# The development of two types of emulsifier-free ice cream

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# Abstract

The objectives of this study were to seek and evaluate novel, effective methods to produce emulsifier-free ice cream whilst maintain equivalent quality and functionality. Based on literature analysis of fat structuring and partial coalescence mechanisms in ice cream, two hypotheses were proposed for creating equivalent structuring and functionality in ice cream without the need for chemically manufactured emulsifiers. Methodologies for characterising overrun, particle size distribution, meltdown and microscopy tests were developed to determine product properties.

The first hypothesis was based on split stream mixing of non-homogenised cream to homogenised ice cream mixes prior to freezing, with the non-homogenised cream providing a fraction of fat droplets structurally pre-disposed towards partial coalescence. Non-homogenised cream was added to mixes to make up between 2 and 10% of the fat content in ice cream. A positive correlation between the contents of mixed cream and extent of partial coalescence could be observed based on the amount of non-homogenised cream added, along with related changes to ice cream functionality.

The second hypothesis was to replace commercial monoglycerides in the formulation with selected long chain free fatty acids at comparable concentrations, on the basis that naturally derived fatty acids could impart equivalent surface-active functionality to monoglycerides. From the results of particle size distribution, meltdown and microscopy tests, oleic fatty acid provided the closest equivalence to monoglyceride functionality, whilst stearic and palmitic fatty acids were found to be ineffective.

Findings indicate that ice cream microstructures associated with the inclusion of monoglycerides in ice cream formulations can be successfully replicated using naturally derived raw materials with minimal changes to processing.

# Contents

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# 1. Introduction

Producing healthy and nutritionally balanced, natural foods has been one of the key drivers of the food industry (Carocho et al., 2014). For many years ice cream has proven to be a highly popular fast-moving consumer good (FMCG). As part of consumer choice in this area, there already exist many examples of organic and healthy ice creams in the marketplace, for example, variants with low-fat, non-fat and sugar-reduced/free attributes. In addition, ice creams designed for special consumer needs have also appeared in the freezer, such as lactose-free and dairy-free products. As part of broader consumer drivers, there is an increasing desire to manufacture ice creams with a lower additive footprint (Patil & Banerjee, 2017; Rinaldi et al., 2014). In this regard, synthetic emulsifiers, stabilisers, and artificial colours colourants and flavours should be excluded from formulations. However, the technical functionality provided by these additives provides a critical contribution to product quality, based on optimising the microstructure of the ice cream. Emulsifiers, in particular, play an important role in improving texture, enhancing meltdown stability and imparting dryness on extrusion (Huppertz et al., 2012). How to keep a stable and firm microstructure for ice cream without the use of emulsifier additives, such as monoglycerides, is a critical problem in production.

The term “emulsifiers” is generically used for a range of highly functional ingredient and additive components. The terminology is quite broadly applied, but also indicative one of their key roles in food production, that of emulsion stabilisation. Normally immiscible liquid phases of oil and water can be kinetically, homogeneously mixed. In emulsions, one liquid phase will exist as small droplets known as the dispersed phase, which is distributed in a surrounding continuous phase of the other liquid phase (Whitehurst, 2004). The stability of the emulsion is provided by emulsifiers. Ice cream is a particularly complex food colloids comprising an emulsion, a foam and a dispersion of solid ice crystals in a highly concentrated, viscous continuous phase. Milk proteins present in dairy ice creams can act as effective natural emulsifiers providing stability to both emulsion droplets and air bubbles. However, texture and stability (both melt and storage) are known to be greatly improved when small amounts of additive emulsifiers are added to the formulation. Inclusion of emulsifiers is able to improve the structural contribution to ice cream properties. In particular, prior research shows that ice cream without emulsifiers tend to produce ice creams with larger air and less stable bubbles (McClements & Dickinson, 1996). Ice cream texture can be correlated to air bubble size, with reductions in bubble size improving creaminess, and conversely, larger bubbles decreasing creamy texture. Emulsifier-free ice cream formulations also tended to display poorer meltdown properties compared to similar formulations comprising emulsifiers. One of the key research questions within this study is how to replicate the technical functionality provided by additive emulsifiers using natural ingredients. In order to achieve this goal, we need to consider the principles of microstructure formation of ice cream.

The particular ice cream structural characteristics imparted by the inclusion of emulsifiers are based on two key interactions provided by the fat globules. The first of these is the adsorption of

fat droplets onto the surface of the air bubbles, thereby reducing bubble size and improving bubble stability via a Pickering type mechanism compared to protein-stabilised bubbles. The second is the generation of partially coalesced agglomerates of fat in the continuous or matrix phase of the ice cream. These agglomerates improve melt stability by reducing the rate of drainage from the foam as the ice cream melts (Ghosh & Rousseau, 2010; Koxholt et al., 2001). Emulsifiers play vital roles in these mechanisms by replacing proteins from fat globule interface during the manufacture of the ice cream mix. The formation of a mixed protein-emulsifier interfaces decreases the shear stability of fat globules and allows them to stick together during collisions during the freezing process. Internal fat crystals prevent full coalescence into larger droplets, instead, globules coalesce and partially retain their individual integrity to form agglomerated structure. This mechanism does not occur to any great extent when emulsifiers are excluded from the formulation, as fat globules entirely stabilized by milk proteins are considerably more stable under shear (Cheng et al., 2020). Furthermore, protein stabilised emulsions do not show any great propensity to adsorb to the surface of air bubbles during freezing, hence the larger size of bubbles in formulation in the absence of emulsifiers (L. Y. Lee et al., 2018).

How to generate partial coalescence and promote droplet adsorption to the surface of air bubbles without the use of additive emulsifiers is the main research question of this project. Generally, many factors could influence the process of partial coalescence, such as temperature, processing or fatty acid composition of milk fats (Fredrick et al., 2010). All these factors can provide possibilities to enhance partial coalescence from another mechanism rather than emulsifier addition.

In this project, two distinct approaches have been considered. The first of these is the incorporation of varying levels of non-homogenised cream into the formulation, based on the hypothesis that the composition and interfacial properties of non-homogenised cream droplets can provide both structural aspects of partial coalescence and Pickering foam stabilisation during ice cream manufacture, and as previously observed in the preparation of whipped cream. The second approach is the replacement of monoglycerides with naturally derived long chain fatty acids, based on the hypothesis that these fatty acids are able to impart droplet surface properties equivalent to monoglycerides during the manufacture of ice cream. To determine the effectiveness of these two approaches, overrun, particle size distribution, meltdown and microscopy tests will be applied to ice cream.

# 2. Literature review

## 2.1 Ice cream

Ice cream is a very popular product and is loved by food consumers around the world for its cool and smooth sensory properties. It is especially popular in New Zealand, which has the highest ice cream consumption of over 23 litres per capita. From the information of Statistics New Zealand, kiwi families will spend $1 for ice cream in every $44 food consumption ("The New Zealand Ice Cream Industry.," 2019). New Zealand ice cream industry exports about 9000 tons product with values of NZD $41 million to overseas market ("New Zealand Ice Cream Exports and Imports.," 2019).

Different governments have their own specific regulations for ice cream formulation and production. Ice cream production in New Zealand conforms to guidelines which are developed by the New Zealand Ice Cream Manufacturers Association, Ministry for Primary Industries and Ministry of Health. Based on the descriptions from the joint Australia New Zealand Food Standards Code 2.5.6, ice cream is a type of aerated frozen food which is made from cream or dairy products and other foods. The milk fat content should be higher than 100g/kg and food solids should not lower than 168g/L ("Australia New Zealand Food Standards Code," 2015). Specific definitions are applied for other particular types of ice cream, such as low-fat ice cream.

Ice cream is well known for its unique and complex structure which continues to intrigue food researchers. Its structure and composition comprise multiple colloidal states including air bubbles, fat globules, ice crystals as well as proteins, emulsifiers, stabilizers, and sugars. The size of air bubbles are in the range of 20-100 µm (Goff, 2002) and ice crystals vary from 1-50 µm (Cook & Hartel, 2010). Lactose crystals should ideally be absent in the product (Bhandari & Roos, 2012), while the size of fat droplets and clusters can vary according to formulation, being typically as low as 1µm, but being able to form clusters of over 50 µm in size (Hartel, 2019; Patel, 2017). The dispersed phases of fat droplets, air bubbles, ice crystals are surrounded by a concentrated, continuous serum phase, which contains water soluble materials, such as sugars, stabilizers, whey proteins, colourants and flavours. The size of these phases highly influences the quality and shelf life of ice cream.

## 2.2 Ingredients of ice cream

### 2.2.1 Fats

Fats are often considered as the main ingredient in ice cream production that determines overall sensory quality, and which are able to contribute to multiple functional roles. Milk fats naturally provide desirable flavours, act as flavour carriers and provide a smooth texture for the product. Milk fats can influence ice crystal growth by due to low heat conduction rate and optimise the melting behaviour of ice cream by its high melting temperature (Goff & Hartel, 2013). There is nutritional value to be considered as well, providing some fatty acids, energy and acting as a carrier for some fat-soluble vitamins (Tharp & Young, 2012). Arguably, the most important contribution of milk fats on the properties of ice cream is based on their interactions during processing (notably in combination with emulsifiers), such as droplet agglomeration and adsorption to air bubbles. This has significant impact on ice cream structure build-up, appearance, texture and melting-behaviour. The forming of this unique, complex and delicate structure is achieved through a specific manipulation of the fat globule emulsion system.

In natural milks, milk fat exists in a globule state made up of a triglycerides core and a complicated natural membrane that is formed during the secretion process. The composition of milk fat is complex and mainly composed of triacylglycerides, and also contains some free fatty acids, phospholipids and traces of monoglycerides and diglycerides (Table 1) (Jensen et al., 1991).

Table 1. Lipid composition of milk fat globules and membranes (Jensen et al., 1991).

| Fatty Acid | % of Total Fatty Acid Content | Melting Point °C |
|---|---|---|
| butyric | 3.0 – 4.5 | -7.9 |
| caproic | 1.3 – 2.2 | -1.5 |
| caprylic | 0.8 – 2.5 | 16.5 |
| capric | 1.8 – 3.8 | 31.4 |
| lauric | 2.0 – 5.0 | 43.6 |
| myristic | 7.0 – 11.0 | 53.8 |
| palmitic | 25.0 – 29.0 | 62.6 |
| stearic | 3.0 – 7.0 | 69.3 |
| oleic | 30.0 – 40.0 | 14.0 |
| linoleic | 2.0 – 3.0 | -5.0 |
| linolenic | < 1.0 | -5.0 |
| arachidonic | < 1.0 | -49.5 |

Table 2. Fatty acid composition of milk fat (Fee & Chand, 2006).

The fatty acid content in milk fat has a wide melting range of -49.5 to 69.3 °C (Table 2) (Fee & Chand, 2006). When assembled into triglycerides, the primary lipid component in milkfat, the resulting melting range spans approximately -40 to 40 °C (Versteeg et al., 2016). The structure and crystallinity of milk fat droplets contributes to production of various dairy products, such as whipped cream and cheese. The milk fat globule membrane (MFGM) is very effective in dilute systems (such as milk) at keeping fat globules in an independent and stable state, and protecting them from physical destabilization mechanisms, such as coalescence, flocculation, etc., and to a degree from detrimental effects of biochemical destabilisation mechanisms, such as the actions of lipase enzymes. The MFGM is mainly comprised of phospholipids, with smaller amounts of proteins, however, the specific composition varies with the origins of milk (H. Lee et al., 2018; Smoczyński et al., 2012). The natural fat globule membrane is constructed by 1 inner monolayer of protein and phospholipids and outer bilayer of phospholipids (Figure 1) (Jukkola & Rojas, 2017; Michalski & Januel, 2006). The structure of MFGM is fragile against mechanical agitation, and both structure and composition may change during processing, such as heating and homogenization. Such changes to MFGM structure will further influence the properties of fat globules in dairy products processing.

Figure 1. The conformation of the milk fat globule membrane (Michalski & Januel, 2006).

Milkfat remains the primary lipid type used in the manufacture of ice cream (and is a requisite for being able to label ice cream as dairy). Sources of milkfat for ice cream include fresh cream, frozen cream, plastic cream, butter, anhydrous milk fat and ghee. Fresh cream is arguably the best raw material but is often limited by its high cost and high perishability. Frozen cream can improve the storage stability of cream, however, undesirable flavours from rancidity are occasional deficiencies for this material, and the structure and properties of milkfat globules can be impacted during freezing. Butter is sometimes considered as an appropriate fat source for ice cream production. Extending this further, anhydrous milk fat or butter oil is usually the most appropriate source of milkfat, particularly for recombined ice cream formulations. It has low moisture content, less tendency of oxidation, as well as the easiness of handling in production

(Goff & Hartel, 2013). It also has the benefit of containing no milk solids non-fat component, which allows for simpler mix formulation calculations. Although butter and AMF are easy for transportation and processing, their solid properties require pre-heating and melting before further processing during ice cream manufacture. A less common source of milkfat used in western ice cream manufacture, ghee, is a traditional Indian food ingredient and not commonly used in ice cream production, except for some particular personal or product preferences. Because of the fermentation and heating stage during ghee production, its composition is much more complex than pure butter or AMF (Sserunjogi et al., 1998). The formation of particular ingredients may exert some unexpected effects on ice cream structure formation.

Non-dairy fats are also selected to be used in ice cream mix for healthy, cost and functionality considerations (Güven et al., 2018). However, due to their various compositions and consequently different melting ranges, the choice of fats available for application in ice cream production are limited. The various proportions of liquid and solid fats in freezing temperature significantly affect the formation of ice cream structure. Solid fats are necessary to build the "skeleton" of ice cream microstructure by partial coalescence mechanism, while if too much are introduced, ice cream structure will become weak. Too much liquid fat can also compromise ice cream structure formation by covering air bubbles leading to antifoam effects and collapse of air bubbles. Excess liquid fat can decrease the rate of crystallization and reduce the amounts of crystals piercing into adjacent fat globules, thereby reduce the extent of partial coalescence (Sung & Goff, 2010). Accordingly, the three main non-dairy fats used in ice cream manufacture are coconut, palm kernel (both lauric fats) and palm oil.

It is worth also considering the utilisation and role of fat replacers, that are becoming increasingly applied in low-fat ice cream formulations for health concerns. In low-fat ice cream, how to supplement the functionality of fat when the source is in shortage is the purpose of fat replacers' application. There are various types of fat replacers available to achieve different purposes, for example, emulsifiers and gums can enhance fat destabilization, methylcellulose can simulate mouth feelings with fat by increasing creaminess and lubricating properties, and poly-dextrose can increase viscosities of solution (Akbari et al., 2019; Baer et al., 1999; Veena et al., 2016).

### 2.2.2 Milk solids-not-fat (MSNF)

Milk solids-not-fat (MSNF) contains milk proteins, lactose and minerals. These contribute to textural quality, higher overrun, etc, as well as contributing to sensory properties by imparting dairy notes to the product. Among all available commercial sources, skim milk powder is the most widely used in ice cream production, being considered the highest quality source of MSNF. Some producers also choose to use other MSNF materials to fully or partly replace skim milk powder for cost or functional considerations (Hui & Sherkat, 2005).

From a technical perspective, milk protein is the main surface-active component in ice cream responsible for its emulsifying and foaming capacity. There are two types of milk proteins, caseins and whey proteins. Casein account for 80% total protein content in bovine milk and the rest are whey proteins (Głąb & Boratyński, 2017). Caseins are insoluble at pH 4.6 and normally exist as self-assembled colloidal particles termed micelles, whilst whey proteins are fully soluble at pH 4.6 and show some limited quaternary self-association. The interior structure of the micelle comprises predominantly α-casein and β-caseins interacting together through hydrophobic associations and through formation of electrostatic linkages with colloidal calcium phosphate. The κ-casein fraction tends to be concentrated at the surface of the micelle. Some parts of κ-casein molecules are able to interact with the interior caseins inside with the more hydrophilic parts protrude outside and in contact with the serum. κ-caseins located on the surface are negatively charged and provide stability to the micelle through a combination of electrostatic and steric repulsion.

The whey proteins are notable for being soluble in the serum solution, even at their isoelectric point. They have high nutritional values, especially α-lactalbumin (which is predominant in maternal milk). The primary milk sugar, lactose, can contribute to some extent of sweetness, but nowhere near as effective as sucrose or corn syrup in this capacity. The main defect of lactose in ice cream is its crystallization, which imparts sandiness to product (Nickerson, 1962), although this is an unusual occurrence within modern ice cream manufacturing. Another issue relating to lactose, is lactose intolerance, which is increasingly a consideration in product design. Finally, the minerals in MSNF are general at low concentrations; these can slightly affect the product flavour but do have some impact on freezing point depression.

In commercial production, a wide range of sources of MSNF can be chosen to suit different purposes. Skim milk powder can be regarded as the most traditional source for MSNF. It provides excellent sensory properties, is easy to handle and store due to its fine and powdery state. That said, there is some risk of protein oxidization and caking (particularly for longer stored material) (Fitzpatrick et al., 2010; Scheidegger et al., 2013). Plain concentrated skim milk is another traditional choice for its freshness, desirable flavour, easy handling, but limited for its high perishability. Whey powders can also be selected in the ice cream mix. These have traditionally been considered a lower cost ingredient compared to SMP, however, high lactose contents in some of these products can raise some concerns, as well as imparting some undesirable flavours at high concentrations, so these are rarely used in isolation in ice cream formulations (Goff & Hartel, 2013).

### 2.2.3 Sweeteners

Sweeteners are a very important ingredient component in ice cream, imparting obvious characteristics of sweetness, but also having an essential functional contribution on the

properties of the ice cream. Generally, the dose in standard ice cream mixes is 12-16% on the basis of total solids of 28-40%. Besides enhancing the palatability of the product, sweeteners can also enhance texture and body, extend shelf-life, and increase the solid content of ice cream. The most important technical function is decreasing the freezing point of ice cream, which ultimately determines the ice content in the product at -18°C storage environment. This provides a mechanism for controlling the overall firmness of an ice cream and can be used to give the ice cream good scooping properties (C. Clarke, 2015).

The most popular sweetener combination in ice cream is the mixture of sugar and corn syrup(s), typically in proportions of 9-12% and 4-6% respectively. This percentage chosen is to achieve the balance among total solids, sweetness and freezing point (McSweeney & O'Mahony, 2015). Powdered sucrose from sugar beets or sugarcane is common, with glucose syrups often in a viscous liquid form. As a rule of thumb (and depending on sugar type), every 1 % increase in the concentration of sugar will cause a 0.1 °C freezing point depression in the ice cream mix. Another important impact of sugar is the control of ice crystal size. Ice crystal size will decrease by 25% when sugar content rises from 12 to 18% (Hagiwara & Hartel, 1996).

