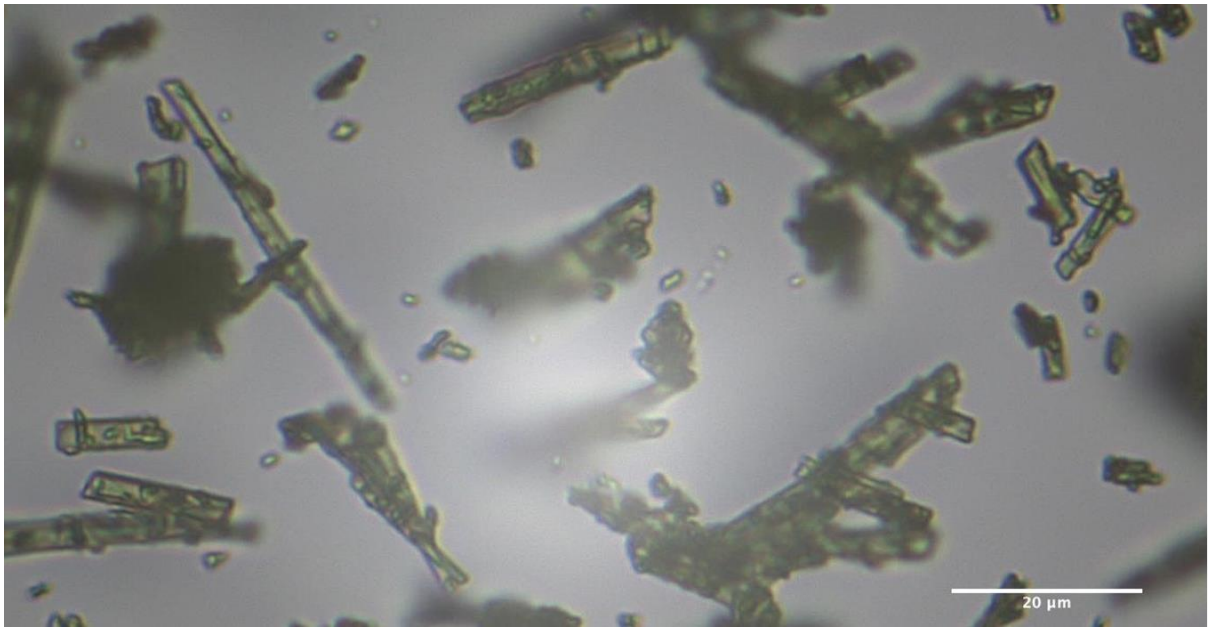


Figure 4.1 (b) Quercetin crystals under a 40 x magnification lens with a 20 µm scale.

4.3.1.2 Effect of grinding polyphenol crystals

Following the finding that the native sizes of the polyphenols were large, the manual grinding of the crystals aimed to reduce their size for further application as suitable surfactants in W/O emulsions. Table 4.1 presents the mean crystal sizes (± standard deviation) and polydispersity indices (PDI) for each grinding duration (0,2,5 and 10 minutes). Due to the non-normal distribution of the particle size data (confirmed via Shapiro-Wilk test), the non-parametric Kruskal-Wallis test was used to assess the effect of grinding time on particle size for each polyphenol. For curcumin, the Kruskal-Wallis test revealed a highly significant difference across the four grinding durations (χ^2= 541.53, df = 3, P < 0.05) indicating that grinding significantly affected particle size.

Post-hoc analysis using Dunn's test with Bonferroni correction showed significant differences between most pairwise comparisons (P < 0.05), except between the 5-minute and 10 minute treatments (P > 0.05), suggesting a plateau effect beyond 5 minutes. This indicates that 5 minutes of manual grinding may represent the practical threshold for curcumin size reduction under the applied conditions. This plateau is likely due to the polyhedral shape of curcumin crystals, which may resist further breakdown past a certain size during manual grinding.

In contrast, quercetin crystals also showed a significant reduction in size with increased grinding duration (Kruskal-Wallis = χ^2 = 524.06, df = 3, P < 0.05). Dunn's test indicated that while most pairwise comparisons were significantly different (P < 0.05), no significant

difference was found between the control and the 2-minute grind ($P > 0.05$). This suggests a slower initial rate of size reduction, likely due to the rod-shaped morphology of quercetin crystals. Unlike curcumin, quercetin continued to reduce in size up to 10 minutes, indicating that a grinding threshold was not yet reached within the tested range.

Table 4.1 Summary of mean crystal sizes for each grinding treatment *

	Grinding duration (mins)	Mean size (μm)	PDI
CURCUMIN	0	6.76 ± 3.34[a]	1.53 ± 0.04
	2	4.36 ± 3.14[b]	2.17 ± 0.17
	5	2.09 ± 1.27[b]	1.42 ± 0.41
	10	1.92 ± 1.13[c]	1.45 ± 0.20
QUERCETIN	0	7.22 ± 6.36 [a]	3.22 ± 0.95
	2	4.67 ± 3.69 [a]	3.73 ± 1.16
	5	3.07 ± 3.05 [b]	2.41 ± 0.16
	10	1.98 1.80 [c]	1.74 ± 0.11

*Data presented as mean ± standard deviation from three independent measurements. Due to non-normal distribution, the effect of grinding crystal size was assessed using the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction. Different superscript letters within each polyphenol indicate statistically significant differences between grinding durations ($P < 0.05$).

Both polyphenols displayed a general trend of decreasing crystal size and polydispersity index with increased grinding. However, the rate of reduction and threshold effects varied, likely influenced by the distinct crystal structures. These results underline the importance of crystal morphology in determining mechanical breakdown behaviour during manual processing.

Figure 4.2 shows the crystal size distribution by a number frequency, expressed as a percentage. The samples were considerably polydisperse, with a PDI ranging from 1.42 to

2.17 for curcumin and 1.74 to 3.73 for quercetin. However, the data indicates a noticeable shift to the left in size distribution when increasing the grinding time, indicating the presence of smaller crystals.

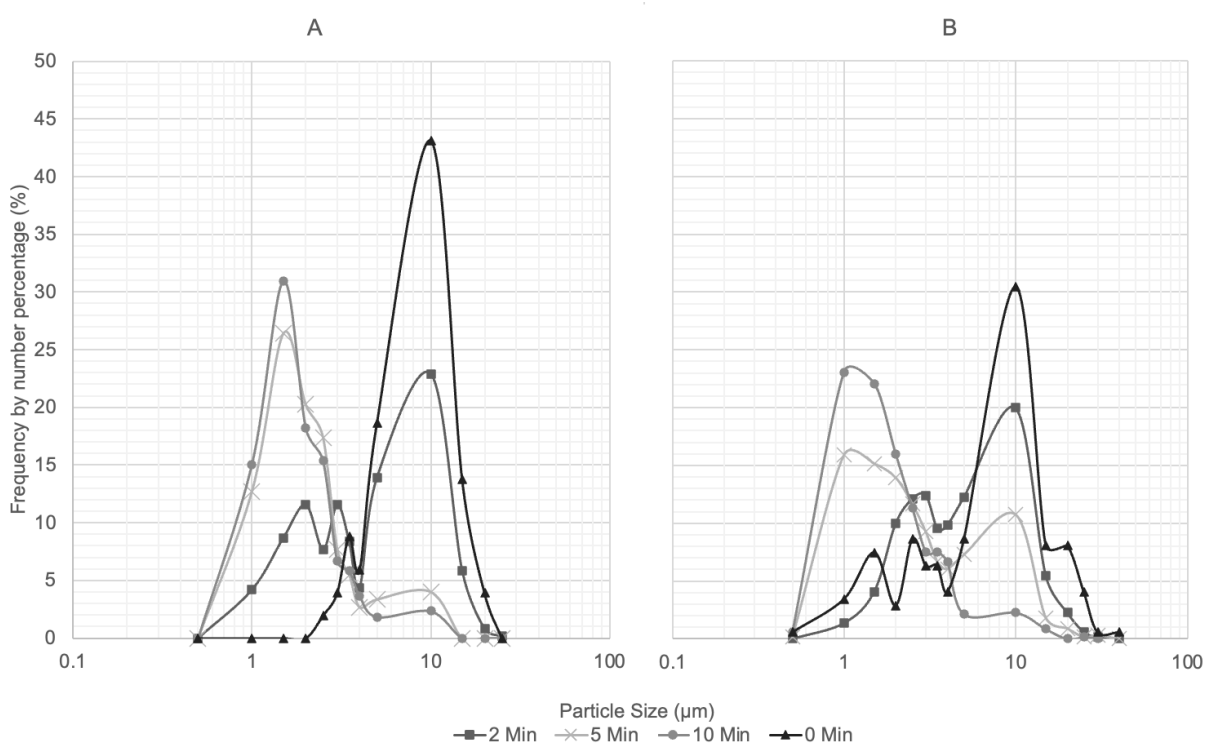


Figure 4.2 (A) Crystal size distribution of curcumin; (B) crystal size distribution of quercetin. Number frequency is expressed as a percentage. Data represent two independent experiments, with approximately 600 individual crystals measured in total

Overall, these findings indicate that as grinding time increased, a decrease in crystal size was found in both polyphenols. Duffas *et al.* (2016) demonstrated that sonication of flavonoids Rutin hydrate and Naringin reduced crystal sizes from 11.22 μm in their native state to 10.52 μm after sonication. Ideally, Pickering emulsion stabilisers should be significantly smaller than the target droplet size – typically by at least one order of magnitude, to promote effective stabilisation (Yusoff and Murray (2011). This enables dense particle packing at the oil – water interface, thereby reducing interfacial tension and improving droplet stability (Wu and Ma, 2016; Ortiz, 2020). Although achieving a a crystal size in the nanometre range was essential for further applications this was unfortunately not attained.

Polyphenol samples were submitted to the University of Leeds and processed using an analytical mill to achieve particle sizes smaller than those obtained manually. However, the

smallest size achieved was 0.9 μm (data not shown). Alternative milling methods, such as liquid-assisted grinding in a cryogenic mill, have been suggested to further reduce crystal sizes (González-González *et al.*, 2020; Habuš *et al.*, 2021). Nonetheless, these methods may compromise the suitability of crystals for food production by employing chemicals or temperature extremes that could contravene food safety regulations. Additionally, sonication may damage crystals, affecting their functionality. There is insufficient evidence to confirm whether smaller particles can be attained or if they would effectively stabilise water droplets to the desired size of less than 1 μm.

4.3.1.3 W/O emulsion using polyphenol crystals

After emulsification, the droplet size was measured by calculating the $D_{4,3}$, of the emulsion droplets (Table 4.2). The distribution of droplet sizes was assessed using the Shapiro-Wilk test, which indicated non-normality. Therefore, the non-parametric Kruskal-Wallis test was used to evaluate the effect of grinding time on droplet size for each polyphenol.

For quercetin, no significant difference in $D_{4,3}$ was observed across grinding times (Kruskal-Wallis χ_2= 4.92, df = 3, P > 0.05), suggesting that grinding duration did not affect emulsion droplet size. In contrast, curcumin showed a borderline significant effect of grinding time, (Kruskal-Wallis χ_2= 7.62, df = 3, P = 0.05), indicating a marginal influence on droplet size. Post-hoc Dunn's test with Bonferroni correction showed no significant pairwise differences between grinding durations. Longer grinding time generally corresponded to a reduced emulsion droplet size, except for quercetin ground for 2 minutes, which yielded an unusually large emulsion droplet of 320.86 μm ($D_{4,3}$).

Table 4.2 Mean volume-weighted diameter ($D_{4,3}$) for emulsions using ground polyphenol crystals*

	Grinding Time (mins)	$D_{4,3}$ (μm)
CURCUMIN	0	245.97 ± 200.04
	2	137.14 ± 19.92
	5	67.76 ± 20.09
	10	68.55 ± 7.30
QUERCETIN	0	234.46 ± 116.77
	2	320.86 ± 191.90
	5	149.47 ± 43.81
	10	81.81 ± 45.31

*Values are the mean and ± standard deviation from triplicate measurements

Zembyla *et al.* (2019) reported varying droplet sizes, with the smallest achieved being $D_{3,2}$ 10.3 μm for curcumin, which is notably smaller than observed in this study, where sizes exceeded for 60 μm for $D_{4,3}$. However, Luo *et al.* (2019) utilising Tp-P to stabilise W/O droplets, managed to achieve smaller droplets (3.07 μm) than those reported by Zembyla *et al.* (2019). Although Luo's study did not specify particle sizes, it suggests that different polyphenols may yield varying particle sizes to support droplets of that size. Tong *et al.* (2021) also employed green tea polyphenols in high internal phase Pickering emulsions (O/W) and noted a particle range of 122 to 712 nm, capable of stabilising emulsion droplets ranging from 100 to 400 nm. While this suggests a relatively close particle to droplet size ratio, it is possible that smaller fractions within the particle size range played a dominate role in stabilisation, or that other stabilising mechanisms were involved. Consistent with the findings of this study, larger droplets sizes were associated with increased susceptibility to coalescence and phase separation.

A notable correlation emerged between the grinding time of crystals and the droplet size ($D_{4,3}$) of emulsions containing curcumin, indicating that as grinding time increases, the volume of emulsion droplets decreases. This finding aligns with the notion proposed by Duffas *et al.* (2016) that smaller crystal sizes facilitate faster adsorption to the droplet

surface, thereby enhancing stability. The quercetin sample ground for 2 minutes appeared as an outlier. This discrepancy may arise from inadequate dispersion of crystals in the continuous phase resulting in instability, incomplete grinding during the 2-minute period, or a production error. Removing this sample from the experiment would likely result in quercetin following the same trend as curcumin. Furthermore, a comparison of quercetin and curcumin crystals in the grinding experiment revealed a significant difference between polyphenols, as expected due to their differing native shapes. However, when incorporated into emulsions, there was no significant difference in droplet sizes between the two polyphenols, suggesting that polyphenol type did not influence emulsion properties.

Despite observing this apparent relationship between crystal size and achieved emulsion droplet size, the emulsions remained unstable, with phase separation occurring in all samples. Two studies focusing on polyphenol-stabilised Pickering emulsion highlighted that larger droplet sizes increase the risk of coalescence and phase separation (Yang *et al.*, 2014; Tong *et al.*, 2021). It is plausible that coalescence phenomenon occurred in the samples, potentially due to inadequate coverage of the droplets. Furthermore, an increase in surfactant concentration was found to impact droplet stability (Tong *et al.*, 2021). However, Ilyasoglu Buyukkestelli and El (2019) reported no effect of the flavanol Caffeic-acid on creaming stability in O/W emulsions, thus no influence on phase separation. Notably the concentration of 0.14 % in Zembyla *et al.* (2019) was sufficient for droplet coverage, in this study employed this concentration (0.14 %) may have been insufficient for achieved (larger) droplet sizes.

Figures 4.3 (a) and 4.3 (b) illustrate the droplet distribution in the W/O emulsions for both curcumin and quercetin treatments, respectively. While there is an evident decrease in size,

with increasing grinding time, it is important to note that both emulsions exhibit significant polydispersity, which can be correlated with Table 4.3.

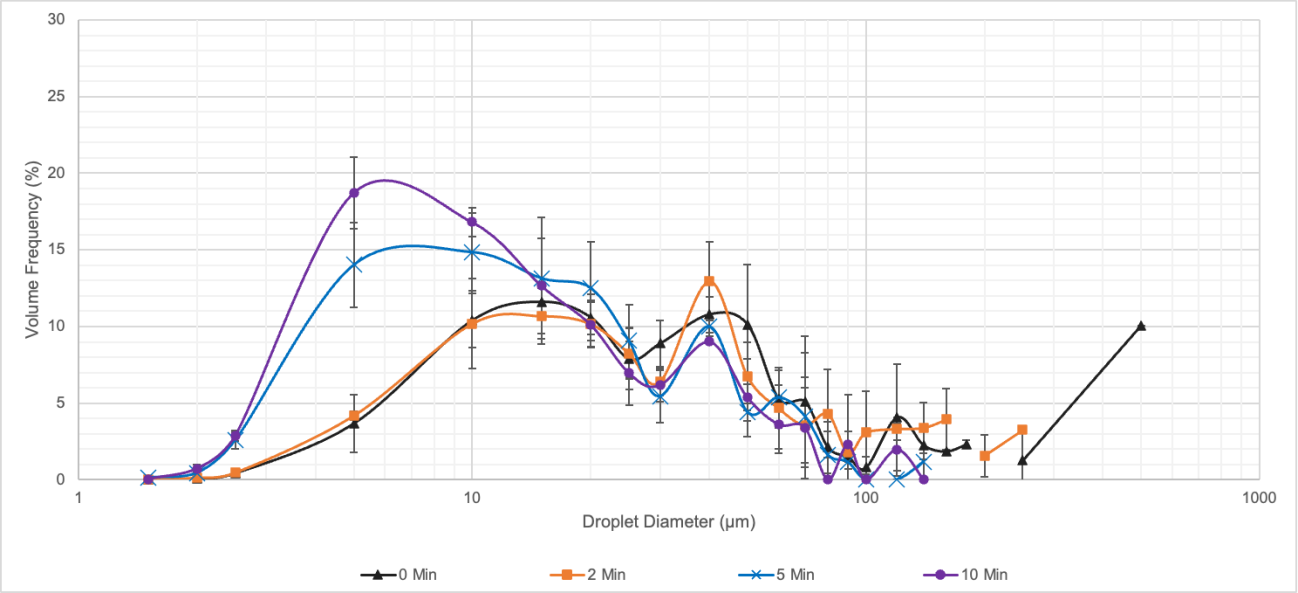


Figure 4.3 (a) Mean droplet size distribution by volume frequency (%) for Curcumin stabilised W/O emulsions. Error bars represent standard deviation of the mean volume frequency at each droplet diameter

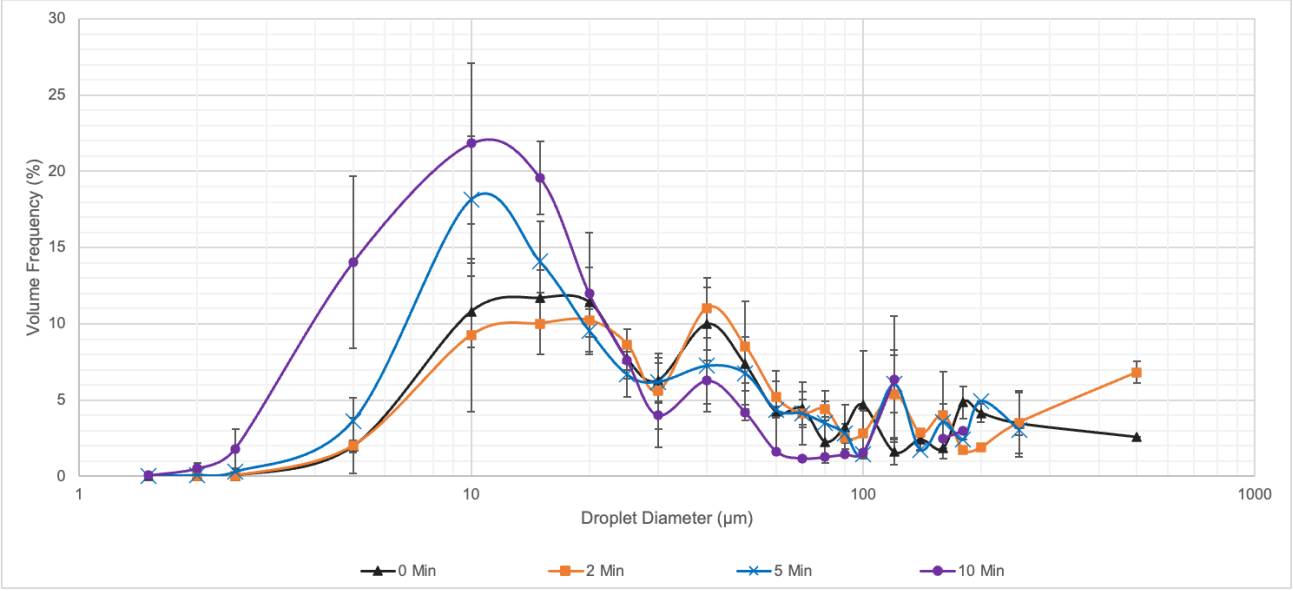


Figure 4.3 (b) Mean droplet size distribution by volume frequency (%) for Quercetin stabilised W/O emulsions. Error bars represent standard deviation of the mean volume frequency at each droplet diameter.

Table 4.3 displays the polydispersity index (PDI) values for the emulsion treatments, all of which significantly exceeded 1.0, indicating a high degree of polydispersity in droplet sizes. Specifically, curcumin at 0 minutes and quercetin at 5 minutes had the highest PDI values and the greatest variation in droplet sizes.

Table 4.3 – Mean polydispersity index (PDI) values for each emulsion treatment with polyphenols*.

	Grinding time (mins)	PDI
CURCUMIN	0	5.21 ± 4.23
	2	4.07 ± 1.17
	5	3.08 ± 0.09
	10	3.30 ± 0.39
QUERCETIN	0	4.95 ± 0.74
	2	4.42 ± 0.63
	5	5.31 ± 0.51
	10	3.57 ± 1.79

*Values are the mean and ± standard deviation from triplicate measurements

Serum index (SI) measurements were conducted at one hour and on days 1, 3, 5, 7 and 10 (Figure 4.4). All emulsions showed visible phase separation within 5 minutes post-emulsification. Although slight variation in SI was observed during the early storage period, values stabilised after day 3 with all samples remaining separated. Due to the minimal change over time, and the early onset of instability, the plotted SI values appear essentially constant. As replicate values were near identical, standard deviation was negiligible and is not shown in the figure.

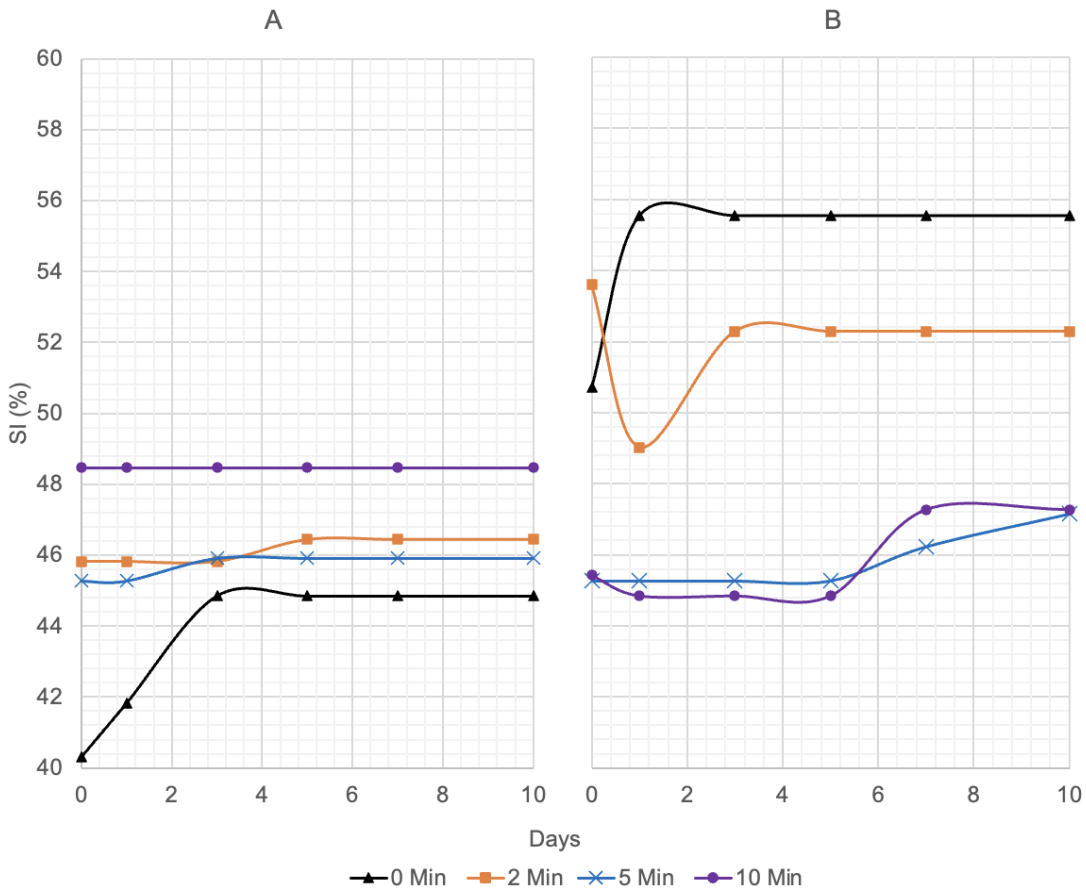
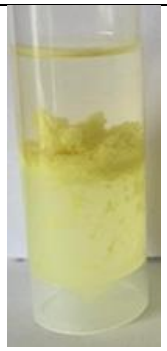


Figure 4.4 Serum index (SI %) for curcumin (A) and quercetin (B) emulsions over 10 days of storage at room temperature. Measurements were taken in triplicate; variation between replicates was minimal and is therefore not displayed.

Figure 4.5 displays images of the emulsions taken on day 0 and compares them with those captured on day 10 for all samples. It is evident that crystals accumulated at the centre between the two phases. This occurred due to the inadequate retention of crystals in the oil phase and the subsequent separation of the two phases, indicating a lack of stabilisation by Pickering crystals. Quercetin exhibited a greater quantity of powder between the two interfaces compared to curcumin, likely due to differences in crystal size, with quercetin crystals generally being larger than those of curcumin.

Figure 4.5 – Images of the stability of polyphenol emulsions one hour after production (day 0) and day 10

	Curcumin		Quercetin	
	Day 0	Day 10	Day 0	Day 10
Control				
2 minutes				
5 minutes				
10minutes				

Grinding of the crystals is believed to contribute to reducing the size of emulsion droplets, particularly noticeable in the case of curcumin. However, despite this process, the emulsions displayed large droplet sizes making them vulnerable to the destabilisation phenomenon, as seen in Figure 4.5. It is plausible that other factors influenced the outcomes, such as the dispersion of crystals in the continuous phase. As previously mentioned, there was some preliminary investigation into phase preparation, but the high temperature posed a risk of altering the crystals. Zembyla *et al.* (2019) utilised a Silverson high shear mixer during phase preparation, while Duffas *et al.* (2016) employed ultrasonics to aid in particle reduction, thereby preventing sedimentation of crystals, which was observed in the emulsions by day 10. Notably, quercetin with a 0- minute grind exhibited a substantial accumulation of polyphenol powder at the oil-water interphase.

Figure 4.6 and Figure 4.7 illustrate the microstructure of various emulsion treatments containing curcumin (Figure 4.6) and quercetin (Figure 4.7). The images depict water droplets suspended in the oil phase, indicating successful stabilisation and formation of a Pickering emulsion. Polydisperse systems can be seen amongst all samples, in particular the 0 minutes for both polyphenols, and referring to Table 4.3 the PDI being 5.21 and 4.95, for curcumin and quercetin, respectively. Over time, differences in droplet sizes could lead to varying levels of polyphenol crystal coverage, rendering certain areas of the droplet vulnerable to coalescence. Initial samples at 0 minutes displayed larger droplets, being around 245.97 μm for curcumin, compared to 10 minutes where the droplet had reduced to 68.55 μm. quercetin-stabilised droplets appeared very large (320.86 μm) compared to those stabilised by curcumin, though this observation lacks statistical validation. This discrepancy may be attributed to differences in crystal sizes with quercetin crystals being larger than curcumin as demonstrated by the Kruskal-Wallis p-value in the grinding experiments.

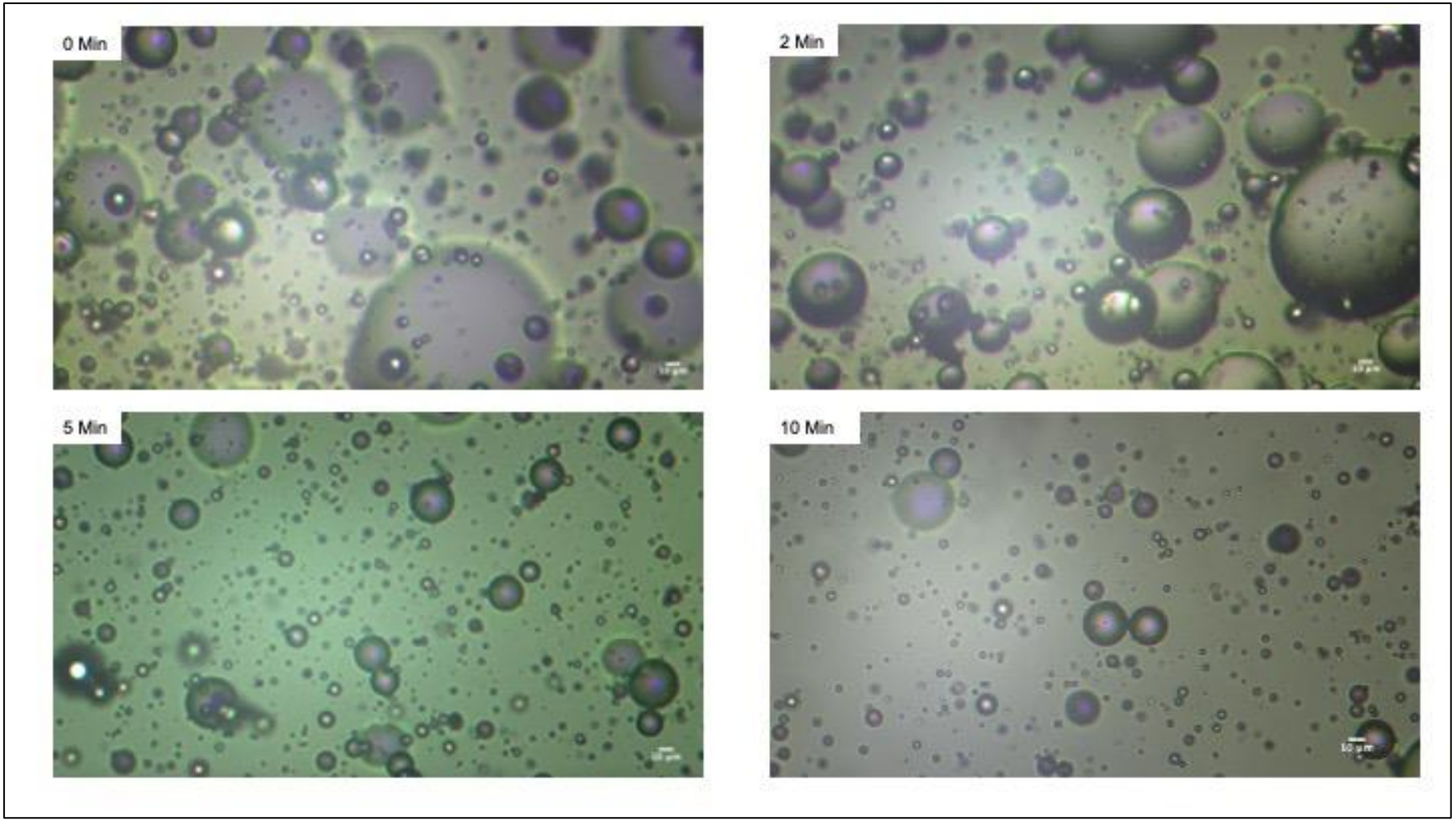


Figure 4.6 Microstructure of curcumin emulsions prepared with different grinding treatments observed under light microscopy at 40 x magnification (scale bar = 10 μm). Pictures show emulsions stabilised with curcumin crystals ground for 0 min, 2 min, 5 min and 10 min. Images represent typical microstructures from triplicate samples, showing changes in droplet size and distribution with increasing grinding duration.

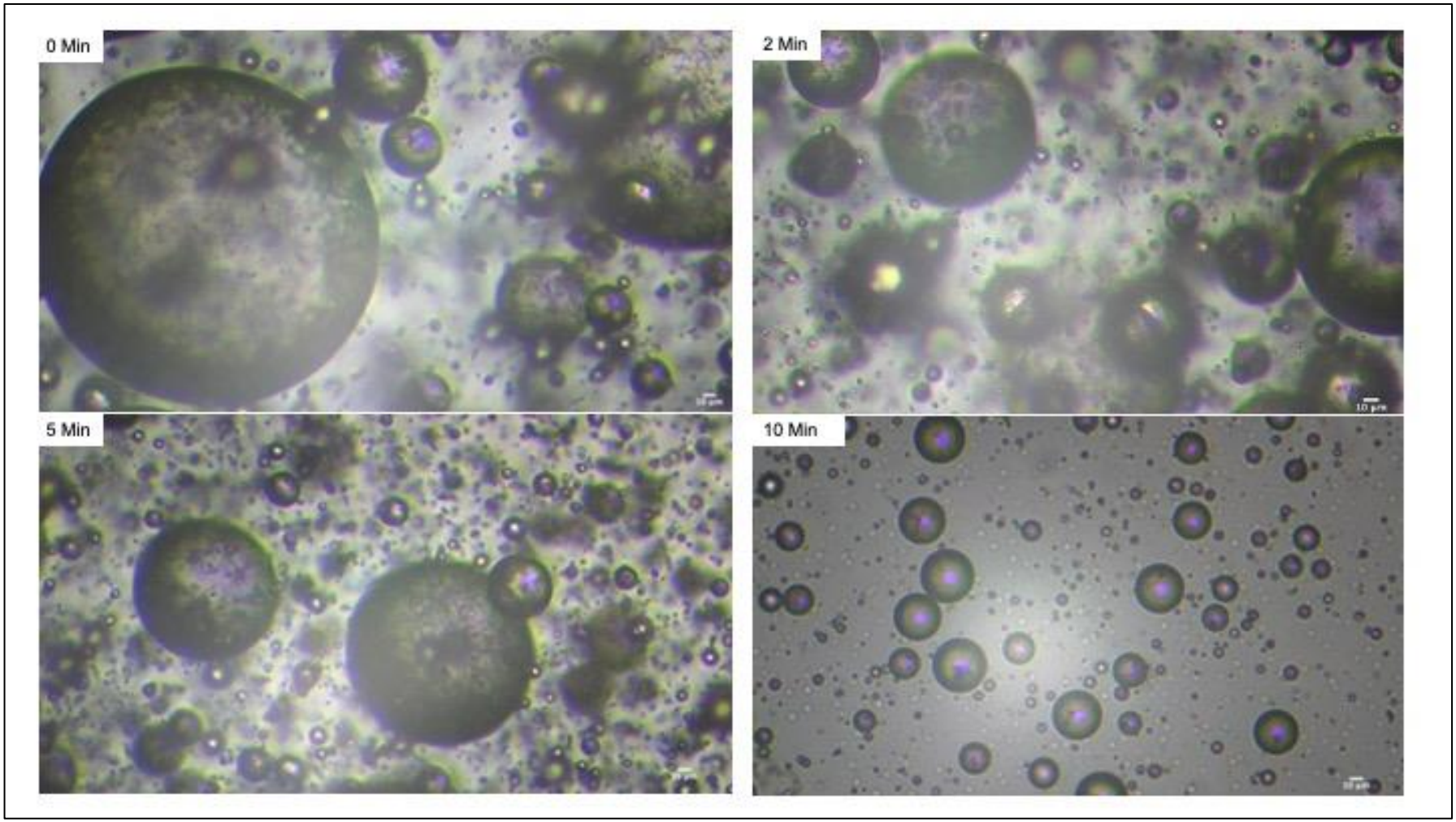


Figure 4.7 Microstructure of quercetin emulsions prepared with different grinding treatments observed under light microscopy at 40 x magnification (scale bar = 10 μm). Pictures show emulsions stabilised with quercetin crystals ground for 0 min, 2 min, 5 min and 10 min. Images represent typical microstructures from triplicate samples, showing changes in droplet size and distribution with increasing grinding duration.

Polyphenol stabilised emulsions exhibited significant polydispersity, contributing to their instability, as indicated by the presence of two separate phases in Figure 4.5 and the microstructure images displaying a range of droplet sizes. Polydisperse emulsions result in imbalanced forces, causing smaller droplets to interact and potentially leading to flocculation and coalescence if the droplet film is compromised. Although no obvious signs of aggregation were apparent in the microscope images of any of the samples, phase separation occurred, indicating that aggregation likely took place. Comparing these results with other studies is challenging due to limited research on the use of plant polyphenols as Pickering emulsifiers in W/O emulsions. Instead, many studies focus on the encapsulation of polyphenols for their health benefits. It is possible that using a different polyphenol, such as Tp-P, could yield emulsions with smaller droplet sizes.

