Characterizations of oil-in-water (O/W) emulsions containing different types of milk fats prepared using rhamnolipids as emulsifiers

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Abstract

Emulsions containing three different types of milk fat fractions (MF13, MF27 and MF42) and anhydrous milk fat (AMF) were prepared at oil to water (O/W) ratios of 1:9, 3:7, 5:5 and 7:3 using rhamnolipids as emulsifiers. The prepared emulsions were analyzed for their storage stability and properties (colour, particle size, zeta potential and rheology). The effects of various factors (freezing/thawing, heating, pH, salts and ionic strength) on the stability of emulsions were also investigated. All emulsions prepared with an O/W ratio of 7:3, regardless of the type of milk fat, rendered a highly condensed, semi solid and cream-like substance whereas other emulsions containing less oil were in a liquid form. Among the four different O/W ratios tested, the highest emulsion stability during the storage of 12 weeks was observed from the emulsions containing 1:9 O/W ratios, due to a combine effect of smaller emulsion particle size and lower collision frequency between droplets. Interestingly, the emulsions with 7:3 O/W ratios were found to be more stable than the ones with 5:5 O/W ratios. This might be due to the limited movements of closely-packed emulsion droplets induced by the high oil concentration of 7:3 O/W ratios. The emulsion stability was significantly affected by low pH, especially at lower than pH 4, due to the loss of electrostatic repulsions between droplets leading to droplet coalescence and also possibly due to hydrolysis of rhamnolipid molecules. The presence of salts (NaCl, KCl and $CaCl_2$) also rendered the emulsion unstable. The degree of instability was gradually increased with increasing salt concentrations. $CaCl_2$ had the most significant effect even at a very low concentration. The viscosity of emulsions increased with increasing oil concentration but was not affected by the types of milk fats. Emulsions with 3:7, 5:5 and 7:3 O/W ratios exhibited non-Newtonian and shear thinning flow behaviour. At 7:3 O/W ratios, MF13 exhibited gel-like properties whereas both MF42 and AMF emulsions became more solid-like at higher frequency.

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Chapter 1. Introduction

Biosurfactants are surface active compounds produced by microorganisms. They are amphiphilic molecules containing both hydrophobic and hydrophilic groups, thus having the ability of reducing the interfacial tension between oil and water (Rosenberg and Ron 1999). Biosurfactants are increasingly recognized as important natural products that have significant potential for their use in a vast variety of applications. As the society become more environmentally conscious, biosurfactants are regarded as potential replacements for chemically synthesized surfactants because they are biodegradable, low or non-toxic natural compounds, hence less impact on the environment (Banat and others 2000). It has been predicted that 10% of the surfactant market will be occupied by biosurfactants by 2010, reaching $200 million US dollars in sales (Nitschke and others 2005).

Among different types of biosurfactants, rhamnolipids are types of glycolipid biosurfactants produced by the bacteria, *Pseudomonas aeruginosa*. They are one of the best studied biosurfactants and have shown high production yields and large potential for commercial developments (Maier and Soberon-Chavez 2000; Nitschke and others 2005). At the moment, rhamnolipids are mainly used in bioremediations and biological controls (Maier and Soberon-Chavez 2000). Although there are patented studies (Ishigami and others 1988a; Ishigami and others 1988b; Van Haesendonck and Vanzeveren 2004) using rhamnolipids as additives in cosmetic products and liposome formation and as softening agents in bakery products, there is no specific information on using rhamnolipids as emulsifiers in preparing oil-in-water emulsions available.

Emulsions are dispersed systems in which one liquid phase is dispersed in another liquid phase by the aid of emulsifiers. In oil-in-water emulsions, oil is the dispersed phase and water is the continuous phase whereas in water-in-oil emulsions, water is in return dispersed in an oil phase. Many products in our everyday life exist in the form of emulsions such as milk, ice-cream, mayonnaise and cosmetic cream. In an emulsion system, emulsifier is one of the most important substances as it determines the formation, stability and physicochemical properties of emulsions. Proteins are currently the single most abundantly used class of emulsifiers in the food industry (Wilde and others 2004). Some other commonly used emulsifiers are sorbitan fatty acid esters

(Span), Tweens and polysaccharides (Owusu Apenten and Zhu 1996; Quintana and others 2002a; Quintana and others 2002b; Granger and others 2003).

In this study, the ability of rhamnolipids as an emulsifier in the making and stabilizing oil-in-water emulsions was investigated for the potential of their use in food applications. Different types of milk fat fractions, including low melting temperature fraction (MF13), high melting temperature fraction (MF42) and anhydrous milk fat (AMF), were used as an oil phase in the preparation of emulsions at various ratios of oil to water. The emulsions prepared were evaluated for their properties (particle size and size distribution, zeta potential, colour and viscosity), stability during storage and changes in the presence of external factors (pH, salt, ionic strength, heating and freeze-thawing).

Chapter 2. Literature Review

2.1 Biosurfactants

Biosurfactants have been recognized as important microbial products that can be used in a variety of industrial applications as alternatives for chemically synthesized surfactants. Major usages of biosurfactants up to date have been in petroleum-related activities such as enhancing oil recovery, cleaning oil spills and oil emulsifications (Kosaric and others 1987). Applications of biosurfactants are still under development and there are potential applications of biosurfactants in a number of fields, including agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing, textile manufacturing, laundry supplies, metal treatment and processing, pulp and paper processing and paint industries (Banat and others 2000).

2.1.1 Types of Biosurfactants

Biosurfactants have the capability of lowering the surface tension of water and the interfacial tension of oil-water mixtures due to their amphiphilic structures consisting of both hydrophilic and hydrophobic groups. The hydrophobic part consists of saturated, unsaturated and/or hydroxylated fatty acids or fatty alcohols, whereas the hydrophilic part consists of mono- oligo- or polysaccharides, peptides and proteins (Lang 2002). Biosurfactant molecules are able to form micelles or reverse micelles, rod-shaped micelles, bilayers and vesicles (Fiechter 1992). The parameters used to assess the effectiveness of biosurfactants are the minimum surface tension value reached and the critical micelle concentration (CMC) needed (Fiechter 1992).

Biosurfactants are characterized, based on their functional groups, into
a) glycolipids
b) lipoamino acids and lipopeptides
c) polymers, which include lipoproteins and lipopolysaccharides
d) phospholipids, mono-, di-glycerides and fatty acids (Lang and Wullbrandt 1999).

They can also be divided into two categories according to their molecular mass: low-molecular-mass biosurfactants (e.g. glycolipids and lipopeptides) and high-molecular-mass biosurfactants (e.g. polymers and phospholipids). Low-molecular-mass biosurfactants can lower surface and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective in stabilizing oil-in-water emulsions (Rosenberg and Ron 1999).

Glycolipids

Glycolipids are low-molecular-mass biosurfactants, which include rhamnolipids, trehalolipids and sophorolipids. They are acylated disaccharides with long chain or hydroxyl fatty acids (Rosenberg and Ron 1999).

Rhamnolipids are produced by *Pseudomonas aeruginosa*. The chemical structures, production, application, chemical and physical properties of rhamnolipids are explained more in details in section 2.2.

Sophorolipids are glycolipids produced by yeast. Several species of yeast *Candida (Torulopsis)* are capable of producing sophorolipids. Figure 2.1 shows an example of the structure of sophorolipids. They consist of two glucose units linked by a β-1, 2 linkage and the 6- and 6′-hydroxyl groups are generally acetylated. The lipid portion and the reducing end are connected by a glycosidic bond and the terminal carboxyl group exhibits in a lactonic form (Rosenberg and Ron 1999). High yields of sophorolipids are obtained by *Candida (Torulopsis) bombicola* ATCC 22214 when cultivated on glucose and oil. Most biosurfactants are generally produced during the late exponential phase. Sophoroselipids are capable of lowering surface and interfacial tensions but are not effective emulsifying agents (Cooper and Paddock 1984). They can lower the surface tension of water from 72 mN/m to 30-60 mN/m (Lang 2002).

Mannosylerythritol lipids are glycolipids produced by yeast *Pseudozyma antarctica*. It is one of the most promising biosurfactants and mainly produced from vegetable oils (Lang 2002). They have the ability to reduce the surface tension of water to 32 mN/m (Adamczak and Bednarski 2000) and exhibit a good emulsifying activity (Kitamoto and others 2001).

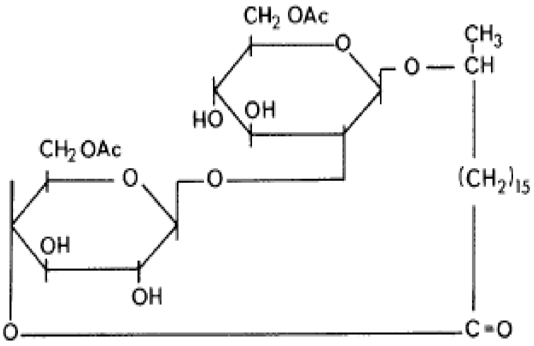


Figure 2.1 Structure of lactonic sophoroselipid produced from *Candida torulopsis bombicola* (Rosenberg and Ron 1999)

Lipopeptides

Examples of lipopeptides are surfactin, polymixin, iturin and fengycin. The general structure of the lipopeptide consists of a peptide cycle of amino acids esterified to a fatty acid chain (Lang 2002). Figure 2.2 shows the molecular structure of surfactin. They are produced by *Bacillus subtilis* and have antibiotic properties. Surfactin is the most well-known lipopeptide and exhibits a wide range of antibacterial spectrum, whereas iturin and fengycin have anti-fungal properties. Surfactin is also able to lower the surface tension of water from 72 mN/m to 27 mN/m (Lang 2002).

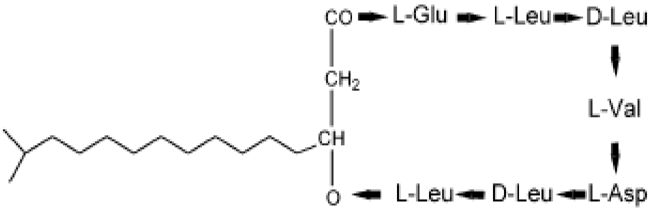


Figure 2.2 Structure of surfactin from *Bacillus subtilis* (Lang 2002)

Bacillus polymyxa and related *bacilli* produce polymixins, which are a group of related lipopeptide antibiotics (Rosenberg and Ron 1999). An example of polymixins - polymyxin B is shown in Figure 2.3.

Polymers

Some bacterial species produce polymeric substances such as polysaccharides, proteins, lipopolysaccharides and lipoproteins. These extracellular surfactants are high-molecular-weight biosurfactants and are called bioemulsans (Rosenberg and Ron 1999).

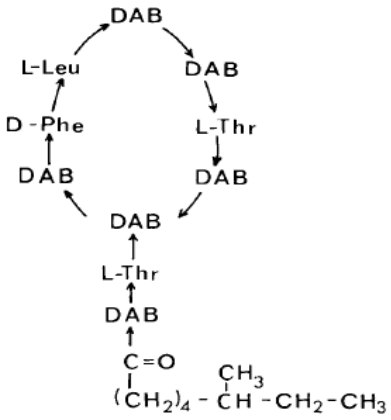


Figure 2.3 Polymyxin B. DAB 2, 4-diaminobutyric acid (Rosenberg and Ron 1999)

The most widely studied bioemulsan is a lipopolysaccharide isolated from *Acinetobacter calcoaceticus* RAG-1 ATCC 31012 (Lang 2002). It has a large molecular weight and is an effective emulsifier at low concentration. The fatty acids account for about 15% of the emulsan's dry weight and are the major contributors to the surface activity (Rosenberg and Ron 1999). A maximum amount of emulsan is produced when RAG-1 cells are grown in medium containing 2% ethanol as carbon and energy sources (Rosenberg and Ron 1999). The majority of the emulsans produced are excreted during the stationary phase of cell growth (Goldman and others 1982).

Alasan is a bioemulsan produced by *Acinetobacter radioresistens* KA 53. It is a high-molecular weight complex composed of proteins and polysaccharides. The protein portion is responsible for the emulsifying activity as the pure polysaccharide portion (apo-alasan) has very low emulsifying activity (Toren and others 2001). Hot phenol or specific proteinase treatments of alasan lead to deproteinization and a loss of emulsifying activity. Temperature has a large influence on the viscosity and emulsifying activity of alasan. At temperatures between 30°C and 50°C, the viscosity of alasan increases by 2.6 times but there is no change in emulsifying activity. At higher temperatures (50°C to 90°C), the viscosity of alasan solution decreases 4.8 times and there is a five-fold increase in emulsifying activity. These changes are due to the interactions of proteins and polysaccharides of the complex mixture (Rosenberg and Ron 1999).

Yeast *Candida lipolytica* produces an extracellular emulsifier called liposan when it is grown on glucose (Cirigliano and Carman 1984). It is a complex biopolymer containing proteins, carbohydrates and lipids. When grown in a batch culture containing glucose as a carbon source, the maximum production activity of liposan occurs during the stationary phase of cell growth (Sarubbo and others 2001).

Biodispersan is an extracellular anionic polysaccharide biopolymer produced by *Acinetobacter calcoaceticus* A2. It is capable of dispersing limestone and titanium dioxide effectively (Rosenberg and others 1988a; Rosenberg and others 1988b).

Phospholipids and Fatty Acids

Some fatty acids are recognized to have surfactant properties. The length of fatty acid hydrocarbon chain determines the hydrophilic/hydrophobic balance. Fatty acids in the range of C12-C14 are most active for lowering the surface and interfacial tensions (Rosenberg and Ron 1999). Some of the surface active fatty acids produced by microorganisms are not simple straight-chain acids but are complex branched-chain isomers (Macdonald and others 1981). Corynomycolic acids are potent surfactants produced by *Corynebacterium lepus* (Macdonald and others 1981). Their surface properties are not greatly influenced by pH and ionic strength (Rosenberg and Ron 1999).

Phospholipids are the major components of cell membranes (Rosenberg and Ron 1999). The phospholipids of *Rhodococcus erythropolis* DSM 43215 exhibit surfactant properties when the bacteria are grown on n-alkanes as the sole carbon source. Among the extracted surfactant mixture, phosphatidylethanolamine is the most potent surfactant and capable of lowering the interfacial tension of the water-hexadecane system to below 1 mN/m (Kretschmer and others 1982). Phospholipids produced by *Thiobactillus thiooxidans* have a superior wetting ability of sulphur (Beebe and Umbreit 1971).

2.1.2 Natural Roles of Biosurfactants

As described above, biosurfactants are produced by a wide range of various microorganisms and exhibit very different chemical structures and properties. It is possible that different groups of biosurfactants have different natural roles in the physiology of microorganisms (Ron and Rosenberg 2001). However, the natural roles of biosurfactants are not clearly understood although some theories have been suggested for the reasons of the generation of biosurfactants by microorganisms (Rosenberg and Ron 1999).

One of the possible reasons that microorganisms produce biosurfactants is to enhance the emulsification and solubilization of hydrocarbon substrates in aqueous environment, which are needed for their growth. Microorganisms that grow on non-polar medium as a sole carbon source excrete biosurfactants to ensure the supply of carbon source (Lin 1996). The interfacial surface between water and oil influences the growth of microorganisms. The growth rate is reduced when the surface area of oil phase becomes limited (Ron and Rosenberg 2001). This means an increase in the bioavailability of hydrophobic substrates. The low water solubility of hydrophobic compounds limits the availability of water-insoluble substrates to microorganisms for its biodegradation. By producing surfactants that lower the surface tension of water and oil, microorganisms create more hydrophobic compounds to become available for biodegradation (Ron and Rosenberg 2001).

The cell surface structures of microorganisms regulate the attachment and detachment of microbes to a proper surface is another possible reason to account for the production of biosurfactants. This is essential to their survival and growth (Rosenberg and Ron 1999). The influences of biosurfactants on the interactions between bacteria and interface have been studied (Neu 1996). Surfactants excreted by microorganisms can form a conditioning film on interface and stimulate or prevent the attachment of certain other microorganisms. They can also regulate the detachments of cells from surfaces when necessary (Rosenberg and Ron 1999). Some cell-bound biosurfactants are also capable of transporting hydrocarbon that are required for growth and changes of the hydrophobicity of surfaces of microbial cells (Lin 1996; Rosenberg and Ron 1999).

Some biosurfactants are potent antibiotics, such as surfactin produced by *Bacillus subtilis* and polymixins produced by *Bacillus polymyxa*. The release of antibiotics ensures better chance of survival in the environment (Lin 1996).

2.1.3 Applications of Biosurfactants

Biosurfactants produced by microorganisms play important roles in many different industrial sectors and have been used in many industrial applications, including oil industry, soil and marine bioremediation, textile and paper industry, agricultural industry and food industry (Banat and others 2000). They are regarded as potential substitutes to the petroleum–based synthetic surfactants as there have been a growing awareness of the importance of protecting the ecosystem and environment and complying with environmental regulations (Banat and others 2000). The increasing consciousness of reducing artificial ingredients in food by consumers increases the needs of natural food additives. Some natural food emulsifiers, such as lecithin and gum arabic, suffer processing and climatic limitations. Therefore, there is also a huge potential for biosurfactants from microorganisms to be utilized in the food industry (Shepherd and others 1995).

Oil Industry

Petroleum and oil industry is the biggest possible market for biosurfactants (Banat and others 2000). There is a large potential for biosurfactant applications in microbial enhanced oil recovery (MEOR) (Banat and others 2000). Oil recovery by conventional pumping technique can only achieve 30-50% recovery of oil from petroleum resource. Much higher values may be achieved when steam and fire flooding techniques are used. The major factor in oil recovery is to lower the interfacial and surface tension between water and oil in the ground. Desirable biosurfactants can replace petroleum sulfonates and lignosulfonates, which are commonly used at the moment but are relatively expensive (Fiechter 1992). Microorganisms for MEOR should be able to grow in conditions such as high temperature, high pressure, high salinity and low oxygen level (Cameotra and Makkar 1998). Several bacteria which favour these conditions have been successfully isolated, including two strains of *Bacillus subtilis,* MTCC 1427 and MTCC 2423 (Banat and others 2000).

Biosurfactants are also useful for bioremediation, such as removing oil from oil spill incidents and combating oil pollution in seas and beaches (Banat and others 2000). The effectiveness of hydrocarbon-degrading microorganisms in removing oil pollution has been reported by several authors (Gutnick and Rosenberg 1977; Mattei and others 1986;

Harvey and others 1990; Leahy and Colwell 1990; Atlas 1991). Biosurfactants enhance the degradation of hydrocarbons by emulsifying them in water.

Other oil industry-related applications of biosurfactants include the removal of oil sludge in oil storage tanks/pipes and the recovery of hydrocarbons from oil sludge, which are economical, environmental friendly and less hazardous for the people involved (Banat and others 1991; Linnéa and others 1992; Banat and others 2000). Studies have also shown that biosurfactants can enhance the transportation and pipelining of crude oil by reducing the viscosity (Zajic and others 1974; Hayes and others 1986; Banat and others 2000).

Agricultural Industry

Some biosurfactants have been studied as alternatives to chemical pesticides due to concerns about pesticide pollution. Rhamnolipids produced by *Pseudomonas aeruginosa* have been shown to have the potential to biologically control zoosporic plant pathogens (Stanghellini and Miller 1997; Banat and others 2000). Biosurfactants are also used in the formulation of poor water soluble organophosphorus pesticides, and the hydrophilization of heavy soils to achieve good wettability, thus enabling an equal distribution of fertilizers/pesticides (Banat and others 2000).

Cosmetic Industry

There are potentials for biosurfactants in the cosmetic and skin care market. There is a skin moisturizer containing sophorolipid, which is also used as a humectant in cosmetics (Yamane 1987; Banat and others 2000).

Food Industry

Emulsifiers play important roles in many food applications. Some biosurfactants can be used as an emulsifier in the food industry and have the same or better emulsifying ability than the commonly used food emulsifiers, such as gum arabic and carboxymethylcellulose (Shepherd and others 1995). A lipophilic polysaccharide biosurfactant produced by *Candida utilis* has been tried to be incorporated into salad cream making. It showed excellent emulsifying properties and the salad cream prepared with this biosurfactant was more stable than the control samples, which were prepared with a reduced amount of egg and no stabilizer (Shepherd and others 1995).

Rhamnolipids has been added to bakery products to improve the dough stability, such as texture and volume, and claimed to improve the quality of butter cream and frozen confectionary products (Van Haesendonck and Vanzeveren 2004; Nitschke and Costa 2007).

A biosurfactant exopolysaccharide (EPS 71a) from *Enterobacter cloacae* is considered to have a potential as a viscosity enhancing agent in the food industry. EPS 71a showed a high and stable emulsifying ability at various pHs. This is because the emulsions stabilized with EPS 71a showed stability over a range of pH 2 - 10. It could be used in food products containing edible acids such as citric acid and ascorbic acid. However it has not been approved yet as a food grade additive for its use in food applications (Iyer and others 2006).

2.2 Rhamnolipids

Pseudomonas aeruginosa produces two main forms of rhamnolipids. They are monorhamnolipids and dirhamnolipids (Nitschke and others 2005). Monorhamnolipids are L-α-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate, known as RL_1. It consists of one molecule of rhamnose and two molecules of β-hydroxydecanoic acid. Dirhamnolipids are L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate, known as RL_2. It is composed of two molecules of rhamnose and two molecules of β-hydroxydecanoic acid (Lang and Wullbrandt 1999). There are two other forms of rhamnolipids that are similar to RL_1 and RL_2 but contain only one β-hydroxydecanoic acid unit. These are known as RL_3 and RL_4, respectively (Nitschke and others 2005). There are also two other forms designated as rhamnolipid A and rhamnolipid B. These are similar to RL_1 and RL_2 with the exception that they have an additional acylation on a rhamnose ring structure by α-decenoic acid (Yamaguchi and others 1976; Lang and Wullbrandt 1999). The chemical structures of RL_1, RL_2, RL_3, RL_4, rhamnolipid A and rhamnolipid B are shown in Figure 2.4.

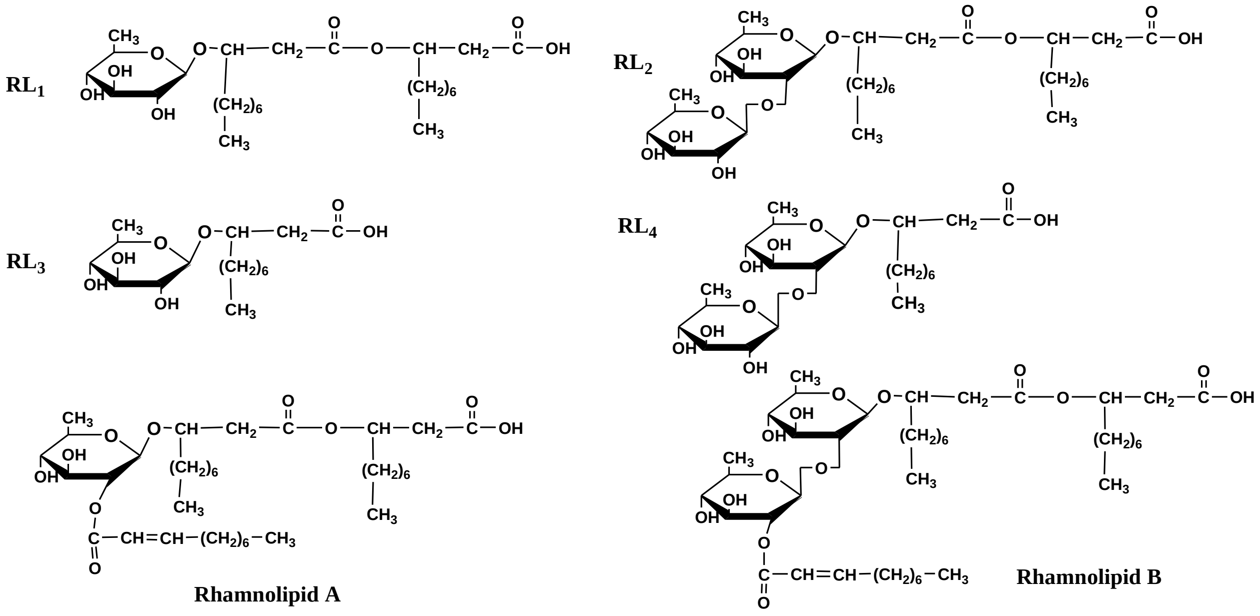


Figure 2.4 Structures of different types of rhamnolipids

2.2.1 Properties of Rhamnolipids

Rhamnolipids are amphiphilic molecules consisting of both hydrophobic and hydrophilic groups. The rhamnosyl and carboxylic groups of rhamnolipids are hydrophilic, whereas the fatty acid chain situated between the rhamnosyl and the carboxylic groups have the hydrophobic characteristics (HelvacI and others 2004). Rhamnolipids are an ionic type of biosurfactants (Cohen and Exerowa 2007). The carboxylic group determines the anionic character of rhamnolipids and makes rhamnolipids sensitive to pH and ionic strength (Ishigami and others 1993). Rhamnolipids are soluble in water and exist as anions at pH above 6.8 (Ozdemir and others 2004). At pH 5, rhamnolipids exist in the non-ionized form. A decrease in the pH of aqueous rhamnolipid solutions leads to an increase in the packing density and a decrease in the surface area of molecules, which results in the lack of electrostatic repulsion between molecules (Ozdemir and others 2004; Cohen and Exerowa 2007).

In other words, the morphology of rhamnolipids changes with the pH of the surrounding environment (Ishigami and Suzuki 1997). Increasing pH increases the negative charges of the carboxylic polar head groups of rhamnolipid molecules, which leads to stronger repulsive forces between the head groups and results in larger head group diameters.

The increase in ionic strength in the surrounding environment also leads to a decrease in the electrostatic repulsions between the carboxylate head groups of rhamnolipid molecules due to the shielding of counter ions. Therefore the rhamnolipid molecules exhibit non-ionic behavior (HelvacI and others 2004).

One of the most well known functions of rhamnolipids is their ability to lower the surface tension of water, due to their amphiphilic structures (Nitschke and others 2005). They reduce the surface tension of water from 72 mN/m to less than 30 mN/m and the water/oil surface tension from 43 mN/m to less than 1 mN/m (Parra and others 1989). The surface active property of rhamnolipids is determined by the ratio of different types of rhamnolipids, the presence of unsaturated bonds, the length of alkyl chain and the size of the hydrophilic group (Nitschke and others 2005). Rhamnolipids are also shown to have emulsifying properties (Patel and Desai 1997). They are capable of lowering the interfacial tension of water and hydrocarbon mixture by forming micro-emulsions. The emulsions formed with a variety of hydrocarbons from hexane to octadecane using rhamnolipids as emulsifiers have been shown to be stable for up to 48 hours (Patel and Desai 1997).

2.2.2 Production of Rhamnolipids

There are several methods available for the production of rhamnolipids via a microbial fermentation process by *Pseudomonas aeruginosa,* such as (Fed-) batch cultivation under growth limiting conditions, batch cultivation under resting cell conditions, semi-continuous production with immobilized cells (without nitrogen source) and continuous cultivation and production with free cells (Lang and Wullbrandt 1999). Various substrates are used as a carbon source (glycerol, glucose, n-alkanes and triglycerides) and a nitrogen source (ammonium and nitrate ions) (Syldatk and Wagner 1987).

A high amount of rhamnolipids is produced by growing the bacteria cells under growth-limiting conditions, such as a restrained source of nitrogen and multivalent ions (Guerra-Santos and others 1986; Nitschke and others 2005). The synthesis of rhamnolipids starts when the nitrogen source is depleted and the microbial cells enter the stationary phase (Ramana and Karanth 1989; Lang and Wullbrandt 1999). Carbon sources either induce or repress the synthesis of rhamnolipids (Nitschke and others 2005). It was discovered that the production of rhamnolipids was much higher in *Pseudomonas aeruginosa* UG2 grew on water immiscible substrates such as corn oil and long chain alcohol than those that grew on water soluble substrates, such as glycerol and glucose (Mata-Sandoval and others 2000; Nitschke and others 2005).

The microbial growth conditions, such as temperature, pH, agitation and oxygen are also factors affecting the production of rhamnolipids (Nitschke and others 2005). The production of rhamnolipids is optimal at pH 6 - 6.5 and drops significantly at pH above 7 (Guerra-Santos and others 1984; Nitschke and others 2005). The optimum temperature for the production of rhamnolipids is 37°C (Robert and others 1989; Nitschke and others 2005).

Using low-cost substrates is important in facilitating the industrial development of biosurfactants (Nitschke and others 2005). Rhamnolipids can be produced from renewable resources such as vegetable oil. There are other alternate substrates suitable for their production that are cheaper, including residues from vegetable oil refinery, which is the most commonly used low cost substrate (Nitschke and others 2005), olive oil mill effluent (Mercadé and others 1993) and used frying oils (Abalos and others 2001).

2.2.3 Applications of Rhamnolipids

Rhamnolipids are one of the most important classes of biosurfactants because of its high production yield, use of relatively cheap substrates, such as carbohydrates, vegetable oils and some waste from the food industry and large potential for commercial developments

Chemical

Rhamnolipids are a main source of L-rhamnose, which is a starting material for the synthesis of certain organic compound and flavor compounds (Linhardt and others 1989). Rhamnose can also be obtained from other sources, such as naringin from citrus peels, oak bark and rutin from a variety of plants. However, production from other sources showed many limitations when compared to production from rhamnolipids (Maier and Soberon-Chavez 2000).

Bioremediation

Many researches have been carried out in utilizing rhamnolipids for the treatment of contaminants. The addition of rhamnolipids enhances the breakdown of hexadecane, octadecane, n-pradaffin and phenanthrene in liquid systems, and mixtures of hexadecane, tetradecane, pristine, creosote and hydrocarbon in soils (Maier and Soberon-Chavez 2000; Mulligan 2005). It has been suggested that there are two possible mechanisms involved in the enhancement of biodegradation by rhamnolipids. The first one is that rhamnolipids increase the hydrocarbon solubility, thus increasing the bioavailability to degrading cells (Zhang and Miller 1992; Maier and Soberon-Chavez 2000). The second one is that rhamnolipids interact with the degrading cells and increase the hydrophobicity of cell surface, thereby the cells associate with hydrophobic substrates more easily (Zhang and Miller 1994; Maier and Soberon-Chavez 2000).

Polynuclear aromatic hydrocarbons (PAHs) are produced from petroleum refinement, coal production and wood preservation (Mulligan 2005). The biodegradation of PAHs is slow and sometimes infeasible. Rhamnolipids can be used as flushing agents in soil washing or soil flushing to remove such substances (Maier and Soberon-Chavez 2000). It has been shown that rhamnolipids are found to be more effective in removing hydrocarbons from soils than the chemical surfactants Triton X-100 and Tween 60 (Scheibenbogen and others 1994; Maier and Soberon-Chavez 2000). Rhamnolipids have also been studied for the removal of heavy metals in contaminated soils (Maier and Soberon-Chavez 2000).

Agricultural Industry

Rhamnolipids have antimicrobial and antifungal activities. They are effective antifungal agents against *Aspergillus niger, Gliocadium virens, Chaetonium globusum, Penicillium crysogenum* and *Aerobacidium pullulants*, and inhibit the growth of *Botryis cinerea* and *Rhizotecnia solani* (Abalos and others 2001; Nitschke and others 2005). Rhamnolipids are also highly effective against zoosporic plant pathogens, *Pythium aphanidermatum*, *Phytophthora capsici* and *Plasmopara lactucae-radicis* (Maier and Soberon-Chavez 2000). A biofungicide formulation containing rhamnolipids which was approved by

FDA has been developed to prevent plant pathogenic fungi. It can be used in fruit, vegetables and legume crops because of its low acute mammalian toxicity and non-mutagenicity (Nitschke and Costa 2007).

Cosmetic Industry

It has been shown that rhamnolipids have emulsifying properties and are capable of emulsifying hydrocarbons and stabilizing emulsions (Nitschke and others 2005). However, they are low-molecular-mass biosurfactants and therefore are not as effective as the high-molecular-mass biosurfactants, such as bioemulsans. The cosmetic industry uses a large quantity of chemically synthesized surfactants in a variety of products. Rhamnolipids offer several advantages over the synthetic ones, including low irritancy, anti-irritating effects and compatibility to skin (Kleckner and Kosaric 1993).

Food Industry

There have been several studies on the use of rhamnolipids for their applications in bakery products through improving the dough or batter stability by increasing their shock resistance to the mechanical operation of bread-making process. It has been shown that the volume of bake products, such as bread, cake and sponge cake and the structure of the crust and crumb during the process of baking can be improved and the microbial deterioration of bakery products can be reduced. Rhamnolipids have been shown to improve the properties of butter cream, decoration cream, non dairy cream filling and other fresh or frozen fine confectionery products (Van Haesendonck and Vanzeveren 2004).

2.3 Emulsions

An emulsion is a colloidal dispersion of two immiscible liquids, such as oil and water, in which one liquid is dispersed in another (McClements 2005). There are two types of simple emulsion systems, oil-in-water (O/W) emulsions and water-in-oil (W/O) emulsions. In O/W emulsions, oil is dispersed in a continuous water phase, whereas in W/O emulsion, water is the dispersed phase in an oil phase. Food products existing in

O/W emulsions include milk, mayonnaise, salad dressing and sauces. Butter and margarines are W/O emulsions.

Emulsifiers are essential in the formation and stabilization of emulsions. They lower the surface tension of an oil and water interface by a forming protective layer around emulsion droplets. Energy is needed to deform and break up the droplets and is usually provided by intense agitation (Walstra 1996). High pressure homogenizers are the most commonly used device in producing emulsions (Stang and others 2001). Usually a high speed mixer is used to prepare a coarse emulsion before processing by a high pressure homogenizer. The high pressure homogenizer produces emulsions with smaller droplets by pumping and forcing the coarse emulsion through a narrow valve and breaking down the large droplets by a combination of intense disruptive forces (Phipps 1985; McClements 2005). Figure 2.5 shows the breakdown of coarse emulsion droplets into fine emulsion droplets by a high pressure homogenizer.

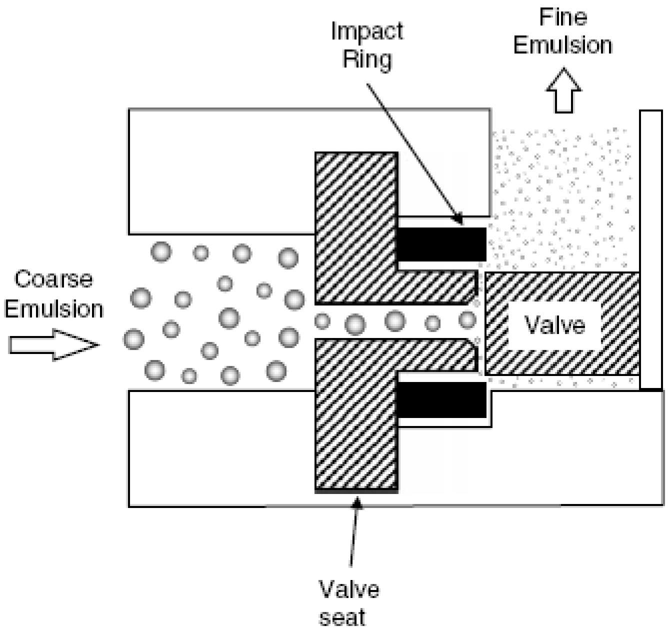


Figure 2.5 Breakdowns of coarse emulsion to emulsion with fine droplets (McClements 2005)

During the emulsification process, oil is broken up into smaller droplets by a mechanical force created from agitation. The newly created interfaces of the small droplets are then quickly coated by emulsifiers. The migration of emulsifiers to the interfaces is done by convection. Sufficient amount of emulsifiers is required for the full coverage of the interfaces of the newly formed droplets, otherwise the droplets may coalesce. A self-stabilization mechanism called Gibbs-Marangoni effect prevents the coalescence of oil droplets that are partially coated with emulsifier and it is only effective when the emulsifiers are present in the continuous phase (Walstra 1996). Gibbs-Marangoni effect is the mechanism in which the rapidly moving emulsifiers at the interface drags the associated continuous phase and restore the presence of fluid between droplets, preventing the coalescence of droplets. It requires emulsifiers to diffuse at the interface rapidly, in order to reduce the surface tension (Wilde and others 2004).

2.3.1 Emulsion Stability

Emulsions are thermodynamically unstable (Kontogiorgos and others 2004). There are both physical and chemical forces affecting the stability of emulsions. The physical instabilities of emulsions are Ostwald ripening, creaming, flocculation, coalescence, partial coalescence and phase inversion (Rousseau 2000). The chemical instabilities include oxidation and hydrolysis of lipids (McClements 2005). Some of the physical instabilities are illustrated in Figure 2.6.

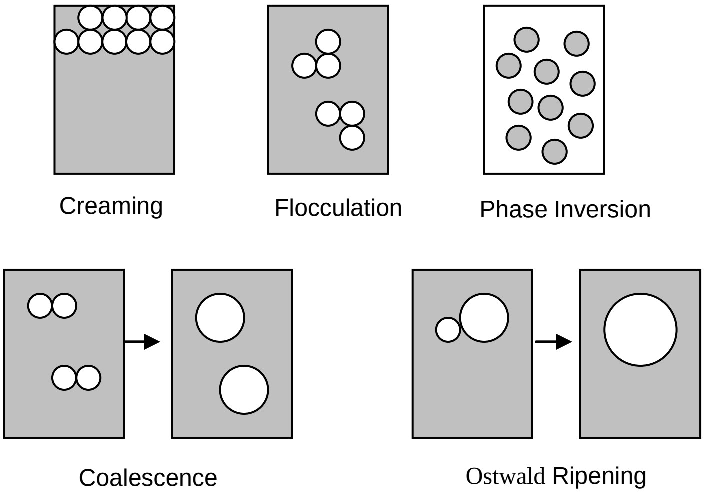


Figure 2.6 Schematic illustrations of the types of instabilities in emulsions

Ostwald Ripening

Ostwald ripening refers to the growth of large droplets by the diffusion of emulsified monomers from small droplets to larger droplets through the continuous phase. This results in a decrease in the number of smaller droplets and an increase in the size of larger droplets, thus leading to the destabilization of emulsions by creaming. Ostwald ripening usually does not occur in O/W emulsions in which the molecules of the dispersed oil phase have a low solubility in water. However, it can be significant in O/W emulsions containing flavor oil or when the continuous phase contains alcohol (McClements 2005).