High fructose corn syrups are also increasingly used these days and can be used at lower solid levels whilst imparting higher sweetness than sugar. These can improve flavours, provide good meltdown properties and firmer texture. It can also help to protect from heat shock effects, shrinkage defects and extend the shelf life. Corn syrups (both glucose and high fructose) are made from corn starch by enzymatic techniques which hydrolyse the starch into glucose (via amylase enzymes), followed by isomerisation to fructose through used of a second enzyme (glucose isomerase). In order to monitor the extent of hydrolysis, we use dextrose equivalent (DE) as the parameter for assessment. Different extents of hydrolysis will result in products with various DE values (M. A. Clarke, 2003). Products with higher DE values will have higher sweetness but lower strength of decreasing freezing point and achieving higher viscosity and firmer texture. Therefore, to achieve a syrup with an optimum balance of all these properties is of importance in hydrolysis. Ice cream producers usually choose syrups with 28-42 DE (Bass et al., 2017).

Finally, it is interesting to note that low sugar products have started to gain a larger share of the ice cream market in recent years. Sugar alcohols are frequently chosen in low sugar products. These non-nutritive sweeteners can provide low calories and are liked by diabetics and dieters alike (Asghar et al., 2013). However, while sugar alcohols can provide good freezing point depression and effective sweetening in their own right, they are associated with some digestive issues, so there some limitation in how much of these materials can be used in formulations.

### 2.2.4 Stabilizers

Most of the stabilizers used in ice cream processing are hydrocolloids. Hydrocolloids are a group of high molecular weight macromolecules that contain large amounts of hydroxyl groups within

their structure, and thus possess hydrophilic properties. This means they have a good affinity with water and water structuring capacity. During hydration, they can bind water and swell to occupy a large volume in solution. The most important function of hydrocolloids is water-binding capacity, and thus small concentrations can significantly increase the viscosity of solutions (Saha & Bhattacharya, 2010), which can enhance mouthfeel and slow down rate of melt. Arguably, however, the most important role of stabilizers in ice cream is to maintain quality during storage, particularly during fluctuations in temperature.

The shelf-life of ice cream is a topic which needs some further context when considering the particular role of stabilizers. Shelf-life encompasses many aspects, such as textural, body, microbiological, compositional changes. As ice cream is normally kept in the frozen environment, the microbial activity has already been limited to a large extent. Accordingly, the most important factor of the shelf-life of ice cream is physicochemical changes that alter quality over time. The most common factor affecting quality during commercial ice cream storage is iciness as a result of temperature fluctuation (Goff & Hartel, 2013).

In considering the microstructure of ice cream, ice crystals and air cells are the two elements that are most prone to change during storage. Growth of both of these during storage is associated with a loss of quality, manifested through behaviours such as coarse texture, increased iciness and coldness, and at extreme level in defects, such as shrinkage. In addition to such defects, lactose crystallization, gumminess, flavour changes can also take place during the storage of ice cream. Stabilizers can help mitigate against many of these defects. Arguably, the most important function of stabilizer in ice cream is to provide its smooth property by controlling ice recrystallization process. Ice recrystallization is the most vital factor to consider for ice cream storage. It is induced by temperature fluctuations during processing, storage, delivery or consumption stage. With changes in temperature, some ice crystals may melt and deposit on the existing surrounding ice crystals. Thus, small ice crystals fuse into bigger crystals without any extra re-nucleation. The control of these defects can be achieved by many ways, such as fast freezing to produce large amounts of tiny ice crystals in the initial stage, low-temperature storage, optimize composition and stabilizer is a very effective and direct way among all of these approaches (Jeremiah, 1996).

The mechanism of stabilizers in controlling ice recrystallization is still obscure. One proposed mechanism is the capacity of increasing viscosity of ice cream mix and the unfrozen phase. Because of the good water-binding and water-holding capacity, the fully swelled stabilizer molecules will attach large amounts of water molecules instead of staying in an unbound state. These attached water molecules will be difficult to diffuse and participate in the ice re-crystallization process. As the viscosity of mix increases (noting that freeze concentration will lead to a significant contribution to unfrozen matrix viscosity when stabiliser is present), the mobility of molecules will be further limited. All these factors can contribute to limiting ice re-crystallization process (Miller-Livney & Hartel, 1997).

As noted earlier, stabilizers can also have beneficial effects at other stages of ice cream production. These include stabilizing ice cream mixes during the preparation and freezing stage, improving whippability, and imparting good resistance to melting in the final product. Importantly, stabilizers do not contribute to taste, flavour or appearance at typical dosage levels. Usually 0.1-0.5% is the dosage level of stabilisers applied in the ice cream mixes. If an excessive amount is introduced in ice cream mix, some undesirable results will be triggered, such as excessive viscosity, chewiness, gumminess and slow melt-down rates, as well as some off flavours associated with gums such as guar (Bahramparvar & Mazaheri Tehrani, 2011).

The most common stabilisers are polysaccharide materials, such as alginate, carrageenan, guar gum, locust bean gum and xanthan gum. Of these, locust bean gum and guar are almost universally used in the ice cream industry, noting that a combination of these two is generally considered most effective in retarding ice recrystallization. One particular issue in the use of these stabilizers is the undesirable wheying-off phenomenon, which is induced by the incompatibility with protein in the mix. As a result of the dissolution competition between stabilizers and proteins, some of the proteins, especially casein micelles will phase separate, leading to destabilisation of mixes during storage, and release of clear serum during melting. Carrageenan is a very effective stabilizer to retard such defect by binding with proteins from detrimental effects by other stabilizers. In many commercial formulations, carrageenan is normally used as a complementary stabilizer in ice cream mix to have a synergistic effect with other stabilizers (Syed et al., 2018). Nowadays, some novel stabilizers have been discovered, such ice structuring proteins and hydrophobins, and which can have very good ice re-crystallization control properties and be very complimentary with traditional stabilizers to produce smooth and uniform product (Aleong et al., 2008).

### 1.2.5 Emulsifiers

The main function of emulsifiers is to generate an even dispersion of two immiscible phases. Generally, two immiscible phases will exist in two separate layers, while emulsifiers can increase the contacting surface area of the two phases by generating tiny spheres of one phase dispersing into the other phase. The two most simple emulsion forms are oil-in-water (O/W) and water-in-oil (W/O) emulsions. Ice cream itself is an example of an O/W emulsion. Emulsifiers are amphiphilic molecules which possess both hydrophobic and hydrophilic parts. The dairy system contains a variety of natural emulsifiers, such as casein micelles, whey proteins and phospholipids. They can participate in the stabilization of both fat globules and air bubbles. However, it is well known that ice cream quality can be improved through partial destabilisation of the protein stabilised ice cream emulsion during freezing, through a mechanism termed partial coalescence.

This is one of the main structuring pathways that can be used to modify ice cream quality through manipulation of the product's microstructure. In order to achieve appropriate levels of partial coalescence, synthetic emulsifiers (i.e. those listed as food additives) are used. Partial

coalescence of fat globules normally happens under shearing and the main sign of occurrence is the breakage of the globule membrane. The membrane formed by protein adsorption during homogenisation gives globules high stability during freezing. This protein layer is partly displaced through the addition of synthetic emulsifiers, such as monoglycerides, rendering it fragile and prone to rupture and fusion through collision with other droplets under the high shearing conditions in the ice cream freezer (Goff, 1997b). Partial coalescence can optimise certain properties of ice cream, such as whipping qualities, meltdown characteristics, the dryness, smoothness and stiffness of texture. In production, emulsifiers start to affect the properties of ice cream from the ageing step until the end of shelf-life. A second mechanism associated with addition of emulsifiers is the tendency of fat droplets to adsorb to the surface of air bubbles during freezing, providing a Pickering type stabilising effect to the foam. This effect has the ability to make air bubbles smaller (thus enhancing the creamy perception of the ice cream) as well as greatly enhancing the stability of the air phase during storage, which helps maintain quality more effectively (Goff & Hartel, 2013).

In commercial production, the types of emulsifiers normally used to displace protein are small molecule emulsifiers derived from lipids. These are made by esterification of specific fatty acids and glycerol or sorbitol or another appropriate hydrophilic moiety. The sites of attachment can be controlled by adjusting conditions. The two main types of emulsifiers in ice cream production are the monoglycerides and polysorbates (C. Clarke, 2015). They both use fatty acids as fat-loving or hydrophobic parts. The different types, numbers and positions of fatty acids in triglycerides can generate different extents of emulsifying properties. For example, diglycerides which are also produced during monoglyceride manufacture, only contains one hydrophilic –OH group, and behave more like triglycerides, possessing little in the way of surface active properties (Hasenhuettl & Hartel, 2008). The glyceride product normally contains 40 – 55% monoglyceride, and rest is diglyceride. Therefore, this product is often named as MDG, the mixture of mono- and diglycerides. Monoglyceride content in distilled monoglyceride can reach to over 90%, but the cost is higher, and the high concentration will cause a hard dispersion in mixing. It is also worth noting that unsaturated monoglycerides can give ice cream higher frozen stability than the saturated, and that often combinations of emulsifiers can provide synergistic benefits, such as the mixture of saturated monoglycerides and polysorbate 80 (L. Y. Lee et al., 2018). Food emulsifiers can be liquid, powders, or flakes. The different physical states are mainly due to the properties of the attached fatty acids. Long-chain and saturated fatty acids attached will generate emulsifiers in the solid-state. They can be sprayed into powders and mix with other ingredients to make ice cream powder (Goff & Hartel, 2013; Whitehurst, 2004).

Polysorbates are water soluble and known to be highly effective at displacing proteins at the oil-water interface, which allows them to be used at relatively low concentrations. The typical dosage levels of monoglycerides is around 0.2-0.5% (usually determined by the amount of fat in the product), while the equivalent polysorbate concentration is 0.04 - 0.07% (Dar & Light, 2014). The two common types of polysorbates used in ice cream production are PS 80 and PS 65. PS 80 is more effective and widely used. As described above, polysorbates are often mixed with MDG

and the ratio is 20/80. Polysorbate use and dosage levels in ice cream is often more tightly regulated than monoglyceride use and is not permitted at all in certain regions.

Other synthetic emulsifiers contain polyglycerol esters, sucrose esters, propylene glycol esters (PGMS) and ethoxylated mono- and diglycerides. They are less commonly used in ice cream production. Nowadays, both pure emulsifiers like glycerides or mixed blends are provided in the market to achieve more stable and controllable emulsifying functions. For New Zealand manufactured ice creams, it is increasingly common to see a combination of monoglyceride and PGMS on ingredient listings.

The application of natural emulsifiers in ice cream is increasingly attracting food researchers', manufacturers' and consumers' interest. This contributes to a wider trend directed towards the increasing demand for additive-free foods. Additive-listed emulsifiers often perceived as synthetic or chemical. Although the emulsifiers account for a very small amount in ice cream mix, they are still unacceptable in terms "all-natural foods" perception. Natural emulsifiers are classified into 4 categories, which are proteins, polysaccharides, phospholipids and miscellaneous natural surface active compounds such as saponins (Ozturk & McClements, 2016). Proteins can be sensitive to the environment and susceptible to pH, salt levels, temperatures and protease activity. Polysaccharides are generally regarded as stabilizers and those emulsifying functions tend to be either exudate gums that are naturally cross-linked with proteins (e.g. gum Arabic, which is generally only used for compositions with low oil phase volumes), or gums have been chemically cross-linked with hydrophobic groups, such as hydroxypropyl cellulose or propylene glycol alginate. Phospholipids are widely used natural emulsifiers, and commonly termed as lecithin in the context of food manufacturing. However, in ice cream production, phospholipids are unable to provide equivalent surface properties and functionality when compared to synthetic emulsifiers, although they are occasionally used in high fat premium products (Figure 2). Their surface behaviour tends to be quite complex, and they can even exist together at the interface with adsorbed caseins (Dalgleish, 1997).

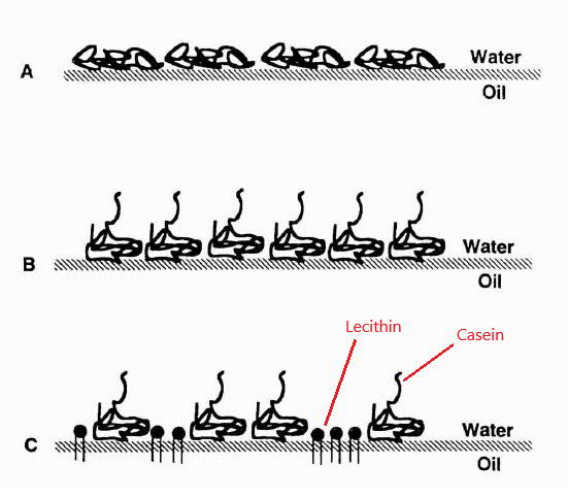

Figure 2. A. Caseins at the W/O interface in low concentration. B. A. Caseins at the W/O interface in high concentration. C. The co-existence of caseins and lecithin at the W/O interface (Dalgleish, 1997).

In general terms of ice cream formulation, the main functions of macromolecular emulsifiers, such as proteins are emulsion forming and stabilization. The role of lipid-derived emulsifiers is displacement and destabilization. Up to now, there is no definitive alternative to synthetic emulsifiers that provides equivalent functionality, noting that monoglycerides have been used in ice cream since the 1940s, and continue to provide effective performance. However, food clean-label ice cream remains a goal of many manufacturers. The challenge in resolving this issue is to replace the role of synthetic emulsifiers in microstructure formation of ice cream. In order words, it is how to promote and enhance the partial coalescence mechanism of fat globules in ice cream without emulsifiers.

## 2.3 Ice cream processing

The production of ice cream is both simple and complex. The simple part is that all the important structural changes happen at one stage, i.e. the freezing process itself; however, these structural changes are by nature complex in their dynamics and reliant of additional processing operations (which can be divided into 4 stages, ingredients mixing, ageing, freezing and hardening to be appropriately achieved) (Goff, 1997a). The three key phase transitions that take place during freezing are ice crystallization and foam formation and stabilisation, and fat agglomeration, all of which impact on the final properties and quality of the ice cream. A more detailed consideration of the overall manufacturing process follows.

Mix preparation process contains 3 steps, which are mixing, pasteurization and homogenization. At first, all ingredients will be mixed up at least 50 °C to make sure all completely dissolved (and to ensure that fats are fully molten). The combined aqueous and oil phases form a crude emulsion pre-mix, which will be pasteurized at specific temperatures. The temperature should be related to the type of pasteurization method of the batch, high-temperature short-time (HTST), Higher-Heat Shorter-Time (HHST) or ultra-high-temperature (UHT) (Goff & Hartel, 2013). The main purpose of pasteurization is to inactive pathogens. Besides this, whey proteins can also be denatured to enhance the water-holding capacity and emulsifying properties. The hydration of sweeteners and minerals will be improved. After pasteurization, the ice cream mix will be homogenised in two stages, to generate smaller fat globules. Some ice cream production methods start homogenization before pasteurization. In homogenization, the size of large raw fat globules decreases rapidly, and surface area increase dramatically at the same time. To complement the newly formed surface area, milk proteins in the serum phase will adsorb onto the fat globule membrane to stabilize the new fat globules (Figure 3). All these changes happen in a short transition time, which is only 10 – 15 microseconds (TetraPak, 2019). On cooling post-homogenisation, any added synthetic emulsifiers will begin to compete with milk proteins at the

surface of fat droplets, based on the fact that small molecule emulsifiers tend to possess greater surface activity relative to proteins (Figure 4).

Figure 3. The changes in the composition of milk fat interface after homogenization (Michalski & Januel, 2006).

Figure 4. The replacement of milk proteins by emulsifiers at fat globule interface (Goff, n.d.).

Emulsifier displacement of surface proteins proceeds during ageing of the ice cream mix at 4 °C for 4 – 24 hrs. Partial removal of the protein layer causes droplets to become more prone to break and cause destabilization during the subsequent freezing step of the process (Goff, 1997a). Aging also promotes fat crystallisation, noting that solid fat droplets are key for making a stable product. Additionally, stabilizers and milk proteins in the serum phase will continue to hydrate in the ageing stage. After this, the ice cream mix will be pumped into the freezer for a concomitant whipping and freezing. The ice cream freezer is essentially a scraped surface heat exchanger. During freezing, mechanical agitation will cause fat globules to partially coalesce with each other to form clusters. This is one of the most important microstructural aspects of ice cream and

sufficient amounts of such structure can only be achieved by conducting whipping and freezing at the same time (Koxholt et al., 2001). During the freezing process, water in the serum phase also starts to crystallize and all ingredients in the continuous phase will be concentrated. About 50% of the water has been frozen in this stage. This causes a thickening of the ice cream mix that helps trap air bubbles incorporated as part of foaming. After the freezing process, ice cream is extruded from the freezer at about -4 to -6 °C, which allow it to be pumped and shaped into containers prior to hardening. The hardening process is operated at temperatures between -30 °C -40 °C to fix the ice cream structure as quickly as possible before returning to storage and distribution temperatures of -18 °C at which point about 85% of the water will be frozen (Tharp & Young, 2012).

## 2.4 Ice cream structures and properties of ingredients

For consumers, ice cream is best characterised by its cold, smooth and creamy mouthfeel; however, from the food researcher's point of view, its microstructural complexity and the synergistic interactions imposed by different ingredients are of particular fascination. Structurally, ice cream can be described as a complex colloid, comprising separate phases of partially coalesced fat, ice crystals, air bubbles and freeze concentrated serum phase, noting that all these components are formed simultaneously in production, and are maintained during the shelf life of the product (C. Clarke, 2015). Ingredients like fats, milk proteins, sugar, emulsifiers and stabilizers all have their own functional effects and participate in the structural formation during the low-temperature processing. Different formulation designs can be used to achieve the optimum properties of ice cream, as well as giving different sensory experiences for consumers. Nowadays, many novel ice cream formulations are developed to meet different consumer demands, such as non-dairy (vegan), lactose free, as well as low fat and low sugar compositions. All of the above factors require a deep understanding of microstructure formation and interactions of ingredients in ice cream, and particularly those ingredients with specific contribution to the structural elements of fat partial coalescence, ice crystallisation, air bubbles stabilisation and the composition of the freeze-concentrated serum phase (Adapa et al., 2000; Pei & Schmidt, 2010).

### 2.4.1 Partial coalescence

Partial coalescence is a very important and specific type of colloidal destabilisation and has already been proved to be one of the main fat structuring mechanism in ice cream (Méndez-Velasco & Goff, 2012a), providing product benefits such as dryness on extrusion and improved meltdown stability. Beyond ice cream, the partial coalescence mechanism is an important

structuring component in the production of other dairy products, such as whipped cream, and butter (Rybak, 2016).

