Studies on the Shear Stability of Partially Crystalline Oil-in-Water Emulsions

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Abstract

Partial coalescence or fusion of fat globules is a common form of destabilisation in food-related partially crystalline oil-in-water emulsions that generally causes thickening of the emulsion, reduced functionality and phase separation. Partial coalescence is particularly exacerbated by shear, temperature fluctuations and air incorporation. Each of which the food product may be exposed to during manufacture, storage, transport or end use leading most often to deleterious effects. One notable example of partial coalescence being desirable is during the manufacture of ice cream.

In order to better understand the factors affecting partial coalescence and their interactions, a systematic series of studies was undertaken on the shear stability of a model food-related partially crystalline oil-in-water emulsion. Shear-induced aggregation of 35 wt% partially crystalline oil-in-water emulsions was studied under conditions causing jamming of partially coalesced fat globule aggregates formed under steady shear. Emulsions with different interfacial compositions and solid fat content (~25–90%) were studied to probe their effect on the generation of a jamming transition over a range of shear rates (500–2000 s^{-1}). By displacing adsorbed sodium caseinate from the oil-water interface using Tween 20, partial coalescence sensitive emulsions were prepared with either mixed sodium caseinate-Tween 20 or Tween 20 dominated interfacial film compositions using 0.5 or 1.5 wt% Tween 20 respectively. Quiescently, the emulsions were stable with 0.5 wt% Tween 20 regardless of solid fat content. At 1.5 wt% or above, stability decreased with increasing solid fat content and Tween concentration. Under shear, partial displacement of adsorbed sodium caseinate by Tween 20 generally resulted in the formation of relatively stable aggregates whereas high displacement of protein led to the formation of less stable aggregates under shear.

The aggregation time of emulsions with Tween 20 dominated interfaces decreased with increasing solid fat content whereas for mixed sodium caseinate-Tween 20 emulsions it increased with increasing solid fat content. The extent of fat globule aggregation at low solid fat content was relatively low irrespective of Tween concentration, whereas at high solid fat content, the extent of aggregation varied considerably when interfacial composition was altered. Cryo-TEM micrographs of the fat globules revealed a relatively smooth surface regardless of composition indicating that fat crystal protrusion was not a major factor affecting the stability. These findings showed that interfacial protein functions both to regulate the formation of partially crystalline aggregates as well as the aggregate stability under shear. Furthermore, high solid fat content emulsions although low in liquid oil content were shown to be highly susceptible to partial coalescence when the adsorbed protein load was low.

The effect of polyoxyethylene sorbitan fatty acid esters (Tween) alone or in combination with sorbitan fatty acid esters (SPAN) on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat) was studied. Low molecular weight emulsifiers like Tweens and SPANS are often used to regulate the susceptibility of partially crystalline oil-in-water emulsions to aggregation and partial coalescence. It was found that emulsion stability increased with increasing chain length of the saturated Tween emulsifiers, while unsaturated Tween 80 was comparatively much more unstable than the saturated types of Tween. The effect of 1 wt% SPAN (SPAN 20, 40, 60 and 80) in the dispersed phase of emulsions containing different concentrations of Tween 80 (0.2–0.6 wt%) was also investigated. The emulsions showed sharp stability transitions from stable to unstable over the range of Tween 80 concentrations tested. All SPAN containing emulsions with 0.2 wt% Tween 80 were stable under shear however all emulsions with 0.6 wt% Tween 80 immediately aggregated when shear was applied. At

0.4 wt% Tween 80, the saturated long fatty acid chain length emulsifiers (SPAN 40 and 60) were in general much more stable compared to SPAN 20 and 80 containing emulsions. This study indicated that oil soluble SPAN emulsifiers with low and high melting points were similarly poor at preventing partial coalescence. Therefore, phase transitions were likely not a major contributing factor to emulsion stability.

Finally, the effects of Tween 20, 40, 60 and 80 on the stability of partially crystalline oil-in-water emulsions prepared with and without 1 wt% sodium caseinate were studied. Generally, 1 wt% sodium caseinate emulsions with the unsaturated emulsifier Tween 80 were the most unstable followed by the saturated emulsifiers Tween 20, 40 and 60 in order of increasing fatty acid chain length. Long chain saturated Tween emulsifiers (Tween 40 and 60) improved emulsion stability regardless of whether sodium caseinate was present indicating that alone these emulsifiers form more robust interfacial films compared to the shorter chain length Tween 20 and the unsaturated Tween 80. The Tween type dependent effect on supercooling and fat crystallization caused by interfacial heterogeneous nucleation was also studied using pulsed nuclear magnetic resonance. With sodium caseinate, the degree of supercooling decreased and the crystallization rate diminished with increasing saturated fatty acid chain length but only negligible changes were found without sodium caseinate. These findings indicate that long chain saturated Tweens improve emulsion stability by forming robust interfaces but with sodium caseinate also improve stability through interfacial heterogeneous nucleation. These novel findings provide guidance on how combinations of proteins and emulsifiers can be used to modify and control the stability of partially crystalline oil-in-water emulsions through their combined effects on fat crystallization and interfacial film properties.

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Fuller, G. T., Considine, T., Golding, M., Matia-Merino, L., MacGibbon, A., & Gillies, G. (2015). Aggregation behaviour of partially crystalline oil-in-water emulsions: Part I – Characterization under steady shear. *Food Hydrocolloids, 43*(0), 521-528.

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Fig. 7.1. Schematic diagram showing potential structural changes of two interfacial films in response to high and low solid fat content.

List of Abbreviations

HPKO – Hydrogenated palm kernel oil

T20 – Tween 20

T40 – Tween 40

T60 – Tween 60

T80 – Tween 80

SDS – Sodium dodecyl sulfate

PGFE – Polyglycerol fatty acid esters

SPAN – Sorbitan monoesters

Lactem – Lactic acid esters of mono and diglycerides

GMS – Saturated monoglyceride

GMU – Unsaturated monoglyceride

SSL – Sodium stearoyl lactylate

$d_{4,3}$ – Volume weighted mean diameter

$d_{3,2}$ – Surface weighted mean diameter

SFC – Solid fat content

HLB – Hydrophilic-lipophilic balance

TEM – Transmission electron microscopy

Cryo-TEM – Cryogenic transmission electron microscopy

AFM – Atomic force microscopy

NMR – Nuclear magnetic resonance

Chapter 1: Thesis introduction

1.1 Introduction

Aqueous liquid and soft-solid materials containing emulsified lipid are notoriously prone to physical destabilisation through a variety of mechanisms due to thermodynamic instability (Dickinson, 2012; McClements, 2005; McClements & Rao, 2011). These mechanisms include lipid droplet coalescence, flocculation, coagulation, Ostwald ripening and systemic phase separation to name a few (McClements, 2005). When the dispersed lipid droplets also contain crystalline fat, a slew of additional destabilisation pathways leading to partial coalescence or fusion of the lipid droplets may also occur (Walstra, 2003). Although each pathway tends to lead to partial coalescence, each is caused by different perturbations such as shear, temperature fluctuations or air incorporation. The purpose of the research presented herein is to shed light on the complex and often interdependent effects of the fat globule interfacial film composition, solid fat content and fat crystallization nucleation mechanism on the stability, structural and material properties of model partially crystalline oil-in-water emulsions for food applications.

The partial crystallization of emulsified fat typically transforms a spherical oil droplet into a globular-shaped semi-solid particle (Walstra, 2003). In general, this transition reduces the deformability of an oil droplet due to crystal network formation and results in the presence of fat crystals at the droplet surface. Together, the lower deformability and crystals render the globules prone to partial fusion during collision or close contact due to the increased local stress on the tip of a protruding crystal (Walstra, 2003). In this way, a protruding crystal may rupture an interfacial film through a so called “pin” effect

and allow for liquid oil to flow between the globules leading to partial coalescence (Golemanov, Tcholakova, Denkov, & Gurkov, 2006).

In the food industry, partial coalescence is often required as part of a food manufacturing process so the goal is often to control when partial coalescence occurs rather than completely prevent it. To regulate partial coalescence, one needs to restrict the protrusion of fat crystals and/or increase the thickness or strength of the interfacial film (Fredrick, Walstra, & Dewettinck, 2010; Pelan, Watts, Campbell, & Lips, 1997; Walstra, 2003). Unfortunately, there is no customary way to measure the protrusion distance of crystals from fat globules. Therefore, it is difficult to determine if, for example, an increase in emulsifier concentration leads to an increase in the susceptibility to partial coalescence through an increase in fat crystal protrusion distance or rather the thinning of the interfacial film due to protein displacement by an emulsifier. Furthermore, the ability to measure the concentration of interfacially adsorbed protein (a routine measurement for liquid oil emulsions) is hampered by the presence of fat crystals (Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014; Segall & Goff, 1999). Centrifugation at low temperatures where the fat is crystalline often results in partial coalescence due to concentration of the fat globules into a densely packed phase. Heating above the melting point of the fat is a possible workaround however the interfacial composition may be altered due to the temperature dependence of competitive displacement between proteins and emulsifiers (Dickinson & Tanai, 1992), emulsifier phase transitions (Krog & Larsson, 1992), and possibly the change in globule surface from liquid to partially solid.

Despite the difficulties of measuring some of the basic properties of partially crystalline emulsions, researchers have sufficiently worked out which combinations of proteins and emulsifiers are appropriate for controlling emulsion stability. These ingredients often

include at least one emulsifier and one protein but are more often than not more complex. Ingredients often include both water and fat soluble emulsifiers, a blend of proteins, stabilizers and sugars. Without enumerating all the possible interactions that may take place in such a complex food product, it is obvious that if one desires to understand how all of these components interact and affect emulsion stability, one would need to take a systematic approach. By starting with the simplest ingredient blend and progressively adding and studying the effects of each ingredient on emulsion stability, a clearer understanding of each ingredient's functional contribution can be determined. Only with this knowledge and information can rational design of such a complex food product be undertaken.

1.2 Objectives and research overview

It was the aim of the authors to conduct a systematic series of studies on a model emulsion system using ingredients well characterised in the literature. The desire was to establish a clearer baseline understanding of the primary factors affecting partial coalescence with a particular emphasis on establishing the relative importance of different factors when they affect stability in combination. It is desired that this work can be built upon by researchers in a systematic way so that the factors affecting the stability of more complex emulsions can be better understood.

The initial research question driving this work was around the combined effect of solid fat content and interfacial film composition on emulsion stability. To this end, sodium caseinate and Tween 20 were used as emulsifiers since the interfacial behaviour of these ingredients alone and in combination have been well investigated in partially crystalline oil-in-water emulsions (Thivilliers-Arvis, Laurichesse, Schmitt, & Leal-Calderon, 2010; Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt, 2008), liquid oil-in-water

emulsions (Dalgleish, Srinivasan, & Singh, 1995; E. Dickinson, Radford, & Golding, 2003) and in both air-water and oil-water interfacial films (Mackie, Gunning, Wilde, & Morris, 2000; Woodward, Gunning, Mackie, Wilde, & Morris, 2009). By adjusting the Tween 20 concentration, emulsions with interfacial films containing either a mixture of sodium caseinate and Tween 20 or predominately Tween 20 were prepared. The solid fat content was also independently adjusted in order to determine how different combinations of solid fat content and interfacial film composition impacted shear-induced partial coalescence.

Following this work, a more complex emulsion system containing different Tween emulsifiers as well as combinations of Tween 80 and oil soluble SPAN emulsifiers was investigated. Different Tween emulsifiers were studied for the purpose of understanding the effect of fatty acid chain length on emulsion stability. Tween emulsifiers were specifically chosen because the emulsifiers do not undergo any phase transitions (Gulseren & Coupland, 2008). Most often, increasing fatty acid chain length of an emulsifier encourages self-assembly into liquid crystalline or crystalline phases especially at low temperatures (Krog, et al., 1992). Therefore, Tween emulsifiers were chosen to decouple these effects on emulsion stability. The addition of SPAN emulsifiers, which are essentially Tween emulsifiers with smaller hydrophilic headgroups, was done to understand if the addition of SPAN (some types of which may crystallize) could improve emulsion stability by forming a crystalline interfacial film or by improving the packing density. Both mechanisms could potentially hinder partial coalescence by inhibiting liquid oil migration between globules upon collision and by creating a more robust interfacial film.

Finally, the effect of changing Tween type and concentration on emulsion stability was studied more in depth. In particular, the role of interfacial heterogeneous nucleation on

emulsion stability was investigated to determine if it could inhibit partial coalescence. Long chain fatty acid emulsifiers including Tween 40 and 60 are often reported to decrease the degree of supercooling required for crystallization. Arima, Ueno, Ogawa, & Sato (2009) found that interfacial heterogeneous nucleation promoted the alignment of fat crystals with the curvature of the fat globule-water interface. Alignment of the fat crystals may better reduce crystal protrusion and hence reduce the susceptibility to partial coalescence.

Through these investigations, it was hoped to identify strategies to design partially crystalline oil-in-water emulsions with improved or novel functionality. With these new insights, perhaps the design of partially crystalline oil-in-water emulsions can move from more of an art to that of a science.

Chapter 2: Literature review

2.1 Introduction

A variety of food products comprise a metastable dispersion of oil-in-water stabilized by surface active molecules like proteins and low molecular weight emulsifiers. Examples of these food emulsions include salad dressing, mayonnaise, milk, cream and ice cream mix (McClements, 2007; Shen, Guo, & Zhu, 2011). When a portion of the dispersed oil is crystalline, the dispersed phase may undergo a unique type of aggregation called partial coalescence due to the presence of both fat crystals and liquid oil. The partial coalescence process is an integral part of the manufacture of food products like butter (King, 1953) and ice cream (Mendez-Velasco & Goff, 2011) as well as in the process of whipping cream (van Aken, 2001). Excessive partial coalescence can be detrimental in some products such as natural cream where it leads to undesirable thickening and reduced functional performance. Conversely, inadequate partial coalescence leads to poorer meltdown properties in ice cream. Therefore, partial coalescence is a process that needs to be managed by the food scientist; prevention in some food products and regulation in others. In order to meet these challenges, it is critical that the partial coalescence process be well described by a general model(s) for each food product in which it may occur. A clear understanding of the factors influencing partial coalescence is necessary to rationally design food products that meet consumer needs.

2.2 The partial coalescence mechanism

The term partial or arrested coalescence is used to describe the predominantly irreversible state of aggregation of partially crystalline fat droplets which are connected

through a “neck” of liquid oil. To arrest the coalescence process, a crystalline fat network must be present in at least one droplet to serve as a barrier against full coalescence. Without the crystal network, the two liquid droplets simply coalesce into one. Shown in Fig. 2.1, the mechanism by which the liquid “neck” of oil is formed is thought to occur when a fat crystal protruding from one globule ruptures the interfacial film of another globule. Once a fat crystal from one globule contacts the fat phase of another globule, liquid oil can flow between the globules and form a junction if the contact time is sufficient (Walstra, 2003).

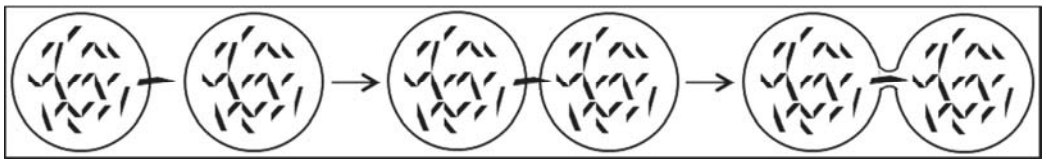


Fig. 2.1. Highly schematic representation of the partial coalescence mechanism, after Boode (1992). Reprinted from Advances in Colloid and Interface Science, 153/1-2, Fredrick, E., Walstra, P., & Dewettinck, K., Factors governing partial coalescence in oil-in-water emulsions, pp. 30-42, Copyright (2009), with permission from Elsevier.

The many factors influencing partial coalescence of partially crystalline fat droplets were investigated by van Boekel (1980), Melsen (1987), Boode, Bisperink, & Walstra (1991), Boode & Walstra (1993), Boode, Walstra, & Degrootmostert (1993), and more recent findings have been reviewed by Fredrick, Walstra, & Dewettinck (2010). The role of crystallization at the oil-water interface has also been recently reviewed by Douaire, et al. (2014). These factors are explained in the following sections.

2.3 Fat crystal formation in oil droplets

2.3.1 Nucleation and crystal growth

Triglycerides undergo a phase transition from liquid to crystalline upon cooling below the melting point through a two-step process of nuclei formation and crystal growth (Smith, Bhaggan, Talbot, & van Malssen, 2011). Homogeneous nucleation only occurs when catalytic impurities are not present in a fat droplet which leads to spontaneous nuclei formation after cooling below the melting point of the fat (Kloek, Walstra, & van Vliet, 2000). In "real" food emulsions, however, catalytic impurities are more numerous so heterogeneous nucleation typically occurs unless the number of fat droplets exceeds the number of nuclei (Kloek, Walstra, & van Vliet, 2000). Heterogeneous nucleation may occur in emulsions through three types of nucleation. In interfacial heterogeneous nucleation, the hydrophobic groups of surfactants adsorbed at the oil-water interface function as nucleation sites (Arima, Ueno, Ogawa, & Sato, 2009). In volume heterogeneous nucleation, foreign matter like dust may serve as the nucleating site. Since larger droplets are expected to contain more foreign matter (nucleation sites), volume heterogeneous nucleation may occur more often in larger droplets than smaller ones. Due to the lack of nucleation sites in small droplets, the onset crystallization temperature is generally lower than that of large droplets. Here, the term supercooled or undercooled is commonly used to describe oil droplets which remain liquid below the melting point of the bulk fat (Coupland, 2002). Lastly, interdroplet heterogeneous nucleation may occur when a protruding crystal from a partially or fully crystalline droplet penetrates a supercooled liquid oil droplet upon collision (McClements, Dickinson, & Povey, 1990). Once a crystal forms, it serves as a nucleation site for

further crystallization throughout the volume of the oil droplet in a process called secondary nucleation (Rousseau, 2002).

After nucleation, triglyceride molecules generally arrange into one of three crystal types known as α, β' and β which differ in terms of their subcell structure (Sato & Ueno, 2011). The tendency of fat crystals to initially crystallize in one of the less stable crystal forms (α or β') is due to their lower nucleation activation energy (McClements, 2012). Reflecting the greater thermodynamic stability of β-crystals, α (least stable) and β'-crystals may convert to β-crystals over time. This is a uni-directional process though so the conversion from β to α is not possible without remelting the crystals (Himawan, Starov, & Stapley, 2006). The dominant crystal polymorph formed in a blend of triglycerides or fat however depends on several factors including palmitic fatty acid content, distribution and position of the palmitic and stearic fatty acids on the triglyceride molecule, degree of saturation and degree of randomization since they influence the packing arrangement of the molecules within the crystals (O'Brien, 2009). For example, milk fat and palm kernel oil tends to form more β'-crystals whereas cocoa butter and lard form more β-crystals (O'Brien, 2009). The polymorphic form may alter the texture of some foods. For example, β-crystals tend to form relatively large plate-like crystals which are not desirable in fats and spreads (Sato & Ueno, 2011). Despite different the crystal polymorphs having different properties, no relationship between crystal polymorph type and the susceptibility to partial coalescence has been clearly established (Fredrick, et al., 2010).