Creaming

Creaming is the separation of emulsion droplets as a cream layer separates from the aqueous continuous phase via a gravitational force. It can be described as the upward movements of the dispersed droplets, which are usually due to the density difference between the dispersed phase and continuous phase (Walstra 1996). There are methods to minimize the rate of creaming, such as reducing droplet size, modifying continuous phase rheology and increasing droplet concentration. Emulsions containing smaller droplets are more stable than those containing large droplets. Creaming can also be suppressed by increasing the viscosity of the continuous phase or the volume fraction of the dispersed phase as this will limit the movements of droplets (McClements 2005).

Flocculation

Flocculation is a type of droplet aggregations in emulsions. It is caused by the frequent encounter of droplet particles in liquid by Brownian motion (Walstra 1996). In flocculation, two or more droplets stick together but retain their individual structures (McClements 2005). There are two types of flocculates, in terms of their structures, open packing and close packing. In open packing structure, droplets attach to each other and do not alter their positions when they come in contact with each other, subsequently forming flocculates with large amount of continuous phase entrapped (Bremer and others 1993; McClements 2005). In close packing structure, droplets rearrange their positions after they come in contact with each other, resulting in flocculates that have more compact structure and less continuous phase entrapped (McClements 2005).

Emulsions containing flocculated particles exhibit either shear-thinning or shear-thickening flow behaviour. In the case of shear thinning behavior, flocculates are broken down by shear forces and the apparent viscosity of emulsion decreases (Bujannunez and Dickinson 1994; McClements 2005). In the case of shear-thickening behaviour, flocculation of droplets is promoted by shear forces and leads to an increase in the apparent viscosity of emulsion (Spicer and Pratsinis 1996; McClements 2005).

Coalescence

Coalescence is the phenomenon that two droplets merge together and become one large droplet due to the rupture of the thin film (lamella) between them (Walstra 1996). It is an irreversible process and requires the emulsion droplets to be in close proximity for a long period of time, which occurs in creamed or flocculated emulsions. In O/W emulsions, droplet coalescence leads to the formation of oil layer on top, which is also called oiling off (Damodaran 2005). The rate of coalescence of emulsion droplets is highly dependent on the nature of emulsifiers and the chemical properties of continuous phase as they have a significant influence on the properties of the interfacial membrane surrounding emulsion droplets (McClements 2005). Physical forces, such as freezing, drying and centrifuging can also cause the interfacial membrane to stretch and tear, which result in an exposure of emulsifier depleted surface area of droplets. Therefore, it increases the chances of coalescence taking place when two droplets come close to each other (Walstra 1996; van Aken 2004).

Partial Coalescence

Partial coalescence is the phenomenon that a portion of oil within emulsion droplets crystallizes and pierce through the interfacial membranes, causing linkages between the droplets when they come into contact, thus leading to the formation of irregular clusters and hence the instability of emulsions (Benjamins and others 2009). Partial coalescence of oil droplets leads to an increase in the apparent volume fraction of the dispersed phase. A gel-like network of partially coalesced oil droplets can be formed when the original volume fraction of the dispersed phase is high (Walstra 1996). The volume fraction of dispersed phase, fat crystallization, oil droplet size, and the type and concentration of emulsifiers are the main factors contributing to the degree of partial coalescence in emulsions (Palanuwech and Coupland 2003).

Partial coalescence is common in many dairy products containing an emulsion system, as milk fat partly crystallizes over a wide range of temperatures, thus contributing to the emulsion instability (McClements 2005).

Phase Inversion

Phase inversion occurs when an O/W emulsion system changes to a W/O emulsion system or vice versa. Phase inversion is a complex process, which involves flocculation, coalescence and partial coalescence (Brooks and others 1998; McClements 2005). Phase inversion is necessary in some food productions, such as butter and margarine. It is induced by the partial coalescence of emulsion droplets and shearing force, which lead to the formation of fat crystal network that entraps the water phase within (Dickinson and Stainsby 1982; McClements 2005).

2.3.2 Factors Influencing Emulsion Stability

Stability of emulsion is the most important factor in emulsion technology (Franco and others 1997). It is mainly controlled by the three phases in the emulsion system: the dispersed phase, the continuous phase and the interfacial materials (emulsifiers) (Dalgleish 2004). Other factors, such as temperature, pH and ionic strength, also influence the stability of an emulsion system.

Dispersed phase – Fat and Oil

In an O/W emulsion, the dispersed phase is made up of oil or fat which is dispersed as droplets in the continuous water phase. Fat and oil are lipid compounds, which by definition are soluble in organic solvents but are insoluble or sparingly soluble in water (McClements 2005). The terms fat and oil are often used interchangeably. Strictly speaking, fat is normally defined as lipids that are solid at room temperature whereas oil is liquid at room temperature (Nawar 1996; McClements 2005).

Fat and oil influence the characteristics of emulsions in various ways. The flavour and aroma of emulsions are influenced by the type and concentration of fat or oil present as lipids carry certain flavour compounds. The oil or fat content influences the color and opacity of emulsions. The viscosity of emulsions increases with an increase in the dispersed droplet concentration (McClements 2005). Fat and oil also influence the functionality of emulsions by their degree of crystallinity (Dalgleish 2006). The physical state of lipid is important to the texture, mouthfeel, stability, and appearance of many food emulsions. It is determined by the arrangement of triglyceride molecules that can exist in three crystalline forms, α, β' and β (McClements 2005). Crystallization of triglycerides first occurs in the α-form, which is the least stable form. Increasing the temperature to slightly above the melting temperature of α-form allows it to melt and crystallize to β'-form. In the same way, melting and crystallization of β'-form occur and form the most stable β-form (Roos 1995). Crystallization of fat within emulsion droplets could lead to partial coalescence, which increases the overall viscosity and decreases the stability of emulsions (McClements 2005).

Milk fat is an important component in many products. It is composed of mainly triglycerides and small amount of other lipids, including diglycerides, monoglycerides, fatty acids, phospholipids, cholesterol, cholesteryl esters and hydrocarbons (Zegarska 2003). Milk fat has a broad range of melting temperatures, ranging from -40°C to +40°C, due to the complexity of fatty acid composition and triglyceride structure,. Therefore it could exist in a mixture of different physical states at a temperature that it is used. It is in the form of crystals at solid state. In liquid state, it could be in the form of free liquid, liquid in crystal matrix, or liquid adsorbed on crystal surface (Kaylegian 1995).

Specialty milk fats are manufactured to meet specific uses in a variety of applications. They are fractionated milk fats with a relatively narrow melting temperature that are produced by fractionation processes. In this process, whole bulk milk fat is separated and fractionated into different components that have different physical and chemical properties (Kaylegian 1999). Specialty milk fats can be divided into different categories based on their melting characteristics, very high melting fractions (VHMF) that melt above 45°C, high melting fractions (HMF) that melt between 35 and 45°C, medium melting fractions (MMF) that melt between 25 and 35°C and low melting fractions (LMF) that melt between 10 and 25°C (Kaylegian 1999). HMF contains mainly long-chain saturated fatty acids, whereas MMF has two long-chain saturated fatty acids and one short-chain unsaturated fatty acids and LMF consists of one long chain saturated fatty acid and two short-chain unsaturated fatty acids (Timms 1980; Marangoni and

Lencki 1998). The solid fat contents of fractionated milk fats and anhydrous milk fat (AMF) used in this study at various temperatures are shown in Table 2.1.

Table 2.1 Solid fat contents (%) of fractionated milk fats and anhydrous milk fat (AMF) at given temperatures (Product specifications, Appendix A)

Temperature (°C)	Typical Solid Fat Content (%)			
	MF13	MF27	MF42	AMF
0	30	76.1	86.8	66.9
5	21	73.4	85.4	63.3
10	9	68.2	82.4	56.4
15	-	57.6	75.4	41
20	-	38	64.5	22.5
25	-	12.7	54.9	12.4
30	-	-	43.5	5.9
35	-	-	30.1	1

Continuous Phase – Water

In the O/W emulsion, water makes up the continuous phase. Any components present in the continuous water phase influence the properties and stability of emulsions (Bergethon 1998; Norde 2003; McClements 2005; Sosa-Herrera and others 2008). As described, the instability mechanisms such as creaming, flocculation and coalescence are due to the movements of emulsion droplets, which are affected by the viscosity of the continuous phase. The more viscous the continuous phase, the less chance of emulsion droplets coming into contact with each other to induce the instability phenomenon. The viscosity of the continuous phase also affects the production of small droplets during homogenization. Some other factors, such as ionic strength, dielectric constant and refractive index of the continuous phase, affect the strength of attractions between emulsion droplets, which in turn affect the flocculation and coalescence ability of emulsion droplets (McClements 2005).

The Interfacial Membrane – Emulsifiers

Emulsifier is the most important ingredient in an emulsion system as it determines the overall appearances, texture and stability of emulsions (Demetriades and others 1997b). Emulsifiers are amphiphilic substances that are capable of lowering the surface tension of an oil and water interface and promote the formation of emulsions. They adsorb onto oil droplets and act as interfacial barriers to prevent them from aggregating by providing repulsive forces and steric hindrance. The important characteristics of a desirable emulsifier to be used in forming and stabilizing emulsion are its ability of reducing the surface tension of an oil and water interface significantly, adsorbing onto an oil/water interface rapidly during homogenization, and forming an effective interfacial layers to prevent droplet aggregations (McClements 2005). The emulsifiers commonly used in the food industry are proteins and low molecular weight surfactants (Bos and Vliet 2001).

Types of Emulsifiers

Emulsifiers can be classified by the hydrophile-lipophile balance (HLB) value. It indicates the affinity of emulsifiers for oil or water phases. A HLB value is given to each emulsifier according to their chemical structures (McClements 2005). An emulsifier with HLB value of 7 indicates it is equally soluble in water and oil. A HLB value less than 7 indicates the emulsifier is more soluble in oil, whereas a HLB value greater than 7 indicates the emulsifier is more soluble in water (Walstra 1996). According to the Bancroft’s rule (Davis 1994; Bergenståhl 2008), a water soluble emulsifier should be used to in O/W emulsions, and an oil soluble emulsifier should be used in W/O emulsions (McClements 2005).

Most emulsifiers used in the food industry can also be divided, based on their charge, into three groups, nonionic, ionic and zwitterionic (McClements 2005). They form compact adsorbed layers at the oil and water interface (Wilde and others 2004). Examples of these emulsifiers are listed in Table 2.2.

Lecithin is a naturally occurring zwitterionic emulsifier and can be found in various sources, such as soybeans, rapeseed and egg yolk. Lecithin extracted from soybeans is most commonly used in the food industry due to its economic advantage. Lecithin from egg yolk is used to stabilize mayonnaise and salad dressing but is not extracted as a specialized ingredient due to the high cost (McClements 2005).

Table 2.2 Examples of some emulsifiers used in the food industry (McClements 2005)

Nonionic	Ionic	Zwitterionic
Acetic acid esters of monoglycerides	Sodium stearoyl lactylate (SSL)	Lecithin
Lactic acid esters of monoglycerides	Diacetyl tartaric acid esters of monoglycerides (DATEM)	

Nonionic emulsifiers stabilize emulsions by short range repulsive forces, such as steric repulsion, hydration and thermal fluctuation interactions. Emulsions stabilized by non-ionic surfactants are less sensitive to pH and ionic strength (McClements 2005). Ionic emulsifiers stabilize emulsions with electrostatic repulsion by causing emulsion droplets to have the same surface charges (McClements 2005). The electrostatic repulsion is greatly affected by the ionic strength of the aqueous phase. Electrostatic repulsion is only effective at low ionic strength condition. When the ionic strength is increased in the aqueous phase, emulsion droplets lose the repulsive forces between them and come close to each other, which promote the instability mechanisms such as flocculation and coalescence (McClements 2005).

Proteins are the most commonly used emulsifying agents in the food industry (Wilde and others 2004). They stabilize emulsion droplets by forming a viscoelastic layer at the oil and water interface (Wilde and others 2004). Proteins tend to have a particular molecular orientation at the interface, with the non-polar portions facing the aqueous phase and the polar portions facing the oil phase (McClements 2005). Also it is often that proteins undergo structural arrangements at the interface to maximize the contact between the non-polar portion and the oil phase (Norde 2003).

Proteins stabilize emulsions with a combination of electrostatic and steric interactions, which provide strong repulsive forces between emulsion droplets (McClements 2004). The outer segments of adsorbed protein molecules generate an osmotic pressure gradient, which prevents droplets from coming together too closely (Damodaran 2005).

Emulsions with small droplet sizes can be produced using proteins as emulsifiers and the interfacial membranes formed are usually thin (McClements 2005). Therefore they become unstable when subjected to mechanical stresses such as shearing and turbulent flow (Dickinson 1997; Mohan and Narsimhan 1997; Dickinson and Davies 1999; van Aken 2002; McClements 2005), due to the clumping or tearing of the interfacial membrane, which expose the oil region of the droplet and promote coalescence (McClements 2005).

pH and Ionic Strength

The major mechanism stabilizing an emulsion system is the electrostatic repulsion between droplets. The magnitude of the surface charge on emulsion droplets is determined by the type of emulsifier, the concentration of the emulsifier at the interface and the surrounding aqueous conditions (pH, types of salts and ionic strength) (McClements 2005). In protein stabilized emulsions, flocculation occurs at pH close to the protein's isoelectric point (Demetriades and others 1997a; Kulmyrzaev and others 2000). This is because the droplet net charge is zero at the protein's isoelectric point and therefore the electrostatic repulsion is not sufficient to prevent droplet flocculation, which could lead to other types of instability of the emulsion system, such as droplet coalescence.

Some emulsion products are fortified with minerals, such as infant formulas. The presence of minerals changes the ionic strength, which affects the stability of emulsion systems (Kulmyrzaev and Schubert 2004). The ionic strength of an emulsion system is determined by the concentration and valence of ions it contains. The electrostatic repulsion between droplets is weaken with increasing ionic strength until it is no longer strong enough to keep droplets apart and leads to droplet flocculation (McClements 2005). The reduction of the electrostatic repulsion is due to the contraction of the electrical double layer around the charged droplets with the increased ion concentration (Kulmyrzaev and Schubert 2004). Different types of ions induce the instability of emulsion by different mechanisms (McClements 2005). Monovalent ions such as K^+, Na^+ and Cl^- cause flocculation by the screening of the electrostatic interactions, whereas multivalent ions such as Ca^{2+} cause flocculation by a combination of both ion adsorptions on droplet surfaces and electrostatic screening (Kulmyrzaev and others 2000; Kulmyrzaev and Schubert 2004). Therefore, multivalent ions have more significant impact on the instability of emulsions than do monovalent ions (Keowmaneechai and McClements 2002).

Temperature

Globular protein-stabilized emulsions are sensitive to temperature. That is because proteins denature and unfold when the temperature exceeds the critical value, thus exposing the interior hydrophobic and reactive groups. This in turn causes the interactions between the denatured protein molecules that are adsorbed on the same or different emulsion droplets, hence inducing flocculation or coalescence mechanisms (McClements 2004). Temperature also determines the physical state of oil droplets in O/W emulsions, which influences the emulsion properties such as viscosity and stability.

Some emulsions become unstable when they are frozen and thawed. The emulsion instability caused by freezing is due to the physicochemical changes occurring in the emulsion system as follows: the formation of ice crystals in the aqueous phase forces the oil droplets closer together; the ionic strength increases in the aqueous phase due to the ice crystallization and the electrostatic repulsions between droplets are screened which promote droplet coalescence; the disruption of the interfacial membranes of oil droplets by penetration force during ice crystallization, thus the oil droplets are more susceptible to coalescence during thawing; the loss of functional properties of emulsifiers at freezing temperature (McClements 2004). The stability of an emulsion during freeze-thaw is also influenced by the type and concentration of the fat phase. Highly concentrated emulsions are more susceptible to destabilization because of greater stresses on the dispersed phase during the frozen state (Ghosh and Coupland 2008).

2.3.3 Characterization of Emulsion

Emulsion Colour

The appearance of an emulsion product plays an important role in consumer perceptions of the product. It is determined by the interactions between the emulsion and the radiation in the visible region of the electromagnetic spectrum (Clydesdale 1978;

Chantrapornchai and others 1998). The colours of O/W emulsions are affected by droplet size and droplet concentration (McClements 2005). The study carried out by Chantrapornchai and others (1998) showed that in O/W emulsions containing 0.005 wt% dye, the increased of oil droplet concentration from 0-5 wt% led to a significant increase in lightness and a decrease in the intensity (chroma) of the emulsion. The changes were less significant at higher droplet concentrations. On the other hand, the lightness of the emulsion colour decreased and the intensity (chroma) increase with increasing in the droplet size.

The colour of emulsions can be described by a tristimulus coordinates concept, which means the colour is characterized by three mathematical parameters. The most commonly used system is the CIE (Commission International de l'Eclariage) L*a*b* system (McClements 2005).

Emulsion Droplet Size

The particle size of emulsions is usually defined as a diameter of the internal phase globule (Harrison and Cunningham 1985). The size of emulsion droplets is vital to the whole emulsion system as it influences the color, texture and stability of emulsions. Good stability is usually associated with fine, uniform particle size (Harrison and Cunningham 1985). The particle size is affected by a number of factors, such as type and concentration of emulsifier, homogenization pressure and temperature and the properties of oil and aqueous phases (McClements 2005). A particle size of 0.5 to 5 μm gives emulsion an opaque appearance (Harrison and Cunningham 1985). The emulsion particle size can be determined by several methods, including static light scattering, dynamic light scattering, diffusing wave spectroscopy and electrical pulse counting.

Zeta Potential

Emulsion droplets carry electrical charges, which are acquired from the emulsifiers adsorbed at the interfaces. The electrical charges are important to emulsion stability and they are often characterized by zeta potential (ζ). The charged surfaces of emulsion droplets are surrounded by a cloud of counter ions in order to achieve the overall electrical neutral of the emulsion system. This distribution of ions at the droplet surfaces is called the electrical double layer (McClements 2005), as illustrated in Figure 2.7.

ζ–potential is the potential generated between the electrical double layer of the droplet and the surrounding bulk phase during the movement of droplets (Hunter 1981). The magnitude of ζ–potential can provide information on the interactions between emulsion particles and hence the stability of the emulsion system. Large positive or negative ζ-potential values indicate strong repulsion between droplets and it is reduced by the presence of salts. The ζ–potential of an emulsion system can be measured by methods such as particle electrophoresis and electroacoustics (McClements 2005). The presence of minerals in an emulsion system increases the ionic strength of the aqueous phase and reduces the magnitude of ζ–potential, therefore reducing the electrostatic repulsions between droplets and lowering the emulsion stability (Hunter 1986).

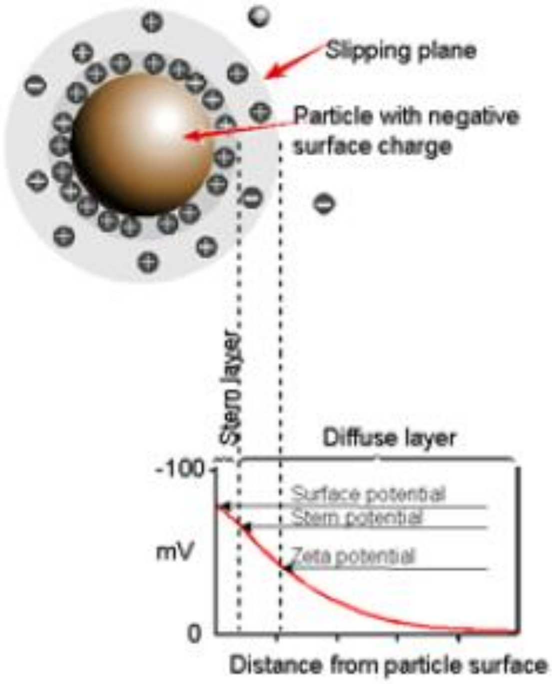


Figure 2.7 A schematic representation of zeta potential (adapted from Technical Notes, Malvern Instruments Ltd)

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a thermal analysis technique that monitors the energy supplied to or released from a sample in relation to a reference material when subjected to a programmed temperature regime (Schenz 2003). DSC is widely used in the food industry for analysis of water, starch, lipid and proteins (Gekko and Satake 1981; Simpson and Hagemann 1982; Zeleznak and Hoseney 1984). It is useful for studying the thermal and structural properties of milk fat. The changes in polymorphic behaviour of milk fat during crystallization and melting can be determined using DSC coupled with microscopic techniques (Lopez and others 2000; Lopez and others 2001; Amara-Dali and others 2007).

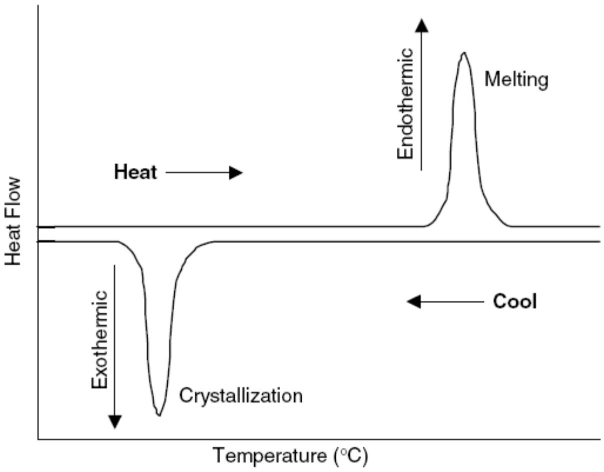


Figure 2.8 An example of thermogram from DSC analysis (adapted from McClements 2005)

The rearrangement of molecules during phase transitions of a substance causes the release or absorption of energy, which can be recorded on a thermogram (Figure 2.8). Melting of milk fat is an endothermic event because energy is needed for the closely-packed triglyceride structures to break apart, whereas crystallization is exothermic due to the energy released from molecules forming crystalline structures (McClements 2005). Crystallization of oil droplets can lead to partial coalescence, which has a major influence on the property and stability of emulsions. DSC can be used to provide insight information on the effects of different factors, such as oil composition, dispersed phase volume fraction, temperature and process condition, on the thermal behaviour of emulsions (Walstra and van Beresteyn 1975; Simoneau and others 1993; Clausse 1998; Vanapalli and others 2002; Cramp and others 2004).

Crystallization of milk fat occurs when the free energy of the solid state is lower than the free energy of the liquid state (Hartel 2001). The crystallization mechanism of milk fat in the emulsified state is different from the crystallization mechanism of bulk liquid fat. In the bulk liquid of pure milk fat, crystallization is triggered by heterogeneous nucleation because impurities in the bulk liquid can act as starting points for crystallization (i.e. heterogeneous crystallization). In emulsions, milk fat is dispersed as fine droplets and the number of droplets is larger than the number of impurities. Therefore homogeneous crystallization takes place, which requires crystals arising from the fat itself instead of foreign particles (Coupland 2002). Homogeneous crystallization requires a higher degree of super cooling than heterogeneous crystallization (Skoda and Van den Tempel 1963; Walstra and van Beresteyn 1975). However, homogeneous nucleation only occurs in emulsions with small particle size so the emulsions have less than one nucleus per droplet. Therefore the crystallization mechanism of emulsions is also influence by the composition and the structure of the emulsions (Coupland 2002).

There are also other crystallization mechanisms, such as surface heterogeneous nucleation and interdroplet nucleation. In surface heterogeneous nucleation, the nucleation rate is accelerated if the hydrophobic portion of surfactant is similar to the oil. In the interdroplet nucleation, nucleation is induced by collisions of liquid droplets and solid droplets (Skoda and Van den Tempel 1963; Dickinson and others 1993; Dickinson and others 1996; McClements and Dungan 1997; Coupland 2002).

Emulsion Rheology

Rheology can be defined as the study of deformation and flow of matters under the influence of an applied stress (Rao 2007). Deformation usually applies to solid-like materials, whereas flow usually applies to liquid-like materials (Bourne 2002). The rheological properties of an emulsion are dependant on the composition and properties of the emulsion and the environment that the emulsion is subjected to, such as temperature (McClements 2005). The emulsion viscosity increases with increasing the number of droplet particles or the dispersed phase volume fraction (Mewise and Macosko 1994; Larson 1999; McClements 2005). When the dispersed phase volume fraction is really high, the emulsion droplets are packed very closely to each other and not much movement can be generated. At this stage, the emulsion exhibits viscoelastic gel-like properties (McClements 2005). The emulsion viscosity is also affected by the physical states of the emulsion droplets (McClements 2005). It is greater if the droplet particles are rigid, due to the disruption of flow by friction (Hunter 1986; Mewise and Macosko 1994; McClements 2005). The viscosity of an emulsion is also determined by the viscosity of the continuous phase (McClements 2005). Increase in the viscosity of the continuous phase increases the viscosity of the whole emulsion system. Therefore the viscosity of an emulsion can be modified by adding hydrocolloids into the continuous phase (Sosa-Herrera and others 2008).

The rheological properties of emulsions are largely influenced by the interactions between emulsion droplets. Flocculation is due to a loss of the repulsive force provided by electrostatic repulsion between emulsion droplets and leads to an increase in the emulsion viscosity (McClements 2005). As mentioned earlier in section 2.3.2, the interactions between emulsion droplets are determined by the nature of the interfacial layer (emulsifiers) and the ionic condition of the continuous phase, such as pH and ionic strength.

Emulsion droplet surface charge can also affect the rheological properties of an emulsion through primary, secondary and tertiary electroviscous effects (McClements 2005). The primary electroviscous effect is caused by the attraction between droplets and counterions in the surrounding phase lag behind when the droplets move through the fluid (Pal 1996; Larson 1999; Rubio-Hernández and others 2004; McClements 2005). However the primary electroviscous effect is not significant in most colloidal dispersions (Hiemenz and Rajagopalan 1997). The secondary electroviscous effect is due to an increase in the effective diameter of emulsion droplets caused by the electrostatic repulsion between droplets (Hiemenz and Rajagopalan 1997; McClements 2005). The electrostatic repulsion between droplets is determined by the type of emulsifiers used. Some emulsions stabilized by certain emulsifiers have higher viscosity than those stabilized by others (Dickinson 1998; McClements 2005). The tertiary electroviscous effect is due to changes in the thickness of the interfacial layer caused by changes in the environmental conditions, such as pH and ionic strength (Hiemenz and Rajagopalan 1997; McClements 2005). It is more pronounced in emulsions that stabilized with proteins, which form a viscoelastic interfacial layer on the emulsion droplets (McClements 2005).

For the measurements of rheological properties of materials, three basic parameters, shear rate (γ), shear stress (σ) and viscosity (η) are used to study the flow behaviour of fluids and solid materials and have the units of s^{-1}, Pa and Pa.s, respectively. The relationship between these three parameters is described by the equation below.

$$\sigma = \eta\gamma$$

The flow behaviour of fluid can be divided into two main categories – Newtonian and non-Newtonian. Non-Newtonian flow can be further divided into pseudoplastic, dilatant and plastic flow behaviour (Bourne 2002). Illustrations of these flow behaviours are shown in Figure 2.9. Newtonian fluid has the most simple flow properties. The viscosity (η) of a Newtonian fluid is independent to the shear rate and the shear rate is directly proportional to the shear stress (Bourne 2002). Hence the viscosity of a Newtonian fluid is the same at any given shear rate. Examples of Newtonian fluid are water, coffee and milk.

Non-Newtonian fluid does not have one single viscosity value as the viscosity changes with the shear rate applied. Therefore apparent viscosity is usually used. Pseudoplastic fluid has ‘shear thinning’ flow behavior. Hence the viscosity of the fluid decreases with increasing shear rate (Bourne 2002). Examples of fluid exhibiting pseudoplastic behaviour are salad dressing and concentrated fruit juice. Opposite to pseudoplastic fluid, dilatant fluid has ‘shear thickening’ flow properties, which means the viscosity of the fluid increases with increasing shear rate (Bourne 2002). An example of dilatant fluid is concrete or liquid containing a high proportion of insoluble particles. A plastic fluid is the fluid with ‘yield stress’, which is the minimum stress that must be exceeded before the fluid begins to flow (Bourne 2002). Ideal plastic fluid is also called ‘Bingham plastic’. Ideal plastic fluid exhibits Newtonian flow behavior above the yield stress. It can be seen as Newtonian fluid with a yield stress. Non-ideal plastic fluid is the fluid that does not exhibit Newtonian flow behavior above the yield stress (McClements 2005).

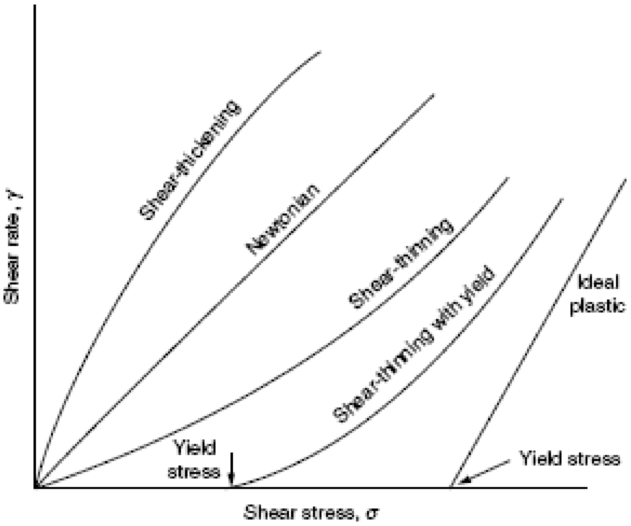


Figure 2.9 Flow behaviours of fluids. Adapted from (Rao 2005)

Thixotropic is used to describe the types of fluid which exhibit time dependant shear thinning flow behaviour, whereas rheopectic depicts fluid with time dependant shear thickening behaviour. When the shear stress versus shear rate curves obtained from increasing and then decreasing shear rate do not coincide, a hysteresis loop is formed. The values of the latter curve are lower than the former one in thixotropic fluid and the complete opposite happen in rheopectic fluid (Rao 2007).

The flow behaviour of fluid can be determined by shear measurements, which are carried out in mechanical rheological instruments with different measuring systems, including concentric cylinder, parallel plate and cone and plate (Bremer and others 1993). In the concentric cylinder system, a sample to be measured is placed between the gaps between two cylinders (Brummer 2006). Measurements can be done by rotating the cylinder. The force required to rotate the cylinder reflects the viscosity of the sample, hence the flow behaviour of the sample can be defined. In the parallel plate system, a sample is placed between two parallel plates. Measurements can be done by rotating the upper plate while keeping the lower plate stationed (McClements 2005). The cone and plate system is similar to the parallel plate system except the upper plate is replaced with a cone that has a slight angle. The cone and plate system has a more uniform stress over the sample than the parallel plate system (McClements 2005). Hence more accurate measurements can be made.

For viscoelastic materials, the rheological properties can be studied using the dynamic rheological method (oscillatory tests), in which materials are subjected to small amplitude oscillatory force without altering the structures (Rao 2007).

$$\tan(\delta) = G''/G'$$

G’ is the elastic modulus, which measures the energy stored and recovered. G’’ is the viscous modulus that measures the energy dissipated or loss as heat during a test cycle (Venugopal and Muthukumarappan 2003). When G’>G’’, materials behave more solid like. If G’’>G’, materials are more liquid like. Phase angle, δ is a dimensionless value range between 0° and 90°. It provides indications that whether the elastic or viscous properties predominate in a material. In general, a material exhibits elastic behaviour at low frequencies (Quintana and others 2002b).

Chapter 3. Materials and Methods

3.1 Materials

Milk Fat

Three types of milk fat fractions – Speciality Milk Fat 13 (MF13), Speciality Milk Fat 27 (MF27), Speciality Milk Fat 42 (MF42), and Anhydrous Milk Fat (AMF) were supplied by Fonterra Co-operative Group Limited, New Zealand. MF13, MF27 and MF 42 have specific melting temperatures of 13°C, 27°C and 42°C, respectively. AMF contains a broader range of milk fat fractions and has the melting point of 31-34°C. The milk fat product specifications are attached in Appendix A.

Rhamnolipids

An aqueous solution of rhamnolipids (JBR425) at 25% (JBR425, Jeneil Biosurfactant Co. LLC, Saukville, WI, USA) was supplied by Flavorjen New Zealand Limited (Auckland, New Zealand) and was used as an emulsifier in this study. The specification of rhamnolipids (JBR425) is included in Appendix A.

Water

Distilled water was prepared using Aquaton A8000 Water Distillation Unit (Bibby Sterinlin Ltd, UK) and was used as the continuous phase in the preparation of rhamnolipid-based emulsions containing different types of milk fats.

3.2 Emulsion Preparation

Oil-in-water emulsions were prepared by emulsifying each of the four fractionated milk fats into an aqueous phase (i.e. distilled water) containing rhamnolipids (0.5% of the final emulsion weight) at different oil to water (O/W) ratios of 1:9, 3:7, 5:5 and 7:3. The amounts of each component required are illustrated in Table 3.1. Prior to emulsification, the milk fats were melted using a microwave (Delioe 1000 Watt, Menumaster Commercial). Depending on the type of milk fat, distilled water was also heated to the temperature above the melting point of milk fat to be emulsified using the microwave in order to prevent the solidification of milk fat during emulsion preparation.

Table 3.1 Amounts of milk fat, water and 25% rhamnolipid solution needed to make up 100 g of emulsion with different O/W ratios stabilized with 0.5% rhamnolipids

Emulsion (g)	Oil : water ratio	Oil (g)	Water (g)	25% rhamnolipid solution (g)
100	1:9	9.65	88.35	2
100	3:7	28.95	69.05	2
100	5:5	48.25	49.75	2
100	7:3	67.55	30.45	2

The emulsification was carried out in a two-step process. In the first step, the mixture of milk fat, water and rhamnolipid was roughly homogenized to prepare a coarse emulsion using a VirTis Homogenizer (Model: Tempest 500W, The VirTis Company Inc, New York) operated at 12,000 rpm for 1 minute. In the second step, the coarse emulsion was homogenized 4 times using a two-stage high pressure homogenizer (APV-2000, Invensys® APV®, Denmark) operated at 50 MPa (500 Bar) and 5 MPa (50 Bar) for the first and second stages, respectively. The homogenization temperature was kept at above the melting point of milk fat.

3.3 Color Measurements

The colours of emulsions were measured using a colorimeter (CR-300, Minolta, Japan) equipped with a 2° observer, a CIE standard illuminant C and an 8 mm diameter measuring area. A glass Petri dish was used as a sample cell. The colorimeter was calibrated against the empty glass Petri dish with a standard white plate before the sample measurements. The colour measurements were carried out at 20°C.

The emulsions were poured into the glass Petri dish. The head of colorimeter measuring probe was placed at the bottom of the glass Petri dish containing the samples and the measurement was taken three times and averaged by using the ‘Multi Measure’ function. The colour measurements were displayed as Yxy (CIE 1931), Lab (CIE 1976), LCh, Hunter lab and XYZ tristimulus values by a data processor Dp-30. All emulsion samples were measured in triplicates. The colours of bulk milk fats were also measured. The milk fats were melted into liquid and poured into the glass Petri dish and then carefully put into a freezer at -18°C and stored for 24 hours to achieve the complete solidification. The colour measurements were carried out immediately after the milk fat was taken out from the freezer to prevent any changes in physical states (e.g. melting).

3.4 Microscopic Analysis

The emulsions containing AMF with O/W ratios of 3:7 and 7:3 were analyzed for their microscopic images using a light microscope (Zeiss Axiophot Compound Light Microscopic) attached with DFC 320 camera and a confocal laser scanning microscope (TCS SP5, Leica Microsystems GmbH, Wetzlar, Germany). Emulsion samples were diluted with distilled water before the microscopic examination. A portion (2 mL) of the diluted samples was stained by mixing it with 3 drops of a 1% Nile Blue stain solution. The stained and unstained emulsion samples were then examined using the microscopes.

3.5 Particle Size and Zeta Potential Analysis

The particle size of emulsions was analyzed by the dynamic light scattering technique using a Malvern Zetasizer (Zen3600, Malvern Instruments, USA). The emulsions were diluted with distilled water prior to the measurements. All measurements were carried out at 25°C. The results of the particle size were expressed as Z-average size, also known as the cumulants mean. The zeta potentials of emulsions were also determined using the Malvern Zetasizer.

3.6 Storage Stability

The stability of emulsions containing MF13, MF27 and MF42 at four different O/W ratios (1:9, 3:7, 5:5 and 7:3) during a 12 weeks storage period was determined using a centrifugal method. The instability of emulsions was measured by the extent of droplet coalescence as exhibited by a layer of yellow coloured free oil. A 10mL of each freshly made emulsion was put into 15mL PE cylindrical shaped centrifuge tubes, tightly sealed with caps and stored at two different temperatures of 4°C and 20°C. The stability of emulsions was assessed every week for the first four weeks and every two weeks for the rest of the storage period. The emulsions were taken out and left at room temperature (20°C) for 1 hour and then put into 50°C water bath for 5 minutes to melt any solidified free oil (if present) for the easy separation from the stable portion of emulsions during the subsequent centrifugation. The emulsions were then centrifuged at 20°C and 1,000 x g for 10 minutes using a centrifuge (Labofuge 400R, Heraeus).