Emulsions are colloidal materials based on the immiscibility of the separate oil and water phases, For mixtures of oil and water, thermodynamic equilibrium is represented as full phase separation into distinct layers, which represent the minimum free energy and the lowest surface area of the system (Goff, 1997b; Truong et al., 2016). Emulsions, in which one material state is dispersed in the other, are kinetically rather than thermodynamically stable. Consequently, destabilisation of emulsions starts immediately after formation and can only be slowed down rather than stopped completely. For many of emulsions, dispersed fat globules tend to coalesce each other, form larger globules and rise to the top resulting of phase separation. Phase separation can happen in raw milk and is typically called creaming (Walstra et al., 2005). Fat globule coalescence in milk may be exacerbated when the globules' membrane is ruptured by air input or whipping actions. Additional colloidal destabilisation mechanisms include flocculation, and Ostwald ripening. While Ostwald ripening of emulsion droplets in ice cream is not generally considered important, it can be a causative effect of ice crystal growth during storage (Fox, 1983; Walstra et al., 2005).

Partial coalescence is a particular manifestation of the coalescence mechanism and which occurs in emulsions comprising partially crystalline droplets. During cooling, tri-acyl-glycerides of particular saturated fats (such as butter, coconut and palm) will start to crystallise. While this can occur throughout a droplet, crystals located at the droplets surface can be radial or tangential to the interface. As crystals form at the o/w interface and parts of membrane at which emulsifiers are preferentially located are relatively thin and poorly stabilised. As a consequence, crystals will protrude through the membrane itself. Fat crystallisation enables fusion of droplets, but also serve to inhibit full droplet coalescence, in that solid fat within the droplets themselves prevents coalescing droplets from fully flowing together. Therefore, the globules will stay in a condition of partial coalescence and form irregular aggregates (Walstra et al., 2005). When temperature rises, the fat crystals inside the irregular aggregates will melt to liquid phase and the true coalescence between fat globules will happen. The interesting part of partial coalesced fat aggregates is, the integrity of globules will be partially kept, whilst any oil phase can flow between droplets. The protruding crystals can also pierce into adjacent globules to enhance the partial coalescence mechanism. Some liquid lipids may exude out of the membrane to allow wetting between fat globules, which can also contribute to the partial coalescence (Fox, 1983). Partial coalescence of fat globules will proceed continuously and form an extensive network eventually to support the overall structure and entrap other ingredients to give the ice cream good physical properties and desirable texture and appearance.

Partial coalescence is a very interesting mechanism and plays vital roles in forming structures of many food products. However, the microstructure formation in practice varies with different types of foods, such as whipped cream, ice cream and butter, particularly in regard to fat content. Both butter and whipped cream have higher fat contents than ice cream. Butter has the highest fat level, comprising typically 80% of the product, whilst whipped cream usually has fat contents

between 30 and 40% of the formulation. The structure formation of butter is mainly based on churning process of non-homogenised high fat cream. Generally, when liquid fat globules get close to air bubbles, the membrane of fat globule will merge with the o/w interface and the internal liquid oil will spread over air bubbles. Liquid oil will cause the local thinning of bubbles and they will break down eventually. This causes the coalescence of fat globules, as well as acting as an antifoam. However, in a freezing barrel, globules will become partially crystallised, and the oil spreading will minimise. Instead, crystallised globules will keep attaching to air bubbles by crystals piercing and wetting the air-water interface, and mechanical agitation can enhance such actions by both incorporating air bubbles and driving fat globules to the surface of these. The fat coverage of air bubbles will cause large amounts of aggregation among crystallised fat globules, which is called the flotation mechanism (Figure 5). An increasing number of large fat granules will form and cause phase inversion eventually. Further working stages are still needed to squeeze out moisture content. The cooling conditions are very important in this process, for if excessive liquid fat exists, flotation will not happen and tiny fat droplets will form after air bubbles breakdown (Walstra et al., 2005).

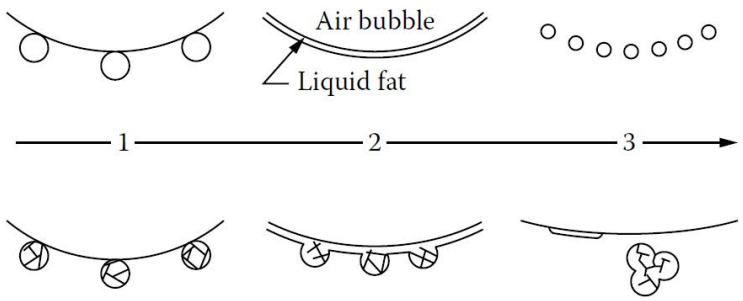


Figure 5. The changes happen at the air bubble surface during butter churning (Walstra et al., 2005).

Whipped cream structure formation starts from foaming in which bubbles are stabilised by milk proteins. During the whipping process, air bubbles will initially be covered by caseins and small amounts of whey proteins. As whipping continues, fat globule membrane will be disrupted, and the internal hydrophobic crystalline fat will be exposed. At the same time, liquid oil can also exude out of the ruptured globules and assist globules to stick onto the air bubble interface. Fat globules will increasingly cover the air bubble interface and this mechanism can also accelerate partial coalescence, with fat aggregates forming an extensive network. Such a network can trap air bubbles and provide mechanical stability for the whole structure of whipped cream. However, excessive whipping will cause the butter granules forming and lower the strength of whipped cream(Brooker, 1993; Rybak, 2016). Normally in frozen desserts, the proper degree of fat destabilization should be in the range of 2.6 -55.3% and the value in average is 21.9% (Hartel et al., 2017).

In ice cream production, because of its lower fat content, fat globules will not form a percolating network as in whipped cream, tending to form localised agglomerates dispersed within the serum

phase. However, extensive fat globule adsorption onto bubble surfaces is observed, providing good stabilisation. Formed fat aggregates will act as a bridge to connect air bubbles together, which is necessary for good microstructural properties (Figure 6). Fat aggregation formation happens in the concomitant whipping and freezing process. (Norn, 2015).

Figure 6. The stabilised air bubble structure of ice cream. FG: fat globules; WP: whey protein; FA: fat globule agglomerate; CM: casein micelles; CSM: casein submicelle; **β**-C: **β**-casein (Koxholt et al., 2001).

The fat aggregates formed by partial coalescence mechanism play vital roles in building ice cream structures and good properties. As stated earlier, they contribute to dryness on extrusion, which contributes to shaping or moulding and good meltdown properties for both consumption and storage. Fat agglomeration and the formation of small stable bubbles due to fat adsorption can also provide a smooth texture and good creaminess for ice cream. Therefore, the extent of fat destabilisation needs to be controlled precisely. Insufficient fat destabilisation will cause poor shaping and quick melting properties; excessive fat destabilisation due to over quantity of surfactants added or strong shearing will result of butter granules formation, which will affect sensory properties and make ice cream very difficult to melt or crack into large pieces during meltdown process (Fox & McSweeney, 2006). The exact behaviour can vary according to emulsifier type.

### 2.4.2 Factors affecting the extent of partial coalescence

Generally, there are various compositional and processing factors that may affect the extent of partial coalescence. With this knowledge, we can pre-design the formulation and processing conditions to target a desirable extent of partial coalescence. The factors affecting the rate and the extent of partial coalescence are based on two aspects: the encounter frequency and capture efficiency of fat globules in collisions (Fredrick et al., 2010). The encounter frequency defines the possibility of the fat globules to get close enough and collide, and the capture efficiency is the probability for the fat globules to stick together and form aggregates as a consequence of these collisions.

#### *2.4.2.1 Fat globule membrane composition*

In milk, fat globules are naturally stable, their exterior membranes, which are constructed by 1 inner monolayer of protein and phospholipids and outer bilayer of phospholipids provide their stable properties. This membrane can keep milk globule in a very stable state and protect it from destabilisation during the lifetime of the milk. After homogenization, the size of milk fat globules is dramatically decreased, typically by an order of magnitude, thereby increasing to total fat surface area. The native milk fat globule membrane constituents are not enough to fully cover this increase in droplet surface area, so milk proteins in the serum phase provide stabilisation to the droplets. Both caseins and whey proteins can adsorb onto the naked globule membrane formed during homogenisation. Research shows that caseins make up the predominant protein group of the newly formed membrane (Cano-Ruiz & Richter, 1997). This may be due to high amphiphilicity of the caseins, which can provide excellent emulsifying properties. Generally, during interacting with fat globules, casein micelles will have some changes in conformation, their interior hydrophobic parts will spread and contact with fat globules, while the hydrophilic parts will protrude into the continuous phase (Damodaran & Paraf, 1997).

The surface layer formed by an excessive amount of whey protein can provide higher emulsion stability than the caseins (Pelan et al., 1997). When whey proteins are applied in limited quantity, the fat globule membrane will be more prone to partial coalescence than caseins. After heating treatment, the emulsifying properties of whey proteins can be highly improved. This phenomenon is mainly due to the denaturation of whey proteins with heating. The hydrophobic parts of whey protein are curly and normally wrapped inside. Heating will make the structure to unfold and become exposed. This phenomenon is most apparent when the heating treatment is conducted before the emulsification. Research shows that the time for whipping UHT cream is 40% longer than the raw or pasteurised cream (Goff, 1997b).

Generally, protein adsorption will provide higher stability for homogenised emulsions relative to non-homogenised fat droplets with intact MFGM. There are two reasons for this issue, the first is the higher thickness of membrane and the second is the static and steric repulsions provided by proteins. Pasteurisation process in dairy production can enhance the emulsifying property of whey protein and increase the emulsion stability, while little effects on caseins where this is done prior to homogenisation (Sarkar & Singh, 2016).

Highly stable droplets do not have a lot of functionality during ice cream manufacture, and as such protein stabilised emulsions tend to melt faster and have poorer sensory quality than those comprising emulsifiers (noting that this observation is highly dependent on the actual amount of fat in the formulation. In order to promote partial coalescence mechanism, small molecular emulsifiers are widely used in food production. Emulsifiers primarily promulgate protein displacement but can also influence the crystallinity of the droplets. There are two theories relating to the mechanism by which protein is displaced by emulsifiers. First is the existence of an interfacial tension gradient between proteins and emulsifiers at the oil-water interface.

Research shows that for specified concentrations typical of the ice cream system, milk proteins can reduce the interfacial tension from 8.26 dynes/cm to 5.5 dynes/cm, while emulsifiers can achieve an even lower value of 2.24 dynes/cm (Goff, 1997c), thereby leading to preferential adsorption of the emulsifier. Another theory is surfactants will accumulate at the vacant spots in the surface of the protein membrane and exert a pressure on proteins, pushing them into the serum phase. Then surfactants will occupy on the fat globule surface. No matter in which theory, the protein replacement by emulsifiers leads to a decrease in protein surface coverage. For example, protein surface loading assessments have determined that the protein loading decreases from 15.9% to 7.8% with as a consequence of polysorbate 80 addition (Fredrick et al., 2010; Goff, 1997c).

The relative ability of displacing proteins varies according to both protein and emulsifier type. Caseins are arguable more readily displaced from the interface compared to whey proteins. The membrane formed by whey proteins are firmer and more thoroughly covered, which make such membrane difficult be displaced by emulsifiers. Therefore, the ratio of caseins and whey proteins in ice cream mix should be carefully controlled, particularly if whey is being used to supplement a formulation (Hasenhuettl & Hartel, 2008).

The second function of emulsifiers is to influence fat crystallisation. Some emulsifiers can change the contact angle of fat crystals to improve protrusion efficiency. This can alter the wettability of the crystals exposed to the aqueous phase as well as modifying the protrusion distance of fat crystals at the interface. Some oil-soluble surfactants can also affect the crystallisation behaviour of fats, such as nucleation, crystal growth and polymorphic transitions. Excessive amounts of emulsifiers can cause the excessive fat destabilisation, in which coalescence happens more instead of partial coalescence. This will have a large detrimental effect on properties of ice cream Generally in ice cream, the optimum emulsifier use is in the range of 0.1-0.2% (Fredrick et al., 2010).

In addition, as introduced in the section above, the degree of saturation of emulsifiers may also have large effects on the degree of partial coalescence. For instance, unsaturated monoglyceride can help to promote fat destabilisation and form large fat networks. This is mainly due to its promotion of large fat crystal formation and changes on the conformation of fat globules, which turns spherical shape into platelet structure. The relative freezing stability of emulsions is therefore very emulsifier specific, noting that high concentrations of saturated monoglycerides can actually re-stabilise emulsion droplets during freezing. The main reason is the interface formed by saturated monoglyceride becomes increasingly crystalline and mechanically stable at higher surface coverages, which may decrease the potential of fat destabilization (Méndez-Velasco & Goff, 2012b).

#### *2.4.2.2 Composition effects – solid fat content*

As one of the main components of the partial coalescence mechanism, the characteristics of the fat source are very important. Notably, the relative amount of solid fat in emulsion should be sufficient to induce and maintain partial coalescence. As described above, fat crystals formed in fat globules play dominant roles in partial coalescence. They help fat globules connect together and form an expansive network providing series of desirable properties to dairy frozen desserts. Research shows that the rate of PC formation can be largely increased by a factor of $10^6$ due to the presence of fat crystals (Walstra, 2002). As freezing stage is the main part for most of the partial coalescence happening, sufficient fat crystals should also form in emulsion at this stage. Research shows that fat crystallisation will reach the maximum level after 4-5 hours of ageing (Goff & Hartel, 2013). For milk fat globules, they naturally contain a certain amount of saturated triacylglycerol molecules and will become partially crystallised in chilling conditions. Generally, solid fat content in ice cream formulation should accounts for between 1/3 to 2/3 to generate a sufficient degree of partial coalesced structure (Sung & Goff, 2010). Excessive saturated fat in the fat source is also undesirable because insufficient liquid oil is available to stick the fat aggregates together.

In seeking to make ice cream products healthier, there has been recent research relating to the replacement of the dairy fat with more unsaturated plant-based alternatives (e.g. sunflower oil), or by increasing the polyunsaturated fat content in formulation, such as Alpha-linolenic acids (Goh et al., 2006). Additional research has focussed on decreasing fat content in ice cream through the application of fat replacers to produce healthier variants (Akbari et al., 2019). The biggest challenge to develop such ice creams is to achieve the same structures as products in normal formulations (i.e. those based on fat contents typically at 10wt%). Based on the general rule of fats in ice cream as discussed above, the fat source selected for ice cream production, no matter whether dairy or non-dairy source, should have 50-70% fat crystallised at 4°C to achieve a desirable fat destabilisation property. Higher concentrations of unsaturated triglycerides can lead to increasing coalescence rather than partial coalescence. For example, (Sung & Goff, 2010) compared palm kernel oil (PKO) and high oleic sunflower oil (HOSO) and their combinations as the fat source in ice cream formulations. They found that in order to produce stable ice cream, the mix at least should contain 60 to 80% of the more saturated fat. Therefore, in commercial production, saturated plant-based fat can be selected to mix with liquid oil for a good structural formation to a degree. Researchers also found the application of two-stream production can also help this issue. They divided the mix into two parts, one comprising emulsifiers with the solid fat and the other comprising protein with liquid fat. Both two blends were mixed together after being homogenised separately. The outcome of this approach was to make liquid fat droplets fully stable during subsequent freezing. (Méndez-Velasco & Goff, 2011).

### *2.4.2.3 Composition effects – arrangement of crystals*

As mentioned earlier, the arrangement of crystals formed during crystallisation is important. Even highly crystalline droplets can be stable to partial coalescence, particularly when few crystals are able protrude out of the membrane and pierce into adjacent globules (as is the case for protein stabilised emulsions). The protrusion efficiency is determined by the contact angle of crystals against aqueous phase ($\theta_w$) or oil phase ($\theta_o$). The contact angle is mainly controlled by three interfacial tensions in three different directions, which are the interfacial tension between water and solid phase, oil and water phase and solid and oil phase. They can be expressed as $\gamma_{ws}$, $\gamma_{ow}$ and $\gamma_{so}$ (Binks & Horozov, 2006). For example, when $\theta_w$ is smaller than 90°, crystal is preferably wetted by the aqueous phase, which means that the crystal can protrude into the aqueous phase. When $\theta_w$ is equal to 90 °, crystals will not protrude into any phase, and when $\theta_w$ is larger than 90°, crystals will preferentially protrude into the oil phase. This theory is very important, especially for some specific types of fat globules. According to Walstra's theory, based on the difference in the morphology and arrangement of crystals in globule, fat globules can be divided into 6 types (Figure 7). For the L and M type of fat globules, the $\theta_w$ of crystals is the main factor in the efficiency of protrusion, so the formulation of mix and other processing conditions should be adjusted to achieve the appropriate $\theta_w$. However, for N1 and N2 type, the size of fat crystals is the critical factor. The K type of fat globules has the highest capture efficiency (Fredrick et al., 2010). Therefore, processing conditions should be properly controlled as far as manageable in order to produce structurally desirable globules to enhance partial coalescence. In addition, tempering could change the morphology of fat globules. Research shows that the type of fat globules can change from N2-type to M-type in the tempering process (Figure 8). This finding can also be applied in real production and is of particular importance to the whipping process for creams.

Figure 7. The different morphology of crystallized fat globules (Fredrick et al., 2010).

Figure 8. The changing process of the morphology of crystallized fat globules during tempering (Fredrick et al., 2010).

#### *2.4.2.4 Crystal size and its morphology*

Crystal size is mostly affected by the temperature rate at the beginning and the time-temperature program after nucleation. A high cooling rate can enhance the supercooling mechanism to achieve a higher extent of nucleation and the fat globules in smaller sizes. Then the following time-temperature program will eventually determine the size of crystals. The secondary, time-temperature program can also affect the crystal polymorphism, α, β and β'. Crystals in different morphology will have different polarity. Research shows that β crystals are the only non-polar type and more preferable to be wetted by oil phase (Fredrick et al., 2010). Therefore, we can produce more effective fat crystals by adjusting the time-temperature program.

#### *2.4.2.5 Fat globule size distribution*

The fat globule size distribution after homogenisation can also affect the degree of fat destabilisation. A larger quantity of fat globules can be obtained after high-pressure homogenization and this can increase the possibility of the collision among fat globules. In addition, in small fat globules, the fat crystal will be more prone to protrude out because of the small diameter. This theory has been verified for a 15% fat content ice cream mix. The experimental result shows the meltdown rate of ice cream decreases when higher homogenisation pressure is applied. However, the sample with a fat content of 10% shows the exactly opposite result. A faster meltdown rate is observed after increasing the homogenisation pressure (Goff & Hartel, 2013). This phenomenon reflects that the extent of fat destabilisation is complex and related to various factors, such as fat content, protein content and size of fat globules. In this case, fat content seems to be more dominant. When higher homogenization pressures are applied, globules in smaller sizes are obtained. For low-fat content formulations, protein is more readily available to stabilize fat globules. As a result, fat globules will be more stable against partial coalescence, which is the main reason for the poor meltdown property of 10% ice cream when under higher pressure homogenization. In another aspect, homogenization in low pressure may also increase the degree of fat destabilization. Fat globules in larger size will be produced and they will have a higher colliding possibility in comparison to smaller fat globules. In total, to have a proper homogenisation plays a vital role in the properties of final product. The key is to find the perfect condition to balance all factors.