4.3.2 Sunflower Lecithin

4.3.2.1 Concentration of sunflower lecithin

Table 4.4 presents the mean droplet size ($D_{4,3}$) and PDI of emulsions formulated with various concentrations of sunflower lecithin. The analysis explored the relationship between $D_{4,3}$ and the concentrations of sunflower lecithin. It was found that the mean droplet size decreased as the concentration of sunflower lecithin increased, suggesting a trend towards smaller droplet sizes at higher concentrations, particularly up to 1.5 %. Beyond 1.5 %, further increases in lecithin concentration (up to 3 %) did not result in significant changes in droplet size. The $D_{4,3}$ values at 2 % and 3 % were relatively stable (ranging from 12.32 to 12.80 μm), suggesting a plateau effect. PDI remained low and consistent, except for a slight increase at 3 % (1.48), possibly due to excess emulsifier destabilising the system. Statistical analysis using the Kruskal-Wallis test revealed no significant differences in droplet size across lecithin concentrations (χ_2 = 7.55, df = 5, P = > 0.05). Post hoc pairwise comparisons using Dunn's test with Bonferroni correction also found no statistically significant differences between any specific concentrations (P > 0.05 for all comparisons).

Table 4.4 – Summary of emulsion volume-weighted droplet means and polydispersity index (PDI) for the varying concentrations of sunflower lecithin in water-in-oil-emulsion*

Sunflower lecithin concentration (%)	**$D_{4,3}$ (μm)**	**PDI**
0.5	19.29 ± 6.90	2.87 ± 0.54
1.0	17.42 ± 8.02	1.44 ± 0.18
1.5	12.32 ± 0.62	1.18 ± 0.11
2.0	12.44 ± 2.48	1.00 ± 0.33
2.5	12.80 ± 2.28	1.12 ± 0.25
3.0	12.48 ± 3.10	1.48 ± 0.43

*Values are the mean and ± standard deviation from triplicate measurements for both parameters

When comparing the results outlined in Table 4.4 with the droplet size distribution depicted in Figure 4.8, a clear trend emerges where lower concentrations are associated with higher PDI values. For instance, at a concentration of 0.5 %, the PDI significantly rises, surpassing 1 with a value of 2.87, while at 2 %, the PDI reaches its lowest at 1. Those with a higher PDI exhibit a more diverse graph shape, whereas those with a lower PDI display a smoother distribution curve, which can be seen in Figure 4.8.

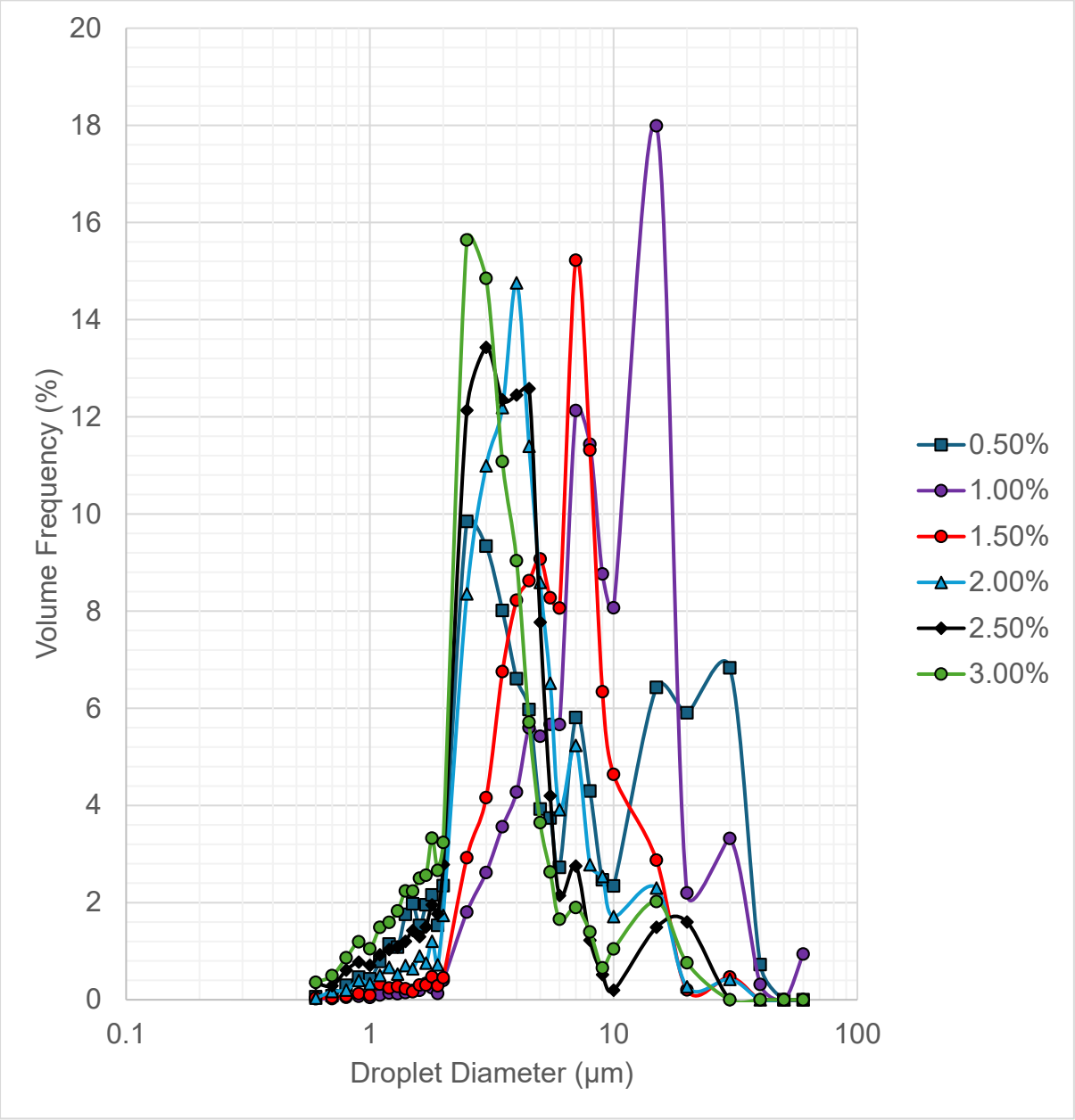


Figure 4.8 – Droplet size distribution across different concentration of sunflower lecithin in the water-in-oil emulsion.

Serum index (SI) from day 0 to day 30 can be seen in Figure 4.9, with the biggest jump in SI seen between day 0 and day 5. From day 5 to 30, the SI gradually increases with a clear serum layer seen visually at the base of the emulsion. Concentrations 0.5 % and 1 % had the highest SI values overall, which can be attributed to the larger droplets, being 19.29 µm and 17.42 µm. Similarly, concentrations 1.5 % to 3 % were similar in SI values during the period, which again could be attributed to the similar droplet size.

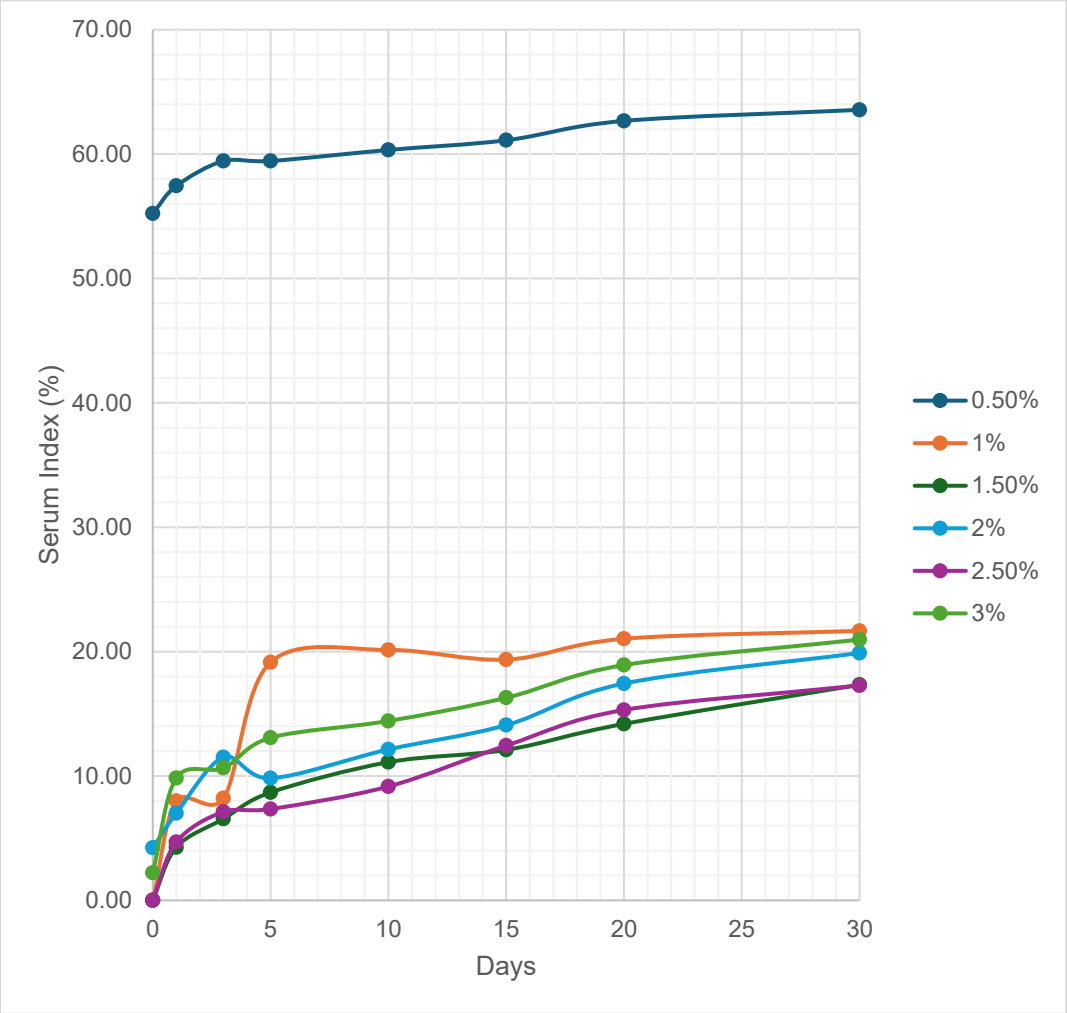


Figure 4.9 Serum index for sunflower lecithin concentrations 0.5 % to 3 % Measurements were taken in triplicate; variation between replicates was minimal and standard deviation is therefore not displayed.

While numerous studies have examined sunflower lecithin, there is limited research on its ability to stabilise W/O emulsions independently. Pan, Tomas and Anon (2002) explored the impact of sunflower lecithin on both O/W and W/O emulsions, investigating different ratios of phospholipids within the lecithin type. They observed varied droplet sizes in 30:70 W/O emulsions, ranging from 4 μm to 11 μm at concentrations of 0.1 % to 1 %. These droplet sizes were smaller than those observed in our study, where droplet diameters ranged from 19.29 μm at 0.5 % to 17.416 μm at 1.5 %.

However, the findings presented in this study were based on mean values calculated using the volume-weighted diameter, which tends to skew toward larger droplet sizes. When comparing these results to those of other studies, differences in methodological approaches

can significantly influence droplet size outcomes. For instance, while this study employed ultrasonic homogenisation, another study utilised an Ultra-Turrax with an attachment operating at a speed of 10,000 rpm, which could explain the variations observed. Similarly, Sui *et al.* (2017) reported smaller droplets (3.815 μm) in soy lecithin stabilised emulsions, notably smaller than those observed in the initial investigation of droplet size using varying concentrations, but more comparable when altering ultrasonication time to reduce droplet sizes. Additionally, Rivas, Schneider and Rohm (2016) explored sunflower lecithin in W/O emulsions, both with and without partial replacement of PGPR. They found that lecithin only emulsions ranged from 12 μm to 25 μm, results comparable to those of this study, which found larger sizes compared to the studies previously mentioned.

The investigation into varying concentrations of sunflower lecithin, ranging from 0.5 % to 1.5 %, revealed a noticeable decrease in droplet sizes. This reduction aligns with literature suggesting that higher surfactant concentrations lead to improved stability by ensuring sufficient coverage of droplets (Genot, Kabri and Meynier, 2013). Additionally, when considering both droplet size and serum index (SI), it becomes apparent that the larger SI values observed for concentrations of 0.5 % and 1 % may contribute to the observed droplet sizes. Larger droplet sizes have been associated with increased recoalescence tendencies, leading to phase separation and compromised emulsion stability (Sui *et al.*, 2017). Understanding these relationships is crucial for optimising emulsion stability and functionality.

However, the observed decrease in droplet sizes was not supported by statistical analysis, as droplet sizes remained similar from 1.5 % to 3 %, being around 12 μm. This consistency could potentially be attributed to the duration of ultrasonic homogenisation. It is plausible that during the 5-minute duration, droplets within these concentrations were sufficiently covered by the surfactant. Further investigation, if resources and time permitted, could have measured the critical micelle concentration. This parameter determines the optimal surfactant concentration required to adequately cover the droplets (McClements, 2016). Establishing the critical micelle concentration would provide insights into whether the observed droplet sizes are indeed influenced by surfactant coverage.

4.3.2.2 Effect of ultrasonic time on emulsion droplet size and stability.

Table 4.5 presents the mean $D_{4,3}$ for sunflower lecithin stabilised emulsions across three concentrations (2 %, 2.5 % and 3 %) and four ultrasonication times (5, 10, 15 and 20 minutes). In general, droplet size decreased with increasing sonication time across all concentrations. For example, droplet size decreased from 12.44 μm at 5 minutes to 6.63 μm at 20 minutes at a 2 % concentration.

A one-way ANOVA was conducted within each concentration group to determine whether ultrasonication time significantly affected droplet size. Although none of the p-values were below the 0.05 significance threshold, a decreasing trend was observed in all groups, with the 3 % concentration approaching significance (P = 0.053).

Table 4.5 - Volume weighted droplet diameter ($D_{4,3}$) for the three chosen sunflower lecithin concentrations and varying ultrasonication time*.

Sunflower concentration (%)	**$D_{4,3}$ (μm) and Ultrasonication time (minutes)**				
	5	**10**	**15**	**20**	**P Value**
2.0	12.44 ± 2.48[a]	8.94 ± 5.21[b]	7.42 ± 0.60[b]	6.63 ± 0.86[b]	*0.223*
2.5	12.80 ± 2.28[a]	9.14 ± 2.46[b]	7.66 ± 0.83[b]	6.88 ± 3.16[b]	*0.079*
3.0	12.48 ± 3.10[a]	10.68 ± 6.02[b]	7.08 ± 0.13[b]	5.71 ± 0.72[b]	*0.053*
P Value	*0.875*	*0.430*	*0.066*	*0.707*	

*Values are the mean ± standard deviation from triplicate measurements (n = 3). P- values are from one-way ANOVA comparing ultrasonication times within each concentration. Superscripts indicate significant differences between ultrasonication times based on Tukey's HSD test (P < 0.05). Values sharing the same letter are not statistically different.

To assess the combined effects of ultrasonication time and lecithin concentration, a two-way ANOVA was also performed. The analysis revealed a significant main effect of time (P < 0.05), indicating that increasing ultrasonication time significantly reduced droplet size. There was no significant main effect of concentration (P = 0.362) and no significant interaction between time and concentration (P = 0.987), suggesting that the influence of ultrasonication on droplet size was consistent across all concentrations.

Post hoc comparisons using Tukey's HSD test showed that droplet sizes at 5 minutes were significantly larger than at 10, 15 and 20 minutes. However, differences beyond 15 minutes were not statistically significant, indicating a possible plateau effect in droplet size reduction after a certain duration of ultrasonication.

These findings support the hypothesis that increasing ultrasonication time generally reduces droplet size. For example, at 2% concentration, the mean droplet size decreased from 12.44 μm at 5 minutes to 6.63 μm at 20 minutes. This outcome aligns with existing literature which suggests that longer ultrasonic duration results in increased cavitation effects, facilitating the further breakdown of larger droplets into smaller ones (Maghamian, Goli and Najarian, 2021; Yue, 2022).

Despite the absence of statistically significant differences within each concentration group, the overall decreasing trend across all treatments implies that ultrasonic processing can effectively reduce droplet size, enhancing the stability of emulsions. The ability of all tested concentrations (2 %, 2.5% and 3%) to stabilise emulsions at varying times also supports the idea that these levels provide sufficient interfacial coverage.

Other studies investigating ultrasonic time and emulsion droplet sizes have similarly reported a trend of decreasing droplet size and improved particle distribution with longer ultrasonic durations, thereby enhancing stability. For example, Kaltsa *et al.* (2014) observed a reduction in droplet size from 1.141 to 0.891 μm in olive oil O/W with ultrasonic durations ranging from 1 minute to 4 minutes. Similarly, Sui *et al.* (2017) achieved a decrease in droplet size from 3.815 μm to 3.369 μm using soy protein isolate and lecithin with ultrasonic treatments lasting 12 to 24 minutes. However, it is worth noting that excessive ultrasonication poses a risk of droplet recoalescence and may negatively impact emulsion stability as cautioned in previous studies (Kaltsa *et al.*, 2014; Sui *et al.*, 2017; Maghamian, Goli and Najarian, 2021).

Serum index comparing the chosen three concentrations (2 % , 2.5 % and 3 %) over the different ultrasonic durations can be seen in Figure 4.10. Like results previously discussed, the biggest jump in SI is seen between day 0 and day 5. Results are generally clustered by percentage concentration of surfactant, with those of 20 minutes generally clustered towards lower SI values compared to those of 5 minutes.

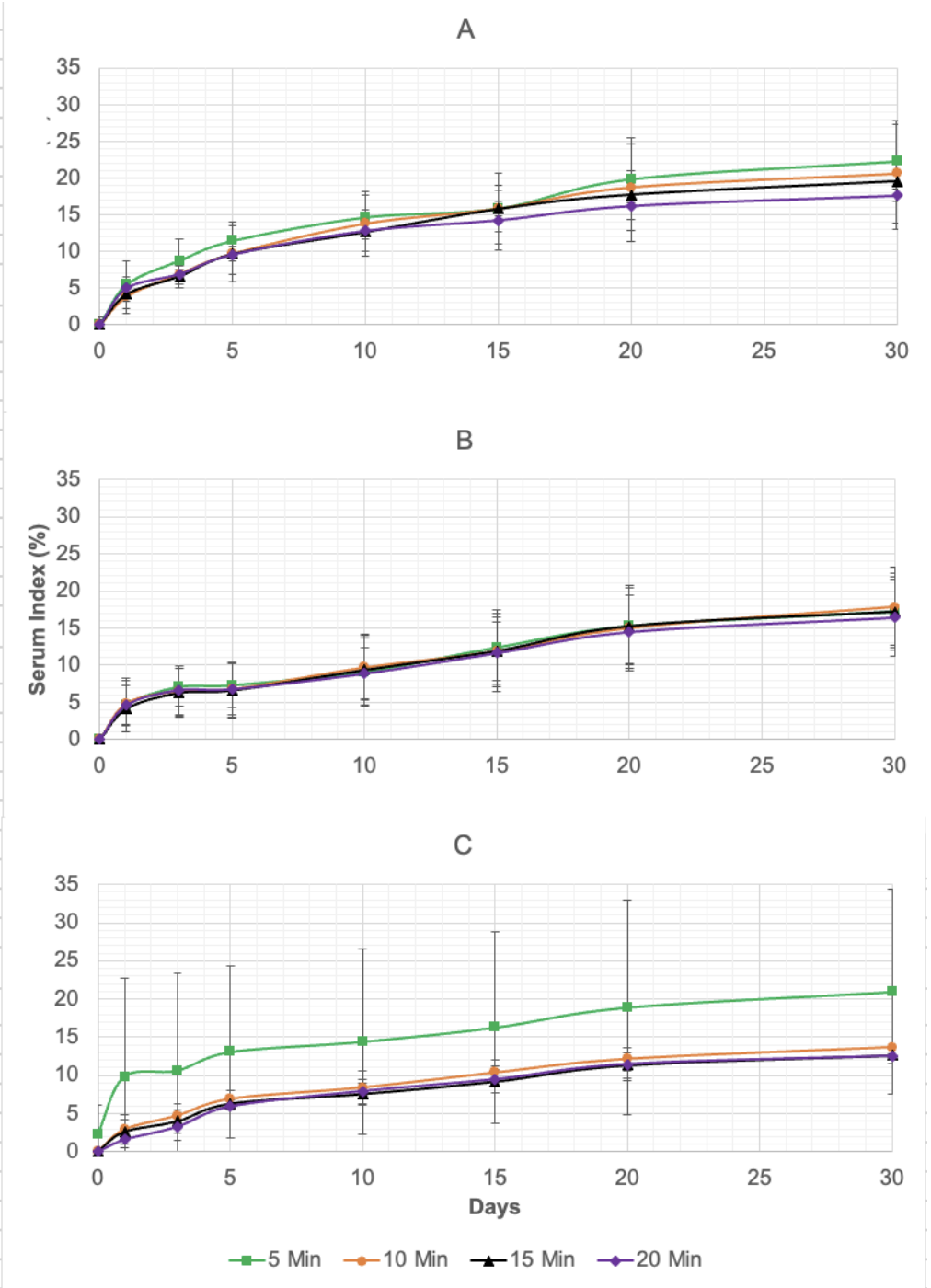


Figure 4.10 - Serum index for sunflower lecithin stabilised W/O, comparing ultrasonic time and concentrations (A – 2 %, B – 2.5 %, C – 3 %). Error bars represent standard deviation of the mean serum index from triplicate measurements.

The analysis of the serum index over the storage period reveals distinct trend that shed light on the emulsion's stability dynamics. Initially, from day 0 to day 5, there is rapid increase in the SI, indicating the presence of larger droplets. This phenomenon is likely attributable to the polydispersity of droplet sizes, where larger droplets coalesce, forming a serum layer at the bottom of the sample. Subsequently, from day 5 to day 30, a gradual increase in the

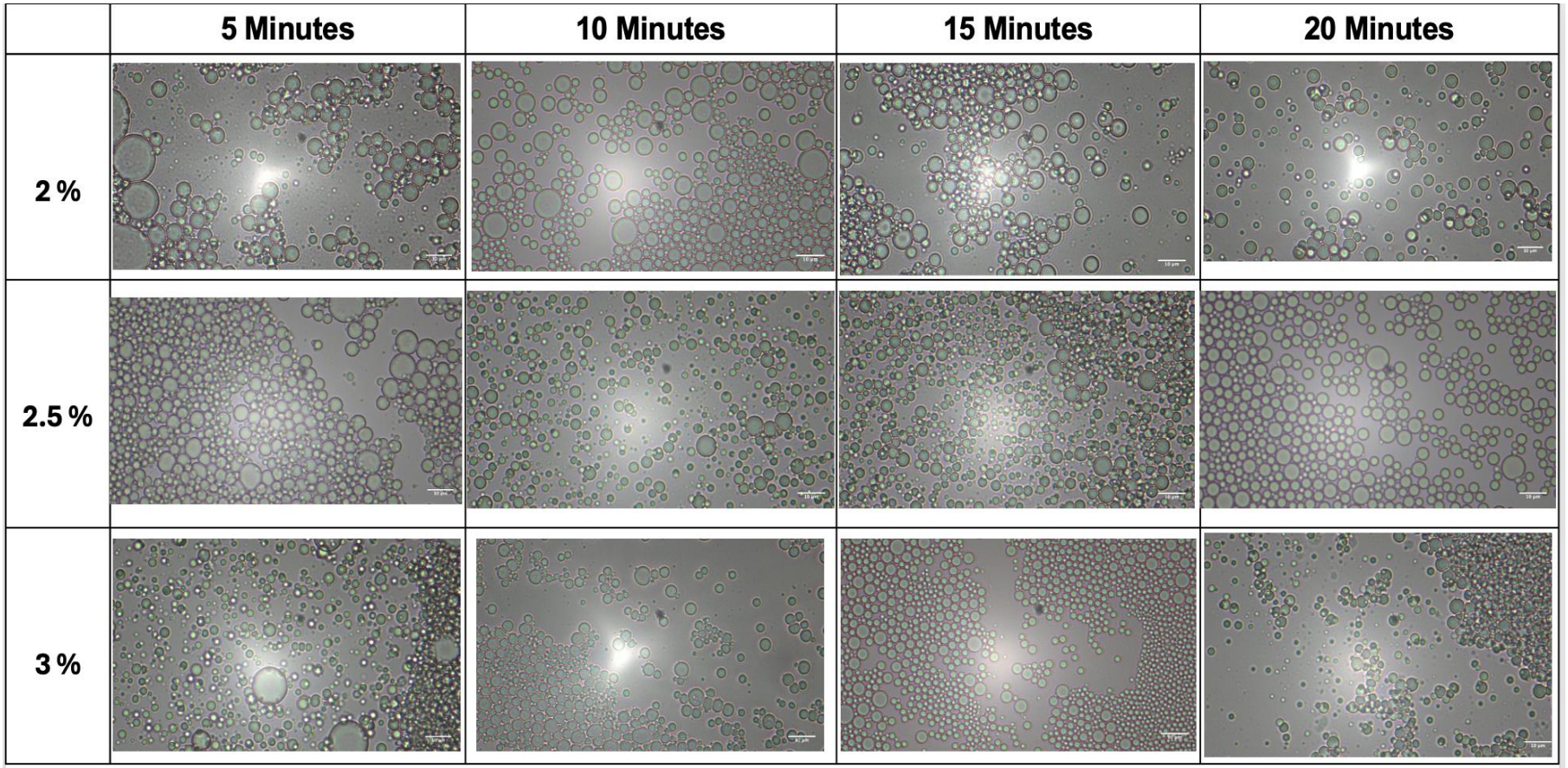
serum index is observed, suggesting a prolonged process wherein droplets gradually come into proximity and eventually coalesce. This delay in coalescence may be due to factors such as molecular interactions and steric hinderance (McClements, 2016). Additionally, gravitational separation contributes to this phenomenon, as larger droplets, having a higher mass, sink to the bottom, leading to their separation from the bulk emulsion phase. These findings underscore the importance of understanding emulsion stability mechanisms, which can inform strategies for optimising shelf life and maintaining product quality over time. Figure 4.11 shows the microstructure of the emulsions, with the range of droplet sizes in the images related to the PDI results, which can be seen in Table 4.6, below.

Table 4.6 Polydispersity index (PDI) for the three chosen sunflower lecithin concentrations and varying ultrasonication time*.

Sunflower Concentration (%)	**PDI and Ultrasonication time (Minutes)**			
	5	**10**	**15**	**20**
2.0	1.184 ± 0.328	1.165 ± 0.119	1.364 ± 0.171	1.095 ± 0.070
2.5	1.123 ± 0.428	0.732 ± 0.079	1.082 ± 0.101	1.043 ± 0.022
3.0	1.341 ± 0.262	1.077 ± 0.125	1.382 ± 0.238	1.059 ± 0.045

*Values are the mean and ± standard deviation from triplicate measurements

Figure 4.11 Microstructure of W/O emulsions over varying ultrasonic times



Notably, the results from the ultrasonic experiment indicate that concentrations ranging from 2 % to 3 % achieved smaller droplet sizes with longer ultrasonication exposure. This suggests the influence of surfactant concentration and ultrasonic treatment duration on droplet size reduction. The trend observed in the serum index analysis complements these findings, as smaller droplet sizes achieved through optimal ultrasonication can enhance emulsion stability, reducing the likelihood of coalescence and phase separation over time.

4.4 Conclusion

Based on the findings and discussion presented in this chapter, the grinding process of polyphenol crystals impacted the overall size of the emulsion droplets. Despite efforts, emulsions with a 40:60 (water to oil) ratio and a 0.14 % concentration did not exhibit long-term stability. Additionally, the size of the crystals post-grinding did not reach below the 0.1 μm threshold. Consequently, it was concluded that the manually obtained crystal size at Harper Adams University was insufficient for effectively reducing droplet size, even with modifications to methods involving ultrasound, ratios and concentration in the emulsion. Therefore, the investigation into polyphenols was discontinued within the scope of this project. However, the availability of an analytical mill capable of reducing particle size to 0.2 μm or below presents an intriguing opportunity to utilise these natural surfactants in Pickering W/O emulsions. Such polyphenols could be explored for fortification with double emulsions to harness their health-promoting properties.

To conclude, the investigation into the effects of sunflower lecithin concentration and ultrasonication time on droplet size with emulsions revealed notable trends. The chosen surfactant concentration of 2 % demonstrated a correlation with a decrease in droplet size, while prolonged ultrasonication also showed similar trends, though these observations were not statistically significant. Nonetheless, the study highlights the promising potential of sunflower lecithin as a natural surfactant in primary W/O emulsions. Although a droplet size of < 1 μm was not successfully achieved, the surfactant showed positive stabilisation effects indicating potential for further development. Moving forward, further exploration of this surfactant's capabilities, particularly in conjunction with milk fat, holds promise for application in skimmed milk double emulsions and the development of reduced fat cheese.

CHAPTER FIVE: PARTIAL REPLACEMENT OF PGPR WITH SUNFLOWER LECITHIN IN WATER-IN-MILK EMULSIONS

5.1 Introduction

Building on the previous chapter, 2 % sunflower lecithin was used in water-in-sunflower oil emulsions, although a droplet size of < 1 μm was not achieved, this chapter will develop parameters to attempt to achieve this size threshold. The progression to a milk fat system from sunflower oil is necessary for further applications such as reduced fat cheese. However, transitioning from liquid oil to milk fat introduces its own challenges.

The molecular structure of the fat and the packing of these structures significantly impacts the melting point (McClements, 2016). Triglyceride molecules that are branched are typically unsaturated fatty acids, meaning they are unable to pack tightly. This results in a lower melting point, making them liquid at room temperature, such as those in sunflower oil. In contrast, saturated triglycerides found in milk fat are often straight, enabling tight packing. With milk fat containing numerous fatty acids (Sánchez-Vega *et al.*, 2021), which contribute to a wide melting point range from – 40 °C to 40 °C (Patel, 2020). The variability of fatty acids and structure influences the interaction and crystallisation of fat when emulsifiers are added and can disrupt the structure (Panchal *et al.*, 2020). A combination of the molecular structure and physicochemical properties, such as the attractive interactions involved with van der Waals forces, polarity and the dielectric constant of the oil and fat which will ultimately impact the colloidal interactions in emulsions (McClements, 2016).

Finding an alternative to synthetic surfactants can prove difficult, as found in Chapter 4, polyphenols were not suited for this thesis. However, sunflower lecithin showed some potential. A study by Aktar and Dickinson (2001) showed that the use of lecithin in a combination of rapeseed oil and anhydrous milk fat in W/O emulsions, created stable droplets of < 1 μm ($D_{4,3}$). The conclusion from their study was that lecithin would be suitable for use in dairy-based food emulsions, which provides positive potential for this chapter. Limited studies have shown the complete replacement of PGPR with lecithin, often stabilisation by lecithin alone is unsuccessful due to the production of large droplets (Pang *et al.*, 2022; Silva *et al.*, 2023). Tekin, Sahin and Sumnu (2017) found that a blend of PGPR and lecithin was the best solution for reducing PGPR in low fat ice cream. Interestingly Killian and Coupland (2012) observed that over a six hour period, lecithin stabilised

emulsions increased from 43.1 μm to 77 μm, while PGPR stabilised emulsions exhibited a much smaller size increase over a longer duration. Therefore, there is potential for the partial replacement of PGPR with sunflower lecithin to reduce the total amount of PGPR needed. Okuro *et al.* (2019) found that it was possible to use lecithin to decrease the use of synthetic surfactants, with a combination of PGPR and lecithin successfully producing stable emulsions. However, lecithin alone was unable to create an emulsion with small droplets, and separated after 7 days of storage, with a reduction found in backscattering results from 10 % at the bottom to 80 % at the top, indicating clear serum and cream layers.

5.1.1 Aim and objectives

The aim of this experimental study was to investigate sunflower lecithin as the lipophilic emulsifier in water-in-milk fat emulsions for their ability to be utilised further in skimmed milk double emulsions. The objectives were:

- Evaluating the use of sunflower lecithin as a complete replacement for PGPR in water-in-milk fat emulsions.
- Evaluating the use of a partial replacement of PGPR with sunflower lecithin in water-in-milk fat emulsions.
- Developing the ultrasonic homogenisation process to produce <1 μm water-in-milk fat emulsions using a PGPR to sunflower lecithin ratio at a total of 2 % surfactant level to create stable emulsions.

5.2 Materials and methods

All materials and methods are described in Chapter 3 – general materials and methods. As discussed in the previous chapter, PGPR and sunflower lecithin the chosen surfactant concentration was 2 % of the total emulsion and the following surfactant ratios and emulsion codes used in this experiment are presented in Table 5.1.

Table 5.1 – Codes for the varying emulsion surfactant ratios of PGPR to sunflower lecithin

Emulsion code	Surfactant ratio (PGPR : Sunflower lecithin)
P2:L0	2:0
P1.5:L0.5	1.5:0.5
P1:L1	1:1
P0.5:L1.5	0.5:1.5
P0:L2	0:2

The experiment started with progression from the previous Chapter with solely sunflower lecithin as the emulsifier (section 5.3.1). Heat camera images were taken prior and post ultrasonication using a FLIR C2 Compact Thermal Imaging camera (Cheshire, UK) borrowed from an external company. Although the temperature scale on the camera was slightly inaccurate, the colour-coded areas consistently reflected the relative differences in heat and cool zones. This made the camera useful for visualising temperature variations. The actual temperatures were verified using a calibrated temperature probe.

Following this saw the development of the ultrasonic parameters (section 5.3.2) and the movement towards partially replacing PGPR with sunflower lecithin (section 5.3.3) to create stable water-in-milk fat emulsions suited for further application in skimmed milk double emulsions.

When analysing data, the Shapiro-Wilk test was used to assess whether the data was normally distributed. Shapiro results were above the threshold ($P > 0.05$) therefore suggesting the data was normally distributed, enabling the use of Analysis of Variance (ANOVA). ANOVA was employed to test the difference between the emulsion groups. A linear regression was also employed to model the relationship between certain variables to identify specific trends.

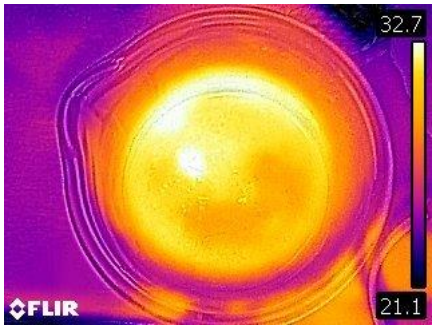
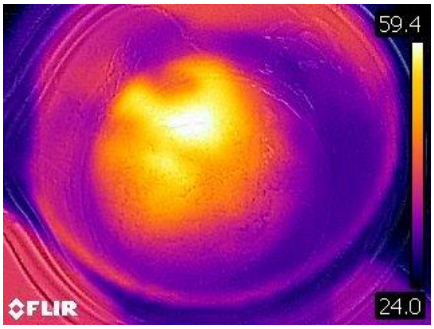
5.3 Results and discussion

5.3.1 Milk fat, sunflower lecithin and the runaway heat phenomenon

During emulsion homogenisation, milk fat presented some challenges when sunflower lecithin was used exclusively. The sample not only showed phase separation but also

experienced severe variability in temperature. The images in Figure 5.1 demonstrate variability, with the first image (before sonication) showing an even spread of heat, while the second image (post-ultrasonication) shows significant temperature differences. The hottest location was the ultrasonic probe (+ 80 °C) and the cooler areas (30 °C) were closest to the ice bath.

Figure 5.1 - Distribution of heat images of the P0:L2 emulsion after ultrasonication

Before sonication	After sonication
	

The significant temperature difference within the sample supports the theory of runaway heat, where warmer zones heat faster (Chandrapala *et al.*, 2013; Muñoz *et al.*, 2018). Milk fat is susceptible to runaway heating (Muñoz *et al.*, 2018). There are limited research papers solely using milk fat as the continuous phase, one study Aktar and Dickinson (2001) found that lecithin was successful in stabilising W/O emulsions. However, in this study, the continuous phase was a combination of rapeseed oil and anhydrous milk fat in a 1:1 ratio, which would have altered the crystallisation of the fat and thus influenced the nature and performance of the continuous phase.