After centrifugation, the height of the separated free oil layers was measured and converted as a volume of oil, which was then calculated as a percentage of the initial amount of oil emulsified. The following equation was used for calculation which indicated the extent of emulsion stability (or instability).

$$\text{Emulsion instability} = \frac{\text{Amount of free oil}}{\text{Total amount of oil present in the emulsion}} \times 100\%$$

3.7 Effects of Freezing/Thawing and Heating on Emulsion Stability

The effects of freezing/thawing and heating on the stability of emulsions containing MF13, MF42 and AMF at the O/W ratios of 3:7, 5:5 and 7:3 were determined. A 10mL of the freshly made emulsions was put into 15mL PE centrifuge tubes. The samples for freezing and thawing were stored -18°C for 48 hours and then allowed to stand at room temperature (20°C) for 2 hours or until completely thawed. They were then put into 50°C water bath for 5 minutes in order to melt any solidified free oil (if present), followed by centrifugation at 1000 x g for 10 minutes at 20°C. The emulsion samples for heat treatment were place in 80°C water bath for 30 minutes. They were then centrifuged under the same condition as the freezing/thawing samples. After centrifugation, the height of the separated free oil layers was measured and then calculated as a percentage of the total amount of oil present in the original emulsion samples. All samples were analyzed in duplicates.

3.8 Effects of pHs on Emulsion Stability

The effects of pHs on emulsion stability were investigated with the emulsions containing MF13, MF42 and AMF at the O/W ratio of 3:7. A 10mL of the freshly made emulsions was adjusted to the desired pH levels (pH 2, 3, 4, 5, 6, 8 and 12) by adding either 0.1M or 1M HCl or NaOH solutions. The pHs were monitored at 20°C using a pH meter (Sartorius Basic Meter PB-11, Sartorius AG, Germany). After the pH adjustments, the emulsions were transferred to 15 mL cylindrical shaped PE centrifuge tubes and were then put into 50°C water bath for 5 minutes prior to centrifugation. This allowed the melting of any solidified free oil (if present) in order for its easy separation from the remaining stable emulsion during the subsequent centrifugation step. The emulsions were centrifuged at 1,000 x g and at 20°C for 10 minutes. After centrifugation, the height of the separated free oil layers were measured and calculated as a percentage of the total amount of oil in the original emulsion samples. All samples were analyzed in duplicates.

3.9 Effects of Salts and Ionic Strength on Emulsion Stability

The effects of type of salts and its concentration on the stability of emulsions were studied. Three types of salts, NaCl (M = 58.44, SAXA® Sea Salt, Cerebos Skellerup Ltd), KCl (M = 74.56, Panreac Quimica SA) and $CaCl_2.2H_2O$ (M = 147.02, Scharlau Chemie S.A. Spain), were used at various concentrations ranging from 1mM to 600 mM for NaCl and KCl and from 0.5 mM to 5 mM for $CaCl_2$. The emulsion samples tested were MF13, MF42 and AMF emulsions with the O/W ratio of 3:7.

Stock salt solutions of NaCl and KCl were both prepared at 2.5 M and 1 M, whereas the $CaCl_2$ solution was prepared at 1 M. Appropriate aliquots were added to the emulsions to obtain the required final salt concentrations, as illustrated in Table 3.2. The salt solutions and emulsions were gently mixed in 15mL PE centrifuge tubes and left at room temperature (20°C) for 30 minute before putting into 50°C water bath for 5 minutes to melt any released free fat from the emulsions. They were then centrifuged at 1,000 x g for 10 minutes at 20°C. After centrifugation, the height of the separated free oil layers were measured and calculated as a percentage of the total amount of oil in the original emulsion samples. All samples were analyzed in duplicates.

Table 3.2 The amounts of stock salt solution and emulsion mixed to obtain the required salt concentrations in 10 g of the final emulsions

Salt	Salt (mM)	Emulsion (g)	Salt Solution (g)	
			1M	2.5M
NaCl or KCl	600	7.6	-	2.4
	450	8.2	-	1.8
	250	9	-	1
	100	9	1	-
	25	9.75	0.25	-
	10	9.9	0.1	-
	5	9.95	0.05	-
	1	9.99	0.01	-
$CaCl_2$	5	9.95	0.05	-
	2	9.98	0.02	-
	1	9.99	0.01	-
	0.5	9.995	0.005	-

3.10 Rheological Properties of Emulsions

The rheological properties of emulsions were measured with a rheometer (AR 550, TA Instruments, Waters LLC, Great Britain), equipped with a cone and plate system (2°, 60 mm) The rheological tests carried out included steady flow, thixotropic loop, temperature sweep and dynamic oscillation. The rheometer was operated using the software Rheology Advantage Instrument Control AR (version v5.6.0).

Steady Flow

The flow behaviour of emulsions was analyzed at 20°C using steady flow analysis, in which emulsion samples were subjected to increasing shear force provided by increasing shear rate. The shear rate was increased from 0 s^{-1} to 500 s^{-1} and the shear stress/viscosity reading was taken every 60 seconds.

Thixotropic Loop

Thixotropic loop analysis was to further define flow behaviour of non-Newtonian emulsions. In the thixotropic loop analysis, the shear rates were first increased from 0 s^{-1} to 500 s^{-1} and then decreased from 500 s^{-1} to 0 s^{-1}. The shear stress/viscosity reading was taken every 60 seconds and the measuring temperature was kept at 20°C.

Temperature Sweep

Temperature sweep analysis was carried out to evaluate the changes of viscosity in emulsions with temperature changes. The emulsions were kept at a constant shear rate of 50 s^{-1} while the temperature was increased from 4°C to 80°C and decreased from 80°C to 4°C at the rate of 2°C/minute. The shear stress/viscosity reading was taken every 60 seconds.

Dynamic Oscillation

Dynamic oscillation test was done to obtain information on the structural breakdown and regeneration of highly viscous emulsions (i.e. viscoelastic properties). This test was carried out on the emulsions with 7:3 O/W ratios. *Amplitude sweep* analysis was carried out to determine a linear viscoelastic region (LVR) at the strain range from 0.01 to 2% and the frequency at 1 Hz. *Frequency sweep* analysis was conducted from 1 to 40 Hz at 0.5% strain (LVR). All measurements were carried out at 20°C and the parameters measured were the elastic modulus (G'), viscous modulus (G'') and phase angle (δ).

3.11 Differential Scanning Calorimetry (DSC) Analysis

A differential scanning calorimeter (Q-100, TA Instruments, Waters LLC, Great Britain) was used to analyze the melting and crystallization behaviour of emulsions. Distilled water, bulk milk fats and 25% rhamnolipid solution were also analyzed. The emulsion samples analyzed were emulsions containing MF13, MF42 and AMF at O/W ratios of 3:7. 5:5 and 7:3. The aluminium pans containing samples were placed inside the DSC alongside an empty reference pan. Samples were first heated up to 60°C to erase all the thermal history of the milk fat and cooled down to -50°C then heated back up to 60°C. The rate of heating and cooling was 5°C per minute. All analyses were done in duplicate.

3.12 Statistical Analysis

One-way analysis of variance (ANOVA) and Turkey’s post tests were performed using SPSS (Version 16.0) to determine if there were any significant differences between samples.

Chapter 4. Results and Discussions

4.1 Visual Characterizations of Emulsions

Emulsions containing MF13, MF42 and AMF stabilized by 0.5% rhamnolipids at 1:9, 3:7, 5:5 and 7:3 O/W ratios were visually examined. All emulsions prepared were white and opaque with a pale yellow tint (Figure 4.1). No pronounced visual difference was observed between the emulsions containing different types of milk fats at a given O/W ratio. However, the fat concentration had a significant impact on the physical appearance of the emulsions. An increase in the fat concentration gave rise to a slight increase in yellowness and a large increase in the emulsion viscosity. These visual observations were confirmed by the instrumental analysis of emulsion samples.

The highest O/W ratio (7:3) used in this study induced the formation of emulsions with a semi-solid texture, whereas the emulsions of 1:9, 3:7 and 5:5 O/W ratios existed in a liquid form (Figure 4.1). The 7:3 O/W ratio emulsions had a very fine, condensed and soft texture similar to other high dispersed phase ratio emulsions such as mayonnaise. Such characteristic results from the large volume of dispersed oil phase relative to the smaller volume of continuous aqueous phase. In a highly concentrated emulsion, emulsion droplets are packed closely together, thereby enabling the emulsions to form a gel-like texture (Harrison and Cunningham 1985; Chanamai and McClements 2000; McClements 2005).

When the emulsions were stored at 4°C after the preparation, the liquid emulsions did not show any distinctive change that could be observed by visual inspection. However, the emulsions containing MF42 and AMF at 7:3 O/W ratio exhibited a dramatic change in their textural properties after storing at 4°C from the soft, cream-like substance to the sticky, crumbly semi-solid mass (Figure 4.2 c and 4.2 d). In contrast, the MF13 emulsion containing the same fat content did not show such change and remained the same at 4°C (Figure 4.2 a and 4.2 b).

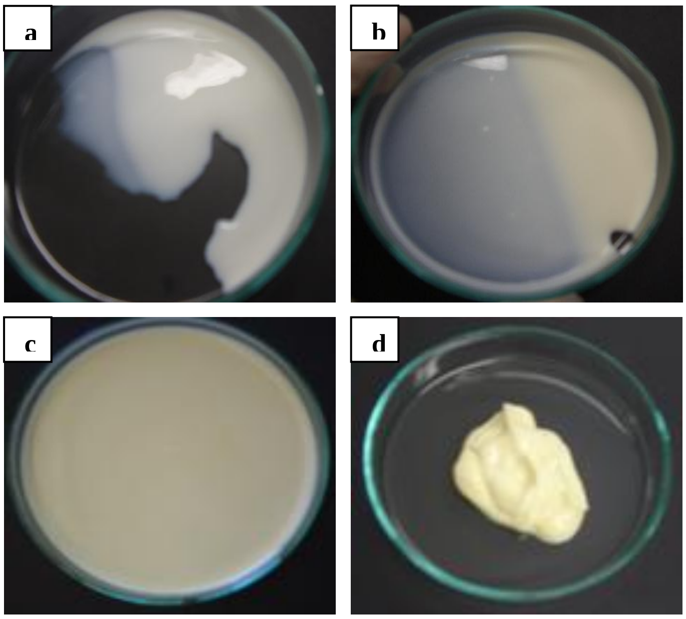


Figure 4.1 MF13 emulsions stabilized with O/W ratio of (a) 1:9; (b) 3:7; (c) 5:5 and (d) 7:3

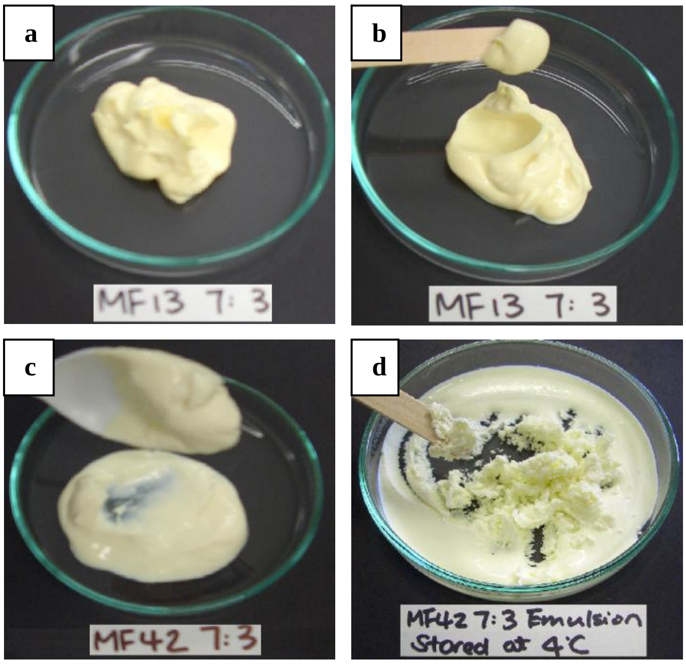


Figure 4.2 Physical appearances of emulsions with 7:3 O/W ratio (a) MF13 emulsion at 20°C; (b) MF13 emulsion at 4°C; (c) MF42 emulsions at 20°C and (d) MF42 emulsion at 4°C

This phenomenon could be due to the crystallization (solidification) of MF42 and AMF fat droplets with higher melting points coupled with the partial coalescence of fat droplets in the emulsions containing a high fat content. Whereas in MF13 emulsions, the melting point of droplets are relatively lower. At 4°C, the emulsions droplets in AMF and MF42 emulsions could be a mixture of liquid, semi-solid and solid as AMF and MF42 have an average solid fat content of 63.3% and 85.4% at 5°C, respectively (Table 2.1). Therefore, when the emulsions were kept at 4°C, the fat within the emulsion droplets would undergo crystallization and the crystalline fat formed might penetrate the interfacial membranes formed by rhamnolipid molecules. This could have resulted in the formation of linkages between fat droplets, leading to a partial coalescence and clustering between the fat droplets (Benjamins and others 2009).

4.2 Colour Measurements

The colours of emulsions containing MF13, MF42, and AMF at three different O/W ratios (3:7, 5:5 and 7:3) were measured at 20°C using a tristimulus colorimeter. The results obtained were expressed as CIE L*, a*, b* and are shown in Table 4.1. In the CIE L*, a*, b* colour notation, 'L' represents lightness, ranging from black to white (0-100), 'a' represents redness when positive or greenness when negative and 'b' represents yellowness (positive) to blueness (negative).

Table 4.1 The CIE L* a* b* values of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

Emulsion	O/W ratio	L*	a*	b*
MF13	3:7	85.37 ± 1.17	-4.21 ± 0.36	15.06 ± 0.33
	5:5	86.17 ± 1.45	-4.06 ± 0.23	17.85 ± 1.16
	7:3	84.60 ± 0.95	-3.94 ± 0.16	22.41 ± 0.89
MF42	3:7	87.66 ± 0.41	-2.84 ± 0.17	7.90 ± 0.31
	5:5	87.28 ± 0.20	-2.98 ± 0.09	9.76 ± 0.49
	7:3	83.27 ± 2.07	-4.13 ± 0.85	12.96 ± 0.04
AMF	3:7	85.27 ± 0.73	-3.86 ± 0.29	10.45 ± 0.48
	5:5	87.46 ± 1.61	-3.48 ± 0.23	13.96 ± 0.08
	7:3	85.65 ± 1.59	-3.48 ± 0.03	18.14 ± 0.29

In all cases, the L* values were between 85 and 87, indicating the emulsions were whitish (i.e. bright) in colour. In terms of colour hue, the emulsions had negative a* values from -3 to -4, which indicates green in colour. The b* values were all positive ranging from 7 to 22, indicating that the emulsions also had a yellow hue. In other words, all emulsions had a yellow-green hue tending towards yellow because of the b* values were relatively higher than the a* values.

Among the different emulsions, the major difference affected by the types of milk fats and the O/W ratios was observed to be the b* values. On the other hand, no pronounced variation was observed in the L* and a* values between samples. It has been reported that the colour and opacity of emulsions are dependent on the oil droplet concentration (McClements 2005). This indicates that emulsion becomes brighter with a higher droplet concentration due to an increase in light scattering. However, this is only the case when the emulsions are not highly concentrated. There was no significant difference in the lightness values between all the emulsion samples used in this study, therefore they could be considered as highly concentrated even at the 3:7 O/W ratio.

As illustrated in Table 4.1, the b* values (i.e. yellowness) increased gradually as the oil concentration increased in all the emulsions containing the different types of milk fats. Among all the emulsions, the ones containing MF13 had the highest b* values while the ones containing MF42 had the lowest. It is not clear why the MF13 emulsions had the highest b* value among the three types of milk fat emulsions.

In order to identify the effect of the original colour of milk fat on the corresponding emulsion colour, the milk fats (MF13, MF42 and AMF) were measured for their colours when they existed in solid and liquid state (Table 4.2) as the colour measurement is influenced by the physical properties of objects. However, it should be noted that the colour appearance of liquid oils exhibiting transparency or translucency is determined by the transmitted light. Therefore the results obtained do not represent the true colour of liquid oil but they still provide some insight information on different colour characteristics between the different liquid milk fat samples.

The results in Table 4.2 indicate that when the milk fats were liquid, their colours were similar and had no significant differences in any of the three colour parameters.

However, the solid milk fats resulted in a significant colour difference. MF13 had lower L* and a* values than AMF and MF42, unlike their emulsions, which had no major difference in the L* and a* values. On the other hand, the b* value was the highest in AMF while it was similar between MF13 and MF42, indicating AMF was more yellow than MF42 and MF13. However, as mentioned above, the MF13 emulsions were more yellow than the emulsions containing AMF and MF42. Based on the results obtained, any correlation between the emulsion colours and the milk fat colours could not be established, suggesting that the difference in b* values between MF13, MF42 and AMF emulsions was probably due to some other physical factors rather than the physical state of the emulsified milk fats.

Table 4.2 The L*, a*, b* values of milk fat MF13, AMF and MF42 in solid and liquid state

	Milk fats	L*	a*	b*
Solid	MF13	44.58 ± 5.37	-1.19 ± 0.85	29.88 ± 3.57
	AMF	68.70 ± 0.72	-5.63 ± 0.53	41.92 ± 3.09
	MF42	69.69 ± 0.34	-6.63 ± 0.18	29.25 ± 0.26
Liquid	MF13	28.79 ± 0.39	0.79 ± 0.44	9.45 ± 0.66
	AMF	29.75 ± 1.34	0.04 ± 0.03	8.93 ± 1.11
	MF42	29.01 ± 0.02	-0.82 ± 0.07	9.41 ± 0.04

Attempts were made to identify a correlation between the emulsion particle size and the colour parameters (i.e. L*, a* and b*). As shown in section 4.4, the emulsion particle size was not different between the emulsions containing MF13, MF42 and AMF at a given oil concentration. However, in all cases, it was increased as the oil concentration was increased, being about 200 nm, 240 nm and 340 nm for the emulsions at 3:7, 5:5 and 7:3 O/W ratios, respectively.

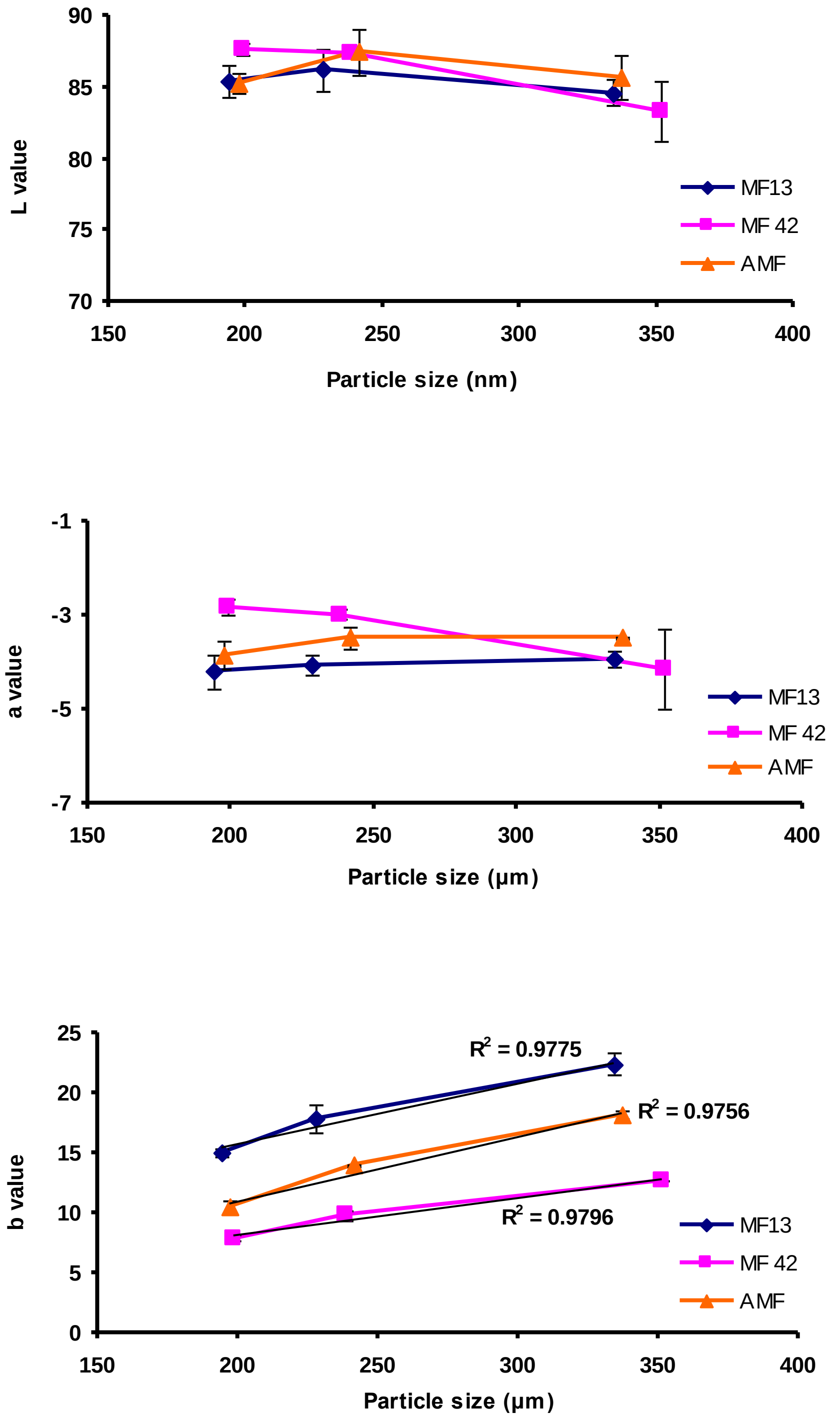


Figure 4.3 Changes in L*, a*, b* values with droplet size in emulsions containing MF13, MF42 and AMF

Figure 4.3 illustrates that the L* and a* values were not affected by the size of the emulsion droplets and the types of milk fats they contained. However, gradual increases in the b* values with increasing droplet sizes were detected in all the emulsions, indicating the emulsions became more yellowish with larger emulsion droplets.

4.3 Microscopic Analysis

The appearance of emulsions was examined using a light microscope and a confocal lazer scanning microscope. Prior to the examination, the samples were diluted with distilled water and stained with Nile blue. The Nile blue staining enabled a clear visualization of emulsion droplets with a more defined surrounding edge. However, it seemed to alter the nature of emulsion, causing an increase in the emulsion particle size. Therefore, the emulsions without staining were also examined. No noticeable differences in the emulsions attributable to the types of milk fats emulsified was observed from the microscopic images. Figure 4.4 shows the representative images captured from the AMF emulsions with 3:7 and 7:3 O/W ratios. The emulsion droplets were fine spherical particles and distributed in various sizes.

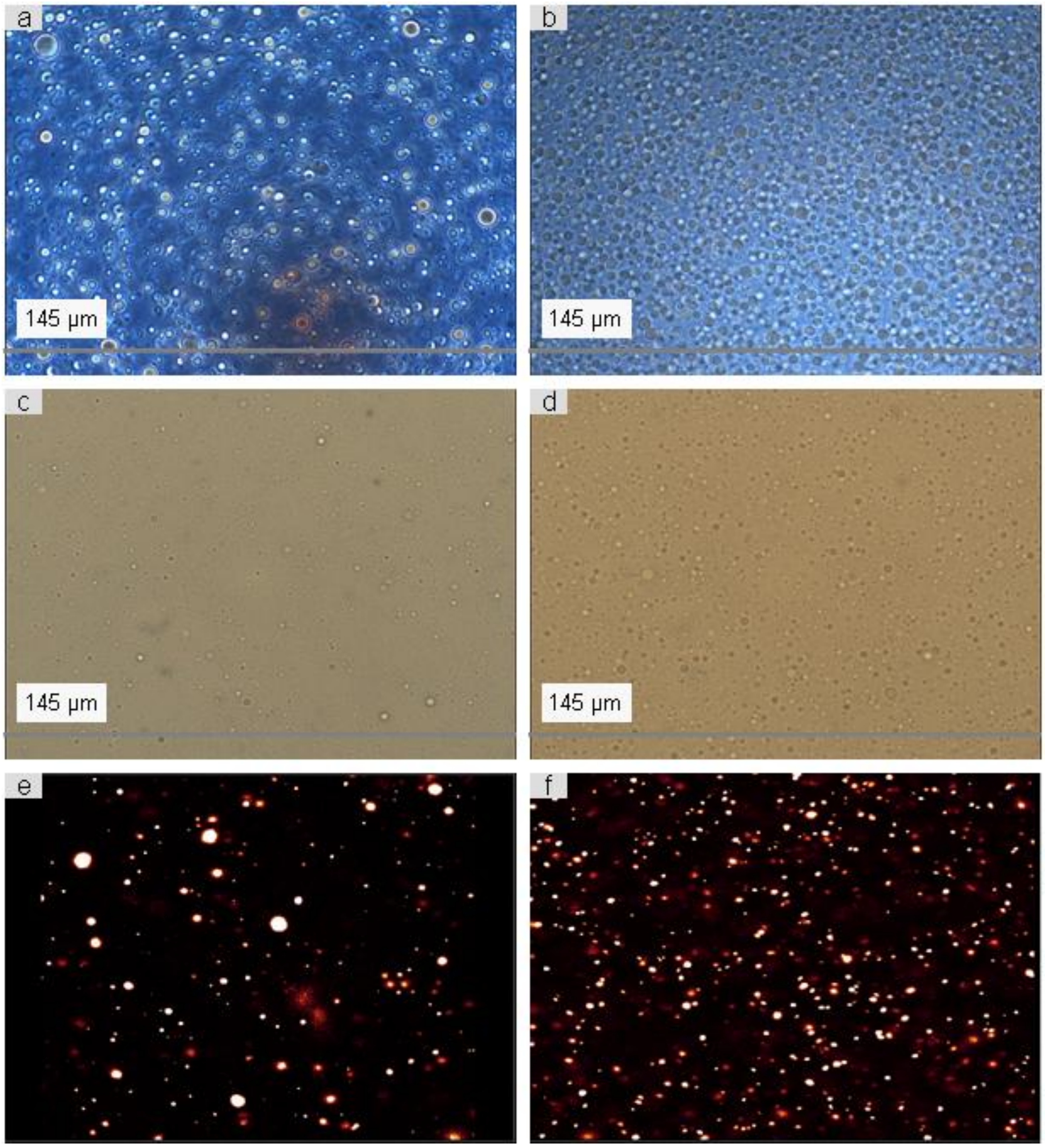


Figure 4.4 Microscopic images of emulsions containing AMF at O/W ratios of 3:7 (a, c and e) and 7:3 (b, d and f). a, b, c and d: light microscope images. e and f: confocal lazer scanning microscope images. a, b, e and f: with Nile blue stain. c and d: without Nile blue stain.

4.4 Particle Size and Zeta Potential Analysis

The emulsions containing MF13, MF42 and AMF with 3:7, 5:5 and 7:3 O/W ratios were analyzed for the particle size and size distribution. The particle size distributions in all the emulsions were monomodal, ranging from 60 nm to 1000 mm (Figure 4.5). The distribution curves for the emulsions containing a higher milk fat concentration shifted to the bigger particle sizes, thereby resulting in a larger average particle size.

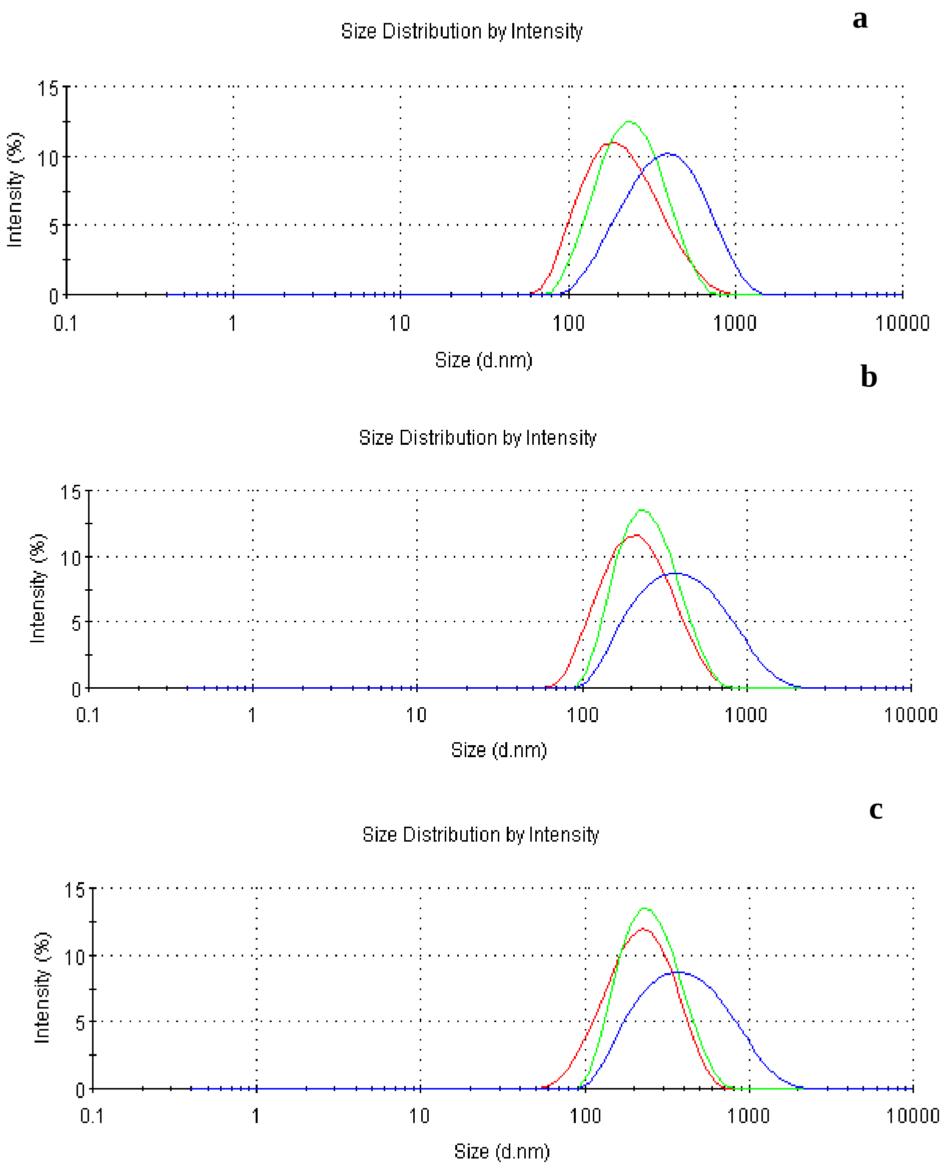


Figure 4.5 Size distributions of particles in emulsions containing (a) MF13, (b) MF42 and (c) AMF at O/W ratio of 3:7 (red), 5:5 (green) and 7:3 (blue).

Table 4.3 shows the average particle diameters of emulsions containing different types of milk fat. They were in the ranges of 194-202 nm, 228-242 nm and 334-352 nm when the emulsions were prepared at the O/W ratios of 3:7, 5:5 and 7:3, respectively. The particle diameters increased with increasing oil concentration, which was consistent with findings of studies by others (Pandolfe 1995; Floury and others 2000). It was observed that the average particle size was not affected by the type of milk fat used. The emulsions containing the different types of milk fats but with the same O/W ratio had similar particle diameters.

Table 4.3 The average particle size of emulsions containing MF13, MF42 and AMF with O/W ratios of 3:7, 5:5 and 7:3

Emulsion	3:7 O/W	5:5 O/W	7:3 O/W
	Particle size (nm)		
MF13	194 ± 1	228 ± 6	334 ± 2
MF42	202 ± 3	238 ± 8	352 ± 8
AMF	197 ± 6	242 ± 6	337 ± 5

The zeta potentials (ζ-potentials) of emulsions were also measured. ζ-potential provides information on the interactions between droplets and can be used to predict the stability of emulsion systems. Normally emulsions that have ζ-potentials higher than +30 mV or lower than -30 mV are considered stable (Technical Notes, Malvern Instruments Ltd). As illustrated in Figure 4.6, the zeta potentials of all emulsions were lower than -30 mV and became more negative as the milk fat concentration increased, except for the AMF emulsions with a 7:3 O/W ratio. Therefore, it could be considered that all the emulsions prepared had a good stability and the repulsive forces between droplets were larger in the emulsions containing higher oil concentrations (i.e. bigger particles). The reason for the sudden decrease in ξ-potential of the AMF emulsion containing 7:3 O/W ratios was not clear.

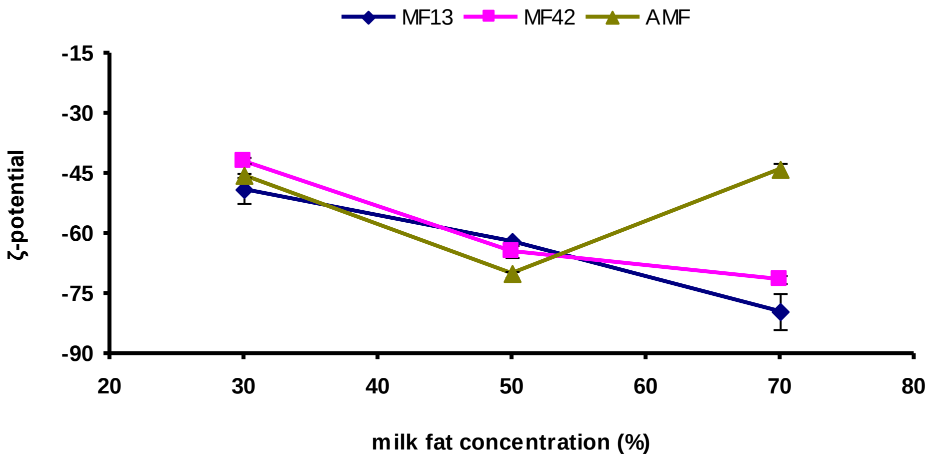


Figure 4.6 Zeta potential of emulsions containing MF13, MF42 and AMF at O/W ratios of 3:7, 5:5 and 7:3

4.5 Storage Stability of Emulsions

The stability of emulsions containing MF13, MF27 and MF42 with four different O/W ratios (1:9, 3:7, 5:5 and 7:3) was monitored by measuring the amount of free oil separated during storage at 4°C and 20°C for 12 weeks. The results, which were expressed as a percentage of total emulsified oil, are shown in Table 4.4. It should be noted that MF27 was used instead of AMF.

The emulsions with the 1:9 O/W ratios appeared to be the most stable, irrespective of the types of milk fats, as no free oil separation was observed after 12 weeks at both 4°C and 20°C. On the other hand, the emulsions with the 5:5 O/W ratios were the least stable. They showed the earliest onset and the highest level of instability, especially at 20°C. This was probably due to the effect of the larger particle size and number in the 5:5 O/W emulsions, which could render a higher collision frequency and interactions between droplets, especially at higher temperature.

A higher instability in emulsions containing larger droplets was also reported by Chanamai and McClements (2000). Coalescence between emulsion droplets is the major mechanism in the formation of free oil. Coalescence is a type of emulsion instability that two emulsion droplets come in contact with each other due to Brownian motion and become a larger droplet. Collision frequency is one of the factors determining the rate of coalescence. It is defined as 'the total number of droplet encounters per unit time per unit volume of emulsions' (McClements 2005).

However, the emulsions containing the high milk fat concentration (70%) were more stable than those with 5:5 O/W ratios. At the 4°C storage temperature, the emulsions did not show any instability throughout the entire 12 weeks storage period, same as those with 1:9 O/W ratios. At the storage temperature of 20°C, the same emulsions however had later onset of instability and less amount of oil separation than the emulsions containing 5:5 O/W ratios with the exception of MF42 emulsions, which might be due to experimental errors.

The decrease in the rate of instability of emulsions containing high milk fat concentrations was probably due to the particle-particle interactions and crowding effects described by Chanamai and McClements (2000). In highly concentrated emulsions, droplets are packed closely together, thus limiting their movements and enabling them to remain stable as long as the stabilizing force is stronger than the attractive force between droplets.

The emulsions stored at 4°C were generally more stable than those at 20°C. This could be due to the mobility of droplets were higher at higher temperature, thus increasing the rate of droplet collisions and instability.

Table 4.4 The percentages of oil separation emulsions containing MF13, MF42 and MF27 with different O/W ratios over 12 weeks at two storage temperatures (4°C and 20°C)

O/W ratio	**Emulsion**	**1**	**2**	**3**	**4**	**8**	**10**	**12**
		Storage (weeks) at 4°C						
1:9	MF13	0	0	0	0	0	0	0
	MF27	0	0	0	0	0	0	0
	MF42	0	0	0	0	0	0	0
3:7	MF13	0	0	0	0	0	0	5.6
	MF27	0	0	0	0	0	0	2.3
	MF42	0	0	0	0	0	0	1.4
5:5	MF13	0	0	0	0	3.3	6.7	6.7
	MF27	0	0	0	0	0	0	3.3
	MF42	0	0	0	0	0	0	2.5
7:3	MF13	0	0	0	0	0	0	0
	MF27	0	0	0	0	0	0	0
	MF42	-	-	-	-	-	-	-
		Storage (weeks) at 20°C						
1:9	MF13	0	0	0	0	0	0	0
	MF27	0	0	0	0	0	0	0
	MF42	0	0	0	0	0	0	0
3:7	MF13	0	0	0	0	0	5.6	5.6
	MF27	0	0	0	0	2.8	11	11
	MF42	0	0	0	0	0	0	8.3
5:5	MF13	0	0	10	20	20	25	25
	MF27	0	0	0	8.1	18	25	20
	MF42	0	0	0	0	0	0	0
7:3	MF13	0	0	0	0	2.5	3	5.1
	MF27	0	0	0	0	7.3	7.3	7.3
	MF42	0	0	0	0	0	0	22

4.6 Effects of Heating and Freezing/Thawing on Emulsion Stability

Protein stabilized emulsions are sensitive to temperature as protein denatures once a critical temperature is reached, thus leading to emulsion instability (McClements 2004). Rhamnolipid molecules are stable to 121°C for at least one hour (Product specifications, Appendix A). In this study, the stability of emulsions after heating at 80°C for 30 minutes was investigated. The results are presented in Table 4.5.