#### *2.4.2.6 Processing parameters – flow conditions*

It is worth noting that partial coalescence can also occur quiescently through Brownian motion or density differences (Goff, 1997b). The rate and the extent of fat agglomeration will be more pronounced as the interfacial stability of emulsions is progressively weakened. When external agitation is applied, both encounter frequency and capture efficiency will be increased and enhance partial coalescence. Research shows that under simple flow condition, the extent of partial coalescence can be a function of shearing rate. The reasons are three, first is the continuous shearing makes fat globules to move around each other to achieve higher collision possibilities, and the second is the added shearing forces can push globules together, decreasing the intermolecular distance and helping globules to overcome repulsions. The third reason is the shearing effects can enhance the fat crystal functions in partial coalescence. In general, turbulent shearing is believed to enhance the partial coalescence the most. However, excess shearing may break the fat globule aggregates which just formed into individual globules (Fox & McSweeney, 2006). In ice cream production, agitation always combine with the whipping action. The air introduced can also enhance fat clustering, because the air bubbles can push fat globules to get closed (Goff, 1997a). As a consequence, destabilisation in aerated, sheared emulsions is generally faster and more extensive than for similar shear conditions in the absence of aeration.

#### *2.4.2.7 Processing parameters – temperature*

Freezing temperature is a necessary condition of partial coalescence. In the ice cream making process, temperature fluctuation history should be carefully controlled for a desirable product. Temperature can affect the solid content in fat globules, the arrangement of fat crystals, the morphology, polymorphism and size of fat crystals (Fredrick et al., 2010). In processing methods, there is another important section called tempering which also shows large effects on partial coalescence. As all knowledge related to fat already been introduced, tempering will be the main topic in this section. While tempering is rarely encountered in ice cream manufacturing, it can be applied during the manufacture of creams to create products with variable viscosity.

Tempering easily happens in a non-homogenised or poor homogenised system. In simple words, tempering just means to provide temperature fluctuations to products. Tempering has a close relation with partial coalescence and sometimes is called as thermally induced partial coalescence (Leal-Calderon, 2012). In tempering process, temperature will rise first and cool down again, this will result of a more viscous, gel or even solid like texture (Thivilliers et al., 2008). This process is also often called as re-bodying. Actually, the more specific explanation is more related to the fat crystallisation in globules. As crystallisation happens, a compact fat crystal network will form in globule, and fat crystals will be prevented from moving to the interface. When the temperature rises, fat crystals start to melt and the crystal network will become more flexible, fat crystals can be more freely to interact with o/w interface. As the environment chills

again, new occurrences of nucleation tend not to happen, while the size of small crystals will increase to have further protrusion into the adjacent globules and partial coalescence is enhanced (Moens et al., 2016). The morphology and crystal arrangements in fat globules are also changed. The effects of tempering are dependent on both overall fat content and relative solid fat content. Generally the fat content should be higher than 25% and solid fat content should be 1.5-8% (Moens et al., 2016). Another factor is the rigidness of interface membrane. Tempering effects will be large if fat globule membrane comprises small molecule surfactants (either completely, or having partially displaced a protein adsorbed layer), noting that fully protein-coated emulsion droplets tend to be resistant to tempering. The resident time at the highest temperature is called as $T_{max}$. $T_{max}$ and should be long enough to get efficient tempering. The re-cooling process should be slow, otherwise a secondary nucleation may possibly occur instead of crystal growth (Fredrick et al., 2010).

Therefore, tempering effects can enhance the whipping properties, shorten the whipping time, to achieve a good overrun, firmer and more stable texture, and more stable in storage. However, repeating tempering will transfer the quantity of partial coalesced clusters into coalesced clusters and cause phase separation eventually.

## 2.5 The assessment of partial coalescence

To have a reliable, precise and rapid assessment partial coalescence can provide us a good correlation between the microstructure and the qualities of food products. Assessment of partial coalescence structure presents a number of challenges. First is sample treatment. The partial coalesced fat structures can be very fragile and easy to be disrupted or destroyed by processing or sample handling. Some treatments on samples may even increase the amounts of partial coalesced fat clusters. The second is to achieve a precise estimation of the quantity of partial coalesced clusters in the sample. As discussed before, various types of destabilised fat structures are formed during the whipping process, such as coalesced clusters and flocculated clusters. To discover the objective structures, extract them and measure the total quantity of them is indeed a challenge for scientists. The most effective and reliable methods for quantitative and qualitative measurements of dairy products are the combination of particle size assessment by laser diffraction and microscopy (Thivilliers et al., 2008). However, some brief introductions will still be made for some other representative methods that have been applied in the past.

### 2.5.1 Turbidity

Turbidity is the measurement of the extent of the particulates in solution. When light is trying to pass through a solution, it can be disrupted or absorbed by the particulates and cause light scattering. The concentration and size of particulates is correlated with the extent of light

scattering. In the stage of sample treatment, samples are firstly diluted by a factor of 200 or 500, then they will be centrifuged to separate out the destabilised fat content. After sample treatments, samples will be put into vials and assessed by spectrophotometer with visible light at 540 nm (Goff & Hartel, 2013).

### 2.5.2 Solvent extraction

This is the one of the earliest methods for determining “free fat”. Free fat describes structures formed through agglomeration of emulsion droplets. Such destabilised fat clusters are easier to be extracted by specific solvents than stable, emulsified fat globules. The extraction conditions and the specificity of the solvent are very important in this method. Heptane, hexane or petroleum ether are normally used as solvents for triglyceride oils. In practical applications, the weighted sample will be mixed with the solvent first, the organic phase will be transfer to the evaporator to remove the solvents. The rest after evaporation will be the proportion of destabilised fats (Petrut et al., 2016). However this method is no longer believed to be reliable in estimating the partially coalesced fat content in dairy products, however it can be applied for a rough estimate of the destabilised fat content (Fox & McSweeney, 2006; Koxholt et al., 2001).

### 2.5.3 Dye dilution

The dye dilution method is similar to the solvent extraction method. In this method, oil soluble dyes are mixed with non-polar solvent to visualise destabilised fat contents. After centrifugation, destabilised fats will rise up with dyes, be diluted and their relative concentration determined by absorbance testing. As both solvent extraction and dye dilution method need centrifugation, the extent of separation should be properly controlled for precise measurement, since centrifugation can influence the structure and stability of the emulsion. Additionally, the dye selected should have a good performance in absorbance testing (Peng et al., 2018).

### 2.5.4 Laser diffraction – particle size measurement

Particle size distribution measurement by laser diffraction technique is regarded as one of the most effective and reliable quantitative testing methods of partial coalescence. This technique is also based on the light scattering mechanism induced by particles in solution when a beam laser is passing through the sample. Particles in bigger size have smaller scattering angles. The scattered light is then received by series of detectors installed in a variety of angles. After complicated process of signal transmission and transfer, particle size distribution of sample will be expressed as a plot on the computer screen after complicated mathematical computation

(Malvern Instruments Limited, 2015). In practice, particles are in a variety of shapes and give large difficulties to measurement techniques. However, laser light scattering assume that all particles are in a spherical shape, which can be described by the diameter only. There are a variety of options in testing, which are based on the same weights, volume, surface area, etc. The most frequently used two parameters in the report are the D [3,2], which represents the Surface Area Moment Mean; and D [4,3], which is the Volume or Mass Moment Mean. Which parameter is more appropriate in testing process mainly depends on which property of particles we are focusing on (Malvern Instruments Limited, 1998). Emulsions samples, particularly those with high oil phase volumes, are generally diluted before introduced into the sample chamber, noting that the water in the measuring cell will give a further dilution in a factor of approximately 1000 (Williams & Phillips, 2004).

### 2.5.5 Microscopy

Microscopy is commonly used to identify the specific microstructure of foods across a range of length scales and can be used to visualise partial coalescence in dairy products. Generally, electron microscopy (EM) and confocal microscopy (CSLM) are most widely applied in the characterisation of emulsion structures. There are two types of electron microscopy widely used for whipped dairy products, cryo scanning electron microscopy (Cryo-SEM) and transmission electron microscopy (TEM). Both Cryo-SEM and CLSM can generate 3D structure, with SEM providing higher structural resolution (Caillet et al., 2003) (Figure 9). TEM can be operated in low temperature and the thin sections should be prepared before the observation (Caldwell et al., 1992).

Figure 9. Ice cream microstructure by scanning electron microscopy (Caillet et al., 2003).

In total, different types of methods can be applied for specific purposes. They all have their strengths and weaknesses in different aspects, so in order to generate more reliable results, different methods are usually applied in combination. Arguably, for measuring ice cream structure, cryo-SEM is considered best practice, on the basis of being able to visualise the trapped ice cream structure at freezer temperatures across a range of length scales, and which allows all phases (ice, air and fat) to be fully characterised. Particle size distribution and microscopy method are believed to be the best tests to give good descriptions of whipped dairy products both quantitatively and qualitatively.

## 2.6 Air bubble formation

Air bubbles are an important feature of the ice cream structure. The size of air bubbles and ice crystals are very similar in the range of 20-100 μm, while air accounts for the highest volume of ice cream (Goff, 1997a). They mainly provide a softening of the texture and impart creaminess to the product. They are also very important in achieving good melt-down properties and provide a good stand-up property to other whipped dairy products, such as cream. Air bubbles are formed during the whipping process. The introduction of air is different among different freezing methods. In some batch freezers and for most soft ice machines, air is drawn into the viscous mix from the atmosphere by continuous agitation. For most commercial batch and continuous freezers, air is instead incorporated into the mix in the form of small air bubbles under controlled pressures, which allows much greater control over the total volume of air incorporated. The shearing action in the freezer serves to reduce bubble size (Marshall et al., 2003).

The lamellae of air bubbles can change dynamically during the freezing process. As discussed in section 1.4.1, fat globules and fat clusters are the main elements to stabilise air bubbles, β-casein, casein micelles, casein submicelles and emulsifiers also participate in lamellae formation. The effect of emulsifiers to enhance fat destabilization is also highly impactful on the properties of the air phase, noting that fat globule adsorption to the surface of bubbles during freezing has a significant impact on bubble size and stability. This is most apparent based on structural changes during the hardening process. Research has shown that, for an ice cream sample without emulsifiers and stabilisers added, bubble growth can be observed after 60 mins of hardening (Pei & Schmidt, 2010). However, for the ice cream samples with proper content of emulsifiers and stabilisers in the formulation, destabilisations of air bubbles are effectively retarded. It is worth noting that the properties of the freeze concentrated serum can also have a major effect on air bubble stabilisation. The main effect of the freeze concentrated serum is the high viscosity, which can limit air bubble recoalescence and/or growth through disproportionation. In this regard, stabiliser addition can be effective in improving whipping properties, but arguably the presence of destabilised fat in the highly concentrated serum phase may also serve a similar purpose.

Finally, the ice crystals formed from the serum can also enhance the shear forces to decease sizes (Goff & Hartel, 2013).

The application of novel processing methods can also help to produce small, uniform and stable air bubbles in ice cream. Pre-aeration in the mix is a good technique. In commercial production, it can combine with the product recirculation technique for desirable air bubble and ice crystallization properties. Lowering extrusion temperature can also help generate small air bubbles and ice crystals. Research proved that an obvious decrease of air bubble size can be observed when extrusion temperature decreases from -4.7 to -13 °C (Shrivastav & Goswami, 2017; Tharp & Young, 2012). Another issue is the contradiction existing in the manipulation of the sizes of air bubbles and ice crystals in the scraped surface freezer. Short residence times in the freezer can produce small ice crystals, while small air bubbles need longer residence time. However, this contradiction can be perfectly resolved by the two novel techniques above (Goff, 2002).

There are some notable defects related to loss of air phase stability in ice creams that include air coarsening and shrinkage. They are both due to the changes in air bubble sizes. Air bubble stabilisation is important, especially in hardening and storage stages. The main phenomena influencing air bubble stability during manufacturing and storage are: Ostwald ripening, coalescence, drainage and air bubble distortion by ice crystals. Drainage means the air bubbles will rise in ice cream especially under warmer temperature, and arguably is only encountered in ice creams that have become fully melted. Disproportionation, in which large bubbles increase in size at the expense of smaller bubbles, can occur during hardening as well as during temperature cycling of stored ice creams. Coalescence occurs due to film rupture between adjacent bubbles, and again can occur during both manufacture and storage, and in the case of ice cream can be influenced by the presence of ice crystals (noting that ice crystal growth during storage can exacerbate coalescence). Ice cream formulation and the environmental temperature play vital roles in air bubble stabilization (Goff & Hartel, 2013). In order to prevent such defects, enough non-emulsified protein should be in the formulation to fully coat air bubbles. In this regard, the type of protein and its structural and mechanical properties and the air-water interface can be important. Recent studies show that a new protein formed by filamentous fungi (hydrophobin) can highly effective at stabilising air bubbles particularly against Ostwald ripening. From a commercial perspective, emulsifier addition to formulations, and the subsequent adsorption of fat droplets to bubble surfaces remains the most effective way of protecting bubbles against both coalescence and disproportionation, as the adsorbed fat droplets provide a Pickering type stabilising mechanism. This stabilisation mechanism is particularly important for ice creams undergoing temperature fluctuations, noting that air bubbles will become smaller with lower temperature. When the temperature decreases to -15 °C, most of the changes in air bubble sizes will be almost trapped due to high viscosity of the serum phase. And all changes will become rapid over -6 °C (Goff & Hartel, 2013). Accordingly, ice creams kept at freezer storage conditions of -18°C are structurally fixed but will start to destabilise when removed from these

conditions and allowed to warm up. Ice cream quality can be most effectively maintained by minimising temperature fluctuations during handling, distribution and storage.

Where air coarsening does occur, it can cause various defects. The size distribution of air bubbles in ice cream will become heterogeneous, which will influence melting properties and consumption experience. When air bubbles start to coalesce, they can ultimately begin to form air channels will allow the air to escape from the ice cream, causing a shrinkage defect. Shrinkage is a complex phenomenon and the most common reason is the changes in environmental pressure. The most direct appearance of shrinkage is the separation between ice cream and walls of cartons. Thus, factors which can promote the coalescence of air bubbles will also contribute to shrinkage, noting that poor temperature handling is the biggest contributor to this particular problem (Hui & Sherkat, 2005).

## 2.7 Ice crystallisation

Ice crystallisation can be divided into two steps, nucleation and ice crystals growth. Nucleation is induced by supercooling and crystal nucleuses can form in this process. Then nuclei will grow into large crystals in both freezing and hardening stages (Adapa et al., 2000). Nucleation plays a dominant role in the properties of ice crystals in final product. In the freezing barrel, nuclei will form at the interior steel wall of the freezer barrel. After nucleation, ice crystals will start to form and accumulate into a slushy layer, which also contains a large amount of concentrated unfrozen solute. Then dashers scrape the ice slush off, then disperse them into the bulk of ice cream mix. These Ice crystals will start to melt when contact with the warmer mix, which absorbs the heat of mix. The mix starts to be cooled down and eventually ice crystals scraped off the surface of the freezer will no longer melt when reincorporated into the mix, leading to an increase in viscosity. Once ice content and viscosity reach a critical level, the ice cream is extruded out of the freezer. Research shows that the size of ice crystals being extruded from the freezer should be lower than 50μm for a desirable quality (Park et al., 2015).

In order to produce ice crystals with appropriate properties, all processing stages need to be carefully controlled. The first requirement is to produce nuclei in the nucleation stage. In order to make nucleation happens quickly the surface of the freezer barrel needs to be cold. Refrigerants such as liquid ammonia, freon, can rapidly decrease the wall temperature to – 30°C, which allows for almost immediate freezing for any of the mix that comes into contact with the barrel surface. The second requirement is to control ice crystal growth. In order to achieve this purpose, it is necessary to limit the crystal residence time in the freezer barrel and to avoid temperature fluctuations. The third is the agitation actions in barrel should be as effective as possible. Ice crystals in the bulk do not equivalent heat conduction as steel, so agitation can be used to promote heat transfer and contribute to small crystal formation. Fourth, the role of ingredients should also be considered. Some ingredients can decrease the freezing point of mix

and ice cannot form in a large amount as before. So, less ice will cause a soft ice cream texture. Some ingredients like stabilisers and milk proteins can increase the viscosity of serum phase around ice crystals to control the size growth in storage (Sun, 2012).

Changes to ice crystal size due to handling, distribution and storage can also be highly impactful on product quality. Large crystals formed in storage due to changes in the storage environment are very undesirable and can cause significant defects in ice cream. Increasing ice crystal size occurs due to recrystallisation based on storage temperature and/or temperature fluctuations. After hardening, ice cream will be stored at -18°C. Research shows that relative storage temperature has a significant effect on ice crystal growth. The shelf life of ice cream at -10°C is only a week, while stored at – 15°C can extend the shelf-life to a month. The growth rate of ice crystals is highly affected by temperature variations. The actions of water molecules will increase at high temperature while freezing temperature will retard the molecular movements. When the temperature decreases to -32°C, ice cream texture will become highly viscous, most of the molecular movements will be trapped and both chemical and physical changes rarely happen. This temperature is called the glass transition temperature and is expressed as Tg'. Stored under Tg' is certainly very effective to give a good shelf-life of ice cream, however, the costs will be unacceptable and the product too cold and hard to eat when removed straight from the freezer. Another parameter is Tm', which is around -20 - -25°C, which represents the end of the freezing point curve. Storage at Tm' can also provide a very good shelf-life of ice cream and the commercial storage temperature is generally under -20°C. Traditional freezer temperatures of -18°C are generally considered acceptable for maintaining ice cream structure and quality, however even slight warming above this can begin to compromise product quality (Goff & Hartel, 2013).

As noted, inadvertent temperature fluctuations in storage and transition process can greatly impact quality due to ice recrystallisation. After production, ice cream will be firstly kept in manufacturing plants, then transported to the distribution centres, after this, products will be sent to retail outlets and finally bought by consumers (during which the time for transportation home can also impact on product temperature). Total storage period is can be up to two months. During the long storage, there are lots of opportunities of causing temperature fluctuations and most of them cannot be eliminated by more cares or good regulations. Therefore, other factors should be enhanced to diminish the extents of ice recrystallisation. Research shows that some ice structuring proteins (ISP) isolated from some arctic fish and winter wheat have very effective effects on ice crystallisation. The proteins can tightly absorb onto the surface of ice crystals to prevent further growth. Research shows that the added ISP can decrease the potential of ice recrystallisation by about 46% with the content of 0.0037% (Regand & Goff, 2006). The emulsifier propylene glycol monostearate have also been found to have similar functions (as well as performing as a typical ice cream emulsifier such as monoglyceride. In addition, the changes in parameters like pressure and temperature in the freezing stage can also limit the ice crystal sizes (Cook & Hartel, 2010). For processing, fast freezing and effective heat conduction can generate smaller ice crystals. Dasher type, design and speed can also affect ice crystals. During storage,

some phase-change materials can be used in the freezing chamber to minimise the temperature fluctuations.