Additionally, lecithin has been found to influence lipid crystallisation by affecting crystallisation kinetics (Schubert, Schicke and Müller-Goymann, 2005; Rigolle *et al.*, 2015; Silva *et al.*, 2023). This may be the explanation to why emulsions P0.5:L1.5 and P0:L2 saw phase separation. Previous research discussed in Chapter 4 using sunflower oil did not encounter this issue, indicating that the polarity of the oil compared to fat and the interactions between lecithin and milk fat would have impacted the emulsion.

5.3.2 Effect of ultrasonic time on W/O properties in milk fat

Using ANOVA to compare the impact of emulsion surfactant ratio and ultrasonication time on the average droplet size ($D_{4,3}$). The results indicated no significant difference between time and concentration on droplet size, meaning together they did not impact droplet size. Figure 5.2 shows the $D_{4,3}$ across each time iteration for each surfactant ratio. Although there was no significant effect of ultrasonic time on $D_{4,3}$ ($P > 0.05$), a weak trend of decreasing droplet size with increased ultrasonic time was only observed for emulsion P1.5:L0.5 with a $D_{4,3}$ of 3.69 ± 0.66 μm after 5 minutes, reducing to 2.61 ± 0.77 μm after 15 minutes sonication.

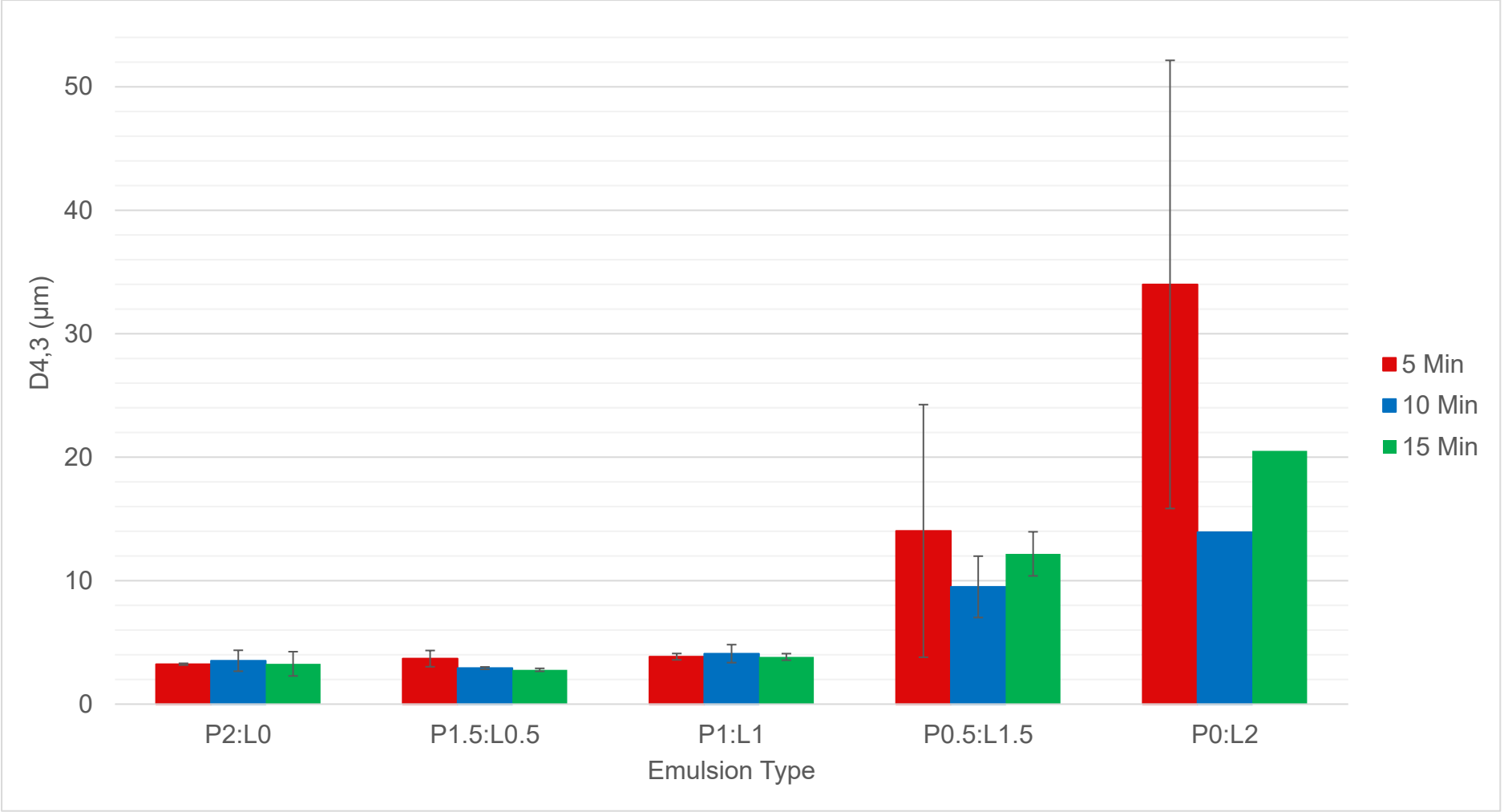


Figure 5.2 – Droplet size of each ultrasonic time, grouped by each emulsion surfactant ratio. Values Error bars represent standard deviation of the mean $D_{4,3}$ from triplicate measurements.

Some papers as discussed in Chapter 4 from O'Sullivan *et al.* (2015) and Sui *et al.* (2017) supported this phenomenon, as the increased and constant expansion and retraction mechanism caused larger droplets to break into smaller ones. However, the risk of recoalescence with excessive ultrasonication was discussed by Maghamian, Goli and Najarian (2021), which may explain the patterns observed within emulsions P0.5:L1.5 and P0:L2, where increasing time from 5 to 10 minutes caused a drop from 14.03 ± 10.30 μm for emulsion P0.5:L1.5, followed by an increase to 12.17 ± 1.79 μm after 15 minutes. Interestingly emulsions P2:L0 and P1:L1 had some varying levels of droplet sizes across the times but no identifiable difference.

Table 5.2 contains the polydispersity index (PDI) for each emulsion group at the different times tested. Generally, there was no significance across time iterations, or identifiable trends. However, emulsions P1.5:L0.5 and P1:L1 saw an increase in PDI over time, meaning a larger range of droplet sizes were identified, despite that within emulsion P1.5:L0.5 the droplet sizes on average tended to reduce in size. Emulsion P0:L2 exhibited reduction in PDI over the time iterations, this could have been due to the instability of the emulsion and the larger droplets being broken down during ultrasonication and creating a more evenly spread of droplet sizes rather than after 5 minutes with some very large droplets skewing the data.

Table 5.2 – Polydispersity index (PDI) for each emulsion ratio at each time iteration*

EMULSION	**TIME (Minutes)**		
	5	**10**	**15**
P2:L0	0.83 ± 0.08	1.11 ± 0.20	0.96 ± 0.18
P1.5:L0.5	0.90 ± 0.07	0.93 ± 0.09	0.94 ± 0.07
P1:L1	1.09 ± 0.11	1.13 ± 0.10	1.29 ± 0.17
P0.5:L1.5	1.34 ± 0.13	1.00 ± 0.64	1.30 ± 0.35
P0:L2	2.08 ± 1.14	1.07 ± 1.40	1.69 ± 0.93

*Values are the mean and ± standard deviation from triplicate measurements

5.3.3 Effect of PGPR to sunflower lecithin ratio in W/O emulsions

A significant difference was found between surfactant ratio and $D_{4,3}$ ($P < 0.05$), suggesting that the surfactant ratio significantly impacts droplet size. Specifically, a higher proportion of sunflower lecithin resulted in larger droplets (3.84 μm for P1:L1 to 14.03 μm for P0.5:L1.5

and 33.99 μm for P0:L2). This was corroborated by the PDI displayed alongside $D_{4,3}$ in Table 5.3, showing a greater size range of droplets with higher $D_{4,3}$ values for emulsions with more sunflower lecithin.

Table 5.3 – Volume-weighted mean droplet ($D_{4,3}$) and polydispersity index (PDI) results for 5-minute ultrasonication across varying emulsion ratios*

Emulsion Code	$D_{4,3}$ (μm)	PDI
P2:L0	3.24 ± 0.07	0.83 ± 0.08
P1.5:L0.5	3.69 ± 0.66	0.90 ± 0.07
P1:L1	3.84 ± 0.26	1.09 ± 0.11
P0.5:L1.5	14.03 ± 10.23	1.34 ± 0.13
P0:L2	33.99 ± 18.15	2.08 ± 1.14

*Values are the mean and ± standard deviation from triplicate measurements

This is consistent with the findings of Okuro *et al.* (2019) who reported a $D_{3,2}$ of 2.4 μm for emulsion P2:L0, the result in this study was larger compared to surfactant ratios with higher PGPR. Okuro *et al.* (2019) also observed that emulsions with higher PGPR concentrations had a more monodisperse structure, leading to greater stability, as backed by their back scattering results. They hypothesised that reducing lecithin to 0.5:1.5 (lecithin to PGPR) allows PGPR to migrate to the droplet interface, where it forms a strong steric barrier by filling gaps between lecithin molecules, thereby enhancing kinetic stability.

When comparing their analysis to the results in this chapter, it is possible that similar behaviour occurs in emulsions with higher PGPR. In cases where lecithin is present in greater amounts it likely migrates to the droplet interface first, with PGPR forming a secondary layer on top. This results in larger droplet sizes and a weaker interfacial layer, which can lead to coalescence

and phase separation, evident in the serum index observed both in this study and in Okuro *et al.* (2019). The packing of molecules around the droplets and the interaction between surfactants suggest that when lecithin is dominant, it may act more as a competitor rather than as a co-surfactant, which can be attributed in PGPR heavy emulsions.

Similarly, Pang *et al.* (2022) found that samples with 2 % lecithin were unstable, showing the largest droplet sizes in maize oil with a $D_{3,2}$ of 15.968 μm. The concentration of surfactant may not have been sufficient to cover all droplets, leading to coalescence and eventual phase separation. Rivas, Schneider and Rohm (2016) found a bimodal distribution in lecithin based emulsions compared to that of PGPR at similar diameters, concluding that sunflower lecithin alone was unable to create small droplet sizes and stable emulsions. Yao *et al.* (2024) at a slightly higher surfactant percentage total of 4 %, also found that emulsions with higher amounts of lecithin in the ratio lead to larger droplets and had a bimodal distribution, contributing to the gravitational separation and instability of the emulsion.

The inclusion of PGPR in the ratio enhanced droplet stability due to its small molecular weight, allowing it to quickly absorb at the oil-water interface and form a tightly packed, protective film. Conversely, lecithin was found to migrate slowly to the oil-water interface (Ushikubo and Cunha, 2014), contributing to larger droplet sizes, which were observed here.

From analysing droplet size and examining microstructure, emulsion P0:L2, showed significantly larger droplets, compared to emulsions P2:L0, P.15:L0.5 and P1:L1, which can be correlated to the results from $D_{4,3}$. The aggregation seen in lecithin-rich emulsions, such as emulsion P0.5:L1.5, might result from the diverse structures that lecithin can create (Ushikubo and Cunha, 2014; Silva *et al.*, 2023), which in turn affects the overall structure of the emulsion. Structural changes in an emulsion, such as the presence of large or aggregated droplets, can affect its viscosity (Kasinos *et al.*, 2014; Panagopoulou *et al.*, 2017).

Figure 5.3 compares the apparent viscosity of each of the W/O emulsions. Those with higher amounts of lecithin had a greater apparent viscosity with 1000 mPas for emulsion P0.5:L1.5, compared to that of emulsion P1.5:L0.5 with 150.34 mPas.

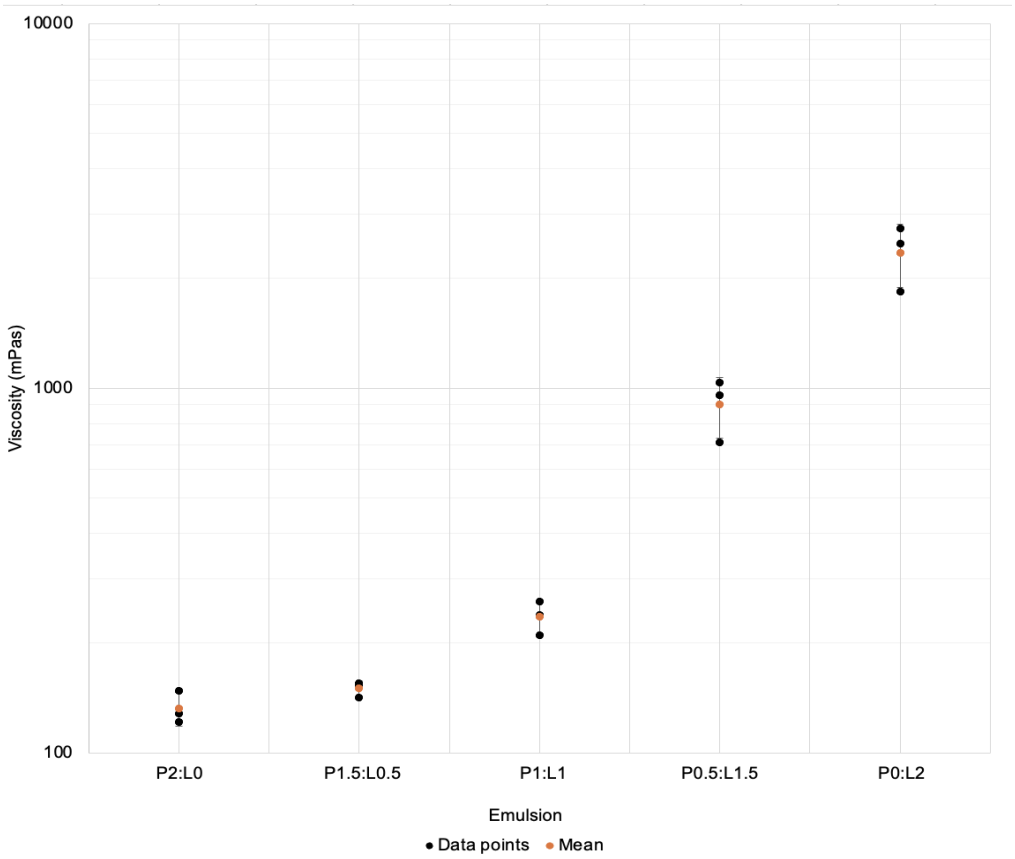


Figure 5.3 – Apparent viscosity of the milk fat emulsion with varying ratios. Scatter plot of data points and mean values from triplicate measurements using a log scale.

A linear regression model was used to examine the relationship between the ratio of surfactants (PGPR and sunflower lecithin) and the apparent viscosity of the emulsions. In this model, the ratio was defined as the proportion of PGPR relative to the total amount of both PGPR and sunflower lecithin combined. The viscosity measurements were treated as the dependent variable, while the surfactant ratio served as the independent variable.

The linear model was selected based on the assumption of continuous, linear trend between the increasing proportion of PGPR and decreasing viscosity, as PGPR is a low viscosity surfactant compared to lecithin. The analysis indicated a statistically significant ($P < 0.05$) negative relationship between PGPR proportion and emulsion viscosity. This finding suggests that increasing the proportion of PGPR results in lower apparent viscosity, highlighting the influence of surfactant composition on the rheological behaviour of emulsions

Comparing both viscosity results and microstructure (Figure 5.4 (a) to (e)), which identified an aggregated network for those with higher amounts of lecithin, will have influenced resistance against the moving spindle, creating the higher apparent viscosity values than those of PGPR. Several studies found that lecithin forms a gel-like and aggregated network

contributing to increased viscosity (Knoth, Scherze and Muschiolik, 2005; Rivas, Schneider and Rohm, 2016; Leong *et al.*, 2018; Balcaen *et al.*, 2021; Pang *et al.*, 2022). Interestingly Yao *et al.* (2024) found that emulsions with a higher level of lecithin had lowered droplet mobility, which they believed was due to the formation of aggregates and a gel like serum, which agrees with this study and described studies above. The addition of PGPR improved the emulsion's flow by reducing yield stress, observed in chocolate production (Schantz and Rohm, 2005; Su, De Meulenaer and Van der Meeren, 2023), which could be exhibiting these characteristics in milk fat and aiding droplet formation and creating a kinetically stable emulsion.

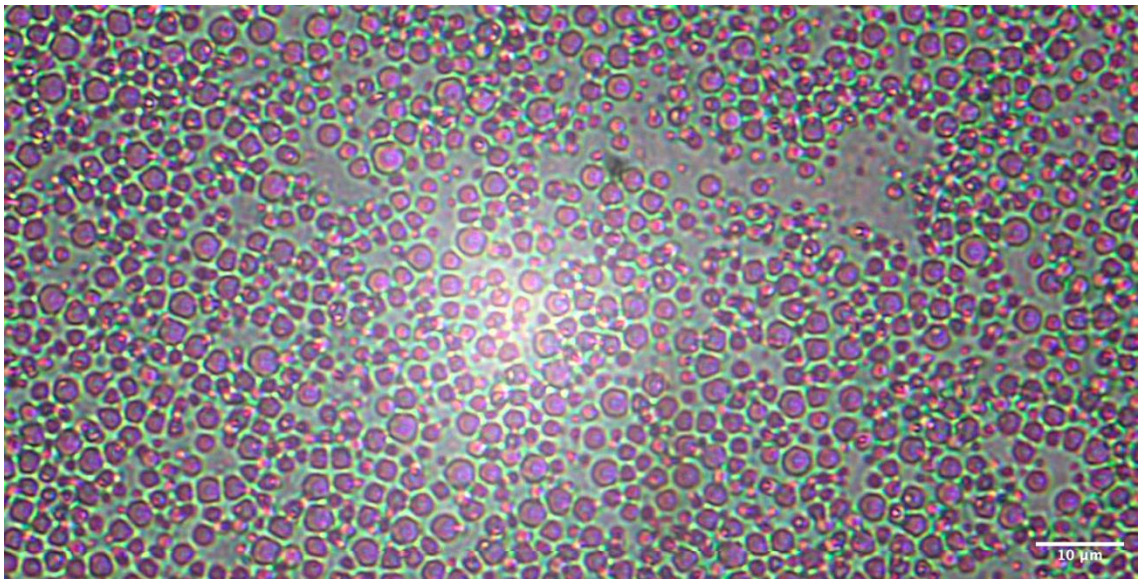


Figure 5.4 (a) – Microstructure of the P2:L0 emulsion under the optical microscope.

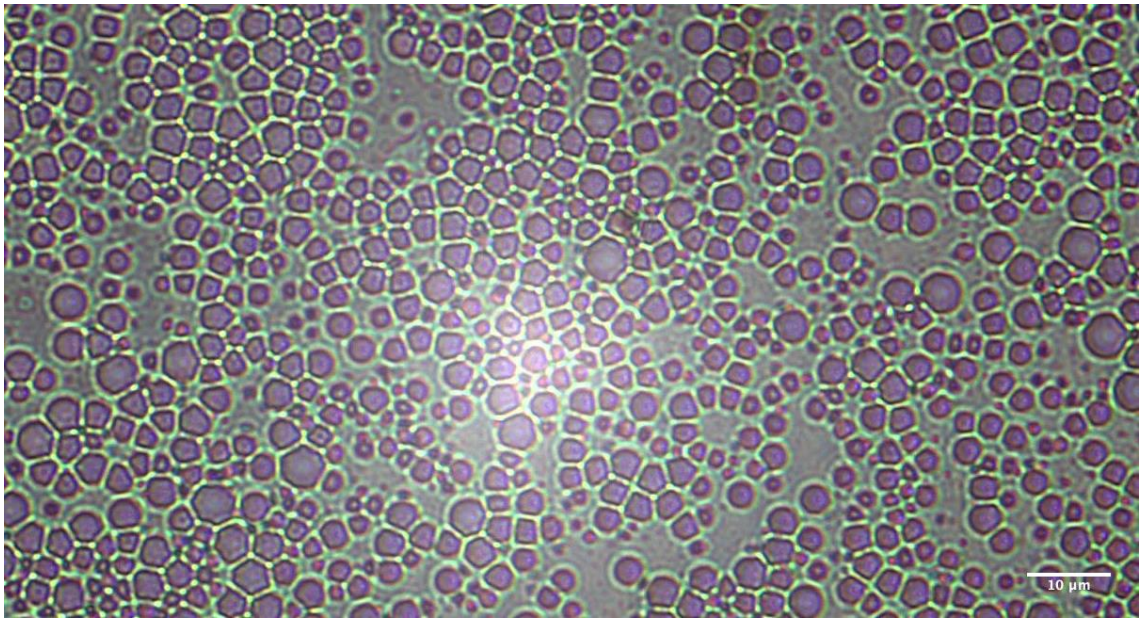


Figure 5.4 (b) – Microstructure of the P1.5:L0.5 emulsion under the optical microscope.

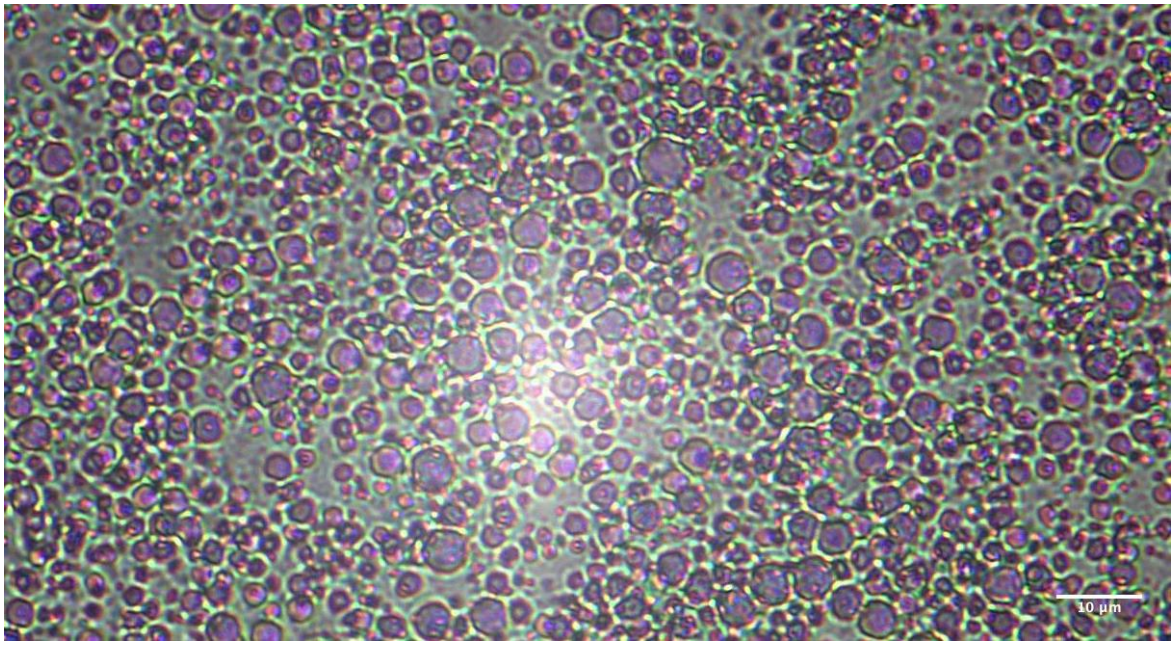


Figure 5.4 (c) – Microstructure of the P1:L1 emulsion under the optical microscope.

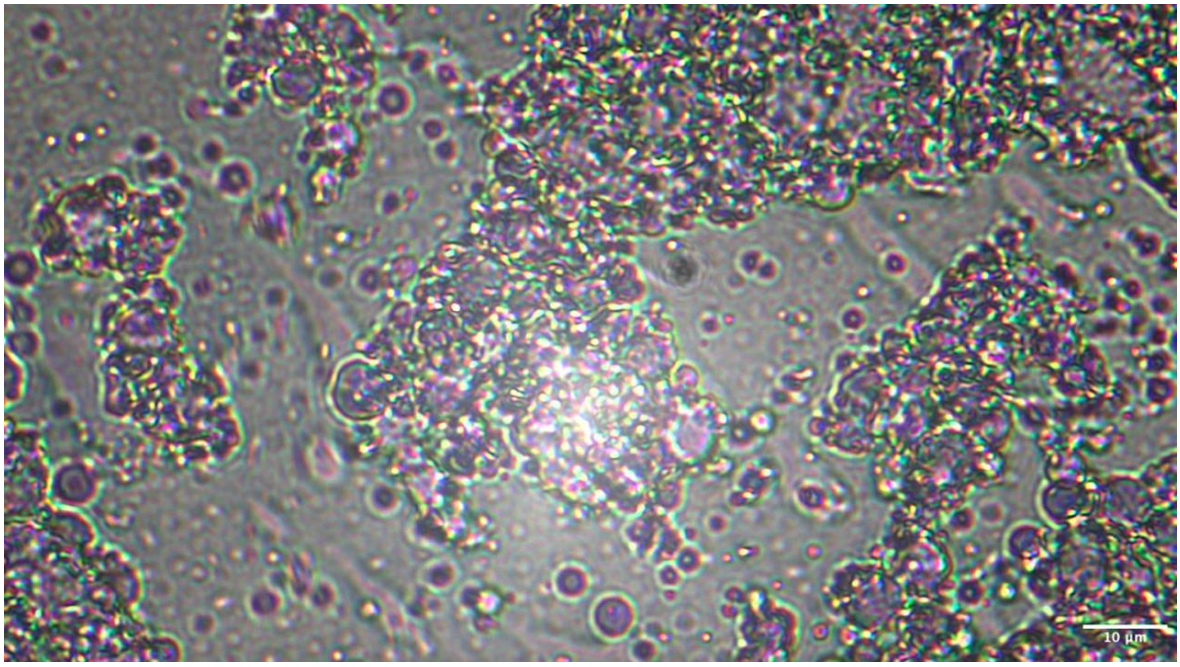


Figure 5.4 (d) – Microstructure of the P0.5:L1.5 emulsion under the optical microscope.

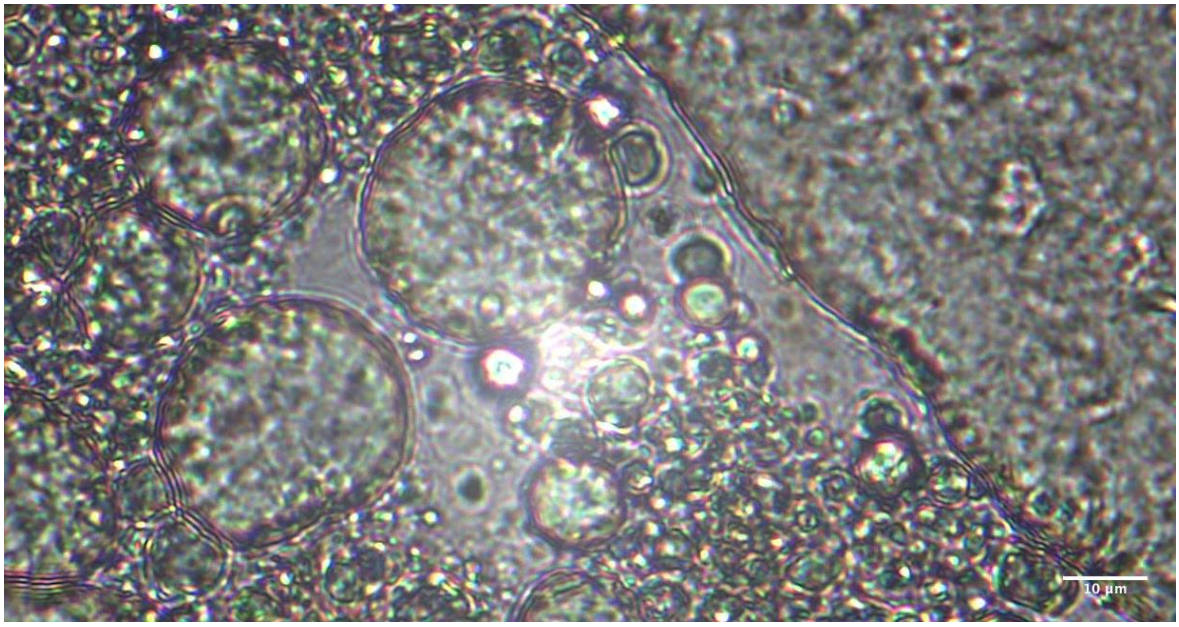


Figure 5.4 (e) – Microstructure of the P0:L2 emulsion under the optical microscope.

The serum index (SI) is used as an indicator of gravitational separation of emulsions. Crystallisation of milk fat retards gravitational separation (McClements, 2016) which could have been the reason why SI was very low and remained low throughout the 30-day period at 0 %, with little clarity of the differing serum layers. However, crystallisation was not measured. Klojdová, Troshchynska and Štětina (2018) compared the use of milk fat to

canola oil in double emulsions and found that milk fat produced emulsions with higher stability due to partial crystallisation, which was also mentioned in Panchal *et al.* (2020).

Emulsion P0:L2, which contained only sunflower lecithin, showed signs of destabilisation with a separated appearance immediately after homogenisation. However, during storage the crystallisation of milk fat may have prevented the sedimentation of water droplets. Interestingly on day 30, phase separation occurred when the samples were heated to 40 °C. Figure 5.5 compared the samples on day 30 at storage temperature (4°C) and after heating to 40 °C, revealing separation upon heating, likely due to the crystallisation of milk fat during refrigeration.

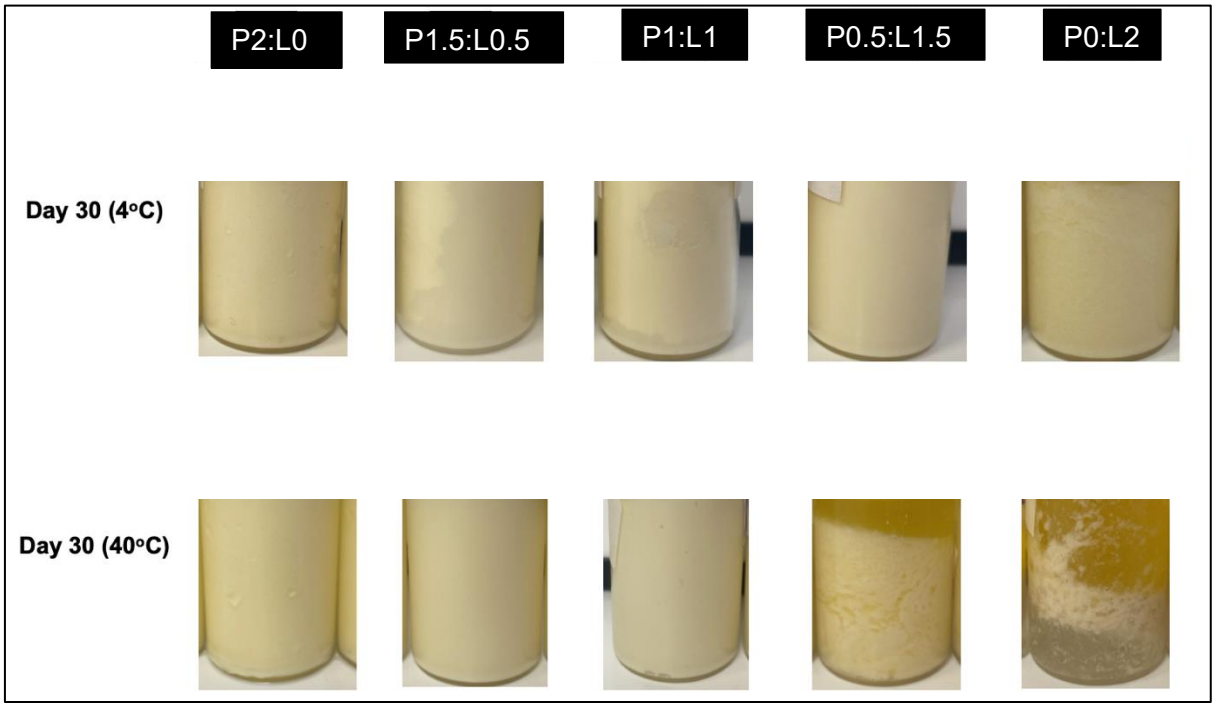


Figure 5.5 – Images of W/O emulsions at day 30, straight from storage at 4°C and after being heated for 30 minutes at 40 °C.

Emulsions P0.5:L1.5 and P0:L2 both exhibited separation. Specifically, emulsion P0:L2 (solely sunflower lecithin) formed three distinct layers: (i) a water at the bottom; (ii) a thick gel-like middle layer of lecithin; and (iii) a fat layer creamed at the top. After being heated for 30 minutes, emulsion P0:L2 showed a serum index of 50 %, indicating clear layer formation.

Investigating the use of further applications of W/O emulsions into double emulsion production, it is fundamental to understand the properties and interactions within a W/O emulsion (Chevalier, Gomes and Cunha, 2021). When considering the rate at which the surfactant adsorbs to the interface, it is important to note that there are specifically two parts: (i) the movement of the surfactant to the interface from the bulk phase followed by (ii)

adsorption to the interface (McClements, 2016). Although adsorption is typically fast, if it is not or is insufficient to cover the droplet, coalescence can occur.

The SI is a good indication of stability over time for W/O emulsions. Additionally, an investigation was conducted to observe the droplet size increase over a short period after homogenisation. When subjecting primary W/O emulsions to two-step emulsification for double emulsion production, excessive homogenisation can destroy the primary emulsions, preventing double emulsion formation.

The droplet size of emulsion P1:L1 was monitored after homogenisation, then at one hour, two hours and four hours post homogenisation. At four hours, the milk fat began to solidify. It is important to identify the optimal time to create double emulsions, to ensure the surfactant has settled around the droplet to withstand further emulsification.

Figure 5.6 shows the $D_{4,3}$ across the time points after emulsification. Using ANOVA and post hoc Tukey HSD, the biggest change was observed between homogenisation and two and four hours later ($P < 0.05$), suggesting that the emulsion undergoes kinetic movement during this period, with some droplets not having fully settled surfactant, leading to coalescence. There was no significant difference between the two- and four- hour marks, indicating that within this time span, emulsion droplet size stabilised and the emulsifier settled around the droplets.

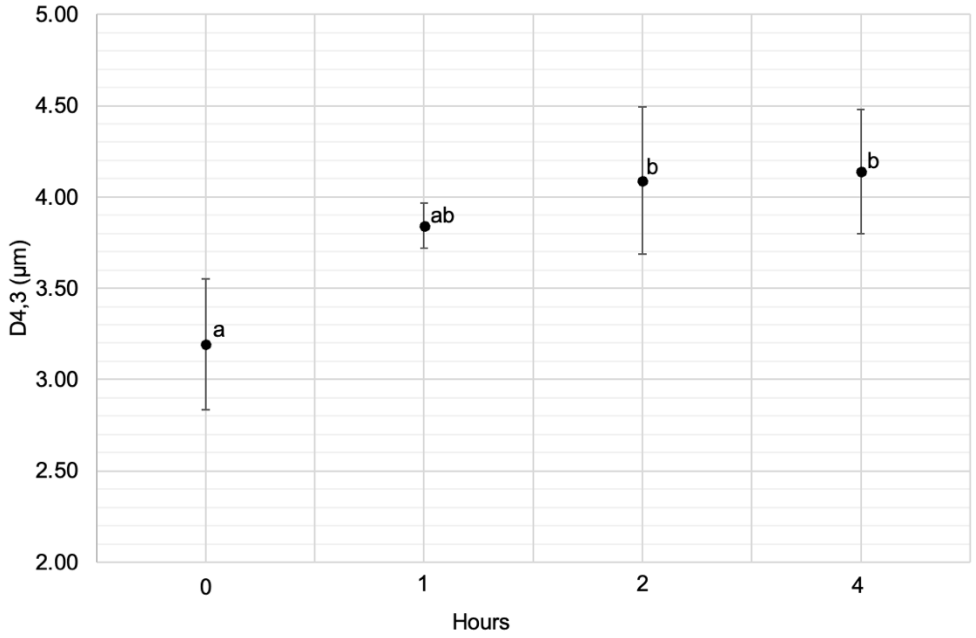


Figure 5.6 – Volume-weighted droplet mean ($D_{4,3}$) of emulsion P1:L1 over a period to monitor the size increase. Error bars represent standard deviation of the mean $D_{4,3}$ from triplicate measurements. Different letters indicate significant differences using Tukey's HSD, $P < 0.05$

Research in this area is limited, especially regarding milk fat W/O emulsions and the optimal time before further processing. Understanding the precise timing and conditions needed for surfactant stabilisation can significantly impact the efficiency and stability of double emulsion production. Due to the lack of equipment available at Harper Adams University to analyse the emulsion (using a Turbiscan or Malvern Mastersizer), measuring the droplet size manually over different time points was the only feasible parameter. However, to obtain a more comprehensive understanding, future studies should incorporate more advanced scientific equipment capable of measuring surfactant adsorption (Surface tensiometers or Spectroscopy methods) and other critical parameters. Addressing this gap is crucial for developing emulsification processes for food applications. Future research should focus on surfactant adsorption and the structural changes and interactions in emulsions over time.