Table 4.5 Percentages of oil separation in emulsions containing MF13, MF42 and AMF after heat treatment

O/W ratio	% of oil separation in emulsions		
	MF13	MF42	AMF
3:7	0.00 ± 0	0 ± 0	0 ± 0
5:5	0.00 ± 0	0 ± 0	0 ± 0
7:3	0.00 ± 0	27.36 ± 5.02	0 ± 0

The emulsions containing MF13 and AMF remained stable after heating, regardless of the O/W ratio. However, MF42 emulsions remained stable only when the O/W ratios were 3:7 and 5:5. MF42 emulsions containing 70% oil exhibited instability with 27% free oil separation. This suggests that the instability observed in the MF42 emulsions with 7:3 O/W ratio was not due to the loss of emulsifying properties of rhamnolipids but rather due to a more severe mechanical change that the emulsion droplets might undergo during the heat treatment.

MF42 has a much higher solid fat content than AMF and MF13 at a given temperature below their melting points (Table 2.1). At 20°C, the solid fat contents of MF42, AMF and MF13 are 64.2%, 22.5% and 0%, respectively. This means the MF42 emulsions would have a large amount of crystallized fat whereas AMF and MF13 emulsions contained only a small portion or no crystallized fat before heating. Partial coalescence increases with increasing solid fat content (Boode and others 1993). The collisions between these crystallized droplets in the highly concentrated MF42 emulsions with the O/W ratio of 7:3 could lead to partial coalescence. Heating the emulsions at 80°C would have melted and disrupted the milk fat crystal net work, which provides the mechanical strength for the irregularly shaped partially crystallized droplets (Cramp and others 2004). As a result, full coalescence between droplets might take place, resulting in the formation of free oil. The instability was not observed in MF42 emulsion with 3:7 and 5:5 O/W ratios, probably because no partial coalescence occurred between the semisolid emulsion droplets due to the lower droplet concentration. The collision frequency of emulsion droplets decreases with the decrease in droplet concentration and partial coalescence only takes place when the droplets get close together (McClements 2005).

When the same emulsions containing MF13, MF42 and AMF with O/W ratio of 3:7, 5:5 and 7:3 were frozen at -18°C for 48 hours and thawed, all emulsion samples appeared to destabilize, regardless of the types of milk fats and the O/W ratios. The emulsion system was broken down completely as the oil separation was observed to be 100%. It separated into two phases, with the yellow free oil phase on top and the translucent aqueous phase at the bottom after centrifugation (Figure 4.7).

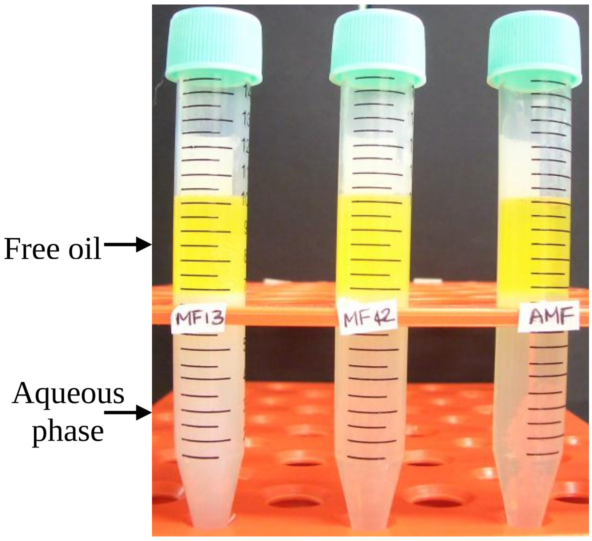


Figure 4.7 The separations of oil from the stable emulsions (MF13, MF42 and AMF) with 5:5 O/W ratio after freezing and thawing

The instability of emulsions occurred during freezing/thawing could be due to several physicochemical processes. First of all, the milk fat within the oil droplets crystallized at -18°C, which might lead to partial coalescence. Partial coalescence is a type of emulsion instability that the fat crystals within oil droplets pierce through the interfacial membranes and form linkages with other partial crystallized droplets, forming irregularly shaped aggregates (Walstra 2003). At -18°C, these aggregates would remain intact by the strength of the fat crystal network. Upon thawing, they rapidly could have coalesced and resulted in the formation of free oil at the increasing temperature. Secondly, the formation of ice crystals in the aqueous phase during freezing might force oil droplets to come into close proximity and increase the volume fraction of the oil phase dramatically (Ghosh and Coupland 2008). Droplets stabilized by electrostatic repulsions are susceptible to coalescence when they are sufficiently close to each other (McClements 2005). Thirdly, the formation of ice crystals penetrates the interfacial membranes surrounding the oil droplets. Thus they are more prone to coalescence when thawing (McClements 2005).

4.7 Effects of pHs on Emulsion Stability

The pH values of emulsions containing different types of milk fats, MF13, MF42 and AMF at 3:7 O/W ratios were all around 7.2 (Table 4.6). It was observed that all the prepared emulsions at pH 7.2 remained stable without creaming or oil separation. As rhamnolipids molecules are anionic type of biosurfactants with the pK_a value of 5.6 (Ishigami and others 1987; Cohen and Exerowa 2007), the emulsion stability observed could be attributed to the electrostatic repulsive forces between the negatively charged droplets at pH 7.2.

Table 4.6 The pHs of emulsions containing MF13, MF42 and AMF at 3:7 O/W ratio

Emulsions	pH
MF13	7.19 ± 0.02
MF42	7.19 ± 0.02
AMF	7.17 ± 0.05

The effects of pHs on the emulsion stability were investigated by measuring the amount of oil separated after adjusting the pH of emulsions to pH 2 – 12 followed by centrifugation (Figure 4.8). The results, expressed as a percentage of oil (i.e. free oil) separated from the stable emulsions, are shown in Figure 4.9. The amount of free oil increased as the emulsion pH decreased from pH 7.2. However no oil separation occurred at the pH values higher than 7.2, indicating that the emulsion stability was significantly affected by acidic conditions but not by alkaline conditions. As anticipated, no difference was observed between the emulsions containing different types of milk fats at the given same pH levels.

The instability of emulsions observed at pH lower than 7.2 could be related to the coalescence of emulsion droplets. As the pH decreases, the carboxylic groups of rhamnolipid molecules are protonated and the rhamnolipids exhibited nonionic behaviour. Hence the electrostatic repulsions between droplets are reduced and the droplets can come into contact with each other readily, leading to coalescence and emulsion destabilization (Ozdemir and others 2004). On the other hand, the emulsions remained stable at pH levels higher than 7. This was because as pH increases, the negative charges on the carboxylate groups of rhamnolipid molecules increase and create larger electrostatic repulsions between them, therefore increasing the electrostatic repulsive forces which are stabilizing the emulsion droplets (Champion and others 1995).

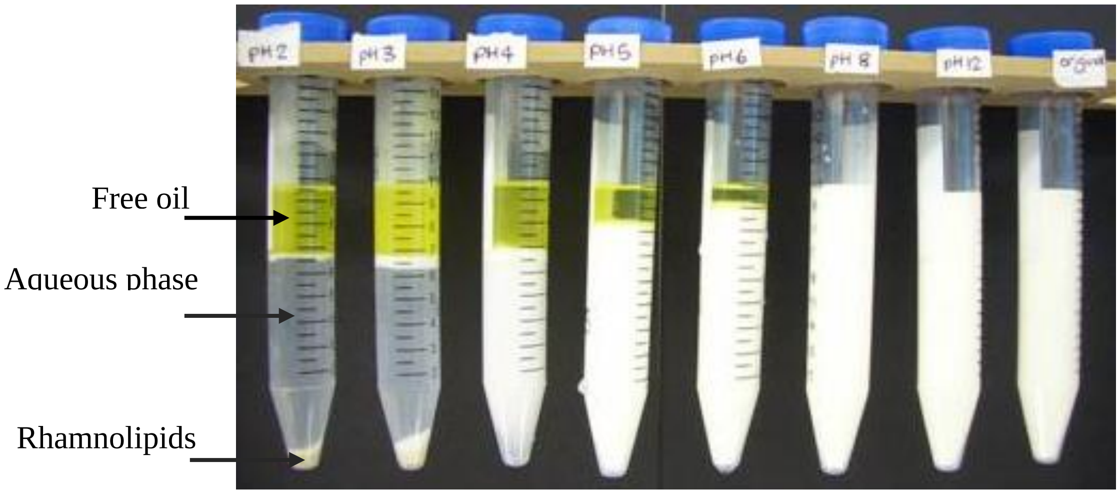


Figure 4.8 Oil separations in MF 13 emulsion with 3:7 O/W at pH 2 to pH 12

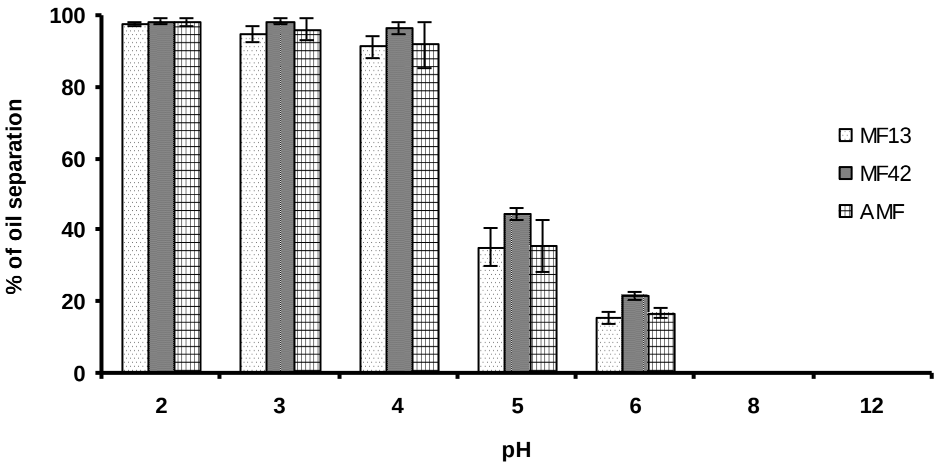


Figure 4.9 The percentages of oil separation in emulsions (O/W 3:7) containing MF13, MF42 and AMF at different pH levels

It was interesting to observe that at pH 2, almost 100% of oil was separated out by completely destabilizing the emulsion systems. A separated layer of free oil and translucent aqueous phase with a brown coloured precipitate were noted. Although it has not been analyzed, the brown color precipitate is believed to be rhamnolipid molecules. The similar results were also obtained in the emulsions at pH 3 but with a little cream layer below the free oil phase. This suggests that a small portion of the emulsion still remained stable. However, both the sedimentation of rhamnolipids and the formation of the translucent aqueous phase were not observed in the emulsions at pH 4-6 even when the separation of oil took place. Instead, the emulsions revealed two separated layers – the yellow free oil layer on top and the white emulsion layer at the bottom. The degree of oil separation at pH 4 was still high, being around 93% as compared to 40% and 20% at the pH levels of 5 and 6, respectively. The clear separation of two phases observed only under the extreme pH levels such as pH 2-3 could be partly due to the hydrolysis of rhamnolipid molecules at very low pH levels as the glycosidic linkages of rhamnolipids can be hydrolysed by acid. This needs to be further investigated.

The MF13 emulsions with adjusted pH were also measured for their ζ-potentials (Table 4.7). The emulsion at pH 12 had the highest ζ-potential value of -58 mV, whereas sample at pH 4 had the lowest value of -44.4 mV. Although the magnitude of ζ-potential tends to decrease as the pH decreases, no certain trend could be established between them. The ζ-potentials of the emulsions of pH 4, pH 5 and pH 6 appeared to be relatively high being -44.4 mV, -49.8 mV and -49.1 mV, respectively. This could be due to the fact that the ζ-potentials of these emulsions were measured from the stable parts of emulsions after removing the free oil. Therefore it should be noted that the ζ-potential values obtained using this method do not provide the 'true' stability estimation of emulsions.

Table 4.7 Zeta potentials of MF13 emulsions with 3:7 O/W ratio adjusted to different pH levels

pH	ζ-potentials (mV)
4	-44.4
5	-49.8
6	-49.1
7.2	-54.4
8	-48.1
12	-58.0

4.8 Effects of Salts and Ionic Strength on Emulsion Stability

The effects of types of salts and ionic strength on the emulsion stability were studied. The emulsions (3:7 O/W) containing MF13, MF42 and AMF were added with NaCl, KCl and $CaCl_2$ at various concentrations. The concentrations of NaCl and KCl used were ranged from 1 to 600 mM, whereas the $CaCl_2$ concentrations were between 0.5 mM and 5 mM. The instability of emulsions was measured by the amount (%) of free oil separated from the emulsions. The results are shown in Figures 4.10 to 4.13.

The results shown in Figure 4.10 indicate that the presence of NaCl modified the behaviour of emulsions by inducing free oil separation. As the concentration of NaCl increased gradually from 1 to 450 mM, the emulsions became increasingly unstable, regardless of the types of milk fats they contained. A maximum oil separation of 70% occurred at 450 mM. When the salt concentration was further increased from 450 mM to 600 mM, the amount of oil separated was however significantly lower than the emulsions containing lower salt concentrations (250 mM and 450 mM). This phenomenon was even more pronounced in the emulsions containing AMF. In contrast, at lower salt concentration ranges up to 100 mM NaCl, the emulsions containing AMF rendered a relatively high instability with larger amounts of oil separation compared to the emulsions containing MF13 and MF42 ($p<0.05$).

The reason for the lower oil separation observed at 600 mM NaCl compared to at 450 mM seemed to be associated with creaming as shown in Figure 4.11a, which was not observed in the other emulsions with lower NaCl concentrations. There were three layers formed in the emulsions when 600 mM NaCl was added: the free oil layer on top, the yellowish cream layer in the middle and the stable emulsion layer at the bottom. Interestingly these cream layers disappeared over night (approximately 18 hours) when stored at both 4°C and 20°C, whereas the free oil layers increased substantially (Figure 4.11b). This phenomenon suggests that the oil droplets in the cream layers could have undergone coalescence and became destabilized, leading to the separation of oil.

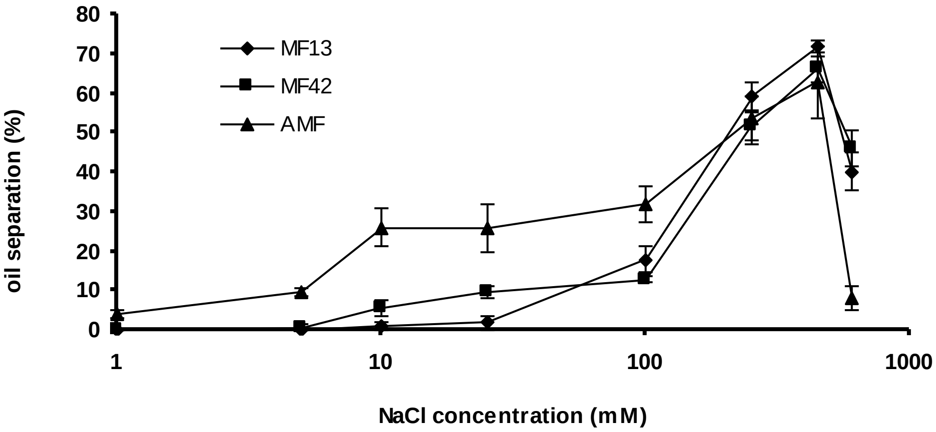
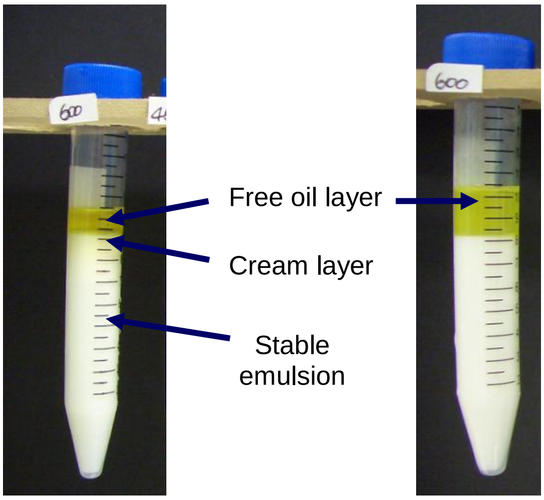


Figure 4.10 The effect of NaCl on the stability of emulsions (3:7 O/W) containing MF13, MF42 and AMF

The patterns of emulsion instability induced by the different concentrations of KCl were very similar to the observed effects of NaCl concentrations (Figure 4.12). The percentage of oil separation at 450 mM was also around 70%. Similarly, at low KCl concentrations (1, 5, 10 and 100 mM), the AMF emulsions appeared to be less stable than MF13 and MF42 emulsions as the percentage of oil separation was significantly higher ($p<0.05$). However, as the KCl concentration increased, the difference in the amount of oil separated between these three types of emulsion became small and insignificant.



(a) (b)

Figure 4.11 (a) the formation of free oil and cream layers in the emulsions (MF13 3:7 O/W) after the addition of 600 mM NaCl; (b) the disappearance of the cream layer and the substantial increase in the amount of free oil after 18 hours.

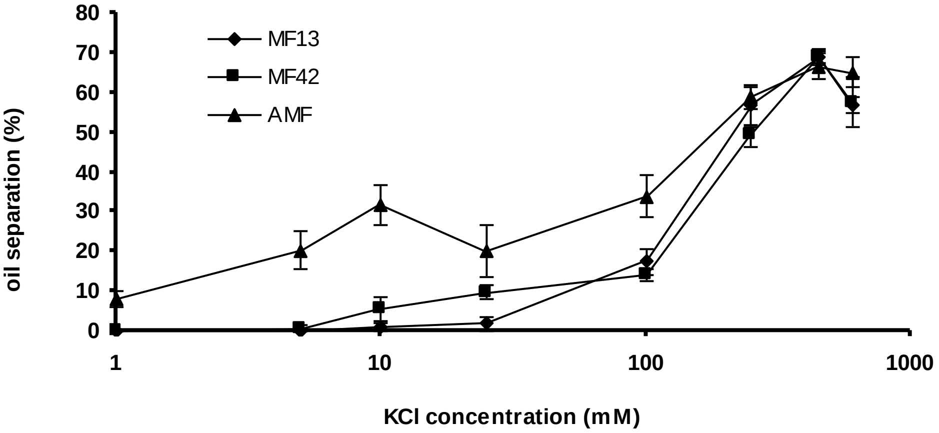


Figure 4.12 The effect of KCl on the stability of emulsions (3:7 O/W) containing MF13, MF42 and AMF

The effect of $CaCl_2$ and its concentration on the stability of emulsions were more significant even at a very low salt concentration as it induced a relatively higher oil separation than did NaCl and KCl (Figure 4.13). The similar results were reported by Keowmaneechai and McClements (2002). However, unlike the AMF emulsions that exhibited a significantly higher instability than the MF13 and MF42 emulsions at the low salt concentrations of NaCl and KCl, the addition of $CaCl_2$ resulted in the MF13 emulsion being more stable than the other two types of emulsions. As the results of this inconsistent variation, it is rather difficult to make conclusive statement on whether the types of milk fats had any influence on the salt-induced emulsion instability.

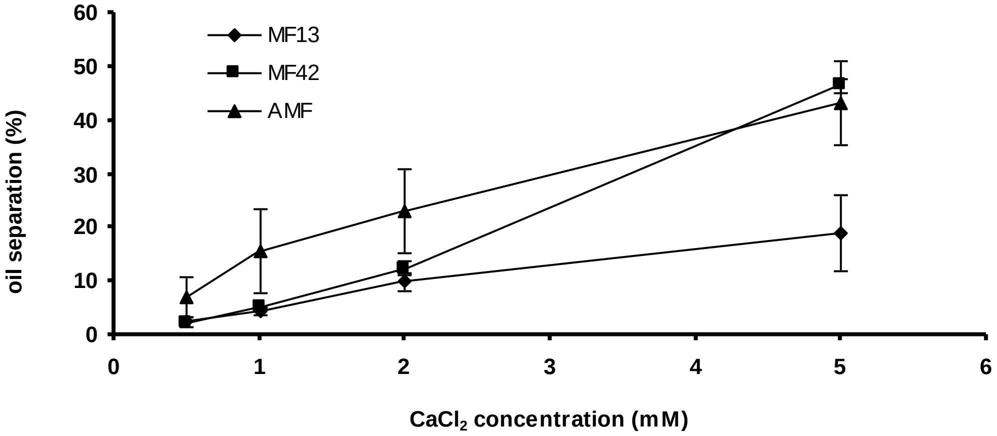


Figure 4.13 The effect of $CaCl_2$ on the stability of emulsions (3:7 O/W) containing MF13, MF42 and AMF

The formation of free oil observed when salts were added to the emulsions could be resulting from the coalescence of oil droplets, which is the mechanism that two emulsion droplets merge together and become a larger droplet. The rate of coalescence is determined by the nature of the interfacial membranes surrounding the droplets and it is influenced by the ionic strength of the aqueous phase (McClements 2005). As already described, the stability of rhamnolipids stabilized emulsions is attributed to the repulsive electrostatic forces provided by the negatively charged rhamnolipid molecules at the droplet interfaces. The addition of NaCl, KCl and $CaCl_2$ increases the ionic strength in the aqueous phase of the emulsions, which causes the shielding of the negative charges of the carboxylate head groups by counter ions (Na^+, K^+, Ca^{2+}) (Hunter 1981). This thus results in the reduced electrostatic repulsions between emulsion droplets (Demetriades and others 1997a; HelvacI and others 2004). Coalescence of droplets take place when the electrostatic repulsion is no longer strong enough to keep them apart, leading to the separation of oil.

The monovalent ions (Na^+, K^+ and Cl^-) and the divalent ion (Ca^{2+}) induce the instability in emulsions by different mechanisms. Na^+, K^+ and Cl^- caused the coalescence of droplets by screening of electrostatic interactions (Kulmyrzaev and Schubert 2004), whereas Ca^{2+} causes the coalescence of droplets by a combination of binding of counter ions to droplet surfaces and screening of electrostatic interactions (Kulmyrzaev and others 2000). Therefore, it is believed that the latter case can induce a higher degree of droplet coalescence. This could be accounted for the relatively higher oil separation observed in emulsions with the addition of $CaCl_2$ in this study.

4.9 Rheological Properties of Emulsion

Steady State Flow Measurements

The flow behaviour of emulsions containing MF13, MF42 and AMF at O/W ratios of 3:7, 5:5 and 7:3 was analyzed by registering shear stress at shear rates from 0 to 500 s^{-1} and at the constant temperature of 20°C. The flow curves, plotted as log-log viscosity versus shear rate, are shown in Figure 4.14. The results indicate that the viscosity increased with increasing fat content. However, no pronounced difference in viscosity was observed between emulsions containing different types of milk fats. It has been reported that the physical states of the emulsion droplets affect the viscosity of emulsions because of the disruption of flow by friction from rigid particles (Hunter 1986; Mewise and Macosko 1994; McClements 2005). However this was not observed in this study despite the fact that the physical states of milk fats within the oil droplets at 20°C could be different between the MF13, MF42 and AMF emulsions. This is because MF13 has a melting point of 13°C, therefore the milk fats within the emulsion droplets would have been liquid, whereas the milk fats droplets in MF42 and AMF emulsions could have been solid or mixtures of semi-solid and liquid.

All emulsions exhibited non-Newtonian flow behaviour as the emulsion viscosity decreased with the increasing shear rate, hence a shear-thinning flow behaviour. However, the shear thinning effect was more pronounced for the emulsions with the higher O/W ratios (5:5 and 7:3). Some fluctuations in viscosity values observed below shear rates of 1 s^{-1} are suggested to be due to the sensitivity of the equipment sensors used (Lorenzo and others 2008). The shear thinning behaviour of emulsions is usually due to the presence of aggregated or flocculated emulsion droplets, which increases with increasing milk fat concentration. For emulsions have high dispersed phase concentrations, especially those containing 70% of oil, the droplets come into close contact with each other, thus causing them to exist as flocculates rather than single individual droplets. As the shear increases, the flocculated droplets are disrupted and lead to the decrease in the viscosity of emulsions (Floury and others 2000).

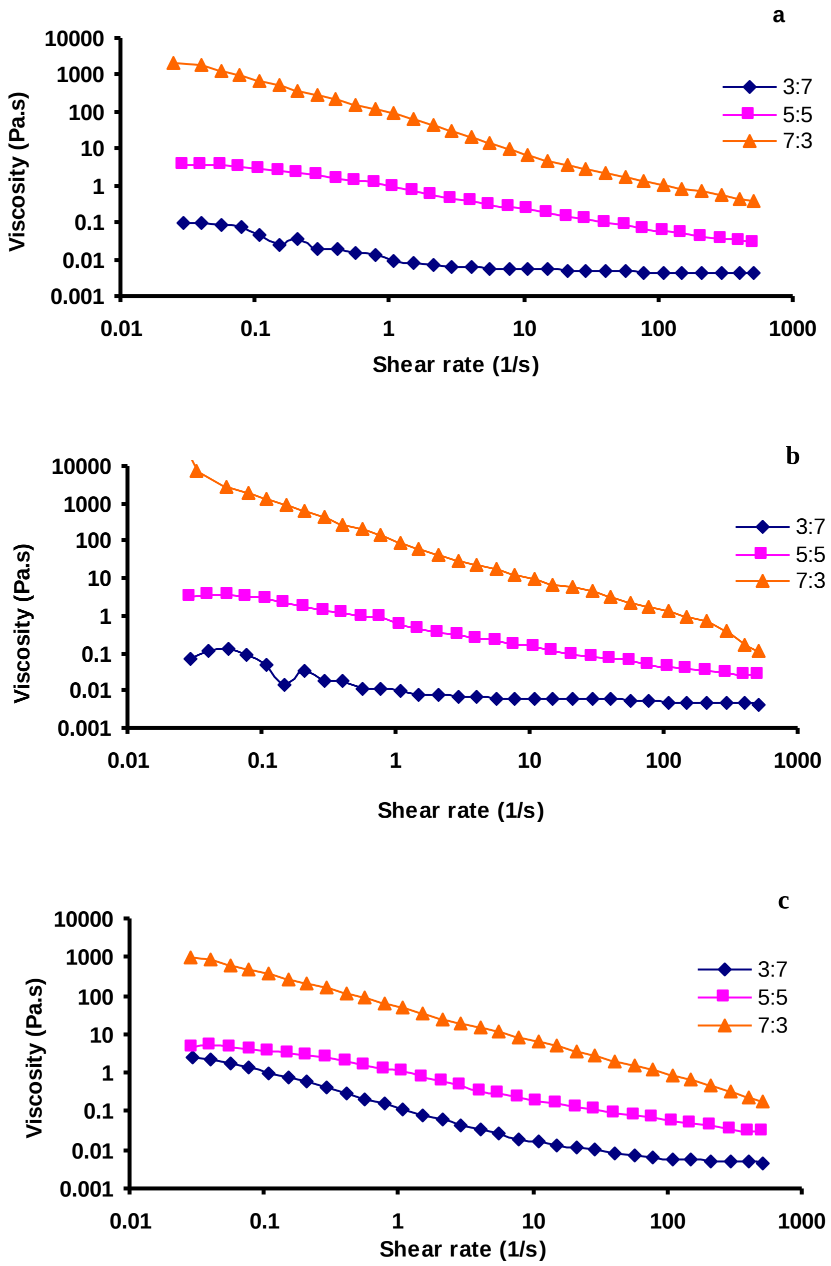


Figure 4.14 The flow behaviour of emulsions containing (a) MF13, (b) MF42 and (c) AMF at O/W ratios of 3:7, 5:5 and 7:3

The emulsions containing different types of milk fats with 5:5 and 7:3 O/W ratios were further analyzed for their thixotropic characteristics. They were subjected to increasing and then decreasing shear rate in the range of 0 to 500 s^{-1}. The changes in the emulsion viscosity as a function of shear rate were plotted logarithmically as shown in Figure 4.15.

For the emulsions with 5:5 O/W ratios, the change in the viscosity with shear rates followed a similar pattern. The viscosity decreased with the increasing shear rate during the ‘up cycle’. During the ‘down cycle’, the viscosity remained the same at shear rates above 10 s^{-1}. However at shear rates lower than 10 s^{-1}, the viscosity was slightly higher than the viscosity observed during the ‘up cycle’. This behaviour is thought to be that in the ‘up cycle’ stage, as the shear rate increases the aggregated droplets are broken down by the more intense shear stress applied to the emulsions, thus reducing their viscosity. In the subsequent ‘down cycle’ stage, as the shear rate decreases to a certain level, the shear stress applied is no longer effective at breaking down any aggregated structures and promotes the chance of droplet aggregation by the increased collision frequency between droplets.

The MF13 emulsions containing O/W of 7:3 showed a slight decrease in the viscosity at shear rates lower than 10 s^{-1} during the ‘down cycle’. However for MF42 and AMF emulsions with the same O/W ratios, the viscosity observed during the ‘down cycle’ was considerably lower. This suggests that emulsion droplets were packed closely together and formed a three dimensional network in the highly concentrated emulsions. The three dimensional network are broken down by the increasing shear rate and cannot be rebuilt when the shear rate is reduced. Hence, the viscosity of the emulsions is lower due to the disruption of the structures (McClements 2005).

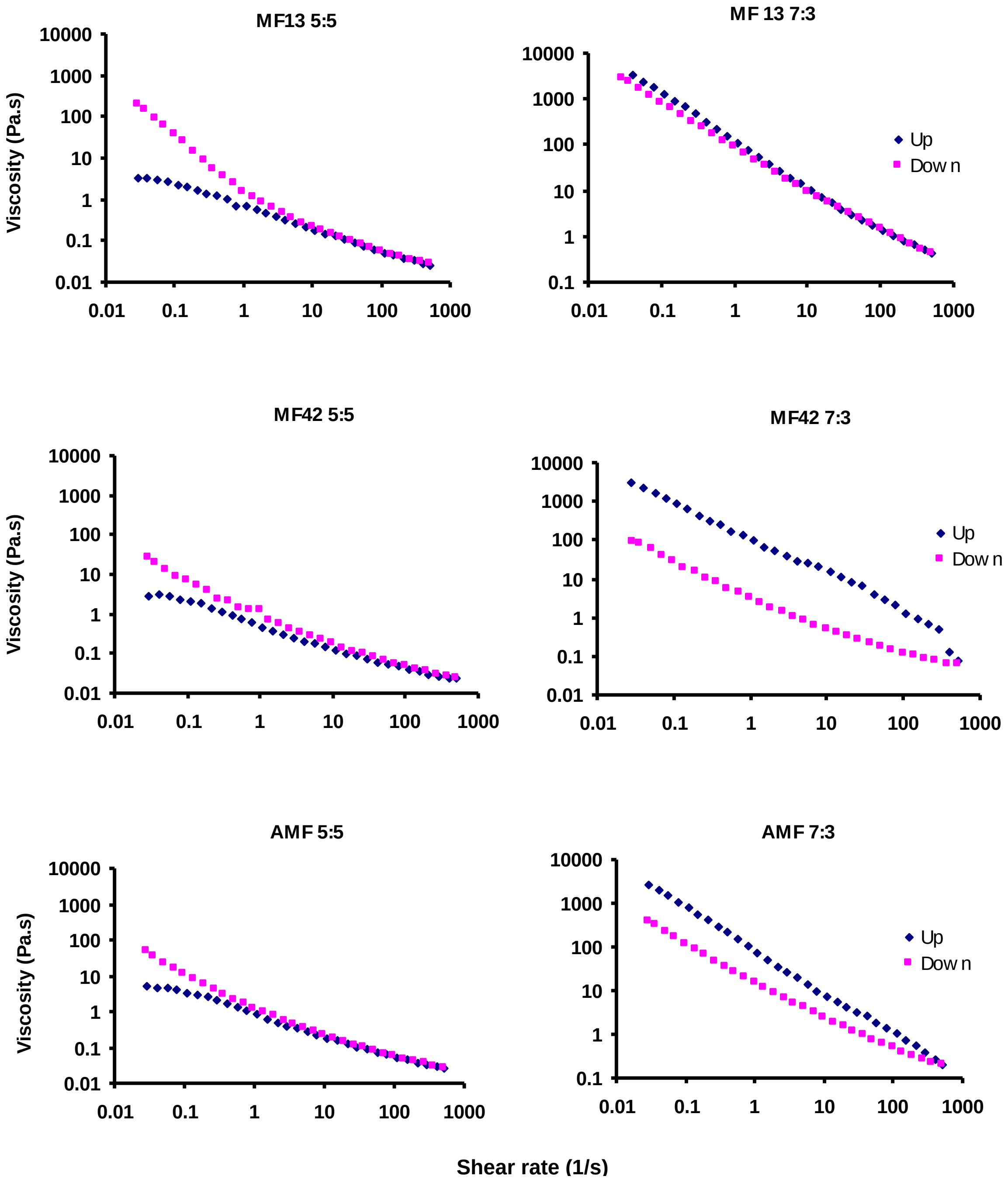


Figure 4.15 Thixotropic measurements of emulsions containing MF13, MF42 and AMF at O/W ratios of 5:5 and 7:3

Temperature Sweep Test

The changes in rheological properties of emulsions containing MF13, MF42 and AMF with temperature were analyzed. For this test, the emulsions were subject to a constant shear rate of 50 s^{-1} and the temperature was first increased from 4°C to 80°C and then decreased back to 4°C at the rate of 2°C per minute. The viscosity of emulsions was plotted as a function of temperature logarithmically as illustrated in Figure 4.16.

The viscosity of emulsions with the O/W ratio of 3:7 remained relatively constant with a slight decrease when the temperature increased from 4°C to 80°C, irrespective of the type of milk fat. As temperature decreased from 80°C to 4°C, the emulsions became slightly more viscous compared to the viscosity measured during increasing temperature. Similar results were observed in the emulsions with 5:5 O/W ratios. However the increase in emulsion viscosity during the cooling cycle was more pronounced when compared to the emulsions containing 3:7 O/W ratios. At 5:5 O/W ratios, the viscosity of the emulsions containing MF13 remained the same during the heating cycle. On the other hand, the initial viscosity during the heating cycle at low temperature was high for the emulsions containing MF42 and AMF. It dropped when the temperature reached around 40°C and 30°C, respectively and stayed relatively constant until 80°C. The initial high viscosity was clearly due to the physical state of milk fat droplets, which are solid at low temperature due to the high melting point of MF42 and AMF. For the highly concentrated emulsions with 7:3 O/W ratios, the viscosity during the cooling cycle was lower than during the heating cycle, which was opposite to the trends observed in emulsions containing 3:7 and 5:5 O/W ratios. This could be due to the fact that the MF42 and AMF emulsions containing 70% of milk fat were highly viscous at low temperature and the viscosity was reduced once the temperature reached the melting points of MF42 and AMF, which is approximately 42°C and 34°C, respectively.