The role of surfactants and other oil soluble additives on fat crystal nucleation and crystal growth has been studied extensively in bulk and emulsified fat. In bulk fat, surfactants may influence fat crystal nucleation by either inhibiting or promoting nucleation and/or crystal growth (Smith, et al., 2011). For nucleation, the effect(s) may

be a change in nucleation time or temperature and change in the number and composition of nuclei (Smith, Bhaggan, Talbot, & van Malssen, 2011). Furthermore, the initial polymorphism and the change from unstable to stable crystal types ($\alpha \rightarrow \beta' \rightarrow \beta$) over time may be greatly affected by surfactant type and concentration. It is difficult to predict how a surfactant or other additive will change the crystallization properties of a triglyceride because different components of a surfactant affect the fat in different ways (Smith, et al., 2011). Recently Smith, et al. (2011) published a diagram listing and describing the many modes of action additives have on nucleation, crystal growth and polymorphic crystal transitions (Fig. 2.2). In general though, there are only two rules that predict whether a surfactant will affect fat crystallization: 1) Similarity in triglyceride composition between the additive and fat. 2) Affinity of the surfactant for the crystal matrix.

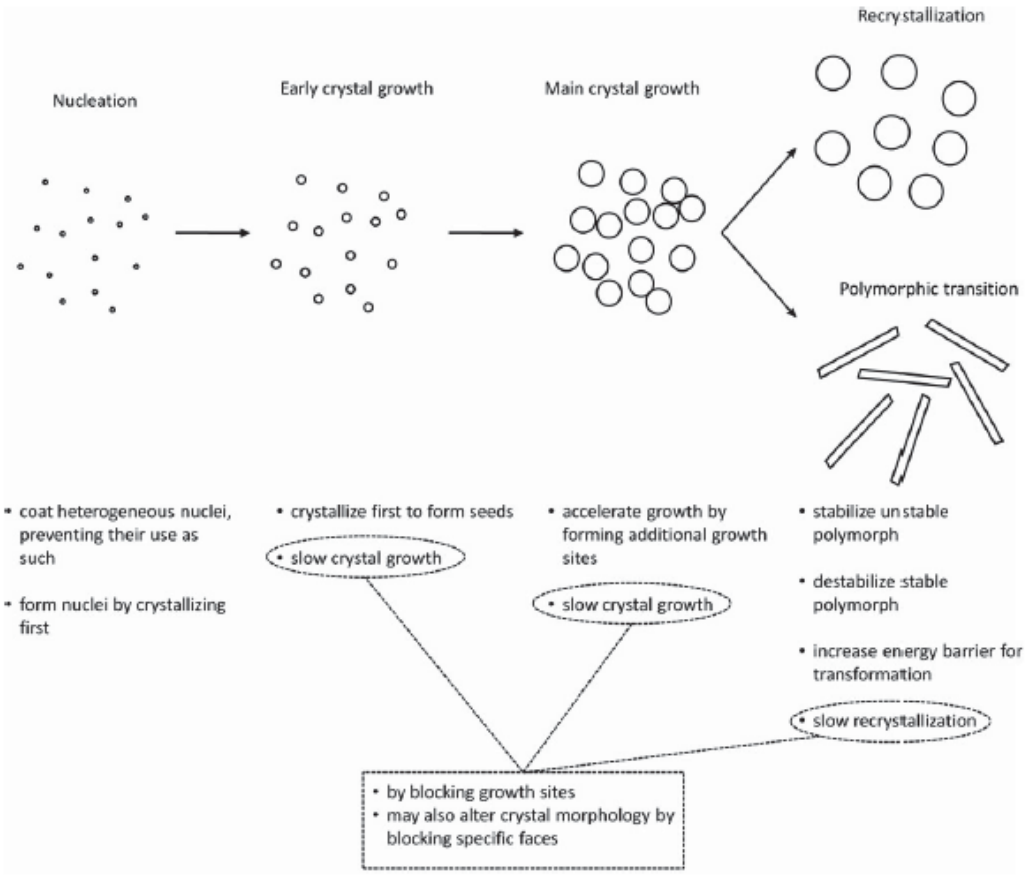


Fig. 2.2. Modes of action of additives. Journal of the American Oil Chemists' Society, Crystallization of Fats: Influence of Minor Components and Additives, 88, 2011, p. 1094, Smith, K. W., Bhaggan, K., Talbot, G., & van Malssen, K. F. Copyright (AOCS 2011) with permission of Springer. The figure highlights the potential effects of surfactants and additives on triglyceride nucleation and crystal growth.

In emulsified fat, the same general rules apply to nucleation and crystal growth but there is an additional effect caused by surfactants adsorbed at the oil-water interface. This topic is discussed in more detail in Section 2.4.3.2.

2.3.2 Effect of solid fat content on partial coalescence

The ratio of liquid oil to solid fat has a significant effect on the susceptibility to partial coalescence. According to McClements (2007), the rate of partial coalescence reaches a maximum around 20% solid fat content (SFC) and gradually decreases with increasing SFC (Fig. 2.3). At low SFC, droplets tend to coalesce more once aggregated due to greater liquid oil content while at high solid fat content, the lack of liquid oil hinders the degree to which fusion can occur. Ultimately though, the effect of solid fat content on the susceptibility to partial coalescence is formulation specific since simply increasing the interfacial protein load can prevent partial coalescence entirely (Pelan, Watts, Campbell, & Lips, 1997). Furthermore, the effect of SFC on partial coalescence may be determined by testing an emulsion at different temperatures or by preparing separate emulsions with different SFC at a constant temperature. Testing an emulsion over a range of temperatures is dubious however, if one seeks to understand the general effect of SFC on partial coalescence. This is because other factors that affect partial coalescence such as interfacial protein load, interfacial film viscoelasticity and surfactant phase behaviour are also temperature dependent. Likely due in part to the interdependent nature of the factors affecting partial coalescence, the effect of SFC on partial coalescence has not been thoroughly investigated in the literature.

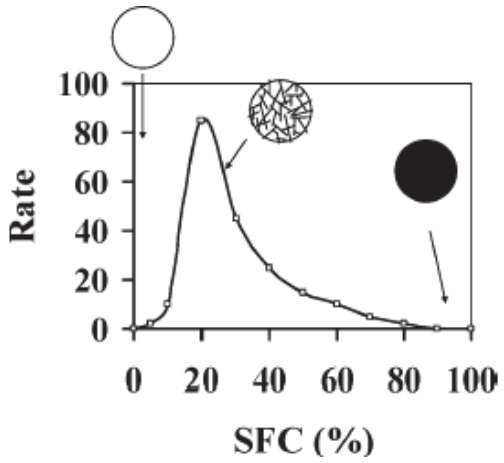


Fig. 2.3. Relationship between the rate of partial coalescence and solid fat content. Reprinted with permission from Critical Reviews in Food Science and Nutrition, 47/7, McClements, D.J., Critical review of techniques and methodologies for characterization of emulsion stability, pp. 611-649, Copyright (2007), with permission from Taylor and Francis.

2.3.3 Fat crystal adsorption and protrusion from the fat globule-water interface

The partial coalescence mechanism described by Walstra (2003) supports the hypothesis that partial coalescence is driven by fat crystal protrusion from an interface. In general, partial coalescence is reported to occur more readily as fat crystals increase in size and become more needle-shaped in appearance. Larger crystals tend to promote partial coalescence since they can protrude further than smaller crystals from the globule. Fat crystal size can be reduced by lowering the fat globule size since fat crystals typically do not increase in size beyond the diameter of the globule (McClements, Dungan, German, Simoneau, & Kinsella, 1993). Alternatively, rapid cooling is well known to produce smaller crystals compared to slow cooling (Lopez, et al., 2002).

Fat crystals in oil droplets are thought to orient themselves at the oil-water interface to minimize the interfacial free energy (van Boekel, 1980). Intuitively, fat crystals with a higher affinity for the interface will protrude from the interface more leading to increased susceptibility to partial coalescence. van Boekel (1980) measured the contact angle of crystals (solid paraffin or tristearate) at the paraffin oil-water interface. Measured from the aqueous phase, a contact angle of 180° meant a crystal was completely wetted by the oil phase while 0° meant a crystal was completely wetted by the aqueous phase. At 90°, both phases equally wetted a crystal. Although predicted, the contact angle measurements showed that crystals may or may not orient at the oil-water interface and several examples of emulsions where crystals did not adsorb were identified (van Boekel, 1980). In response to the unexpected results, the author discussed why both the measured contact angle values and those predicted may disagree. Factors such as variability in crystal shape and type, irregular shape of the interface and cooling rate were claimed to influence the contact angle significantly. In addition, fat crystal flocculation leading to network formation or an energy barrier promoted by the interface were also proposed as explanations for the lack of crystal adsorption at the interface.

Johansson, Bergenstahl, & Lundgren (1995) and Johansson & Bergenstahl (1995) studied the effects of water and fat soluble emulsifiers and oil type on the adsorption of fat crystals (α, β' and β) from tristearin and palm stearin at the oil-water interface. Their method measured the receding contact angle so the angles represent wetting by the opposite phase than results reported by van Boekel (1980). The α-type crystals with contact angles in the range of ~20–30° were all located at the oil-water interface. On the other hand, the β-type crystals from tristearin were completely wetted by the oil phase (contact angle 0°) whereas the palm stearin β-crystals were similar to the α-crystals in

contact angle. The enhanced adsorption of palm stearin β-crystals in the presence of most of the emulsifiers tested may be due to their slight polarity (measured by a higher contact angle without emulsifier present) compared to the nonpolar tristearin crystals. In addition, the presence of surface active impurities in palm stearin β-crystals, compared to the likely more pure tristearin, could explain the differences between the 2 types of β-crystals tested. Generally, the authors found the addition of emulsifiers promoted the adsorption of the crystals to the oil-water interface due to a reduction in surface tension. Emulsifiers which lowered the interfacial tension to a greater extent generally resulted in greater contact angles many of which were greater than 90° indicating better wettability by the aqueous phase.

2.4 Interfacial film composition and properties

2.4.1 Protein–based interfacial films

Partially crystalline oil-in-water emulsions stabilized by proteins are well known to be very resistant to partial coalescence (Pelan, et al., 1997; Walstra, 2003). Adsorbed proteins provide a thick steric barrier on the aqueous phase side of the oil-water interface thus hindering close contact between fat globules which is required for partial coalescence to occur (Xu, Nikolov, & Wasan, 2005b). Protein interfacial films can roughly be divided into two categories. Films formed by globular proteins like β-lactoglobulin have greater protein-protein interfacial interactions leading to rigid or capsule-like films (Erni, Windhab, & Fischer, 2011) while random coil protein films like β-casein have poorer protein-protein interactions leading to weaker, more viscous films. As a consequence of the intermolecular interactions at the interface, the response of a protein film to deformation varies from yielding (viscous) to fracture (elastic) behaviour (Martin, Stuart, Bos, & van Vliet, 2005). Sodium caseinate stabilized films,

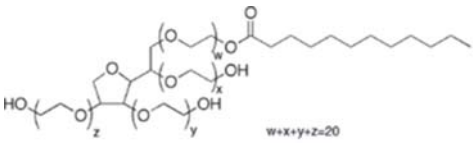
which are composed of a mixture of casein proteins, were found to be stronger than that of a pure β-casein film but slightly weaker than a pure κ-casein film (Dickinson, Murray, & Stainsby, 1988).

The influence of the strength of the protein film has been extensively studied. Wierenga, Kosters, Egmond, Voragen, & de Jongh (2006) studied the surface shear elasticity of undenatured ovalbumin adsorbed at an air-water interface. Comparing the surface shear elasticity of ovalbumin with and without sulfhydryl blocking, the authors concluded that disulfide bonding did not enhance the film elasticity. The authors' work supports the hypothesis that the strength of the films originates from dense packing of the protein particles at the interface rather than a 3-dimensional gel-like structure. However, they do also support that the adsorbed proteins can be "gelled" by heating or compression for example which may further strengthen the interfacial film (Wierenga, et al., 2006). Once gelled and interacting by intermolecular bonding, lateral film interactions due to hydrogen bonding, hydrophobic interactions, electrostatic interactions and covalent bonding should play a greater role in the film properties (Bos & van Vliet, 2001). For example, Dickinson & Hong (1994) showed how heating a β-lactoglobulin stabilized emulsion increased the protein film's resistance to displacement by Tween 20.

Using a Langmuir trough, Hotrum, Stuart, van Vliet, & van Aken (2003) studied the spreading behaviour of β-casein stabilized oil droplets at the air-water interface. When subjected to deformation, the film flowed and oil was released from the droplet which then spread on the interface in a radial fashion. Showing different behaviour, a β-lactoglobulin film fractured and the oil spread throughout the fragments (Hotrum, et al., 2003). This work provides strong evidence that differences in the interfacial film composition impact the spreading of oil released from fat globules at the air-water interface.

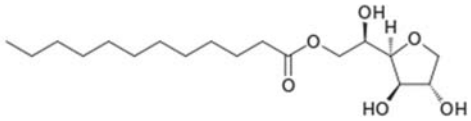
2.4.2 Food grade low molecular weight surfactants

Low molecular weight surface active molecules (surfactants) also referred to as emulsifiers are commonly added to natural and recombined partially crystalline oil-in-water emulsions to promote fat globule destabilisation. Food grade surfactants generally consist of a lipophilic fatty acid “tail” group linked to a hydrophilic “head” group. The hydrophilic head group can vary significantly in terms of molecular weight thus imparting varying degrees of hydrophilicity which in turn affects the interfacial activity of the molecule. Additionally, anionic surfactants carry a negatively charged hydrophilic group and thus may impart electrostatic stabilization between neighbouring fat globules. Lecithins, an example of a more complex surfactant, may possess both positively and negatively charged groups (McClements, 2005). The chemical structures of several common food grade surfactants are shown in Figure 2.4.



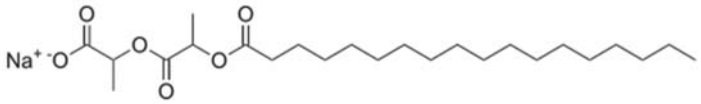
Polysorbate 20

(Polyoxyethylene 20 sorbitan monolaurate)

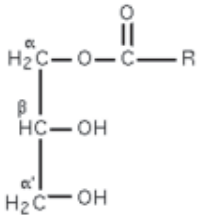


SPAN 20

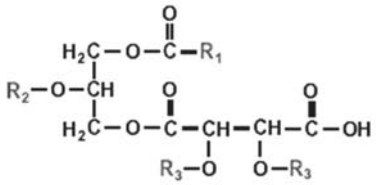
(Sorbitan monolaurate)



Sodium stearoyl lactylate



1-Monoglyceride



DATEM

(Diacetyl tartartic acid esters of mono- and diglycerides)

Fig. 2.4. Chemical structure of several food grade surfactants. R-groups: (R) Fatty acid group; (R_1) Stearoyl group; (R_2) Hydrogen or acetyl group; (R_3) Acetyl group.

Polysorbates or Tweens are a group of nonionic surfactants commonly used to modify the stability of partially crystalline oil-in-water emulsions. Shown in Figure 2.4, polysorbates contain a fatty acid tail and a polyethoxylated sorbitan ester head group making the surfactant highly water soluble. Sorbitan monoesters (SPAN) are non-ethoxylated derivatives of polysorbates and are thus primarily oil soluble (Krog, 1997). Commercial polysorbates and SPAN emulsifiers vary by the primary type of fatty acid tail i.e. lauric (C12:0), palmitic (C14:0), stearic (C16:0) or oleic (C18:1) fatty acids.

These are denoted by the numbers 20, 40, 60 and 80 respectively. Some varieties of Tweens also contain different degrees of ethoxylation and both SPANs and Tweens may contain more than 1 fatty acid e.g. sorbitan tristearate (SPAN 65).

Stearoyl-lactylates are emulsifiers formed by reacting stearic acid with sodium or calcium hydroxide. As a result of containing a stearic acid tail group, the surfactants have a high melting point. Sodium stearoyl-lactylate (SSL) generally crystallizes into the α-crystal form and hence provides unique functionality to partially crystalline oil-in-water emulsions like other α-crystal tending emulsifiers. SSL is an effective stabiliser of oil-in-water emulsions and is generally soluble in hot fat and dispersible in hot water. High concentrations of free fatty acids as well as tri- and polylactic acids are commonly present in commercial products (Krog, 1997).

Monoglyceride emulsifiers and their derivatives form the largest group of emulsifiers used in foods. Monoglycerides consist of a glycerol molecule linked to one fatty acid tail through an ester bond (Fig. 2.4). The free hydroxyl groups can also be esterified with different types of organic acids to form emulsifiers with significantly altered functionality caused by changes in interfacial activity, charge and crystallization temperature (Krog, 1997). Some examples of commercial varieties include ACETEM from acetic acid, LACTEM from lactic acid and DATEM from diacetylated tartaric acid anhydride, collectively known as the organic acid esters of monoglycerides. Notably, monoglycerides are formed from a series of chemical reactions which produce several by-products including diglycerides, free glycerol and free fatty acids. Additionally, the fatty acid tail varies depending on the source of lipid, both in terms of chain length and degree of saturation/unsaturation. Commercially, so called crude mono- and diglyceride blends are available which contain ~50–60% monoester content. Distilled monoglycerides contain higher monoester content (~90%) (Krog, 1997). Ultimately, the

ability to manipulate the head and tail groups opens up the possibility to tailor mono- and diglyceride emulsifiers for many food applications.

2.4.3 Mixed protein and low molecular weight surfactant films

Adsorbed protein films at the oil-water and air-water interfaces are partially displaced by low molecular weight surfactants due to competitive adsorption at the interface (Dickinson, Euston, & Woskett, 1990). By studying planar interfaces with atomic force microscopy (AFM), Mackie, Gunning, Wilde, and Morris (1999, 2000) found that the displacement of proteins follows a general orogenic mechanism. The mechanism is thought to occur in three stages: surfactant adsorption, expansion of surfactant domains and domination of the interface by surfactant (percolation) (Mackie, 2004). These stages are shown in Fig. 2.5 for the displacement of sodium caseinate by Tween 20 (Fig. 2.5 a–c) or Tween 60 (Fig. 2.5d–f) from an air/water interface. At sufficient concentration of low molecular weight surfactant, gaps or weak areas in the protein films are filled by surfactant. With time, the areas expand and compress the protein film. Given enough time and sufficiently high concentration of surfactant, the protein film can be fully displaced. The displacement of adsorbed proteins by surfactants leads to a much greater susceptibility of a partially crystalline emulsion to partial coalescence (Pelan, et al., 1997).

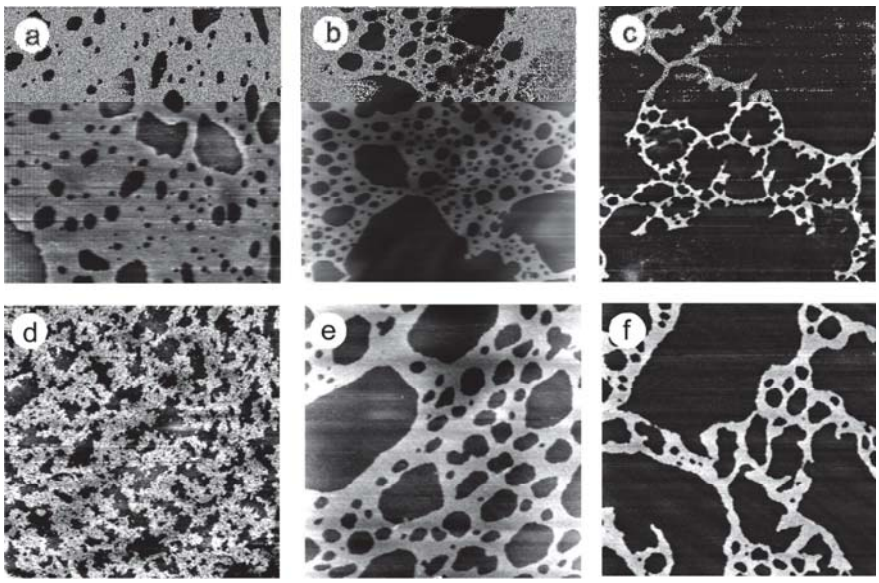


Fig. 2.5. Atomic force microscopy (AFM) images showing the displacement of sodium caseinate by Tween 20 (a–c) or Tween 60 (d–e) from an air-water interface. Reprinted with permission from {Woodward, N. C., Gunning, A. P., Mackie, A. R., Wilde, P. J., & Morris, V. J. (2009). Comparison of the orogenic displacement of sodium caseinate with the caseins from the air-water interface by nonionic surfactants. *Langmuir, 25*(12), 6739-6744}. Copyright {2009}. American Chemical Society. The protein films were initially spread to a surface pressure (π) of 12 mN m^{-1}. The images show the changes in the film structure after successive adsorption of the surfactants. (a) π = 20.3 mN m^{-1}, image size 12.5 μm. (b) π = 22.3 mN m^{-1}, image size 10 μm. (c) π = 23.7 mN m^{-1}, image size 15 μm. (d) π = 19.3 mN m^{-1}, image size 10 μm. (e) π = 19.8 mN m^{-1}, image size 15 μm. (f) π = 22.0 mN m^{-1}, image size 15 μm.