5.4 Conclusion

This research chapter investigated lecithin as an emulsifier in milk fat-based W/O emulsions but found it was unsuccessful due to runaway heat, likely causing the detected instability. Further research is required to understand the interactions occurring with milk fat compared to sunflower oil, although this was not progressed due to lack of resource and time at Harper Adams University. Future work could incorporate the investigation into milk fat. Consequently, after these issues with milk fat, a shift was made towards reducing synthetic surfactants by partially replacing PGPR with lecithin at a 2 % total using varying ratios (P2:L0, P1.5:L0.5, P1:L1, P0.5:L1.5 and P0:L2).

Comparative analysis revealed that lecithin-heavy emulsions had more aggregated structure and higher viscosity, while PGPR-based emulsions exhibited lower viscosity, smaller droplets and a greater monodispersed structure, indicating better emulsion stability and uniformity. Further examination showed that ultrasonic time did not influence droplet size ($P > 0.05$); thus, a 5-minute ultrasonication period was deemed adequate for producing a kinetically stable emulsion with a droplet size ($D_{4,3}$) of below 3 μm. Although the desired < 1 μm was not achieved, it was deemed adequate to progress further into application, due to the reduction in PGPR that was achieved and other papers which found similar sizes and a lack of papers to support the complete use of sunflower lecithin with the desired droplet size.

Based on these findings, emulsions with different ratios of PGPR to sunflower lecithin (P2:L0, P1.5:L0.5 and P1:L1) will be investigated further to explore the potential of reduced synthetic lipophilic surfactants in double emulsions, particularly for cheese production applications.

CHAPTER SIX: FORMULATION OF SKIMMED MILK DOUBLE EMULSIONS USING CHOSEN PRIMARY SURFACTANTS FOR FUTURE APPLICATION

6.1 Introduction

Progressing from Chapter 5, where three emulsion treatments with partially replaced amounts of PGPR by sunflower lecithin as the lipophilic surfactants were chosen (P2:L0, P1.5:L0.5 and P1:L1), this chapter focuses on the formulation of skimmed milk double emulsions, with the reduced amounts of synthetic surfactant.

Double emulsions, particularly water-in-oil-in-water ($W_1/O/W_2$) types, can be utilised in numerous ways, such as improving the structure and function of reduced fat and low fat products and as a method of fortification (Leong *et al.*, 2018). Despite being thermodynamically unstable, these emulsions can be stabilised using hydrophilic surfactants which stabilise the oil droplets in the secondary water phase (O/W_2) (McClements, 2016; Gamlath *et al.*, 2023). Chapter 2 discussed various natural surfactants that have been proven effective in stabilising O/W_2 emulsions.

Skimmed milk is a potential secondary phase for stabilising the primary emulsion in a double emulsion due to its naturally abundant casein and whey proteins. These proteins adsorb to the droplet interface during homogenisation, forming a stabilising layer around the O/W_2 droplets. An additional benefit of using skimmed milk is its compatibility with dairy products, such as low fat yogurt and cheese production.

The size of the milk fat globule impacts the viscosity and processing of milk into products, ultimately influencing the sensory and 'creaminess' sensation of these products. Meaning that the droplet size in a double emulsion is crucial. In addition, varying sizes of O/W_2 droplets can lead to gravitational separation. Ideally, for applications in dairy and cheese, double emulsion droplets should be within the same range as milk fat globules, averaging between 4 to 6 μm (Truong and Bhandari, 2020; Fox, 2022). Giroux *et al.* (2013) successfully created double emulsions with skimmed milk using a high-pressure homogeniser, creating droplet sizes of 6 to 7 μm ($D_{4,3}$). Similarly Paximada, Howarth and Dubey (2021) created double emulsion droplets of 4 to 6 μm ($D_{4,3}$). Both studies had achieved a droplet like the milk fat globule, but that was due to the inner droplet sizes being below < 1 μm. Giroux *et al.* (2013) also successfully incorporated slightly larger double emulsion droplets of 30 μm using a Ultra-Turrax into cheese and Gamlath *et al.* (2023) saw

double emulsion droplet sizes ranging from 280 μm to 380 μm. Both of these studies were much larger than the natural milk fat globule which generally ranges from 2 to 20 μm. However, the larger droplets of 30 μm and 280 μm to 380 μm were able to incorporate them into milk for dairy application. El Kadri *et al.* (2018) used double emulsions in set-type yogurt for the delivery of probiotics, with droplet sizes of 10 to 15 μm for the inner water droplets compared to 50 to 70 μm for the outer droplets. These were successfully incorporated into yogurt, but sensory evaluation was not undertaken to see the true impact of these larger droplet sizes compared to the native milk fat globule. The size of the double emulsion droplets is not only dependent upon the droplet size of the primary emulsion but also the interaction of the primary emulsion with the secondary phase and processing parameters. For example the viscosity of the primary emulsion which can influence the droplet formation of double emulsions (Silva *et al.*, 2020).

The alteration of the homogenisation method for double emulsions can influence the size of the droplet produced and can be manipulated to create the researchers' desired outcome. Excessive homogenisation may have a negative effect on the primary emulsion resulting in the prevention of double emulsion formation. A study by Leong *et al.* (2018) used an Ultra-Turrax and ultrasound, double emulsions made with ultrasound were much smaller than those of the Ultra-Turrax, ranging from 10 to 100 μm compared droplets ranging from 1 to 50 μm. However, the concern with ultrasound is excessive and prolonged processing times which can cause re-coalescence of droplets and ultimately impair double emulsion stability. The manipulation of processing techniques can benefit double emulsion production and help to create the desired emulsions for further application in food research.

6.1.1 Aim and objectives

The aim of this experimental study was to investigate the chosen primary emulsion treatments from Chapter 5 in their ability to create stable skimmed milk double emulsions.

The objectives include:

- Evaluating the impact of the primary emulsion treatments (varying ratio of PGPR to sunflower lecithin) on $W_1/O/W_2$ droplet size and encapsulation efficiency.
- Optimising the processing parameters of double emulsions to create droplets suited for further application in dairy products, such as cheese. This involves:
    - Assessing the impact of processing speed and time on the Silverson High Shear Mixer during double emulsion production
    - Determining the optimal ratio of the primary emulsion to the secondary phase (skimmed milk) for creating double emulsions.

6.2 Materials and methods

All materials and methods are described in Chapter 3 – general materials and methods. After a decision was made to partially replace PGPR with sunflower lecithin the chosen surfactant concentration was 2 % of the total emulsion and the following surfactant ratios and emulsions codes used in this experiment are presented in Table 6.1.

Table 6.1 – Sample codes for the varying primary emulsion surfactant ratios of PGPR to sunflower lecithin

Primary emulsion code	Surfactant ratio PGPR:Sunflower lecithin
P2:L0	2:0
P1.5:L0.5	1.5:0.5
P1:L1	1:1

When investigating the initial speed and time on the Silverson high shear mixer, emulsions were created to a 20:80 ratio of (W_1/O) to secondary phases (W_2) [double emulsion code WOW 20). Then for the following experiments, codes in Table 6.2 identify all the ratios for the double emulsions.

Table 6.2 – Sample codes for the varying ratios of primary emulsion (water-in-milk fat) to the secondary aqueous phase (skimmed milk)

Double emulsion code	Primary (W_1/O)	Secondary (W_2)
WOW 20	20	80
WOW 25	25	75
WOW 30	30	70
WOW 35	35	65
WOW 40	40	60

Encapsulation efficiency was calculated using an adaptation of equations from Piacentini (2016) the calculation of droplet volume by McClements (2016). This equation (Equation 17) was used as an estimation of inner droplet encapsulation into the double emulsion.

Equation 17 – Encapsulation Efficiency

$$EE\ (\%) = \frac{V_{encapsulated}}{V_{outer\ droplets}} x\ 100$$

The volume of encapsulated (inner droplet) was calculated by multiplying the number of droplets by volume of a droplet. The radius in the equation was the average radius of all the inner droplets in that image. The outer droplets were calculated in the same way, and although this assumes that all droplets were the same size, which in the microstructure they were not, this provides an indication to the average double emulsion encapsulation. The size of the droplet was measured using Image J software (Java, NIH Image) from images collated from the use of the optical microscope. Multiple images were measured to estimate the encapsulation efficiency for each emulsion.

6.3 Results and discussion

6.3.1 Effect of processing conditions on the properties of double emulsions homogenised with high-shear mixer (Silverson)

The investigation into varying speeds and times aimed to optimise production parameters to create double emulsion droplets similar in size to milk fat globules, which range from 2 μm to 20 μm, with an average of 4 to 6 μm (Truong and Bhandari, 2020; Fox, 2022). Achieving this size is critical for application in cheese production and other dairy products. Figure 6.1 displays the $D_{4,3}$ of each of the double emulsion, categorised by the primary emulsion treatments, across the three speeds and times used on the Silverson high-shear mixer.

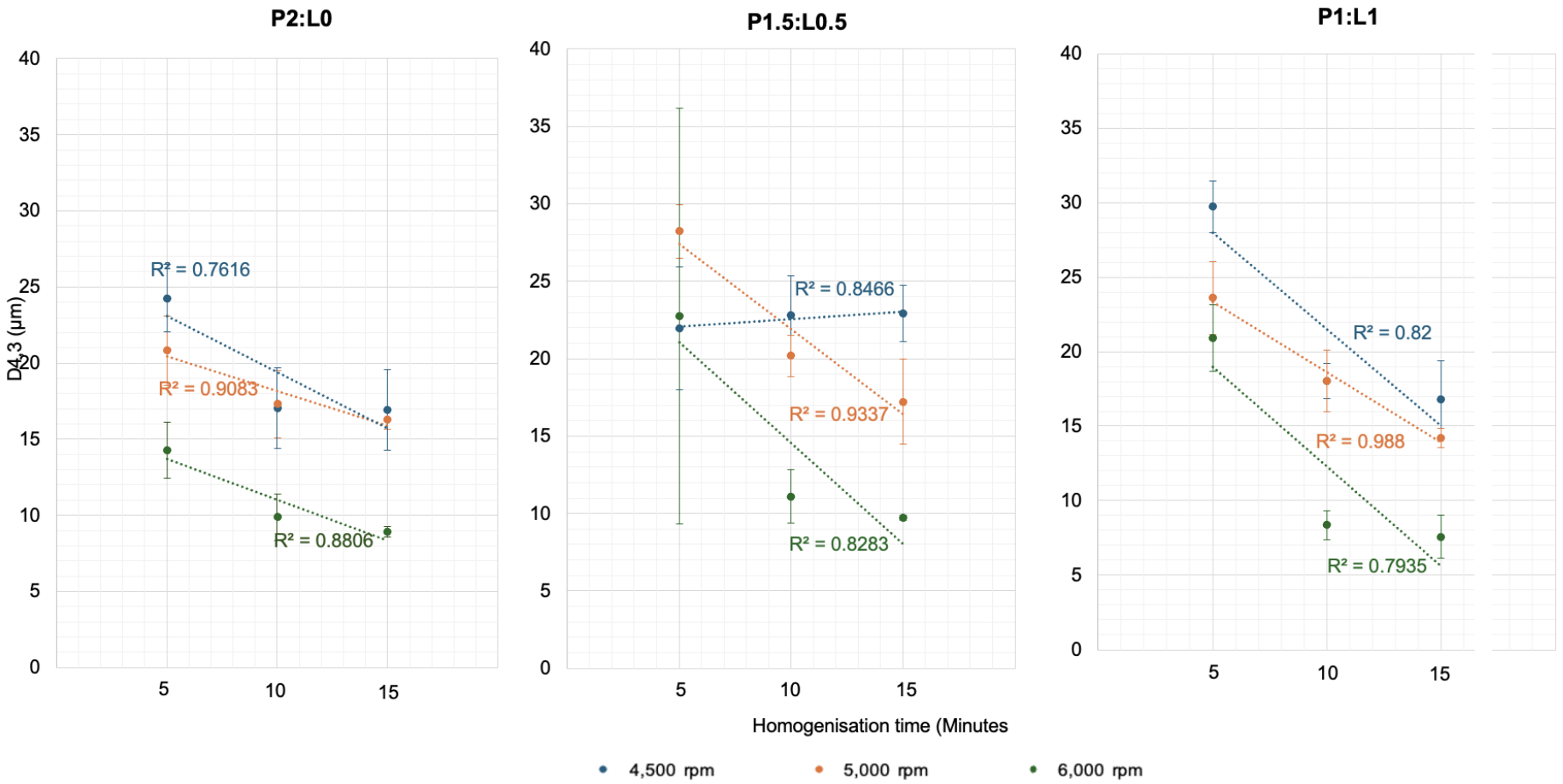


Figure 6.1 Volume-weighted droplet mean ($D_{4,3}$) values for each primary emulsion treatment, split across each speed and each time iteration on the Silverson high shear mixer. Error bars represent standard deviation across triplicate samples and a linear trend line has been fitted , with the coefficient of determination (R^2) calculated and displayed.

Generally, as the speed during double emulsion increases, the droplet size decreases. For example at 5 minutes on the Silverson, P2:L0 at 4,500 rpm is 24.27 μm and reduces in size to 14.27 μm at 6,000 rpm. Similarly, P1:L1 shows a droplet size of 29.71 μm at 4,500 rpm reduced to 23.62 μm at 5,000 rpm and 20.92 at 6,000 rpm. This trend was supported by regression analysis, with the coefficient of determination (R^2) values for P2:L0 indicating a moderatley strong to strong correlation between speed and droplet size (R^2 = 0.7616 at 4,500rpm, 0.9083 at 5,000 rpm and 0.8806 at 6,000 rpm).

Similar correlations were found for P1.5:L0.5 (R^2 = 0.8466, 0.9337 and 0.8283 for 4,500, 5,000 and 6,000 rpm respectively) and P1:L1 (R^2 = 0.82, 0.988 and 0.7935, for 4,500, 5,000 and 6,000 rpm respectively), indicating that as speed increases, droplet size consistently decreases across all primary emulsion formulations.

However, an exception was observed for P1.5:L0.5 at 4,500 rpm, where droplet sizes increased slightly over time, which contrasts with the overall decreasing trend seen in other conditions. This anomaly may be attributed to experimental variability or minor emulsion instability during processing at this speed, but given the strong correlations at other speeds and forumlations, it does not undermine the general trend observed.

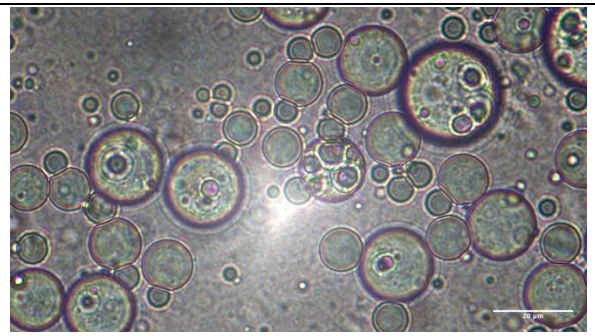
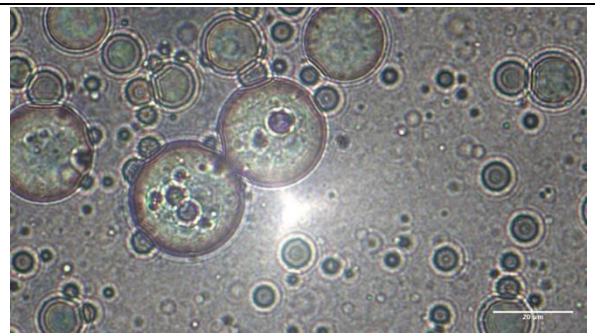
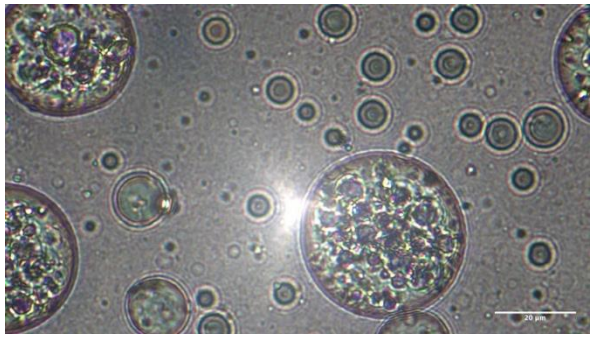
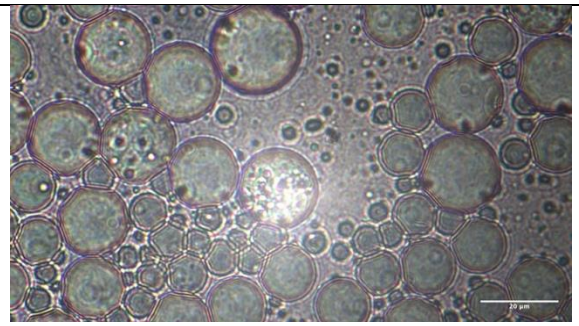
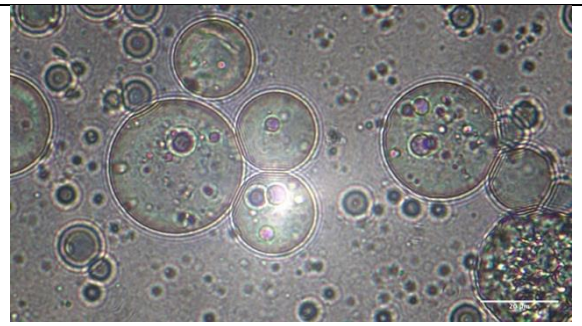
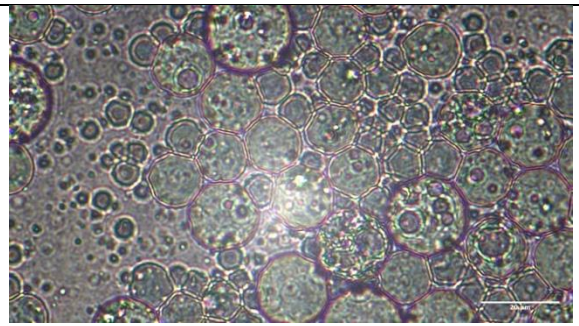
Incresing the time on the Silverson also resulted in smaller droplet sizes. For instance, P2:L0 at 5,000 rpm decreased from 20.83 μm, at 5 minutes to 17.30 μm at 10 minutes, and 15.28 μm at 15 minutes. P1.5:L0.5 at 6,000 rpm showed a reduction from 15.03 μm to 9.72 μm over the same time span. These findings were further supported by ANOVA, which confirmed a significant difference between both speed and droplet size, and time and droplet size ($P < 0.05$). The combined effect of primary emulsion, time and speed on $D_{4,3}$ was also significant ($P = 0.0167$), indicating that all parameters meaningfully influenced final droplet size.

Lower speeds and shorter times resulted in larger droplets, between 23 to 29 μm. These findings are consistant with several studies using a similar homogenisation method with an Ultra-Turrax rotor-stator mixer. For example, Leong *et al.* (2018) found $W_1/O/W_2$ droplets ranging from 10 to 100 μm at a ratio of 5:95 ($W_1/O:W_2$) using an Ultra-Turrax. Similarly, Giroux *et al.* (2013) using a ratio of 35:65 ($W_1/O:W_2$) identified droplet sizes over 30 μm using comparable methods in skimmed milk, compared to those produced by high pressure homogenisation, which were less than 13 μm. Pérez, Wagner and Márquez (2017) achieved double emulsion droplets of 31.01 μm at a 20:80 ratio ($W_1/O:W_2$) with a 2 % PGPR concentration and 4.9 % gluclose in the inner droplet. The larger droplets require more surfactants to be able to cover the droplet, which leads to coalescence and phase

separation. Despite limited research securing double emulsion droplets within the size range of milk fat globules, Paximada, Howarth and Dubey (2021) successfully achieved droplets within the 4 to 6 μm range at a ratio of 5:95 (W_1/O:W_2). This was accomplished using a whey protein-fortified water droplet, which influenced the stability and formation of the emulsions, as whey protein and the lipophilic emulsifiers surrounding the droplet would interact creating a strong steric barrier. Giroux *et al.* (2013) using a ratio of 35:65 (W_1/O:W_2) achieved a smaller droplet, similar to the milk fat globule with high pressure homogenisation being 6 to 7 μm. In this experiment, the only droplets achieving similar sizes were those at 6,000 rpm for durations of 10 and 15 minutes. The higher speeds and longer homogenisation times can damage and destabilise the emulsion, as excessive force can disrupt and break the droplets destroying the inner droplets of the primary emulsion but also encourage recoalescense, as found in previously mentioned studied with excessive ultrasounds times in Chapter 4.

Further investigation into the microstructure, displayed in Figure 6.2, 6.3 and 6.4, demonstrate a range of droplet sizes and the ones with a larger lecithin content (emulsion P1:L1) were slightly more flocculated compared to emulsion P2:L0. The images also show that at greater speeds and longer times, there were fewer encapsulated water droplets. This outcome defeats the purpose of double emulsions, as the absence of encapsulated water droplets results in an O/W rather than a true double emulsion. This finding underscores the importance of optimising both the speed and duration of homogenisation to maintain the integrity of the double emulsion structure, crucial for their intended application in dairy products.

Figure 6.2 – Microstructure of the double emulsions after production at 4,500 rpm on the Silverson high shear mixer over different time iteration and each primary emulsion treatment.

	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
5 Minutes			
10 Minutes			

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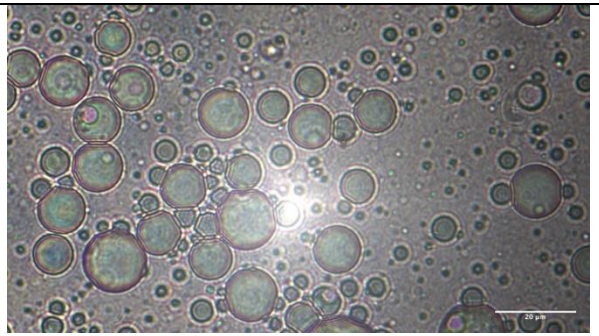
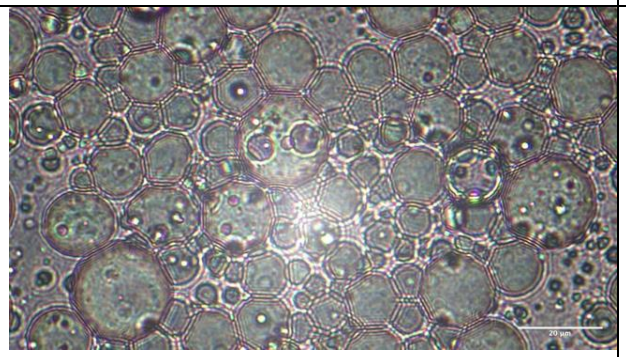
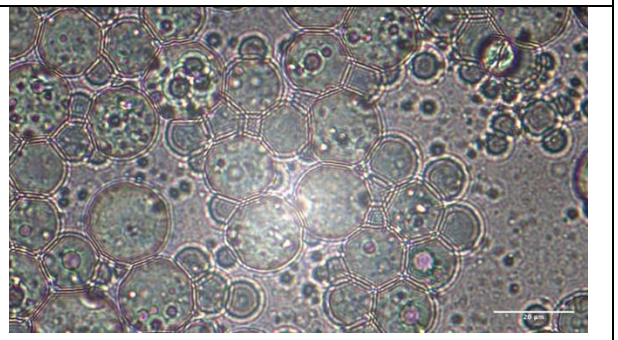
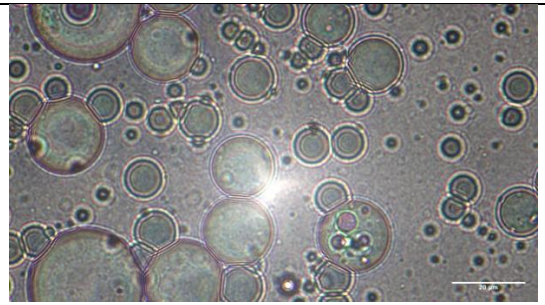
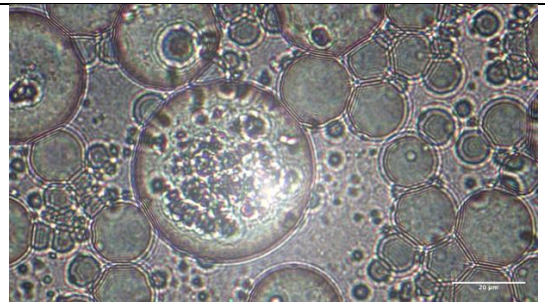
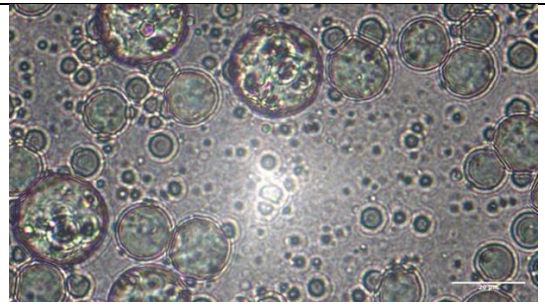
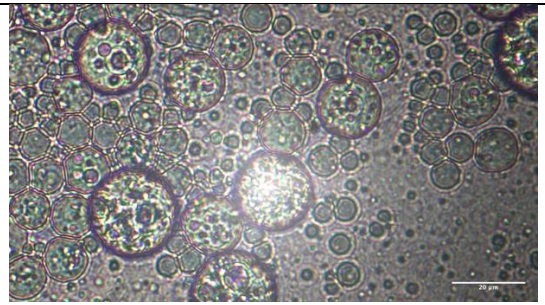
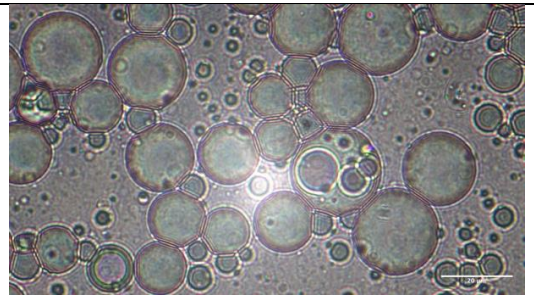
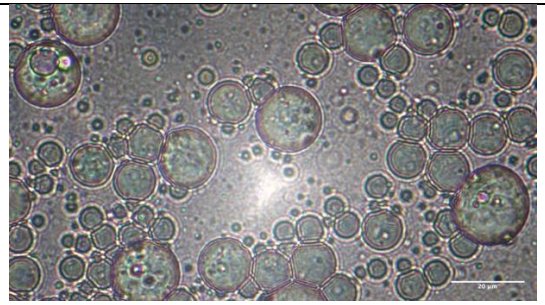
	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
15 Minutes			

Figure 6.3 – Microstructure of the double emulsions after production at 5,000 rpm on the Silverson high shear mixer over different time iteration and each primary emulsion treatment.

	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
5 Minutes			
10 Minutes			

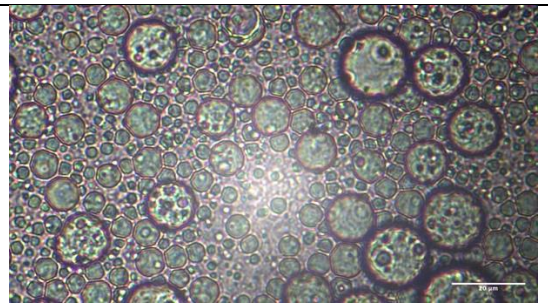
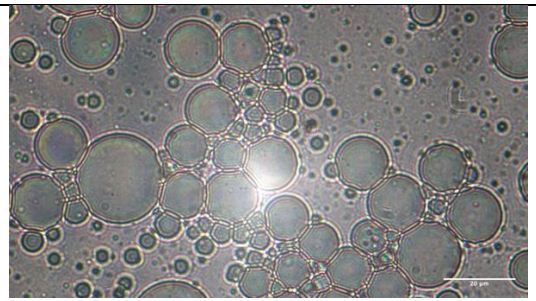
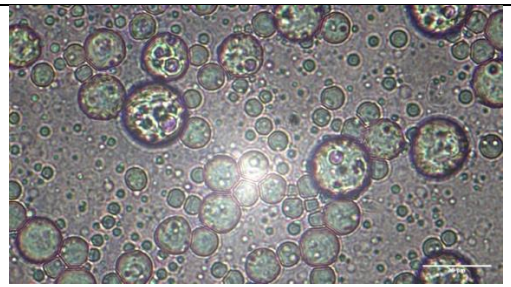
	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
15 Minutes			

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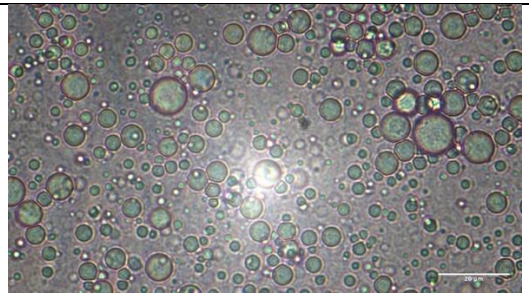
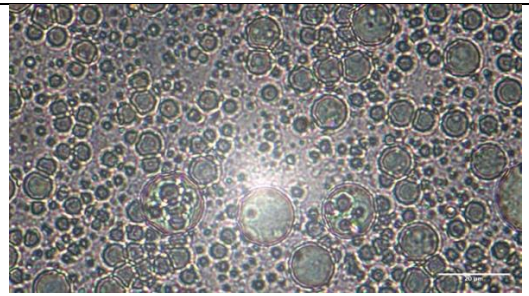
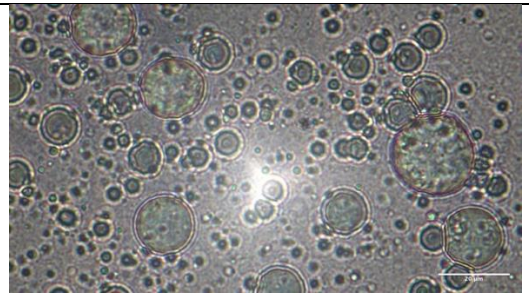
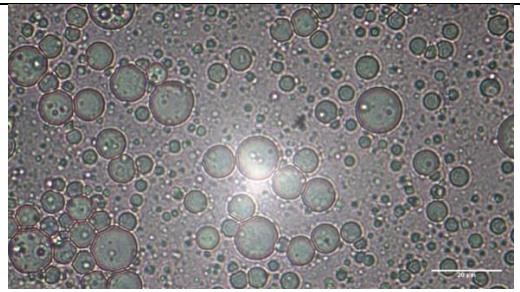
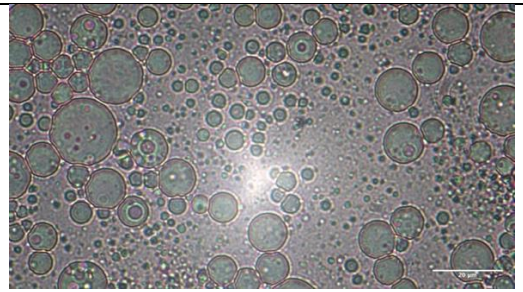
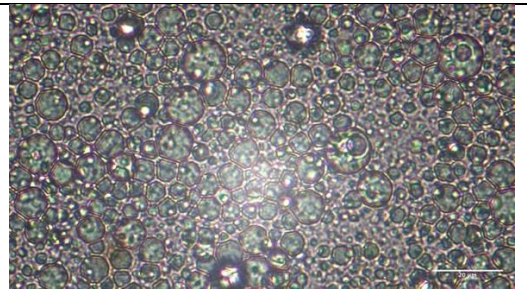
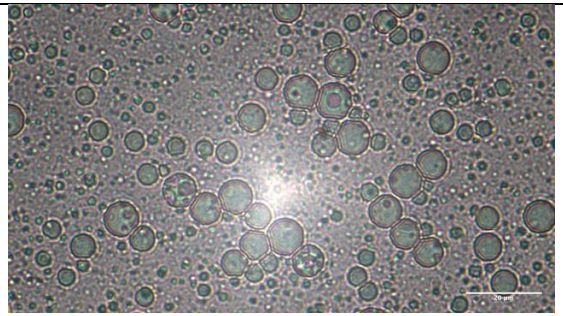
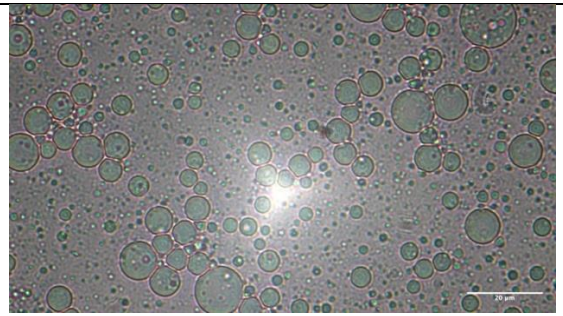
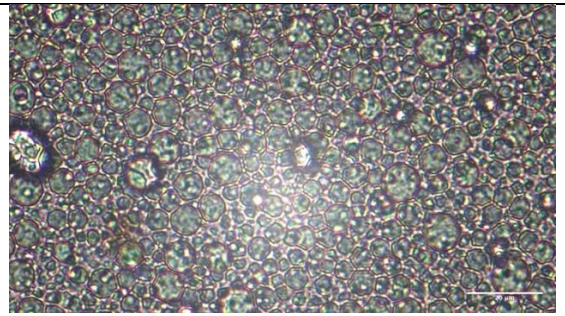
Figure 6.4 – Microstructure of the double emulsions after production at 6,000 rpm on the Silverson high shear mixer over different time iteration and each primary emulsion treatment.

	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
5 Minutes			
10 Minutes			

	Emulsion P2:L0	Emulsion P1.5:L0.5	Emulsion P1:L1
15 Minutes			

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Figures 6.5 to 6.7 display the double emulsions under a confocal fluorescence microscope, specifically the samples created at a speed of 5,000 rpm for 10 minutes. The images reveal fluorescent, red-stained fat globules and black circles within the globules, indicating the retention of inner water droplets, thus double emulsions have been achieved. Leong *et al.* (2020) also used this fluorescence microscope and identified the production of double emulsions, by identifying black circles within the red fat droplets. Some differences were observed between the confocal images obtained at Warwick University and the optical microscopy images taken at Harper Adams University. As the emulsions were prepared at Harper Adams and transported for approximately two hours before imaging, it was hypothesised that time-related changes during transport may have influenced the emulsion structure. This explanation is supported by the fact that the experiment was repeated on two separate occasions, and similar differences were observed each time, consistently following the transport. Given that optical microscopy was conducted shortly after emulsion preparation, while confocal microscopy occurred after a delay, it is likely that structural changes over time — such as droplet coalescence or phase separation — contributed to the observed discrepancies. Nonetheless, inherent differences between confocal and optical microscopy techniques may also have played a role in the visual variation. During transport, the emulsions began to separate due to the relatively large initial droplet size (~20 μm), which likely promoted coalescence and an increased serum inddex. As a result, the samples were shaken prior to confocal imaging, which may further contribute to the formation of larger, aggregated droplets exceeding 30 μm in diameter.