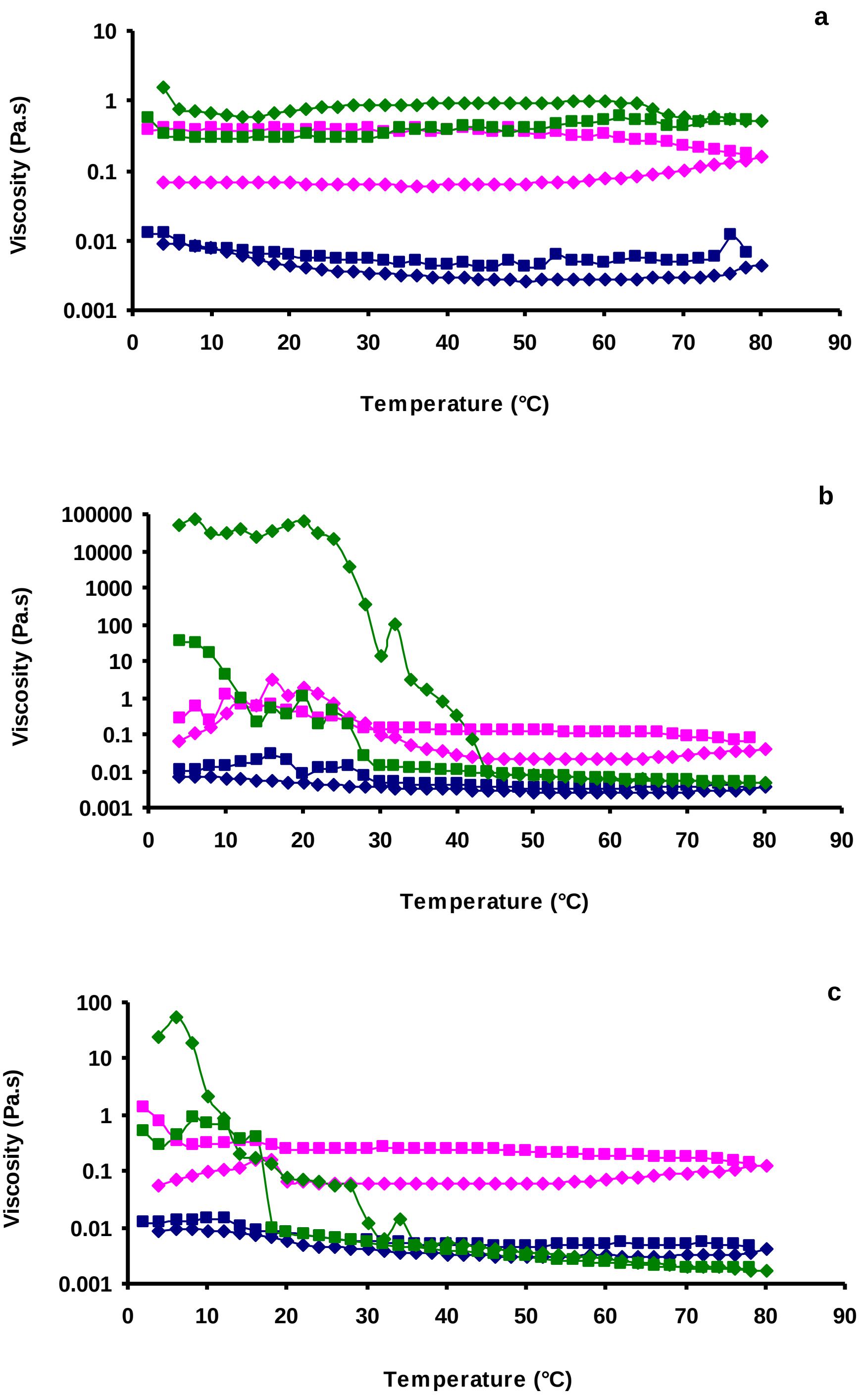


Figure 4.16 Changes in viscosity with temperature in emulsions containing (a) MF13, (b) MF42 and (c) AMF at O/W of 3:7 (blue), 5:5 (pink) and 7:3 (green). (♦) 4-80°C; (■) 80-4°C;

Dynamic Tests

The amplitude sweep tests were carried out to determine the linear viscoelastic regions of highly concentrated emulsions (70% milk fat) containing MF13, MF42 and AMF at a frequency of 1Hz and varying stress amplitudes. The elastic modulus (G'), viscous modulus (G'') and phase angle (δ) were measured and displayed in Figure 4.17.

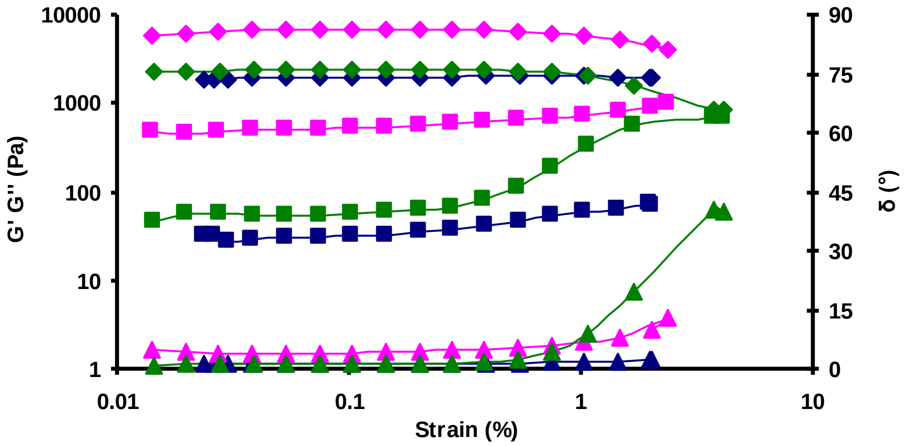


Figure 4.17 Elastic modulus (G', ♦), viscous modulus (G'', ■) and phase angle (δ, ▲) as a function of strain (%) for emulsions with 7:3 O/W ratios (MF13, blue; MF42, pink; AMF, green).

Emulsions containing MF13 and MF42 showed larger linear viscoelastic regions than the one containing AMF. In fact, the elastic modulus (G') and the viscous modulus (G'') of MF13 and MF42 emulsions stayed constant for almost the whole stress amplitude range tested. It has been suggested that weakly flocculated and stable emulsion systems have longer linear regions than those that are coagulated and strongly flocculated (Tabilo-Munizaga and Barbosa-Canovas 2005). Therefore, emulsions containing AMF could be slightly less stable than those containing MF13 and MF42.

Emulsions containing AMF exhibited structural changes at strain values above 1%, demonstrated by the changes observed in the dynamic modulis G' G'' and δ. The decrease in G' and increase in G'' indicated the emulsions changed from a solid-like material to a liquid like material, which was also confirmed by the dramatic increase in δ. δ has a value range from 0° to 90° with 0° representing absolute solid and 90° representing absolute liquid (Bourne 2002). The maximum stress amplitude is the stress at which the elastic modulus (G’) stays constant. It also represents a type of yield value. The structure of the material will be destroyed if the stress exceeds the maximum stress (Davis 1973; Munoz and Sherman 1990). In this case, the maximum strain for the emulsions containing MF13, MF42 and AMF was 1%. Hence the linear viscoelastic regions for MF13, MF42 and AMF emulsions were below the strain of 1%.

As illustrated in Figure 4.18, the elastic modulus G’ was always higher than the viscous modulus G’’ in the emulsion containing MF13 in the range of frequency studied, indicating it had a weak gel structure (Quintana and others 2002b; Sosa-Herrera and others 2008). The phase angle δ was approximately 45° at frequency ranged between 0.01 and 1 Hz. It dropped significantly to approximately 6.5° at 5 Hz, which indicates the elastic modulus became more dominant at frequency higher than 5 Hz. Although G’ was higher than G’’ at frequency between 0.01 and 2.5 Hz, the difference was small.

MF42 and AMF emulsions had different viscoelastic properties to MF13 emulsions. Both MF42 and AMF emulsions exhibited liquid like characteristics initially, as the viscous modulus (G’’) was greater than the elastic modulus (G’). The phase angle (δ) had values between 80° and 90° for both emulsions before the solid-liquid transition took place. The MF42 emulsions slowly became more solid like at 20 Hz, indicated by the decrease in the phase angle (δ). The transition of viscoelastic behaviour from more liquid like to more solid-like for the AMF emulsions took place at approximately 4 Hz.

It is not clear why the emulsions containing MF13 displayed such different viscoelastic properties to the emulsions containing MF42 and AMF. However, they shared one common characteristic, which was the decrease in the values of δ at higher frequency. Hence the elastic modulus became more dominant at higher frequency. Materials exhibit more liquid-like characteristics at low frequency as the molecules within materials move and rearrange during the experiment. At high frequency, structures within materials do not have sufficient time to break down and rebuild, hence the elasticity is more pronounced (Everett and Auty 2008).

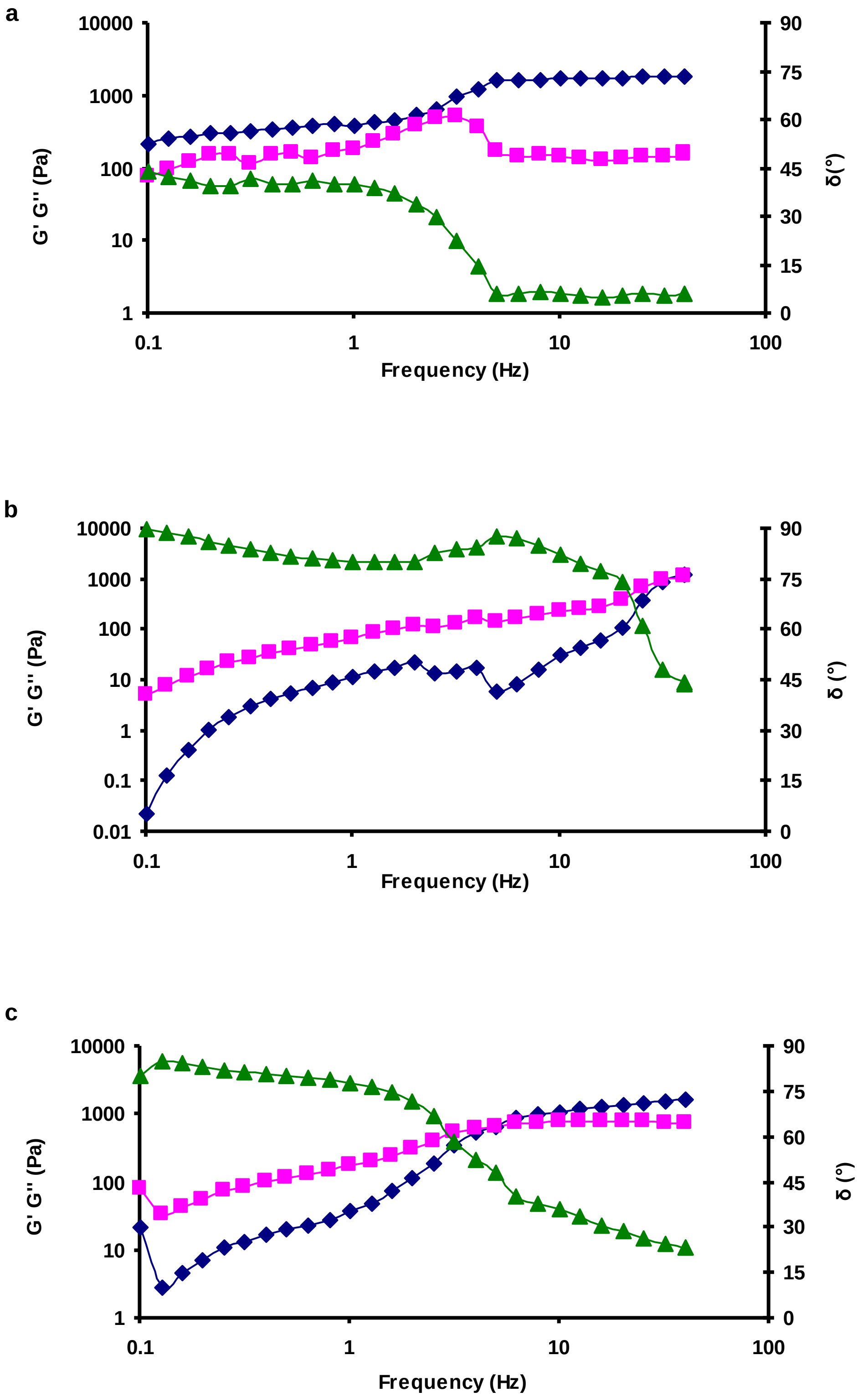


Figure 4.18 Elastic modulus (G’, ♦), viscous modulus (G’’, ■) and phase angle (δ, ▲) as a function of frequency (Hz) for emulsions containing (a) MF13, (b) MF42 and (c) AMF at 7:3 O/W ratio

4.10 DSC

The thermograms of the cooling and heating of three different bulk milk fats (MF13, MF42 and AMF) from DSC, corresponding to milk fat crystallization and melting are shown in Figure 4.19. The crystallization and melting points of the milk fats were taken from the onset temperature of the peaks. During the cooling process, which was when the liquefied milk fats were subjected to decreasing temperature from 60°C to -50°C, MF13, MF42 and AMF started crystallizing at temperatures 2°C, 28°C and 20°C, respectively (Figure 4.19a). MF13 showed a single exothermic peak at approximately -1°C, whereas the other two fats exhibited two exothermic peaks at 24°C and 9°C for MF42 and 16°C and 8°C for AMF.

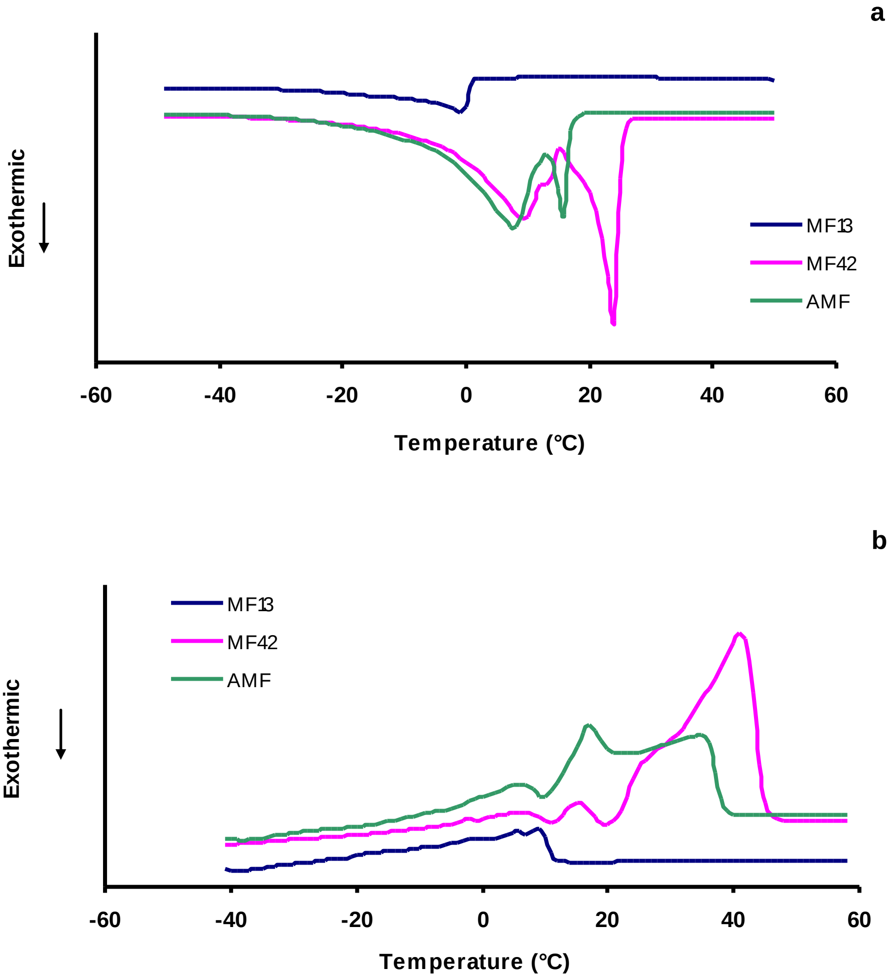


Figure 4.19 Thermograms of crystallization (a) and melting (b) properties of three different types of bulk milk fats (MF13, MF42 and AMF) analyzed using DSC

According to Table 2.1, which illustrates the solid contents of each milk fat at various temperatures, the melting points of MF13, MF42 and AMF are 11-15°C, 43°C and 31-34°C, respectively. The crystallization temperatures observed in bulk milk fats were lower than their melting points. This was because usually the nucleation of fat crystals does not occur until the free energy barrier is overcome, which takes a substantial period of time (Hartel 2001; Cramp and others 2004).

During the subsequent heating process, the solidified fats were heated from -50°C to 60°C. The melting transitions of MF13, MF42 and AMF all started at -40°C but the major phase transitions occurred at the temperatures around their known melting points (Figure 4.19 b). MF13 showed a single broad endothermic peak at 9°C. On the other hand, MF42 exhibited two small endothermic peaks at 7°C and 15°C and one large peak at 41°C. The large peak at 41°C confirms the melting point of MF42 known as around 43°C. The two small peaks indicate that although MF42 contains mostly high melting point triglycerides, there is still a small amount of low melting point portion present in MF42. AMF showed three partially overlapped endothermic peaks corresponding to low-, medium- and high melting fractions during melting, which was a typical melting curve of milk fat according to other authors (Timms 1980; Shukla and others 1993; Lopez and others 2001). Based on these results, the melting peak of MF13 could be corresponding to the peaks of the low-melting fractions of MF42 and AMF.

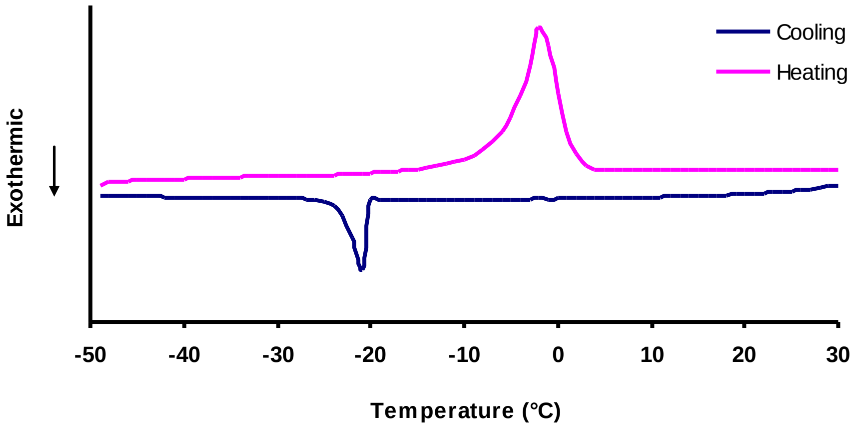


Figure 4.20 Thermogram of rhamnolipids (25%, w/w) during cooling and heating

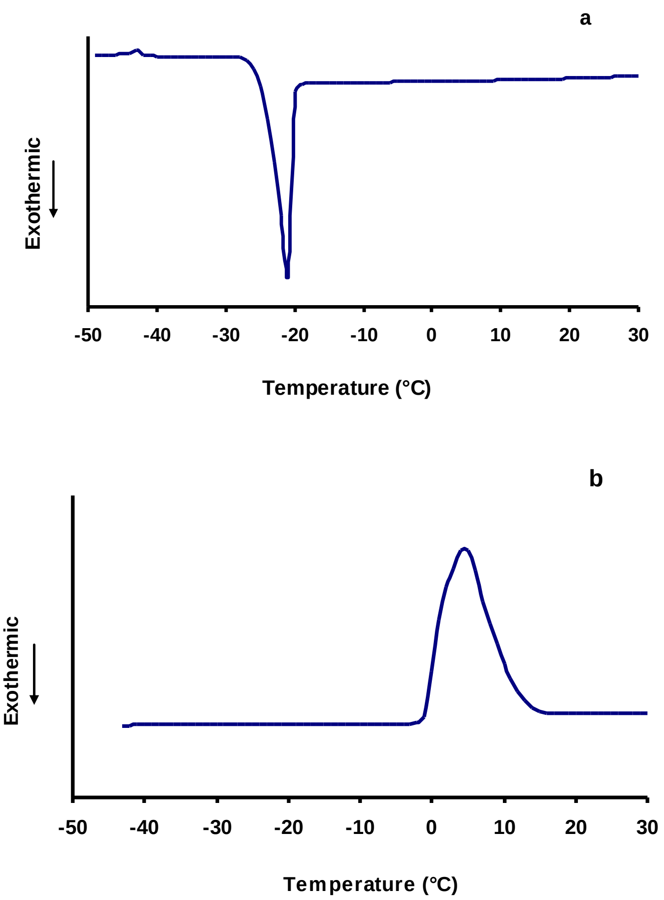


Figure 4.21 Thermograms of pure water during (a) cooling and (b) heating

The emulsions containing milk fats (MF13, MF42 and AMF) at three different ratios of O/W (3:7, 5:5 and 7:3) were also analyzed for their thermal properties using DSC. The MF13 emulsions with different O/W ratios showed similar crystallizing and melting behaviours, as shown in Figure 4.22 and Figure 4.23. In the cooling process, the MF13 emulsion containing 70% fat was characterized by two broad exothermic peaks at -2°C and -13°C (Figure 4.22b) and one large exothermic peak at -17°C (Figure 4.22a). When compared to the exothermic peak occurred during the cooling of pure water (Figure 4.21a), it can be concluded that the large exothermic peak at -17°C represents the crystallization of water and the broad peaks at -2°C and -13°C represent the crystallization of milk fat droplets.

The melting of MF13 emulsions containing different O/W ratios are shown in Figure 4.23. Only single endothermic peaks around 1°C were observed in all emulsions. When compared to the thermograms obtained from the melting of water (Figure 4.21b), it can be concluded that the singles peaks in Figure 4.23 represents the melting of water. The melting peaks of emulsion droplets were not observed.

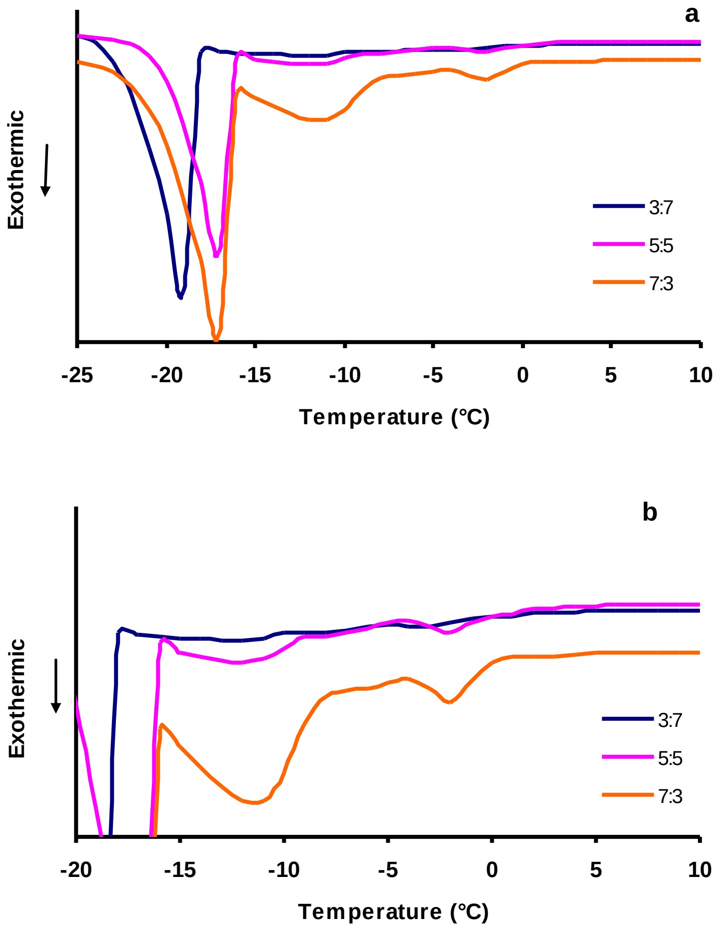


Figure 4.22 Thermograms of MF13 emulsions with O/W ratios of 3:7, 5:5 & 7:3 during cooling from 60°C to -50°C. (a) Temperature range:-25°C to 10°C; (b) -20°C and 10°C

However, heat absorption (endothermic) can be observed when the melting curves were focused at the temperature range of -45°C and 5°C (Figure 4.23b), which indicates the melting of milk fat droplets starting at -40°C. As illustrated in Figure 4.19b, the melting of pure MF13 started at approximately -40°C and completed at approximately 12°C. This suggests the melting peaks caused by emulsion droplets were masked by the melting peaks of water and the melting of emulsified MF13 would have continued on to the temperature of 12°C.

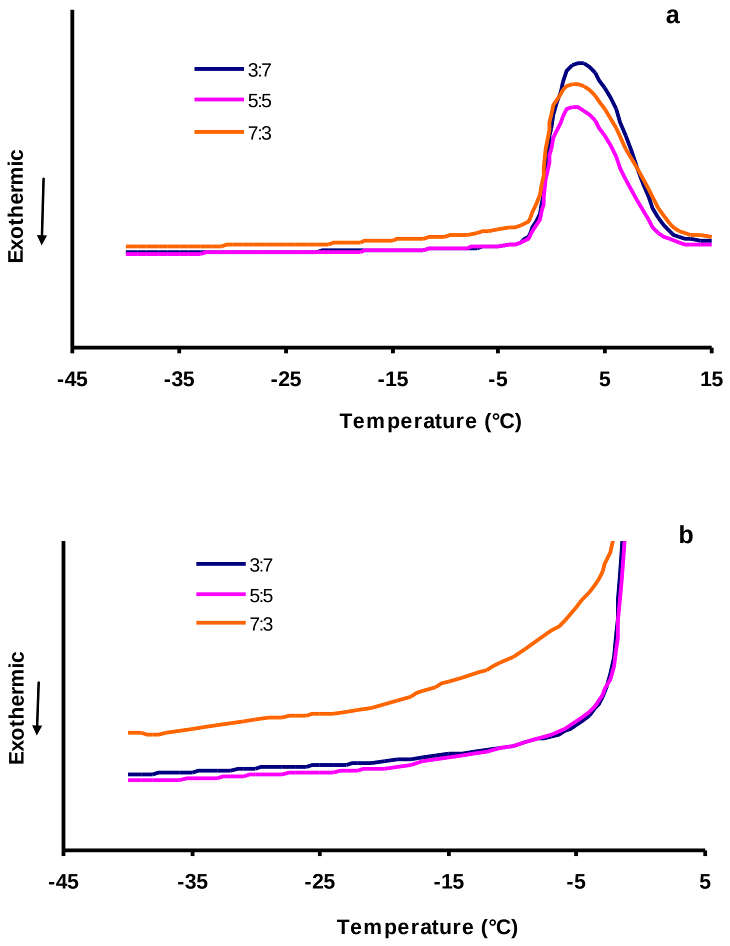


Figure 4.23 Thermograms of MF13 emulsions with O/W ratios of 3:7, 5:5 & 7:3 during heating from -50°C to 60°C. (a) Temperature range:-45°C to 15°C; (b) -45°C and 5°C

In MF42 emulsions, the crystallization of milk fat droplets occurred at approximately 28°C, followed by the crystallization of water when the temperature reached -15°C (Figure 4.24a). During the subsequent heating cycle, the endothermic peaks from the melting of milk fat droplets were observed at approximately 42°C (Figure 4.24b).

The cooling curves of AMF emulsions containing different O/W ratios are shown in Figure 4.25a. Only the exothermic peaks from the crystallization of AMF droplets are shown as the exothermic peaks from water crystallization are similar to those observed in MF13 and MF42 emulsions (Figure 4.22a and 4.24a). The crystallization curves were slightly different between emulsions with different O/W ratios. The earliest onset of milk fat droplets crystallization was observed in emulsion containing 50% of milk fats, which was at 20°C. When the temperature reached 15°C, milk fat droplets in all AMF emulsions started to crystallize and showed broad exothermic peaks.

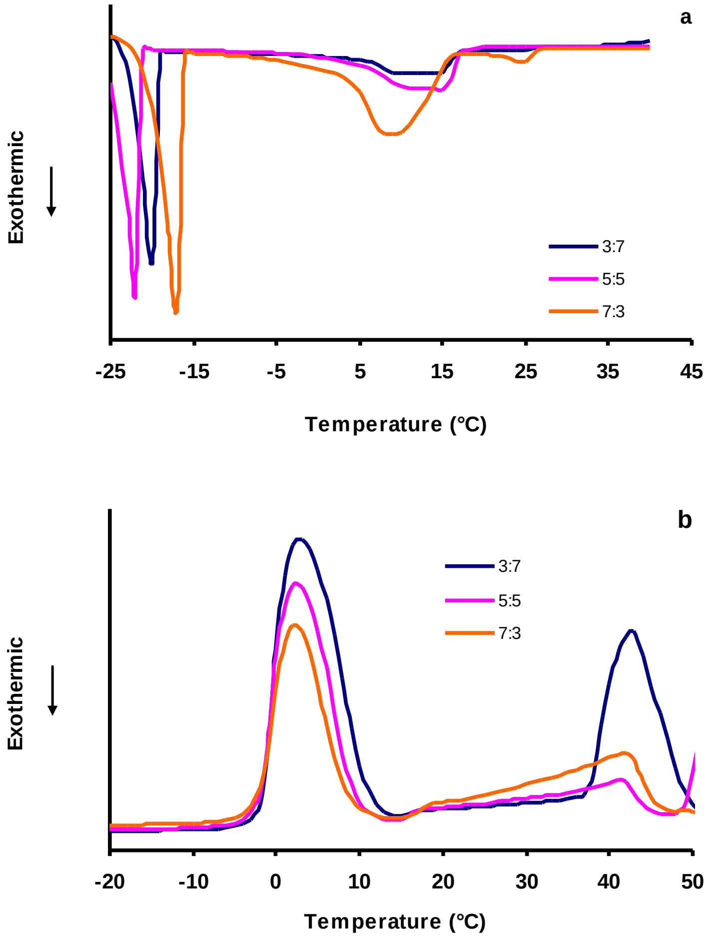


Figure 4.24 Thermograms of (a) cooling and (b) melting of MF42 emulsions with different O/W ratio

The melting curves of AMF emulsions are shown in Figure 4.25b. Only the endothermic peaks from AMF droplets are shown here as the melting peaks from water are similar to those observed in MF13 and MF42 emulsions (Figure 4.22b and 4.24b). For each of the three O/W ratios, two endothermic peaks were observed with one at 15°C and the other one at a temperature range between 30 and 35°C.

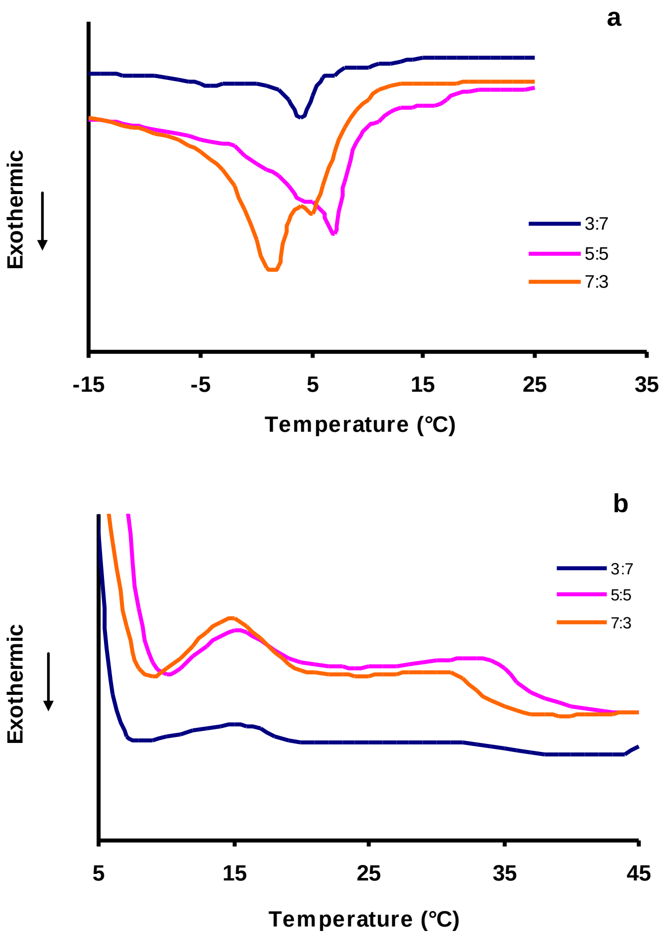


Figure 4.25 Thermograms of AMF emulsion milk fat droplets during (a) cooling and (b) heating

The crystallization temperatures of MF13, MF42 and AMF in bulk and emulsified states were similar in this study. It has been reported that emulsified fats require a higher degree of super cooling than bulk fats due to different crystallization mechanisms (Walstra and van Beresteyn 1975; Coupland 2002; Vanapalli and others 2002; Tippetts and Martini 2009). In bulk fats, heterogeneous crystallization takes place due to the presence of impurities, whereas the fat crystallization mechanisms are homogeneous in emulsions (Skoda and Van den Tempel 1963; Walstra and van Beresteyn 1975). However, homogeneous nucleation only occurs in emulsions with small particle size so less than one nucleus per droplet (Coupland 2002). Therefore, it could be concluded that the particle sizes of the emulsion droplets in this study were large enough to include some impurities, hence heterogeneous crystallization took place in all MF13, MF42 and AMF emulsions, as suggested by McClements and others (1993).

McClements and others (1993) found that the crystallization of emulsion droplets is affected by the droplet size. The onset temperature of crystallization is higher in emulsions containing larger droplets than those containing smaller droplets. Another study carried out by Tippetts and Martini (2009) suggests that crystallization of lipids in emulsions is inhibited by small droplet size and low oil content. However, neither of the phenomenon was observed in this study. The onsets of crystallization for emulsions containing different milk fat concentrations, which led to different droplet sizes, were similar to each other.

Cramp and others (2004) and Vanapalli and others (2002) studied the effects of crystallization on the stability of emulsions by putting emulsion samples through multiple cooling-heating cycles. They observed homogeneous nucleation in emulsions and concluded that emulsions were destabilized by crystallizations when heterogeneous nucleation took place in the subsequent cycles. This technique could not be applied to this study as heterogeneous nucleation was observed in the emulsions. However, all MF13, MF42 and AMF emulsions were destabilized by freeze-thawing (section 4.6). Therefore, DSC can be coupled with other techniques such as microscopic analysis for a more in depth study on the structural changes in emulsions during cooling and heating.

Chapter 5. Conclusions

The ability of rhamnolipids as emulsifiers in preparing oil-in-water emulsions at various ratios of oil to water (O/W) was investigated. The properties of rhamnolipids-stabilized emulsions containing different types of milk fat fractions (MF13, MF42) and anhydrous milk fat (AMF) were analyzed. All emulsions were white opaque liquids with a hint of yellowness at oil concentrations less than 50%. However, the emulsions containing 70% oil existed as semi solid cream like substances and had a higher intensity of yellowness. The particle size of emulsion droplets increased with increasing oil concentration, ranging from 0.19 to 0.35 μm. Microscopic images from a light microscope and a confocal laser light microscope analysis of emulsions confirmed that the emulsion droplets were spherical.

All emulsions were stable at 20°C after preparation due to the electrostatic repulsions between droplets, suggesting that rhamnolipids are effective in forming and stabilizing emulsions even at high oil concentrations up to 70%. This was also confirmed by the zeta potential values, ranging from -40 mV to -80 mV, depending on the O/W ratio. The emulsions containing a larger average particle size due to the higher oil concentration tended to give rise to an increase in their zeta potential values, although one emulsion sample skewed from this trend and it was probably due to experimental errors. Although all emulsions containing 10% milk fat (1:9 O/W ratio) were stable for 12 weeks at both 4°C and 20°C, the other emulsions containing higher oil concentrations revealed instabilities over the storage period, especially at 20°C. Among the emulsions containing 30-70% milk fat (3:7, 5:5 and 7:3 O/W), the instability was higher in the emulsions containing 30% and 50% milk fat, probably due to the higher collision frequency between droplets. The emulsions containing 70% milk fat were relatively more stable, which could be due to their static state by closely packed droplets.

All the MF13 and AMF emulsions remained stable after heating at 80°C for 30 minutes, regardless of the O/W ratio. However, in the MF42 emulsions, only those containing less than 50% of milk fats remained stable, whereas the ones containing 70% of milk fats exhibited oil separation. On the other hand, freezing and thawing destabilized all the emulsions, irrespective of the type of milk fat and the O/W ratio.

As per pH effect, the emulsion stability was significantly decreased as the emulsion pH decreased from the original pH 7.2, resulting in the free oil formation. This could be due to the reduction of electrostatic repulsions between emulsion droplets. At pH below 4, almost complete separation of oil was observed from all emulsions. Hydrolysis of rhamnolipid molecules might also be attributed to the instability of emulsions at very low pH levels. The addition of salts (NaCl, KCl and $CaCl_2$) also led to emulsion instability. The instability of emulsions when added with salts or under the acidic condition could be due to the screening of electrostatic repulsive forces between droplets, hence they were prone to coalescence since rhamnolipid molecules are anionic and sensitive to pH and ionic strength.

The rheological properties of emulsions measured indicated that all the emulsions containing different types of milk fats and various O/W ratios exhibited non-Newtonian flow behaviour. The emulsion viscosity increased with increasing O/W ratio. However, the type of milk fat had no influence on the emulsion viscosity at a given O/W ratio, suggesting the viscosity was not significantly affected by the physical characteristics of milk fat as to their different solid fat contents when the viscosity was 20°C. The overall rheological tests of emulsions showed that at 7:3 O/W ratio, the AMF emulsion was more flocculated and less stable than the emulsions containing MF13 and MF42. The MF42 and AMF emulsions however had different viscoelastic properties to the MF13 emulsions. From the DSC analysis, it was found that the crystallization and melting characteristics of bulk and emulsified milk fats were similar.

Overall, rhamnolipids were found to be highly effective in forming and stabilizing oil-in-water emulsions even at high O/W ratio, indicating that rhamnoilpids have excellent emulsifying properties. However, due to their anionic characteristic, the rhamnolipid-stabilized emulsions are sensitive to pH and ionic strength.

Chapter 6. Future Recommendations

Based on this study, there are several aspects could be further studied.

- The amount of rhamnolipids adsorbed onto the fat surfaces in emulsions containing different O/W ratios.

- The behaviour and physicochemical properties of rhamnolipid molecules affected by pH, salt and ionic strength. Their impact and influence on the property and ability of rhamnolipids as emulsifiers.

- The relationship between the emulsion particle size and zeta potential.

- The effect of presence of other compounds (proteins and carbohydrates) on the behaviour and property of rhamnolipids as an emulsifier.

- Changes in emulsion structures during crystallization and melting of emulsified milk fat using DSC coupled with microscopic analysis.

- The ability of rhamnolipids as foaming agents under various conditions.

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Appendix A. Product Specifications

JENEIL BIOSURFACTANT CO., LLC
400 N. Dekora Woods Blvd.
Saukville, WI 53080
(262) 268-6815

August 16, 2001

JBR425
PRODUCT DATA SHEET

Product Description:

JBR425 is an aqueous solution of rhamnolipids at 25% concentration. It is produced from sterilized and centrifuged fermentation broth which has had all protein removed. Two major rhamnolipids, RLL (R1) and RRLL (R2), are present. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and ?-hydroxydecanoic acid.