## 2.8 Serum phase

The serum phase, also known as the matrix, is the unfrozen component of ice cream and, from a colloidal perspective, acts as the continuous phase for all the other dispersed states. Its properties can influence the shelf-life, softness and coarseness of ice cream. Sweeteners, stabilisers, milk proteins and mineral salts are all solubilised in the serum phase. Its properties can be modified through the water-binding action of added stabilizers and to a lesser extent by the milk proteins. Furthermore, the freeze-concentration of sugars and minerals is determinant for overall freezing point depression and ice crystal content. The freeze-concentrated serum phase has a very important stabilizing effect on air bubbles and ice crystals. As most of the mechanisms have already been introduced above, two topics will be mainly discussed in this section, the milk protein – stabiliser complex formation and lactose crystallisation.

As briefly introduced above, the stabilisers which are commonly used in ice cream manufacture, like guar gum, locust bean gum or carboxymethyl cellulose are all incompatible with milk proteins, especially the casein micelles. Milk proteins can form a complex with stabilisers by electrostatic interactions and this can cause phase separation in ice cream, and the phase separation is more easily detected in soft-serve ice cream. The newly formed complex is actually desirable to the ice cream structure. They can effectively retard ice recrystallisation and contribute to forming a more rigid and solid texture of ice cream. However, the phase separation induced by such complex formation is another unavoidable defect. Fat and the complex of proteins and stabilisers will concentrate in an opaque part and separate from the aqueous phase. This defect mostly happens at high ionic level or high pH level which is higher than the isoelectric point of protein (Williams & Phillips, 2004). Research shows that such complex formation cannot be effectively prevented, but this phenomenon can be relieved. K-carrageenan is used as a secondary stabiliser for this case. The minimum amount of k-carrageenan is in the range of 0.012 – 0.02% to resolve this problem. K-carrageenan can form complexes with caseins to separate the large complex into tiny pieces, like emulsification, and the phase separation will be minimised. Another way is to adjust the ratio of casein micelles and whey proteins, as caseins are the main reason for this defect, it is also feasible to reduce casein micelles content to minimise the complex formation (Goff & Hartel, 2013).

Lactose crystallisation is another defect to affect the quality of ice cream. As the melting properties of ice and lactose crystals are different, they will provide different tastes in mouth. Generally, when the size of lactose crystals exceeds 15 μm, they can be detected by mouth (Boom, 2008). Formulation and storage temperature are the main effects on this defect. Most lactose crystallisation happens at -10 - -12 °C. An MSNF content of 15.6 - 18.5 % can effectively prevent

lactose crystallisation, for enough proteins and stabilizers in the formulation. Agitation can enhance lactose crystallisation by promoting the nucleus formation. The extra addition of nuts or fruits can also cause sandiness. Overall, in order to prevent sandiness, the lactose content, storage time and temperature fluctuation should be controlled (Hui & Sherkat, 2005).

As mentioned above, the main action of the serum phase is to provide the stabilization of microstructure of ice cream by increasing the viscosity. Besides the manipulation of formulation, novel processing methods can also contribute to this purpose. High-pressure processing is a good method to increase the viscosity of serum without nutritional or sensory effects on ice cream. Whey proteins in the mix will be denatured and form complexes with casein micelles, beyond this, the hydration of casein micelles is also enhanced by the high-pressure actions on the mix. Such changes contribute to a more stable structure and good meltdown properties. This new technique could be an effective replacer of stabilizers and provide possibilities of an additive-free ice cream production (Huppertz et al., 2011).

## 2.9 The background of the Masters project

Synthetic emulsifiers are increasingly becoming a target for reformulation and replacement with natural alternatives in food products. This trend is motivated by recent reports that some particular emulsifier additive may harmful to human health. For example, Gewirtz demonstrated that certain artificial emulsifiers could alter the mucus layer of gut allowing bacteria to more easily attack gut cells. This could create digestive and metabolic issues (Reardon, 2015).

To develop synthetic emulsifier-free products is naturally of interest to the ice cream industry. Generally, in order to produce emulsifier-free ice cream, the principle is to seek for ways to replace the functions of emulsifier in ice cream processing, or simply, to promote enough degree of partial coalescence in ice cream structure without emulsifiers. Therefore, emulsifier-replacer can be applied or complementing actions of emulsifiers by applying different processing methods. Maestrello et al. (2017) applied chia seed mucilage in ice cream as emulsifiers and found ice cream with 0.6% mucilage can have a good meltdown property. Prapasuwannakul et al. (2014) selected green coconut pulp to replace dairy fats, milk and stabilisers and found similar properties as the regular formulation. Musselwhite and Walker homogenised ice cream mix in the presence of caseins and gelatine, a good stand-up ice cream had achieved without emulsifier using (Moran & Rajah, 1994). As discussed in previous chapters, low-temperature extrusion technique can enhance partial coalescence (Bolliger et al., 2000). Besides, two-stream processing can also promote fat agglomeration. One stream contains fat source, whey protein isolate and the other contains skim milk powder, sweeteners and stabilizers. They are pasteurized individually and mixed just before freezing (Segall & Goff, 2002). Sofian-Seng and co-authors also explored the use of enzymatic lipolysis as a biochemical route for producing monoglycerides during ice cream manufacture.

Our attempts of designing an emulsifier product start from a preliminary study in 2017. In that protocol, non-homogenised milk fat from cream was used as the base for the emulsifier-free ice cream formulation design. The complete use of non-homogenised cream would be expected to lead to excessive agglomeration during freezing leading to buttering in the final product. Instead, split streaming of non-homogenised cream and homogenised cream at varying ratios was hypothesised as providing control over the extent of partial coalescence, allowing benefits associated with partial coalescence to be achieved without leading to excessive agglomeration and butter grain formation in the final product. However, some unexpected results were observed in these preliminary experiments. The control sample, which was stabilised with protein, had a higher particle size result than for positive control (which contained monoglyceride) and the 2% sample had better meltdown testing results than 3% and 4%. We suspected and confirmed that the ghee used to make homogenised droplet in this experiment was responsible for this, determining that it contained notably higher than expected free fatty acid (FFA) concentrations, that were believed to acting as emulsifiers and thus influencing emulsion stability in the combined system.

On the basis of these findings the objective of this Masters study were:

(i) to determine whether split stream processing of non-homogenised cream (based on fresh cream) and protein stabilised emulsions (based on fresh AMF with low FFA content) could be used to improve product properties in the absence of monoglycerides.

(ii) to use the findings of the preliminary study and determine whether fatty acids could be used as natural emulsifiers in ice cream formulations in lieu of conventional monoglyceride emulsifiers.

# 3. Materials and methods

The ice cream formulations developed for this study were made without any flavours or colours. The reason for this design is just for a specific research purpose without any interference factors. As seeking for novel applications to enhance the partial coalesced structures is the main purpose, specific formulations are designed, and series of physical tests are prepared. There are two hypotheses in this project, first is blending unhomogenised cream into mix and the second is using fatty acids instead of emulsifiers in mix. Tests involve overrun, particle size distribution, meltdown and microscopy test. As discussed in the literature review, the combination of particle size test and microscopy is the best option for quantitative and qualitative measurements of ice cream.

## 3.1 Raw materials, equipment and formulations

| Ingredient | Manufacturer |
|---|---|
| Anhydrous milk fat | Fonterra – NZMP |
| Instant skim milk powder | Fonterra – NZMP |
| Sucrose – White Sugar | Davis trading |
| Glucose syrup (DE: 38-44) | Davis trading |
| Mono-diglyceride – GRINDSTED® MONO-DI HP 40-M | DANISCO |
| Guar gum | DANISCO |
| Locust bean gum | DANISCO |

Table 3. The raw materials applied in the ice cream mix.

| Equipment | Manufacturer |
|---|---|
| Ultra-high temperature processing (UHT pasteuriser) | |
| Laminar Flow Cabinet | Air Care Technology Ltd : AC 1100 |
| Homogeniser | Rannie : Serial # 1.88234 |
| Ice cream machine | Tetrapak |
| Laser analyzer Mastersizer 2000 + Hydro 2000 MU | Malvern Panalytical |
| Leica SP5 DM6000B Scanning Confocal Microscope | Leica Microsystems |

Table 4. The equipment applied in ice cream production and physical analysis.

All ice cream formulations were based on a standard composition comprising 10% milkfat. Full details of formulations, including design of split stream compositions and fatty acids dosing can be found in Appendix 1.

## 3.2 Ice cream making

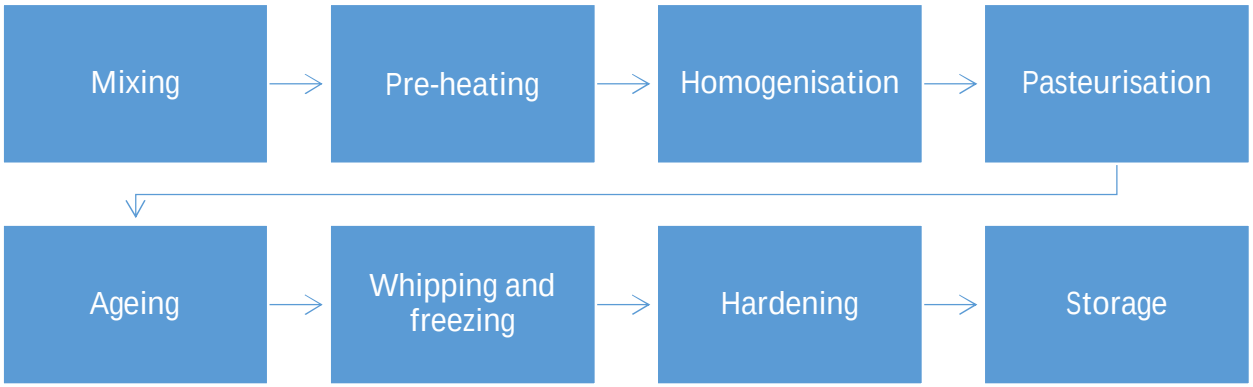


Figure 10. Flowchart of ice cream production.

A standard ice cream production procedure was chosen for this project (Figure 10). All processing steps were conducted in the pilot plant at Massey University and followed the Standard operating procedures (SOP) of each equipment.

Figure 11. Mixing of ice cream ingredients in a steel bucket.

All fresh ingredients were mixed in a steel bucket with a mechanical mixer (Figure 11). In order to minimise errors, the preparation of each sample was strived to be made simultaneously. With the limitations of the working area and labour, the maximum numbers of sample to be done in the lab was 5. In practice, particular mixing methods were applied to fit the physical properties of different ingredients. Glucose syrup is the stickiest ingredient in the formulation. It was firstly mixed in hot water after weighing. The skim milk powder was then added. AMF solids of required weight were placed into a steel bucket and put into a steam jacket heater to melt them > 40°C. As solid AMF melted, monoglyceride emulsifiers were also added into the steel bucket to melt together. This action was mainly for effective emulsification of AMF. Both monoglycerides and fatty acids were treated in this way. Sugars dissolve easily and they were normally mixed with stabilizers before adding into the bucket to prevent gum clusters formed in solutions.

Two-stage homogenization was then applied to the ice cream mix. The pressures of the two valves were 200 bar and 50 bar respectively. This is mainly for the avoidance of the formation of fat clusters after homogenization (Bylund, 2003).

In order to achieve a sufficient pasteurization, sterilizer is designed as containing two heating parts, pre-heating and heating. As the same, cooling and chilling parts constitute the temperature decreasing section before collecting. The temperature of pre-heating and heating parts for self-sterilization was 85 °C and 142 °C respectively. The whole plant will be sterilized for 30 mins. After this, the pasteurization of ice cream mix started. By applying the classic pasteurization condition of the sterilizer, preheating temperature was set at 50 °C and heating temperature was decreased to 83 °C, cooling and chilling temperature were set at 0 °C. The flow rate was set at 1.6L/min and holding time was 30 s. The sterilized product was then collected and packed manually in a sterilised chamber. Timers were used to identify the position of the product in pipes. Based on the flow rate of the product, it is estimated that the product can be collected after 2.5 mins. Sterilizing chemicals were sprayed on technician's hands and the plastic buckets before collecting. Products were collected in a laminar flow cabinet. After pasteurization, the sterilizer was washed with caustic chemicals and rinsed for 30 mins respectively.

After this, the buckets will be kept in the chiller of pilot plant at 4 °C for 24 hrs, which is called the ageing stage. After ageing, the mix will be introduced into the ice cream machine. The mix was pumped into the machine at the rate of 20 L/h and the extrusion temperature was set at -6 °C. The dasher inside the freezing barrel rotated at the rate of 600 rpm. Overrun was set at 100% and viscosity was 70%. Ice cream extruded was collected with labelled sampling plastic containers. The paper square box was also used for producing samples in the same volume for meltdown test. As no colours contained in ice cream formulation, all ice creams were plain. In practice, it is difficult to distinguish the different types of ice cream during production. As a result of this, drops of red dye were applied in the interval of two mixes. When coloured ice cream came out of the spout, this was the sign of the next formulation coming. Once production was completed, ice cream machine will be rinsed by hot water and washed by caustic solution. Another rinsing was

also needed after washing. Collected ice cream was stored in a freezing chamber at -30 °C for hardening.

## 3.3 Testing methods

There are 4 classic methods for testing its physical properties of ice cream have been applied in this project. With the limitation of budget, some novel techniques such as Cryo Scanning Electron Microscopy were not included.

### 3.3.1 Overrun test

The overrun test is for the determination of the air volume in ice cream samples. The formula is below:

Overrun% = (Wt. of the mix – Wt. of sample vol. of ice cream)/Wt. of sample vol. of ice cream x 100%

This test was performed during the running of ice cream. A plastic container was used to hold ice cream mix and ice cream samples. The weights were measured immediately. Three tests were made for each sample and an average value was calculated.

### 3.3.2 Particle size distribution test

The principles of this test have already been discussed in the literature review. The chamber which is named 2000 MU was selected in this test. 2000 MU is mainly for achieving a rapid and complete dispersion for a sample in a flexible volume. In this project, large and sticky particles of ice cream samples may easily cause the contamination of the sample cell when using the small size dispersion chamber. In contrast, a more complete dispersion can be achieved with 2000 MU. Before measuring, samples were melted at temperature of 20 °C for half-hour, then diluted with the equal weight of DI water. The refractive index (RI) of particles was set at 1.460 and the dispersant RI was 1.330. Each sample was set by measuring 12 times repeatedly and the delay was 10 seconds. An average value of all results was calculated after discarding unreasonable results. Pump speed was set at 1800 rpm for even dispersion. After measurement, all graphs and data will be generated by the software.

### 3.3.3 Meltdown test

Ice cream cubes were taken out of the cubic paper box and placed on a steel net. As stored in paper boxes, ice cream was moulded in the same dimension with the length of 8cm, width of 4 cm, and the height of 3.5cm (Figure 12). This net is weaved by crimped steel wire with a mesh size of 1cm. Plastic boxes were put under the ice cream cube to collect the drops of melted ice cream. In order to minimize the instrument error, only one balance was chosen to weigh all plastic boxes regularly in every 5 minutes. As all plastic containers were of the same size and the difference among them was smaller than 0.5 g, they can be weighted by one balance after one time of tare at the beginning. In this project, this test normally lasted for 4 hours in a temperature-controlled lab at 20℃.

Figure 12. In meltdown test, ice cream cubes were all placed on a steel net.

### 3.3.4 Microscopy test

The confocal laser scanning microscope applied in this project is own by the Manawatu Microscopy and Imaging Centre. The model is Leica SP 5. The objective used was 63X, NA 1.4. After melting in room temperature for 20 minutes, ice cream was collected with a plastic dropper and then transferred onto a microscope slide. The slide with cavity was selected in order to protect the fragile microstructure of ice cream. Nile Red and Fast Green were dropped in the ice cream sample for staining fat globules and proteins. After putting down the cover slip, the glass slide with sample will be put on the platform of microscope and a drop of immersion oil will be placed on the cover slip to achieve a higher magnification. When sample can be clearly observed from eye lens by manipulating the two focus knobs, lasers will be ignited and the microstructure of ice cream in high resolution will appear on the two monitors. In this project, two lasers were used to correspond to the two types of dyes. Nile Red was excited at 561 nm and the scanning frequency for collecting was set at 570-611 nm. Fast Green was excited at 633 nm and collected

in the range of 643-743 nm. The brightness and contrast of image can be adjusted by turning smart gain and smart offset knob. 3 images were captured and re-edited by ImageJ software for each sample.

# 4. Results and Discussion

## 4.1 Overview of preliminary findings

This short section summarises some of the preliminary work undertaken prior to the results presented in this thesis. This focusses mainly on the blending of non-homogenised and homogenised creams as the fat source for the ice cream and uses the same formulation as outlined in the materials and methods (Muir, 2017). Total fat content was set at 10%, with non-homogenised cream comprising 2, 3, 4 and 5% across four formulations, with the balance comprising a protein-stabilised emulsion with ghee as the source of milkfat. The principle hypothesis was that increasing levels of non-homogenised cream in the formulation would lead to higher levels of partially coalesced fat, and that performance attributes such as meltdown would correlate with this change. It was also expected that the control sample (based on a fat phase comprising a fully protein stabilised ghee emulsion) would display minimal droplet destabilisation during freezing, and that the formulation comprising entirely non-homogenised cream would lead to excessive agglomeration and buttering.

Without presenting specific findings, it was observed that the control emulsion displayed an unusually high degree of droplet destabilisation and demonstrated a markedly higher melt stability than would be expected, based on prior observations of ice creams using this particular formulation.

It was hypothesised that the ghee, which was applied in ice cream formulation as the fat source, might be the main reason for such abnormal results, noting that the sample used in the formulation was quite old. When a fresh ghee sample was used, the resulting ice cream sample displayed considerably fewer fat droplets destabilisation compared to the older ghee sample.