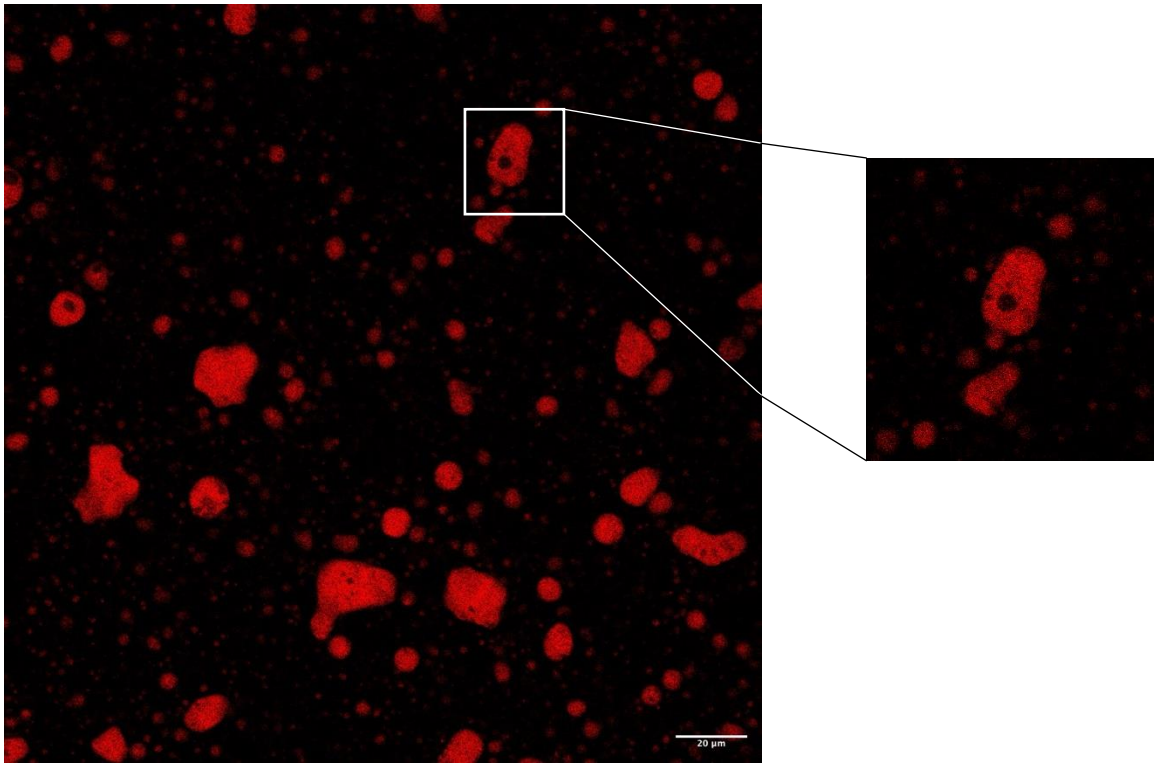


Figure 6.5 – Confocal microscope of double emulsion P2:L0 after 10 minutes Silverson high shear mixer at 5,000 rpm, showing fat droplets (in red) and inner water droplets (seen as black circles within the fat globules).

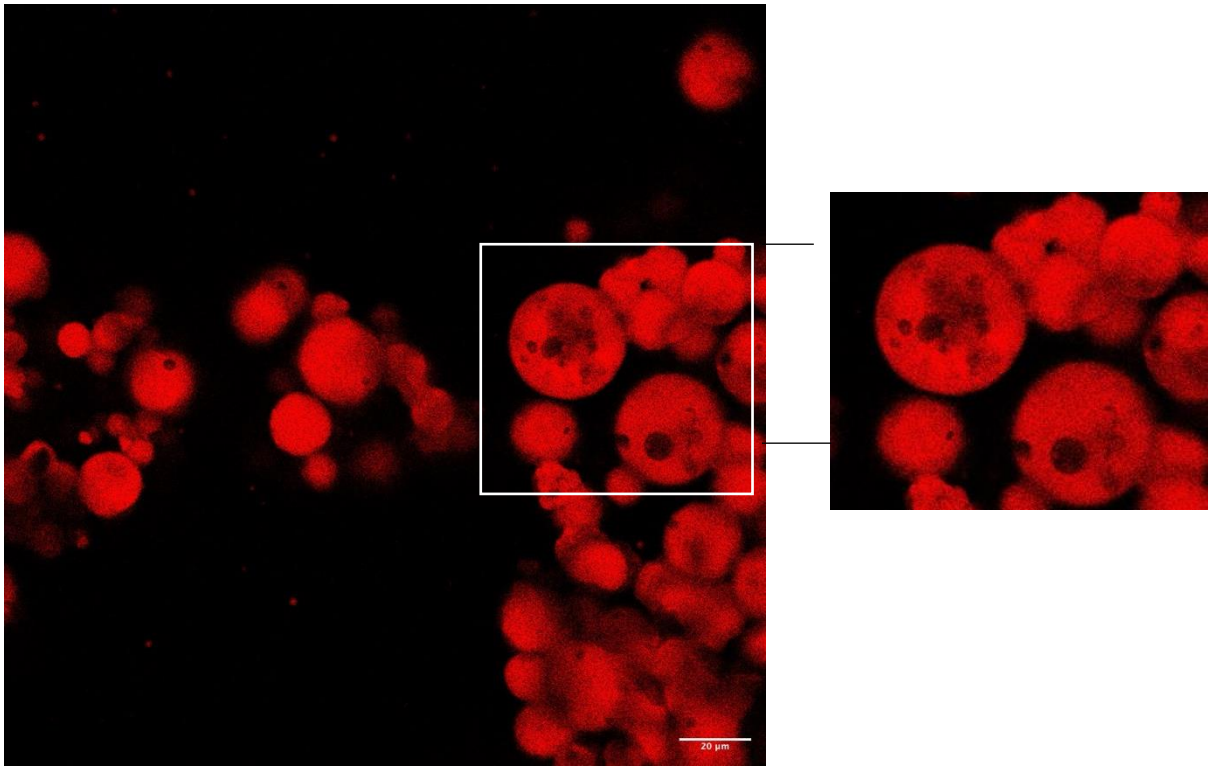


Figure 6.6 – Confocal microscope of double emulsion P1.5:L0.5 after 10 minutes Silverson high shear mixer at 5,000 rpm, showing fat droplets (in red) and inner water droplets (seen as black circles within the fat globules).

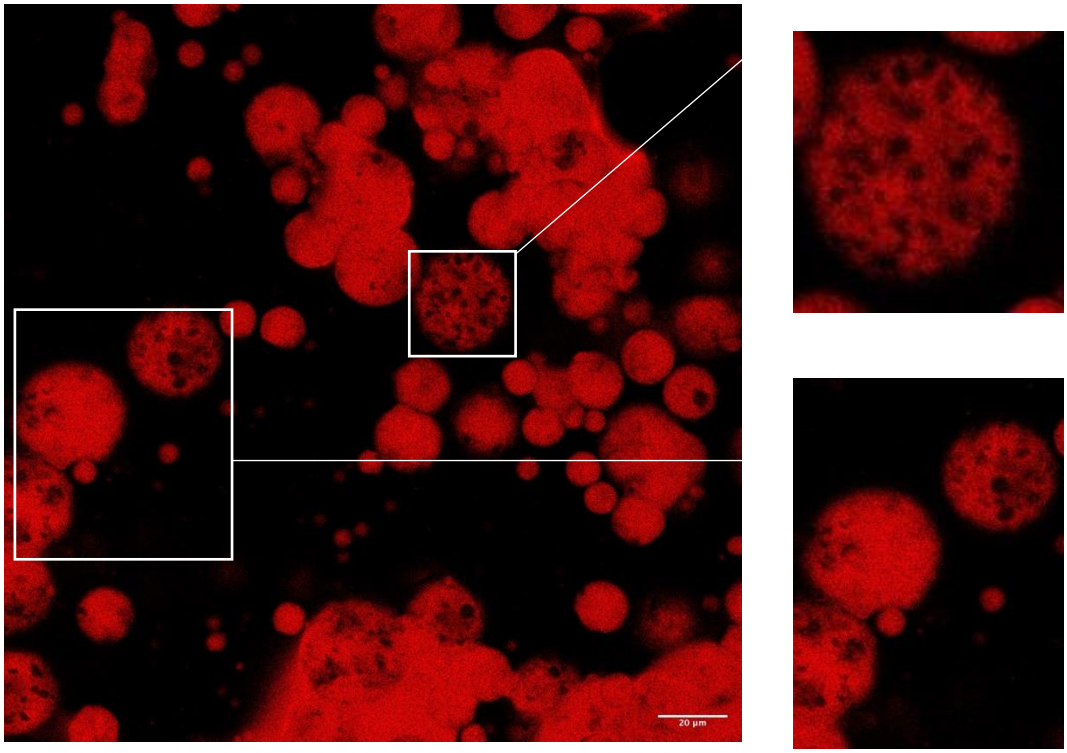


Figure 6.7 – Confocal microscope of double emulsion P1:L1 after 10 minutes on the Silverson high shear mixer at 5,000 rpm, showing fat droplets (in red) and inner water droplets (seen as black circles within the fat globules).

6.3.2 Investigating the primary emulsion to the secondary phase ratio on skimmed milk double emulsions.

Following the previous experiment, it was decided to use 6,000 rpm on the Silverson high shear mixer to produce double emulsions. This speed was chosen to achieve droplet sizes closer to those of milk fat globules, having achieved droplets of 7 to 9 µm and were stable for the longest time. The next step was to investigate the effects of homogenisation time by comparing 10 minutes and 15 minutes on the Silverson high shear mixer, the aim was to optimise double emulsion production further. Additionally, the ratio of the primary emulsion to the secondary phase was altered to improve the encapsulation of the inner water droplets. It has been hypothesised that increasing the primary to secondary phase ratio results in larger droplet sizes (Maghamian, Goli and Najarian, 2021). Other studies have also described the effect of the inner phase viscosity on encapsulation efficiency in double emulsion, where a greater viscosity of the primary emulsion leads to a larger double emulsion droplet (Leong *et al.*, 2018; Hu and Van der Meeren, 2024)

.Figures 6.8 and 6.9 shows a scatter plot comparing the $D_{4,3}$ value against estimated encapsulation efficiency percentage across the five double emulsion ratios per primary emulsion. These plots were designed to investigate the hypothesis that larger droplets are associated with greater encapsulation efficiency, as more internal phase may be retained within each droplet. The graphs indicate a general trend; as ratio increases, both encapsulation efficiency and droplet size also increased. For example, for WOW 20 with a primary emulsion of P1:L1 at 10 minutes had an estimated encapsulation efficiency of 0.178 % and a droplet size of 9.885 μm, compared to WOW 35, with an estimated encapsulation efficiency of 1.305 % and a droplet size of 14.246 μm.

ANOVA identified a highly significant difference ($P < 0.05$) between emulsion ratios $D_{4,3}$ and encapsulation efficiency. This proves that a change in primary emulsion to secondary ratio results in a difference in droplet size and encapsulation efficiency. This finding is supported by other studies, which describe how increasing the ratio of primary to secondary phases results in larger droplet sizes (Maghamian, Goli and Najarian, 2021), with the primary droplet size influencing encapsulation efficiency, retention and O/W_2 size. For example, Gamlath *et al.* (2023) describe how the inner water droplets of 1 to 3 μm contribute to much larger double emulsion droplets of 300 μm.

In the current study, despite the primary emulsions' droplets ranging from 3.2 to 3.5 μm, droplet sizes of 10 to 15 μm for WOW 30 at 10 minutes at 6,000 rpm were achieved, but the estimated encapsulation efficiency varied from 0.6 % to 1.5 %. In comparison, lower ratios such as WOW 20, produced smaller droplet sizes of 8 to 11 μm, with encapsulation efficiencies under 0.3%. WOW 40, the ratio with the highest primary to secondary proportion, showed the largest droplet sizes (16 to 19 μm) and encapsulation efficiencies ranging from 1.1 % to 1.5 %. This suggests that a higher ratio provides more opportunity for inner water droplets to be encapsulated, though at the expense of increased O/W_2 size.

To better understand the relationship between droplet size and encapsulation efficiency, linear trend lines with R^2 values were fitted to Figures 6.8 and 6.9. For 10 minute homogenisation (Figure 6.8), R^2 values were 0.55 for P2:L0, 0.652 for P1.5:L0.5, and 0.066 for P1:L1, indicating moderate to strong correlations in the first two emulsions and a very weak correlation in the third. For 15 minutes (Figure 6.9), the R^2 values were 0.68 (P2:L0), 0.477 (P1.5:L0.5), and 0.304 (P1:L1). These results confirm that in some emulsions, particularly P2:L0, droplet size and encapsulation efficiency are closely linked, whereas in others, like P1:L1, this relationship is weaker – potentially due to the formulation differences or destabilisation effects.

The goal is to strike a balance between encapsulating as many primary emulsion droplets as possible without producing excessively large droplets, which increases the risk of coalescence and phase separation. Although time on the Silverson high shear mixer had a significant impact ($P < 0.05$), suggesting that $D_{4,3}$ and encapsulation efficiency changes over time, the combined effects of emulsion ratio and time, and emulsion type and time, had no significant impact ($P < 0.05$) on $D_{4,3}$ or encapsulation efficiency. These effects were evaluated using ANOVA. Based on this statistical analysis, the difference between 10 minutes and 15 minutes on the Silverson high shear mixer did not have a significant ($P > 0.05$) impact when all factors are considered. Therefore, the shorter time of 10 minutes would be more suitable as it reduces production time, without significantly affecting the double emulsions. This time was selected as the optimal processing conditions for the next chapter.

There was a significant impact of primary emulsion type (P2:L0, P1.5L:L0.5 and P1:L1) on the $D_{4,3}$ and encapsulation efficiency ($P < 0.05$), indicating that the primary emulsion affected these variables and consequently the overall double emulsion. Hu and Van der Meeren (2024) found that higher lecithin content in the primary emulsion composition caused an increase in inner droplet size, which in turn impacted and doubled the O/W_2 droplet size. Leong *et al.* (2018) also observed that an increased amount of lecithin resulted in a greater number of primary emulsion droplets encapsulated, due to the viscosity. Although the number of primary emulsion droplets to secondary in this study was not investigated, some of the microstructure images suggest a similar outcome

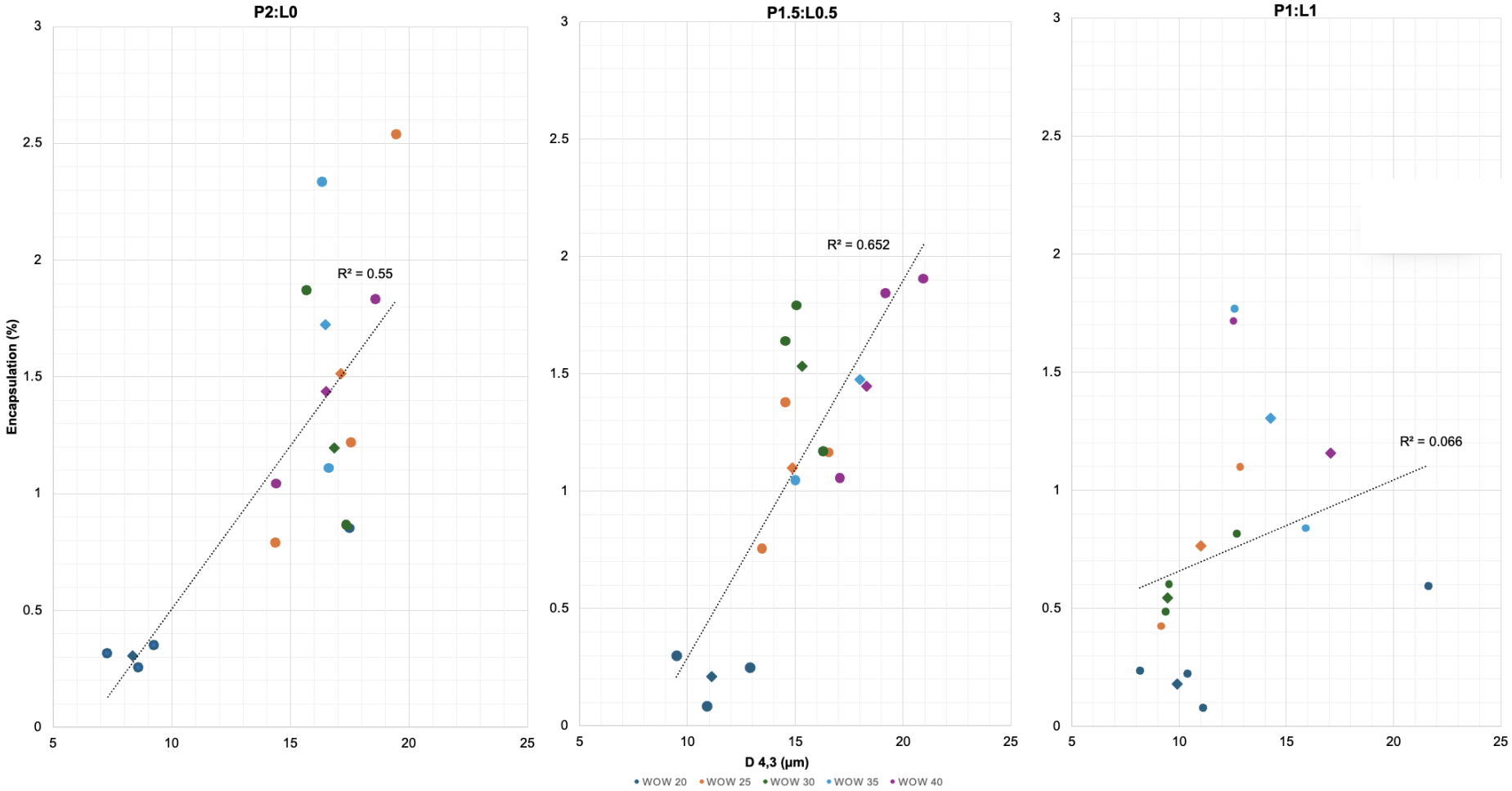


Figure 6.8 – Scatter plot comparing volume-weighted diameter ($D_{4,3}$ /μm) and encapsulation efficiency for each of the double emulsion ratios, and separated by primary emulsion groups for 10 minutes on the Silverson Mixer. (●) Data points and (♦) mean of triplicate measurements with a linear trend line fitted with R^2 values .

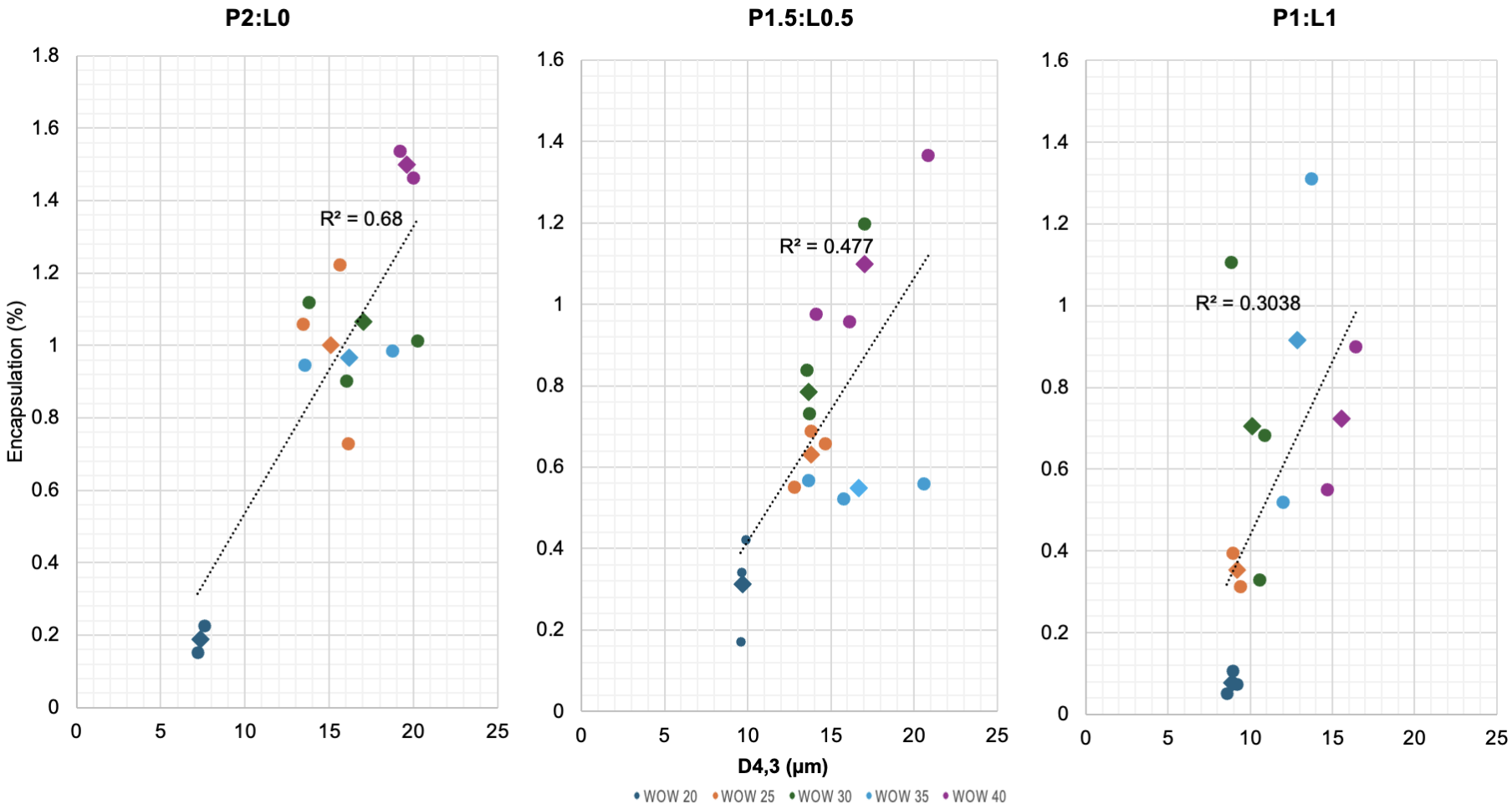


Figure 6.9 – Scatter plot comparing volume-weighted diameter ($D_{4,3}$ /µm) and encapsulation efficiency for each of the double emulsion ratios, and separated by primary emulsion groups for 15 minutes on the Silverson Mixer. (●) Data points and (♦) mean of triplicate measurements with a linear trend line fitted with R^2 values .

Figure 6.10 shows the serum index of each double emulsion ratio over a period of seven days. These emulsions began to destabilise after 2 hours, with a gentle increase and then begin to plateau and after being in the fridge there was a compact layer of fat at the top. Although not published, Klojdová, Troshchynska and Štětina (2018) mention about their previous work in their published paper, where upon cooling a compact layer of milk fat was found in their double emulsion, which was also experienced in this study. The reason for the compact layer could be the reliance on the natural caseins and whey proteins in the skimmed milk. There may not be enough to cover the droplet, therefore causing coalescence and eventually phase separation. This leads to the fat to cream to the top, and because it is not suspended in the milk, it causes a firm layer. Several studies supported the results found here, in Figure 6.10, where the double emulsions were not stable for seven days (Leong *et al.*, 2018; Maghamian, Goli and Najarian, 2021). This could be associated with larger droplets and the potential for coalescence. For example in the study by Klojdová, Troshchynska and Štětina (2018) they found that droplets went from 30 μm to 85 μm in 0 to 4 weeks, which could have been due to coalescence over time. All SI values reached beyond 40 % after 2 hours, similarly, Giroux *et al.* (2013) found 46 % SI for skimmed milk double emulsions.

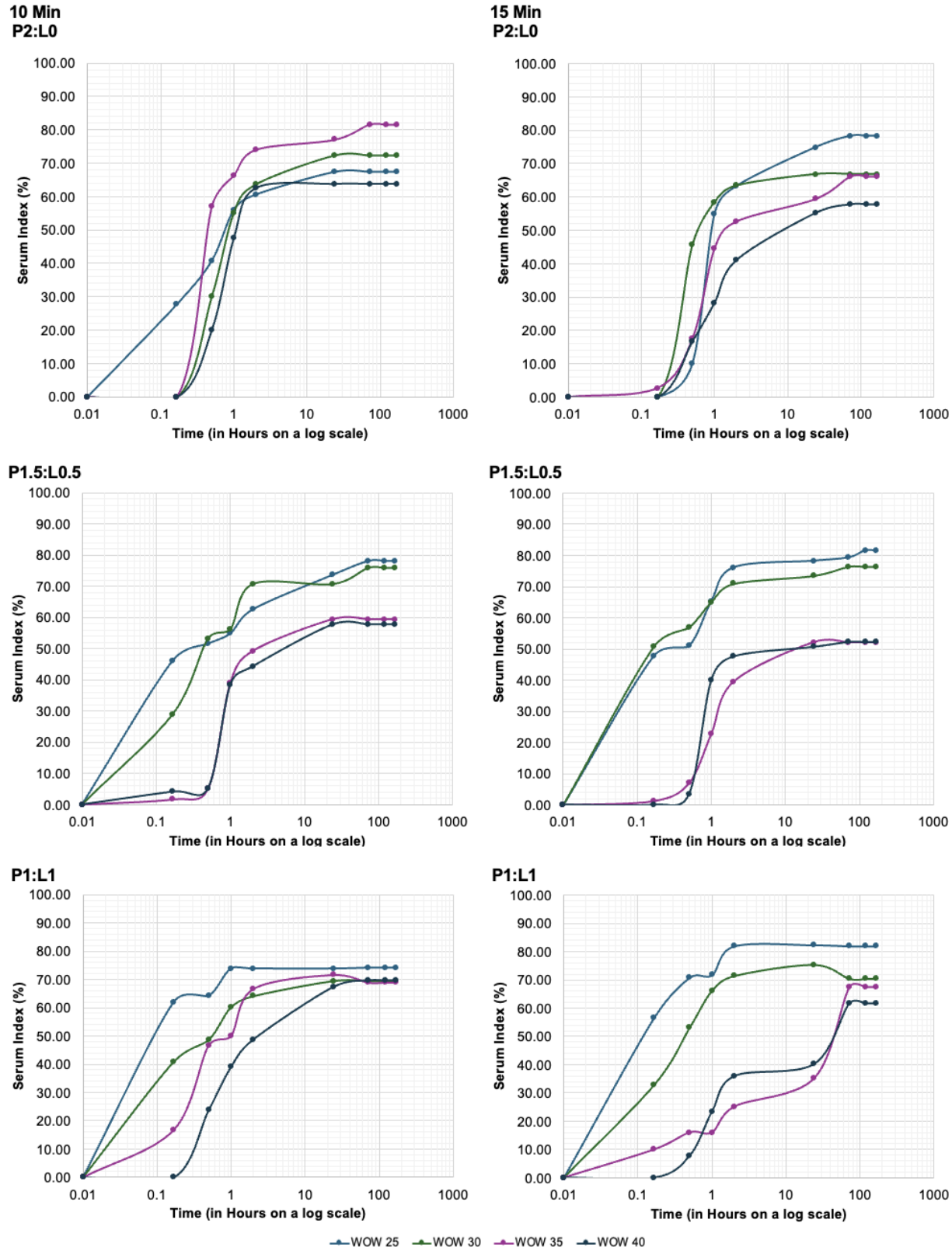


Figure 6.10 – Serum index over a week (in hours) for 6,000 rpm for the varying double emulsion ratios, separated by each primary emulsion. Values are mean values over triplicate measurements.

Following on from evaluating the ratio and droplet size, WOW 35 balanced droplet size and encapsulation efficiency, with droplets being from 14 to 18 μm and the encapsulation efficiency being 1.3 to 1.7 % across the three primary emulsion ratios (P2:L0, P1.5:L0.5 and P1:L1). Other papers which have progressed from double emulsion production into cheese or dairy products have used droplets much larger than these, such as El Kadri *et al.* (2018), who had droplets of around 50 to 70 μm in yogurt. Gamlath *et al.* (2023) incorporated double emulsion droplets of up to 380 μm. Both Giroux *et al.* (2013) and Leong *et al.* (2020) reached droplets of 30 μm which were incorporated into cheese. There are a limited number of studies which achieved small droplets, similar to the milk fat globule, the notable studies that did were; Giroux *et al.* (2013) with high pressure homogenisation achieving 6 to 7 μm and Paximada, Howarth and Dubey (2021) with droplets of 4 to 6 μm.

Comparing the homogenisation time of 10 minutes to 15 minutes, statistical analysis (ANOVA, $P > 0.05$) suggested that a combination of all factors had no impact on the droplet size ($D_{4,3}$) and encapsulation efficiency. Further examination of the droplet distribution, shown in Figure 6.11, the difference in distribution between the two times is minimal, so a lower production time of 10 minutes is justifiable for cheese making to reduce the total production time. The distribution has some slight variation in droplet size but generally monomodal with the largest proportion (25 %) being around the 10 μm to 20 μm and not a huge variation in droplet sizes.

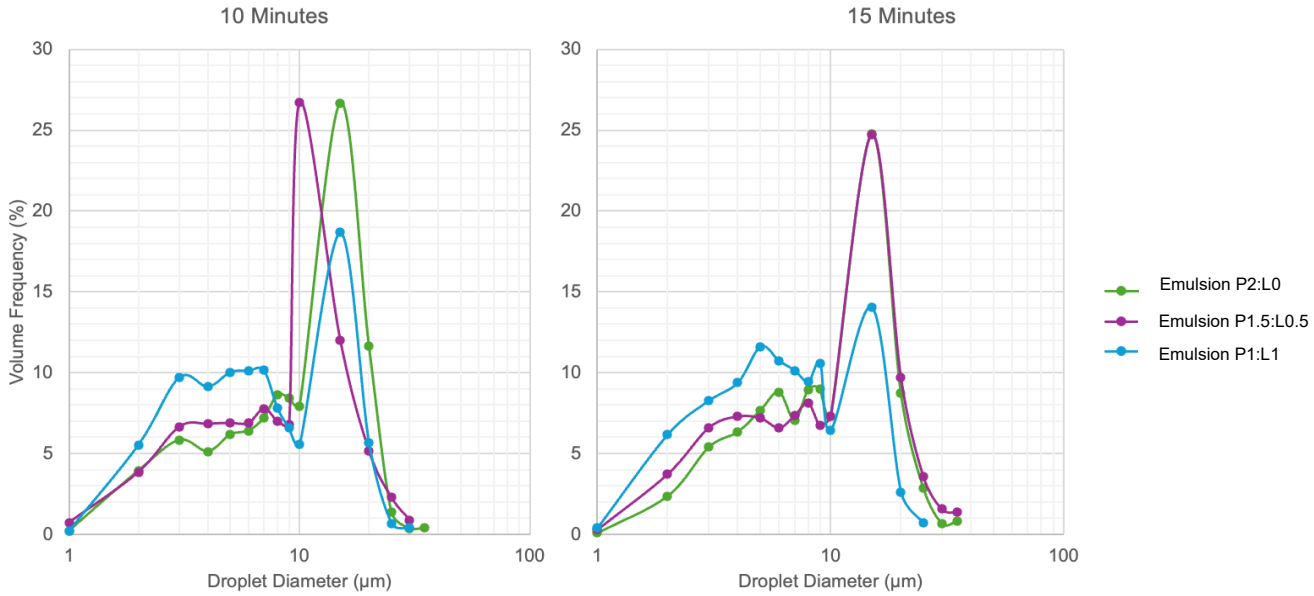


Figure 6.11 Droplet size distribution by volume frequency (%) for the chosen emulsion (WOW 35) over the two time iterations (10 and 15 minutes).

Droplet size, encapsulation efficiency and serum index of the double emulsions have all been explored and discussed in relation to the different production parameters. The effect of the primary emulsion, from Chapter 5, by partially replacing PGPR with sunflower lecithin, has been explained in each of the elements in some detail, with the impact of viscosity and size of the inner droplets, which ultimately will have an impact on the overall size.

To assess the individual effects of the primary emulsion treatments (P2:L0, P1.5:L0.5 and P1:L1) on double emulsion droplet size ($D_{4,3}$). One-way ANOVA was performed for each combination of double emulsion ratio and homogenisation time. Where a significant overall effect was observed ($P < 0.05$), post hoc Tukey HSD tests were used to identify which specific primary emulsion groups differed. While the majority of treatments showed no statistically significant differences between primary emulsions, a small number of conditions did yield significant pairwise differences, as summarised in Table 6.3. These outcomes suggest that the effect of primary emulsion composition on droplet size may be condition-dependent, and while lecithin inclusion can influence inner droplet formation, its impact on final droplet size was not universally significant across all formations.

Table 6.3 Tukey HSD P-value results for the primary emulsion treatments and their impact on $W_1/O/W_2$ volume-weighted droplet mean ($D_{4,3}$)[1]

Double emulsion ratio	Time on Silverson high shear mixer (mins)	Overall ANOVA P-Value	Significant pairwise comparisons (Tukey HSD)
WOW 20	15	0.005**	P2:L0 vs P1.5:L0.5 ** P2:L0 vs P1:L1 ** P1.5:L0.5 vs P1:L1 **
WOW 25	15	0.005**	P2:L0 vs P1:L1 ** P1.5:L0.5 vs P1:L1 *
WOW 35	10	0.003**	P2:L0 vs P1:L1 ** P1.5:L0.5 vs P1:L1 *
WOW 35	15	0.031*	P2:L0 vs P1:L1 *

[1] *P < 0.05; ** P < 0.01; *** P < 0.001. Only statistically significant pairwise comparisons are shown.

Where the P-values are significant, the differences are between emulsions P2:L0 and P1:L1, which would be expected, as P2:L0 has no lecithin and primary emulsion P1:L1 has a ratio of 1:1 PGPR to sunflower lecithin. Following on from Chapter 5, water droplets were the largest for this emulsion which could impact the double emulsion, and also the viscosity of the primary emulsion with greater concentrations of lecithin leading to a greater apparent viscosity. Therefore, this all links to previous comments on the viscosity and droplet size impacting the double emulsion, which contributes to this theory.

6.5 Conclusion

Using the chosen primary emulsions (P2:L0, P1.5:L0.5 and P1:L1) from Chapter 5, this study demonstrated that the partial replacement of PGPR with sunflower lecithin as the lipophilic surfactant can be used in skimmed milk double emulsion production, resulting in stable double emulsions suitable for further application. Additional developments in the production method – including adjustments to Silverson speed and time and the ratio of primary to secondary emulsions – were also found to significantly influence double emulsion size and encapsulation efficiency.

Although the produced double emulsions did not reach droplet sizes equivalent to milk fat globules, they were comparable to those reported in previous studies and showed acceptable stability and encapsulation potential for dairy applications. The WOW 35 formulation achieved a good balance between droplet size and encapsulation efficiency using a method of 6,000 rpm for 10 minutes, producing droplets stable for over 2 hours.

Future investigations could involve the inclusion of functional ingredients in the inner water phase, not only to explore further application but also study the effects of osmotic gradients and potential water migration between phases. Additionally, investigating alternative homogenisation methods such as micro fluidisation or membrane emulsification – could be valuable. These methods are known to produce smaller and more uniform droplets and could potentially enhance stability and encapsulation efficiency. Thus, it is reasonable to expect that a change in homogenisation method would lead to different emulsion properties and may help to better mimic the size of natural milk fat globules.

CHAPTER SEVEN: EVALUATING THE POTENTIAL OF DOUBLE EMULSIONS WITH REDUCED AMOUNTS OF SYNTHETIC SURFACTANTS TO IMPROVE REDUCED FAT CHEDDAR CHEESE

7.1 Introduction

Double emulsions have been widely explored for their potential to enhance the sensory and functional properties of reduced fat or low fat food products. These emulsions have been successfully used in various applications to improve both texture and flavour profiles (section 2.6.2). For example, Rakshit and Srivastav (2022) demonstrated that incorporating double emulsions in 40% reduced fat short dough biscuits led to improved sensory attributes. Similarly, several studies have focused on the use of double emulsions in cheese (section 2.7) , finding positive effects on texture and functionality, though sensory evaluations were not extensively covered (Sharma Khanal et al., 2019; Gamlath et al., 2023; Giroux et al., 2013). Building on the developments discussed in previous chapters, this study optimizes double emulsion parameters while minimizing the use of synthetic lipophilic surfactants for application in reduced fat Cheddar cheese. The goal was to achieve emulsion droplet sizes comparable to milk fat globules (4 to 6 μm), though the emulsions produced had a larger average size ($D_{4,3}$) ranging from 14 μm to 18 μm (as seen in Chapter 6). Despite the deviation in droplet size, these emulsions can be incorporated and applied in cheese production to assess their impact on reduced fat cheese functional and sensory characteristics. This approach aims to further explore the utility of double emulsions in improving food products, with a focus on optimizing their application in cheese.

7.1.1 Aim and objectives

The aim of this experimental study was to investigate the chosen double emulsions with reduced amount of synthetic lipophilic emulsifiers, from Chapter 6 in application in reduced fat cheese. The objectives include:

- To evaluate the impact of double emulsions containing lecithin as the lipophilic surfactant on the nutritional composition and visual appearance of reduced fat cheese
- To assess the effectiveness of double emulsions in improving texture, compared to conventional reduced fat
- To investigate the functional properties of double emulsion cheeses, including oil loss and meltability compared to reduced fat and full fat cheeses.