Low molecular weight surfactants affect competitive adsorption with proteins in different ways depending in large part on their charge. Dickinson (1999) described how low molecular weight surfactants displace adsorbed proteins from the oil-water interface

through two mechanisms. In the solubilisation mechanism, surfactants bind to protein molecules causing an increase in its solubility in the continuous aqueous phase leading to an increased ease of desorption of the protein-surfactant complex from the interface. In the replacement mechanism, the presence of the surfactant at the interface lowers the interfacial free energy of the system to a greater extent than the protein alone. Since ionic surfactants tend to interact strongly with proteins, protein desorption generally results from the solubilisation mechanism while non-ionic surfactants desorb proteins by the replacement mechanism (Bos, Nylander, Arnebrant, & Clark, 1997). For sodium caseinate planar films, it has been shown that α-casein and β-casein are preferentially displaced from the interface in lieu of κ-casein (Woodward, et al., 2009).

Several mechanisms have been proposed to explain why κ-casein rich protein films better resist displacement compared to α- and β-casein films. Structurally, κ-casein maintains a more native, folded structure upon adsorption to interfaces compared to α-casein and β-casein (Boyd, Mitchell, Irons, Musselwhite, & Sherman, 1973; Mitchell, Irons, & Palmer, 1970). This may allow κ-casein to produce more protein-protein interactions with neighbouring molecules since it interacts less with the interface compared to α- and β-casein. This logic helps in explaining the lower surface viscosity of α- and β-casein films compared to κ-casein films (Benjamins, Feijter, Evans, Graham, & Phillips, 1975). Furthermore, κ-casein can produce more disulfide linkages with neighbouring proteins compared to α- and β-casein. Boyd, et al. (1973) showed that reduction of κ-casein with mercaptoethanol resulted in films with decreased surface viscosity and increased susceptibility to displacement.

Together, the above work indicates mixed protein and surfactant films are generally inhomogeneous i.e. contain surfactant-rich domains within a continuous protein film. Furthermore, some proteins are preferentially displaced compared to others. Therefore,

the stability of partially crystalline oil-in-water emulsions will greatly depend on the blend of proteins and surfactants. These ingredients must be considered in tandem in order to design an interfacial film suitable for food emulsions where controlled destabilisation is desired.

2.4.3.1 Effect of fat crystallization on the adsorption and displacement of mixed interfacial films

In general, the transition from liquid oil to crystalline fat has been shown to decrease the surface coverage of adsorbed proteins on oil droplets (Barfod, Krog, Larsen, & Buchheim, 1991; Chen & Dickinson, 1993; Euston & Mayhill, 2001). However, protein and surfactant interfacial activity changes with temperature so the effects of crystallization and temperature reduction are difficult to separate. Chen & Dickinson (1993) found a lower surfactant-to-protein molar ratio was required to displace β-lactoglobulin from an *n*-hexadecane-water interface after crystallization. In model ice cream mix emulsions, Barfod, et al. (1991) found that the adsorbed protein content decreased rapidly during the first 2 h of storage at 5 °C before levelling off. The authors also found that oil soluble emulsifiers (monoglycerides) reduced the protein load greater than in the control ice cream mix prepared without any emulsifier (only skim milk powder). It was suggested that greater displacement of proteins by surfactants with decreasing temperature was due to greater emulsifier surface activity at low temperature (Barfod, et al., 1991; Krog, 1991).

Since crystallization of the oil phase is linked to temperature reduction, it is not clear whether crystallization or temperature reduction promote the change in adsorbed protein content. Reports on non-crystallizing oils do not offer clear insight either. For example, Dickinson & Tanai (1992) reported the adsorbed protein content of a soy oil emulsion

decreased with decreasing temperature from ~20–10 °C, then increased upon further cooling from ~10–0 °C. On the contrary, Euston, et al. (2001) reported that the adsorbed protein content increased with decreasing temperature in a soy oil emulsion over the range of 40–5 °C. Furthermore, oil soluble emulsifiers such as mono- and diglycerides may undergo a phase change as temperature decreases (Krog & Larsson, 1992). Such changes have immediate and time dependent effects on the affinity of protein for the oil-water interface.

One may also expect that a protein would bind more strongly to an oil-water interface compared to a crystalline fat-water interface because the non-crystalline interface is smoother and the non-polar protein side chains can penetrate its liquid surface (Dickinson, 1999). However, the displacement is likely dependent on the relative binding ability of the surfactant to the two surfaces rather than the protein. Elwing, Askendal, & Lundstrom (1989) and Elwing & Golander (1990) studied the difference in adsorption between proteins and Tween 20 on a surface with a gradient of hydrophobicity. Displacement of the protein by Tween 20 was greatest on the more hydrophobic surface owing to the enhanced affinity of Tween 20 to the hydrophobic surface rather than the binding ability of the protein.

Lastly, methods for measuring the adsorbed protein content may involve heating the emulsion above the melting point of the fat (Segall & Goff, 1999). This temperature change may also alter the adsorbed protein load by melting fat crystals, changing the phase of emulsifiers and altering the interfacial activity of both proteins and surfactants. For example, very large differences in adsorbed protein loads (~3–4 fold) have been reported using different adsorbed protein methods despite not too dissimilar formulations (Fredrick, et al., 2013; Pelan, et al., 1997).

2.4.3.2 Effect of adsorbed surfactants on fat crystallization

Some surfactants with a hydrophobic fatty acid tail that penetrates into the oil phase, can serve as sites for nucleation and crystal growth (Douaire, et al., 2014). Illustrated in Fig. 2.6, the surfactant acts as a template for crystal formation through interfacial heterogeneous nucleation (Sakamoto, et al., 2004). These crystals can then serve as a nucleation site for more crystals through volume heterogeneous nucleation (Sakamoto, et al., 2004). Sakamoto, et al. (2004) used polyglycerol fatty acid ester (PGFE) prepared from either palmitic, stearic or behenic acid to study the template effect on Tween 20 stabilized palm mid-fraction emulsions. Crystallization was enhanced in the presence of hydrophobic PGFE especially those with long chain saturated fatty acids. In general, the template effect is greatest when the fatty acid composition of the surfactant and the oil phase is similar (Smith, et al., 2011).

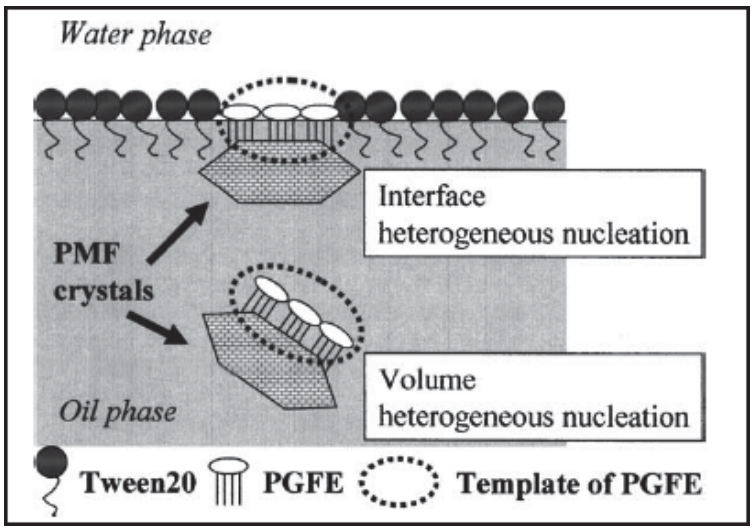


Fig. 2.6. Diagram of heterogeneous nucleation mechanisms caused by polyglycerol fatty acid esters (PGFE). Reprinted from Colloids and Surfaces B: Biointerfaces, 37/1-2, Sakamoto, M., Ohba, A., Kuriyama, J., Maruo, K., Ueno, S., & Sato, K., Influences of fatty acid moiety and esterification of polyglycerol fatty acid esters on the crystallization of palm mid fraction in oil-in-water emulsion, pp. 27-33, Copyright (2004), with permission from Elsevier.

It has long been speculated that water soluble emulsifiers like sodium dodecyl sulfate (SDS) or polysorbates promote a change in the orientation and protrusion length of fat crystals at the oil-water interface. Perhaps the most extreme example is called the Lanza process where complete removal of fat crystals from liquid oil is accomplished by a high concentration of a water soluble wetting agent such as SDS (Haraldsson, 1984). The crystals are better wetted by the aqueous phase than the liquid oil and pulled into the aqueous phase as a result. Recently, Spicer & Hartel (2005) showed how a similar process can expel fat crystals from oil droplets in rather dramatic fashion where "comet-like" expulsion of the crystals was observed (Fig. 2.7C). Using light microscopy, Boode & Walstra also found fat crystals protruded from fat globules dispersed in SDS (Fig.

2.7A and B). To verify that surfactants increase the protrusion distance, Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt (2008) used AFM to compare the surface topography of an anhydrous milk fat interface coated with sodium caseinate or Tween 20. The authors found that surface roughness was greater in the presence of Tween 20 (5 wt% in aqueous phase). One caveat of all of these reports was the need for high concentrations of surfactant which would not be found in "real" food emulsions. To the author's knowledge, no study has reported on an increase in fat crystal protrusion length caused by surfactant concentrations typically found in food emulsions. However, any increase in the fat crystal protrusion length is expected to increase the rate of partial coalescence by improving the capture efficiency (Walstra, 2003).

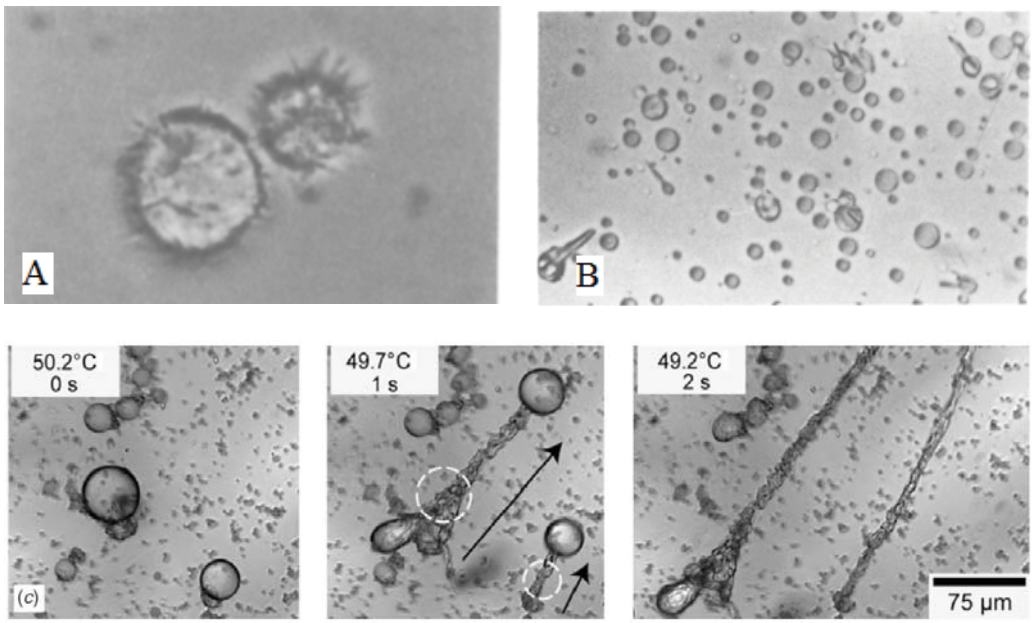


Fig. 2.7. Light microscopy images of fat crystal growth from triglyceride droplets. (A) Triglyceride blend (96.7% triglyceride, 3.2% diglyceride and 0.1% monoglyceride) dispersed in 14 mM SDS at 4 °C showing fine crystals protruding from fat globules (magnification 3000x). (B) Triglyceride blend (same as A) dispersed in 10 mM SDS showing occasional growth of large crystals from oil droplets (magnification 800x). A and B above are reprinted from Colloids and Surfaces A: Physiochemical and Engineering Aspects, 81, Boode, K. & Walstra, P., Partial coalescence in oil-in-water emulsions 1. Nature of the aggregation, pp. 121-137, Copyright (1993), with permission from Elsevier. (C) Time-lapse images of tristearin droplets dispersed in 4 wt% SDS and 0.4% decanol crystallizing during cooling at 30 °C min^{-1}. Reproduced from Spicer & Hartel (2005), with permission from CSIRO Publishing.

Additional support for the interaction of surfactants with fat crystals comes from research on emulsions containing both crystallized (solid) fat droplets and supercooled

oil droplets (Dickinson, Kruizenga, Povey, & Vandermolen, 1993; McClements, Dickinson, et al., 1993). Here, a lack of nucleation sites in the oil droplets caused them to supercool i.e. remain liquid below the crystallization temperature of the bulk fat. Once sheared in the presence of solid droplets, however, the supercooled droplets crystallized slowly over time presumably due to interactions with the solid droplets. The mechanism was thought to be penetration of the supercooled droplets by fat crystals protruding from the solid fat droplets. Importantly, McClements, et al. (1993) found the crystallization rate increased with increasing surfactant concentration but it also depended on the surfactant type in the following order: Tween 20 > SDS > β-lactoglobulin > β-casein. Assuming that crystal protrusion distance was the primary cause for the differences in crystallization rate, the authors proposed that several factors could have altered the protrusion distance. Since interfacial tension decreases with increasing surfactant concentration, the fat crystal-interface contact angle may also increase leading to greater crystal protrusion (Section 1.4). In addition, differences in nucleation and crystal formation, dependent on surfactant type (discussed previously in this section) could also alter the crystal protrusion distance. Dickinson, et al. (1993) also found that ionic strength did not impact the crystallization rate. Since ionic strength only affected the electric double layer thickness and not crystallization, the fat crystal properties and protrusion distance may be the main factors affecting the crystallization rate found in these studies.

2.4.3.3 Effect of monoglycerides on interfacial properties and emulsion stability

In addition to displacement of proteins from an oil-water interface, monoglycerides exhibit a temperature dependent polymorphism (Vereecken, et al., 2009). They may form liquid, liquid crystalline or crystalline structures due to interactions between the

fatty acid chains and water. The phase change may impart unique functionality to partially crystalline emulsions such as improved fat globule destabilisation during ice cream manufacture (Krog, et al., 1992).

Krog, et al. (1992) also showed that monoglyceride stabilized emulsions with increasing fatty acid chain length displayed an increasing sensitivity of surface tension to temperature. With decreasing temperature, the relative change in the reduction of surface tension was greater with increasing chain length (Krog, et al., 1992). Furthermore, longer chain length monoglycerides raised the temperature at which a rapid decrease in surface tension occurs. The authors reported that the abrupt decrease in interfacial tension was caused by a phase transition from liquid to either liquid crystalline or crystalline phase. By comparing the interfacial tension with temperature for an interface stabilized by protein only, monoglyceride only and monoglyceride plus protein, the authors also showed that interfacial tension measurements support the theory that monoglycerides desorb proteins from the interface because of a lack of additivity in the interfacial tension of the monoglyceride plus protein interface. The lack of additivity indicated the interface was covered predominantly by monoglycerides.

In an effort to determine if interactions had occurred between monoglycerides and proteins adsorbed at an interface, Doxastakis & Sherman (1986) measured the interfacial viscoelasticity using a biconical disc rheometer. The authors found a greater than additive increase in viscoelasticity occurred between mono- and diglycerides and sodium caseinate in combination which implies that some form of concentration dependent interaction was taking place.

Heertje (1993) reported saturated monoglycerides were better at displacing adsorbed protein compared to unsaturated monoglycerides. At low concentration ($\leq 0.25\%$),

monooleoylglycerol (unsaturated) better displaced sodium caseinate compared to monostearoylglycerol (saturated) whereas at higher concentrations the saturated surfactant was much better. The author proposed the higher adsorption free energy of the unsaturated monoglyceride aided in the displacement at low concentration. At high concentration, the authors proposed the much tighter packing of the saturated monoglyceride overrode the difference in adsorption free energy leading to complete protein displacement at a lower concentration of saturated monoglyceride.

Studies on sodium caseinate stabilized oil-in-water emulsions containing tristearin crystals show a general trend of increased stability with increasing chain length and greater overall stability of saturated monoglyceride compared to unsaturated types (Davies, Dickinson, & Bee, 2000; Davies, Dickinson, & Bee, 2001). Pelan, et al. (1997) also found ice cream mix emulsions showed better stability when stabilized by saturated monoglyceride compared to unsaturated monoglyceride. Notably, the authors also found that protein desorption alone did not explain the increased sensitivity of the emulsions to shear. The authors only measured a small change in adsorbed protein content despite a relatively large change in extractable fat after shearing emulsions containing unsaturated monoglyceride. Extractable fat was measured using a solvent extraction technique and is commonly used to determine the extent of fat globule destabilisation. The work of Pelan, et al. (1997) indicated the change in fat globule stability could not be explained by protein desorption alone.

Recently, Munk, et al. (2013) studied the stability of palm kernel oil-in-water emulsions with combinations of added monoglycerides viz.: lactic acid ester of monoglyceride (LACTEM), diacetyl tartaric acid ester of monoglyceride (DATEM), saturated monoglycerides (GMS) and unsaturated monoglycerides (GMU). In general, the stability of emulsions containing GMS were much improved over GMU emulsions

which is in agreement with the works discussed previously. Interestingly, the authors found that the changes in stability were not primarily caused by changes in crystallization behaviour as the different monoglycerides only affected crystallization in a minor way. However, the authors noted that GMS decreased the degree of supercooling substantially compared to the rest of the monoglycerides. However, it was not clear from this study if crystallization alone was the cause of the lowered degree of supercooling. Golemanov, Tcholakova, Denkov, & Gurkov (2006) also found a correlation between less supercooling and increased stability to partial coalescence. Notably though, this study only used emulsifiers which do not crystallize at low temperatures. This can be explained by the production of smaller crystals due to an increased number of nucleation sites caused by interfacial heterogeneous nucleation (Campbell, Goff, & Rousseau, 2001). Therefore, less supercooling may be induced in general for long chain saturated fatty acid containing emulsifiers regardless of their phase behaviour.

2.4.4 Summary

From the literature reviewed above, some general findings about partial coalescence can be summarized. In order for partial coalescence to occur, protein layers need to be predominantly removed so that close approach of fat globules is possible (Walstra, 2003). Upon close approach, the surface roughness may dictate the susceptibility to partial coalescence since only crystals able to span the gap between the droplets may rupture the interface leading to interaction between the globules either hydrophobically between crystalline fat surfaces or physically by a liquid oil bridge (Golemanov, Tcholakova, Denkov, & Gurkov, 2006). Surfactants may play a large role in controlling the surface roughness via either crystallizing directly (Krog, et al., 1992), templating

crystal formation (Sakamoto, et al., 2004) or altering the degree of droplet supercooling by increasing the number of nucleation sites (Golemanov, et al., 2006; Munk, et al., 2013). Direct crystallization of a saturated fatty acid emulsifier at the interface likely leads to a more rapid onset of fat crystallization (less supercooling) as well but even without emulsifier crystallization, longer chains lower the degree of supercooling compared to short chain lengths. It is very likely that changes in the interfacial layer topography caused by surfactant-dependent crystallization changes will affect the susceptibility to partial coalescence. For example, if liquid oil is involved in securing the connection between fat globules then a layer of crystalline emulsifier should drastically alter the ability of liquid oil to secure the connection. This may lead to modification of the texture and functionality of partially crystalline food emulsions.