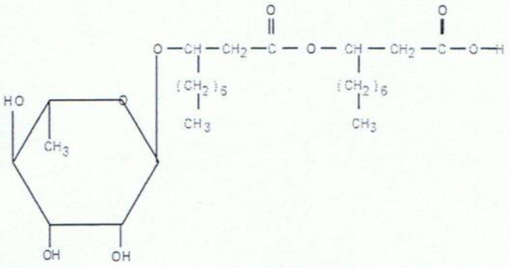
Formal Chemical Names:

RLL or R1:	?-L-Rhamnopyranosyl-?-hydroxydecanoyl-?-hydroxydecanoate
RRLL or R2:	2-O-?-L-Rhamnopyranosyl-?-L-rhamnopyranosyl-?-hydroxydecanoyl-?-hydroxydecanoate

CAS Number: **147858-26-2**

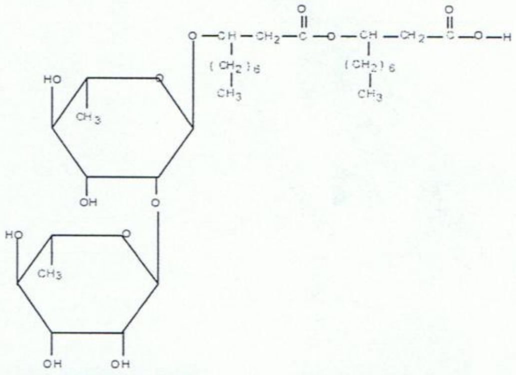
Structure:

Formal Structure and Chemical Names:



Formula: R1 or RLL: $C_{26}H_{48}O_9$
Molecular Weight: 504

RLL or R1
?-L-Rhamnopyranosyl-?-hydroxydecanoyl-?-hydroxydecanoate



Formula: R2 or RRLL: $C_{32}H_{58}O_{13}$
Molecular Weight: 650

RRLL or R2
2-O-?-L-Rhamnopyranosyl-?-L-rhamnopyranosyl-?-hydroxydecanoyl-?-hydroxydecanoate

TYPICAL PROPERTIES	
Appearance:	Amber solution
Odor:	Soapy
Specific Gravity:	1.05 – 1.06
PH:	6.5 – 7.0
Solubility in Water:	Soluble at neutral pH
Suitable Diluents:	Water, most common alcohols
Suggested Starting Concentrations:	Active Rhamnolipid Ingredient: 1.0, 0.1, 0.01%

JBR425
PRODUCT DATA SHEET

Surface and Interfacial Tension:

Surface: 29 dynes/centimeter (Surfactant diluted in water; concentration >50 ppm, Surface tension measured by ring method of du Nuoy using Fisher tensiometer).

Interfacial: 0.3 dynes/centimeter (Surfactant diluted in water, concentration >40 ppm, paraffin oil layered on top of solution, interfacial tension measured by pulling ring from water phase).

Volatility:

Not volatile

Stability:

Stable at room temperature

Stable to 121° C for at least one (1) hour.

Unstable at extreme pH due to hydrolysis of the glycosidic linkage between sugar and lipid.

Biodegradability:

Readily biodegradable

Environmental Characteristics:

Tests were accomplished in accordance with OECD (Organization for Economic Co-operation and Development) 301D, OECD 209, and OECD 202 for ready biodegradability, Activated Sludge Respiration Inhibition (ASRIT) and aquatic toxicity to daphnia. These results were to determine whether or not the chemical has the potential to cause problems in waste treatment plants or in the environment.

OECD 301D "Ready Biodegradability"
The biosurfactant showed ready biodegradability by demonstrating a biodegradability rate of 68.4% on day 10 of the 28-day test cycle. This is an excellent test result clearly demonstrating that JBR425 is readily biodegradable.

OECD2O9 "ASRIT"
The biosurfactant showed no toxicity to activated sludge biomass with an $EC_{50} > 1000$ mg/l. This is the best test result possible. This means that this surfactant could be discharged to a waste treatment plants at concentrations >1000 PPM or 0.1% with no adverse effects.

OECD 202 "Aquatic Toxicity to the Water Flea, *Daphnia* Magna"
The biosurfactant demonstrated acute toxicity to *Daphnia* Magna with an EC_{50} of 36.1 mg/l. This is an extremely low toxicity for a commercial surfactant.

JBR425
PRODUCT DATA SHEET

Toxicity:

Toxicity testing was done in accordance with U. S. EPA guidelines by an independent laboratory. A 9.5% rhamnolipid concentration aqueous solution was tested except as noted. Results follow:

ACUTE ORAL TOXICITY

The Acute Oral LD_{50} is greater than 5,000 mg/kg. Toxicity Category IV.

ACUTE DERMAL TOXICITY

The Acute Dermal LD_{50} is greater than 5,000 mg/kg. Toxicity Category IV.

ACUTE INHALATION TOXICITY

The Acute Inhalation LC_{50} is greater than 2.05 mg/L. Toxicity Category IV.

DERMAL IRRITATION

Slightly irritating. Irritation cleared by 72 hours. Toxicity Category IV.

OCULAR IRRITATION

Moderately irritating. Toxicity Category I. At 1% rhamnolipid concentration, result was Toxicity Category III.

Surface and interfacial tension:

Surface: 29 dynes/centimeter (Surfactant diluted in water; concentration > 50 ppm, surface tension measured by ring method of du Nuoy using Fisher tensiometer)

Interfacial: 0.3 dynes/centimeter (Surfactant diluted in water, concentration > 40 ppm, paraffin oil layered on top of solution, interfacial tension measured by pulling ring from water phase).

Volatility:

Not volatile

JBR425
PRODUCT DATA SHEET

Application Data

In aqueous solutions, JBR425 has a very low Critical Micelle Concentration indicating the strong surface activity shown at low concentrations, characterized by low surface tension for water and electrolyte solutions with very low interfacial tensions for water/hydrocarbon systems.

JBR425 can produce stable close-celled foams in aqueous solutions and acts as a foam stabilizer for other surfactants, particularly anionic and amphoteric compounds. JBR425 also exhibits good tolerance to Calcium ions and is an effective foaming agent for incorporation into soap-based products.

JBR425 is an excellent emulsifier and co-emulsifier for a wide range of organic solvents producing emulsions of greatly enhanced stability. JBR425 also exhibits corrosion inhibition properties in aqueous solutions, particularly towards ferrous metals.

The general chemical and physical characteristics of JBR425, with the high degree of surface activity produced at very low concentrations, indicates the use of JBR425 as a performance enhancing additive in a wide range of application areas.

Application Examples:

Typical examples utilizing the performance enhancing effects of JBR425 in various surfactant-based applications are:

Agriculture:
Anti-viral agent to control mosaic viruses
Wetter, emulsifier and adjuvant for herbicidal and pesticidal systems
Dispersant for wettable powders

Environmental Remediation
Soil washing to remove hydrocarbons and heavy metals
Wastewater treatment to remove hydrocarbons and heavy metals
Chelating agent
Oil slick dispersant

Food Industry:
Emulsifier and co emulsifier for natural fats and oils
Wetter and detergent for fruit and vegetable washing
Cleaning and sanitizing food processing equipment & areas

JBR425
PRODUCT DATA SHEET

Application Examples: (Continued)

Metal Processing:
Concentration of ores
Emulsifier and corrosion inhibitor for metal working fluids for cutting and forming
Wetting, emulsification, lubrication and corrosion inhibition in rolling oils, cutting oils and lubricants
Mold release additive in casting manufacture
Pickling and electrolytic cleaning, coating and plating
Corrosion inhibitor
Wetting and foaming in electrolytic plating

Paper Processing
Pulp derinsinification and washing agent

Personal Care:
Foam booster for bubble baths, shampoos, toothpastes, shaving cream/foam/gel, toothpaste, and other soap-based products.
Emulsifier for formulated skin creams, conditioners, degreasers & cleansers.
Wetter and detergent for contact lens cleaners and soaking solutions.

Other application include:
Elastomers and plastics
Electronics cleaners
Environmental remediation
Fire containment
Foam fractionation
Industrial and institutional cleaning
Leather processing
Paint and protective coatings
Petroleum production and products

Anhydrous Milk Fat (AMF)

PRODUCT BULLETIN

PB.098
Version 2.0906

NZMP AMF from Fonterra gives a natural dairy flavour and creaminess to finished products and is the perfect high quality fat ingredient.

NZMP AMF is pure milk fat, produced by separation and inversion of fresh cream. It has excellent natural keeping qualities.

NZMP AMF is virtually free of moisture and contains no chemical additives, preservatives, flavours, foreign fats or other impurities.

PRODUCT CHARACTERISTICS

- 99.9% pure milk fat made from 100% pure cream.
- Imparts good dairy flavour to product.
- NZMP AMF is full of natural goodness – it contains no additives.
- Is produced in a sophisticated processing plant to ensure product consistency.
- Good shelf life in high quality packaging.

SUGGESTED USES

NZMP Anhydrous Milk fat is particularly suitable for use in:

- Frying, grilling, roasting.
- Sauces.
- Recombined dairy products.
- Ice cream.
- Processed cheese.
- Confectionery.
- Bakery applications

TYPICAL COMPOSITIONAL ANALYSIS

The analysis results listed in this product bulletin are typical. Refer to the selling specification for minimum & maximum limits by parameter.

Fat (g/100g)	99.9
Moisture (g/100g)	<0.1

PACKAGING

Packs are available in**: ***

1. A round closed-head drum, lined with food grade resin and headspace filled with nitrogen.

Net Weight	210 kg
Gross Weight	226.8 kg
Cubic measure – per drum	0.255 m^3

2. A 1000 kg fiberboard bin, coated with a weatherproofing compound enclosing an internal plastic laminate polyethylene bag with an oxygen barrier. This unit has an integral heating element (230VAC or 110VAC), enabling the milk fat to be melted prior to use.

Net Weight	1000 kg
Gross Weight	1075 kg
Cubic measure (including pallet)	1.46 m^3

3. A 1250 kg Goodpack. The Goodpack IBC is a metal-framed unit which when empty, and the sides folded down, can be stacked to save storage space. The Goodpack is a reusable unit, and is de-hired when empty, and collected by agents once notified. An electric heating element, either 110VAC, or 230VAC (to suit the Customer requirement), may be fitted inside the bottom of the Goodpack, on which the 3-ply metallised bag liner sits.

Net Weight	1250 kg
Gross Weight	1367 kg
Cubic measure	1.79 m^3

4. A 20kg steel closed head pail, lined with food grade resin.

Net Weight	20 kg
Gross Weight	21.4 kg

***** Not all packaging formats are available in every region.

NZMP™
New Zealand... making it happen!

Fonterra™
Dairy for life

Anhydrous Milk Fat (AMF)

PRODUCT BULLETIN

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STORAGE AND HANDLING

Anhydrous milk fat may be transported and stored without refrigeration. However, the storage life will depend on the storage temperature. To maintain quality, it is recommended the product is stored in a cool dry area away from direct sunlight. Provided the seal is unbroken and the above storage conditions are followed, the milkfat will be suitable as a dairy ingredient for at least 12 months after the date of manufacture.

TYPICAL MINERAL ANALYSIS

Iron (mg/kg)	<0.2
Copper (mg/kg)	<0.05
Sodium (mg/100g)	<1
Potassium (mg/100g)	<1
Calcium (mg/100g)	<1

TYPICAL CHEMICAL ANALYSIS

Free fatty acids as Oleic acid (%m/m)	0.2
Peroxide Value (meq O_2/kg)	0.2
Iodine value	29 - 39
Reichert Meissl value	22.4 – 31.8
Polenske value	1.5 – 3.4
Kirchner value	17 - 27
Saponification value	225 - 235

Typical Fatty Acid Composition

(g fatty acid / 100g fatty acid)

	Fatty Acid	**Min%**	**Max %**
Butyric	C4:0	3.0	6.8
Caproic	C6:0	1.5	2.9
Caprylic	C8:0	0.7	1.7
Capric	C10:0	1.7	3.8
Lauric	C12:0	2.0	4.2
Myristic	C14:0	8.9	12.8
Palmitic	C16:0	22.3	31.4
Stearic	C18:0	10.4	16.8
Oleic	C18:1	18.3	29.9
Linoleic	C18:2	0.8	2.9
Linolenic	C18:3	0.8	2.0

TYPICAL NUTRITIONAL ANALYSIS

Energy (kJ/100g)	3700
Calories (kcal/100g)	880
Total Fat (Milk Fat) (g/100g)	99.9
Moisture (g/100g)	<0.1
Protein (g/100g)	<0.01
Carbohydrate (g/100g)	<0.01
Sugars (Lactose) (g/100g)	<0.01
Dietary Fibre (g/100g)	Nil
Cholesterol (mg/100g)	240

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Dairy for life

Anhydrous Milk Fat (AMF)

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FATTY ACIDS (g / 100g Product)	
Saturated fatty acids	60.2
Total Unsaturated fatty acids	22.6
Mono unsaturated fatty acids	20.6
Poly unsaturated fatty acids	2.1
Trans[1] fatty acids[2]	4.9
Vitamins	
Vitamin A (mg retinol/100g)	1.0
Vitamin A potency (IU/g) (retinol + β carotene)	35 - 50
Vitamin D (mg/100g)	Trace
Vitamin E (mg/100g)	3.8
Vitamin C (mg/100g)	<0.1

TYPICAL PHYSICAL PROPERTIES

Melting Point (Mettler)	31 - 34℃
Refractive Index	1.4534 - 1.4549
Specific Heat at 40℃ (kJ/kg)	2.1
Solubility of water in AMF (40℃)	0.20
Viscosity at 40℃ (mPa.s)	31
Viscosity at 50℃ (mPa.s)	22

[1] Methylene interrupted *trans* .

[2] Please refer to individual country regulations for *trans* fatty acid labelling requirements

Density (Typical Seasonal Range) kg/dm^3

Temperature	Range
10℃	0.935 - 0.956
20℃	0.922 - 0.942
30℃	0.909 - 0.925
40℃	0.900 - 0.912
50℃	0.893 - 0.902
60℃	0.888 - 0.893
70℃	0.885 - 0.888

Solid Fat Content (Typical Seasonal Range) %

Temperature	Mean	Typical Range
0℃	66.9	58.5 – 70.5
5℃	63.3	55.0 – 67.1
10℃	56.4	48.5 – 60.9
15℃	41.0	33.5 – 46.6
20℃	22.5	17.1 – 27.8
25℃	12.4	8.4 – 14.7
30℃	5.9	3.8 – 7.8
35℃	1.0	0.0 – 1.9

TYPICAL MICROBIOLOGICAL ANALYSIS

Aerobic Plate Count (cfu/g)	<1000
Listeria (/25g)	Absent
Salmonella (/25g)	Absent
Refer to individual Fonterra product selling specification for specific microbiological limits.	

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Dairy for life

Anhydrous Milk Fat (AMF)

PRODUCT BULLETIN

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QUALITY ASSURANCE

Strict quality control procedures are enforced during manufacture. The manufacturing environment is also subject to regular monitoring and control.

Product is pasteurised.

Final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures.

During storage and shipment, precautions are taken to ensure that the product quality is maintained. Each package is identified enabling trace back.

COMPLIANCE

- Meets CODEX requirements for Anhydrous Milk Fat. http://www.codexalimentarius.net/web/index_en.jsp
- Meets Halal requirements.

SUGGESTED LABELLING

Anhydrous Milk Fat or Milk Fat
Allergens: Contains Milk and Dairy Products.

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient.

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Speciality Milk Fat 13

Milk Fat Fraction

PRODUCT BULLETIN

PB.107
Version 2.1006

NZMP Speciality Milk Fat 13 from Fonterra gives a natural dairy flavour and creaminess to finished products and is the perfect high quality fat ingredient.

NZMP Speciality Milk Fat 13 is pure milk fat, produced by separation, inversion and fractionation of fresh cream. It has excellent natural keeping qualities.

NZMP Speciality Milk Fat 13 is virtually free of moisture and contains no chemical additives, preservatives, foreign fats or other impurities.

The product is a milk fat with a melting point of 13℃,
offering functional and economic advantages in applications such as recombining, spreadable butter manufacture, bakery

PRODUCT CHARACTERISTICS

- 99.9% pure milk fat made from 100% pure cream.
- Has a melting point of about 13℃.
- Imparts good dairy flavour to product.
- NZMP Speciality Milk Fat 13 is full of natural goodness – it contains no additives.
- Is produced in a sophisticated processing plant to ensure product consistency.
- Good shelf life when stored frozen.

SUGGESTED USES

NZMP Speciality Milk Fat 13 is suitable for use in:

- Bakery – melted fat applications.
- Blended bakery margarines.
- Spreadable butter when blended with speciality mil fat 42.
- Spreadable milk fat vegetable oil blends.
- Sauces.
- When changing from AMF to a speciality milk fat Fonterra recommends that the speciality milk fat should be trialed for the specific product formulation and process.

TYPICAL COMPOSITIONAL ANALYSIS

The analysis results listed in this product bulletin are typical. Refer to the selling specification for minimum & maximum limits by parameter.

Fat (g/100g)	99.9
Moisture (g/100g)	<0.1

PACKAGING

1. A round closed-head drum, lined with food grade resin and headspace filled with nitrogen.

Net Weight	210 kg
Gross Weight	226.8 kg
Cubic measure – per drum	0.255 m^3

STORAGE AND HANDLING

Speciality Milk Fat 13 should be stored refrigerated or frozen. However, the storage life will depend on the storage temperature. Provided the seal is unbroken and the above storage conditions are followed, the milkfat will be suitable as a dairy ingredient for at least 12 months after the date of manufacture.

TYPICAL MINERAL ANALYSIS

Iron (mg/kg)	<0.2
Copper (mg/kg)	<0.05

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TYPICAL CHEMICAL ANALYSIS

Free fatty acids as Oleic acid (%m/m)	0.2
Peroxide Value (meq O_2/kg)	0.2
Iodine value	43 - 48
Saponification value	233 – 238
Refer to individual Fonterra product selling specification for specific chemical limits.	

Typical Fatty Acid Composition

(g fatty acid / 100g fatty acid)

Fatty Acid		Typical %
Butyric	C4:0	4.4
Caproic	C6:0	2.7
Caprylic	C8:0	1.6
Capric	C10:0	3.4
Lauric	C12:0	3.7
Myristic	C14:0	10.2
Palmitic	C16:0	20.0
Stearic	C18:0	6.7
Oleic	C18:1	30.8
Linoleic	C18:2	3.6
Linolenic	C18:3	1.3

TYPICAL NUTRITIONAL ANALYSIS

Energy (kJ/100g)	3700
Calories (kcal/100g)	880
Total Fat (Milk Fat) (g/100g)	99.9
Moisture (g/100g)	<0.1
Protein (g/100g)	<0.01
Carbohydrate (g/100g)	<0.01
Sugars (Lactose) (g/100g)	<0.01
Dietary Fibre (g/100g)	Nil

FATTY ACIDS (g / 100g Product)*

Saturated	51.2
Mono unsaturated	32.0
Poly unsaturated	4.4

*Refer to standard AMF bulletin for typical *Trans* mono unsaturated Fatty Acid Composition.

TYPICAL PHYSICAL PROPERTIES

Melting Point (Mettler)	11 - 15℃
Refer to individual Fonterra product selling specification for specific physical property limits.	

Typical Solid Fat Content (%)

Temperature	Mean
0℃	30
5℃	21
10℃	9

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TYPICAL MICROBIOLOGICAL ANALYSIS

Aerobic Plate Count (cfu/g)	<1000
Listeria (/25g)	Absent
Salmonella (/25g)	Absent
Refer to individual Fonterra product selling specification for specific microbiological limits.	

QUALITY ASSURANCE

Strict quality control procedures are enforced during manufacture. The manufacturing environment is also subject to regular monitoring and control.

Product is pasteurised.

Final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures.

During storage and shipment, precautions are taken to ensure that the product quality is maintained. Each package is identified enabling trace back.

COMPLIANCE

- Meets CODEX requirements for Milk Fat. http://www.codexalimentarius.net/web/index_en.jsp
- Meets Halal requirements.

SUGGESTED LABELLING

Anhydrous Milk Fat or Milk Fat
Allergens: Contains Milk and Dairy Products.

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient.

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Speciality Milk Fat 27

Milk Fat Fraction

PRODUCT BULLETIN

PB.108
Version 2.1006

NZMP Speciality Milk Fat 27 from Fonterra gives a natural dairy flavour and creaminess to finished products and is the perfect high quality fat ingredient.

NZMP Speciality Milk Fat 27 is pure milk fat, produced by separation, inversion and fractionation of fresh cream. It has excellent natural keeping qualities.

NZMP Speciality Milk Fat 27 is virtually free of moisture and contains no chemical additives, preservatives, foreign fats or other impurities.

PRODUCT CHARACTERISTICS

- 99.9% pure milk fat made from 100% pure cream.
- Has a melting point of about 27℃.
- Lighter in colour than standard anhydrous milk fat.
- Imparts good dairy flavour to product.
- NZMP AMF is full of natural goodness – it contains no additives.
- Is produced in a sophisticated processing plant to ensure product consistency.
- Good shelf life in high quality packaging.

SUGGESTED USES

NZMP Speciality Milk Fat 27 is suitable for use in:

- Bakery – melted fat applications, cookies and cakes.
- Blended bakery margarines.
- Recombined dairy products.
- NOT suitable for recombined consumer butter.
- Cheese milk extension.
- Recombined sweetened condensed milk and evaporated milk.
- Ice cream.
- Whipping creams.
- Sauces.
- When changing from AMF to a speciality milk fat Fonterra recommends that the speciality milk fat should be trialed for the specific product formulation and process.

TYPICAL COMPOSITIONAL ANALYSIS

The analysis results listed in this product bulletin are typical. Refer to the selling specification for minimum & maximum limits by parameter.

Fat (g/100g)	99.9
Moisture (g/100g)	<0.1

PACKAGING

1. A round closed-head drum, lined with food grade resin and headspace filled with nitrogen.

Net Weight	210 kg
Gross Weight	226.8 kg
Cubic measure – per drum	0.255 m^3

STORAGE AND HANDLING

Speciality Milk Fat 27 may be transported and stored without refrigeration. However, the storage life will depend on the storage temperature. To maintain quality, it is recommended the product is stored in a cool dry area away from direct sunlight. Provided the seal is unbroken and the above storage conditions are followed, the milkfat will be suitable as a dairy ingredient for at least 12 months after the date of manufacture.

TYPICAL MINERAL ANALYSIS

Iron (mg/kg)	<0.2
Copper (mg/kg)	<0.05

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TYPICAL CHEMICAL ANALYSIS

Free fatty acids as Oleic acid (%m/m)	0.2
Peroxide Value (meq O_2/kg)	0.2
Iodine value	27 - 30
Reichert Meissl value	26 – 30
Polenske value	1.8 – 2.9
Kirchner value	23 - 24
Saponification value	233 – 236
Refer to individual Fonterra product selling specification for specific chemical limits.	

Typical Fatty Acid Composition

(g fatty acid / 100g fatty acid)

Fatty Acid		Typical %
Butyric	C4:0	4.0
Caproic	C6:0	2.4
Caprylic	C8:0	1.2
Capric	C10:0	2.4
Lauric	C12:0	2.5
Myristic	C14:0	9.8
Palmitic	C16:0	32.8
Stearic	C18:0	13.2
Oleic	C18:1	19.5
Linoleic	C18:2	2.5
Linolenic	C18:3	0.7

TYPICAL NUTRITIONAL ANALYSIS

Energy (kJ/100g)	3700
Calories (kcal/100g)	880
Total Fat (Milk Fat) (g/100g)	99.9
Moisture (g/100g)	<0.1
Protein (g/100g)	<0.01
Carbohydrate (Lactose) (g/100g)	<0.01
Sugars (g/100g)	<0.01
Dietary Fibre (g/100g)	Nil

FATTY ACIDS (g / 100g Product)*	
Saturated	65.2
Mono unsaturated	20.1
Poly unsaturated	2.8
*Refer to standard AMF bulletin for typical *Trans* mono unsaturated Fatty Acid Composition.	

TYPICAL PHYSICAL PROPERTIES

Melting Point (Mettler)	25.5 – 28.5℃
Refractive Index	1.4535 - 1.4543
Specific Heat at 40℃ (kJ/kg)	2.1
Solubility of water in AMF(40℃)	0.20
Viscosity at 40℃ (mPa.s)	31
Viscosity at 50℃ (mPa.s)	22
Density at 40℃ (kg/dm^3)	0.906
Refer to individual Fonterra product selling specification for specific physical property limits.	

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Typical Solid Fat Content (%)		
Temperature	Mean	Range
0℃	76.1	73.3 – 77.7
5℃	73.4	70.2 – 75.2
10℃	68.2	64.7 – 70.1
15℃	57.6	52.1 – 59.5
20℃	38.0	30.8 – 40.6
25℃	12.7	8.0 – 16.4

TYPICAL MICROBIOLOGICAL ANALYSIS

Aerobic Plate Count (cfu/g)	<1000
Listeria (/25g)	Absent
Salmonella (/25g)	Absent
Refer to individual Fonterra product selling specification for specific microbiological limits.	

QUALITY ASSURANCE

Strict quality control procedures are enforced during manufacture. The manufacturing environment is also subject to regular monitoring and control.

Product is pasteurised.

Final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures.

During storage and shipment, precautions are taken to ensure that the product quality is maintained. Each package is identified enabling trace back.

COMPLIANCE

- Meets CODEX requirements for Anhydrous Milk Fat. http://www.codexalimentarius.net/web/index_en.jsp
- Meets Halal requirements.

SUGGESTED LABELLING

Anhydrous Milk Fat or Milk Fat
Allergens: Contains Milk and Dairy Products.

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient.

Speciality Milk Fat 42

Milk Fat Fraction

PRODUCT BULLETIN

PB.110
Version 2.1006

NZMP Speciality Milk Fat 42 from Fonterra gives a natural dairy flavour and creaminess to finished products and is the perfect high quality fat ingredient.

NZMP Speciality Milk Fat 42 is pure milk fat, produced by separation, inversion and fractionation of fresh cream. It has excellent natural keeping qualities.

NZMP Speciality Milk Fat 42 is virtually free of moisture and contains no chemical additives, preservatives, foreign fats or other impurities.

PRODUCT CHARACTERISTICS

- 99.9% pure milk fat made from 100% pure cream.
- Has a melting point >41.8℃.
- Lighter in colour than standard anhydrous milk fat.
- Imparts good dairy flavour to product.
- NZMP AMF is full of natural goodness – it contains no additives.
- Is produced in a sophisticated processing plant to ensure product consistency.
- Good shelf life in high quality packaging.

SUGGESTED USES

NZMP Speciality Milk Fat 42 is particularly suitable for use in:

- Chocolate Confectionery.
- Confectionery centres.
- Blended milk fat vegetable oil margarines for bakery and spreads.
- Recombined spreadable butter when blended with speciality milk fat 11.
- Standardising seasonal differences in milk fat hardness.
- When changing from AMF to a speciality milk fat it is recommended that the speciality milk fat be trialed for the specific product formulation and process.

TYPICAL COMPOSITIONAL ANALYSIS

The analysis results listed in this product bulletin are typical. Refer to the selling specification for minimum & maximum limits by parameter.

Fat (g/100g)	99.9
Moisture (g/100g)	<0.1

PACKAGING

Packs are available in**:**

1. A round closed-head drum, lined with food grade resin and headspace filled with nitrogen.

Net Weight	210 kg
Gross Weight	226.8 kg
Cubic measure – per drum	0.255 m^3

STORAGE AND HANDLING

Speciality Milk Fat 42 may be transported and stored without refrigeration. However, the storage life will depend on the storage temperature. To maintain quality, it is recommended the product is stored in a cool dry area away from direct sunlight. Provided the seal is unbroken and the above storage conditions are followed, the milkfat will be suitable as a dairy ingredient for at least 12 months after the date of manufacture.

TYPICAL MINERAL ANALYSIS

Iron (mg/kg)	<0.2
Copper (mg/kg)	<0.05

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TYPICAL CHEMICAL ANALYSIS

Free fatty acids as Oleic acid (%m/m)	0.2
Peroxide Value (meq O_2/kg)	0.2
Iodine value	25 - 33
Reichert Meissl value	14– 19
Polenske value	1.5 – 2.5
Kirchner value	15 - 24
Saponification value	220 – 233

Refer to individual Fonterra product selling specification for specific chemical property limits.

Typical Fatty Acid Composition

(g fatty acid / 100g fatty acid)

Fatty Acid		Typical %
Butyric	C4:0	2.0
Caproic	C6:0	1.3
Caprylic	C8:0	0.8
Capric	C10:0	2.2
Lauric	C12:0	3.0
Myristic	C14:0	11.8
Palmitic	C16:0	34.8
Stearic	C18:0	15.2
Oleic	C18:1	17.0
Linoleic	C18:2	2.1
Linolenic	C18:3	0.5

TYPICAL NUTRITIONAL ANALYSIS

Energy (kJ/100g)	3700
Calories (kcal/100g)	880
Milk Fat (Milk Fat) (g/100g)	99.9
Moisture (g/100g)	<0.1
Protein (g/100g)	<0.01
Carbohydrate (g/100g)	<0.01
Sugars (Lactose) (g/100g)	<0.01
Dietary Fibre (g/100g)	Nil

FATTY ACIDS (g / 100g Product)*	
Saturated	68.2
Mono unsaturated	17.3
Poly unsaturated	2.3

*Refer to standard AMF bulletin for typical *Trans* mono unsaturated Fatty Acid Composition.

TYPICAL PHYSICAL PROPERTIES

Melting Point (Mettler)	43℃
Refractive Index	1.4535 - 1.4543
Solubility of water in AMF (40℃)	0.20
Viscosity at 40℃ (mPa.s)	31
Viscosity at 50℃ (mPa.s)	22
Density at 40℃ (kg/dm^3)	0.906

Refer to individual Fonterra product selling specification for specific physical property limits.

Speciality Milk Fat 42

Milk Fat Fraction

PRODUCT BULLETIN

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Typical Solid Fat Content %		
Temperature	Mean	Range
0℃	86.8	83.8 – 88.4
5℃	85.4	82.3 – 86.9
10℃	82.4	78.6 – 84.4
15℃	75.4	74.4 – 78.0
20℃	64.5	58.9 – 67.6
25℃	54.9	49.3 – 58.3
30℃	43.5	38.2 – 46.6
35℃	30.1	35.2 – 32.6

TYPICAL MICROBIOLOGICAL ANALYSIS

Aerobic Plate Count (cfu/g)	<1000
Listeria (/25g)	Absent
Salmonella (/25g)	Absent
Refer to individual Fonterra product selling specification for specific microbiological limits.	

QUALITY ASSURANCE

Strict quality control procedures are enforced during manufacture. The manufacturing environment is also subject to regular monitoring and control.

Product is pasteurised.

Final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures.

During storage and shipment, precautions are taken to ensure that the product quality is maintained. Each package is identified enabling trace back.

COMPLIANCE

- Meets CODEX requirements for Anhydrous Milk Fat. http://www.codexalimentarius.net/web/index_en.jsp
- Meets Halal requirements.

SUGGESTED LABELLING

Anhydrous Milk Fat or Milk Fat
Allergens: Contains Milk and Dairy Products.

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient.

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Appendix B. Experimental Raw Data

Table A1. Raw data of steady flow analysis of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

	MF13						MF42						AMF					
	3:7		5:5		7:3		3:7		5:5		7:3		3:7		5:5		7:3	
shear rate 1/s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s
0.029	0.001	0.099	0.110	3.810	46.85	2241	0.002	0.074	0.095	3.290	56.82	162141	0.079	2.751	0.147	5.083	27.76	983.7
0.040	0.003	0.105	0.159	4.020	75.45	1905	0.005	0.123	0.151	3.795	126.52	8300	0.098	2.453	0.207	5.212	35.08	887.6
0.056	0.005	0.095	0.216	3.896	77.09	1393	0.007	0.130	0.202	3.638	152.92	2852	0.107	1.914	0.272	4.900	37.73	680.6
0.077	0.006	0.083	0.268	3.498	79.14	1033	0.008	0.100	0.252	3.279	154.25	1938	0.112	1.470	0.344	4.471	39.86	519.5
0.107	0.005	0.050	0.334	3.127	81.21	760	0.006	0.052	0.308	2.875	147.10	1342	0.115	1.078	0.424	3.959	41.63	388.6
0.148	0.003	0.025	0.401	2.715	83.00	561	0.002	0.015	0.360	2.433	142.63	946.1	0.118	0.801	0.511	3.448	42.55	287.0
0.205	0.007	0.036	0.477	2.320	84.87	412.6	0.007	0.036	0.391	1.897	133.96	638.1	0.123	0.600	0.614	2.985	44.18	214.8
0.286	0.006	0.020	0.564	1.973	86.62	303.01	0.006	0.020	0.432	1.510	125.26	434.8	0.120	0.418	0.734	2.562	47.35	165.4
0.398	0.008	0.021	0.657	1.652	89.51	225.03	0.008	0.019	0.479	1.205	117.23	294.9	0.120	0.302	0.824	2.070	49.40	124.1
0.553	0.009	0.016	0.759	1.373	92.24	166.81	0.007	0.012	0.551	0.995	115.31	207.5	0.122	0.220	0.886	1.604	51.67	93.35
0.768	0.010	0.013	0.901	1.173	96.66	125.95	0.008	0.011	0.723	0.941	112.69	146.6	0.124	0.162	0.999	1.301	52.09	67.82
1.068	0.010	0.010	1.012	0.948	99.21	93.00	0.010	0.010	0.656	0.615	99.47	92.97	0.126	0.118	1.161	1.088	53.97	50.58
1.483	0.012	0.008	1.089	0.734	100.2	67.62	0.013	0.009	0.679	0.458	91.92	62.00	0.129	0.087	1.127	0.760	54.03	36.39
2.061	0.015	0.007	1.226	0.595	99.30	48.13	0.016	0.008	0.757	0.368	93.77	45.45	0.134	0.065	1.251	0.607	53.85	26.11
2.864	0.019	0.007	1.361	0.475	90.42	31.55	0.021	0.007	0.886	0.309	91.49	31.94	0.138	0.048	1.348	0.471	56.72	19.81
3.979	0.025	0.006	1.577	0.397	85.58	21.50	0.027	0.007	1.021	0.257	93.42	23.49	0.143	0.036	1.455	0.366	60.74	15.26
5.529	0.034	0.006	1.785	0.323	82.42	14.90	0.037	0.007	1.187	0.215	98.37	17.80	0.149	0.027	1.634	0.296	66.57	12.04
7.682	0.044	0.006	2.039	0.265	76.22	9.923	0.051	0.007	1.379	0.179	101.38	13.20	0.159	0.021	1.855	0.241	71.15	9.261
10.67	0.060	0.006	2.458	0.230	76.22	7.139	0.069	0.006	1.593	0.149	105.86	9.92	0.179	0.017	2.099	0.197	74.93	7.020
14.83	0.080	0.005	2.830	0.191	76.68	5.169	0.093	0.006	1.823	0.123	108.36	7.31	0.213	0.014	2.41	0.163	77.80	5.245
20.61	0.109	0.005	3.230	0.157	81.16	3.940	0.127	0.006	2.067	0.100	121.83	5.91	0.249	0.012	2.82	0.137	81.36	3.947
28.64	0.146	0.005	3.712	0.130	85.33	2.980	0.176	0.006	2.385	0.083	135.18	4.72	0.296	0.010	3.31	0.115	85.03	2.969
39.79	0.195	0.005	4.295	0.108	90.29	2.269	0.243	0.006	2.853	0.072	136.18	3.42	0.356	0.009	3.92	0.098	86.22	2.167
55.29	0.269	0.005	4.936	0.089	97.75	1.768	0.326	0.006	3.564	0.064	134.30	2.43	0.430	0.008	4.68	0.085	92.68	1.676
76.82	0.364	0.005	5.550	0.072	107.0	1.393	0.423	0.006	4.169	0.054	138.78	1.81	0.531	0.007	5.56	0.072	99.16	1.291
106.7	0.497	0.005	6.455	0.060	120.7	1.131	0.559	0.005	4.861	0.046	158.88	1.49	0.675	0.006	6.52	0.061	102.23	0.958
148.3	0.674	0.005	7.647	0.052	133.8	0.902	0.758	0.005	5.777	0.039	142.50	0.96	0.872	0.006	7.68	0.052	100.73	0.679
206.1	0.924	0.004	9.071	0.044	147.2	0.714	1.026	0.005	6.974	0.034	160.85	0.78	1.144	0.006	9.00	0.044	100.38	0.487
286.4	1.255	0.004	10.81	0.038	162.1	0.566	1.378	0.005	8.492	0.030	112.84	0.39	1.512	0.005	10.60	0.037	100.70	0.352
397.9	1.714	0.004	13.29	0.033	179.4	0.451	1.864	0.005	10.699	0.027	73.68	0.19	2.015	0.005	12.94	0.033	102.33	0.257
500.0	2.113	0.004	15.47	0.031	192.5	0.385	2.284	0.005	13.285	0.027	57.28	0.11	2.465	0.005	15.11	0.030	96.97	0.194

Table A2. Raw data of thixotropic loop analysis (up cycle) of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