- To evaluate the sensory attributes of cheeses formulated with double emulsions, using a trained panel

7.2 Material and methods

Detailed methods for cheese production and analysis can be found in Chapter 3. Cheeses were made in 30 L batches, and each of the 5 cheese samples were made in triplicate for nutritional and functional analysis. Then an additional two batches were made specifically for sensory evaluation.

Samples (250 g) were sent to ALS (Chatteris, UK) for nutritional analysis where fat (by the Gerber method), protein and moisture (loss on drying) were tested. This was outsourced due to time constraints in the project. A 100 g sample from each wheel in each batch was sent for microbiological testing at ALS (Shrewsbury UK). Microbiological testing was outsourced to an accredited laboratory (ALS, Shrewsbury, UK), in accordance with UK Food safety regulations, which require that such analyses be conducted by certified facilities. The microbiological testing was for the following bacteria: *Enterobacteriaceae, Escherichia coli, Listeria spp*. in 25 g, *Salmonella spp.* in 25 g, *Bacillus cereus* and *Sulphite reducing clostridia*). Results were recorded in Table A1 in Appendix 1, samples which exceeded the thresholds were retested but all samples used for sensory evaluation passed the regulatory thresholds as stated in the UK regulations (Commission, 2005), where a reference table can be found in Table 2 in Appendix 2.

Fluorescence confocal microscopy was undertaken at the University of Warwick and more details can be found in Chapter 3 section 3.2.6. Texture analysis was undertaken at Harper Adams University using the P/3 3 mm probe, more details can be found in section 3.2.13. Functionality tests were also undertaken at Harper Adams University and were following the Schreiber method for meltability and oil loss where more details can be found in sections 3.2.11 and 3.2.10. Sensory evaluation was undertaken using the flash profile method, with 14 panellists and 6 cheese samples (3 double emulsion samples, reduced fat, full fat and a commercial sample) where they were coded with three random digits. Panellists attended a training session, which involved tasting cheeses to find and develop similar understanding of cheese characteristics terminology and understand what was required of them during the experiment. The panellists then came back for the 2 hour experiment, which had a break in-between, and they were given 31 characteristics to rank the samples against. A more detailed methodology can be found in section 3.2.15.

Table 7.1 identifies the cheese treatment codes referred to in this chapter. Emulsions used in cheese making had been developed throughout the research chapters. Detailed emulsion production can be found in Chapter 3. In summary from the previous chapters, primary emulsions with anhydrous milk fat and a 2 % lipophilic surfactant concentration (PGPR: sunflower lecithin) at a 40:60 water to oil ratio was used. The emulsion was then homogenised for 5 minutes in the ultrasonic homogeniser and left for 2 hours prior to double emulsification. Double emulsions were made in 1.5 L batches at a ratio of 35:65 for W_1/O to skimmed milk and homogenised in the Silverson high shear mixer for 10 minutes at 6,000 rpm and added to cheese milk prior to starter addition.

Table 7.1 – Cheese sample codes and explanation

Code	Description
FF	Full-fat Cheddar cheese using 3.24 % cheese milk
RF	Reduced fat Cheddar cheese using a combination of skimmed milk and whole milk to obtain the same fat content as the double emulsion cheese milks
DE1	Double emulsion 1, which consists solely of PGPR as the lipophilic emulsifier (P2:L0)
DE2	Double emulsion 2, consisting of 1.5 PGPR to 0.5 sunflower lecithin as the lipophilic emulsifier (P1.5:L0.5)
DE3	Double emulsion 3, consisting of 1 PGPR to 1 sunflower lecithin as the lipophilic emulsifier (P1:L0)
ASDA Half Fat Mature	ASDA Half fat mature Cheddar was used as the commercial comparison in the sensory evaluation, due to being similar in fat content and visual appearance to that of the double emulsion cheeses

7.3 Results and discussion

7.3.1 Nutritional analysis

Table 7.2 outlines the nutritional analysis of the cheese samples, as well as the cheese yield. FF had the highest yield of 10.52 % compared to the RF and double emulsion cheeses, which were similar with yields of 7.4 ± 0.2 %. A strong positive correlation was found between fat content and cheese yield (Pearson's $r = 0.911$, $P < 0.05$). ANOVA with Tukey's HSD confirmed that FF yield was significantly different ($P < 0.05$) from all other treatments, while no significant differences were observed between RF and any of the DE cheeses. Similarly, fat content of FF was significantly higher ($P < 0.05$), than all other samples with no significant pairwise differences between RF and the DE cheeses, which was as expected. These findings were consistent with Sharma Khanal *et al.* (2019) who also identified that yield was directly proportional to fat content. Likewise, Lobato-Calleros *et al.* (2002) observed that a decrease in milk fat content led to reduction in yield. This outcome aligns with the previous results, as the composition of milk, particularly its fat and casein content, significantly impacts cheese yield (Fox *et al.*, 2017).

Table 7.2 Nutritional analysis of each of the sample cheeses produced

	FF	RF	DE1	DE2	DE3
Cheese Yield (%)	10.52 ± 0.03^{a}	7.55 ± 0.33^{b}	7.422 ± 0.14^{b}	7.56 ± 0.33^{b}	7.65 ± 0.31^{b}
Fat (by Gerber) (%)	29.75 ± 1.78^{a}	13.00 ± 1.98^{b}	13.82 ± 3.36^{b}	13.82 ± 3.51^{b}	12.33 ± 0.41^{b}
Protein (g/100g)	28.47 ± 7.29^{a}	32.02 ± 4.68^{a}	34.12 ± 1.06^{a}	32.90 ± 4.17^{a}	32.55 ± 3.66^{a}
Moisture (loss on drying) (g/100g)	38.70 ± 5.46^{b}	43.33 ± 2.83^{ab}	45.53 ± 1.46^{a}	45.30 ± 1.05^{a}	45.87 ± 1.63^{a}

Values are the mean and ± standard deviation from triplicate measurements. Superscript letters $^{(a,b)}$ indicate significant differences means within a row ($P < 0.05$), based on one-way ANOVA followed by Tukey's HSD post hoc test. Means that do not share a letter are significantly different.

There was no significant difference between the fat contents of the double emulsion cheeses and RF, which was as expected as fat content was altered so that it would be comparable. The slight variation in fat between DE3 and the other double emulsion and RF cheeses would most likely be due to loss during whey removal (Lobato-Calleros *et al.* (2002), and potentially the lack of homogeneity of the cheeses tested despite a consistent sampling strategy. There was no significant difference between protein and different cheese samples, although a weak negative correlation (-0.254) was found between fat and protein, but it was not significant ($P > 0.05$).

A significant ($P < 0.05$) negative correlation ($r = -0.694$) was found between fat and moisture content, meaning that as fat content decreased, moisture increased. Similar results were found in other studies with double emulsion cheeses having a greater moisture than those without double emulsions (Totosaus, Rojas-Nery and Franco-Fernández, 2017; Leong *et al.*, 2020). This may be attributed to the water droplets encapsulated within the fat phase of the double emulsion structure, which contribute to total moisture content. As discussed by Cipolat-Gotet *et al.* (2020) such internal water is considered bound or restricted, being physically retained within the fat globules and therefore less available for evaporation or interaction with the matrix.

7.3.2 Texture analysis

Firmness also described as hardness, refers to the deformation of applied stress by the texture analyser machine (Fox *et al.*, 2017). Figure 7.1 displays the firmness results for each of the cheeses. RF was the hardest (684.65 g) and FF the softest (335.91 g), with the double emulsion samples ranging from 564 g to 590 g. The ASDA half fat was softer than both RF and double emulsion samples, despite being similar in fat content, with ASDA half fat being 15 % fat. This discrepancy between firmness values could be attributed to the length of the maturation period, due to time constraints, experimental samples could not be matured for six months, and similar locally produced cheeses with known maturations times were not available during the study period. It can be noted that during maturation enzymatic activities occur within the cheese, including proteolysis, which break down the protein and has been found to reduce firmness. This effect is commonly observed after extended maturation periods, typically ranging from 3 to 6 months or longer (Anvari and Joyner, 2019). The samples in this study were only matured for four weeks, meaning there was limited time for proteolysis compared to that of ASDA half fat mature, which would have matured for at least 6 months commercially.

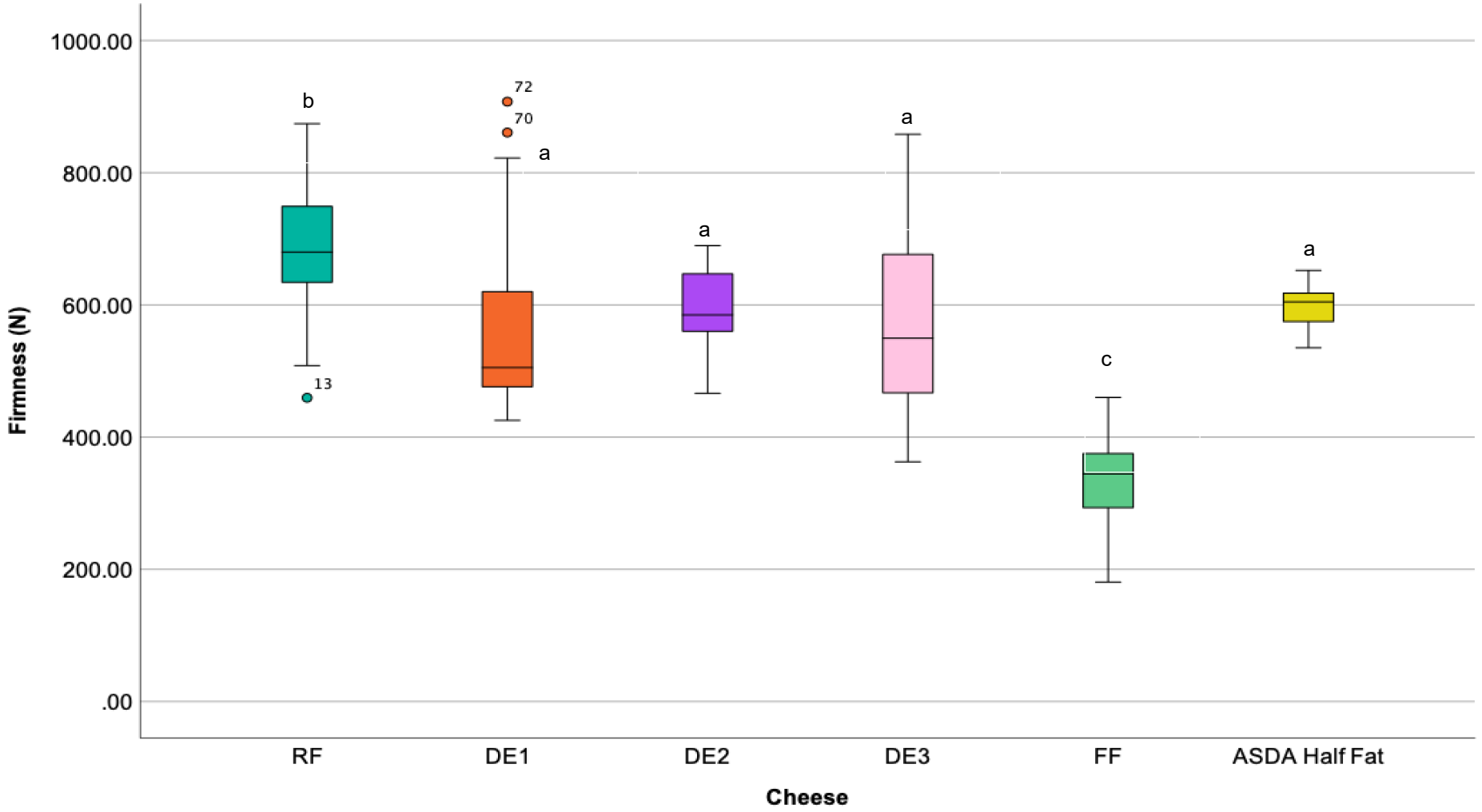


Figure 7.1 Box plot comparing firmness results of each of the cheese samples including the commercial sample used in sensory evaluation, n = 36. Different letters indicate significant differences (P < 0.05) between sample groups based on Dunn's post hoc test with Bonferroni correction following Kruskal Wallis analysis. Groups that share at least one letter are not significantly different.

Delving into the results further the Kruskal-Wallis test returned a significant P-value ($P < 0.05$) between cheese treatments and firmness results. To analyse this further, the Dunn's test with a Bonferroni correction was used to confirm pairwise comparisons between certain samples. No significant difference ($P > 0.05$) within the Dunn's test was found between any of the DE1, DE2 and DE3 samples, meaning that primary emulsion lipophilic surfactant ratio did not have an impact on firmness. Interestingly, DE2 was not found to be statistically different to the RF in terms of firmness. However, since DE2 also did not significantly differ from DE1 and DE3 – both of which were significantly different from RF – this result may be due to variability or sample size rather than a true difference. While DE2 had the largest average droplet size (17.93 μm) compared to the other double emulsion samples (DE1 – 16.49 μm and DE3 – 14.25 μm), the impact of droplet size on firmness in this case remains inconclusive. Coalescence, which is discussed further in section 7.7.3, may also play a role. For DE1 and DE3 there was a significant difference in firmness between them and RF ($P < 0.05$). As expected, there was a significant difference between all samples and FF, with FF being the softest and requiring a smaller amount of force to pierce the cheese. The results to some extent correspond with other findings investigating similar parameters. Paximada, Howarth and Dubey (2021) found that in double emulsion Cheddar cheeses fortified with plant proteins, the full fat cheeses were the softest (20 N) compared to low fat being the hardest (41 N) with the double emulsions' samples between FF and low fat (29 to 35 N). Sharma Khanal *et al.* (2019) also concluded that the FF version was the softest compared to their low fat version being the hardest, in addition the authors believed that the fat droplets were too small and fit within the pores of the casein matrix, whereas larger ones would disrupt the casein structure. Interestingly, Leong *et al.* (2020) found that the double emulsion cheeses were the hardest.

Figure 7.2 compares the springiness results between the different cheese samples where springiness is described as the bouncing property of cheese and its tendency to recover from deformation after removal of deforming stress (Fox *et al.*, 2017). FF had the highest springiness value (33 %) compared to the other samples, due to the higher fat content. The fat which is an inert filler and acts as a spacer in the casein matrix, allowing it to spring back into shape and recover after deformation. Meaning that the RF and DE samples had lower springiness values (around 30 to 32 %) due to the lack of fat disrupting the casein matrix allowing for stronger bonds to form between casein molecules. Using Kruskal-Wallis test, a significant different was found between the springiness results and cheese samples ($P < 0.05$). When Dunn's test with Bonferroni correction was employed to investigate the difference between samples further and the only significant differences ($P < 0.05$) were found between FF and all sample cheeses.

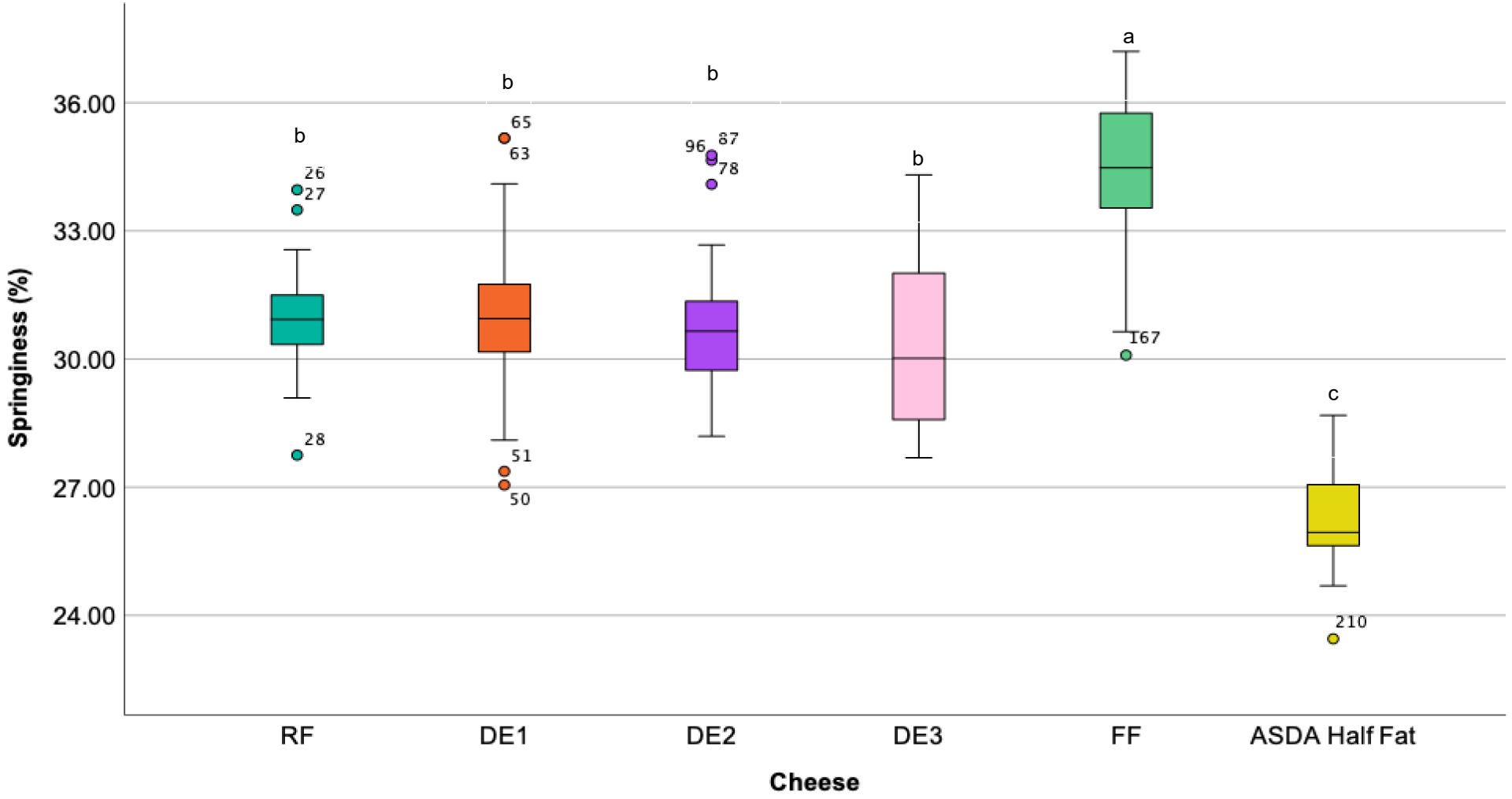


Figure 7.2 – Box plot comparing springiness results of each of the cheese samples including the commercial sample used in sensory evaluation, n = 36. Different letters indicate significant differences (P < 0.05) between sample groups based on Dunn's post hoc test with Bonferroni correction following Kruskal Wallis analysis. Groups that share at least one letter are not significantly different.

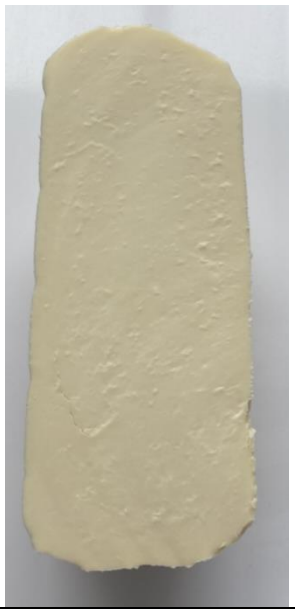
Lobato-Calleros *et al.* (2002) used emulsions made with vegetable oil and substituted these into Manchego cheese. They found that cheeses with emulsions were lower in hardness and springiness but higher in cohesiveness. This could be attributed to the addition of the vegetable oil which has a lower melting point than anhydrous milk fat (McClements, 2016; Patel, 2020; Sánchez-Vega *et al.*, 2021). Similarly, Totosaus, Rojas-Nery and Franco-Fernández (2017) found that using soyabean oil emulsions in Oxaca cheese resulted in a harder texture, but adhesiveness was significantly higher. Both cohesiveness and adhesiveness in these studies could be attributed to vegetable oil used in the fat phase. In contrast, in this study, the lower springiness observed in the double emulsion and RF cheeses is most likely due to the milk fat as the fat phase. Milk fat contains a complex mixture of fatty acids with a wide range of melting points (- 40 °C to 40 °C). This means that at room temperature, some fatty acids are present in a liquid or semi-solid state while others remain solid, influencing the cheese texture. For instance, milk fat contains short-chain fatty acids like butric acid (melting point ~7.9 ºC), as well as long-chain saturated fatty acids such as palmitic acid (melting point ~63 ºC), stearic acid (~70 ºC) and myristic acid (~54 ºC) (Patel, 2020). The mixture of solid and liquid fat fractions at ambient temperature may reduce the elasticity and springiness of the cheese matrix.

Considering both firmness and springiness in the cheeses, the double emulsion samples were not significantly different from each other, meaning that the inner lipophilic emulsifier ratio did not have a significant effect on the cheese. This means that the differing ratios of PGPR:sunflower lecithin did not have a huge impact so reducing the amount of synthetic surfactant will not have detrimental effects on the firmness and springiness of a double emulsion cheese. The double emulsion samples in general did improve the firmness of the cheese compared to that of the RF, meaning that double emulsions could be positively used in research to improve reduced fat cheese. However, they were not successful in totally mimicking full fat cheese properties. In addition, commercially available cheeses, with lower fat contents on the market, appear to be softer in firmness compared to the experimental cheeses, although as discussed this may be due to the difference in maturation period. Other studies report a mixture of results with some positive results with double emulsions improving the cheese, but also those which are negatively portrayed and double emulsion samples being the firmest. This would explain why the double emulsion cheeses were softer in the above discussed studies and why the only other study which used milk fat as the fat phase was Paximada, Howarth and Dubey (2021) followed the same trend as found in this study.

7.3.3 Visual appearance and microstructure

Cheese has a macro structure, which can be easily seen with distinct curd joins and visual appearances (Figure 7.3). The microstructure of cheese is the spatial distribution of major milk components, such as the milk fat globules entrapped within the casein matrix (Guinee, 2016) (identified in Figures 7.4 to Figure 7.8). Figure 7.3 shows the images of the cheeses after four weeks of maturation. In the double emulsion sample cheeses (DE1, DE2 and DE3) it is obvious to see the macrostructure with distinct lines showing the individual curds particles and joins, where the RF and FF cheeses have occasional slits and cracks but less apparent curd joins and appear whiter and smoother compared to the double emulsion samples. The lack of fat, reduces light scattering resulting in a more translucent cheese (Mistry, 2001; Johnson *et al.*, 2009; Ibáñez, Waldron and McSweeney, 2016), which can be seen in the double emulsion samples. The fat aids the smooth look of a cheese, which can be attributed to the FF sample. The RF sample, looks less translucent than the double emulsion cheeses, this could be attributed to the homogenised milk used, whereas a study by Karaman and Akalın (2013) found that homogenised milk cheese had a whiter appearance as well as a firmer texture. So, the even distribution of the fat (although reduced fat) would be more evenly spread than in the double emulsion cheeses and could be attributed to the whiter look compared to the double emulsion cheeses.

Figure 7.3 Images of the cheese following 4 weeks maturation prior to analysis.

RF	FF	
		
DE1	**DE2**	**DE3**
		

Figures 7.4 to Figure 7.8 displays the confocal microscope images of the sample cheeses. The fat droplets which were dyed by Nile red can be seen entrapped within the Fast Green FCF dyed casein network. The black circles within the red fat droplets, suggest the double emulsions were successfully entrapped during the cheese making process. The large

black expanse in places and black holes in the images, suggest pores, cracks or slits, which are naturally found within the macrostructure of cheese and caused by curd particles, curd chips and the way in which they bind together (Fox *et al.*, 2017). Overall, double emulsions were able to be entrapped within the casein matrix. The yellow arrows in Figure 7.4 highlight the double emulsion droplets, where a clear black circle within the fat droplet can be seen. Droplets for DE1 identified in Chapter 6 were 16.485 μm (for the volume weighted mean – $D_{4,3}$).

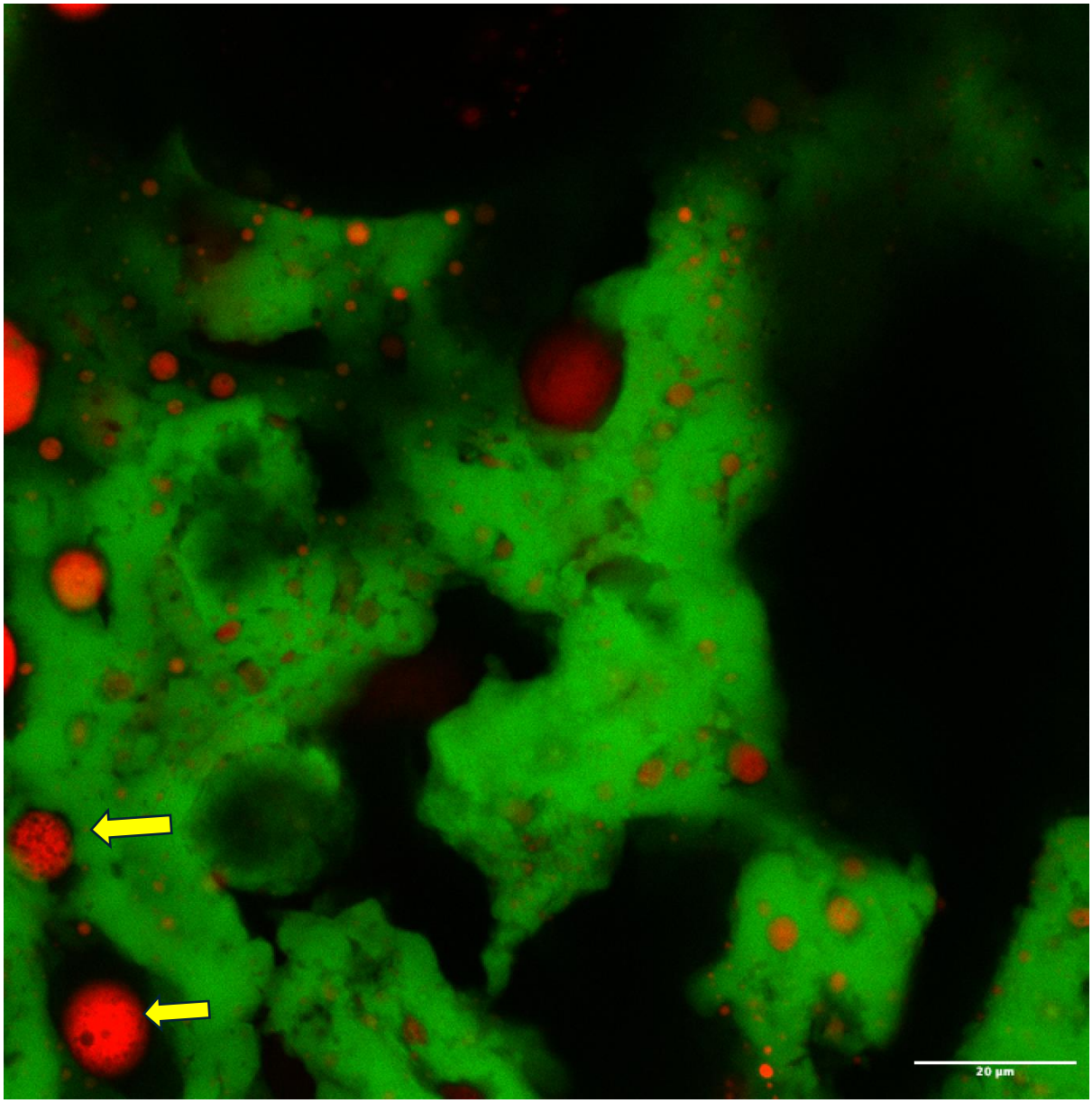


Figure 7.4 – Fluorescence microscope image of sample cheese DE1

In Figure 7.5 there are two fat droplets (identified by a yellow arrow) which are centrally placed in the image, that look to be slightly elongated compared to others in the image and in other cheeses. This could be due to aggregation of fat globules and the mechanical tearing of the curds during the cheese making process (Everett and Auty, 2017).