2.5 Shear-induced partial coalescence

In quiescently stable partially crystalline oil-in-water emulsions, partial coalescence of fat globules is commonly observed after mechanical agitation or shear. In the manufacture of butter for example, agitation of cream leads to formation of clumps of fat globules which can then be easily separated from the aqueous serum called buttermilk (King, 1953). Temperature fluctuations alone, in the absence of shear, may also result in partial coalescence. This type of partial coalescence will be discussed later in Section 2.6. In the following sections, the range of factors affecting shear-induced partial coalescence will be described.

2.5.1 Fat globule collision frequency and efficiency

According to Walstra (2003), two particles (fat globules in this case) are aggregated when they remain in contact for a much longer time than they would in the absence of

colloidal interaction forces. If the particles encounter each other under quiescent conditions due to Brownian motion, diffusion or phase separation for example, it is referred to as perikinetic aggregation. If velocity gradients in the fluid cause the encounters, the aggregation is called orthokinetic or shear-induced aggregation.

When a solution containing particles is sheared, the particles will inevitably encounter each other. Smoluchowski's coagulation equation describes this process wherein two particles collide and coalesce (von Smoluchowski, 1917). The probability that two particles will interact depends on the encounter frequency and efficiency. The encounter frequency F_s due to shear between two particles under simple shear conditions is given by,

$$F_s = \frac{16}{3} G r^3 n^2 \tag{1}$$

where G = shear rate, r = droplet radius and n = droplet volume fraction (Shankaran & Neelamegham, 2001). The equation shows encounter frequency increases with increasing shear rate, increasing volume fraction and increasing droplet size.

Once particles encounter each other, the capture efficiency describes how successful the encounters are. From Walstra (2003), the capture efficiency α is given by,

$$\alpha = 0.6\left(\frac{A}{\eta\, \psi\, d^3}\right)^{0.18} \tag{2}$$

where A = Hamaker constant, η = viscosity, ψ = velocity gradient and d = droplet diameter. The equation predicts higher efficiencies than found in most systems because the formula neglects many aspects of real systems such as the chemical interaction potential between the droplets.

The equations above are only valid under laminar flow conditions. The type of fluid flow greatly impacts the kinetics of orthokinetic aggregation by changing both the encounter frequency and efficiency. For Newtonian fluids under laminar flow, the fluid is envisaged to move in layers like a deck of cards (Mezger, 2011). At higher shear rates, secondary flow may develop due to the increased contribution of centrifugal or inertial forces over the viscous forces. Secondary flow is characterized by deviation from smooth flow into vortices or swirls. Further increasing shear rate results in turbulent flow which is characterized by random or chaotic fluid flow (Bernard, Crouch, Choudhari, Bogard, & Thole, 1998).

2.5.2 Role of fat globule interaction potential

Aside from physical factors like particle size and flow regime, aggregation also depends on the chemical interaction forces between particles (Walstra, 2003). In Fig. 2.8, the sum of the van der Waals attractive force and repulsive electrostatic force (DLVO interaction energy) as a function of distance between two hypothetical droplets is illustrated. Owing to the differences in the strength of the two forces at short and long droplet separation, an attractive secondary minimum (II) may occur in addition to the primary attractive minimum (I). At both attractive minima, flocculation should occur although at the secondary minimum the attractive force is significantly weaker thus flocculation may be reversible. At intermediate separation, the droplets experience a net attractive force. The repulsive force serves as an energy barrier inhibiting the transition to the primary minimum (I) where strong attractive forces cause irreversible droplet aggregation or coagulation (Petsev, 2004).

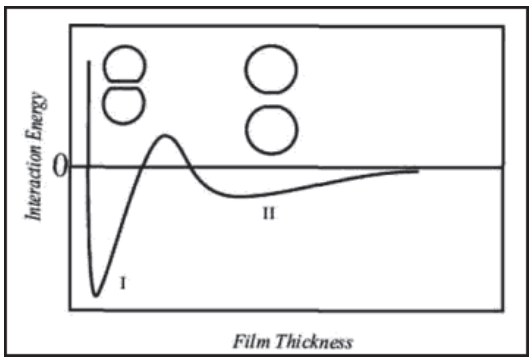


Fig. 2.8. Illustration of the interaction potential between two droplets as a function of distance. Reprinted from Interface Science and Technology, Petsev, D.N., Theory of emulsion flocculation, p. 38, Copyright (2004), with permission from Elsevier.

Fig. 2.8 is however a simplification of the interaction forces experienced by two approaching droplets. For example, steric stabilization forces are strongly repulsive at short distances, which is why proteins stabilize oil droplets so well against coalescence (McClements, 2007). Furthermore, other repulsive forces related to hydrodynamic effects occur upon close approach viz.: solvation, hydration and structural forces (Schowalter, 1984). A summary of the types of interactions along with their respective sign (attractive or repulsive), strength, range and the major factors affecting them has been reviewed by McClements (2005).

For inhibition of partial coalescence, close approach of the fat globules must be prevented to the point that the interfacial fat crystals cannot span the gap between the globules (Walstra, 2003). To this end, adsorbed proteins generally prevent partial coalescence altogether due to their strong steric and/or electrostatic repulsive forces (Xu, et al., 2005b). However, proteins are commonly displaced from the interface by low molecular weight surfactants so that controlled destabilisation can occur during the

whipping of cream or manufacture of ice cream (Fredrick, et al., 2010). In these cases, quiescent stability and shear instability are both required. Thus, prevention of close globule contact using steric stabilization would likely result in insufficient shear instability. Therefore, careful management of the attractive forces, especially depletion attraction, may be necessary to create a quiescently stable yet shear unstable emulsion. For example, Vanapalli, Palanuwech, & Coupland (2002) studied the stability of partially and fully crystalline emulsions flocculated to different degrees by the addition of a depleting agent (xanthan gum). Depletion attraction is the result of two particles (fat globules in this case) existing at a distance smaller than the diameter of another unadsorbed polymer. This leads to the polymer being excluded from the interglobule space which in turn leads to an osmotic gradient that promotes flocculation of the fat globules (Dickinson, Golding, & Povey, 1997). Vanapalli, et al. (2002) found that at concentrations of xanthan gum causing depletion flocculation, confectionary fat emulsions were more prone to partial coalescence. At higher xanthan gum concentrations where flocculation was inhibited by increased viscosity, partial coalescence was much reduced.

2.5.3 Measuring system considerations

For a given shear rate, a fluid will experience a different types of flow depending on the measuring system used e.g. cup and bob, parallel plate or cone and plate (CP) (Mezger, 2011). For CP systems, secondary flow always occurs but the effect is negligible at low shear rates and low cone angles. Under these conditions, primary flow occurs as layers of concentric circles outward from the cone to the sample edge. Due to continuity, the flow then proceeds downwards at the sample edge, inward along the plate and upwards along the cone surface (Fewell, 1977). Non-negligible secondary flow is initiated by an

increase in the ratio of the centrifugal forces to the viscous forces to a critical value. For CP systems, Sdougos, Bussolari, & Dewey (1984) found the ratio of centrifugal to viscous forces can be described by,

$$\tilde{R} \equiv \frac{r^2 \omega \alpha^2}{12v} \tag{3}$$

where $\tilde{R}$ = Reynolds-like number, r = cone radius, ω = angular velocity, α = cone angle and v = kinematic viscosity.

Several authors have studied where the onset of secondary flow begins to be significant. Sdougos, et al. (1984) found that secondary flow is negligible when $\tilde{R} \ll 1$, significant when $\tilde{R} \sim 1$ and flow is turbulent when $\tilde{R} > 4$. Since secondary flow is a local event occurring at first near the perimeter of the cone, the effect on the torque response (a bulk measurement) may be minimal. Therefore, a conservative approach should be taken when selecting a limit for $\tilde{R}$. Taking into account the work of Sdougos, et al. (1984) and the latter work of Ellenberger & Fortuin (1985) and Buschmann (2002), the consensus seems to be that secondary flow effects are negligible when $\tilde{R} < 0.1$. Secondary flow in a CP system can significantly affect the collision frequency by altering the local velocity gradient thereby affecting shear induced aggregation (Shankaran, et al., 2001). Thus, by keeping secondary flow negligible, equations 1 (encounter frequency) and 2 (encounter efficiency) can be used to determine the aggregation rate.

Although equation 3 was developed for non-truncated tip cones, it is still valid for cones with truncated tips (Mezger, 2011). In Fig. 2.9, a schematic of a truncated CP system is shown where the dotted lines correspond to the imaginary cone tip. Modern cones have truncated tips for several practical reasons: prevent wear and abrasion of the cone and

plate, prevent friction between the cone tip and plate from influencing the torque value and allow for particulate containing fluids to be measured (Mezger, 2011). Flow induced by a truncated cone is assumed to be the same as a traditional CP flow to the edge of the truncation. Below the truncation line, the fluid flows in the manner of that induced by a parallel plate. An assumption is made that the transition from parallel plate flow to CP flow is smooth and continuous (Hou, 1981). Since the gap below the cone truncation is small (< 50 μm), any change in flow regime is considered negligible.

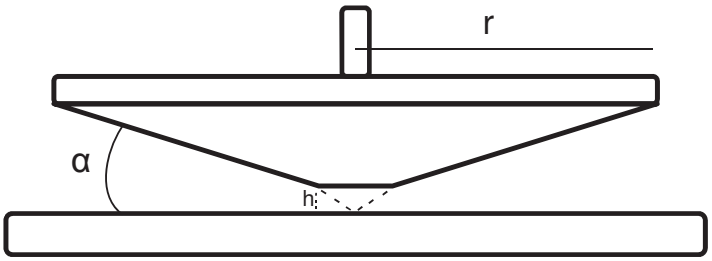


Fig. 2.9. Schematic of a truncated cone-and-plate system where r is the cone radius, α is the cone angle and h is the gap height. The size of the angle is exaggerated for clarity.

Although all geometries can produce laminar flow, the CP system has the advantage of producing a uniform shear rate and shear stress throughout the gap (Mezger, 2011). Furthermore, the entire sample is subjected to shear. Therefore, further testing can be easily performed on the sheared fluid because mixing of sheared and unsheared portions is avoided.

Despite a strong fundamental understanding of the flow behaviour of fluids in different geometries, many questions still remain regarding the movement of particles within the flow field and the structures formed as a result of shear-induced migration. Briefly, particles under shear tend to migrate toward regions of low shear stress and/or low

curvature (Krishnan, Beimfohr, & Leighton, 1996) driven by shear-induced normal stresses (Morris & Boulay, 1999). Some consequences include the formation of particle concentration gradients throughout the sample volume (Morris, et al., 1999) and structures resembling strings of beads attached to the wall or plate (Feng & Joseph, 1996; Pasquino, Snijkers, Grizzuti, & Vermant, 2010). Collectively, these studies indicate that the flow behaviour of partially crystalline emulsions aggregating under shear is likely very complex.

2.5.4 Methods used to study shear-induced aggregation

Researchers have studied shear-induced aggregation of emulsions under primary flow, secondary flow and turbulent conditions (Boode & Walstra, 1993; Chen, Dickinson, & Iveson, 1993; Dickinson, Owusu, & Williams, 1993; Melsen, 1987; Selomulya, Bushell, Amal, & Waite, 2002; van Boekel, 1980; van Boekel & Walstra, 1981). With increasing shear rate, greater flow instabilities result leading to increased secondary flow and ultimately turbulent flow. Furthermore, the onset and rate of shear-induced aggregation occurs earlier and faster with increasing shear rate. To study the effect of flow regime, Melsen (1987) used a couette flow apparatus to measure the aggregation of a recombined milk fat emulsion under laminar flow and flow with Taylor vortices at the same shear rate. Primary flow was generated by rotating the outer cylinder and keeping the inner cylinder fixed while flow with Taylor vortices was generated by rotating the inner cylinder and keeping the outer cylinder fixed. The results showed greater destabilisation occurred during flow with Taylor vortices, possibly caused by elongational flow which occurs within the vortices but not in laminar flow. When secondary flow develops, local velocity gradients cause increased or decreased hydrodynamic forces depending on the location of the particle in the flow field

(Shankaran, et al., 2001). Generally, hydrodynamic forces increase with increasing shear rate and tend to breakdown aggregates once they reach a critical size (Selomulya, et al., 2002), thus the rate of shear-induced aggregation (not just the onset time) is affected by the shear rate. Ultimately, the consequences of increased shear rate and deviation from laminar flow are numerous.

Some researchers have utilized a constant shear rate (Guery, et al., 2006; Legrand, et al., 2005), a stress ramp (Davies, et al., 2000; Davies, et al., 2001) and even oscillatory testing (Giermanska, et al., 2007; Thivilliers-Arvis, Laurichesse, Schmitt, & Leal-Calderon, 2010; Thivilliers, et al., 2008) to induce aggregation. Oscillatory testing has proven useful to study shear-induced destabilisation in relatively unstable emulsions especially those with large droplet sizes (Thivilliers-Arvis, et al., 2010). In the stress ramping experiments, the critical stress value which induces aggregation is determined. Using this approach, Davies, et al. (2001) showed how the relative shear stability of fine, partially crystalline emulsions was influenced by the presence of saturated and unsaturated monoglyceride surfactants. For the constant shear rate approach, the aggregation time is determined. One notable advantage of the constant shear rate approach is that for some emulsions (at least those of interest here), the viscosity and shear stress remain relatively constant before aggregation occurs. This allows for the determination of a characteristic induction time (t_c) (Fig. 2.10) which is primarily dependent on the size of the repulsive energy barrier between the droplets (Guery, et al., 2006). The value of t_c indicates when the onset of significant aggregation begins.

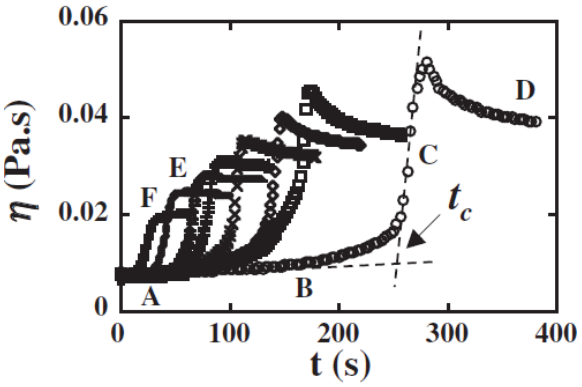


Fig. 2.10. Shear-induced aggregation curves of viscosity versus time. The emulsion is a water-in-oil-in-water double emulsion consisting of an outer droplet layer of crystalline fat. Each curve from left to right represents a different shear rate which varies from 300 to 80 s^{-1}. The induction time (t_c) is indicative of the onset of aggregation. Reprinted with permission from [Guery, J., Bertrand, E., Rouzeau, C., Levitz, P., Weitz, D. A., & Bibette, J., Physical Review Letters, 96, p.198301, 2006.] Copyright (2006) by the American Physical Society.

Several studies on orthokinetic aggregation of emulsions have produced similarly shaped curves of viscosity versus time at constant shear rate like those shown in Fig. 2.10 despite having very different dispersed phases. The dispersed phases include droplets of crystalline triglyceride containing water droplets (double emulsion) (Guery, et al., 2006), bitumen droplets (Legrand, et al., 2005) and latex spheres (Zaccone, Gentili, Wu, & Morbidelli, 2010). As shown in Fig. 2.10, initially the emulsions show relatively little change in viscosity for a period of time (induction time) followed by a sudden and rapid increase caused by aggregation of the dispersed phase. The presence of the induction time implies that an energy barrier is present which prevents the aggregation process from proceeding. After the energy barrier is overcome by the hydrodynamic forces, aggregates form causing a self-accelerating increase in the

aggregation rate. From these curves, the onset of aggregation can be determined which provides information on the relative strength of the energy barrier and the rate at which it proceeds (Guery, et al., 2006). Thus, the induction time can be used as an indication of the relative stability of the emulsion against aggregation.

2.5.5 Shear-induced partial coalescence studies on food emulsions

Lips, Westbury, Hart, Evans, and Campbell (1993) studied orthokinetic aggregation of a model oil-in-water food emulsion (35% coconut/hardened palm kernel) stabilized with UHT treated buttermilk protein and Tween 60. The emulsion was homogenized at 100 bar which yielded a mean droplet size ($d_{3,2}$) of 0.7 µm. The aggregation study was carried out over a temperature range of 5–30 °C in Couette, cone-and-plate and parallel plate geometries. The authors showed that the critical time for the onset of aggregation decreased approximately linearly from 12–27 °C then immediately returned to near the starting value when the temperature was increased above the melting point of the fat. This study illustrates the importance of SFC on the rate of partial coalescence i.e. more liquid oil promotes partial coalescence so long as a minimum crystal content is present. However, the authors came to a different conclusion than van Boekel (1980) on the role of fat crystals in the process. Lips, et al. (1993) proposed the function of fat crystals was to ensure and mediate partial coalescence whereas van Boekel (1980) postulated that they had a direct kinetic role in rupturing droplet films.

Hinrichs & Kessler (1997) studied orthokinetic aggregation by subjecting unpasteurized milk and cream, with varying fat content, to shear rate sweeps. The authors found a linear dependence of the critical aggregation shear rate on fat volume fraction. Also, the critical shear rate was higher at 5 °C than 10 °C implying greater instability with less SFC (Hinrichs, et al., 1997). These results are in agreement with those of Lips, et al.

(1993) described above. Hinrichs, et al. (1997) explained the reduced stability at lower SFC based on the idea that a crystalline shell forms around milk fat globules during crystallization. Under this assumption, the crystalline shell should decrease in thickness with increasing temperature or decreasing SFC and therefore lead to an increased susceptibility to partial coalescence.

The shear-induced aggregation of sodium caseinate stabilized groundnut oil-in-water emulsions containing tristearin crystals and monoglycerides in the dispersed phase was studied by Davies, et al. (2000) and Davies, et al. (2001). Using a shear stress ramping procedure, the authors found the emulsions aggregated at different critical stress values depending on their relative stability. Differences in the shear stability in the presence of the different types of monoglycerides were attributed to differences in the displacement of proteins from the interface and changes in fat crystal properties caused by the monoglycerides. Although not measured directly, the authors conjectured that unsaturated monoglycerides may promote the formation of crystals less aligned with the interface compared to saturated monoglycerides which may promote fat crystal alignment at the interface. These changes in the organization of the fat crystals at the interface could explain the enhanced stability of the emulsions containing saturated monoglyceride.

2.5.6 The stability of fat globule aggregates under shear

2.5.6.1 Aggregate break up and compaction

Once fat globules aggregate or partially coalesce under shear, the aggregation rate is expected to increase due to an increase in encounter efficiency of the aggregate compared to an individual globule (Walstra, 2003). Thus, shear-induced aggregation

tends to be a self-accelerating process (Lips, et al., 1993). However, the size aggregates can grow to under shear, can be limited by the shear hydrodynamic forces if they are sufficiently large enough to break the internal bonds between particles within a growing aggregate (Jarvis, Jefferson, Gregory, & Parsons, 2005). Aggregates may respond to excessive hydrodynamic forces like shear stress by simply compacting into a denser and thus stronger structure more resistant to break up. Tangential shear stresses are reported to lead to surface erosion of aggregates due to the less dense arrangement of particles at aggregate surfaces. Aggregate fragmentation may also occur, especially in low density arrangements composed of weak internal bonds, due to tensile stress on the aggregate (Jarvis, et al., 2005).