	MF13				MF42				AMF			
	5:5		7:3		5:5		7:3		5:5		7:3	
shear rate 1/s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s
0.0290525	0.10353	3.56875	75.095	39771.45	0.081135	2.8345	82.775	3144.5	0.153325	5.317	78.54	2776.75
0.0394375	0.134875	3.41375	136.96	3523.2	0.1245575	3.17025	93.5225	2340.75	0.201275	5.0845	82.455	2089
0.0556975	0.1712	3.074	139.8475	2542.95	0.165	2.994	92.3725	1645.85	0.256225	4.6225	84.595	1528.25
0.0768625	0.20865	2.71775	142.22	1858.4	0.19395	2.51925	92.9775	1208.2	0.317725	4.136	86.4075	1125.075
0.107125	0.2515	2.34525	143.285	1335.95	0.240925	2.259	93.77	875.175	0.38755	3.61775	87.565	817.725
0.147875	0.30405	2.056	142.6325	954.95	0.277875	1.877	94.715	639.175	0.457425	3.0865	88.3375	595.35
0.2054	0.367075	1.78725	141.9725	687.915	0.30205	1.4745	95.3725	463	0.543	2.6365	88.7925	430.4
0.286125	0.4289	1.4985	139.7275	483.9525	0.34695	1.2134	97.2725	340.4	0.625325	2.18525	88.285	307.85
0.398125	0.495675	1.24525	132.06	330.015	0.381775	0.9594	99.5425	250.325	0.6934	1.74375	87.8275	220.4
0.5532	0.57135	1.032675	126.81	228.255	0.421525	0.7625	101.0175	182.61	0.749675	1.35625	87.7125	158.375
0.768575	0.57785	0.751775	125.3925	162.9475	0.47065	0.61335	104.195	135.7275	0.835425	1.088225	83.285	108.32
1.06725	0.805425	0.754775	125.32	117.3975	0.5106	0.478475	107.6475	100.7875	0.926575	0.86815	80.9075	75.75
1.48275	0.89815	0.60565	116.8625	78.61	0.5671	0.38265	101.375	68.235	0.9906	0.667375	79.135	53.295
2.0605	1.022875	0.496375	115.8125	56.2925	0.626325	0.303775	115.615	56.32	1.10275	0.535125	75.4175	36.6375
2.86375	1.1075	0.386725	112.73	39.3825	0.724875	0.2532	119.1	41.5375	1.22725	0.4285	78.2875	27.345
3.97825	1.3285	0.3339	111.99	28.14075	0.8504	0.2137	121.315	30.485	1.389	0.349025	81.2075	20.395
5.52875	1.52525	0.275875	110.395	19.9455	1.0139	0.183375	143.9075	26.06	1.5695	0.2839	77.6925	14.05
7.6825	1.789	0.2329	115.2475	14.9915	1.18325	0.154075	160.425	20.9305	1.77975	0.231675	75.825	9.8695
10.675	2.05225	0.19225	110.6025	10.362	1.39875	0.131	169.43	15.8505	2.04925	0.19195	79.5675	7.45375
14.83	2.37	0.1598	113.6925	7.667	1.60025	0.1078975	166.6	11.2285	2.3575	0.15895	83.1125	5.60225
20.61	2.71875	0.13195	115.655	5.61175	1.85575	0.090045	169.5325	8.23275	2.73575	0.13275	88.6625	4.302
28.6375	3.13325	0.1094375	119.735	4.18175	2.1925	0.0765625	201.67	7.044	3.1675	0.1106125	95.185	3.324
39.79	3.59725	0.0903975	126.0675	3.16775	2.60325	0.0654275	167.13	4.2	3.69325	0.0928225	108.44	2.72525
55.29	4.1805	0.07561	133.315	2.41175	3.09875	0.05605	159.7	2.88975	4.33925	0.078485	107.7775	1.94925
76.82	4.9475	0.0644025	140.385	1.827075	3.7535	0.048855	172.6	2.248	5.08175	0.0661525	113.975	1.48375
106.7	5.80575	0.05439	150.73	1.412125	4.6315	0.0433875	141.475	1.32615	5.97575	0.05598	116.725	1.093375
148.3	6.7945	0.045815	162.16	1.093525	5.4405	0.03668	144.025	0.971075	7.07225	0.0476825	114.16	0.769825
206.1	8.12775	0.0394375	175.7125	0.85245	6.56475	0.031855	152.68	0.741175	8.36725	0.0406	115.175	0.558775
286.4	9.71075	0.0339125	190.05	0.663575	8.08825	0.028245	145.575	0.508175	9.9475	0.0347375	111.65	0.389825
397.9	11.9025	0.02991	205	0.515225	10.40325	0.0261425	52.7525	0.132605	12.2475	0.0307825	107.8	0.270925
500	13.845	0.02769	218.225	0.436475	12.21	0.0244225	40.87	0.081745	14.2725	0.0285475	107.0125	0.214025

Table A3. Raw data of thixotropic loop analysis (down cycle) of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

	MF13				MF42				AMF			
	5:5		7:3		5:5		7:3		5:5		7:3	
shear rate 1/s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s	shear stress Pa	viscosity Pa.s
500	14.0175	0.028035	217.075	0.434125	12.16	0.02432	31.5075	0.0630155	14.355	0.0287075	100.1825	0.200425
359.825	11.495	0.031945	197.925	0.55	10.0105	0.0278175	24.357	0.067693	11.6875	0.032485	82.5825	0.2295
259	9.4715	0.036575	181.9	0.702325	8.13125	0.0313975	19.884	0.0767748	9.59925	0.03707	69.87	0.2698
186.4	7.886	0.042315	167.4975	0.8989	6.875	0.0368925	17.10525	0.09178	7.96475	0.0427325	60.06	0.32225
134.1	6.62825	0.049415	155.2525	1.1573	5.71425	0.0426	14.176	0.10568	6.68625	0.04985	55.52	0.4139
96.53	5.60925	0.0581075	143.625	1.488025	4.99	0.05169	12.12925	0.125695	5.6695	0.0587275	50.18	0.519775
69.47	4.79925	0.06908	134.275	1.9325	3.99175	0.0574575	10.3948	0.14962	4.84775	0.0697775	44.5475	0.64125
49.9975	4.124	0.0824825	124.41	2.4885	3.3995	0.06799	9.109	0.182105	4.175	0.0835	39.585	0.7917
35.98	3.55575	0.0988275	116.9575	3.25	2.99125	0.0831325	8.07725	0.224545	3.62375	0.100695	35.4025	0.9838
25.9	3.1085	0.120025	109.9325	4.245	2.76525	0.106765	7.26225	0.2804425	3.16925	0.1224	31.7975	1.22775
18.64	2.71225	0.1455	105.3225	5.6505	2.14425	0.11507	6.32	0.3391025	2.789	0.14965	28.77	1.5435
13.41	2.4365	0.181625	101.42	7.561	1.89	0.140875	5.61125	0.4183	2.47775	0.1847	26.3025	1.9605
9.65325	2.16825	0.224625	97.3875	10.08675	1.74925	0.181175	4.987575	0.5165975	2.21075	0.229025	24.1225	2.49875
6.946	1.988	0.2862	94.45	13.596	1.5635	0.225125	4.6145	0.664025	1.9905	0.286525	22.4625	3.233
5.00075	1.89825	0.37955	92.675	18.53725	1.4419	0.288325	4.21415	0.843125	1.8035	0.36065	20.875	4.1745
3.599	1.741	0.483725	90.68	25.2	1.272375	0.353575	4.0376	1.121675	1.64625	0.4573	19.595	5.44575
2.59	1.65375	0.638625	89.8775	34.7025	1.074925	0.415125	3.729625	1.4412	1.50625	0.58155	18.37	7.093
1.863	1.6315	0.8758	89.7475	48.19	1.0805	0.580125	3.471175	1.862175	1.411	0.757025	17.055	9.15225
1.3405	1.50625	1.12355	88.155	65.7325	0.967575	0.722525	3.25155	2.425375	1.30175	0.971125	16.02	11.94475
0.965275	1.5309	1.58595	87.7625	90.9175	1.24715	1.293775	3.250225	3.366625	1.2535	1.29825	15.17	15.72
0.6946	1.83935	2.648	87.24	125.5825	0.884575	1.27255	3.1377	4.5194	1.20575	1.73625	14.4925	20.87
0.4994	1.921625	3.84675	87.03	174.325	0.72885	1.45995	2.904	5.8132	1.158775	2.31925	14.10975	28.2375
0.35985	2.058125	5.7205	87.1175	242.175	0.743025	2.072425	2.9686	8.248	1.1098	3.08325	13.33875	37.085
0.2587	2.39375	9.25325	86.935	335.9	0.6158	2.3825	2.78505	10.76225	1.07865	4.1705	12.837	49.6025
0.1866	2.8085	15.05075	86.695	464.375	0.785125	4.20425	2.830925	15.1975	1.08215	5.797	12.53625	67.17
0.133775	3.43575	25.6925	86.385	645.125	0.715775	5.3545	2.746675	20.53175	1.088375	8.13475	12.26625	91.6425
0.0966775	3.88025	40.14	85.77	885.475	0.711775	7.331	2.828975	29.1405	1.1261	11.6435	12.03425	124.41
0.069225	4.30325	62.1775	85.4525	1233.425	0.62115	8.98	2.831825	40.767	1.165975	16.8275	11.92675	172.19
0.0500075	4.88375	97.665	85.3575	1705.475	0.6513	13.03025	3.1012	61.79	1.222925	24.4225	11.8745	237.3
0.0355625	5.407	152.0775	85.3325	2392.5	0.710725	19.9305	2.864475	79.995	1.28545	36.035	11.83575	332.075
0.0286825	6.077	212.075	85.4725	2967.25	0.795675	27.505	2.70895	93.6875	1.38895	48.25	11.8995	413.55

Table A4. Raw data of temperature Sweep (4-80°C) of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

	MF13			MF42			AMF		
	3:7	5:5	7:3	3:7	5:5	7:3	3:7	5:5	7:3
temperature °C	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s
3.8	0.0090	0.0706	1.6121	0.0074	0.0660	55810	0.0093	0.0586	25.35
5.9	0.0091	0.0712	0.7665	0.0072	0.1182	74312.5	0.0094	0.0708	56.92
7.9	0.0086	0.0717	0.7209	0.0069	0.1695	31875	0.0095	0.0895	19.4303
9.9	0.0079	0.0718	0.6876	0.0066	0.4008	34530	0.0090	0.1049	2.1426
11.9	0.0071	0.0716	0.6259	0.0063	0.8492	44325	0.0086	0.1111	0.9126
14.0	0.0063	0.0708	0.6092	0.0061	0.6774	24025	0.0084	0.1196	0.2172
16.0	0.0056	0.0697	0.6158	0.0058	3.4660	38895	0.0075	0.1665	0.1850
18.0	0.0048	0.0687	0.6989	0.0054	1.2136	54317.5	0.0069	0.1626	0.1408
20.0	0.0044	0.0677	0.7552	0.0048	1.9256	70347.5	0.0060	0.0704	0.0792
21.9	0.0042	0.0668	0.7705	0.0045	1.4450	33865	0.0051	0.0682	0.0753
24.0	0.0039	0.0659	0.8423	0.0042	0.7039	21616	0.0048	0.0607	0.0685
25.9	0.0038	0.0652	0.8611	0.0041	0.3097	3965.5	0.0045	0.0600	0.0568
28.0	0.0037	0.0646	0.8691	0.0039	0.2031	393.17	0.0043	0.0613	0.0557
30.0	0.0035	0.0640	0.8935	0.0038	0.1020	15.18	0.0042	0.0616	0.0125
32.0	0.0034	0.0636	0.8980	0.0037	0.0877	108.51	0.0040	0.0619	0.0063
34.0	0.0033	0.0633	0.8981	0.0036	0.0532	3.1203	0.0038	0.0625	0.0140
36.0	0.0032	0.0630	0.9068	0.0034	0.0398	1.7507	0.0037	0.0617	0.0051
38.0	0.0030	0.0630	0.9273	0.0034	0.0351	0.8099	0.0035	0.0610	0.0053
39.9	0.0031	0.0634	0.9313	0.0033	0.0276	0.3361	0.0034	0.0607	0.0054
42.0	0.0030	0.0637	0.9335	0.0032	0.0253	0.0753	0.0033	0.0605	0.0050
43.9	0.0029	0.0640	0.9461	0.0031	0.0236	0.0089	0.0032	0.0606	0.0046
46.0	0.0029	0.0644	0.9491	0.0030	0.0231	0.0082	0.0032	0.0610	0.0043
48.0	0.0028	0.0655	0.9660	0.0029	0.0228	0.0083	0.0031	0.0615	0.0040
50.0	0.0027	0.0665	0.9280	0.0029	0.0226	0.0081	0.0031	0.0615	0.0038
52.0	0.0028	0.0682	0.9561	0.0028	0.0224	0.0077	0.0030	0.0626	0.0036
54.0	0.0028	0.0701	0.9607	0.0028	0.0223	0.0073	0.0031	0.0633	0.0034
56.0	0.0028	0.0711	0.9872	0.0029	0.0222	0.0068	0.0032	0.0651	0.0032
58.0	0.0028	0.0740	1.0440	0.0028	0.0224	0.0066	0.0033	0.0675	0.0030
60.0	0.0029	0.0778	1.0158	0.0028	0.0229	0.0064	0.0033	0.0721	0.0028
62.0	0.0028	0.0808	0.9400	0.0028	0.0235	0.0059	0.0032	0.0765	0.0027
64.0	0.0028	0.0874	0.9345	0.0028	0.0237	0.0061	0.0032	0.0782	0.0025
66.0	0.0030	0.0929	0.7703	0.0028	0.0245	0.0061	0.0032	0.0870	0.0024
68.0	0.0031	0.0987	0.6333	0.0028	0.0255	0.0057	0.0032	0.0953	0.0023
70.0	0.0030	0.1066	0.5853	0.0028	0.0273	0.0054	0.0033	0.0943	0.0021
72.0	0.0030	0.1155	0.5456	0.0029	0.0313	0.0052	0.0033	0.0995	0.0020
74.0	0.0032	0.1247	0.6221	0.0029	0.0347	0.0051	0.0033	0.1045	0.0020
76.0	0.0034	0.1333	0.5617	0.0031	0.0369	0.0051	0.0034	0.1120	0.0019
78.0	0.0042	0.1458	0.5385	0.0033	0.0372	0.0050	0.0037	0.1255	0.0018
79.9	0.0046	0.1627	0.5144	0.0037	0.0404	0.0049	0.0042	0.1322	0.0018

Table A5. Raw data of temperature Sweep (80-4°C) of emulsions containing MF13, MF42 and AMF at 3:7, 5:5 and 7:3 O/W ratios

	MF13			MF42			AMF		
	3:7	5:5	7:3	3:7	5:5	7:3	3:7	5:5	7:3
temperature °C	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s	viscosity Pa.s
78.1	0.0068	0.1756	0.5313	0.0037	0.0749	0.0048	0.0048	0.1428	0.0018
76.0	0.0118	0.1803	0.5254	0.0041	0.0713	0.0050	0.0051	0.1514	0.0019
74.0	0.0059	0.1944	0.5251	0.0042	0.0757	0.0051	0.0052	0.1593	0.0019
72.0	0.0054	0.2059	0.5064	0.0041	0.0841	0.0053	0.0053	0.1751	0.0019
70.0	0.0051	0.2237	0.4303	0.0040	0.0896	0.0055	0.0050	0.1823	0.0020
68.0	0.0052	0.2525	0.4491	0.0038	0.0995	0.0054	0.0051	0.1828	0.0020
66.0	0.0053	0.2684	0.5375	0.0039	0.1086	0.0056	0.0051	0.1840	0.0022
64.1	0.0057	0.2785	0.5417	0.0038	0.1086	0.0058	0.0053	0.1933	0.0022
62.0	0.0053	0.2924	0.5884	0.0036	0.1098	0.0060	0.0053	0.1939	0.0023
60.0	0.0049	0.3282	0.5198	0.0033	0.1166	0.0062	0.0050	0.2009	0.0024
58.1	0.0051	0.3179	0.4895	0.0033	0.1183	0.0064	0.0050	0.1990	0.0025
56.0	0.0051	0.3192	0.4933	0.0033	0.1101	0.0067	0.0050	0.2087	0.0026
54.0	0.0063	0.3595	0.4520	0.0034	0.1152	0.0070	0.0049	0.2176	0.0027
52.0	0.0044	0.3453	0.4076	0.0036	0.1207	0.0073	0.0048	0.2185	0.0029
50.0	0.0043	0.3476	0.4172	0.0036	0.1221	0.0077	0.0048	0.2264	0.0030
48.0	0.0051	0.4056	0.3645	0.0037	0.1284	0.0081	0.0047	0.2378	0.0032
46.0	0.0042	0.3654	0.4131	0.0038	0.1287	0.0085	0.0048	0.2404	0.0034
44.1	0.0043	0.3707	0.4231	0.0040	0.1271	0.0089	0.0049	0.2515	0.0036
42.0	0.0049	0.4126	0.4397	0.0041	0.1334	0.0095	0.0049	0.2496	0.0038
40.0	0.0045	0.3741	0.3940	0.0042	0.1347	0.0101	0.0051	0.2508	0.0041
38.0	0.0046	0.3650	0.4122	0.0044	0.1349	0.0108	0.0052	0.2518	0.0043
36.0	0.0051	0.4010	0.3832	0.0045	0.1376	0.0115	0.0053	0.2531	0.0046
34.0	0.0049	0.3637	0.4177	0.0047	0.1387	0.0123	0.0055	0.2511	0.0049
32.0	0.0050	0.3620	0.3284	0.0049	0.1377	0.0132	0.0056	0.2587	0.0051
30.1	0.0055	0.4100	0.2921	0.0052	0.1418	0.0143	0.0059	0.2557	0.0055
28.1	0.0054	0.3860	0.2905	0.0075	0.1502	0.0265	0.0061	0.2536	0.0060
26.1	0.0056	0.3755	0.2848	0.0139	0.2059	0.1906	0.0064	0.2517	0.0064
24.0	0.0060	0.4045	0.2935	0.0126	0.3233	0.4527	0.0068	0.2471	0.0069
22.1	0.0060	0.3907	0.3268	0.0122	0.2819	0.1879	0.0075	0.2447	0.0075
20.0	0.0062	0.3767	0.2872	0.0087	0.3812	1.1024	0.0081	0.2518	0.0084
18.0	0.0066	0.3961	0.2901	0.0207	0.4663	0.3307	0.0088	0.2961	0.0098
16.1	0.0067	0.3865	0.3071	0.0293	0.6572	0.4840	0.0092	0.3379	0.4039
14.1	0.0070	0.3784	0.2935	0.0194	0.5904	0.2209	0.0108	0.3474	0.3689
12.1	0.0073	0.3916	0.2922	0.0180	0.6380	0.9760	0.0141	0.3145	0.6580
10.1	0.0075	0.3979	0.2935	0.0138	1.2799	4.3845	0.0140	0.3147	0.7384
8.1	0.0081	0.3923	0.2911	0.0130	0.2431	15.6813	0.0135	0.2886	0.8746
6.1	0.0095	0.4081	0.3167	0.0110	0.5435	28.860	0.0130	0.3550	0.4324
4.1	0.0127	0.4104	0.3384	0.0103	0.2566	33.382	0.0127	0.7985	0.3033
2.1	0.0131	0.3876	0.5695				0.0127	1.3810	0.5237

Table A6. Raw data of strain sweep of emulsions containing MF13, MF42 and AMF at 7:3 O/W ratios

MF13				MF42				AMF			
% strain	G'	G"	delta	% strain	G'	G"	delta	% strain	G'	G"	delta
	Pa	Pa	degrees		Pa	Pa	degrees		Pa	Pa	degrees
0.024	1913.53	32.37	1.641	0.014	5854	495.75	4.870	0.014	2318.5	47.27	1.187
0.026	1925.68	33.19	1.655	0.019	6294	473.875	4.393	0.020	2341.75	57.71	1.404
0.030	1940.70	28.23	1.441	0.027	6595.25	486	4.274	0.028	2363.25	57.0225	1.375
0.038	1964.68	29.98	1.430	0.038	6826	502.75	4.239	0.038	2392	55.3975	1.322
0.053	1986.88	30.60	1.426	0.053	6982.75	507.75	4.165	0.053	2411.25	54.33	1.292
0.074	2003.48	31.37	1.425	0.074	7069	518.75	4.200	0.074	2427	56.1	1.325
0.103	2019.88	32.63	1.404	0.102	7120.25	531.475	4.253	0.103	2439.75	57.685	1.355
0.142	2035.80	33.31	1.400	0.143	7126	552.425	4.385	0.143	2447.75	61.21	1.433
0.199	2036.10	36.05	1.448	0.199	7083.75	581.75	4.609	0.200	2445.25	64.355	1.512
0.277	2039.45	38.77	1.502	0.277	7007.25	608.65	4.870	0.275	2438.5	68.5575	1.607
0.384	2046.33	42.64	1.602	0.380	6884.5	641.05	5.177	0.381	2414.5	81.34	1.917
0.535	2055.65	47.63	1.750	0.530	6692.5	676.625	5.607	0.528	2366	114.9975	2.740
0.740	2057.83	53.89	1.884	0.738	6388.75	715.25	6.205	0.740	2270	194.25	4.760
1.027	2051.38	60.13	2.055	1.029	5955.75	755.75	7.041	1.052	2062.5	339.9	9.157
1.419	2025.28	65.32	2.253	1.453	5357	805.25	8.376	1.669	1624.75	581.05	19.580
1.972	1971.55	73.79	2.576	2.346	4171.75	992.7	13.348	3.700	867.525	716.55	40.668
1.989	1990.43	70.10	2.431	1.996	4902.5	907.925	10.371	4.073	882.7	688.625	40.305

A7. Raw data of frequency sweep of emulsions containing MF13, MF42 and AMF at 7:3 O/W ratios

	MF13			MF42			AMF		
frequency	G'	G"	delta	G'	G"	delta	G'	G"	delta
Hz	Pa	Pa	degrees	Pa	Pa	degrees	Pa	Pa	degrees
0.10	221.92	75.66	43.98	0.02	5.08	89.97	21.82	80.11	80.12
0.13	256.57	96.53	42.43	0.13	7.80	89.15	2.94	33.53	85.32
0.16	279.98	121.78	41.10	0.44	11.83	87.93	4.62	43.60	84.41
0.20	302.68	152.63	39.75	1.03	16.61	86.45	7.28	57.05	83.37
0.25	318.04	155.76	39.79	1.90	22.16	85.09	11.34	74.68	82.17
0.32	328.92	118.68	41.80	3.05	28.17	83.81	13.06	86.44	81.85
0.40	348.73	158.71	40.19	4.40	34.78	82.76	17.08	103.23	81.03
0.50	374.68	160.43	40.22	5.62	40.35	81.99	20.18	117.55	80.55
0.63	381.26	138.45	41.40	7.30	48.30	81.32	23.34	129.60	80.01
0.80	407.08	175.93	40.20	9.29	57.83	80.77	27.83	146.83	79.36
1.00	399.05	182.51	40.22	11.78	69.80	80.31	37.22	175.15	78.03
1.26	444.43	231.52	38.94	14.55	83.99	80.19	50.22	207.35	76.74
1.58	468.65	288.70	37.36	18.49	103.00	80.06	75.61	250.45	74.76
1.99	546.43	393.93	33.83	23.22	124.98	80.26	119.26	311.30	71.83
2.50	663.40	492.61	30.18	14.27	113.49	82.99	191.55	402.65	67.17
3.14	961.48	526.96	22.90	15.42	134.91	83.88	364.02	537.95	58.85
3.97	1264.43	372.26	14.59	18.42	162.70	84.44	545.23	606.80	52.45
4.95	1640.40	176.32	6.47	5.97	142.50	87.66	661.75	677.38	48.44
6.21	1690.40	149.92	6.49	8.29	173.75	87.41	881.38	728.58	40.67
7.94	1706.45	153.93	6.73	16.62	200.80	85.35	1000.28	754.40	37.90
9.99	1735.53	145.15	6.19	31.29	226.75	82.33	1095.33	778.25	35.97
12.56	1757.95	137.77	5.69	46.55	252.65	79.71	1200.78	791.45	33.63
15.88	1787.93	131.83	4.92	61.92	283.70	77.75	1309.75	788.25	30.96
19.98	1810.65	139.35	5.45	107.49	382.45	74.39	1408.00	784.55	28.80
25.13	1820.45	147.65	6.22	402.75	678.80	61.51	1504.00	775.45	26.72
31.63	1850.60	143.38	5.71	907.40	995.00	48.09	1577.75	757.15	24.83
39.77	1864.78	153.90	6.04	1205.10	1111.25	44.28	1657.25	753.48	23.60
39.96	1866.90	162.20	6.23	1259.45	1144.45	44.02	1673.75	750.10	23.32

Table A8. Data from DSC analysis of MF13, MF42 and AMF bulk fats

	Heat Flow (mW)							Heat Flow (mW)						
°C	AMF		MF13		MF42		**°C**	AMF		MF13		MF42	
	cooling	heating	cooling	heating	cooling	heating		cooling	heating	cooling	heating	cooling	heating
-49	-2.73	-0.17	-1.03	-0.16	-2.41	0.16	**5**	-7.97	5.55	-1.86	3.17	-6.23	3.69
-48	-2.72	1.81	-2.50	1.45	-2.41	1.56	**6**	-8.48	5.60	-1.86	3.12	-6.64	3.72
-47	-2.71	2.38	-2.52	1.90	-2.41	1.91	**7**	-8.96	5.62	-1.86	3.07	-7.07	3.72
-46	-2.71	2.57	-2.53	2.05	-2.42	2.01	**8**	-9.06	5.34	-1.86	3.01	-7.47	3.62
-45	-2.71	2.64	-2.55	2.13	-2.43	2.05	**9**	-8.35	4.96	-1.85	2.94	-7.75	3.45
-44	-2.71	2.68	-2.55	2.17	-2.43	2.08	**10**	-7.22	4.94	-1.85	2.88	-7.69	3.28
-43	-2.71	2.70	-2.56	2.18	-2.42	2.10	**11**	-5.85	5.23	-1.85	2.83	-7.08	3.20
-42	-2.71	2.69	-2.58	2.10	-2.41	2.12	**12**	-5.33	5.74	-1.85	2.81	-6.00	3.34
-41	-2.72	2.65	-2.60	1.96	-2.41	2.13	**13**	-4.98	6.42	-1.85	2.80	-6.02	3.67
-40	-2.72	2.61	-2.63	1.84	-2.42	2.14	**14**	-5.14	7.18	-1.85	2.80	-5.67	4.08
-39	-2.73	2.58	-2.65	1.76	-2.42	2.16	**15**	-6.24	7.93	-1.85	2.80	-4.11	4.26
-38	-2.75	2.57	-2.67	1.74	-2.43	2.18	**16**	-7.22	8.63	-1.85	2.80	-4.21	4.12
-37	-2.76	2.59	-2.71	1.76	-2.45	2.20	**17**	-4.33	8.84	-1.85	2.81	-4.82	3.86
-36	-2.78	2.63	-2.74	1.88	-2.48	2.23	**18**	-3.01	8.45	-1.85	2.81	-5.32	3.57
-35	-2.79	2.69	-2.78	2.02	-2.46	2.26	**19**	-2.64	7.91	-1.85	2.81	-5.70	3.29
-34	-2.81	2.75	-2.82	2.17	-2.48	2.28	**20**	-2.52	7.52	-1.85	2.81	-6.19	3.21
-33	-2.83	2.81	-2.86	2.29	-2.50	2.31	**21**	-2.50	7.35	-1.85	2.81	-6.96	3.40
-32	-2.85	2.87	-2.91	2.39	-2.50	2.33	**22**	-2.50	7.32	-1.86	2.82	-8.19	3.87
-31	-2.88	2.92	-2.95	2.48	-2.52	2.36	**23**	-2.50	7.31	-1.86	2.82	-10.02	4.63
-30	-2.90	2.96	-3.00	2.56	-2.53	2.38	**24**	-2.50	7.31	-1.86	2.82	-12.26	5.46
-29	-2.93	3.01	-3.05	2.63	-2.55	2.40	**25**	-2.50	7.33	-1.86	2.82	-8.71	6.05
-28	-2.96	3.04	-3.10	2.71	-2.57	2.42	**26**	-2.50	7.39	-1.86	2.82	-3.53	6.42
-27	-2.99	3.08	-3.15	2.80	-2.60	2.44	**27**	-2.50	7.49	-1.86	2.82	-2.65	6.70
-26	-3.03	3.11	-3.20	2.89	-2.62	2.46	**28**	-2.51	7.61	-1.86	2.83	-2.51	6.95
-25	-3.06	3.14	-3.25	2.98	-2.65	2.48	**29**	-2.51	7.72	-1.87	2.83	-2.51	7.20
-24	-3.10	3.17	-3.30	3.07	-2.68	2.50	**30**	-2.51	7.83	-1.87	2.83	-2.52	7.46
-23	-3.15	3.20	-3.36	3.15	-2.71	2.53	**31**	-2.51	7.93	-1.88	2.83	-2.52	7.74
-22	-3.20	3.23	-3.42	3.25	-2.74	2.55	**32**	-2.51	8.04	-1.88	2.83	-2.52	8.07
-21	-3.26	3.27	-3.48	3.36	-2.82	2.58	**33**	-2.51	8.14	-1.88	2.83	-2.52	8.51
-20	-3.32	3.31	-3.54	3.49	-2.81	2.60	**34**	-2.51	8.19	-1.89	2.83	-2.52	9.03
-19	-3.38	3.35	-3.61	3.64	-2.85	2.63	**35**	-2.51	8.11	-1.89	2.84	-2.52	9.56
-18	-3.44	3.41	-3.69	3.80	-2.89	2.66	**36**	-2.51	7.47	-1.89	2.84	-2.53	10.07
-17	-3.49	3.46	-3.77	3.91	-2.93	2.68	**37**	-2.51	5.89	-1.90	2.84	-2.53	10.62
-16	-3.56	3.53	-3.85	3.96	-2.98	2.71	**38**	-2.51	4.61	-1.90	2.84	-2.53	11.20
-15	-3.63	3.61	-3.92	3.99	-3.03	2.75	**39**	-2.51	4.14	-1.91	2.84	-2.53	11.81
-14	-3.71	3.68	-3.99	4.05	-3.08	2.78	**40**	-2.51	4.00	-1.91	2.84	-2.53	12.40
-13	-3.79	3.76	-4.04	4.14	-3.14	2.81	**41**	-2.51	3.97	-1.92	2.84	-2.53	12.80
-12	-3.88	3.83	-4.08	4.23	-3.20	2.84	**42**	-2.51	3.95	-1.93	2.85	-2.53	12.29
-11	-3.98	3.90	-4.15	4.33	-3.26	2.87	**43**	-2.51	3.94	-1.93	2.85	-2.53	9.02
-10	-4.08	3.97	-4.24	4.42	-3.33	2.90	**44**	-2.50	3.94	-1.94	2.85	-2.52	5.59
-9	-4.18	4.03	-4.34	4.50	-3.41	2.94	**45**	-2.50	3.94	-1.94	2.85	-2.52	4.01
-8	-4.29	4.09	-4.44	4.58	-3.51	2.98	**46**	-2.50	3.94	-1.95	2.85	-2.52	3.49
-7	-4.40	4.15	-4.55	4.67	-3.61	3.03	**47**	-2.49	3.95	-1.95	2.85	-2.52	3.34
-6	-4.53	4.23	-4.68	4.77	-3.73	3.08	**48**	-2.49	3.95	-1.96	2.85	-2.52	3.31
-5	-4.69	4.32	-4.81	4.89	-3.86	3.14	**49**	-2.48	3.96	-1.96	2.86	-2.51	3.29
-4	-4.89	4.43	-4.95	5.02	-4.01	3.21	**50**	-2.47	3.96	-1.97	2.86	-2.51	3.28
-3	-5.12	4.55	-5.08	5.15	-4.17	3.27	**51**	-2.47	3.97	-1.98	2.86	-2.50	3.28
-2	-5.38	4.68	-5.08	5.29	-4.29	3.41	**52**	-2.45	3.98	-1.98	2.86	-2.49	3.28
-1	-5.67	4.80	-4.52	5.36	-4.55	3.47	**53**	-2.44	3.98	-1.98	2.86	-2.48	3.29
0	-5.97	4.93	-3.00	5.41	-4.75	3.48	**54**	-2.41	3.99	-1.98	2.87	-2.46	3.29
1	-6.30	5.07	-1.99	5.42	-4.98	3.51	**55**	-2.39	4.00	-1.97	2.87	-2.44	3.30
2	-6.66	5.20	-1.87	5.42	-5.24	3.55	**56**	-2.34	4.00	-1.94	2.87	-2.40	3.30
3	-7.05	5.32	-1.87	5.51	-5.53	3.60	**57**	-2.19	4.01	-1.84	2.87	-2.30	3.31
4	-7.48	5.44	-1.87	5.70	-5.86	3.64	**58**	-1.63	4.01	-1.44	2.87	-1.90	3.31
							59	0.59		0.23		-0.14	

Table A9. Data from DSC analysis of AMF emulsions (O/W 3:7, 5:5 and 7:3)

	Heat Flow (mW)							Heat Flow (mW)					
°C	3:7		5:5		7:3		**°C**	3:7		5:5		7:3	
	cooling	heating	cooling	heating	cooling	heating		cooling	heating	cooling	heating	cooling	heating
-49	-1.22	0.97	-2.31	0.08	-2.13	0.23	**5**	-3.30	20.54	-6.83	31.09	-6.14	18.10
-48	-1.22	1.26	-2.31	1.75	-2.13	1.66	**6**	-2.79	15.33	-6.80	26.14	-5.38	13.52
-47	-1.23	1.33	-2.30	2.22	-2.13	2.04	**7**	-2.64	10.58	-7.07	20.61	-4.52	9.86
-46	-1.20	1.36	-2.28	2.37	-2.13	2.15	**8**	-2.45	7.23	-5.83	15.25	-4.03	7.53
-45	-1.17	1.39	-2.25	2.43	-2.12	2.20	**9**	-2.36	5.34	-4.93	11.40	-3.61	6.43
-44	-1.17	1.40	-2.16	2.46	-2.13	2.23	**10**	-2.36	4.48	-4.61	9.06	-3.27	6.23
-43	-1.17	1.42	-2.41	2.49	-2.13	2.24	**11**	-2.24	4.19	-4.50	7.94	-2.96	6.39
-42	-1.18	1.43	-2.42	2.51	-2.15	2.25	**12**	-2.21	4.15	-4.32	7.59	-2.90	6.76
-41	-1.19	1.44	-2.44	2.53	-2.17	2.25	**13**	-2.19	4.20	-4.24	7.62	-2.84	7.18
-40	-1.24	1.45	-2.49	2.54	-2.21	2.25	**14**	-2.13	4.27	-4.21	7.78	-2.79	7.56
-39	-1.33	1.47	-2.62	2.55	-2.20	2.25	**15**	-2.08	4.29	-4.13	7.90	-2.77	7.75
-38	-1.19	1.48	-2.55	2.55	-2.17	2.27	**16**	-2.06	4.23	-4.05	7.80	-2.76	7.61
-37	-1.17	1.49	-2.46	2.57	-2.17	2.28	**17**	-2.05	4.09	-3.97	7.51	-2.76	7.22
-36	-1.17	1.50	-2.47	2.58	-2.20	2.30	**18**	-2.05	3.90	-3.89	7.16	-2.75	6.76
-35	-1.17	1.52	-2.49	2.59	-2.22	2.33	**19**	-2.04	3.78	-3.88	6.85	-2.75	6.34
-34	-1.16	1.53	-2.50	2.61	-2.25	2.36	**20**	-2.04	3.72	-3.87	6.65	-2.75	6.06
-33	-1.16	1.55	-2.52	2.63	-2.29	2.38	**21**	-2.04	3.69	-3.86	6.52	-2.74	5.89
-32	-1.16	1.56	-2.54	2.65	-2.32	2.41	**22**	-2.04	3.67	-3.86	6.46	-2.74	5.79
-31	-1.16	1.58	-2.55	2.67	-2.31	2.44	**23**	-2.03	3.66	-3.86	6.45	-2.73	5.73
-30	-1.17	1.59	-2.58	2.69	-2.32	2.46	**24**	-2.03	3.66	-3.86	6.47	-2.73	5.70
-29	-1.17	1.61	-2.61	2.71	-2.35	2.48	**25**	-2.02	3.66	-3.85	6.48	-2.73	5.69
-28	-1.17	1.62	-2.64	2.73	-2.42	2.50	**26**	-2.02	4.15	-3.85	6.51	-2.72	5.69
-27	-1.20	1.63	-2.70	2.75	-2.59	2.52	**27**	-2.02	4.92	-3.85	6.54	-2.72	5.71
-26	-1.21	1.65	-2.79	2.77	-2.99	2.55	**28**	-2.02	5.66	-3.84	6.57	-2.72	5.73
-25	-1.24	1.66	-3.02	2.79	-3.85	2.57	**29**	-2.02	6.40	-3.83	6.61	-2.71	5.75
-24	-1.36	1.68	-3.59	2.82	-5.32	2.59	**30**	-2.01	6.95	-3.82	6.70	-2.71	5.77
-23	-1.76	1.69	-4.92	2.84	-7.54	2.61	**31**	-2.01	7.45	-3.81	7.33	-2.71	5.75
-22	-2.84	1.71	-7.38	2.86	-10.23	2.63	**32**	-2.00	8.11	-3.80	8.39	-2.70	5.56
-21	-5.12	1.72	-11.40	2.89	-3.36	2.66	**33**	-2.00	8.84	-3.78	9.18	-2.70	5.19
-20	-9.21	1.73	-16.87	2.91	-4.09	2.68	**34**	-2.00	9.64	-3.77	9.38	-2.69	4.77
-19	-16.06	1.75	-13.63	2.94	-5.67	2.71	**35**	-1.99	10.30	-3.75	9.05	-2.69	4.47
-18	-14.08	1.76	-7.26	2.96	-8.40	2.74	**36**	-1.98	10.68	-3.74	8.29	-2.68	4.28
-17	-2.51	1.77	-9.69	2.99	-12.36	2.77	**37**	-1.96	10.36	-3.72	7.20	-2.67	4.23
-16	-2.51	1.79	-4.69	3.01	-8.02	2.80	**38**	-1.93	9.31	-3.68	6.07	-2.67	4.23
-15	-2.52	1.80	-4.73	3.03	-10.62	2.83	**39**	-1.92	9.14	-3.64	5.49	-2.66	4.10
-14	-2.53	1.82	-4.78	3.06	-3.77	2.86	**40**	-1.87	9.46	-3.63	5.14	-2.65	4.06
-13	-2.54	1.83	-4.83	3.08	-3.83	2.88	**41**	-1.84	8.79	-3.58	4.98	-2.64	4.04
-12	-2.55	1.85	-4.88	3.11	-3.89	2.91	**42**	-1.84	7.87	-3.52	4.89	-2.64	4.04
-11	-2.56	1.86	-4.94	3.13	-3.95	2.95	**43**	-1.76	6.93	-3.43	4.84	-2.63	4.04
-10	-2.58	1.88	-5.01	3.17	-4.02	2.98	**44**	-1.72	7.73	-3.32	4.82	-2.62	4.05
-9	-2.60	1.91	-5.09	3.21	-4.10	3.04	**45**	-1.76	8.96	-3.16	4.80	-2.60	4.06
-8	-2.62	1.94	-5.18	3.28	-4.19	3.12	**46**	-1.75	10.26	-2.95	5.34	-2.59	4.07
-7	-2.66	1.97	-5.30	3.37	-4.29	3.22	**47**	-1.70	11.76	-2.81	6.61	-2.58	4.08
-6	-2.72	2.03	-5.44	3.48	-4.42	3.35	**48**	-1.70	13.73	-2.65	7.26	-2.57	4.10
-5	-2.80	2.12	-5.59	3.62	-4.57	3.52	**49**	-1.49	14.62	-2.56	7.52	-2.55	4.11
-4	-2.83	2.27	-5.73	3.83	-4.74	3.76	**50**	-1.71	14.71	-2.56	7.80	-2.54	4.12
-3	-2.85	2.61	-5.80	4.24	-4.99	4.22	**51**	-1.65	14.52	-2.40	7.95	-2.52	4.14
-2	-2.85	3.75	-5.84	5.53	-5.34	5.65	**52**	-1.56	14.45	-2.20	7.78	-2.50	4.16
-1	-2.89	11.00	-5.96	11.85	-5.98	12.85	**53**	-1.34	15.71	-1.94	7.85	-2.48	4.42
0	-2.85	24.87	-6.14	25.42	-6.82	22.57	**54**	-1.31	17.87	-1.50	8.18	-2.45	4.90
1	-2.87	31.25	-6.41	33.40	-7.80	24.58	**55**	-1.22	19.45	-0.83	8.85	-2.41	4.58
2	-2.98	32.08	-6.76	36.52	-8.08	24.57	**56**	-0.97	21.43	0.07	8.39	-2.33	4.37
3	-3.20	29.99	-7.21	36.63	-7.05	23.41	**57**	-0.55	18.45	1.52	8.24	-2.10	4.31
4	-3.60	25.97	-7.58	34.66	-6.30	21.39	**58**	0.34	12.56	3.94	4.61	-1.35	4.30
							59	3.87		7.51		4.52	