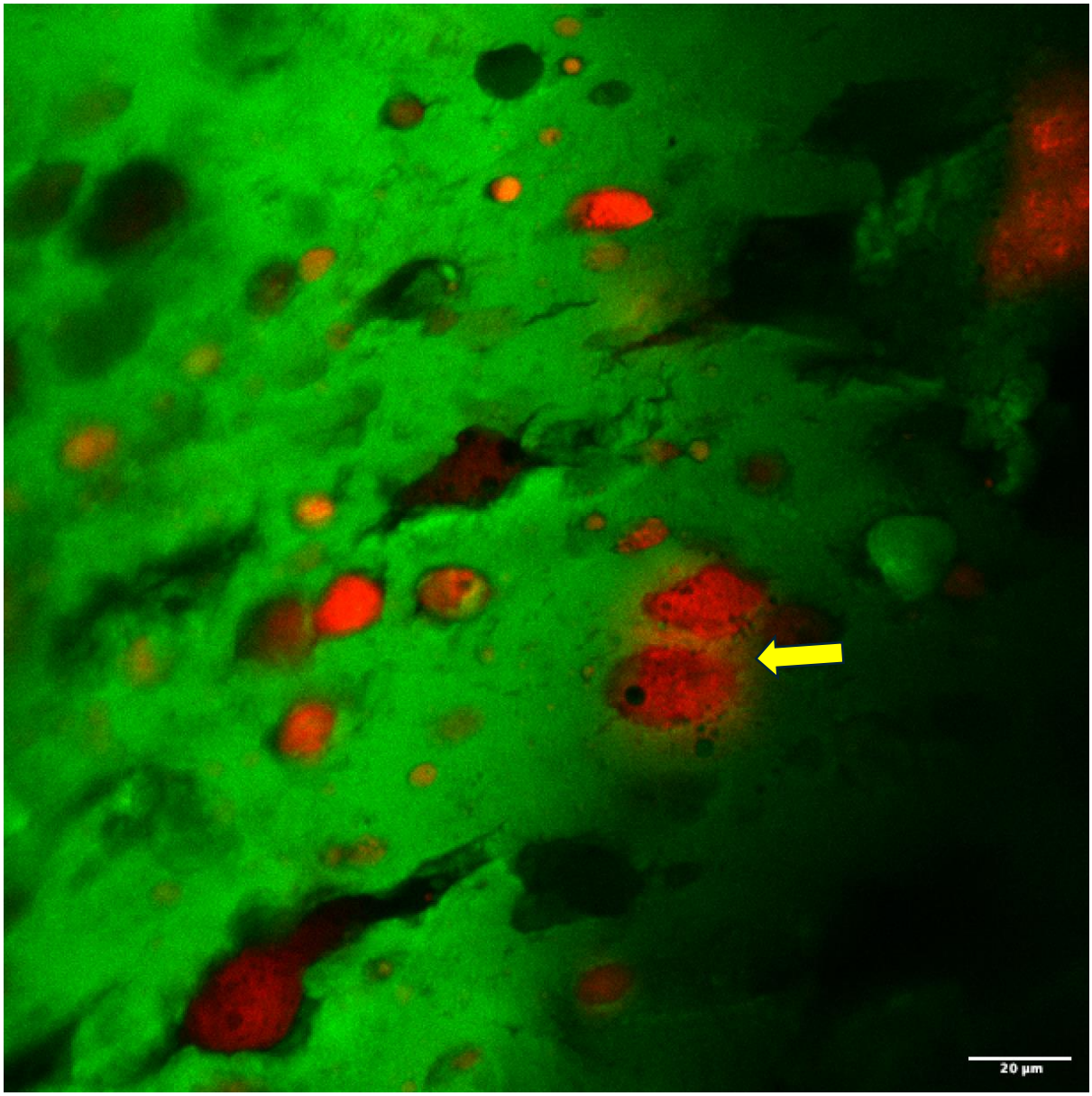


Figure 7.5 – Fluorescence microscope image of sample cheese DE2

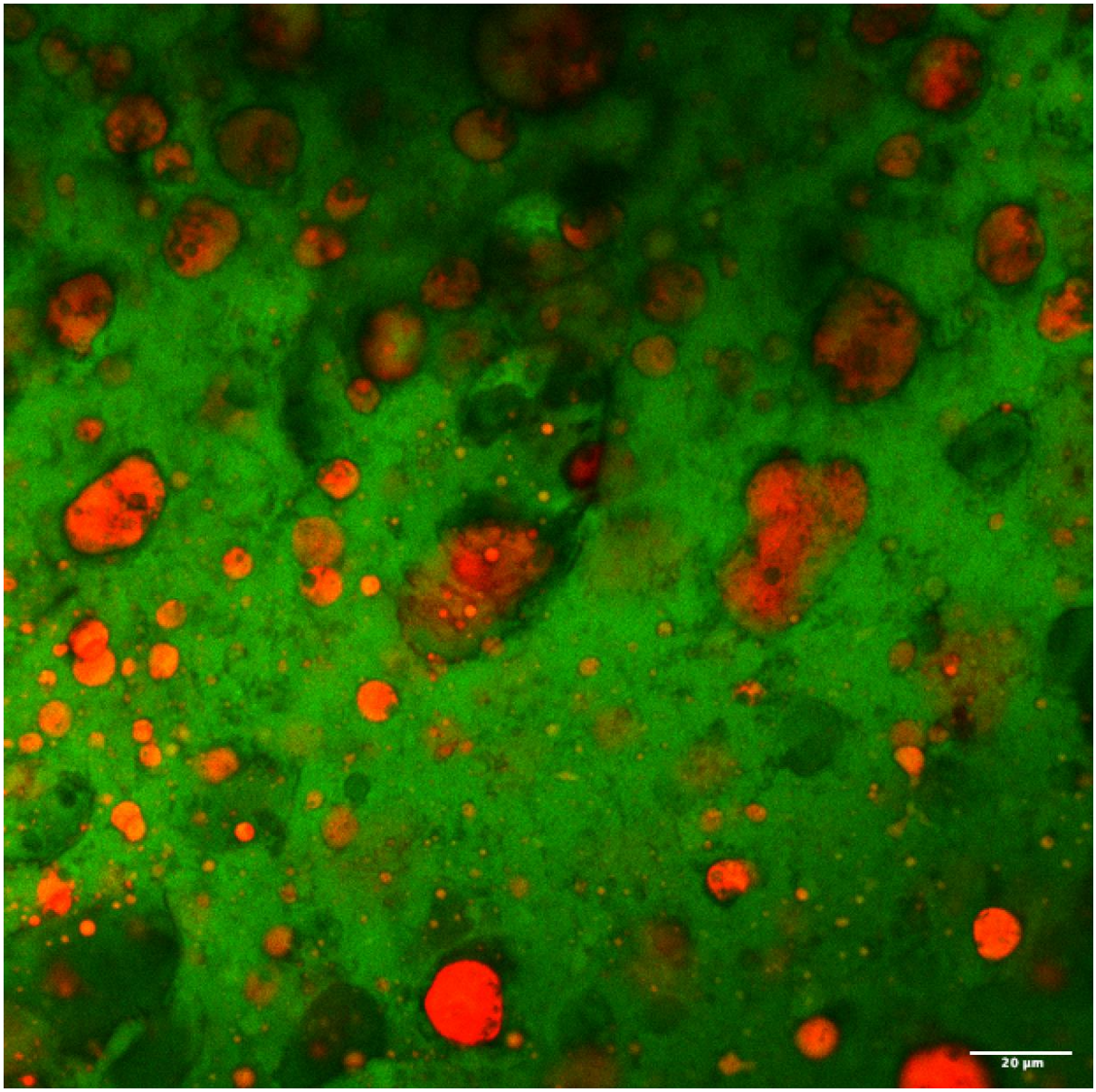


Figure 7.6 – Fluorescence microscope image of sample cheese DE3

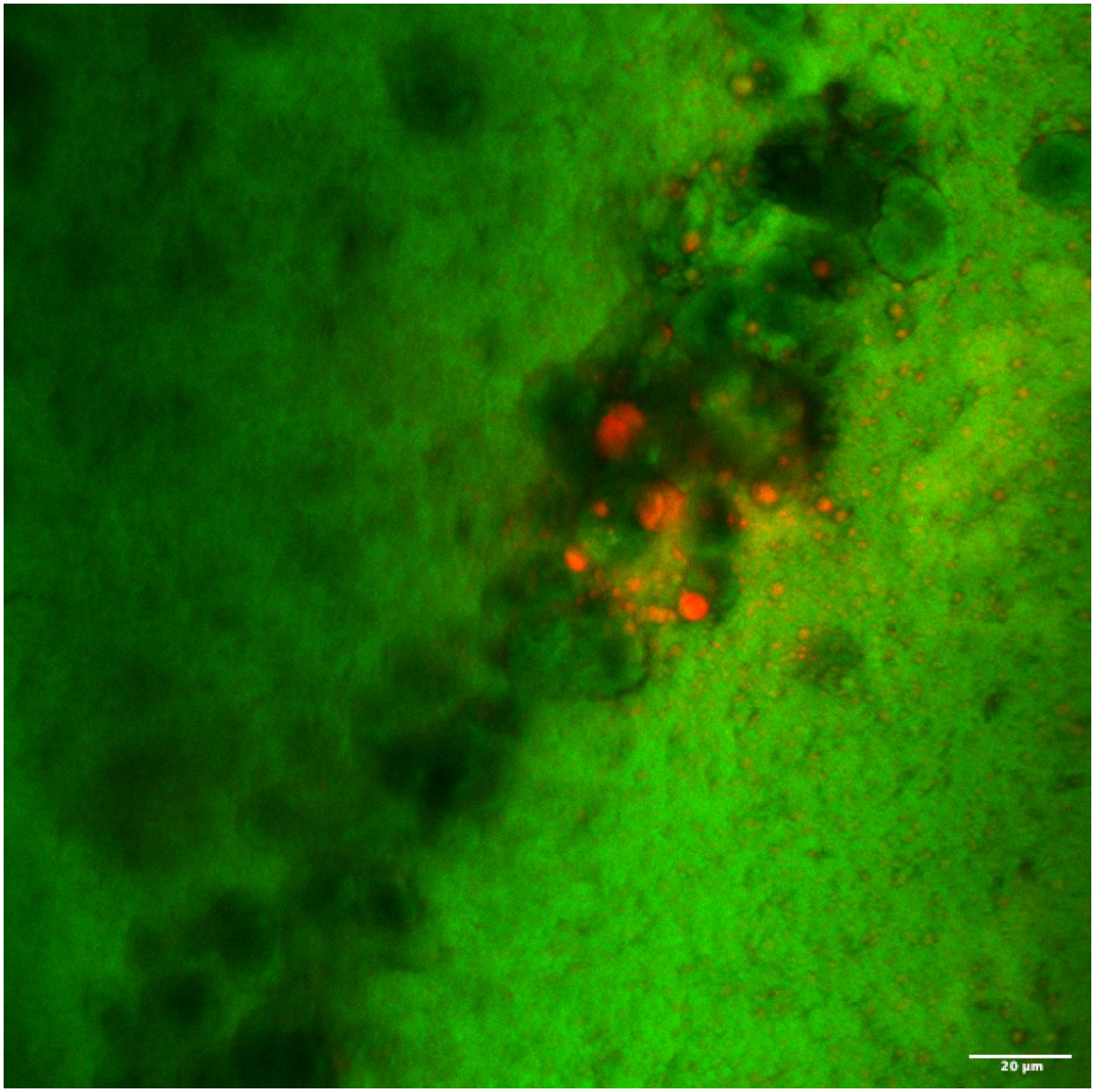


Figure 7.7 Fluorescence microscope image of the reduced fat (RF) cheese

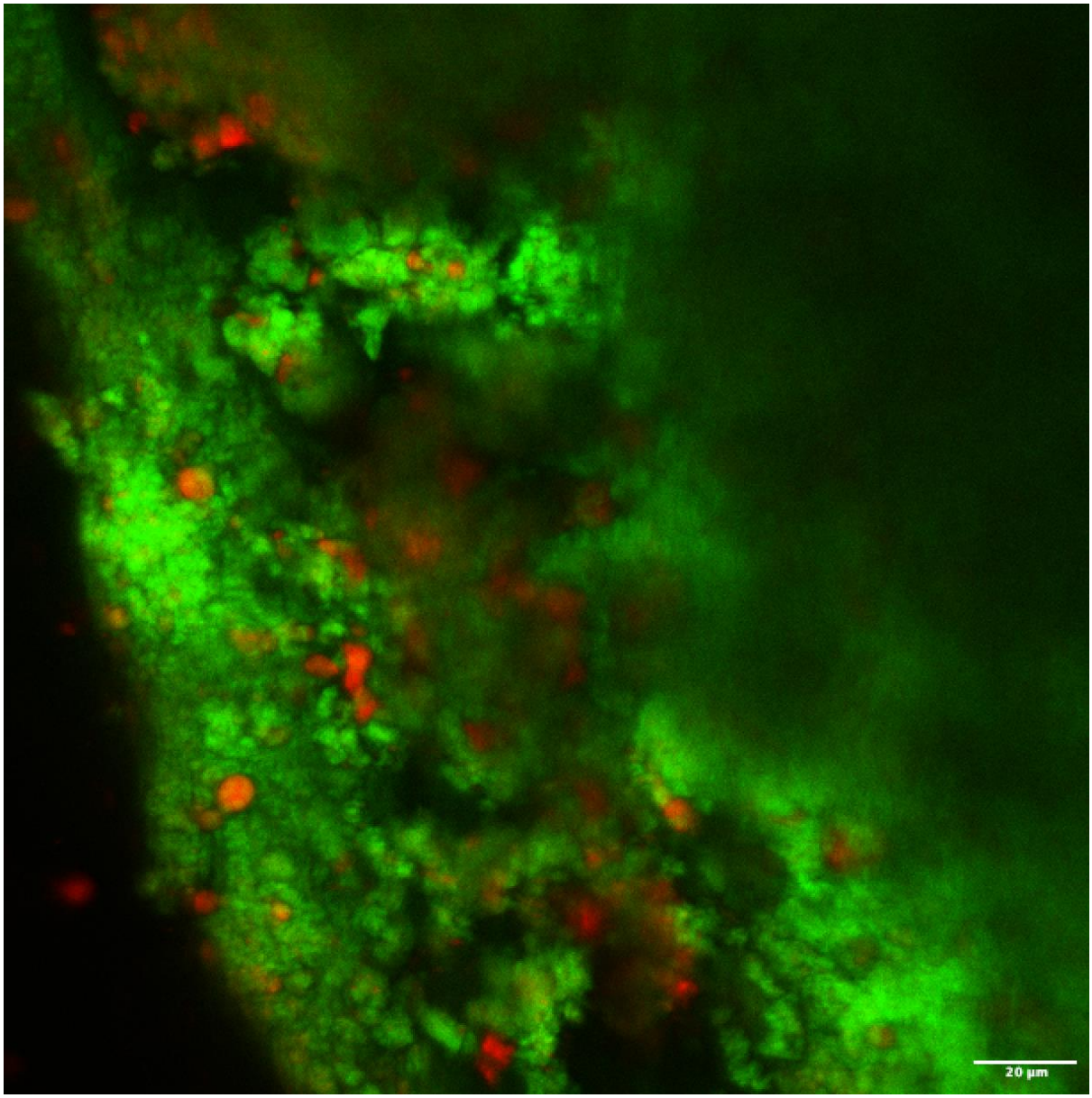


Figure 7.8 – Fluorescence microscope image of the full fat (FF) cheese

The RF (Figure 7.7) and FF (Figure 7.8) have droplets which look small and even due to the milk used being homogenised, compared to those of the double emulsion cheeses. Although homogenised to create the emulsions, the droplet sizes were different, for example the average droplet size for DE2 was 17.930 µm ($D_{4,3}$). This could explain the larger visual droplets entrapped in the casein matrix, seen in Figure 7.5 but also, could have resulted in larger droplets coalescing during the cheese making process and then being lost in the whey. The reduction in fat droplets, which act as an inert filler, could also be a reason why this cheese (DE2) had the highest hardness value (590.243 g) of all the double emulsion. The other fat droplets were 16.485 µm for DE1 (Figure 7.4) and 14.246 µm for DE3 (Figure 7.6).

7.3.4 Functionality analysis

Figure 7.9 shows the mean oil loss as a percentage for each of the cheese samples, which ranged from 6.7 % for RF to 7.5 % for DE1. The oil loss between the sample cheeses was not significantly different ($P > 0.05$). These results are partially contradictory to those found in Paximada, Howarth and Dubey (2021) as their low fat cheese had the highest loss (20 %), which was attributed to less protein available for emulsification allowing the oil droplets to be destabilised and resulting in oil loss. However, their lowest oil loss result was 5 % for both the FF and the double emulsion sample with whey protein. Although this experimental study did not fortify the double emulsions with whey protein, the droplets in the sample cheeses were successfully stabilised and were not lost during the oil loss experiment, it could be speculated that potentially the casein surrounding the fat globules to create the membrane layer to stabilise the droplet also interacts with the casein that is remaining in the milk that creates the casein matrix. Although further investigative work would be required.

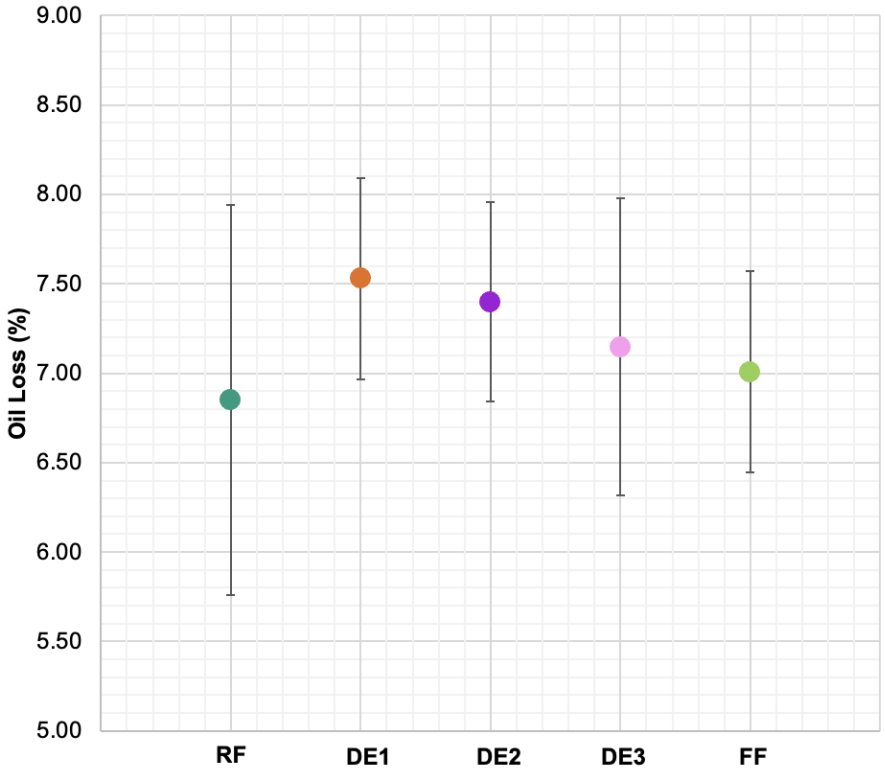
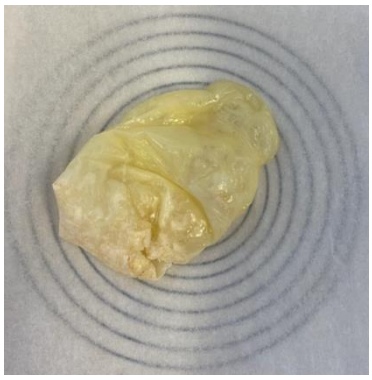
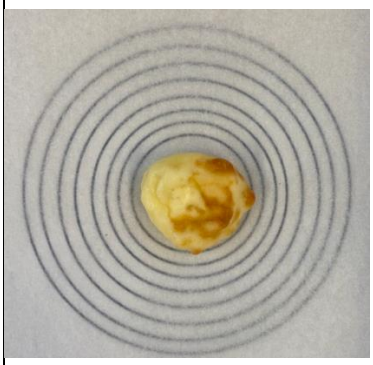
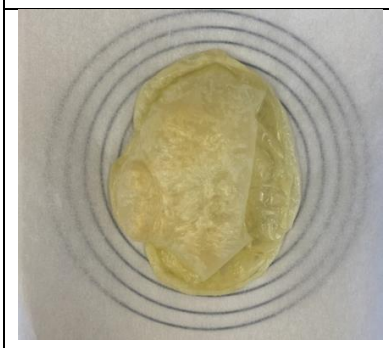
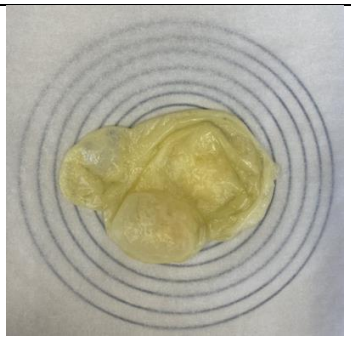
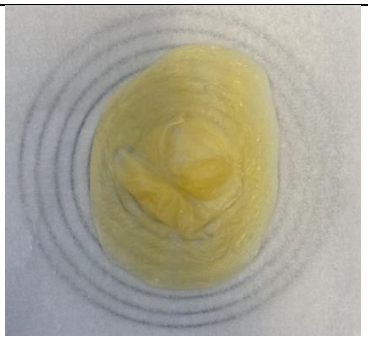


Figure 7.9 – Mean oil loss (%) values across the five cheese samples. Values are from 3 measurements from triplicate experiments and error bars represent standard deviation.

Meltability was assessed as it is related to the melting of entrapped fat and the disruption of the casein matrix upon heating (Van Hekken *et al.*, 2007) and described as the ease of flow on heating (Atik and Huppertz, 2023). Meltability is an important property especially when cheese is used as a cooking ingredient (Atik and Huppertz, 2023). The results are presented

in Figure 7.10, which shows images of each of the melted cheese samples and the average percentage increase in diameter during melting.

Figure 7.10 Images of melted cheese and average meltability increase (%)*

RF - 96.43 % ± 46.75	**FF –** 59.98 % ± 19.79	
		
DE1 – 175 %± 27.02	**DE2 –** 192.00 % ± 15.53	**DE3** - 205.83 % ± 32.67
		

*Values represent the mean and standard deviation from three independent repetitions, each with three replicates (n = 6).

DE3 had the highest percentage increase in meltability diameter (205.83 %) compared to the FF which had the smallest meltability increase. ANOVA revealed a significant difference ($P < 0.05$) between all cheese samples and the increase in diameter during melting. However, when Tukey's HSD was applied, no significant differences ($P > 0.05$) were found between each of the DE samples, indicating no variation among the varying ratios of PGPR to sunflower lecithin in the primary emulsion.

The FF cheese exhibited the lowest percentage increase in diameter, like the findings in Paximada, Howarth and Dubey (2021) who report that their low fat cheese had the highest meltability and concluded that meltability was more influenced by protein content than fat. Another study by Van Hekken *et al.* (2007), investigating the use of microfluidization of milk, found that the smaller oil droplets did not improve meltability by having even distribution of fat droplets, all of which were small and did not disrupt the protein matrix. This can be linked to the FF cheese in this study as using homogenised milk where droplets are all the same size, perhaps these were too small to make a difference in melting.

The double emulsions in this study showed the highest meltability. Although literature suggests that the fat aids in lubricating and stretching the cheese when melted, as fat globules coalesce and facilitate the sliding of casein strands (Fox *et al.,* 2017). The presence of inner water droplets could explain the increased meltability and as seen in Table 7.2 the double emulsion cheeses had the highest moisture content (45 g / 100 g). Everett and Auty (2017) suggest that a higher moisture improves meltability by hydrating casein and allowing easier movement during melting, which can be seen by the double emulsion samples.

Leong *et al.* (2020) observed that emulsion cheeses had a lower melt radius and tended to form a "skin", which they believed limited spread ability due to homogenised oils coated in casein that restricted meltability. Although double emulsion samples did not follow the same trend as Leong *et al.* (2020), a skin was formed on the cheese samples. Drawing from previous studies, the high meltability and skin formation on the double emulsion cheeses could be due to the large double emulsion sizes that coalesce under heat, aiding lubrication of casein strands. Additionally, the higher moisture content helps to hydrate the casein, and the skin formation could be attributed to the skimmed milk used as the secondary phase for double emulsion production, where casein migrates to the droplet surface.

Summarising the oil loss and meltability results, the double emulsion samples had no real effect on the oil loss of cheese, although some literature suggests that similar mechanisms like oleogels were found to reduce oil loss (Dobson and Marangoni, 2024). The results prove

evidence to support the use of this technology to improve the structure and function of reduced fat cheeses, particularly with improving melting properties.

7.3.5 Sensory evaluation

Sensory descriptive analysis methodology was employed utilising the flash profiling method (Petit, 2023) to compare 31 characteristics of six different cheese samples including five experimental treatments (RF, FF, DE1, DE2 and DE3) and a commercial sample (ASDA half fat). Fourteen skilled panellists were recruited and underwent pre-testing sessions to familiarise with the method prior to the flash profiling test sessions.

As this was an exploratory study evaluating novel reduced fat formulations, there were no predefined optimal sensory targets. However, it was anticipated that the DE cheeses would ideally exhibit sensory characteristics similar to FF control, particularly lower scores for hardness and graininess compared to that of the RF. This expectation was based on the intended role of the DE system, although results showed some deviations from the goal.

The cheese samples were simultaneously evaluated in two replications (Rep 1 and 2) of two different sets of labelling codes with a short break between. Generalised Procrustes analysis (GPA) was applied to analyse and interpret the cheese sensory profiles. When visualising the results across the two replications, Procrustes analysis of variance (PANOVA) was used to test the consistency of panellist's performance. PANOVA results reveal that 'panellist' variable is not significantly different ($P > 0.05$) in Rep 1 and in Rep 2. This indicates no significant difference in panellists scoring across the 31 attributes. However, it is noted that p-value of 'panellists' variable is just above the cut-off point of 0.05 ($P = 0.056$) which could stem from sensory fatigue or potential adaptation in ranking methods compared to their initial attempt. Overall panellists ranked the samples in a similar pattern.

The GPA results from both replications can be mainly presented and explained using the first two dimensions (GPA factors one and two) with the total variances explained as high as 83.9 % for Rep 1 and 84.78 % from Rep 2 results (Figure 7.11 and 7.12).

GPA Factor 1 predominantly presents with textural characteristics such as ***hardness, graininess*** and ***creaminess****,* whereas Factor 2 was associated with smell and flavour attributes. In the consensus configuration, Factor 1 explained 64.7% and Factor 2 19.2% of the total variance, giving a cumulative variance of 83.9%. Factors 3 to 5 accounted for smaller proportions: 8.1%, 5.6% and 2.3% respectively, contributing to the remaining variance in the data and relating to more nuanced attributes.

Strong positive correlations were observed between visual appearance (translucent 0.929) and textural characteristics like hardness (0.941), grainy texture (0.990) and powdery mouth feel (0.970). These correlations were consistent across both replications of the cheese sensory profiling.

The GPA Bi-plots (Figures 7.11 and 7.12) display which sensory characteristics dominated the sensory profiles of the different cheese samples evaluated (RF, DE1, DE2, DE3, commercial ASDA half fat and FF). Reduced fat samples and double emulsion samples exhibited similar characteristics with shared strong positive correlations to ***hardness,*** ***graininess*** and ***powdery texture,*** and negative correlations to ***creaminess*** in the first dimensions.

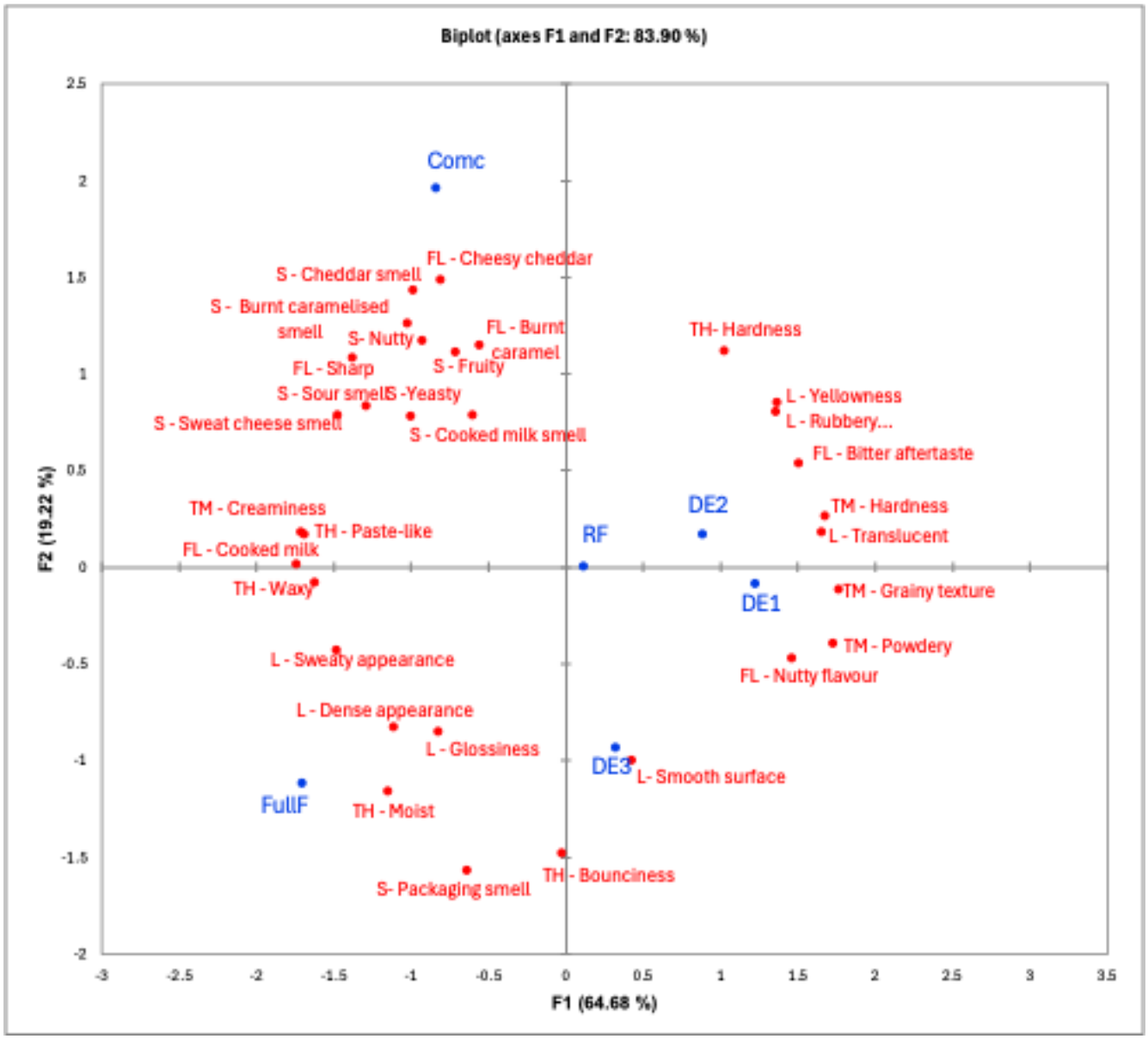
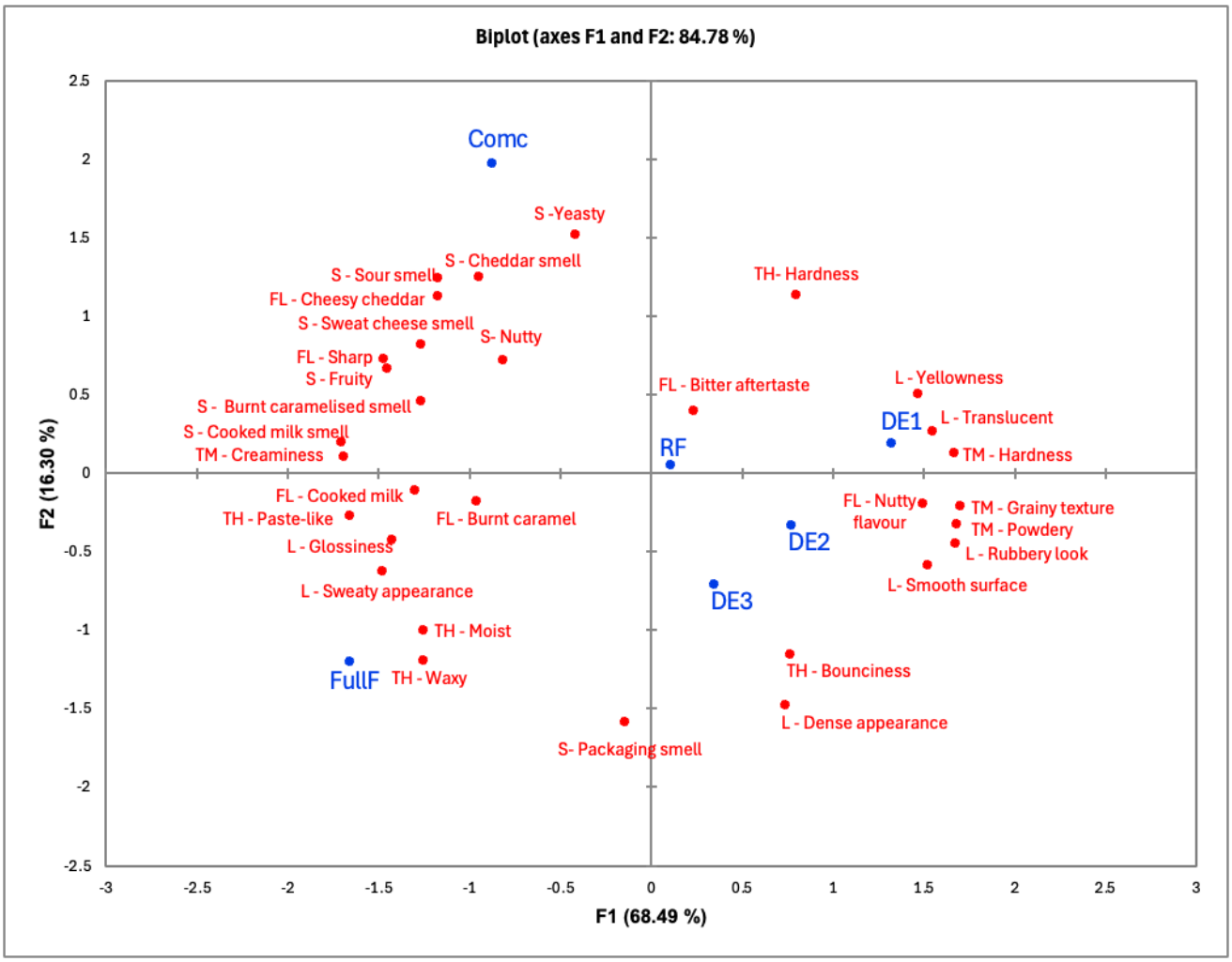


Figure 7.11 – Generalised Procrustes Analysis (GPA) Bi-plot showing Factor 1 (64.7%) and Factor 2 (19.2%) for Replication 1.



3413

3414 **Figure 7.12 Generalised Procrustes Analysis (GPA) Bi-Plot Showing Factor 1 (68.5%)**
3415 **and Factor 2 (16.3%) for Replication 2.**

3416 Both plots (Figure 7.11 and Figure 7.12) display the consensus sensory profiles of cheese
3417 samples. Factor 1 explains 64.7 % (Rep 1) and 68.5 % (Rep 2) of variance, predominantly
3418 representing texture attributes such as hardness, graininess and creaminess. Factor 2
3419 accounts for 19.2 % (Rep 1) and 16.3 %. (Rep 2) of variance, capturing flavour and aroma
3420 characteristics. Together, these two factors explain approximately 84 % of the sensory
3421 variability in both replications.

3422 Based on the mean rank scores of textural attributes perceived in the mouth from Table 7.3,
3423 the DE1 also had the highest rank score for hardness and the lowest for creaminess.
3424 Integrating the information displayed in the GPA Bi-plots (Fig 7.11 and 7.12) with the mean
3425 rank scores, it could also be explained that the FF and ASDA half fat were highly and
3426 positively correlated to ***creaminess*** (with the highest score) and the lowest for ***hardness***.
3427 Interestingly, it can be concluded that DE3 was the softest cheese among the three double

emulsion samples as it had the lowest scores for hardness ($P < 0.05$), grainy texture ($P < 0.05$) and powdery intensities, as well as significantly highest score ($P < 0.05$) for creaminess out of the three double emulsion cheeses, being 3.50 compared to 1.79 and 2.04 for DE1 and DE2, respectively.

Table 7.3 - Mean sensory intensity scores for *texture in the mouth* characteristics*.

Sample	Hardness	Grainy Texture	Creaminess	Powdery
FF	1.0 ± 0.1 ^{a}	1.6 ± 1.0 ^{a}	5.6 ± 1.0 ^{c}	2.1 ± 1.5 ^{a}
ASDA Half Fat	2.4 ± 0.6 ^{b}	2.1 ± 0.7 ^{a}	5.1 ± 0.7 ^{c}	2.1 ± 1.1 ^{a}
DE3	3.2 ± 0.7 ^{c}	3.8 ± 1.1 ^{b}	3.5 ± 0.7 ^{b}	3.9 ± 0.9 ^{c,d}
RF	4.4 ± 0.8 ^{d}	3.8 ± 1.0 ^{b}	3.1 ± 0.8 ^{b}	3.7 ± 1.1 ^{b}
DE2	4.5 ± 0.8 ^{e}	4.4 ± 0.9 ^{c,d}	2.0 ± 0.8 ^{a}	4.3 ± 1.4 ^{c,d}
DE1	5.6 ± 0.5 ^{f}	5.2 ± 0.8 ^{d}	1.8 ± 1.0 ^{a}	4.9 ± 1.2 ^{d}

*Values are expressed as mean ± standard deviation (n = 14). Different superscript letters within a column indicate significant differences between cheese samples (ANOVA, $P < 0.05$)

Table 7.4 shows mean texture scores when panellists evaluated the texture of the cheese in their hand, prior to putting the sample in their mouth. The FF sample was perceived to be the moistest (5.41) and most paste-like (5.57) compared with the other samples ($P < 0.05$), which would be expected as the fat will contribute to the malleability of the cheese when pressed between the two fingers and worked into a paste (Scott, Robinson and Wilbey, 1998). The analysis of hardness in the hand, however, displays some discrepancy to hardness in the mouth. For example, the RF hardness by hand was the highest (5.05) followed by ASDA half fat whereas, with hardness in the mouth, the commercial half fat cheese was close to the experimental FF cheese scores.

For the DE3 cheese the texture in the hand appeared to have a lower rank score (closer to 1), indicating that panellists perceived it as having less firmness and thus a softer texture compared to RF and ASDA half fat samples compared to what was found in the mouth. The explanation behind this requires further investigation, such as an additional repetition. Whilst the sample preparation and serving plans were controlled, to follow the cheese testing protocol, which was trialled and established with the panellists, it would be worth identifying if

this was an anomaly or something else was affecting the difference between the “hardness” experienced in the mouth to the hand.

Table 7.4 – Mean sensory intensity scores for *texture in the hand* characteristics*

Samples	Bounciness	Moist	Paste-Like	Hardness	Waxy
ASDA Half Fat	2.5 ± 1.1 ^{a}	2.9 ± 1.3 ^{a,b}	4.6 ± 1.3 ^{c}	4.2 ± 1.4^{c,d}	3.7 ± 1.7 ^{a}
RF	3.1 ± 1.5 ^{a,b}	2.9 ± 1.2 ^{a,b}	2.7 ± 1.1 ^{a,b}	5.1 ± 1.1^{d}	2.9 ± 1.1^{a}
DE1	3.2 ± 1.4 ^{a,b}	2.3 ± 1.2^{a}	2.3 ± 0.8 ^{a}	3.9 ± 1.2 ^{c}	2.8 ± 1.4 ^{a}
FF	3.7 ± 2.2 ^{b,c}	5.4 ± 1.3 ^{c}	5.6 ± 0.8 ^{d}	1.4 ± 0.9 ^{a}	4.8 ± 1.7 ^{b}
DE2	4.0 ± 1.4 ^{b,c}	3.5 ± 1.4 ^{b}	2.5 ± 1.1 ^{a}	3.6 ± 1.0^{b,c}	3.1 ± 1.5^{a}
DE3	4.6 ± 1.2 ^{c}	3.9 ± 1.4 ^{b}	3.4 ± 1.1 ^{b}	2.9 ± 1.2 ^{b}	3.6 ± 1.1 ^{a}

*Values are expressed as mean ± standard deviation (n = 14). Different superscript letters within a column indicate significant differences between cheese samples (ANOVA, $P < 0.05$)

The sensorial characteristics of the texture in the mouth (Table 7.3) align well with the texture analysis results (Figure 7.1), indicating that the RF and DE samples produced firmer cheeses due to lower fat content (13 %) compared to FF (29 %). The softer texture of the commercial ASDA half fat cheese could be attributed to its longer maturation period (approx. 6 months) leading to increased proteolysis and reduced firmness (Anvari and Joyner, 2019). Similarly, differences in powdery mouthfeel, creaminess and grainy texture were also noted between the commercial sample, FF and all double emulsion and RF cheeses, which could be attributed to the absence of fat in the double emulsion cheeses. Fat is important to taste and mouth feel, as fat coats and lubricates the mouth and is associated with creaminess and smoothness (Everett and Auty, 2017; Metha, 2018; Mattice and Marangoni, 2019; Sharma Khanal *et al.*, 2019; Giha, Ordoñez and Villamil, 2021). The lack of fat in RF and double emulsion cheeses means that there was no fat to coalesce upon chewing and there being a lack of fat to coat the mouth, resulting in this powdery mouth feel.

The commercial cheese was different to other cheeses even though this had a similar amount of fat (15 %, calculated from the nutritional information on the pack) as the double emulsion cheeses. This cheese did not experience the powdery mouth feel to the same extent as the double emulsion cheeses, due to the enzymatic action that has been allowed to occur during the longer maturation period, contributing to mouth fee

l (Muthukumarappan and Swamy, 2023). As during ageing, the proteolytic hydrolysis breaks down the casein which leads to re-organisation and weakening of the protein matrix (Chandrapala *et al.*, 2013) contributing to the lower firmness scores in section 7.3.2 and in the sensorial mouth feel attributes.

Regarding visual appearance, a post-hoc pairwise Tukey's test was applied to test the difference of means, as displayed in Table 7.5. A significant difference ($P < 0.05$) in "rubbery look" was observed between FF (2.304 the lowest) and double emulsion samples. This finding aligns with prior research by Childs and Drake (2009), which indicated that cheeses with reduced fat content were perceived differently in terms of texture and flavour attributes, often classified as rubbery, translucent and lacking flavour, as seen in the results with higher rank values for double emulsion samples. The translucency in cheeses can be attributed to the lack of fat, resulting in a reduced number of aggregates and fat globules to scatter light (Rudan *et al.*, 1999; Mistry, 2001; Pastorino *et al.*, 2002; Johnson *et al.*, 2009; Wadhwani and McMahon, 2012). Interestingly, "yellowness" was also significantly different between cheeses. FF and RF had the lowest scores for yellowness, suggesting these had less yellow appearance and a milkier white colour. Some studies have attributed the opacity of cheese, often found in full fat cheese, to the reduced light scattering ability caused by fat and aggregates.