Very limited information is available on the stability of fat globule aggregates under shear. Intuitively, aggregates with less solid fat would be expected to fuse to a greater extent due to the combination of reduced globule rigidity and greater liquid oil content. More liquid oil should also lead to stronger connections between partially coalesced globules compared to high solid fat content globules.

Xu, Nikolov, & Wasan (2005a) studied the changes in shear-induced fat particle structure variation at different shear rates, shear histories and temperatures using a back-light scattering technique. The authors found increased order of the fat particle structure after 10 min of shear at several shear frequencies but decreasing order thereafter. At 3 °C, the redispersion rate dominated over the aggregation rate leading to a roughly linear increase in the structure factor. At 20 °C, the aggregation rate dominated leading to a brief increase in the structure factor followed by a decrease. The authors proposed that the decrease in stability at 20 °C was due to a change in the balance of factors affecting aggregation i.e. solid fat content and adsorbed protein load. In a follow-up study, Xu, et al. (2005b) studied the effects of different surfactant and protein blends on

partially crystalline emulsion stability. However, the backscattering technique does not provide a direct measure of aggregate size so it is difficult to infer the changes that different surfactants and proteins had on the stability of the aggregates under shear.

2.5.6.2 Fat globule aggregation leading to a jamming transition

Under shear, fat globule aggregates may grow in size and number sufficient to form a space-filling network. In general, aggregation of dispersed particles leads to entrapment of a portion of the continuous phase within the aggregate leading to an effectively greater volume fraction of the dispersed phase (Tadros, 2010). Increased volume fraction leads to an increase in viscosity due to hydrodynamic interactions between the particles and aggregates (Coussot, 2007). If the increase in volume fraction under shear is sufficient, then a jamming transition may occur whereby the fluid emulsion changes to a semisolid or paste due to overcrowding of the aggregates (Coussot, 2007).

Several authors have studied the jamming transition of suspensions such as viscous bitumen emulsions (Legrand, et al., 2005), partially crystalline double emulsions (Guery, et al., 2006) and hard styrene-acrylate suspensions (Zaccone, et al., 2010). These studies have generally found an exponential or power law dependency of jamming time on shear rate. Similar to orthokinetic aggregation (Section 2.1), the jamming time takes longer to occur with decreasing shear rate due to a combination of decreased collision frequency and efficiency due to the decreased shear rate and shear stress (Guery, et al., 2006; Zaccone, et al., 2010). The jamming time also occurs faster with increasing particle size (Legrand, et al., 2005) and volume fraction (Legrand, et al., 2005; Zaccone, et al., 2010).

Interestingly, the jamming transition is simply controlled by the increase in the effective volume fraction which is controlled by the number and density of particles and aggregates (assuming constant temperature and shear conditions) (Liu & Nagel, 1998). Therefore, any change in a factor which regulates the aggregate formation in terms of density or stability, for example, will alter the time at which the jamming transition occurs or when the jamming transition subsides due to aggregate break up or densification. In partially crystalline emulsions, factors like solid fat content and interfacial composition are expected to impact the extent of partial coalescence between fat globules (McClements, 2005) and therefore the jamming time. Unfortunately, the changes in the structure of fat globule aggregates, such as breakup, erosion or compaction, during a jamming transition for instance have not been well described. An improved understanding of these changes would aid in explaining the relationship between fat globule aggregate stability and interfacial composition in addition to the more commonly studied relationship between the susceptibility to aggregation and composition.

Some evidence of differences in bonding within aggregates of partially crystalline fat globules was reported by Thivilliers, et al. (2008) who studied anhydrous milk fat emulsions stabilized by sodium caseinate and Tween 20. By measuring the storage modulus (G') before and after temperature cycling, the authors detected two different modes of aggregation namely partial coalescence and jamming had occurred. Partial coalescence led to much higher values of G' compared to jamming, presumably because of the increased strength of connections between partially coalesced fat globules compared to jammed globules. The authors proposed a diagram where the transition between jammed and partially coalesced globules depended on globule size and surfactant-to-protein molar ratio (R_m) (Fig. 2.11). At high R_m, the authors thought the

displacement of adsorbed protein by surfactant aided in fat globule-globule contact. For small particle sizes, jamming was thought to occur due to weak interactions between the rough globule surfaces. The authors presumed partial coalescence did not occur at small particle sizes because the fat crystals were not large enough to pierce the film of neighbouring fat globules. For large fat droplets and high R_m, a combination of partial coalescence and jamming may occur. This study provides strong evidence that fat globule aggregates may form through different mechanisms which could potentially be useful for altering the texture and/or functionality of partially crystalline oil-in-water emulsions.

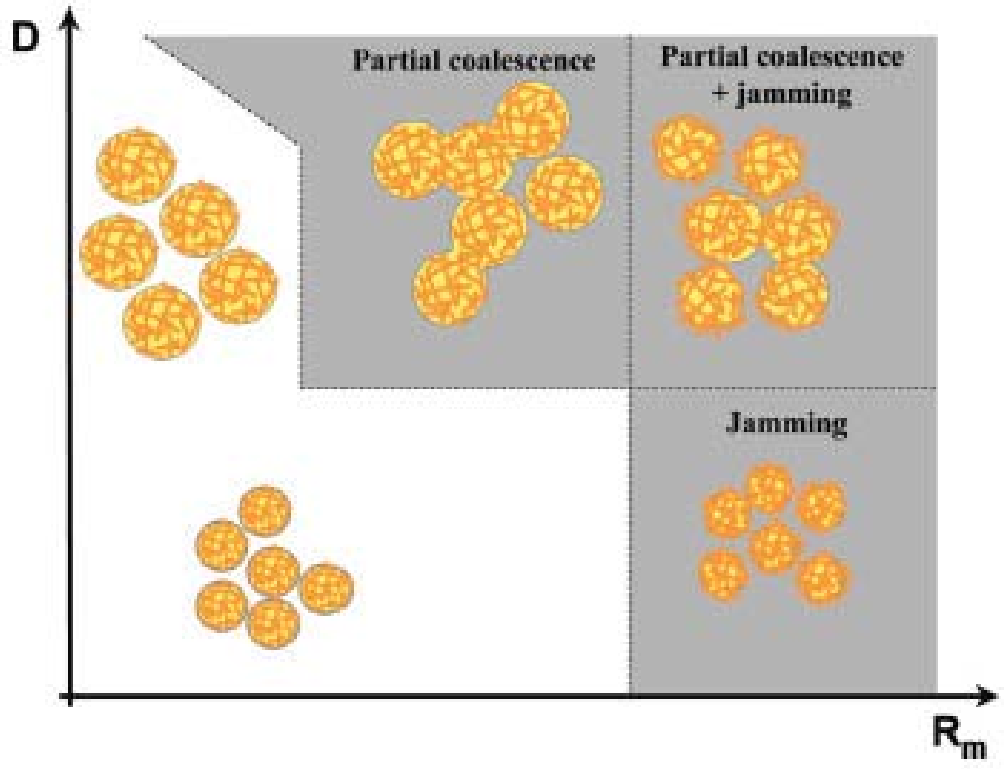


Fig. 2.11. Diagram of the effect of fat globule size (D) and surfactant-to-protein molar ratio (R_m) on the aggregation state of anhydrous milk fat emulsions induced by temperature cycling. The shaded area indicates a strong gel formed. Reprinted with permission from {Thivilliers, F., Laurichesse, E., Saadaoui, H., Leal-Calderon, F., & Schmitt, V. (2008). Thermally induced gelling of oil-in-water emulsions comprising partially crystallized droplets: The impact of interfacial crystals. *Langmuir, 24*(23), 13364-13375}. Copyright {2009}. American Chemical Society.

Previously in this section, the term “jammed” was introduced as a term describing particles which interact to such an extent that a fluid-semisolid phase transition is apparent. For clarification, the above study used the term “jammed” to describe the actual interaction between fat globules in an effort to differentiate it from partial

coalescence. The partially coalesced state likely involved more bonding by liquid oil compared to the “jammed” state.

2.6 Temperature fluctuation-induced partial coalescence

Partial coalescence caused by repeated heating and cooling (temperature cycling) is a common phenomenon in recombined partially crystalline oil-in-water emulsions. Interestingly, partial coalescence of the fat globules commonly occurs without the additional perturbation of shear. Boode, et al. (1991) reported that the mechanism for temperature cycling-induced partial coalescence was similar to that of shear-induced partial coalescence i.e. due to protruding fat crystals rupturing the interface. The authors showed that reheating partially crystalline emulsions increases the susceptibility to partial coalescence. The mechanism is thought to be through an increase in fat crystal size and protrusion distance from the interface after temperature cycling. Upon partial melting, the remaining crystals may migrate to the interface in the reduced solid fat content globule and act as nucleation sites during recrystallization leading to the production of larger crystals. Recently, Thivilliers, et al. (2008) reported that the degree of partial coalescence during temperature cycling depends on the particle size and surfactant-to-protein molar ratio, since these factors regulate the protrusion distance of fat crystals from globules and the contact time between globules upon close approach. The authors noted that partial coalescence is favoured for large particles and high protein displacement. For small particles, jamming may also occur due to interactions between the rough globule surfaces without significant partial coalescence.

As an alternative mechanism for temperature cycling-induced partial coalescence, Roja (2011) reported that partial coalescence may be caused by liquid oil bridging between fat globules that was not facilitated by rupture of the interface by fat crystals. Instead,

the authors directly observed “beads” of liquid oil spontaneously appearing at the fat globule-water interface when the droplets were held at warm temperatures where the fat was primarily molten. Upon re-cooling, the liquid oil droplets at the interface partially crystallized. This indicates the exuded fat contained mid to high melting point fat fractions. This report contradicts the earlier work of Boode & Walstra (1993) who reported that they directly observed fat crystals protruding from fat globules. Interestingly, other reports verifying the protrusion of fat crystals from partially crystalline fat globules using microscopy are virtually non-existent. This may be explained by the choice of sodium dodecyl sulfate (SDS) as a surfactant in these studies and partly by imaging only large globules (several microns or greater in diameter). As Boode & Walstra (1993) noted, SDS may promote migration of fat crystals into the aqueous phase through a “Lanza effect” where the crystals migrate because they are better wetted by the aqueous phase than the oil phase (Section 3.3.2). Recently, Spicer, et al. (2005) showed that by using a similar concept fat crystals could grow or be expelled from oil droplets in a “comet-like” fashion. Notably, the similarities in the optical microscopy images of both studies are rather striking (Fig. 2.7). It is also worth noting that the study of Thivilliers, et al. (2008) discussed in Section 3.2.2., which used AFM to measure the surface roughness of anhydrous milk fat globules, used 5 wt% of Tween 20 as the surfactant. Again it needs to be noted that the high concentration of surfactant may enhance the protrusion of fat crystals from the interface.

2.7 From art to science - interfacial design strategies to control partial coalescence

In the previous sections, partially crystalline emulsions with a broad range of emulsifiers, types of dispersed phases and combinations thereof were discussed in terms of their impact on the susceptibility of fat globules to partial coalescence. In this section,

an attempt is made to distil this research into a coherent strategy for interfacial design of partially crystalline emulsions. To start, the work of Golemanov, et al. (2006) clearly revealed a relationship between fat globule stability and the degree of supercooling caused by differences in emulsifier type and blend. Here, less supercooling led to increased stability. Notably, the authors studied paraffin-in-water emulsions and tetradecane-in-water emulsions where the melting point of the dispersed phase is much narrower compared to a triglyceride. Due to the sharp melting point, the surface roughness is expected to be less than in a triglyceride with a broad range of melting points. Indeed, work by Vanapalli, et al. (2002) which compared the stability of hexadecane emulsions to confectionary fat emulsions supports this conclusion. The authors showed hexadecane emulsions were much more stable to temperature cycling compared to confectionary fat. Since triglycerides in food fats crystallize over a wide temperature range, the time for crystal growth is extended compared to alkanes. Historically, researchers have found increased susceptibility to partial coalescence results from a slow cooling rate presumably due to the reduction in "mixed" fat crystals and the concomitant formation of large crystals with increased purity. Golemanov, et al. (2006) also argued that less supercooling promoted better stability through faster crystallization. In temperature cycling as well, the increased susceptibility is thought to be related to the production of larger crystals grown from the unmelted crystals after recrystallization.

Therefore, it can be assumed that the resistance of fat globules to partial coalescence (ignoring adsorbed protein content and particle size effects) can be improved by promoting rapid crystallization leading to formation of a smoother interface. Methods reported in the literature include using long chain emulsifiers in conjunction with appropriate cosurfactants, (Golemanov, et al., 2006; van Boekel, 1980), interfacial

nucleator addition (Arima, Ueji, Ueno, Ogawa, & Sato, 2007; Awad & Sato, 2002; Sato & Ueno, 2011) and emulsifiers which themselves crystallize at the interface (Krog, et al., 1992). Slowing the polymorphic transition of crystals into unfavourable forms may promote stability as well (Rousseau, 2000).

In summary, while a substantial amount of work has been carried out on the stability of partially crystalline oil-in-water emulsions, the primary factors and mechanism regulating the stability of more complex emulsions is not well described. In this work, an attempt is made to bring this knowledge together to systematically study the stability of a model emulsion with a range of interfacial compositions and solid fat content. Furthermore, the effects of emulsifier chain length, saturation, melting point and HLB will be investigated using Tween and SPAN emulsifiers.

Critical analysis of the literature has enabled a number of key research questions to be defined:

- How does solid fat content affect the stability of partially crystalline oil-in-water emulsions with different interfacial film compositions?
- Is the susceptibility to partial coalescence primarily controlled by the adsorbed protein content?
- Do fat crystals protruding from fat globules promote or initiate partial coalescence?
- Which emulsifier properties, e.g. fatty acid chain length, melting point or degree of saturation, are most important for control of partially crystalline oil-in-water emulsion stability?

- How important are the effects of oil and water soluble emulsifiers on fat crystallization to partially crystalline oil-in-water emulsion stability?
- Can blending high and low melting point emulsifiers increase the stability of partially crystalline oil-in-water emulsions?

Chapter 3: Aggregation behaviour of partially crystalline oil-in-water emulsions: Part I - Characterization under steady shear

Part of the content of this chapter appears in Fuller, et. al., 2015.

3.1 Abstract

Shear-induced aggregation of 35 wt% partially crystalline oil-in-water emulsions ($d_{4,3}$ = ~0.78 μm) was investigated under conditions causing jamming of partially coalesced fat globule aggregates formed under steady shear. Emulsions with different interfacial compositions and solid fat content (~25–90% by volume) were studied to probe their effect on the generation of a jamming transition over a range of shear rates (500–2000 s^{-1}). Shear stable, sodium caseinate stabilized emulsions were rendered shear sensitive by the addition of Tween 20. Partial displacement of adsorbed sodium caseinate by Tween 20 prior to shear generally resulted in the formation of relatively stable aggregates whereas high displacement of protein led to the formation of less stable aggregates under shear. Evaluation of the dependency of aggregation time on shear rate revealed that interfacial composition primarily controlled aggregate formation rather than solid fat content. These findings show that interfacial protein functions both to regulate the formation of partially crystalline aggregates as well as the aggregate stability under shear. Furthermore, the steady shear method utilized here provides excellent utility for the study of a notoriously complex type of shear-induced colloidal aggregation.

3.2 Introduction

Irreversible orthokinetic or shear-induced aggregation of colloidal particles is an active research area in the food, pharmaceutical, cosmetic and petroleum industries because

the functionality of many industrially relevant suspensions are dependent on precise control of shear stability (Coussot, 2007). Aggregate formation and redispersion under shear are commonly studied by monitoring the flow behaviour. Generally, shear-induced aggregation increases the shear viscosity and may promote a fluid-semisolid transition (gelation) if the emulsion becomes effectively concentrated as a result of aggregate growth. Alternatively, aggregate redispersion or compaction reduces the shear viscosity by decreasing the effective volume fraction (Tadros, 2010).

The aggregation mechanism of partially crystalline fat globules in oil-in-water emulsions is particularly complex compared to "hard" or "soft" particles where adsorbed polymers or surfactants, along with the solvent quality, dictate the shear stability (Sonntag, 1993). The stability of partially crystalline fat globules is further complicated by the combination of fat crystals and liquid oil in the dispersed phase. Upon fat crystallization, spherical oil droplets transform into irregularly shaped "globules" due to fat crystal formation and the corresponding density increase. When the interfacial film between fat globules ruptures upon globule collision, liquid oil may flow between the globules and reinforce the connection between them. The crystalline fat provides a physical barrier to full coalescence so the globules form a partially coalesced aggregate (Walstra, 2003). The prevailing viewpoint for many years is that interfacial film rupture is caused by a fat crystal protruding from the globule interface which spans the gap between colliding globules (Walstra, 2003). To summarize, fat crystals are necessary to prevent full coalescence and possibly to initiate partially coalescence whereas liquid oil is necessary to reinforce the connection between globules.

Previous studies have shown that at both low (< 25%) and high (> 60%) solid fat content the rate of partial coalescence is relatively low due to the lack of fat crystals and

lack of liquid oil respectively (Fredrick, Walstra, & Dewettinck, 2010). In terms of interfacial composition, thick protein interfaces have been shown to greatly inhibit partial coalescence by preventing fat globules from approaching one another to a degree where fat crystals can span the gap between them and rupture the interfacial film (Palanuwech & Coupland, 2003). Consequently, low molecular weight surfactants are commonly used by the food industry to displace adsorbed proteins in order to promote partial coalescence when fat globule aggregation is beneficial for functionality. Despite the understanding that solid fat content and interfacial composition both impact partial coalescence, no in-depth study has investigated their combined roles over a range of conditions. Hence, there is only a basic mechanistic understanding of partial coalescence which cannot be directly extended to describe partially crystalline emulsions with complex interfacial film compositions and structures as well as different solid fat contents.

The susceptibility of partially crystalline oil-in-water emulsions to shear-induced aggregation is generally determined using a rheological technique, e.g. steady (Campbell, Norton, & Morley, 1996) or oscillatory shear (Thivilliers-Arvis, Laurichesse, Schmitt, & Leal-Calderon, 2010). In this chapter, the research focused on the application of steady shear to study emulsions designed to undergo a shear-induced jamming transition. A jammed material is one where the mobility of the constituent particles or the dispersed phase is limited to such an extent that solid-like behaviour is exhibited (Liu & Nagel, 1998). Jamming transitions have been studied in a variety of irreversibly aggregating suspensions such as viscous bitumen emulsions (Legrand, et al., 2005), partially crystalline double emulsions (Guery, et al., 2006) and hard styrene-acrylate suspensions (Zaccone, Gentili, Wu, & Morbidelli, 2010). More information on these and related studies is listed in Table 3.1. The wide variability of the dispersed

phases studied and the differences in aggregation mechanism of each highlights the independence of the jamming transition on these factors. Instead, the jamming transition only depends on an increase in the effective volume fraction of the dispersed phase caused by shear-induced aggregation. The aggregate formation kinetics under shear, however, depend on the dispersed phase volume fraction, applied shear stress and interaction potential of the dispersed phase (Guery, et al., 2006; Legrand, et al., 2005; Zaccone, et al., 2010) which makes the jamming process analogous to that of weakly attractive colloidal particles (Trappe, Prasad, Cipelletti, Segre, & Weitz, 2001). This research helps to address the research questions on the role of SFC, adsorbed protein load and their interaction on the susceptibility of partially crystalline emulsions to partial coalescence.