Table A10. Data from DSC analysis of MF42 emulsions (O/W 3:7, 5:5 and 7:3)

	Heat Flow (mW)							Heat Flow (mW)					
°C	3:7		5:5		7:3		**°C**	3:7		5:5		7:3	
	cooling	heating	cooling	heating	cooling	heating		cooling	heating	cooling	heating	cooling	heating
-49	-2.45	0.27	-2.20	0.37	-2.51	-0.26	**5**	-5.95	41.48	-5.71	27.76	-7.23	18.26
-48	-2.45	1.77	-2.20	1.73	-2.50	1.79	**6**	-6.06	37.21	-5.93	21.92	-8.08	13.10
-47	-2.46	2.16	-2.21	2.09	-2.50	2.39	**7**	-6.25	31.83	-6.22	15.64	-9.40	9.39
-46	-2.48	2.27	-2.23	2.19	-2.50	2.59	**8**	-6.68	24.98	-6.77	10.78	-10.95	7.05
-45	-2.48	2.31	-2.19	2.24	-2.50	2.66	**9**	-7.16	17.77	-7.52	7.59	-12.26	5.73
-44	-2.45	2.35	-2.20	2.27	-2.50	2.70	**10**	-7.21	12.40	-7.85	5.73	-12.71	5.02
-43	-2.46	2.38	-2.20	2.30	-2.51	2.74	**11**	-7.25	8.81	-7.91	4.77	-12.49	4.66
-42	-2.48	2.41	-2.20	2.32	-2.51	2.77	**12**	-7.24	6.72	-7.87	4.33	-11.52	4.49
-41	-2.52	2.43	-2.04	2.34	-2.53	2.80	**13**	-7.20	5.68	-7.86	4.15	-9.98	4.42
-40	-2.65	2.45	-2.37	2.36	-2.53	2.83	**14**	-7.31	5.24	-7.98	4.10	-8.12	4.40
-39	-2.92	2.47	-2.53	2.38	-2.53	2.86	**15**	-7.31	5.12	-8.15	4.11	-6.15	4.43
-38	-2.60	2.49	-2.38	2.40	-2.52	2.88	**16**	-5.93	5.43	-7.13	4.62	-4.67	4.60
-37	-2.53	2.51	-2.31	2.41	-2.53	2.91	**17**	-5.07	5.83	-4.75	5.33	-4.31	5.07
-36	-2.57	2.53	-2.30	2.43	-2.53	2.94	**18**	-4.89	5.98	-4.27	5.61	-4.31	5.72
-35	-2.47	2.55	-2.29	2.45	-2.55	2.96	**19**	-4.77	6.04	-4.08	5.74	-4.34	6.13
-34	-2.48	2.57	-2.12	2.47	-2.33	2.99	**20**	-4.77	6.10	-3.95	5.85	-4.37	6.32
-33	-2.50	2.59	-2.39	2.48	-2.63	3.01	**21**	-4.78	6.17	-3.95	5.98	-4.41	6.44
-32	-2.51	2.60	-2.41	2.50	-2.65	3.04	**22**	-4.80	6.24	-3.96	6.15	-4.48	6.58
-31	-2.54	2.62	-2.42	2.51	-2.68	3.06	**23**	-4.82	6.32	-3.97	6.34	-4.63	6.76
-30	-2.64	2.64	-2.44	2.53	-2.71	3.08	**24**	-4.86	6.41	-4.00	6.54	-4.85	6.97
-29	-2.91	2.66	-2.46	2.54	-2.76	3.10	**25**	-4.86	6.50	-4.04	6.77	-4.81	7.21
-28	-3.63	2.68	-2.54	2.56	-2.85	3.12	**26**	-4.70	6.59	-3.96	7.04	-4.15	7.45
-27	-4.13	2.69	-2.74	2.57	-3.05	3.14	**27**	-4.55	6.68	-3.86	7.61	-3.87	7.71
-26	-6.22	2.71	-3.28	2.59	-3.32	3.16	**28**	-4.48	6.78	-3.81	8.46	-3.87	7.97
-25	-9.57	2.73	-4.39	2.61	-3.93	3.18	**29**	-4.44	6.88	-3.79	9.21	-3.89	8.24
-24	-14.74	2.75	-6.50	2.62	-5.14	3.20	**30**	-4.41	7.01	-3.78	10.02	-3.89	8.53
-23	-25.83	2.77	-9.70	2.64	-7.11	3.22	**31**	-4.37	7.17	-3.77	10.72	-3.90	8.85
-22	-8.42	2.79	-14.23	2.66	-9.88	3.25	**32**	-4.30	7.64	-3.77	11.36	-3.90	9.14
-21	-11.73	2.81	-8.20	2.68	-4.81	3.27	**33**	-4.21	8.42	-3.76	12.08	-3.91	9.41
-20	-15.95	2.83	-9.12	2.70	-6.33	3.30	**34**	-4.10	9.86	-3.75	12.74	-3.91	9.70
-19	-5.10	2.85	-4.78	2.72	-8.88	3.33	**35**	-4.08	11.55	-3.73	13.32	-3.92	10.03
-18	-5.10	2.88	-6.19	2.74	-12.53	3.35	**36**	-4.03	12.99	-3.72	13.89	-3.92	10.39
-17	-5.10	2.90	-8.69	2.76	-17.36	3.38	**37**	-3.98	14.34	-3.70	14.53	-3.92	10.79
-16	-5.11	2.92	-10.91	2.78	-11.12	3.41	**38**	-4.01	17.03	-3.68	15.14	-3.93	11.22
-15	-5.11	2.94	-14.04	2.81	-4.38	3.44	**39**	-4.02	22.02	-3.65	15.79	-3.93	11.69
-14	-5.12	2.97	-4.37	2.83	-4.42	3.47	**40**	-3.99	26.33	-3.64	16.44	-3.94	12.16
-13	-5.13	2.99	-4.40	2.85	-4.46	3.50	**41**	-3.92	30.29	-3.62	17.60	-3.94	12.58
-12	-5.15	3.02	-4.42	2.87	-4.50	3.53	**42**	-3.84	33.36	-3.60	22.15	-3.94	12.60
-11	-5.16	3.05	-4.45	2.90	-4.55	3.57	**43**	-3.70	34.82	-3.56	22.95	-3.95	10.62
-10	-5.18	3.08	-4.47	2.93	-4.60	3.61	**44**	-3.49	33.50	-3.49	21.82	-3.95	7.79
-9	-5.20	3.12	-4.50	2.98	-4.65	3.68	**45**	-3.27	31.52	-3.40	21.02	-3.96	6.18
-8	-5.22	3.18	-4.53	3.05	-4.71	3.76	**46**	-3.05	29.85	-3.37	20.18	-3.96	5.44
-7	-5.24	3.26	-4.56	3.15	-4.78	3.89	**47**	-2.67	28.52	-3.27	18.74	-3.96	5.14
-6	-5.27	3.37	-4.60	3.29	-4.87	4.07	**48**	-2.15	26.97	-3.13	17.30	-3.96	5.03
-5	-5.31	3.55	-4.64	3.53	-4.97	4.37	**49**	-1.54	26.00	-2.98	16.44	-3.96	4.99
-4	-5.34	3.86	-4.68	3.97	-5.09	4.94	**50**	-0.76	25.45	-2.77	17.70	-3.96	4.97
-3	-5.38	4.55	-4.74	4.94	-5.22	6.17	**51**	-0.09	25.22	-2.78	18.92	-3.96	4.96
-2	-5.37	6.79	-4.73	7.65	-5.35	9.15	**52**	0.23	24.50	-2.65	19.43	-3.94	4.95
-1	-5.49	15.24	-4.91	15.59	-5.54	15.61	**53**	1.05	24.15	-2.43	20.47	-3.92	4.94
0	-5.54	30.27	-5.00	27.67	-5.71	25.11	**54**	2.04	23.87	-2.05	21.64	-3.89	4.94
1	-5.60	40.75	-5.11	34.50	-5.91	28.84	**55**	2.86	22.52	-1.70	22.07	-3.84	4.94
2	-5.67	45.08	-5.22	36.44	-6.13	28.94	**56**	3.97	21.85	-1.43	22.66	-3.68	4.95
3	-5.75	45.94	-5.38	35.33	-6.40	27.11	**57**	5.91	5.20	-0.63	20.24	-3.21	4.95
4	-5.84	44.51	-5.53	32.25	-6.71	23.52	**58**	10.42	5.19	1.27		-1.95	5.13
							59			5.36		5.32	

Table A11. Data from DSC analysis of MF13 emulsions (O/W 3:7, 5:5 and 7:3)

°C	Heat Flow (mW)					
	3:7		5:5		7:3	
	cooling	heating	cooling	heating	cooling	heating
-49	-1.94	0.65	-2.53	0.28	-2.92	-0.39
-48	-1.94	1.80	-2.53	1.55	-3.06	1.91
-47	-1.94	2.08	-2.54	1.87	-3.07	2.60
-46	-1.94	2.16	-2.55	1.95	-3.09	2.83
-45	-1.95	2.20	-2.57	1.99	-3.11	2.92
-44	-1.96	2.23	-2.59	2.02	-3.13	2.96
-43	-1.97	2.25	-2.60	2.03	-3.16	2.99
-42	-1.99	2.26	-2.62	2.04	-3.19	2.98
-41	-2.02	2.26	-2.64	2.04	-3.23	2.95
-40	-2.17	2.27	-2.70	2.03	-3.31	2.91
-39	-2.38	2.28	-2.79	2.03	-3.45	2.89
-38	-2.12	2.29	-2.72	2.04	-3.36	2.88
-37	-2.06	2.30	-2.74	2.05	-3.38	2.90
-36	-2.08	2.32	-2.76	2.08	-3.42	2.95
-35	-2.09	2.34	-2.79	2.10	-3.48	3.01
-34	-2.11	2.37	-2.82	2.13	-3.53	3.09
-33	-2.12	2.39	-2.85	2.16	-3.58	3.15
-32	-2.13	2.41	-2.88	2.19	-3.65	3.22
-31	-2.15	2.44	-2.91	2.22	-3.71	3.27
-30	-2.17	2.46	-2.94	2.25	-3.79	3.32
-29	-2.20	2.48	-2.99	2.27	-3.84	3.37
-28	-2.24	2.50	-3.05	2.29	-3.94	3.42
-27	-2.39	2.51	-3.19	2.31	-4.02	3.45
-26	-2.85	2.53	-3.14	2.33	-4.11	3.49
-25	-3.88	2.55	-3.24	2.35	-4.37	3.52
-24	-6.07	2.57	-3.40	2.37	-4.86	3.55
-23	-9.72	2.59	-3.77	2.39	-5.83	3.59
-22	-15.45	2.61	-4.64	2.42	-7.34	3.63
-21	-12.94	2.64	-6.56	2.45	-10.17	3.68
-20	-11.00	2.67	-10.21	2.49	-14.52	3.76
-19	-14.08	2.70	-15.87	2.54	-20.42	3.86
-18	-4.48	2.74	-23.32	2.60	-18.18	3.97
-17	-4.53	2.79	-21.78	2.67	-13.27	4.09
-16	-4.66	2.84	-17.20	2.73	-6.81	4.22
-15	-4.73	2.88	-6.31	2.80	-7.40	4.35
-14	-4.74	2.92	-6.50	2.85	-8.14	4.45
-13	-4.81	2.96	-6.69	2.90	-8.88	4.56
-12	-4.81	3.01	-6.74	2.97	-9.44	4.69
-11	-4.74	3.07	-6.51	3.06	-9.44	4.84
-10	-4.47	3.14	-5.88	3.16	-8.37	5.02
-9	-4.42	3.23	-5.29	3.27	-6.65	5.22
-8	-4.41	3.32	-5.18	3.39	-5.69	5.45
-7	-4.35	3.42	-5.05	3.52	-5.50	5.69
-6	-4.23	3.55	-4.88	3.67	-5.36	5.95
-5	-4.13	3.73	-4.64	3.86	-5.20	6.25
-4	-4.19	4.03	-4.53	4.12	-5.26	6.60
-3	-4.23	4.70	-4.85	4.63	-5.64	7.10
-2	-3.99	6.83	-4.94	6.05	-6.01	8.24
-1	-3.89	15.38	-4.47	13.07	-4.88	13.55
0	-3.82	33.68	-4.21	26.54	-4.18	24.92
1	-3.71	42.19	-4.11	31.88	-4.09	30.52
2	-3.63	44.66	-4.01	33.40	-4.06	32.57
3	-3.60	43.54	-3.97	32.89	-4.02	32.62
4	-3.58	40.20	-3.93	31.10	-3.99	31.34
5	-3.55	35.46	-3.89	28.32	-3.95	28.96
6	-3.53	29.52	-3.83	24.61	-3.93	25.48
7	-3.53	23.18	-3.80	19.90	-3.93	21.47
8	-3.52	16.86	-3.79	14.95	-3.93	17.75
9	-3.52	11.72	-3.79	10.49	-3.93	14.10
10	-3.51	8.24	-3.78	7.14	-3.92	10.40
11	-3.51	6.27	-3.78	5.31	-3.93	7.64
12	-3.51	5.33	-3.78	4.53	-3.93	6.18
13	-3.51	4.93	-3.78	4.22	-3.93	5.50
14	-3.50	4.79	-3.77	4.11	-3.93	5.21
15	-3.50	4.74	-3.77	4.08	-3.93	5.10
16	-3.50	4.72	-3.76	4.07	-3.94	5.06
17	-3.49	4.70	-3.76	4.08	-3.94	5.04
18	-3.49	4.70	-3.75	4.09	-3.94	5.03
19	-3.48	4.69	-3.75	4.13	-3.94	5.02
20	-3.47	4.69	-3.74	4.15	-3.94	5.02
21	-3.47	4.70	-3.73	4.15	-3.94	5.02
22	-3.46	4.69	-3.73	4.12	-3.95	5.02
23	-3.45	4.69	-3.72	4.10	-3.95	5.03
24	-3.44	4.68	-3.72	4.07	-3.95	5.03
25	-3.43	4.68	-3.71	4.06	-3.96	5.04
26	-3.42	4.68	-3.70	4.05	-3.96	5.04
27	-3.41	4.68	-3.69	4.05	-3.96	5.05
28	-3.39	4.68	-3.69	4.05	-3.97	5.05
29	-3.37	4.68	-3.68	4.05	-3.97	5.06
30	-3.35	4.68	-3.66	4.05	-3.98	5.06
31	-3.32	4.68	-3.65	4.06	-3.99	5.06
32	-3.30	4.68	-3.64	4.06	-3.99	5.06
33	-3.27	4.68	-3.63	4.06	-4.00	5.07
34	-3.24	4.68	-3.61	4.07	-4.00	5.08
35	-3.20	4.68	-3.60	4.07	-4.00	5.08
36	-3.15	4.68	-3.58	4.08	-4.00	5.08
37	-3.10	4.68	-3.56	4.08	-4.00	5.12
38	-3.03	4.68	-3.54	4.09	-4.01	5.19
39	-2.95	4.68	-3.52	4.09	-4.02	5.15
40	-2.85	4.68	-3.49	4.10	-4.02	5.14
41	-2.74	4.69	-3.46	4.11	-4.03	5.17
42	-2.61	4.69	-3.43	4.15	-4.03	5.14
43	-2.47	4.69	-3.39	4.17	-4.04	5.13
44	-2.31	4.69	-3.34	4.22	-4.04	5.13
45	-2.12	4.69	-3.29	4.17	-4.04	5.31
46	-1.92	4.69	-3.24	4.15	-4.04	5.22
47	-1.72	4.71	-3.17	4.14	-4.03	5.17
48	-1.49	4.70	-3.10	4.13	-4.03	5.35
49	-1.26	4.69	-3.01	4.12	-4.02	5.79
50	-0.94	4.89	-2.91	4.13	-4.01	6.52
51	-0.57	5.29	-2.78	4.13	-3.99	7.10
52	-0.17	5.27	-2.61	4.14	-3.97	7.06
53	0.36	5.71	-2.40	4.15	-3.92	6.58
54	1.09	9.26	-2.13	4.16	-3.86	6.13
55	2.02	11.41	-1.70	4.17	-3.75	5.62
56	3.20	15.79	-1.09	4.18	-3.52	5.45
57	4.93	17.84	-0.16	4.19	-2.99	5.28
58	13.40		1.52	4.20	-1.65	5.26
59					6.13	

Table A12. Data from DSC analysis of pure water and rhamnolipid solution (25% w/w)

Temperature	Heat Flow (mW)				Temperature	Heat Flow (mW)			
°C	water		rhamnolipid		°C	water		rhamnolipid	
	cooling	heating	cooling	heating		cooling	heating	cooling	heating
-49	-3.16	0.86	-1.26	0.49	**5**	-7.38	83.11	-1.95	3.43
-48	-3.15	2.74	-1.28	1.29	**6**	-7.36	75.95	-1.94	3.40
-47	-3.14	3.24	-1.30	1.48	**7**	-7.35	62.12	-1.92	3.40
-46	-3.13	3.41	-1.32	1.55	**8**	-7.33	51.27	-1.90	3.40
-45	-3.11	3.48	-1.34	1.60	**9**	-7.31	41.56	-1.88	3.41
-44	-3.04	3.52	-1.37	1.64	**10**	-7.29	32.83	-1.86	3.42
-43	-2.71	3.56	-1.40	1.70	**11**	-7.27	25.32	-1.84	3.43
-42	-3.27	3.59	-1.43	1.75	**12**	-7.25	19.29	-1.82	3.45
-41	-3.28	3.61	-1.46	1.81	**13**	-7.23	14.88	-1.79	3.46
-40	-3.30	3.64	-1.48	1.89	**14**	-7.22	12.01	-1.76	3.48
-39	-3.39	3.66	-1.51	1.96	**15**	-7.20	10.34	-1.73	3.49
-38	-3.31	3.68	-1.53	2.04	**16**	-7.17	9.44	-1.70	3.52
-37	-3.30	3.70	-1.55	2.11	**17**	-7.14	9.01	-1.67	3.71
-36	-3.31	3.72	-1.57	2.17	**18**	-7.11	8.80	-1.63	3.81
-35	-3.29	3.74	-1.59	2.22	**19**	-7.08	8.72	-1.59	3.83
-34	-3.30	3.76	-1.61	2.26	**20**	-7.04	8.68	-1.54	3.90
-33	-3.30	3.78	-1.63	2.29	**21**	-7.00	8.67	-1.48	3.97
-32	-3.30	3.79	-1.66	2.33	**22**	-6.96	8.67	-1.43	4.04
-31	-3.31	3.81	-1.69	2.36	**23**	-6.92	8.69	-1.37	4.12
-30	-3.33	3.83	-1.72	2.38	**24**	-6.88	8.72	-1.30	4.21
-29	-3.36	3.84	-1.75	2.42	**25**	-6.84	8.75	-1.22	4.29
-28	-3.44	3.86	-1.82	2.46	**26**	-6.79	8.79	-1.12	4.37
-27	-3.69	3.88	-2.00	2.50	**27**	-6.74	8.82	-1.02	4.47
-26	-4.36	3.89	-2.55	2.55	**28**	-6.69	8.86	-0.90	4.56
-25	-5.92	3.91	-3.99	2.60	**29**	-6.64	8.90	-0.77	4.63
-24	-8.68	3.92	-6.89	2.66	**30**	-6.59	8.94	-0.62	4.69
-23	-12.66	3.93	-11.49	2.74	**31**	-6.53	8.98	-0.47	4.76
-22	-17.97	3.89	-5.74	2.82	**32**	-6.47	9.03	-0.31	4.85
-21	-24.60	3.94	-8.89	2.91	**33**	-6.41	9.07	-0.12	4.94
-20	-12.26	3.97	-2.18	3.01	**34**	-6.34	9.12	0.11	5.03
-19	-15.49	4.00	-2.17	3.12	**35**	-6.27	9.18	0.33	5.13
-18	-19.97	4.02	-2.16	3.24	**36**	-6.19	9.24	0.50	5.23
-17	-24.91	4.03	-2.15	3.39	**37**	-6.12	9.30	0.70	5.33
-16	-30.21	4.05	-2.14	3.55	**38**	-6.05	9.39	0.90	5.43
-15	-7.73	4.07	-2.14	3.75	**39**	-5.98	9.51	1.09	5.54
-14	-7.71	4.09	-2.13	3.97	**40**	-5.90	9.63	1.22	5.66
-13	-7.69	4.11	-2.12	4.25	**41**	-5.82	9.75	1.30	5.79
-12	-7.67	4.12	-2.11	4.59	**42**	-5.72	9.89	1.39	5.93
-11	-7.64	4.14	-2.10	5.01	**43**	-5.62	10.07	1.49	6.07
-10	-7.62	4.15	-2.10	5.56	**44**	-5.52	10.83	1.63	6.18
-9	-7.61	4.17	-2.09	6.27	**45**	-5.40	12.19	1.79	6.28
-8	-7.59	4.19	-2.08	7.21	**46**	-5.29	13.14	1.98	6.38
-7	-7.58	4.21	-2.07	8.49	**47**	-5.16	13.84	2.21	6.46
-6	-7.57	4.24	-2.07	10.26	**48**	-5.04	14.46	2.49	6.52
-5	-7.55	4.27	-2.06	12.77	**49**	-4.92	15.09	2.81	6.87
-4	-7.54	4.30	-2.06	16.30	**50**	-4.79	15.73	3.19	6.99
-3	-7.52	4.36	-2.05	21.12	**51**	-4.65	16.39	3.64	7.59
-2	-7.48	4.61	-1.96	26.10	**52**	-4.50	17.18	4.18	7.65
-1	-7.50	8.02	-2.05	20.93	**53**	-4.36	17.96	4.82	8.27
0	-7.47	30.18	-2.02	13.26	**54**	-4.24	18.68	5.60	8.62
1	-7.45	52.90	-2.01	8.08	**55**	-4.06	19.35	6.50	8.84
2	-7.43	68.91	-1.99	5.23	**56**	-3.77	19.99	7.52	9.11
3	-7.41	78.71	-1.98	3.97	**57**	-2.95	12.40	8.69	9.48
4	-7.40	84.36	-1.97	3.54	**58**	-0.63		11.59	5.33
					59	9.47		1.62	

Table A13. Free oil formation in MF13, MF42 and AMF emulsions containing NaCl at various concentrations

	Salt Concentration (mM)	**% of free oil separation**							
		Trial 1		**Trial 2**		**Trial 3**		**Trial 4**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
MF13	**600**	49.58	49.58	42.50	28.33	35.42	35.42	-	-
	450	73.33	73.33	73.33	73.33	65.89	72.48	-	-
	250	65.61	65.61	59.65	59.65	53.68	50.70	-	-
	100	23.86	23.86	17.89	17.89	11.93	11.93	-	-
	25	5.53	5.53	0.00	0.00	0.00	0.00	-	-
	10	2.76	2.76	0.00	0.00	0.00	0.00	-	-
	5	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	1	0.00	0.00	0.00	0.00	0.00	0.00	-	-
MF42	**600**	49.58	28.33	56.67	56.67	35.42	42.50	49.58	49.58
	450	65.89	72.48	59.30	52.71	66.67	72.48	73.33	66.67
	250	53.68	47.72	41.75	41.75	59.65	65.61	47.72	53.68
	100	11.93	11.93	17.89	11.93	11.93	11.93	11.93	11.93
	25	5.56	5.56	11.22	11.22	11.06	11.11	10.90	10.90
	10	2.72	2.70	5.53	5.53	11.06	11.06	2.72	2.72
	5	0.00	0.00	0.00	0.00	2.72	2.72	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AMF	**600**	7.08	7.08	14.17	14.17	3.54	3.54	-	-
	450	40.00	40.00	73.33	73.33	73.33	76.67	-	-
	250	41.75	41.75	65.61	65.61	53.68	53.68	-	-
	100	41.75	35.79	35.79	35.79	17.89	23.86	-	-
	25	33.17	33.17	33.17	33.17	11.06	11.06	-	-
	10	16.59	22.11	32.69	32.69	0.00	0.00	-	-
	5	10.79	10.79	10.79	5.40	0.00	0.00	-	-
	1	5.40	0.00	5.40	5.40	0.00	0.00	-	-

Table A14. Free oil formation in MF13, MF42 and AMF emulsions containing $CaCl_2$ at various concentrations

	Salt Concentration (mM)	**% of free oil separation**							
		Trial 1		**Trial 2**		**Trial 3**		**Trial 4**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
MF13	**5**	32.38	32.38	16.19	16.19	10.50	10.79	-	-
	2	10.79	16.19	8.10	8.10	10.50	10.79	-	-
	1	2.70	2.70	5.40	5.40	10.50	5.40	-	-
	0.5	2.70	2.70	2.70	2.70	10.50	2.70	-	-
MF42	**5**	48.57	48.57	48.57	43.17	48.57	48.57	43.17	43.17
	2	13.49	10.79	16.19	16.19	10.79	10.79	10.79	10.79
	1	5.40	5.40	5.40	5.40	5.40	2.70	5.40	5.40
	0.5	2.70	2.70	2.70	5.40	0.00	0.00	2.70	2.70
AMF	**5**	53.97	53.97	53.97	48.57	21.59	26.98	-	-
	2	37.78	45.87	10.79	10.79	16.19	16.19	-	-
	1	37.78	32.38	10.79	2.70	5.40	5.40	-	-
	0.5	21.59	10.79	2.70	2.70	2.70	2.70	-	-

Table A15. Free oil formation in MF13, MF42 and AMF emulsions containing KCl at various concentrations

	Salt Concentration (mM)	**% of free oil separation**							
		Trial 1		**Trial 2**		**Trial 3**		**Trial 4**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
MF13	**600**	56.67	53.13	56.67	56.67	63.75	57.38	-	-
	450	66.67	66.67	66.67	66.67	73.33	73.33	-	-
	250	47.72	47.72	59.65	53.68	65.61	65.61	-	-
	100	11.93	11.93	14.91	17.89	23.86	23.86	-	-
	25	0.00	0.00	0.00	0.00	5.53	5.53	-	-
	10	0.00	0.00	0.00	0.00	2.76	2.76	-	-
	5	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	1	0.00	0.00	0.00	0.00	0.00	0.00	-	-
MF42	**600**	70.83	63.75	63.75	63.75	35.42	42.50	49.58	70.83
	450	66.67	66.67	65.89	65.89	73.33	73.33	66.67	73.33
	250	47.72	47.72	53.68	53.68	53.68	53.68	41.75	41.75
	100	17.89	11.93	17.89	17.89	11.93	11.93	11.93	11.93
	25	5.56	5.56	11.06	11.06	13.82	13.82	10.90	5.45
	10	2.75	2.75	5.45	5.45	13.82	13.75	0.00	0.00
	5	0.00	0.00	0.00	0.00	2.72	2.72	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AMF	**600**	56.67	64.56	77.92	70.83	56.67	63.75	-	-
	450	66.67	66.67	73.33	73.33	60.00	60.00	-	-
	250	59.65	53.68	65.61	65.61	53.68	53.68	-	-
	100	47.72	35.79	35.79	41.75	23.86	17.89	-	-
	25	33.17	33.17	22.11	22.11	5.53	5.53	-	-
	10	38.70	38.70	27.24	21.65	0.00	0.00	-	-
	5	32.38	21.59	10.79	16.19	0.00	0.00	-	-
	1	10.79	10.79	5.40	5.40	0.00	0.00	-	-

Table A16. Free oil formation in MF13, MF42 and AMF emulsions at various pH

	pH	**% of free oil separation**							
		Trial 1		**Trial 2**		**Trial 3**		**Trial 4**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
MF13	**2**	99.17	99.17	99.17	99.17	96.33	96.33	96.33	96.33
	3	99.17	99.17	99.17	99.17	90.67	90.67	90.67	90.67
	4	96.33	96.33	96.33	96.33	85.00	87.83	73.67	85.00
	5	51.00	48.17	34.00	36.83	22.67	22.67	34.00	34.00
	6	17.00	14.17	17.00	19.83	11.33	11.33	17.00	17.00
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	original	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MF42	**2**	99.73	99.45	96.33	96.33	99.17	99.17	99.17	99.17
	3	99.73	99.45	96.33	96.33	99.17	99.17	99.17	99.17
	4	99.73	99.73	96.33	96.33	99.17	99.17	90.67	92.08
	5	39.67	39.67	45.33	48.17	45.33	45.33	45.33	45.33
	6	17.00	19.83	28.33	25.50	17.00	19.83	22.67	22.10
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	original	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AMF	**2**	99.17	99.17	99.17	99.17	96.33	96.33	-	-
	3	99.17	99.17	99.17	99.17	90.67	90.67	-	-
	4	99.17	99.17	99.17	97.75	73.67	79.33	-	-
	5	51.00	48.17	34.00	36.83	22.67	22.67	-	-
	6	17.00	14.17	17.00	19.83	11.33	17.00	-	-
	8	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	12	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	original	0.00	0.00	0.00	0.00	0.00	0.00	-	-

Table A17. Raw data of colour measurements (L*. a* b*) of emulsions containing AMF, MF42 and MF13 (O/W 3:7, 5:5 & 7:3)

		Trial 1			**Trial 2**			**Trial 3**			**Trial 4**			**Trial 5**		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
AMF 3:7	**L***	83.61	83.58	83.59	83.58	83.58	83.61	86.19	86.15	86.18	85.74	85.77	85.72	87.28	87.25	87.25
	a*	-4.42	-4.42	-4.42	-4.43	-4.43	-4.44	-3.79	-3.79	-3.78	-3.78	-3.79	-3.77	-2.86	-2.87	-2.86
	b*	10.69	10.69	10.69	10.68	10.67	10.7	9.43	9.4	9.46	9.42	9.49	9.41	11.99	12	11.99
AMF 5:5	**L***	89.1	89.05	89.04	85.88	85.83	85.85	-	-	-	-	-	-	-	-	-
	a*	-3.27	-3.25	-3.23	-3.67	-3.73	-3.73	-	-	-	-	-	-	-	-	-
	b*	14.03	14.05	14.04	13.92	13.82	13.89	-	-	-	-	-	-	-	-	-
AMF 7:3	**L***	84.05	84.08	84.06	87.5	87.07	87.15	-	-	-	-	-	-	-	-	-
	a*	-3.57	-3.52	-3.44	-3.44	-3.48	-3.41	-	-	-	-	-	-	-	-	-
	b*	17.76	17.86	17.93	18.49	18.37	18.44	-	-	-	-	-	-	-	-	-
MF42 3:7	**L***	87.78	87.78	87.78	87.13	87.11	87.11	87.51	87.49	87.5	86.7	86.75	86.76	89.18	89.16	89.14
	a*	-2.63	-2.66	-2.67	-3.12	-3.11	-3.11	-2.93	-2.97	-2.96	-3.2	-3.18	-3.2	-2.26	-2.24	-2.29
	b*	8.12	8.14	8.13	7.53	7.53	7.52	8.19	8.21	8.21	6.89	6.93	6.92	8.7	8.71	8.72
MF42 5:5	**L***	87.51	87.45	87.48	87.09	87.06	87.1	-	-	-	-	-	-	-	-	-
	a*	-3.07	-3.07	-3.06	-2.88	-2.89	-2.88	-	-	-	-	-	-	-	-	-
	b*	10.25	10.26	10.25	9.26	9.28	9.28	-	-	-	-	-	-	-	-	-
MF42 7:3	**L***	85.34	85.33	85.33	81.23	81.18	81.18	-	-	-	-	-	-	-	-	-
	a*	-3.3	-3.29	-3.24	-4.58	-5.22	-5.14	-	-	-	-	-	-	-	-	-
	b*	12.99	13	13.02	12.77	13.01	12.97	-	-	-	-	-	-	-	-	-
MF13 3:7	**L***	82.71	82.69	82.71	82.63	82.69	82.69	86.31	86.29	86.33	88.65	88.56	88.7	86.49	86.51	86.52
	a*	-5.02	-5.05	-5.03	-5.04	-5.06	-5.03	-3.71	-3.7	-3.68	-4	-3.99	-3.92	-3.3	-3.3	-3.29
	b*	14.4	14.4	14.4	14.31	14.38	14.38	14.9	14.88	14.92	15.67	15.77	15.67	15.97	15.99	15.91
MF13 5:5	**L***	84.74	84.72	84.7	87.39	87.71	87.74	-	-	-	-	-	-	-	-	-
	a*	-4.31	-4.28	-4.28	-3.99	-3.79	-3.7	-	-	-	-	-	-	-	-	-
	b*	16.75	16.63	16.7	19.88	18.55	18.59	-	-	-	-	-	-	-	-	-
MF13 7:3	**L***	83.72	83.49	83.72	85.57	85.65	85.43	-	-	-	-	-	-	-	-	-
	a*	-4.07	-4.23	-3.97	-3.77	-3.63	-3.94	-	-	-	-	-	-	-	-	-
	b*	21.57	21.25	21.74	23.52	23.3	23.09	-	-	-	-	-	-	-	-	-

Table A18. Raw data of colour measurements (L* a* b*) of bulk AMF, MF42 and MF13 in both liquid and solid states

		Trial 1			Trial 2		
		S1	S2	S3	S1	S2	S3
AMF (liquid)	**L***	30.66	30.72	30.7	28.8	28.84	28.76
	a*	0.01	0.04	0.01	-0.02	0.13	0.07
	b*	8.08	8.27	8.08	9.78	9.67	9.67
AMF (solid)	**L***	69.69	69.22	68.71	68.97	68.03	67.55
	a*	-5.34	-5.24	-5.19	-5.88	-6.11	-6.03
	b*	39.87	39.29	40.04	44.43	43.61	44.25
MF42 (liquid)	**L***	28.99	29	29.06	28.96	28.96	29.06
	a*	-0.83	-0.76	-0.73	-0.91	-0.83	-0.87
	b*	9.37	9.43	9.52	9.33	9.35	9.45
MF42 (solid)	**L***	70.18	69.03	69.13	69.56	69.81	70.41
	a*	-6.3	-6.59	-6.61	-6.7	-6.86	-6.72
	b*	29.26	29.52	29.52	29.64	28.61	28.95
MF13 (liquid)	**L***	28.23	28.22	28.17	29.11	29.31	29.03
	a*	0.89	0.9	0.98	0.18	0.08	0.12
	b*	10.44	10.48	10.38	9.33	9.4	9.44
MF13 (solid)	**L***	49.5	48.34	47.28	41.05	40.86	40.43
	a*	-2.14	-1.87	-1.36	-1.1	-0.43	-0.22
	b*	33.88	32.6	30.75	30.61	26.15	25.31