In analysing the two ghee samples, the older ghee samples showed FFA (free fatty acid) concentrations three times higher than that of than fresh ghee. Therefore, it suspected that the high content of fatty acids is the main reason for higher agglomeration, and that these were acting as surface active components destabilising the interface in a manner analogous to commercial emulsifiers. Accordingly, for this study ice cream formulations were redeveloped using fresh AMF to avoid the influence of FFA on the blended emulsion system. A second objective to this study was added, to further the observations made during the preliminary work, i.e. to determine whether deliberate inclusion of particular FFA into fully homogenised formulations could be used to induce partial coalescence during freezing, thereby providing a naturally derived lipid emulsifier as a potential replacement to commercial monoglycerides.

## 4.2 Blended emulsions comprising Non-homogenised cream and protein-stabilised AMF

Ice cream mixes were prepared using a split stream process that combined two distinctive droplet types. A portion of the mix would comprise fat droplets homogenised using AMF that were stabilised by milk protein, with the remainder of the fat content being provided by non-homogenised cream so that the total fat content in the mix was 10%. The amount of fat provided by non-homogenised cream varied from 0% (i.e. protein-stabilised AMF control emulsion) through to 10% (i.e. fat content was made up entirely of non-homogenised cream).

### 4.2.1 Overrun test

Mixes were frozen and overrun measurements made on samples as they were extruded from the freezer. Findings are shown in Table 5 and Figure 13.

| Non-homogenised cream content in mix | 2% | 3% | 4% | 5% | 10% | NC | PC |
|---|---|---|---|---|---|---|---|
| Overrun value (%) | 60.63 | 79.92 | 85.78 | 129.16 | 121.96 | 65.22 | 87.34 |

Table 5. Results of the overrun test. Sample order indicates the relative amount of non-homogenised fat in the formulation; NC refers to natural control (i.e. protein-stabilised, homogenised 10% AMF formulation); PC refers to positive control (i.e. protein-stabilised, homogenised 10% AMF formulation containing monoglycerides)

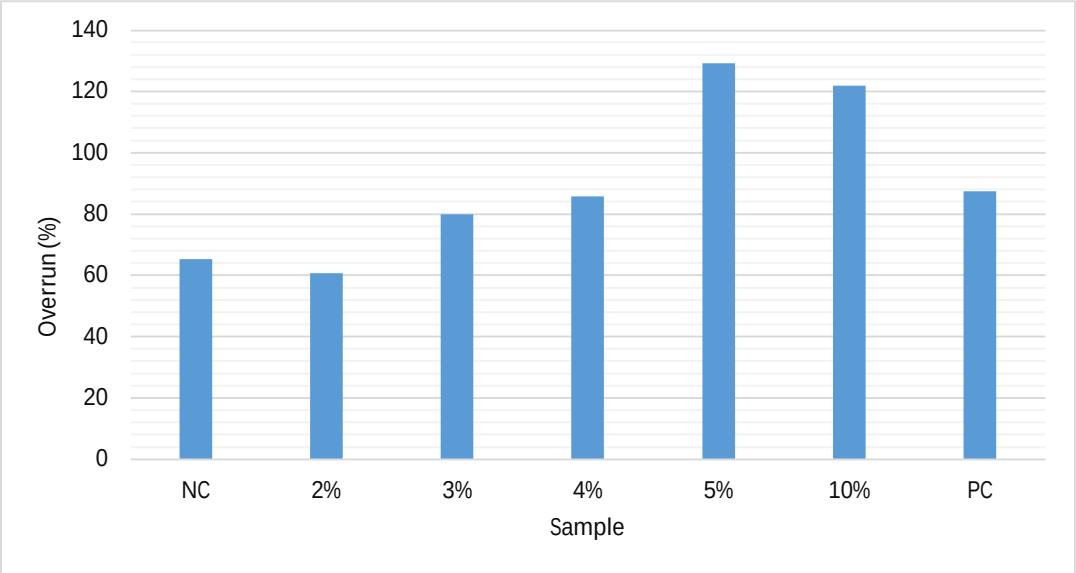


Figure 13. Overrun measurements of ice cream samples containing various concentrations of non-homogenised cream during processing. Sample indicates the relative amount of non-homogenised fat in the formulation; NC refers to natural control (i.e. protein-stabilised, homogenised 10% AMF formulation); PC refers to positive control (i.e. protein-stabilised, homogenised 10% AMF formulation containing monoglycerides)

The data above clearly shows the increasing trend of overrun value with an increasing non-homogenised cream content mixed in ice cream. The fact that a trend appears at all is somewhat unusual since the overrun setting on the freezer was set to 100%, and mixes that are frozen should generally be able to meet the target setting on extrusion. That the range of overruns vary from 60% to 120% provides some indication that structural changes during freezing are affecting the capacity of the mixes to incorporate and stabilise air. Data also show that overrun is maximised at 5% non-homogenised cream content, with the 10% sample shows a slight decrease compared to the 5% sample.

The increasing trend of overrun is possibly due to the increasing degree of fat agglomeration during freezing. As discussed in the literature review, a higher extent of partial coalescence may increase mix viscosity. Increasing bubble stability may also minimise coalescence during processing allowing greater foamability of the mix leading to higher overruns. This is the main reason for the high overrun value of 5 and 10%. Both of them appear to produce a relatively more stable ice cream which can reach the overrun setting of ice cream machine. In contrast, we can deduce that less partial coalescence happens in 2% sample. During production, 5 and 10% samples were observed to have very consistent stable extrusion rate and ice cream is very firm and with desirable appearance (Figure 14). However, the 2% ice cream sample appeared shiny, watery and soft, was less consistent during production with frequent breaks and tears in the ice cream "ropes" on extrusion. The decrease in overrun at 10% non-homogenised cream might be due to the excessive degree of fat destabilisation (noting that the positive control had a lower measured overrun and increased particle size, so the trend may be consistent). As the total fat content is fixed, it will result in fewer numbers but larger sizes of fat clusters. Increasing formation of large agglomerates will provide insufficient coverage to air bubbles and result of lower overrun.

| 2% Sample | 5% Sample |
|---|---|

Figure 14. The comparison of appearance between 2 and 5% sample.

Another hypothesis is due to the poor air bubble holding capacity, air bubbles will coalesce and break during extrusion result of the breakage of the extruded ice cream.

### 4.2.2 Particle size distribution test

In this test, the D (4,3) is selected to indicate the size of fat clusters. D (4,3) is the volume-weighted mean of the diameter of particles. This parameter is commonly used for large particles testing (Malvern Instruments Limited, 1998). Large clusters expressed by higher values mean a higher level of partial coalescence is achieved. In practice, after dilution with DI water, fat clusters in some samples were big enough to be easily observed. Testing result in the table below is the average of 12 replicates of repeated testing. In ice cream samples, higher values mean that much more fat clusters exist, in order words, more partial coalescence happened in production. The distributions of particle size data are shown in Figure 15. Here, it can be seen that most formulations possess a bimodal distribution. In the case of the natural control there is a larger modal distribution centred at ~1µm and a smaller modal distribution centred at ~50µm. With increasing non-homogenised cream concentration there is a clear increase in the relative volume of the modal distribution at the higher particle size range at the expense of the lower size range, indicating a progressive increase in fat destabilisation. In the case of the 10% non-homogenised fat sample, and for the positive control (i.e the formulation comprising added monoglycerides), the smaller particle size modal distribution has almost entirely disappeared.

| Sample order | NC | 2% | 3% | 4% | 5% | 10% | PC |
|---|---|---|---|---|---|---|---|
| Mean of D (4,3), µm | 17.78 | 19.62 | 22.09 | 38.89 | 46.69 | 76.14 | 88.17 |
| Standard deviation of D (4,3), µm | 2.46 | 2.04 | 2.13 | 3.17 | 7.31 | 4.98 | 6.22 |

Table 6. Results of the Particle size distribution test. Sample order indicates the relative amount of non-homogenised fat in the formulation; NC refers to natural control (i.e. protein-stabilised, homogenised 10% AMF formulation); PC refers to positive control (i.e. protein-stabilised, homogenised 10% AMF formulation containing monoglycerides)

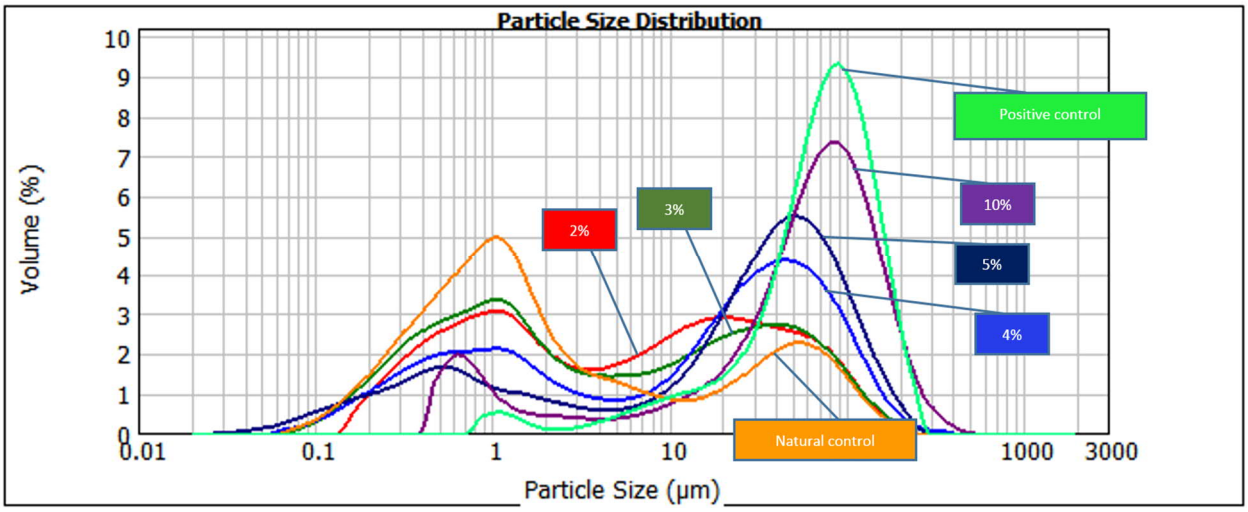


Figure 15. Particle size distributions of melted ice cream samples containing mixtures of homogenised AMF and non-homogenised cream

Data show that NC has the smallest particle size and PC possesses the largest. Besides these two samples, with samples show an increasing trend as the non-homogenised cream content increases in the formulation. Interestingly, the even the protein-stabilised natural control formulation shows some evidence of fat destabilisation, indicating that the interfacial later is not entirely resistant to the shear forces within the freezer. The increase in particle size increasing non-homogenised content is not unexpected, given that the fat droplets stabilised with MFGM are markedly less stable to shearing that the protein stabilised emulsions. It also curious to note that the mean particle size for the formulation comprised entirely of non-homogenised fat droplets is actually smaller than that of the positive control, which may account for why the PC formulation had a lower observed overrun than the 10% cream emulsion (due to excessive fat destabilised as discussed earlier).

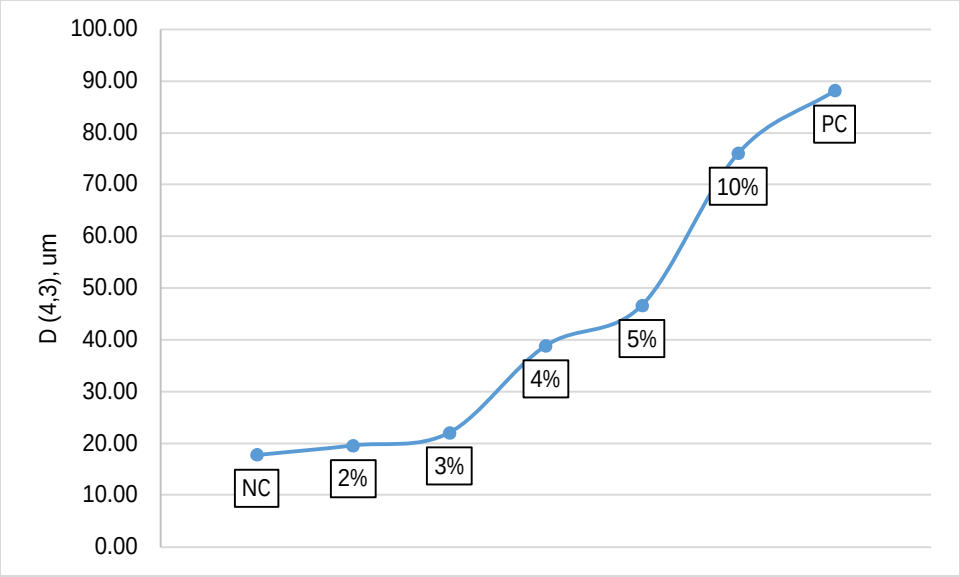


Figure 16. The line graph of the particle size distribution test results. Samples indicate the relative amount of non-homogenised fat in the formulation; NC refers to natural control (i.e. protein-stabilised, homogenised 10% AMF formulation); PC refers to positive control (i.e. protein-stabilised, homogenised 10% AMF formulation containing monoglycerides)

### 4.2.3 Meltdown test

Meltdown testing is an important quality indicator and can provide useful information relating to the structure of ice creams (Goff & Hartel, 2013). Fast meltdown rates will lead to difficulties in handling and such ice creams can be more prone to defects during temperature cycling. Slow meltdown can lead to unattractive or unexpected appearances. Therefore, to have an appropriate meltdown property of ice cream is very important. Meltdown testing typically involves the placement of ice cream blocks of specific size and volume onto wire mesh screens. During melting, liquid falls through the mesh into a balance below, allowing the mass loss to be determined, which can provide an indication of melting rate.

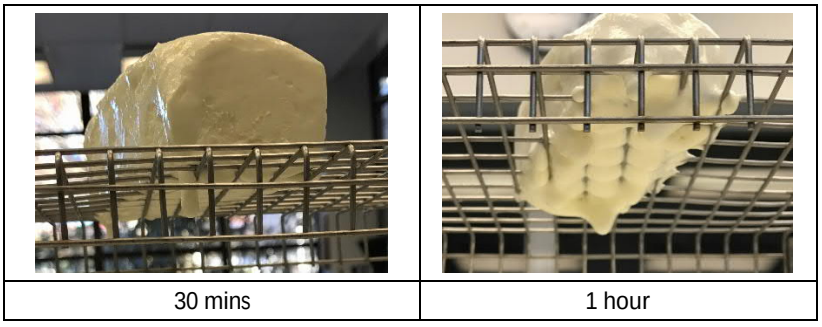


Figure 17. The natural control sample after 30 min and 1 hour.

The melting action which starts from the surface is due to the heat transferring effect. As the outside surface is in direct contact with warmer air, the ice cream inside will have a slow melting rate, because the fat globule and air bubble content can act as good insulating materials. Accordingly, the heat conduction rate into the ice cream inside is relatively slow.

The speed of melting can be influenced by the relative phase volumes of ice and air. The serum phase of the ice cream becomes increasingly dilute and of lower viscosity as the ice cream melts. This serum phase still needs to flow through the tortuous path of air bubbles to actually drain out of the sample and pass through the mesh. From a structural perspective, the foam structure of the ice cream, and its ability to inhibit serum drainage provides the most effective mechanism for controlling meltdown stability.

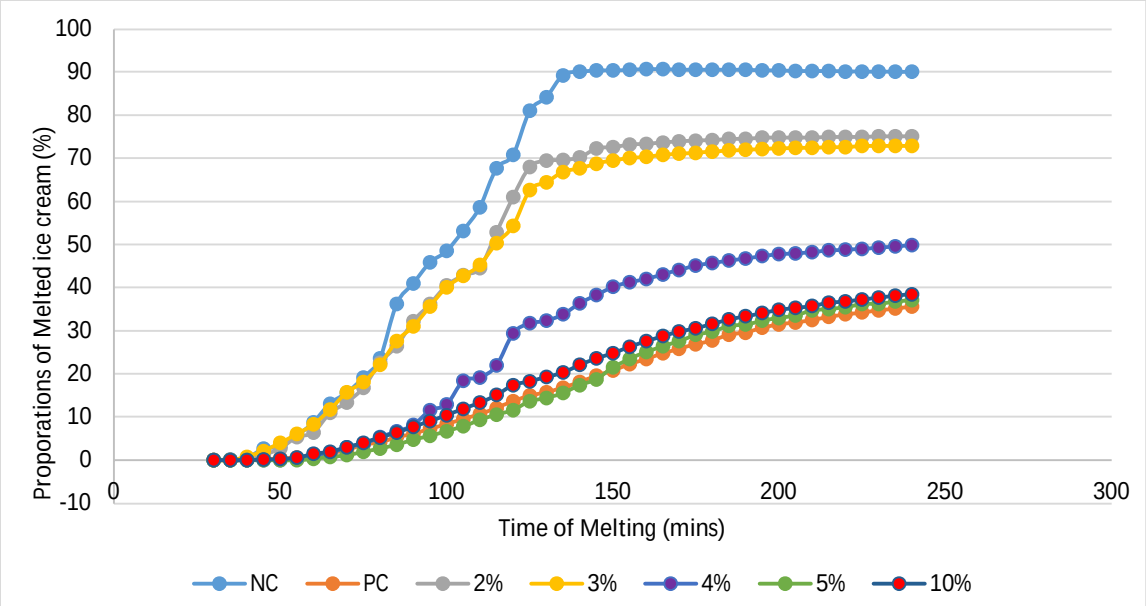


Figure 18. The line graph of meltdown test results. Samples indicate the relative amount of non-homogenised fat in the formulation; NC refers to natural control (i.e. protein-stabilised, homogenised 10% AMF formulation); PC refers to positive control (i.e. protein-stabilised, homogenised 10% AMF formulation containing monoglycerides)

Figure 18 shows the meltdown test of the ice cream samples from this study. It can be seen that melting is quickest for the natural control, noting that the sample has completely drained through the mesh after approximately 2 hours. In comparison, the positive control (i.e. the formulation comprising added monoglycerides) has only shown ~35% mass loss after 4 hours. Adding non-homogenised cream fat droplets to the formulation has a noticeable impact on melt stability, with even the addition of just 2% showing an improvement in melt stability. The rate of meltdown continues to slow down as more non-homogenised cream is added, and remarkably the samples containing either 5% non-homogenised fat droplets, or 10% non-homogenised fat were seen to have almost the same melting profiles as the positive control. Some care should be taken with

analysing these data, noting that there are differences in overrun between the samples which could also impact on the rate of melt.

However, it still seems reasonable to relate these findings to the higher levels of partial coalescence based on increasing non-homogenised cream content. The mechanism by which partial coalescence slows down the rate of melt is by increasing the tortuosity of the foam, making it more difficult for the liquid to drain out (in the same way that whipped creams can fully entrap the liquid continuous phase within the foam structure). Therefore, it can be deduced that the hypothesis of the study is reasonable. By the application of two-stream mixing, the non-homogenised cream added can successfully increase the partial coalescence of fat globules to generate a sufficiently firm and stable microstructure of ice cream and without leading to loss of quality due to excessive agglomeration. From all the three tests above, it can be concluded that, by the application of two-stream mixing, the non-homogenised cream added can successfully give the ice cream a similar microstructure and property as the functions of emulsifiers.