Table 3.1.
Studies on irreversible aggregation of colloidal particles under shear where a jamming transition was induced and utilized for measuring the shear stability.

Dispersed phase composition	Rheological test	Shear rate or stress applied	Geometry	Destabilisation measurement used for assessment of shear stability
Partially crystalline triglyceride	Steady shear	~10 - 8000 s^{-1}	Couette, parallel plate and cone and plate	Critical time - onset of rapid viscosity increase (Lips, Westbury, Hart, Evans, & Campbell, 1993)
Milk fat	Shear rate ramp	≤ 5000 s^{-1}	Couette	Critical shear rate - highest shear rate at which no increase in viscosity was found (Hinrichs & Kessler, 1997)
Groundnut oil + tristearin	Shear stress ramp	0.073─15 Pa	Double gap	Critical destabilizing shear stress (Davies, Dickinson, & Bee, 2000)
Bitumen	Steady shear	50─300 s^{-1}	Couette	Gelation time - time at peak of rapid viscosity increase (Legrand, et al., 2005)
Water-in-crystallized oil (double emulsion)	Steady shear	80─300 s^{-1}	Cone and plate	Induction time - onset of rapid viscosity increase (Guery, et al., 2006)
Styrene-acrylate particles	Steady shear	900─1700 s^{-1}	Couette	Induction time - onset of rapid viscosity increase (Zaccone, Gentili, Wu, & Morbidelli, 2010)
Partially crystalline triglyceride	Steady shear	150 s^{-1}	Starch cell and impeller	Churning time - maximum viscosity reached before churning (Fredrick, et al., 2013)

In the first study of this thesis, the effects of SFC and adsorbed protein load on the shear stability of partially crystalline oil-in-water emulsions were studied. By varying the SFC through manipulation of the fat composition rather than by varying temperature, temperature-dependent changes in the interfacial film composition and structure were avoided. This approach was taken in order to isolate the effect of SFC more clearly. Adsorbed protein load was manipulated by the addition of the low molecular weight emulsifier Tween 20 (0.5 or 1.5 wt%). By creating fat globules coated with either mixed protein-Tween 20 or Tween 20 dominated interfacial films, two compositionally and functionally distinct types of interfacial films could be studied which are representative of food emulsions. In this chapter it will be shown that by manipulating the primary

factors (SFC and interfacial film composition) affecting partial coalescence, the partial coalescence process and jamming transition of partially crystalline oil-in-water emulsions can be altered to generate a variety of aggregation behaviours under shear.

3.3 Materials and methods

3.3.1. Ingredients

Sodium caseinate (92.5 wt% protein, 0.8 wt% fat, 4.66 wt% moisture and 3.4 wt% ash) was provided by Fonterra Co-operative Group Ltd (Auckland, New Zealand). Sodium azide, Tween 20 and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide was purchased from Fisher Scientific (Leicestershire, UK). Hydrogenated palm kernel oil (HPKO) (White Cloud Shortening KL, Goodman Fielder Food Services Ltd, North Ryde, New South Wales, Australia) and refined canola oil (Marsanta Foods, Mt. Wellington, Auckland, NZ) were purchased from a local foodservice supplier. The solid fat content of the HPKO at 5 °C was 90.6 ± 0.3% as measured by low resolution pulsed nuclear magnetic resonance (NMR). Milli-Q water was used in all emulsion formulations.

3.3.2 Emulsion preparation

A stock, protein stabilized oil-in-water emulsion was first prepared for each emulsion. Sodium caseinate (5 wt%) was dispersed by gentle stirring at room temperature overnight. The dispersed phase was comprised of HPKO, canola oil or a combination prepared by mixing the melted fats on a heated stir plate. The sodium caseinate solution, fats and water were separately heated to 65 °C in a water bath before combining along with sodium azide, and then reheated to 65 °C. A coarse emulsion was prepared by high

shear mixing (Ultraturrax T25, Janke & Kunkel GMBH & Co. KG) for 1 min at 13,500 rpm. The emulsions were then reheated to 65 °C in a water bath before homogenization (3 passes) using a pre-heated, two-stage homogenizer (Panda, Niro Soavi, Italy). In order to produce emulsions with similar particle size distributions, the pressure applied to stage 1 was varied from 130–150 bar depending on the % HPKO in the formulation while the pressure on stage 2 was held at 50 bar. All stock emulsions contained 2.4 wt% sodium caseinate, 42 wt% fat and 0.02 wt% sodium azide (as a preservative). Each protein stabilized emulsion was stored overnight at room temperature before further use.

Working emulsions (25 g) were prepared in 50 ml tubes by diluting stock emulsion with Tween 20 (35 wt%) and water. To facilitate mixing, pure Tween 20 was diluted to a 35 wt% solution and adjusted to pH 6.85 with 0.1 M sodium hydroxide before use. The Tween 20 solution was heated to 65 °C and mixed constantly by stir bar, then left to mix overnight at room temperature before use. After combining the stock emulsion and Tween 20 solution at room temperature, the tubes were heated in a 65 °C water bath for 30 min in order to melt any fat crystals that may have formed. The tubes were then transferred to an ice-water bath for 15 min to reduce the temperature to approximately 5 °C. All emulsions were tested after 6 days of storage at 5 °C to allow the SFC and interfacial film composition to reach equilibrium. Each working emulsion contained 2 wt% sodium caseinate and 35 wt% fat with a final pH of 6.85 ± 0.03. All the emulsions studied were prepared and tested at least in duplicate.

3.3.3. Particle size analysis

The particle size distribution (PSD) and volume weighted mean particle diameter ($d_{4,3}$) of emulsions were measured at room temperature by laser diffraction particle size analyser (Malvern Mastersizer 2000, Malvern Instruments Ltd., Malvern,

Worcestershire, UK). Prior to testing, 1 part emulsion was diluted with 4 parts cold water or 1% SDS solution and gently mixed by hand. Since SDS helps to dissociate weakly interacting fat globules but not partially coalesced ones, the types of bonding between fat globules can be inferred by comparing the PSDs in water and SDS. The refractive indices used for water, canola oil and HPKO were 1.330, 1.470 and 1.450 respectively. For emulsions prepared from mixtures of fats, the refractive index was adjusted proportionally. The imaginary refractive index of the dispersed phase was set at 0.001.

3.3.4 Adsorbed protein content

Adsorbed protein content was determined by the depletion method. Following Segall & Goff (1999), centrifugation was performed at 40 °C to reduce the solid fat content in an effort to inhibit partial coalescence during centrifugation. Any partial coalescence during centrifugation would reduce the total fat globule interfacial area leading to erroneous protein surface load measurements.

Emulsions were stored for 24 h at 5 °C before being heated to 40 °C in a water bath and then transferred to a pre-heated (40 °C) centrifuge (Sorvall Evolution RC, Kendro Lab Products, Asheville, NC). Emulsions were spun at 27,750 x *g* for 1 hour to separate the fat globules from the serum phase. The serum phase was collected by syringe and filtered through a 0.2 µm syringe filter. Protein content in the serum phase was determined by micro-BCA analysis (Pierce BCA protein assay, Thermo Fisher Scientific Inc., Rockford, IL) using the standard kit protocol. Total mean interfacial area (S_1) was calculated following Berton, Genot, & Ropers (2011):

$$S_1 = s_{globule} \times \frac{m_{oil}}{V_{globule} \times \rho} \quad (4)$$

where $s_{globule}$ is the surface area (m^2) of one fat globule, m_{oil} is the mass (g) of fat in the same volume of emulsion, $V_{globule}$ is the volume (m^3) of one fat globule and ρ is the density of fat (g m^{-3}). Both $s_{globule}$ and $V_{globule}$ were calculated from the surface weighted mean diameter ($d_{3,2}$). The density of canola oil at 5 °C (0.920 g/cm^3) was determined by extrapolating density measurements from Noureddini, Teoh, & Clements (1992). The density of HPKO at 5 °C (0.987 g/cm^3) was measured using a glass density bottle. The density of fat blends was calculated proportionally based on weight.

Protein surface load (mg protein/m^2) was calculated from the difference between the initial protein concentration in the emulsion and the protein concentration in the serum phase after centrifugation divided by the total mean interfacial area.

3.3.5 Shear stability

Stability under shear was tested over time at a constant shear rate (500–2000 s^{-1}) using a stress-controlled rheometer (MCR 301 Anton Paar GmbH, Germany) in a truncated cone-and-plate geometry (50 mm diameter, 2° angle, 47 µm gap). The plate temperature was maintained at 5 °C by a Peltier plate system. Shear-induced heating of the emulsion was found to be negligible. A solvent trap was used during long testing times to inhibit evaporation of the emulsion and condensation around the rim of the plate and on the cone. The cone and pipettes were pre-chilled before use to help maintain the emulsion temperature at 5 °C.

From the curve of viscosity versus time, the aggregation time of emulsions which destabilised under shear was determined by linearly extrapolating the constant viscosity period and rapid increase following Guery, et al. (2006). The aggregation time was

taken from the crossover point and indicates the onset time of rapid fat globule aggregation which often precedes jamming.

The cone-and-plate geometry was selected so that primarily laminar flow conditions were generated under the shear rates tested here. These conditions were determined by calculating the Reynolds-like number according to Sdougos, Bussolari, & Dewey (1984). However, factors such as truncation of the cone, presence of fat globules i.e. dispersed particles and fat globule aggregate growth or migration during shear have the potential to alter the flow regime. These factors must be considered before making assumptions about the flow regime especially near the jamming transition.

3.4 Results and discussion

3.4.1 Relationship between flow behaviour and aggregation behaviour

The flow behaviour of emulsions with 30, 50, 70 or 100 wt% hydrogenated palm kernel oil (HPKO) and 0.5 or 1.5% Tween 20 were studied under conditions causing a shear-induced jamming transition. In this study, a jamming transition occurs in response to shear-induced aggregation of the fat globules because aggregation leads to an increase in the effective dispersed phase volume fraction due to entrainment of the continuous phase within the aggregates (Tadros, 2010). Once the dispersed phase becomes effectively concentrated, a jamming transition, indicated under steady shear by an abrupt and rapid increase in viscosity, may occur. The rapid increase in viscosity is caused by an increase in hydrodynamic interactions between crowded aggregates (Trappe, et al., 2001). The onset of the jamming transition provides a useful way to measure the relative stability of an aggregating suspension under shear (Guery, et al., 2006; Legrand, et al., 2005; Zaccone, et al., 2010).

Examples of the typical, time-dependent changes in viscosity are shown in Figs. 3.1(A–C). For all three emulsions shown, the viscosity remained relatively constant for a period of time followed by an abrupt increase which is characteristic of a jamming transition. To establish if the flow behaviour was dependent on fat globule aggregation, images of the emulsions were immediately taken after shearing for different periods of time and the corresponding change in particle size was measured after dispersing the emulsion in 1% SDS solution or chilled water. First, the images clearly indicate the sheared emulsions underwent a fluid-semisolid transition which peaked at the jamming transition (Figs. 3.1A–C). The contracted shape of each sheared emulsion on the plate was the result of being drawn towards the centre of the plate as the cone was removed due to the surface tension between the cone and emulsion. The contracted shape indicated a yield stress developed because the emulsion would have otherwise relaxed back to its original shape after the cone was removed. The most extreme example was observed at the jamming transition (image taken at 180 s) in the 70% HPKO, 0.5% Tween 20 emulsion where the sample appeared solid-like (Fig. 3.1A). However, the fluidity was retained after shearing since the emulsion only formed the shape as a result of removal of the cone. In contrast, the final image in Fig. 3.1A shows the sample was pasty rather than fluid. The pasty nature of the sample is indicative of phase inversion due to prolific fat globule aggregation and more than likely significant partial coalescence. Phase inversion also coincided with a sudden decrease in viscosity due to partial expulsion of the sample from the gap.

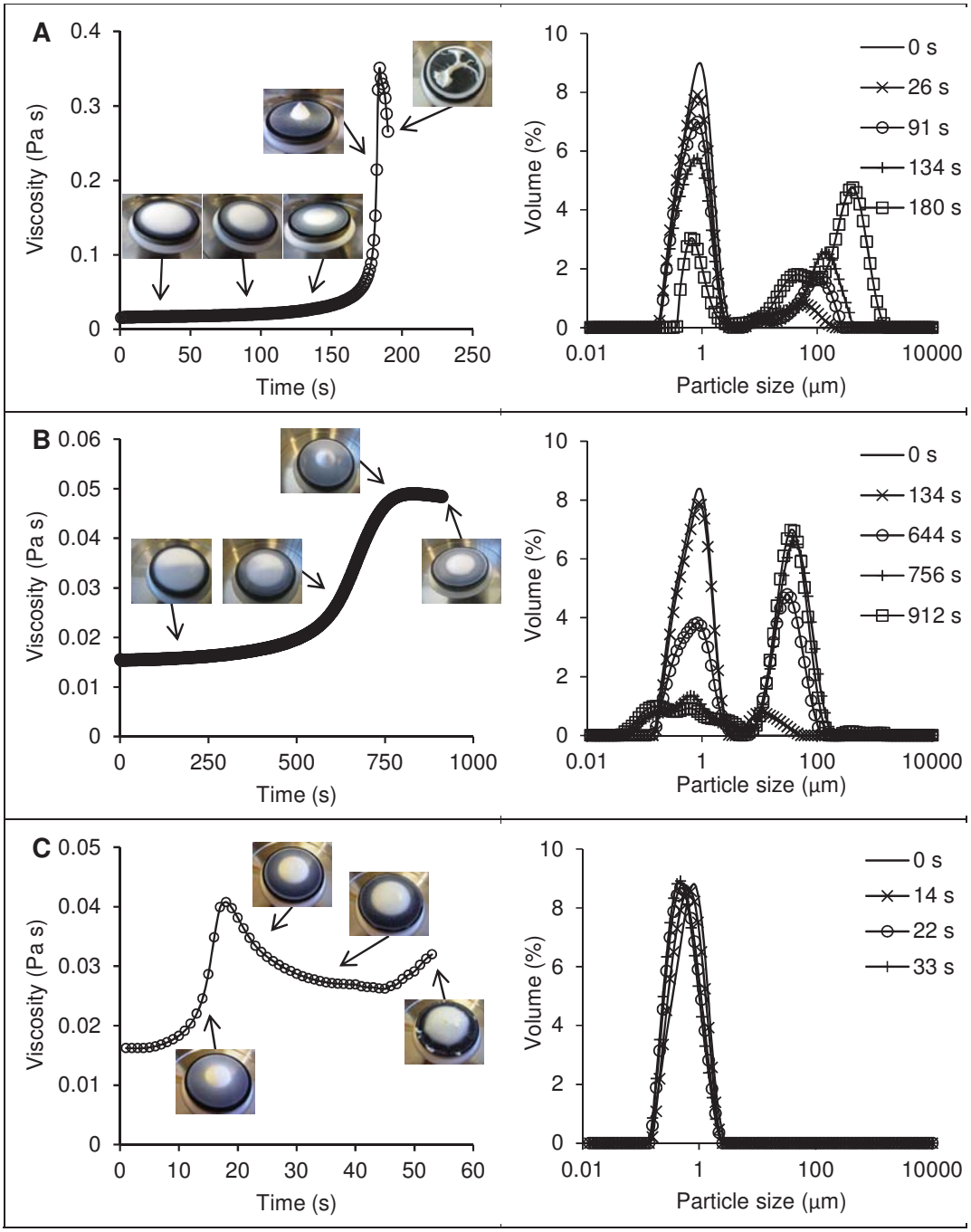


Fig. 3.1. Typical time-dependent change in viscosity of 35 wt% partially crystalline oil-in-water emulsions stabilized by 2 wt% sodium caseinate measured at 5 °C and sheared at 2000 s^{-1}. The dispersed phase contained blends of hydrogenated palm kernel oil (HPKO) and canola oil. Images of the sheared emulsions taken after the cone was removed show the fluid-semisolid transition

which occurred during shear. The particle size distributions of the unsheared and sheared emulsions (same samples shown in images above) were measured after dispersion in 1% SDS solution. Please note the differences in scale of the y-axes. Letters: A) 70% HPKO, 0.5% Tween 20; B) 100% HPKO, 0.5% Tween 20; C) 30% HPKO, 1.5% Tween 20.

Compared to Fig. 3.1A, neither of the other emulsions shown in Figs. 3.1(B) and (C) phase inverted after the jamming transition. Instead, the 100% HPKO, 0.5% Tween 20 emulsion decreased in viscosity after the peak viscosity had been reached and the emulsion was never found to further aggregate even after shearing for several hours (Fig. 3.1B). The 30% HPKO, 1.5% Tween 20 emulsion also decreased in viscosity after the peak viscosity was reached but instead of continuing to decrease, like in Fig. 3.1B, small aggregates were expelled from the gap after a brief period of shearing time whilst the remainder of the emulsion remained fluid (Fig. 3.1C).

Supporting the images described above, the change in particle size measured over shearing time indicated the fluid-semisolid transition was due to fat globule aggregation likely caused by partial coalescence since SDS can dissociate weakly interacting particles (Figs. 3.1(A) and (B)). The initially monomodal particle size distributions became bimodal as a result of shear and the volume of particles greater in size than the largest particle size of the unsheared emulsion, i.e. fat globule aggregates, increased with shearing time at the expense of particles within the size range of the unsheared emulsion. The expected decrease in the % volume of large aggregates following the peak viscosity in Fig. 3.1B, however, was not well captured by laser light scattering when the emulsion was dispersed in 1% SDS. When dispersed only in water though, a decrease in the % volume of large aggregates was found after the peak viscosity.

The particle size distribution during shear was also measured in the emulsion with 30% HPKO, 1.5% Tween 20, however, no large aggregates were detected when dispersed in either SDS or water (Fig. 3.1C). Instead, the mean particle size decreased with increasing shearing time. This unexpected result was also reported by Walstra (2003) on studies of aggregated partially crystalline oil-in-water emulsions heated above the melting point of the fat. Presumably, the aggregates formed in low solid fat content emulsions underwent full coalescence upon measurement of the particle size due to partial melting of the crystalline fat at the measurement temperature for laser light scattering (room temperature). Large oil droplets may cream rapidly and thus not be detectable by laser light scattering. Indeed, the inability to measure large aggregates was only found in emulsions with 30 or 50% HPKO.

3.4.2 Influence of interfacial composition and solid fat content on aggregation behaviour

3.4.2.1 Displacement of adsorbed protein by Tween 20

Shear stable, sodium caseinate stabilized emulsions were destabilized by the addition of Tween 20 which displaced sodium caseinate from the fat globule-water interface (Fig. 3.2). The adsorbed protein concentration at 0% Tween 20 showed a slight dependence on HPKO concentration. The addition of 0.5% Tween 20 decreased the adsorbed protein concentration by approximately 50% and a further increase to 1.5% Tween 20 decreased it roughly another 50%. Above 1.5% Tween 20, no further displacement of protein was found (data not shown). The adsorbed protein loads are in good agreement with Euston, Singh, Munro, & Dalgleish (1995) who studied the displacement of sodium caseinate by Tween 60 at the soy oil-water interface.