Table 7.5 – Mean sensory intensity scores for appearance characteristics*

Sample	Glossiness	Dense Appearance	Yellowness	Rubbery look	Translucent	Smooth surface
DE1	3.2 ± 1.5 [a]	3.2 ± 1.6 [a]	5.4 ± 0.7 [d]	4.0 ± 1.6 [b]	5.3 ± 0.8 [d]	3.7 ± 1.6 [a,b]
ASDA Half Fat	3.3 ± 1.2 [a]	2.9 ± 1.4 [a]	3.9 ± 1.0 [c]	3.3 ± 1.2[a,b]	2.9 ± 0.8 [b]	2.9 ± 1.4 [a]
DE2	3.4 ± 1.5 [a]	3.6 ± 1.6 [a]	4.9 ± 0.9 [d]	4.3 ± 1.5 [b]	5.0 ± 0.8 [d]	3.4 ± 1.7[a,b]
DE3	2.8 ± 1.3 [a]	3.7 ± 1.4 [a]	3.6 ± 0.6 [c]	3.8 ± 1.0 [b]	4.0 ± 1.0[c]	4.3 ± 1.2 [b]
RF	3.4 ± 1.5 [a]	3.7 ± 1.3 [a]	2.2 ± 0.7 [b]	3.3 ±1.5 [a,b]	2.4 ± 0.6 [b]	3.7 ± 1.1 [a,b]
FF	4.9 ± 1.8 [b]	3.8 ± 2.0 [a]	1.0 ± 0.1 [a]	2.3 ± 1.9 [a]	1.3 ± 0.6 [a]	3.1 ± 1.8 [a]

*Values are expressed as mean ± standard deviation (n = 14). Different superscript letters within a column indicate significant differences between cheese samples (ANOVA, $P < 0.05$)

Table 7.6 outlines the mean intensity of flavour characteristics tested in the flash profile, whereas Table 7.7 outlines the mean rank scores for the aroma characteristics. The contribution to cheese flavour is down to the volatile compounds in cheese during breakdown of proteins and fats over maturation (Avsar *et al.*, 2004). The commercial ASDA half fat received the highest for “sharp” and “cheesy Cheddar” flavour scores. This could be explained by its longer maturation period compared to the experimental cheeses, which could have enabled a longer time for lipolysis to occur. In addition, the commercial sample generally had the highest scores for most of the aroma attributes, which could again be linked to the flavour attributes. Often cheese sensory, aroma, flavour and taste are very closely linked and often flavours are aromas (Drake and Delahunty, 2017).

Interestingly “burnt caramel” flavour intensity was similar across all five samples, Drake, Miracle and McMahon (2010) mention the presence of “rosy” and “burnt” flavours in reduced and low fat cheeses in their study, and they believed this was due to the imbalance of phenylethanol and phenylacetic acid. Despite all samples being of similar rank by the panellists, perhaps with a longer maturation period the sample cheeses could experience

slightly higher scores, resulting in a greater difference between them and the FF cheese and be more like the ASDA half fat. Additionally, Bojanic-Rasovic *et al.* (2013) believed that higher moisture content contributed to a distinct milk sugar fermentation, so one may assume that potentially the higher moisture in the double emulsion cheeses could present more burnt caramelised flavours, although none were discovered in this study.

When examining the three double emulsion treatments, most of their flavour characteristics did not show significant differences ($P > 0.05$). However, the nutty flavour was notably prominent across all double emulsion cheeses, with DE2 scoring the highest (4.179), followed by DE1 (3.839). One study explored the origin of the nutty flavour in cheese, though no single cause was identified for Cheddar. It was concluded that certain aldehydes produced from amino acids were linked to enhanced or accelerated nutty flavours (Avsar *et al.*, 2004). The high nutty scores in the double emulsion cheeses may be attributed to the use of PGPR and sunflower lecithin as lipophilic emulsifiers, which could be contributing to these flavours, although no significant differences were found between the double emulsion samples.

Factor 1 (seen in Figures 7.11 and 7.12) highlighted a bitter aftertaste (0.845), yet no significant differences were observed between samples, despite previous studies linking PGPR to bitterness in food products (Jiménez-Colmenero, 2013). Bitterness has also been associated with low fat Cheddar after three months of aging, likely due to higher moisture

content and increased proteolysis, which produces bitter amino acids (Drake, Miracle and McMahon, 2010).

Table 7.6 – Mean sensory intensity scores for flavour characteristics*

Sample	Cooked Milk	Nutty Flavour	Sharp	Cheesy Cheddar	Burnt Caramel	Bitter After taste
DE1	2.2 ± 1.4 ^{a}	3.8± 1.6^{a,b,c}	2.2 ± 1.4 ^{a}	2.1 ± 1.1 ^{a}	2.8 ± 1.4 ^{a}	3.1 ± 1.6^{a}
DE3	3.1 ± 1.6 ^{a,b}	3.9 ± 1.4^{b,c}	3.0 ±1.7 ^{a,b}	3.0 ± 1.2 ^{a,b}	3.5 ± 1.5 ^{a}	2.7 ± 1.6^{a}
DE2	3.3 ± 1.7^{a,b}	4.2 ± 1.8 ^{c}	2.9 ±1.5 ^{a,b}	2.8 ± 1.4 ^{a,b}	3.3 ± 1.5^{a}	3.7 ± 1.7^{a}
RF	3.5 ± 1.3 ^{b}	3.1± 1.5^{a,b,c}	3.2 ±1.4 ^{a,b}	3.4 ± 1.3 ^{b}	3.5 ± 1.4^{a}	3.4 ± 1.5^{a}
ASDA Half Fat	3.7 ± 1.8 ^{b}	2.7 ± 1.9^{a,b}	4.9 ± 1.6 ^{c}	5.7 ± 1.2 ^{b}	3.7 ± 1.9^{a}	3.5 ± 2.0^{a}
FF	4.3 ± 1.8 ^{b}	2.6 ± 1.5 ^{a}	3.9 ±1.7 ^{b,c}	3.3 ± 1.6 ^{b}	3.4 ± 1.5^{a}	3.5 ± 1.8^{a}

*Values are expressed as mean ± standard deviation (n = 14). Different superscript letters within a column indicate significant differences between cheese samples (ANOVA, $P < 0.05$)

Table 7.7 – Mean sensory intensity scores for aroma characteristics (by sniffing)*

Sample	Yeasty	Burnt Caramelised Smell	Cooked milk smell	Sour smell	Fruity	Nutty	Cheddar Smell	Packaging Smell	Sweat Cheese smell
DE3	2.9 ± 1.5^{a}	2.5 ± 1.4 ^{a}	2.7 ± 1.7 ^{a}	2.9 ± 1.6^{a}	3.0 ±1.5 ^{a}	3.5 ± 1.4^{a,b}	2.3 ± 1.2 ^{a}	3.7 ± 1.3^{a}	2.8 ± 1.5 ^{a}
RF	3.1 ±1.4 ^{a,b}	3.4 ± 1.4 ^{a,b}	3.3 ± 1.6^{a,b}	3.3 ± 1.4^{a}	2.9 ± 1.4 ^{a}	2.9 ± 1.4^{a}	3.7 ± 1.3 ^{b}	3.6 ± 1.3^{a}	3.5 ± 1.2^{a,b,c}
DE2	3.2 ± 1.7 ^{a,b}	3.1 ± 1.5 ^{a,b}	3.1 ± 1.4 ^{a,b}	3.5 ± 1.5^{a,b}	3.3 ± ^{a}	3.7 ± 1.4^{a,b}	2.9 ± 1.3 ^{a,b}	3.5 ± 1.4^{a}	3.4 ± 1.4 ^{a,b,c}
FF	3.4 ± 1.5 ^{a,b}	3.9 ± 1.7 ^{b}	4.2 ± 1.7 ^{b}	3.9 ± 1.5 ^{a,b}	3.9 ± 1.6^{ab}	3.6 ± 1.4^{a,b}	3.7 ± 1.5 ^{b}	3.9 ± 1.5^{a}	3.9 ± 1.8 ^{b,c}
DE1	3.5 ± 1.6 ^{a,b}	3.2 ± 1.5 ^{a,b}	2.9 ± 1.6 ^{a,b}	2.9 ± 1.4 ^{a}	3.4 ± 1.5 ^{a,b}	2.8 ± 1.3 ^{a}	2.8 ± 1.3 ^{a,b}	3.3 ± 1.4^{a}	3.1 ± 1.3 ^{a,b}
ASDA Half Fat	4.3 ± 1.9 ^{b}	4.1 ± 1.9 ^{b}	4.0 ± 1.7 ^{b}	4.5 ± 1.7 ^{b}	4.4 ± 1.7 ^{b}	4.5 ± 1.7 ^{b}	5.5 ± 1.1^{a}	3.0 ± 1.6^{a}	4.3 ± 1.8 ^{c}

*Values are expressed as mean ± standard deviation (n = 14). Different superscript letters within a column indicate significant differences between cheese samples (ANOVA, $P < 0.05$)

The sensory evaluation of the cheeses was supported by objective measurements such as instrumental textural results and were as anticipated based on relevant theories. The RF cheeses were the hardest, lacking creaminess, and had a powdery mouthfeel. The double emulsion cheeses were similar in some respects to the RF cheeses, showing comparable scores in certain areas, while some attributes such as specific flavour characteristics were somewhat akin to those of the FF cheeses. However, the cheeses underwent a short maturation period (four weeks), which, had these allowed to mature for longer, could have altered the flavour attributes due to enzymatic action. Generally, the results underscore the functionality of fat in sensory attributes and how its alteration can impact the mouthfeel and softness of the cheese. The double emulsion cheese samples were not significantly different from each other, and despite minor significances discussed, the primary lipophilic emulsifier and the double emulsion did not have a substantial impact on the overall sensory profile. Compared to the other samples, the double emulsion sensorial profiles were generally like the RF cheeses and did not enhance the sensory attributes to the level of the FF cheeses. While double emulsion technology appears to improve the texture according to the texture analyser and functionality, it does not improve the texture and sensory attributes sufficiently to mimic those of full fat cheese.

7.5 Conclusion

To conclude this research chapter, the different double emulsion samples did not result in significant differences in structural, functional or sensorial attributes, indicating that the inner lipophilic emulsifier had limited impact on the final application. The research demonstrated that while double emulsion technology positively influenced the structural and functional attributes of reduced fat cheeses, the sensory evaluation revealed that mimicking full fat cheese was not achievable. The double emulsion treatments were like RF in terms of consumer ranking and perception.

Despite these mixed results on the benefits of double emulsion, there remains potential for double emulsions to be utilized as a fortification method rather than for improving the sensory attributes of reduced fat cheese

CHAPTER EIGHT: GENERAL DISCUSSION

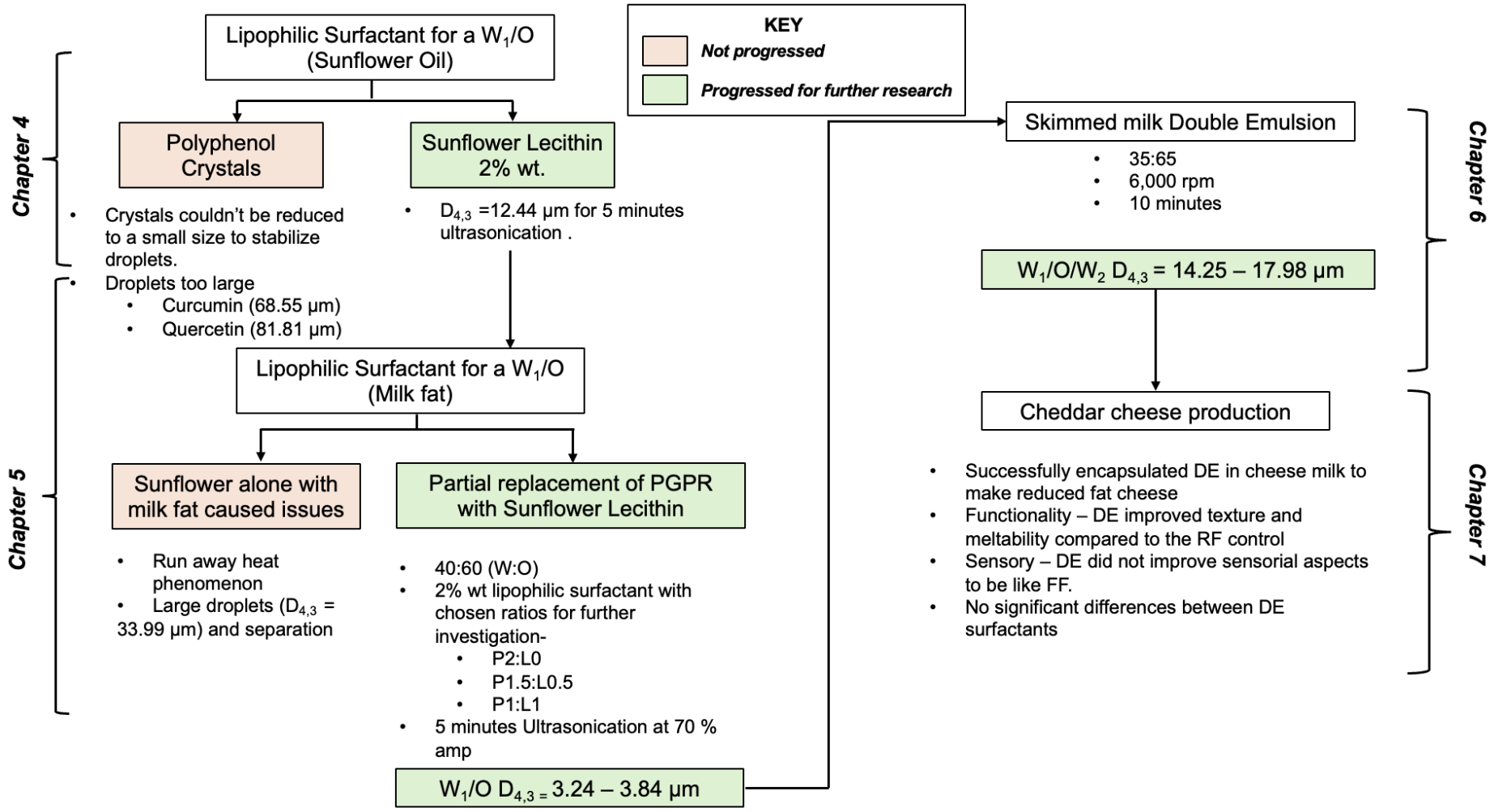
8.1 Introduction

High dairy and cheese consumption has been associated with high dietary intake of saturated fats, which has been linked to adverse health outcomes such as coronary heart disease (CHD) (Beresford, 2023). As health consciousness grows, there is an increasing demand for dietary modifications to mitigate these risks. One significant area of concern is the high fat content in cheese, which contributes to flavour, creaminess mouthfeel and a desirable texture. Efforts to reduce fat content in cheese have often resulted in negative impacts on its functional and sensory characteristics, leading to cheeses with a hard and rubbery mouthfeel and poor meltability. Resulting in consumers looking for reduced fat products which have improved sensorial and functional properties, like their full fat counterparts.

In response to these challenges, double emulsion technology has emerged as a novel approach to enhance the functional and sensory properties of reduced fat cheeses. Several studies have investigated the application of double emulsion technology in cheese production. For instance, Paximada, Howarth and Dubey (2021) explored the use of double emulsion systems fortified with proteins such as whey, pea and rice. Their findings suggested that double emulsion technology could improve the textural and functional characteristics of low fat cheese, although sensory evaluation was not included in their study. Similarly, Leong *et al.* (2020) applied double emulsion technology with canola oil to enhance the unsaturated fatty acid content and lower the fat content in cheese, achieving promising results in terms of functionality.

Despite these advancements, double emulsion technology faces significant challenges, primarily due to its thermodynamic instability, which necessitates the use of surfactants. Traditional surfactants such as PGPR, are synthetic and often do not align with the growing consumer demand for clean label products. Research has explored natural alternatives to synthetic surfactants, with promising results from the use of skimmed milk, which contains casein and whey proteins that can stabilise O/W emulsions. However, the integration of natural lipophilic surfactants to stabilise W/O emulsions remained under explored. Some interesting studies from Zembyla *et al.* (2019) presented opportunity for the use of polyphenol crystals and other studies had investigated the use of sunflower lecithin, solely but also by partially replacing PGPR with sunflower lecithin (Knoth, Scherze and Muschiolik, 2005; Leong *et al.*, 2018; Okuro *et al.*, 2019; Balcaen *et al.*, 2021). The hypothesis tested in this thesis was that double emulsions made with natural or partially natural lipophilic surfactants would influence the sensory and functional properties of reduced fat cheese, with

the potential to make them comparable to their full fat counterparts. Sunflower lecithin was able to partially replace PGPR to stabilise a W/O emulsion and successfully created a double emulsion. Double emulsions were successfully incorporated into cheese with minor improvements in functionality in comparison to the reduced fat cheese, and the sensory evaluation flavour characteristics identified were like the full fat counter parts. However, the sensorial characteristics which were linked to texture in the mouth were not improved by double emulsions. Panellists could identify a difference in these characteristics, such as a lack of creaminess and high rank scores for the attributes hardness and graininess for RF and double emulsion cheeses. Figure 8.1 highlights the flow of the thesis and key findings which link together to reach the conclusion.



3609

3610 **Figure 8.1 Summary flow chart of the thesis**

8.2 Effect of natural lipophilic surfactants on stabilising W/O

The objective of the first two chapters were to identify a natural lipophilic surfactant to stabilise a W/O emulsion and replace the use of synthetic surfactants such as PGPR. Despite investigation using polyphenol crystals, which were a novel opportunity to explore, after research from Zembyla *et al.* (2019) showed the potential use of curcumin and quercetin. Chapter 4's research highlighted a positive outcome in the stabilisation of W/O emulsions with curcumin and quercetin but with large $D_{4,3}$ values, being above 60 μm. Even with manually grinding of the polyphenol crystals to reduce the size, did not aid the production of small stable droplets. As reviewing the literature the shape and size of the stabilising particle in a Pickering emulsion will impact the size of the droplet able to be produced (Xia, Xue and Wei, 2021).

Further refinement using analytical milling might reduce particle size for better emulsification, but this was not feasible due to equipment limitations at Harper Adams University. In contrast, sunflower lecithin however, emerged as a a promising alternative to stabilising W/O emulsions. Although widely used the food industry, its application in milk fat-based W/O emulsions is under researched. Transitioning from sunflower oil to milk fat and sole use of sunflower lecithin caused some interesting interactions between the milk fat and the sunflower lecithin, and the run-away heat phenomenon. Further investigation into the interactions between milk fat and sunflower lecithin would have been beneficial, but due to time and resource availability this was not possible, instead this provides opportunity for future research.

To mitigate the issues encountered, the partial replacement of PGPR with sunflower lecithin was investigated, which had not been undertaken in milk fat before. This enabled the production of stable W/O emulsions particularly with the P1.5:L0.5 and P1:L1 ratios, with droplet sizes of 3.67 μm and 3.84 μm respectively. Emulsions with higher levels of lecithin were like other studies in a similar area, which found that greater volumes of lecithin correlated with larger droplet sizes and more aggregated formations. Increasing the ultrasonication homogenisation times, despite other literature suggesting an increase in time was related to a reduced droplet size, this was not discovered during the formation of these W/O emulsions. Had equipment been available at Harper Adams University then alteration in homogenisation method could have reduced droplet size further, by using high pressure homogenisation. There is limited research on the use of W/O emulsions with a combination of sunflower lecithin and PGPR with milk fat. This thesis provides a method of the development of milk fat W/O emulsions with reduced amounts of synthetic lipophilic

surfactants, which have relatively small droplets that can be utilised into further applications such as double emulsions.

8.3 Development of skimmed milk double emulsions with reduced amounts of synthetic lipophilic surfactants

Building on the findings from the initial research chapters, this thesis also explored the integration of milk fat based primary emulsions with reduced amounts of synthetic surfactants (P1.5:L0.5 and P1:L1) into skimmed milk double emulsions. As highlighted in the literature, the size of the double emulsion is influenced by the size of the encapsulated primary droplets, and varying the ratio of primary to secondary emulsions, which impacts the overall double emulsion size (Maghamian, Goli and Najarian, 2021). With primary droplets approximately 3.6 μm in size, their dimensions will affect the final double emulsion droplet size before the inner droplets are compromised and destroyed during excessive homogenisation. Numerous parameters were investigated in double emulsion production to find a suitable method for further application into reduced fat cheese. The chosen parameters involved a 35:65 W_1/O to W_2 ratio, with homogenisation at 6,000 rpm for 10 minutes which created droplets from 14.25 μm and 17.98 μm for P1:L1 and P1.5:L0.5, respectively. While findings showed an increase in speed and homogenisation duration reduced droplet size, this had to be carefully balanced with encapsulation efficiency and the potential risk of damaging primary droplets, which could impede double emulsion formation and thus application. Some literature does describe the alteration of homogenisation methods which could result in smaller droplet sizes, in which this thesis agreed. However, recent research on balancing the elements to create stable double emulsions for specific dairy application has been underexplored. Therefore, this provided some insight into creating a double emulsion with reduced amount of synthetic surfactant, by replacing PGPR with sunflower lecithin, using milk fat and skimmed milk for further utilisation in dairy application for research.

8.4 Effect of skimmed milk DE in the reduction of fat in cheese on functionality and sensorial characteristics

The objective of this study was to utilise the emulsions developed in the previous three research chapters and incorporating them into cheese production, to improve the functional and sensorial characteristics of reduced fat cheese. DE1, with P2:L0 was used as the control lipophilic surfactant to evaluate the use of the partial replacement of PGPR with sunflower lecithin in other double emulsion samples (DE2 and DE3). Then to evaluate their use as improving the functionality and sensory of reduced fat cheeses.

The double emulsion samples were successfully incorporated into cheese milk, encapsulated into the casein matrix and were identified at one month's storage with the fluorescence confocal microscope. The texture analysis results highlighted that there was no significant difference between double emulsion samples, therefore the replacement of PGPR with sunflower lecithin, did not impact the overall effect of the double emulsion cheeses. The double emulsion cheeses were softer than the RF, with RF being (684.651 g) compared to the double emulsion samples which ranged from 564 g to 590 g and FF being the softest with 335.910 g. This was like Paximada, Howarth and Dubey (2021) which also used milk fat and found that the low fat was the firmest, FF was the softest and double emulsion samples sat in between. Other studies, such as Sharma Khanal *et al.* (2019), found FF to be softer than RF, as expected but then, contrary to this thesis Leong *et al.* (2020) found the double emulsion cheeses to be the firmest, but this was using canola oil rather than milk fat. The commercial comparison, ASDA half fat (15 % fat) was used as it was comparable in fat content to the cheeses (13 %) in the study and was used in the sensory evaluation as comparison to products readily available. The limitation of this study and using this comparison sample was the maturation period, which was longer than one month. A longer maturation time of the thesis cheeses would have been more representative of Cheddar cheese types on the market, but due to time constraints within the PhD this was not possible.

The functionality of cheeses, particularly reduced fat cheese has poor functionality, with a lack of fat to coalesce and lubricate the casein strands during melting. Double emulsion samples in this study improved meltability to RF, this, as discussed could be attributed to the higher moisture content, helping to hydrate the casein strands and the larger double emulsion droplets which coalesce and help to allow casein strands to slide over one another during melting.

Sensory evaluation using a flash profile was undertaken with 14 panellists to rank the five sample cheeses and the commercial sample against 31 sensory characteristics. Both double emulsion and RF cheeses shared similar characteristics, particularly hardness, graininess and powdery mouthfeel characteristics, these linked to the objective results from the texture analyser. However, this does not suggest like theorised that double emulsions improve the sensorial properties of RF cheeses and mimic the full fat counterparts. The flavour characteristics of the double emulsion cheeses were not significantly different from each other, meaning that the inner lipophilic surfactant did not seem to have an impact on flavour attributes. No research using double emulsions in Cheddar cheese has been undertaken to evaluate consumer opinion of double emulsions cheeses compared to the RF control and a FF. Longer maturation could have altered sensory evaluation, as time would have allowed

more enzymatic action had the cheese been left to mature for 6 months. However, a longer duration was not possible during the PhD and future research could benefit from this.

8.5 Conclusion, limitations and future work

Results obtained in this thesis support the hypothesis partially, showing that partial replacement of PGPR with sunflower lecithin at ratios P1.5:L0.5 and P1:L1 can be used to produce stable skimmed double emulsions. These emulsions were successfully incorporated into reduced fat (RF) cheese production, improving certain functional characteristics. Specifically, meltability was enhanced compared to the RF control, and textural analysis confirmed that cheeses made with double emulsions were softer than the RF control but still firmer than full fat (FF) cheese. Sensory analysis revealed that the double emulsions cheeses shared several characteristics with the RF control, particularly in terms of hardness and mouthfeel, suggesting that while functional properties improved, sensory characteristics did not show significant enhancement.

These findings demonstrate the feasibility of using partially natural surfactants to form functional double emulsions, which is an area that has seen little direct application in cheese matrices. While some sensory properties were not enhanced, functional properties such as meltability and texture were improved, laying groundwork for future studies on improved sensory replication and long term maturation.

Limitations included the unavailability of equipment such as a high pressure homogeniser and fine analytical mills. These tools could have refined droplet size and emulsion stability. While these constraints limited the scope of optimisation, they also identify clear technical routes for future investigation. For instance, had an analytical mill been available, further reduction in polyphenol crystal size may have improved W/O emulsion stability. Similarly, a high pressure homogeniser might have further reduced droplet size compared to the ultrasonic method used.

While a longer maturation period (e.g. 6 months) would align better with commercial Cheddar cheese production, this was not feasible due to the fixed duration of the PhD project. Additionally, sourcing comparable cheeses from local producers with controlled maturation timelines was not possible within the study timeframe. Nevertheless, the experimental cheeses were analysed after 4 weeks of maturation – providing valuable early stage insights into functionality and sensory properties.

Double emulsions created with reduced synthetic surfactants were successfully incorporated into the casein matrix of reduced fat cheese, providing a stable system for potential fortification and functional improvement. Given that the emulsions remained stable during

cheese production and storage, there is scope to explore how they could serve as delivery systems for nutraceuticals.

Future work could therefore investigate fortifying Cheddar cheese with vitamins, minerals or antioxidants such as curcumin – as briefly introduced in Chapter 2. This would not only extend the health benefits of cheese but also test the double emulsion system as a controlled delivery mechanism for functional ingredients within a dairy matrix.

This thesis presents novel findings that provide a foundation for future contributions to the literature, particularly around the practical integration of double emulsions using reduced synthetic surfactants in cheese production. While no publications have yet resulted from this work, it opens up valuable avenues for academic dissemination and industrial application.

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Appendices

Appendix 1

Table A1 - Reference criteria for microbiological analysis

	cfu/g
Enterobacteriaceae (presumptive) cfu / g	≤100
Coagulase positive Staphylococci (Presumptive) cfu / g	≤100
Escherichia coli (β-Glucuronidase positive) cfu / g	≤100
Bacillus cereus (presumptive) cfu / g	<20
Sulphite Reducing Clostridia (presumptive) cfu / g	<20
Listeria spp. / 25g	Not detected
Listeria spp Enumeration Count (Presumptive) cfu / g	<100 at end of life
Salmonella sp / 25g	Not detected

(Source adapted from (Commission, 2005))

Appendix 2

Table A2 – Microbiological results from the treatment cheeses prior to sensory evaluation

DATE BATCH MADE	Sample CODE	Enterobacteriaceae (presumptive) cfu / g	Escherichia coli (β-Glucuronidase positive) cfu / g	Coagulase positive Staphylococci (Presumptive) cfu / g	Coagulase positive Staphylococci (Confirmed) .	Bacillus cereus (presumptive) cfu / g	Sulphite Reducing Clostridia (presumptive)	Listeria spp. / 25g	Listeria spp Enumeration Count (Presumptive)	Salmonella sp / 25g	USED FOR SENSORY EVALUATION
	A_LF_W1	260	<10	-	-	<20	<10	ND	<20	ND	
09/05/2024	A_LF_W1 (retake full)	10	<10	50	50	<20	<10	ND	<20	ND	
	B_LF_W1	40	<10	-	-	<20	<10	ND	<20	ND	Y
14/05/2024	B_LF_W1 (retake for CPS)	-	-	<10	-	-	-	-	-	-	
09/04/2024	C_LF_W1	<10	<10	-	-	<20	<10	ND	<20	ND	
	A_DE1_W1	<10	<10	-	-	<20	<10	ND	<20	ND	Y
07/05/2024	A_DE1_W1 (retake CPS)	-	-	10	10	-	-	ND	-	ND	
	B_DE1_W1	<10	<10	-	-	<20	<10	ND	<20	ND	
13/05/2014	B_DE1_W1 (retake CPS)	-	-	<10	-	-	-	ND	-	ND	
08/04/2024	C_DE1_W1	<10	<10	-	-	<20	<10	ND	<20	ND	
	A_DE2_W1	90	<10	-	-	<20	<10	ND	<20	ND	Y
09/05/2024	A_DE2_W1 (retake CPS)	-	-	10	10	-	-	-	-	-	
	B_DE2_W1	>1.50E+03	<10	-	-	<20	<10	ND	<20	ND	
14/05/2024	B_DE2_W1 (retake full)	>1.50E+03	<10	270	270	<20	<10	ND	<20	ND	
08/04/2024	C_DE2_W1	30	<10	-	-	<20	<10	ND	<20	ND	
	A_DE3_W1	150	<10	-	-	<20	<10	ND	<20	ND	Y
13/05/2024	A_DE3_W1 (retake full)	50	<10	10	10	<20	<10	ND	<20	ND	
	B_DE3_W1	>1.50E+03	<10	-	-	<20	<10	ND	<20	ND	
15/05/2024	B_DE3_W1 (retake full)	>1.50E+03	<10	10	10	<20	<10	ND	<20	ND	
09/04/2024	C_DE_W1	<10	<10	-	-	<20	<10	ND	<20	ND	
	A_FF_W1	<10	<10	-	-	<20	<10	ND	<20	ND	
07/05/2024	A_FF_W1 (retake CPS)	-	-	100	80	-	-	-	-	-	
	B_FF_W1	10	<10	-	-	<20	<10	ND	<20	ND	Y
15/05/2024	B_FF_W1 (retake CPS)	-	-	<10	-	-	-	-	-	-	
10/04/2024	C_FF_W1	<10	<10	-	-	<20	<10	ND	<20	ND	

Appendix 3

Conference Abstracts

MIBTP Symposium – 11th April 2022

Title: Double emulsions, their formation, stability, and utilisation in low-fat low–salt cheese – a review.

Abstract: Seventy three percent of adults in the United Kingdom (UK) consume Cheddar (Mintel, 2020). The health consequences of this are substantial as 100g of Cheddar contains 50% and 25% of an adult's recommended daily intake of total fat and sodium respectively. High consumption of fat has been associated with increased risk of cardiovascular disease (Briggs *et al.,* 2017) and obesity. Consumers expect reduced fat products to have the same characteristics of their full-fat counterparts (Nateghi, 2017), but reduced fat and low-fat cheese typically have poor quality textural characteristics (Lobato-Calleros *et al.,* 2007; Khart *et al.,* 2018).

Double emulsions are mechanisms which have been used in numerous studies to reduce the fat content of products and aim to mimic the full-fat structure. However, double emulsions are thermodynamically unstable and require certain mechanisms to stabilise them. The method of production can influence the stability, along with added ingredients such as surface-active ingredients, thickeners or emulsifiers.

One major challenge, with double emulsions in cheese, is the need for these added ingredients to stabilise the emulsions and be 'dairy grade' meaning they would be accepted in the Dairy industry to allow the cheese to still be called cheese. This review outlines the stability mechanisms in double emulsions, their use in previous cheese studies and the challenges involved.

3rd Annual Harper Adams University Research Conference 2023

Date: 3rd September 2024

Location: Harper Adams University

Presentation type: Oral presentation

Title: Is there a natural alternative to synthetic surfactants in double emulsions? - Double emulsions their formation, stability and utilisation in low-fat low-salt cheese.

Authors: Camilla (Millie) Preece, Dr Paraskevi Paximada, Dr Lynn McIntyre, Dr Helen Pittson and Dr Karim Farag.

Seventy-three percent of adults in the United Kingdom (UK) consume Cheddar (Mintel, 2020). The health consequences of Cheddar Cheese (CC) consumption are substantial, as 100g of Cheddar contains 50% and 25% of an adult's recommended daily intake of total fat and sodium respectively. Consumers want reduced fat products to have the same properties as their full-fat counterparts (Nateghi, 2017), but low-fat and low-salt cheese typically have poor quality textural characteristics resulting in a stiff and rubbery texture (Lobato-Calleros et al., 2007; Kharal et al., 2018).

Double emulsions (DE) are mechanisms that have been used in numerous studies to reduce the fat content of products and aims to mimic the full-fat structure. However, DEs are thermodynamically unstable, and require certain mechanisms to stabilise them. Method of production can influence stability, along with added ingredients such as surfactants, thickeners, or emulsifiers.

A major challenge associated with DE formation is the use of synthetic surfactants, is there a 'natural' alternative available? The current research investigates some of the potential 'natural' alternatives to synthetic surfactants and how this will influence the CC production and the potential to fortify DE with vitamins and minerals.

4th Annual Harper Adams University Research Conference 2024

Date: 3rd September 2024

Location: Harper Adams University

Presentation type: Oral presentation

Figure A – Presentation abstract from the Harper Adams Conference booklet

Oral Presentation

Double emulsion technology in reduced fat cheese, does it do what it says on the "tin"?

Millie Preece[1], Dr Lynn McIntyre[1], Dr Evi Paximada[2], Dr Helen Pittson[1], Dr Karim Farag[1] and Dr Wilatsana Posri[1]

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Funded by Harper Adams University and Midlands Integrated Biosciences Training Partnership (MIBTP)

Diets high in fat are linked to health issues such as cardiovascular disease and obesity. In the UK, 73% of adults consume Cheddar cheese regularly, where 100g contributes 50 % of an adult's recommended daily intake of total fat. Developing reduced-fat products to match the characteristics of their full-fat counterparts is however challenging, as reduced-fat cheeses often suffer from poor textural and functional qualities. The use of double emulsions (DE), particularly water-in-oil-in-water, to enhance sensory and functional properties of low-fat foods has demonstrated promising results. However, Des are thermodynamically unstable and typically require surfactants for stabilisation, with a growing interest in natural alternatives.

This study investigates the application of skimmed milk DEs in reduced-fat Cheddar cheese production, using a combination of natural and synthetic surfactants. Functional tests, including texture analysis and meltability, showed that DE-treated cheeses (13 % fat) could enhance functional attributes compared to the control, while sensory evaluation using a flash profile with 14 panellists indicated that DE treatments were ranked similarly to the control. While DE emulsions improve some of the functional attributes of reduced-fat cheeses, the sensory aspect was not so successful, meaning further research is required to improve this area.

EFFOST International Conference 2024

Conference: EFFOST International conference 2024 – Future Food Systems: Innovation through progress at scientific interfaces. 12 – 14 November 2024, Bruges, Belgium

Presentation type: Poster

Aim: The research investigates natural alternatives for synthetic surfactants in double emulsion (DE) within low-fat (LF) Cheddar cheese (CC), with particular emphasis on replacing Polyglycerol polyrincoleate (PGPR) with sunflower lecithin in the primary emulsion. It aims to assess the effects on both DE formation and incorporation in LF CC, while also evaluating functionality and sensory attributes. This investigation addresses the challenges posed by the poor sensory and functional qualities of low-fat cheese, alongside growing consumer demand for healthier foods and a preference for clean label surfactants.

Method: Primary emulsions were prepared by partially replacing PGPR with Sunflower lecithin at 2% (w/w) in 40 : 60 milk fat to distilled water, using ultrasonic homogenisation at 70% amplitude for 5 minutes. Skimmed milk double emulsions underwent method development using a Silverson High Shear mixer to create a DE suitable for incorporation into CC. Chosen DE parameters were 35:65 (W_1/O:W_2) at 6,000 rpm for 10 minutes. These DE were incorporated into 30 L low-fat CC batches, where the cheese milks were standardised to 1.05% wt. and 3.7% wt. for LF control/DE and full fat control respectively. After four weeks maturation at 12°C in vacuum pack bags, CC underwent nutritional analysis, texture analysis, and functionality tests, as well as sensory evaluation using the free-profiling method.
Results: The results demonstrate that the partial replacement of PGPR with Sunflower Lecithin in the primary emulsion was successful at a ratio of 1.5: 0.5 and 1 : 1 producing $D_{4,3}$ of 3.686 μm and 3.843 μm respectively and stable for utilisation in DE. These were then successfully incorporated into skimmed milk DE, producing droplet sizes ranging from 14.246 μm to 17.980 μm. These DE were suitable for the incorporation into low-fat CC. Sensory evaluation and functionality tests indicated promising outcomes, suggesting that the use of these DE can aid improvement of low-fat cheeses using fewer synthetic surfactants in the primary emulsion.

Conclusion: In conclusion, this study suggests that PGPR can be partially replaced with sunflower lecithin in the primary emulsion, offering a natural alternative for stabilising DE in the production of low-fat Cheddar. The successful incorporation of DE into CC with favourable sensory and functionality attributes opens avenues for further developing CC, with enhanced nutritional profiles by using fortification of water-soluble vitamins through the DE system.