### 4.2.4 Microscopy test

Confocal microscopy can provide details of the microstructures of fat globules, air bubbles and their spatial arrangement. While the samples analysed here are of melted ice creams, we can still get some insights of the structural arrangements present in the product. Notably, we can observe residual air bubbles, and the size of the droplets can provide an indication as to the extent of partial coalescence (noting that for the melted samples, these can become fully coalesced entities due to the melting of the fat).

Images of the two control samples, natural control and positive control, are shown in Figure 19. From the observation of these two samples, we can construct the relationships between the extent of partial coalescence and the presentation of microstructures in microscopic images. In confocal microscopic images, fat globules are red, green particles are milk proteins and black circles are air bubbles. For the natural control, the majority of fat globules are relatively small and surrounded by green milk protein rings, along with a few, clearly larger droplets. This means whilst all fat globules are predominantly stabilized by milk proteins, some limited coalescence appears to have taken place, in line with particle size analysis. Air bubble lamellae are also mainly composed of proteins although a few fat globules can be seen adhered to the surface. This fully matches our analysis in the literature review and expectations for an ice cream sample without emulsifiers. For the positive control images, milk fat droplets appear noticeably larger, Fat globules seem naked without membrane. This might be due to the small size of emulsifiers which are too thin to be observed. Air bubbles are mainly coated by fat globules instead of milk proteins. Some denser regions of milk proteins can be seen in the serum phase and concentrate into clusters. There is no immediate explanation for this effect, but it may be a consequence of the replacement of interfacial protein by emulsifiers.

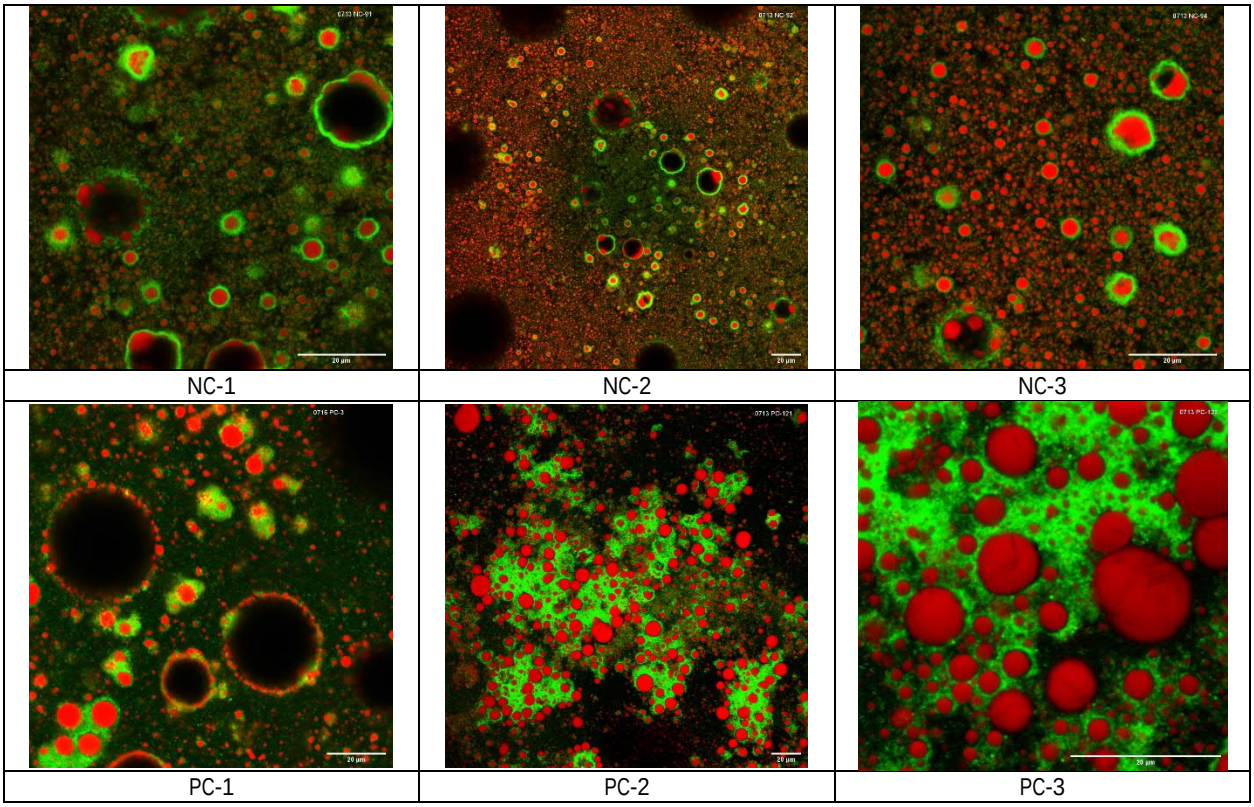


Figure 19. Microscopy test results of NC and PC samples.

Figure 20 shows the effects of increasing non-homogenised cream content on melted ice cream microstructure. Some images do not fully visualise all phases of fat, protein and air, however some observations can still be made. The most obvious trend is an increase in overall fat globule size with increasing non-homogenised fat content, in agreement with Mastersizer date. Air bubbles visualised in samples containing 4, 5 and 10% non-homogenised fat all show decoration with fat droplets, indicating the fat adsorption is taking place in a manner similar to that observed for the positive control. Also similar to the positive control is evidence of more dense regions of protein.

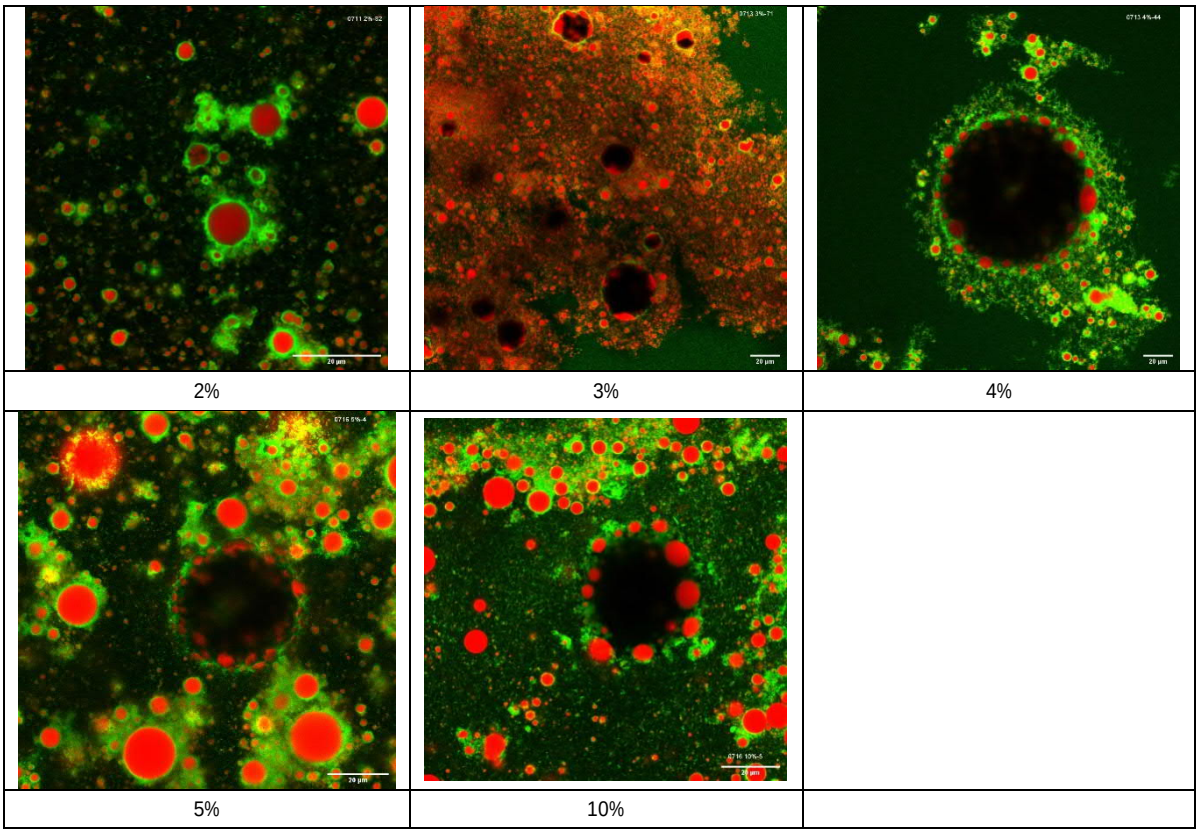


Figure 20. Comparisons of melted ice cream microstructures of samples comprising 2,3,4,5 and 10% non-homogenised milkfat globules.

From mechanistic perspective, the effectiveness of the mixed non-homogenised cream is mainly due to the large size of non-homogenised fat globules. They can strongly enhance partial coalescence of fat globules. Large fat globules in non-homogenised cream have more possibility of colliding with each other to let fat crystals be successfully wetted by oil in adjacent globules. In addition, large fat globules will also generate large fat crystals. They will protrude further and form more partially coalesced structures. All these factors will finally form a firmer, more extensive and stable network of fat globules, and can give ice cream a firmer and more solid texture. Findings indicate that the development of structure arising from the addition of non-homogenised cream is comparable to that of the use of commercial monoglycerides in ice cream formulation and can impart equivalent technical functionality.

## 4.3 Addition of fatty acids and monoglyceride replacement

As indicated earlier, the preliminary work to this study indicated that an older source of milkfat in the form of ghee was causing a higher than expected level of fat destabilisation in the freezing of the natural control ice cream mix. This fat destabilisation was attributed to higher than usual levels of fatty acids in the milk fat, which was confirmed using gas chromatography. This observation necessitated a reformulation of emulsions using AMF as part of this study, but also provided an additional hypothesis for producing ice creams without the need for synthetic monoglycerides. The observations from the preliminary study indicated that free fatty acids were able to act as surface active components in their own right, leading to protein displacement and subsequent partial coalescence. To test this hypothesis, a second set of ice cream mixes was developed in which three types of free fatty acids were added to formulations. These were all long chain molecules, palmitic (C16:0), stearic (C18:0) and oleic (C18:1) fatty acids, on the basis that these are of equivalent chain length to most commercially manufactured monoglycerides, and with the rationale that long chain fatty acids would least impact on sensory properties (even though sensory analysis was not part of this work).

Fatty acids were added at dosage levels of between 0.15 and 0.6% and ice creams manufactured with these formulations were analysed using the same tests as for the prior research.

### 4.3.1 Overrun test

| Sample | 0.3% Palmitic acid | 0.6% Palmitic acid | 0.3% Oleic acid | 0.6% Oleic acid | 0.3% Stearic acid | 0.6% Stearic acid | 0.15% Palmitic acid | 0.15% Stearic acid |
|---|---|---|---|---|---|---|---|---|
| Overrun value (%) | 110.23 | 51.36 | 52.77 | 70.63 | 70.82 | 67.07 | 147.59 | 145.52 |

Table 7. Overrun test results of all fatty acid samples.

Overrun measurements were carried out on samples with findings reported in Table 7. Whilst there was a broad distribution of overruns, ranging from ~50% up to ~150%, it is very difficult to draw any conclusions from these differences. Generally, overrun appears to be lower towards the higher FFA concentrations. Possibly the FFA may possess some specific antifoam capacity which make it difficult to effectively incorporate air, but in the absence of further testing, this is only a speculation.

### 4.3.2 Particle size measurements of melted ice cream

Particle size testing was carried out on melted ice creams, and findings are shown in Table 8, along with the overall distributions shown in Figure 21.

| Sample order | 0.3% Palmitic acid | 0.6% Palmitic acid | 0.15% Palmitic acid | 0.3% Oleic acid | 0.6% Oleic acid | 0.3% Stearic acid | 0.6% Stearic acid | 0.15% Stearic acid | Natural control | Positive control |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean of D (4,3), µm | 5.84 | 5.88 | 3.76 | 38.63 | 43.34 | 5.72 | 7.43 | 3.83 | 12.02 | 68.77 |
| Standard deviation of D (4,3), µm | 1.25 | 1.72 | 1.20 | 9.92 | 9.74 | 0.80 | 1.98 | 0.89 | 1.05 | 7.90 |

Table 8. The particle size test results of all fatty acid samples. The volume-weighted mean of particle size is presented as D [4,3], µm.

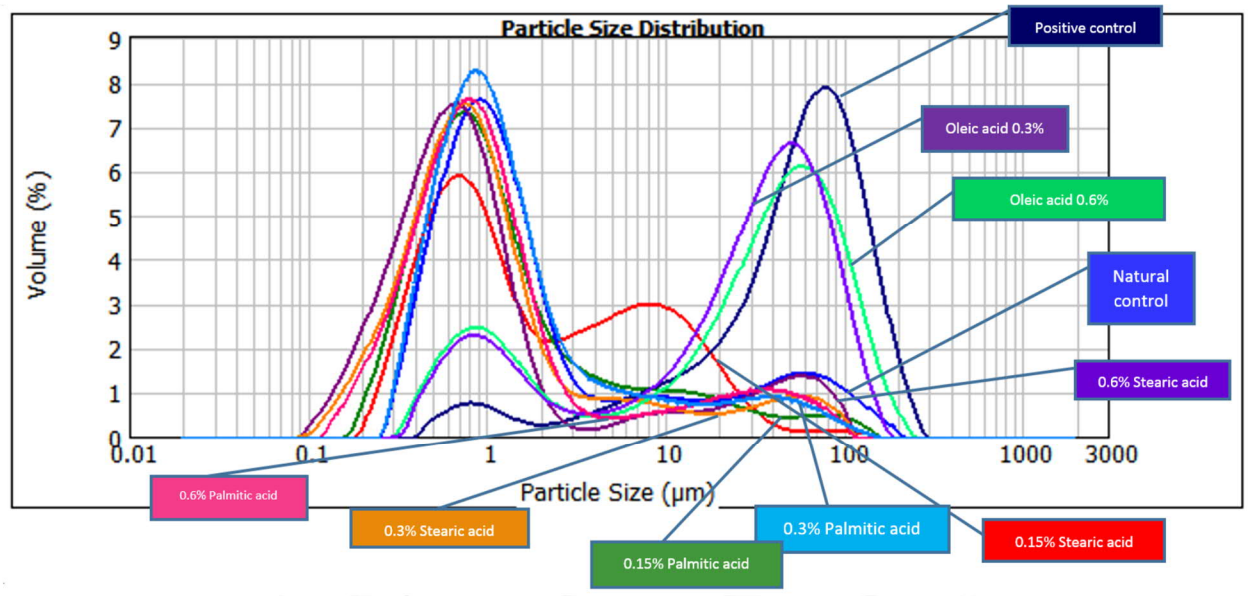


Figure 21. The particle distribution of melted ice cream sample with added fatty acids.

A curious observation was that the droplet size distribution of all ice cream mixes containing saturated fatty acids (i.e. palmitic and stearic) was lower than for the natural control ice cream. As discussed earlier, it is expected that even a protein-stabilised emulsion will undergo a certain amount of destabilisation during freezing; however, it seems unusual that the addition of saturated fatty acids to this emulsion actually serves to improve stability relative to the natural

control. Possibly this is due to formation of lipid mesophase structures at the interface, or maybe due to a reduction in initial droplet size prior to freezing based on lowering of the interfacial tension by the surface active FFA. In contrast, the droplet size of the melted ice cream containing oleic FFA showed a clear increase in particle size, although not to the extent of the positive control. Arguably, the unsaturated oleic fatty acids may have produced weaker patches at the oil-water interface, resulting in droplet destabilisation during freezing.

### 4.3.3 Meltdown test

Meltdown of ice cream samples containing added FFA are shown in Figure 22. Interpretation of these findings is made more difficult due to the large variation in overruns between samples, but it is possible to make some specific observations. We can clearly see that all ice creams comprising added saturated FFA were very quick to melt. In fact, these samples showed worse melt stability than the positive control. This can be related back to the particle size data that showed these samples to have the smallest particle size and least amount of fat destabilisation during freezing. The lack of fat agglomeration allows serum to drain more easily from the foam during melting, and so the foam collapses more quickly. In contrast, the ice cream samples incorporating oleic acid showed an improvement in melt stability compared to the natural control. Again, this can be related to the change in particle size distribution during freezing, with the oleic-based sample showing a higher amount of fat structuring than the natural control. Fat destabilisation for the oleic sample was also noted to be less than that of the positive control containing monoglycerides, and which had the best meltdown performance of all samples. Findings would suggest a clear relationship between the extent of fat destabilisation and melt stability.

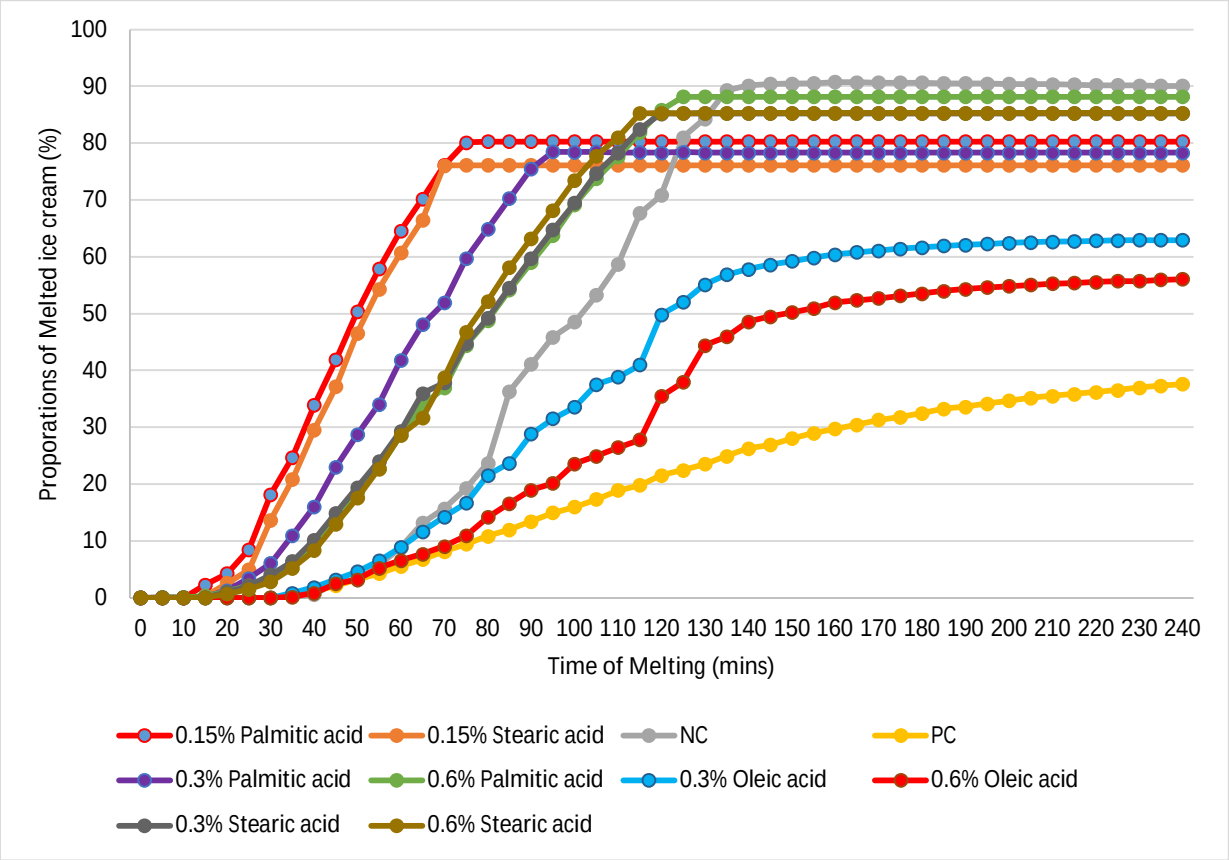


Figure 22. Meltdown results of all fatty acid samples.