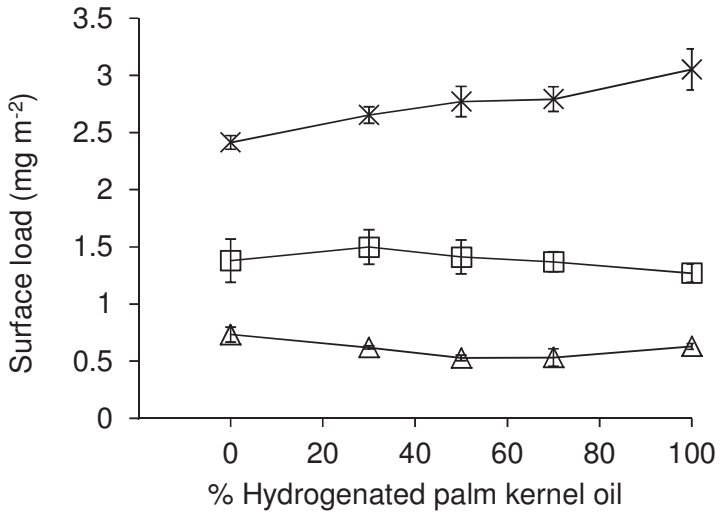


Fig. 3.2. Effect of Tween 20 on the concentration of protein adsorbed at the fat globule-water interface of emulsions with different % HPKO in the dispersed phase. Symbols: (x) 0% Tween 20; (□) 0.5% Tween 20; (Δ) 1.5% Tween 20.

Although standard practice, measurements of adsorbed protein loads in mixed protein-emulsifier emulsions are somewhat misleading since the interfacial film is not homogeneous (Mackie & Wilde, 2005). Woodward, Gunning, Mackie, Wilde, & Morris (2009) found Tween 20 displaced sodium caseinate following a generic, orogenic mechanism whereby displacement results in the formation of an inhomogeneous interfacial film consisting of Tween 20-rich domains or patches distributed within a continuous protein matrix. Once a critical surface pressure was reached by increasing Tween 20 concentration, the mixed interface became Tween 20 dominated and the protein film was predominately, but not completely displaced.

When emulsifier-rich domains or "hotspots" make contact during fat globule collisions, partial coalescence is reportedly more likely to occur (Walstra, 2003). Due to the formation of "hotspots", the adsorbed protein loads reported here should be interpreted

more as an indicator of the susceptibility to partial coalescence rather than a measure of adsorbed protein load. In this study, the Tween 20-rich domains may be more prone to partial coalescence due to the thin nature of the emulsifier film (< 2 nm) (Graca, Bongaerts, Stokes, & Granick, 2007) compared to a sodium caseinate film alone (~10 nm) (Hunt & Dalgleish, 1996).

3.4.2.2 Aggregation behaviour

The changes in flow behaviour in response to different shear rates (500–2000 s^{-1}), Tween 20 concentration and solid fat content are shown in Fig. 3.3. Emulsions with 30, 50, 70 or 100% HPKO and 0.5 or 1.5% Tween 20 were studied. The flow behaviour of each emulsion is shown with the exception of 70 and 100% HPKO prepared with 1.5% Tween 20. These emulsions aggregated immediately when sheared which indicates they were highly susceptible to partial coalescence.

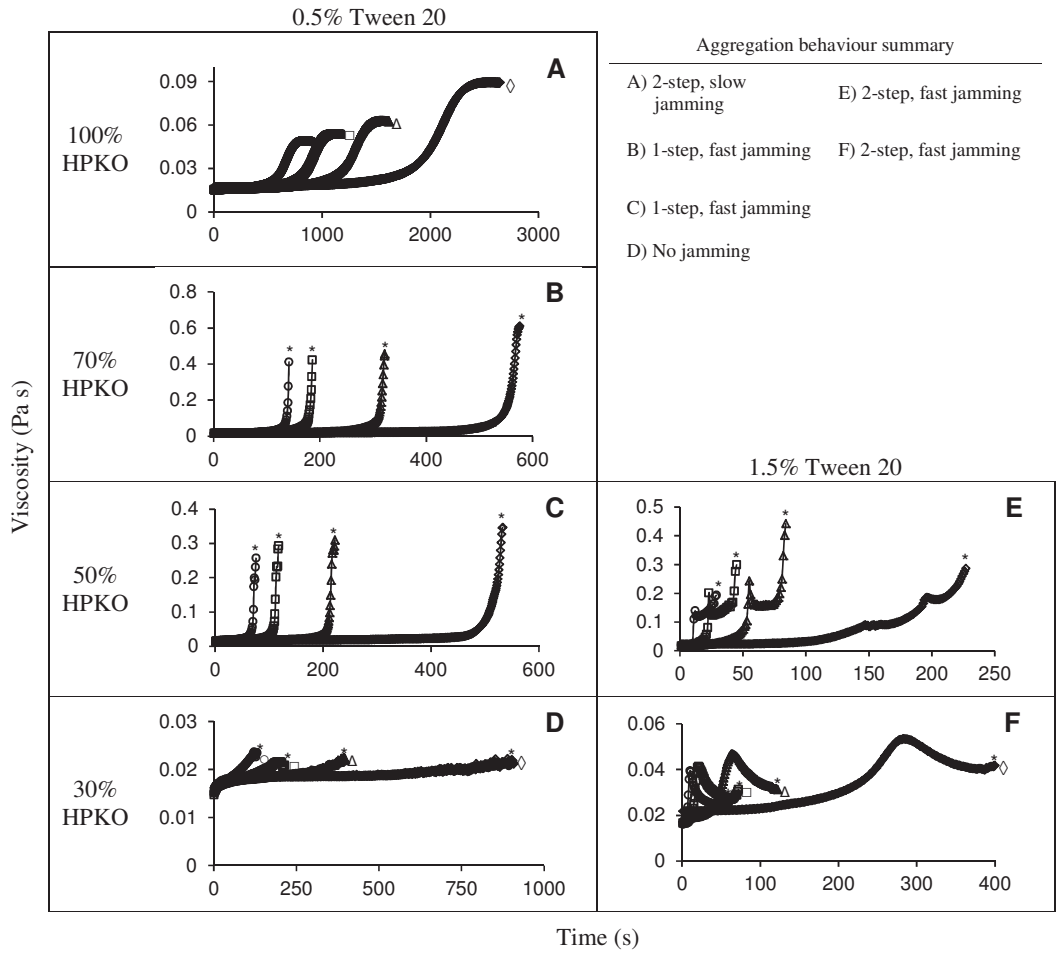


Fig. 3.3. Typical time-dependent change in emulsion viscosity at different shear rates. The measurement temperature was 5 °C. Symbols: (◊) 500 s^{-1}; (Δ) 1000 s^{-1}; (□) 1500 s^{-1}; (○) 2000 s^{-1}. Symbols were inserted at the end of some of the curves for clarity. An asterisk (*) indicates the time at which the emulsion was expelled from the cone and plate gap. Please note the differences in scale of the y-axes. Letters: A) 100% HPKO, 0.5% Tween 20; B) 70% HPKO, 0.5% Tween 20; C) 50% HPKO, 0.5% Tween 20; D) 30% HPKO, 0.5% Tween 20; E) 50% HPKO, 1.5% Tween 20; F) 30% HPKO, 1.5% Tween 20.

Aside from the emulsion with 30% HPKO and 0.5% Tween 20 (Fig. 3.3D), all of the emulsions displayed a characteristic jamming transition (Fig. 3.3). With increasing

shear rate, the onset of the jamming transition occurred at shorter times in all emulsions but the shape of the flow curve over time was generally not altered by shear rate. Aggregation is expected to occur more rapidly with increasing shear rate due to an increase in the collision frequency between fat globules and an increase in shear stress which promotes further fusion of partially coalesced aggregates (Walstra, 2003).

At 0.5% Tween 20, the aggregation behaviour varied broadly with solid fat content. The highest solid fat content emulsion (100% HPKO) displayed a jamming transition at each shear rate and the flow curve displayed a 2-step process of aggregation (Fig. 3.3A). The term 2-step is used to describe the increase in viscosity due to jamming (step 1) followed by a time delay (step 2) prior to either reaching a steady state viscosity or observing macroscopic breakdown of the emulsion. The viscosity in Fig. 3.3A slightly decreased continuously after jamming and was never observed to increase again when sheared for several hours. Additionally, the emulsion never formed a paste nor was any large aggregates expelled from the gap. For 70 or 50% HPKO, aggregation occurred via a 1-step process (Figs. 3.3B and C). The term 1-step is used to describe the continuous increase in viscosity, due to jamming, leading directly to expulsion of the sample from the gap (indicated by the asterisk) due to paste formation/phase inversion caused by prolific fat globule aggregation and partial coalescence. For 30% HPKO, no jamming transition was observed (Fig. 3.3D). The viscosity increased roughly linearly until small aggregates were expelled from the gap whilst the remainder of the emulsion retained its fluidity.

At 1.5% Tween 20, aggregation occurred via a 2-step process at 50 and 30% HPKO (Figs. 3.3E and F). After the jamming transition (step 1), a decrease in viscosity occurred in both emulsions prior to the expulsion of the sample from the gap. Interestingly, the expulsion of each sample was similar to the corresponding emulsion

with 0.5% Tween 20, i.e. the 50% HPKO emulsion was expelled due to phase inversion and the 30% HPKO emulsion due to formation of a few large aggregates.

The aggregation behaviour described above was dependent on both Tween 20 concentration and solid fat content. Comparing emulsions with 100 or 70% HPKO and 0.5 or 1.5% Tween 20, the increase in Tween 20 concentration to 1.5% corresponded with a transition from quiescently stable to quiescently unstable where no shear testing could be performed. At 50 and 30% HPKO, the increase to 1.5% Tween 20 corresponded with a transition from a 1-step aggregation process (50% HPKO) or no jamming (30% HPKO) to a 2-step aggregation process in both emulsions. This result implies that high protein displacement caused by 1.5% Tween 20 (Fig. 3.2) significantly altered the aggregation process at each solid fat content.

In order to explain the cause of the change in aggregation behaviour upon increasing Tween 20 concentration from 0.5 to 1.5%, emulsions with 30, 50, 70 and 100% HPKO emulsions stabilized by only Tween 20 (1–5 wt%), otherwise equivalent in size to those studied with 2% sodium caseinate, were prepared and sheared. Again though, the emulsions prepared with 70 and 100% HPKO were quiescently unstable after chilling so no shearing could be performed. However for 30 and 50% HPKO emulsions which could be sheared, their aggregation behaviour followed a 2-step process at each Tween concentration very similar to those in Figs. 3.3E and F. For example, a 30% HPKO emulsion stabilized by 5% Tween 20 displayed a jamming transition followed by a decrease in viscosity and ultimately expulsion from the gap qualitatively the same as the emulsion stabilized by 2% sodium caseinate and 1.5% Tween 20 (Fig. 3.4).

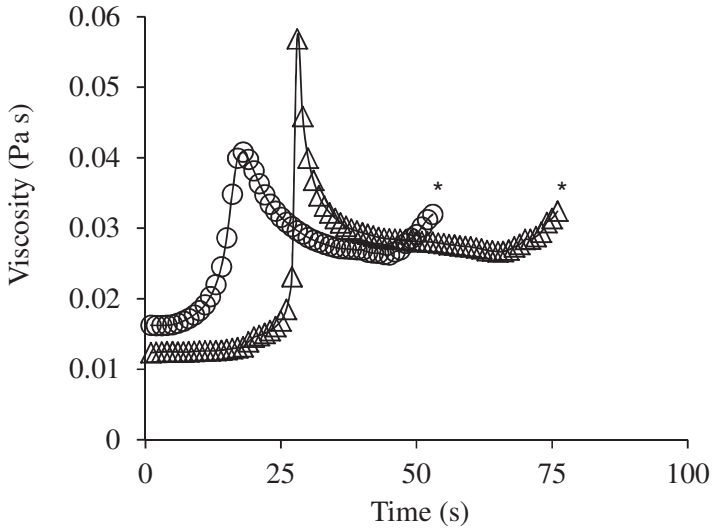


Fig. 3.4. Time-dependent change in viscosity of 30% HPKO emulsions under steady shear (2000 s^{-1}) at 5 °C showing 2-step aggregation behaviour. Emulsions were prepared with and without sodium caseinate. Symbols: (○) 2% sodium caseinate and 1.5% Tween 20; (Δ) 5% Tween 20. An asterisk (*) indicates the time at which the emulsion was expelled from the cone and plate gap.

These findings support an interpretation that the transition from a mixed sodium caseinate–Tween 20 interfacial film (0.5% Tween 20) to a Tween 20 dominated film (1.5% Tween 20) (Fig. 3.2) causes the aggregation behaviour to change from quiescently stable to unstable at 70 and 100% HPKO and from 1-step to 2-step at 30 and 50% HPKO. For the latter case, the change to Tween 20 dominated likely led to formation of weaker aggregates which were not stable under the increased shear stress caused by jamming. In general, aggregates breakdown and/or compact when the external stress exceeds the strength of the internal bonds between the particles (Jarvis, Jefferson, Gregory, & Parsons, 2005). Breakdown occurs through either surface erosion or large scale fragmentation where the type of breakdown is dependent on the location of the weakest bond within the aggregate (Jarvis, et al., 2005). Generally, dense and/or

submicron sized aggregates breakdown through surface erosion due to the weaker connections on the periphery of the aggregate compared to the centre. Larger and less dense aggregates tend to breakdown via fragmentation due to the tensile stress on the aggregate (Jarvis, et al., 2005). Compaction of the aggregates under shear is expected to impact the aggregation behaviour by inhibiting jamming through a decrease in effective volume fraction (Tadros, 2010). In the 100% HPKO emulsion with 0.5% Tween 20, the slow decrease in viscosity after the jamming transition and the detection of small particle sizes not found in the unsheared emulsion (Fig. 3.1B) indicates surface erosion may have occurred. At 1.5% Tween 20 with either 50 or 30% HPKO, the decrease in viscosity after the jamming transition was much more rapid compared to 100% HPKO with 0.5% Tween 20 (Fig. 3.3) and was associated with a macroscopic decrease in thickness presumably caused by a decrease in effective volume fraction (Fig. 3.1C). In the author's opinion, fragmentation and/or compaction are both possible mechanisms for this phenomenon.

Once fat globule aggregates form at 0.5% Tween 20, the sodium caseinate may provide structural support within each growing aggregate leading to 1-step aggregation. On the other hand, at 1.5% Tween 20 the reduction in sodium caseinate may result in a weaker internal structure leading to aggregate breakdown or compaction once a critical shear stress is reached during the jamming transition. Although interfacial layers of sodium caseinate are relatively weak, loosely packed and low in protein-protein interactions compared to β-lactoglobulin for example (Dickinson, 2001), Bressy, Hebraud, Schmitt, & Bibette (2003) showed sodium caseinate films displayed exceptional solid-like character in concentrated emulsions. Thus, the decrease in aggregate stability during jamming at 1.5% Tween 20 was likely caused by the reduction in adsorbed sodium caseinate. Furthermore, compaction should occur more readily with decreasing solid fat

content due to increased liquid oil content. This may explain the lack of a jamming transition at 30% HPKO and 0.5% Tween 20 despite obvious aggregation (Fig. 3.3D).

In this study, it was shown how changes in interfacial composition and solid fat content alter the path (1-step or 2-step) to either phase inversion or aggregate expulsion. These findings are important in terms of understanding the mechanism of partial coalescence. Since liquid oil serves as the means to secure the connections between fat globules, aggregate destabilisation during a 2-step aggregation process is expected to lead to a dramatic change in the aggregation mechanism since aggregate redispersion may result in the release of "free oil" which may promote reconsolidation of the aggregates (Evers, 2004).

Lastly, it is noted that the solid fat content has a strong effect on the flow and aggregation behaviour at both concentrations of Tween 20. The effect of solid fat content will be discussed in more detail in Chapter 4.

3.4.2.3 Dependency of aggregation time on shear rate

To gain further insight into the respective roles of interfacial composition and solid fat content on the aggregation behaviour, the dependency of aggregation time on shear rate was studied. Aggregation time was determined by linearly extrapolating the constant viscosity period and rapid increase following Guery, et al. (2006). The aggregation time measured in this study depends on the hydrodynamic doublet formation rate k (Potanin, 1991). The dependency of k on shear rate $\dot{\gamma}$ is,

$$k \cong \dot{\gamma}^{1-\varepsilon} \tag{5}$$

where ε is a positive coefficient whose value depends on the transport mechanism. The effect of shear rate $\dot{\gamma}$ on a rheologically determined aggregation time may be determined

by taking k to be proportional to the inverse of aggregation time t_a (Guery, et al., 2006; Legrand, et al., 2005). If, $k \propto {}^{1}/_{t_a}$ then it follows,

$$t_a \cong \dot{\gamma}^{-(1-\varepsilon)} \tag{6}$$

Exponents between −1 and −0.75 are reported when convection is the dominant transport mechanism and when repulsive forces are not rate limiting (Potanin, 1991). In this study, exponents near −1 were found for 0.5% Tween 20 emulsions whereas at 1.5% Tween 20 the exponents were much lower (Fig. 3.5). It is useful to compare these results with Guery, et al. (2006) who studied a water-in-oil-in-water double emulsion with a partially crystalline oil phase stabilized by sorbitan monooleate. Their emulsions showed similar behaviour to the emulsions studied here in that they were quiescently stable but rapidly aggregated under shear. Similar to the emulsions studied here with 1.5% Tween 20, the authors found unexpectedly low exponent values ranging from −3.5 to −1.5 and proposed they were the result of the presence of an energy barrier which was overcome by shear. Presumably, the hydrodynamic forces from shear reduced the energy barrier leading to a more exponential dependence of aggregation time on shear rate.

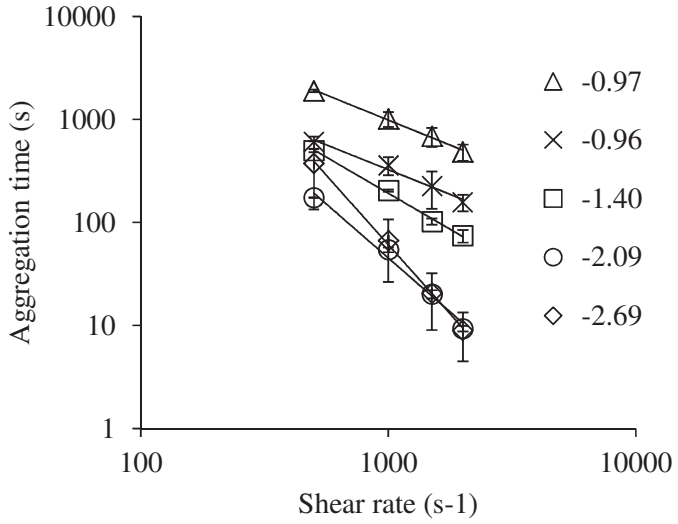


Fig. 3.5. Change in aggregation time with shear rate at 5 °C. Symbols: (Δ) 100% HPKO, 0.5% Tween 20; (x) 70% HPKO 0.5% Tween 20; (□) 50% HPKO 0.5% Tween 20; (○) 50% HPKO 1.5% Tween 20; (◊) 30% HPKO 1.5% Tween 20. Solid lines are power law fits. Exponents from power law fits are listed in the legend. The more lengthy error bars in the bottommost overlapping data sets are for the 30% HPKO 1.5% Tween 20 (◊) sample.

In this study, indeed the repulsive force was likely larger in emulsions with 0.5% Tween 20 compared to 1.5% Tween 20 due to the greater concentration of adsorbed protein (Fig. 3.2). The reduced energy barrier at 1.5% Tween 20 was likely overwhelmed by the hydrodynamic forces generated within the shear rate range studied here and thus contributed to the stronger shear rate dependency as indicated by the more negative exponents in Fig. 3.5. In addition though, the collision efficiency of fat globules is reported to increase with an increase in emulsifier-rich domains or "hotspots" (Walstra, 2003). At 1.5% Tween 20, where the interfacial composition was Tween 20 dominated, the surface area covered by Tween 20 would have been greater compared to emulsions

with 0.5% Tween 20. Therefore, the collision efficiency at a given shear rate is expected to be greater, regardless of the repulsive force, at 1.5% Tween 20 compared to 0.5% Tween 20. Thus, the difference in interfacial surface area covered by Tween 20 must be considered as a mechanism to explain the stronger dependency of aggregation time on shear rate in this study.