### 4.3.4 Microscopy analysis of melted samples

Confocal microscopy results are shown in Figure 23. Oleic acid samples were seen to have the largest fat globules on average, which are around 5-10 μm. In addition, the larger size fat globules are very easily observed in oleic acid samples. For the other samples, only the 0.3% palmitic acid sample contains some larger fat globules of 1-2 μm. For the other saturated fatty acids samples, the fat globules are mostly very small and stable, in agreement with particle size state (Figure 21). In oleic acid sample, more air bubbles can be observed, and they are mostly stabilized by red fat globules. The fat globules at air lamellae are large and covered by milk proteins. Air bubbles can also be observed in other samples, however, most of air bubbles are stabilized by small fat globules and large amounts of milk proteins (Figure 24).

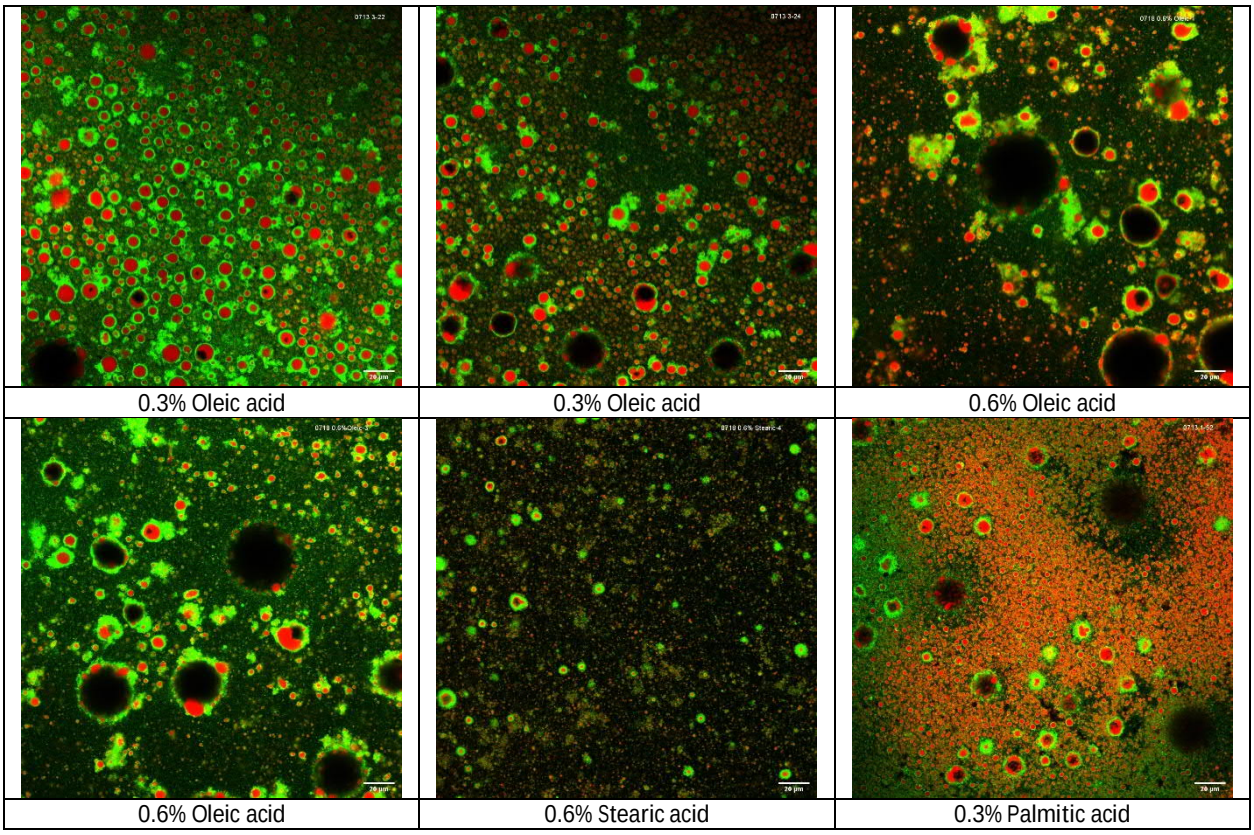


Figure 23. Confocal microscopy images of fatty acid ice cream samples. The large differences in the fat globule sizes among oleic acid, stearic and palmitic acid samples can be clearly observed.

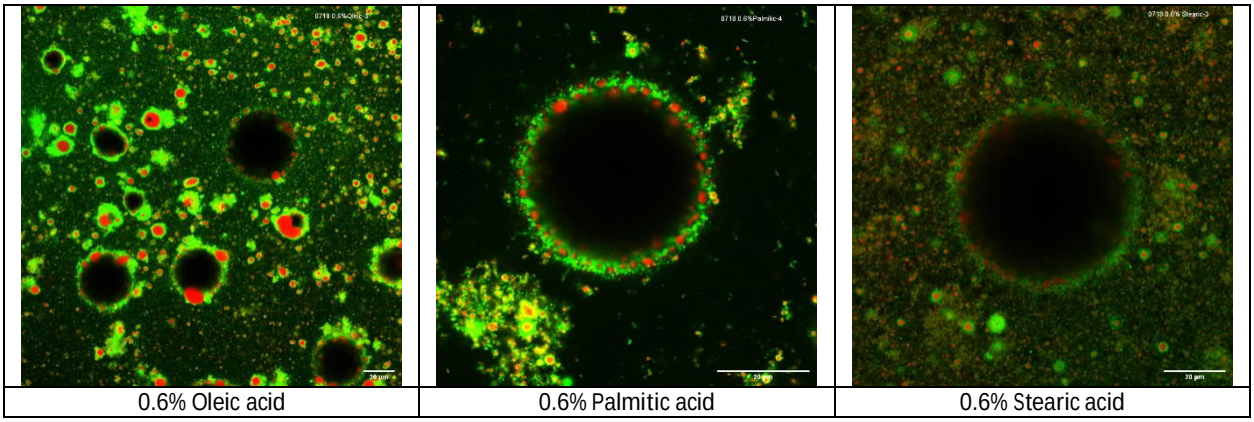


Figure 24. In contrast, the size of fat globules at air bubble interface in 0.6% oleic acid sample is the highest.

The observation the droplets comprising saturated fatty acids may adsorb to the surface of air bubbles (whilst not demonstrating any apparent partial coalescence) may also be impacting on the melt stability of the ice creams by reducing the effective phase volume of emulsion droplets

located in the serum phase. A similar observation has been made when high concentrations of saturated monoglycerides are used in ice cream formulations.

In summary, none of the three fatty acids applied in our ice cream formulation, were seen to be as effective as positive control sample, noting that the saturated fatty acids appeared to make the mix emulsion more stable to subsequent freezing, and with a corresponding increasing in melting rate. Incorporation of oleic acid was found to be closest to the commercial emulsifier function. From a manufacturing perspective, fatty acids are rarely used in food formulations. They are normally esterified with glycerol, sorbitol or sucrose to achieve highly effective emulsifying derivatives. Although there is insufficient information about fatty acids being emulsifiers, we can analyse and deduce the reasons of their performance. At a glance of their chemical formulas, they are very similar except for the double bonds in oleic structure. Their hydrophobic groups are dominant, while the hydrophilic groups are only the carboxylic acid, which may not be regarded as a realistic hydrophilic group. However, the effectiveness of oleic acid may be due to the double bond on the hydrocarbon chain that may influence adsorption and packing at the oil-water interface.

| Palmitic acid | Oleic acid | Stearic acid | GRINDSTED MONO-DI HP 40-M |
|---|---|---|---|
| 53.3 - 62.0 °C (From SIGMA specification) | <10 °C (From SIGMA specification) | 54.5 - 69.0 °C (From SIGMA specification) | 57 °C (melting point) (From DANISCO specification) |

Table 9. Comparisons of melting points of all fatty acids and emulsifiers applied in formulation.

This difference is highlighted when analysing the melting points of the different FFAs (Table 9), noting that both palmitic acid and stearic acid are in solid state during freezing, while oleic acid is liquid at freezer extrusion temperatures. Therefore, we may further suspect that the solidity of the FFA layer at the oil-water interface is the main point of difference in the behaviour of these emulsions during freezing.

However, the effectiveness of fatty acids should not be ignored completely. The functions of oleic acid are very impressive. As there exists co-emulsifiers in food formulations, the synergistic effect of fatty acids may also exist and needs to be proved.

# 5. Conclusions and recommendations for further study

The two hypotheses used in this study were validated to varying degrees as part of developing clean label strategies for the development of synthetic emulsifier-free ice creams. The effects of non-homogenised cream mixing on enhancing partial coalescence is well characterised and some properties of ice cream can reach to the same level as positive control. All tests show reasonable correlations, although the effects on overrun would benefit from further study. In regard to the replacement of monoglycerides with free fatty acids, oleic acid has the most beneficial properties with the saturated fatty acids actually accelerating rate of melt. Particle size test, meltdown test and microscopy test showed some trends in behaviour, while the results of overrun test were again more irregular. Future studies are recommended on the joint effects of fatty acids. More works on ice cream properties of storage and sensory are also recommended.

## Future study recommendations:

a. The compositional analysis of the old ghee should be done deeply to learn its specific composition. Trying to know whether there exist other components which may possess emulsifying functions.

b. Applying different combinations of fatty acids in different contents to study the synergistic effect of fatty acids.

c. Try to study other properties of emulsifier-free ice cream, such as storage stability and sensory assessment.

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# Appendix 1 - Ice cream formulations

In this project, a standard 10% fat formulation was used, and all modifications are based on this. This standard ice cream formulation is as below, and we used this as the positive control group:

## Positive Control

| Ingredients | % of total weight |
|---|---|
| Fat | 10.0 |
| Milk solids-not-fat | 11.0 |
| Sugars | 13.0 – 9% is sucrose and 4% is glucose syrup. |
| Monoglycerides | 0.5% |
| Guar gum<br>Locust bean gum | 0.1%<br>0.1% |
| Water | 65.3% |
| Total solids | 34.7% |

The natural control formulation was based on removal of the monoglyceride component:

## Natural Control

| Ingredients | % of total weight |
|---|---|
| Fat | 10.0 |
| Milk solids-not-fat | 11.0 |
| Sugars | 13.0 – 9% is sucrose and 4% is glucose syrup. |
| Guar gum<br>Locust bean gum | 0.1%<br>0.1% |
| Water | 65.8% |
| Total solids | 34.2% |
| | |

## 1. Modified ice cream formulation by mixing with non-homogenised cream part

For this part, as discussed above, different proportions of the non-homogenised cream will be mixed into the natural control formulation to enhance the partial coalescence activity.

From the ingredient list above, what needs to be kept in mind is, MSNF and water will also be introduced into the ice cream mix when introducing this non-homogenised fat.

| Component | % of the total weight |
|---|---|
| Fat | 35% |
| Protein | 2.2% |
| Sugars | 3.3% |
| Sodium salt | 0.03% |
| MSNF | 5.53% |
| Total solids | 40.53% |
| Water | 59.47% |

Therefore, to mix 2% non-homogenised cream into the natural control formulation, the calculation will be: (suppose the overall weight of ice cream mix is 7kg)

Non-homogenised cream is 2%, therefore the amount of cream introduced will be 2% × 7kg = 140g.

The fat content is 140g × 35% = 49g;

MSNF is 140g × 5.53% = 7.742g;

Water will be 140 – 49 – 7.742 = 83.258 ≈ 83.3g.

So, the amount of other components will be:

Fat: 7kg × 10% = 700g. As the fat content in non-homogenised cream is 49g, the amount of AMF needed will be 700 - 49 = 651g.

Sa the same, MSNF will be 7kg × 11% = 770g. 770 – 7.742 = 762.258 ≈ 762.3g.

Sucrose: 7kg × 9% = 630g.

Glucose syrup: 7kg × 4% = 280g.

Guar gum: 7kg × 0.1% = 7g.

Locust bean gum: 7kg × 0.1% = 7g.

Water: 65.8% × 7kg = 4606g. 4606-83.3 = 4522.7g.

Therefore, the formulation for 2% cream can be summarized as:

| Component | Weight (g) |
|---|---|
| Fat | 651 |
| Fat (from non-homogenised cream) | 49 |
| MSNF | 762.3 |
| MSNF (from non-homogenised cream) | 7.742 |
| Sucrose | 630 |
| Glucose syrup | 280 |
| Guar gum | 7 |
| Locust bean gum | 7 |
| Water | 4522.7 |

As the same, 3,4,5,10% will be (all select 7kg as the total weight):

3% cream

| Component | Weight (g) |
|---|---|
| Fat | 626.5 |
| Fat (from non-homogenised cream) | 73.5 |
| MSNF | 758.4 |
| MSNF (from non-homogenised cream) | 11.613 |
| Sucrose | 630 |
| Glucose syrup | 280 |
| Guar gum | 7 |
| Locust bean gum | 7 |
| Water | 4481.1 |

4% cream

| Component | Weight (g) |
|---|---|
| Fat | 602 |
| Fat (from non-homogenised cream) | 98 |
| MSNF | 754.5 |
| MSNF (from non-homogenised cream) | 15.484 |
| Sucrose | 630 |
| Glucose syrup | 280 |
| Guar gum | 7 |
| Locust bean gum | 7 |
| Water | 4439.5 |

5% cream

| Component | Weight (g) |
|---|---|
| Fat | 577.5 |
| Fat (from non-homogenised cream) | 122.5 |
| MSNF | 750.6 |
| MSNF (from non-homogenised cream) | 19.355 |
| Sucrose | 630 |
| Glucose syrup | 280 |
| Guar gum | 7 |
| Locust bean gum | 7 |
| Water | 4397.9 |

10% cream

| Component | Weight (g) |
|---|---|
| Fat | 455 |
| Fat (from non-homogenised cream) | 245 |

| | |
|---|---|
| MSNF | 731.3 |
| MSNF (from non-homogenised cream) | 38.71 |
| Sucrose | 630 |
| Glucose syrup | 280 |
| Guar gum | 7 |
| Locust bean gum | 7 |
| Water | 4189.7 |

## 2. Modified ice cream formulation by adding fatty acids part

In this part, we applied three different types of fatty acids to replace the monoglycerides in the classic formulation.

In practice, the weights of ice cream mix in different formulations are as below:

| | |
|---|---|
| 0.3% Palmitic acid | 10 kg |
| 0.6% Palmitic acid | 8 kg |
| 0.3% Oleic acid | 8 kg |
| 0.6% Oleic acid | 8 kg |
| 0.3% Stearic acid | 8 kg |
| 0.6% Stearic acid | 10 kg |
| 0.15% Palmitic acid | 8 kg |
| 0.15% Stearic acid | 8 kg |

### 0.3% Palmitic acid: 10 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 1.0 |
| MSNF (SMP) | 11.0 | 1.1 |
| Sugars (Total) | 13.0 | 1.3 |
| - Sucrose | 9.0 | 0.9 |
| - Glucose syrup | 4.0 | 0.4 |
| Palmitic acid | 0.3 | 0.03 |
| Guar gum | 0.1 | 0.01 |
| Locust bean gum | 0.1 | 0.01 |
| Water | 65.5 | 6.55 |
| Total mix | 100 | 10 |
| Total solids | 34.5 | 3.45 |

### 0.6% Palmitic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |

| | | |
|---|---|---|
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Palmitic acid | 0.6 | 0.048 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.2 | 5.216 |
| Total mix | 100 | 8 |
| Total solids | 34.8 | 2.784 |

0.15% Palmitic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Palmitic acid | 0.15 | 0.012 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.65 | 5.252 |
| Total mix | 100 | 8 |
| Total solids | 34.35 | 2.748 |

0.3% Oleic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Oleic acid | 0.3 | 0.024 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.5 | 5.24 |
| Total mix | 100 | 8 |
| Total solids | 34.5 | 2.76 |

0.6% Oleic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |

| | | |
|---|---|---|
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Oleic acid | 0.6 | 0.048 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.2 | 5.216 |
| Total mix | 100 | 8 |
| Total solids | 34.8 | 2.784 |

0.3% Stearic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Stearic acid | 0.3 | 0.024 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.5 | 5.24 |
| Total mix | 100 | 8 |
| Total solids | 34.5 | 2.76 |

0.6% Stearic acid: 10 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 1.0 |
| MSNF (SMP) | 11.0 | 1.1 |
| Sugars (Total) | 13.0 | 1.3 |
| - Sucrose | 9.0 | 0.9 |
| - Glucose syrup | 4.0 | 0.4 |
| Stearic acid | 0.6 | 0.06 |
| Guar gum | 0.1 | 0.01 |
| Locust bean gum | 0.1 | 0.01 |
| Water | 65.2 | 6.52 |
| Total mix | 100 | 10 |
| Total solids | 34.8 | 3.48 |

0.15% Stearic acid: 8 kg of mix.

| Component | % Total weight | Weight (kg) |
|---|---|---|
| Milk fat | 10.0 | 0.8 |
| MSNF (SMP) | 11.0 | 0.88 |
| Sugars (Total) | 13.0 | 1.04 |

| | | |
|---|---|---|
| - Sucrose | 9.0 | 0.72 |
| - Glucose syrup | 4.0 | 0.32 |
| Stearic acid | 0.15 | 0.012 |
| Guar gum | 0.1 | 0.008 |
| Locust bean gum | 0.1 | 0.008 |
| Water | 65.65 | 5.252 |
| Total mix | 100 | 8 |
| Total solids | 34.35 | 2.748 |