The results in Fig. 3.5 also show a lack of influence of solid fat content on the shear rate dependency. This indicates the interfacial film was the primary factor regulating the shear rate dependency. Lips, Westbury, Hart, Evans, & Campbell (1993) studied the shear-induced aggregation of 35 wt% fat emulsions stabilized by buttermilk protein and Tween 60 using a variety of geometries. Supporting the findings of this research, the authors concluded there was no direct kinetic effect of solid fat content or fat crystals on aggregation. Instead, the authors proposed that fat crystals only ensure and mediate aggregation through partial coalescence.

The results described above ultimately portray partially crystalline oil-in-water emulsion aggregation through partial coalescence to be primarily controlled by the interfacial film composition and less by the solid fat content or fat crystals. However, studies show that the protrusion of fat crystals from the oil-water interface may play a more dominant role in stability if fat crystal protrusion is enhanced due to temperature cycling, (Boode, Bisperink, & Walstra, 1991) high aqueous phase emulsifier concentration (Boode & Walstra, 1993; Thivilliers-Arvis, et al., 2010) or fat crystal morphological alteration (Arima, Ueji, Ueno, Ogawa, & Sato, 2007). Further investigation is certainly warranted though, since recent research proposed that fat crystallization may play an indirect role in facilitating the transfer of liquid oil between fat globules during temperature cycling (Ergun, 2011).

3.5 Conclusion

In this study, it was shown that the flow and aggregation behaviour of partially crystalline oil-in-water emulsions ($d_{4,3}$ = ~0.78 μm) under steady shear can be well characterized when a jamming transition is induced. By varying interfacial composition and solid fat content, a variety of aggregation behaviours were described. The extent of displacement of sodium caseinate by Tween 20 from the fat globule-water interface caused considerable changes in the stability of partially coalesced aggregates. Moderate protein displacement led to the formation of relatively stable aggregates whereas high displacement led to the formation of more unstable aggregates under shear. In addition, interfacial film composition was found to regulate the dependency of aggregation time on shear rate while the solid fat content played a minor role. These findings help bring clarity to the respective roles of adsorbed proteins and low molecular weight emulsifiers along with solid fat content on partial coalescence in a model system designed to minimize the effects of secondary factors commonly found in "real" food emulsions. Further assessment of the roles of interfacial composition and solid fat content will be addressed in Chapter 4.

Chapter 4: Aggregation behaviour of partially crystalline oil-in-water emulsions: Part II - Effect of solid fat content and interfacial film composition on quiescent and shear stability

Part of the content of this chapter appears in Fuller, Considine, Golding, Matia-Merino, & MacGibbon, 2015.

4.1 Abstract

Continuing on from Chapter 3, the role of solid fat content and interfacial film composition on the aggregation behaviour of a model food emulsion (35 wt% fat, 2 wt% sodium caseinate, $d_{4,3}$ = ~0.78 µm) was investigated. By displacing adsorbed sodium caseinate from the oil-water interface using Tween 20, partial coalescence sensitive emulsions with interfacial compositions of mixed sodium caseinate-Tween 20 (0.5 wt% Tween 20) and Tween 20 dominated (≥ 1.5 wt% Tween 20) were prepared. Stability was monitored quiescently and under shear over 6 days of storage at 5 °C. Quiescently, the emulsions were stable with 0.5 wt% Tween 20 regardless of solid fat content. At 1.5 wt% or above, stability decreased with increasing solid fat content and Tween concentration. Under steady shear, the aggregation time of emulsions with Tween 20 dominated interfaces decreased with increasing solid fat content whereas for mixed sodium caseinate-Tween 20 emulsions it increased with increasing solid fat content. The extent of fat globule aggregation at low solid fat content was relatively low irrespective of Tween concentration, whereas at high solid fat content, the extent of aggregation varied considerably when interfacial composition was altered. Cryo-TEM micrographs of the fat globules revealed a relatively smooth surface at low SFC but increasingly rough surface containing protuberances with increasing SFC. Notably, the

protuberances were not jagged but rather round indicating that protruding fat crystals may not initiate partial coalescence especially in Tween 20 dominated emulsions. These findings have important implications on how the partial coalescence mechanism changes with solid fat content and interfacial film composition.

4.2 Introduction

Partial coalescence of fat globules in partially crystalline oil-in-water emulsions has been the subject of on-going research since a mechanism was first proposed (Boode, Bisperink, & Walstra, 1991; Boode & Walstra, 1993; Boode, Walstra, & Degrootmostert, 1993; van Boekel & Walstra, 1981). Many factors such as solid fat content, interfacial composition, interfacial film thickness, globule size and viscosity have been identified as affecting the susceptibility of fat globules to partial coalescence (Fredrick, Walstra, & Dewettinck, 2010). These findings have led to an improved understanding of fat globule aggregation in food products such as cream (Hinrichs & Kessler, 1997), ice cream (Goff, Verespej, & Smith, 1999) and whipped toppings (Goff, 1997). More recently, researchers have used emulsions sensitive to partial coalescence as tools to study lipid release during digestion (Golding, et al., 2011) and in-mouth texture perception (Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009; Dresselhuis, de Hoog, Stuart, Vingerhoeds, & van Aken, 2008).

During partial coalescence, rupture of the fat globule interfacial film by a protruding fat crystal is reported to depend on several factors such as size, shape and protrusion distance of the fat crystals as well as the properties of the interfacial film (Fredrick, et al., 2010; Walstra, 2003). However, there is little direct evidence, e.g. micrographs, confirming that fat crystal protrusion plays a direct role in film rupture and partial coalescence (Fredrick, et al., 2013). The available evidence is even contradictory; for

example while trying to confirm the role of fat crystal protrusion in partial coalescence using light microscopy, Boode & Walstra (1993) reported fat crystals protruded from an interface and caused partial coalescence whereas Ergun (2011) reported no crystal protrusion. Instead, molten fat pooled at the oil-water interface during temperature cycling resulting in a potential site for globule-globule association.

Although there is some discrepancy around the role of protruding fat crystals in partial coalescence, the role of solid fat content (SFC) is better described. Studies on the effect of SFC on partial coalescence generally report a maximum rate of partial coalescence around 25–35% SFC (Fredrick, et al., 2010; McClements, 2007; Walstra, 2003). Others report a less conservative range of 10–50% (Davies, Dickinson, & Bee, 2000). At SFC values below and above these values, the partial coalescence rate decreases due to a lack of fat crystals or a lack of liquid oil respectively. As Fredrick, et al. (2010) noted however, the susceptibility to partial coalescence is dependent on many factors in addition to SFC thus deviations from this general rule should be expected.

Partial coalescence can be effectively prevented by adsorbing thick layers of proteins to fat globules (Goff, 1997; Pelan, Watts, Campbell, & Lips, 1997; Segall & Goff, 1999). A thick protein layer provides significant steric stabilization which prevents the close contact between globules necessary for partial coalescence. To induce partial coalescence, low molecular weight emulsifiers are generally added to displace adsorbed proteins from the interface. Mechanistically, protein displacement may increase the occurrence of partial coalescence due to reduced film thickness (Palanuwech & Coupland, 2003), reduced interfacial film elasticity (Dickinson, Owusu, & Williams, 1993) or the formation of emulsifier-rich domains aka “hotspots” within the protein film (Dalgleish, 2006; Walstra, 2003). The latter cause is the result of emulsifiers adsorbing

non-uniformly within the adsorbed protein film (Mackie, Gunning, Wilde, & Morris, 2000).

The fourth chapter of this thesis is an extension of Chapter 3. Having established that the induction of a jamming transition is an excellent way to study the shear-induced aggregation of partially crystalline oil-in-water emulsions, the model emulsion system from Chapter 3 was further studied to better understand the role of SFC in emulsions with different interfacial film compositions of protein and emulsifier. The films studied contained protein only, mixed protein-emulsifier or were emulsifier dominated. Sodium caseinate and Tween 20 were selected for this purpose given the extensive understanding of their effects on partial coalescence (Davies, et al., 2000; Davies, Dickinson, & Bee, 2001; Pelan, et al., 1997; Thanasukarn, Pongsawatmanit, & McClements, 2006) and the displacement mechanism of adsorbed sodium caseinate by Tween 20 (Woodward, Gunning, Mackie, Wilde, & Morris, 2009). To determine the susceptibility of the emulsions to partial coalescence, emulsion stability was examined under both quiescent and shear conditions. Quiescent stability is useful for identifying emulsions which undergo partial coalescence on a timescale of minutes to days, whereas shear stability is useful for accelerating the partial coalescence process in relatively more stable emulsions. Quantitative data on the aggregation time and shear viscosity can also be obtained under shear. The combination of quiescent and shear stability studies allows for the study of a greater range of concentrations of Tween 20 (0.5–3.5 wt%) than possible in Chapter 3.

4.3 Materials and methods

4.3.1 Ingredients

The ingredients used in this chapter were previously described in Section 3.3.1.

4.3.2 Emulsion preparation

The emulsions were prepared in the same manner described in Section 3.3.2.

4.3.3 Particle size analysis

The particle size testing method was previously described in Section 3.3.3.

4.3.4 Solid fat content

The solid fat content (SFC) of bulk fat and emulsions was measured by low resolution pulsed NMR using The Minispec MQ20 (Bruker Optics, GmbH, Rheinstetten, Germany). SFC was determined by the standard direct method using a 90° pulse, 2 s recycle delay time and the standard algorithm supplied by Bruker. Blends of hydrogenated palm kernel oil (HPKO) and canola oil were prepared by gently mixing melted oils at 65 °C. NMR tubes were filled to a height of 35 mm and held at 65 °C for 30 min. Tubes were then stored isothermally at 5 °C for 24 h before analysis. The NMR measurement chamber was cooled to 5 °C to maintain isothermal conditions.

4.3.5 Adsorbed protein content

The adsorbed protein load was measured following the procedure described in Section 3.3.4.

4.3.6 Transmission Electron Microscopy

Both conventional and Cryogenic Transmission Electron Microscopy (Cryo-TEM) were performed in this study. Conventional Transmission Electron Microscopy (TEM) was performed on samples of emulsions embedded in resin. First, the emulsions were fixed after pipetting them into agarose tubes sealed on each end with additional agarose (Allan-Wojtas & Kalab, 1984). The agarose embedded samples were placed in a bijoux bottle containing 3% glutaraldehyde in 0.2 M sodium cacodylate buffer and kept at 5 ^{o}C for 24 h. The agarose embedded samples were rinsed twice with 0.2 M sodium cacodylate buffer rinses over 2 h. The agarose embedded samples were then placed in 1% osmium tetroxide overnight at 5 °C and rinsed twice with buffer for 30 min each the day after. Care was taken throughout the fixation process to keep the sample cold. The dehydration process was carried out at 5 ^{o}C in 25% acetone (15 min) then in 50%, 70% and 90% acetone (30 min each) followed by 100% acetone (3 changes over 90 min). The acetone was then replaced with Procure 812 embedding resin by end-over-end mixing over 36 h. An agarose embedded sample was placed into an embedding capsule filled with resin and cured at 60 ^{o}C for 48 h. The embedded samples were sectioned (90 nm) using the Leica Ultracut microtome. These sections were mounted on 3 mm copper grids and stained with uranyl acetate and lead citrate before examination in a Philips transmission electron microscope at an accelerating voltage of 60 kV.

For Cryo-TEM preparation, samples diluted 1 in 50 with deionized water were applied to freshly glow-discharged holey carbon grids (R1.2/1.3, Quantifoil, Jena, Germany) and vitrified in liquid ethane using an automatic vitrifier (Vitrobot Mk IV, FEI, Eindhoven, The Netherlands). Fat globules were imaged under low dose conditions (10-20 $e^{-}/nm^{2}s$) in a Tecnai 12 TEM (FEI, Eindhoven, The Netherlands) operating at 120

kV. Images were recorded at 2.5 μm defocus using an Ultrascan 1000 digital camera (Gatan, Pleasanton, CA, USA). Prior to vitrification, all procedures were carried out at 4 °C. All Cryo-TEM microscopy was performed at The University of Auckland by Dr. Adrian Turner.

4.3.7 Quiescent stability

After the emulsions were placed in cold storage, quiescent stability was monitored visually over time by tilting the tubes to observe changes in flow. This technique was adopted to determine the broad qualitative differences between formulations during storage since the stability of the emulsions ranged from stable, meaning no visible change in fluidity during storage, to completely unstable meaning the emulsion solidified.

It is important to also note that this is not a true measure of quiescent stability since the emulsions were subjected to shear, albeit low, when the tubes were tilted during testing. Some of the emulsions studied here could maintain fluidity for long periods of time (on the order of days) but immediately gelled or solidified when poured or pipetted. Therefore, the action of tilting the most unstable formulations likely initiated the aggregation process by providing the hydrodynamic energy necessary to bring the globules into close contact.

4.3.7.1 Oiling off

After quiescent testing was completed, each emulsion was transferred to a 65 °C water bath for 24 h. Heating the emulsions above the melting point of the fat allows any partially coalesced fat globules to fully coalesce. If partial coalescence was extensive enough, the large oil droplets creamed to the top of the emulsion and formed a visible

oil layer. The extent of oiling off was determined by measuring the volume of the oil layer. Oiling off (%) was calculated by dividing the volume of the oil layer by the total volume of emulsion multiplied by 100.

4.3.8 Stability under shear

Emulsion stability under shear was measured following the method described in Section 3.3.5 with modifications. In part of this study, the emulsion aggregation time was measured over time at a constant shear rate (2000 s^{-1}) after the emulsions were placed in cold storage at 5 °C. In another part of this study, the peak shear stress reached during the jamming transition was also measured for each emulsion at different shear rates (500–2000 s^{-1}) after 6 days of storage at 5 °C.

4.4 Results and discussion

4.4.1 Particle size distribution

Sodium caseinate-stabilized stock emulsions with different HPKO concentrations were prepared with very similar particle size distributions in order to minimize the effects imparted by differences in particle size on the susceptibility of fat globules to partial coalescence. The volume weighted diameters ($d_{4,3}$) and a representative particle size distribution are shown in Fig. 4.1. For clarity, only one particle size distribution is shown since they all overlap. The particle size distribution of each stock emulsion was monomodal and ranged from approximately 0.2–3.0 μm. Regardless of solid fat content, the stock emulsions showed no signs of partial coalescence or aggregation during storage at room temperature.

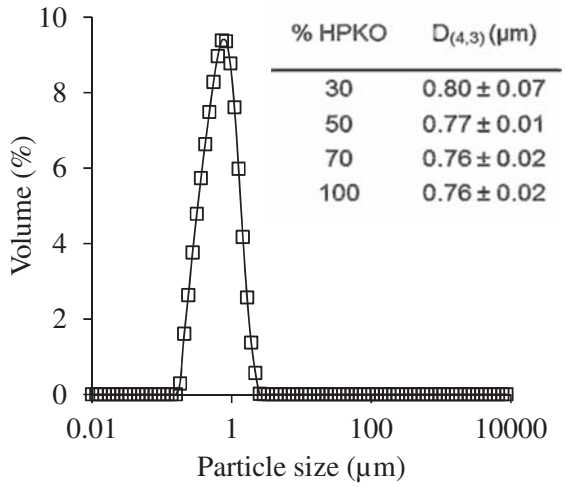


Fig. 4.1. Particle size distribution of a typical stock emulsion stabilized by 2.4 wt% sodium caseinate. The distribution shown contained 70% hydrogenated palm kernel oil. Inset graph shows mean particle sizes ($d_{4,3}$) ± 1 standard deviation of stock emulsions prepared with different concentrations of hydrogenated palm kernel oil in the dispersed phase.

4.4.2 Adsorbed protein content

The addition of Tween 20 (0–3.5 wt%) to sodium caseinate-stabilized emulsions with different HPKO concentrations in the dispersed phase and hence different SFC altered the interfacial film composition (Fig. 4.2). As also described in the previous chapter, the adsorbed protein concentration decreased from approximately 2.5–3.0 mg m^{-2} in the absence of Tween 20 to 0.6 mg m^{-2} with 1.5% Tween 20. At 2.5 and 3.5% Tween 20, a slight decrease in adsorbed protein content was found at 0 and 30% HPKO compared to 1.5% Tween 20. At higher concentrations of HPKO, however, the adsorbed protein loads were quite variable with 2.5 and 3.5% Tween 20 added. The variability in adsorbed protein was likely caused by partial coalescence prior to centrifugation, since

these emulsions were quiescently unstable (Section 4.4.5). Indicating partial coalescence occurred during centrifugation, an oil layer formed at the top of each centrifuge tube containing 2.5 or 3.5% Tween 20 and more prevalently with increasing SFC. However, this study primarily focused on emulsion stability with 0.5% and 1.5% Tween 20, where the protein surface loads were reliably measured, so there should be no complication from partial coalescence interfering with the interpretation of the results.

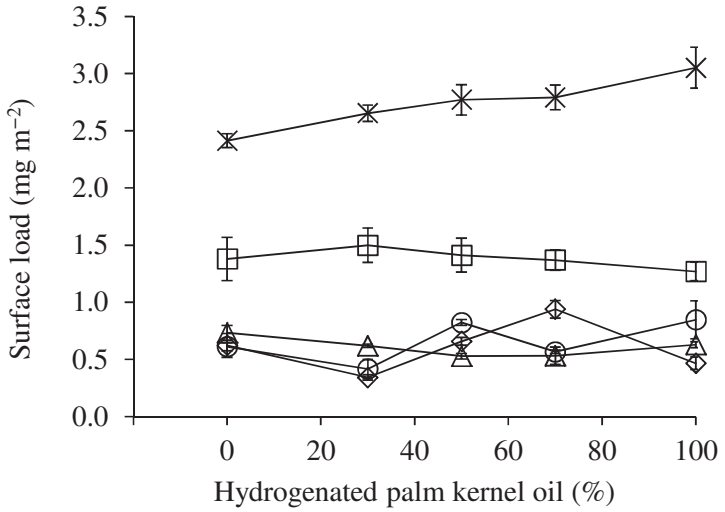


Fig. 4.2. Effect of Tween 20 on the concentration of protein adsorbed at the fat globule-water interface of emulsions with different % hydrogenated palm kernel oil in the dispersed phase. Symbols: (x) 0% Tween 20; (□) 0.5% Tween 20; (Δ) 1.5% Tween 20; (○) 2.5% Tween 20; (◊) 3.5% Tween 20. Solid lines are a guide for the eye.

Tween 20 displaces sodium caseinate following an orogenic mechanism whereby emulsifier-rich domains form within the protein film and expand with increasing emulsifier concentration potentially leading to percolation throughout the interfacial

film (Woodward, et al., 2009). Considering the orogenic mechanism and adsorbed protein measurements (Fig. 4.2), it can be inferred that at 0.5% Tween 20, a mixed sodium caseinate-Tween 20 film was present and at ≥ 1.5% Tween 20, a Tween 20 dominated interface was present. Tween 20-rich domains are the most likely fat globule-globule attachment point for partial coalescence since adsorbed proteins generally provide a strong repulsive force hindering the close approach of fat globules (Walstra, 2003). This hypothesis agreed well with the results in Part 1 of this study where the transition from a mixed sodium caseinate-Tween 20 film to a Tween 20 dominated film was associated with increased shear instability and the formation of more unstable aggregates.

4.4.3 Solid fat content

The solid fat content of the different dispersed phases was manipulated by blending fats with very low (canola oil) and high (hydrogenated palm kernel oil) melting points. The SFC of the fat blends decreased linearly from approximately 91–25% as canola oil content increased (Fig. 4.3). Since canola oil did not contain any crystalline fat at 5 °C, it can also be inferred from the linear change in SFC that canola oil did not interfere with HPKO crystallization in terms of the total crystal content produced.

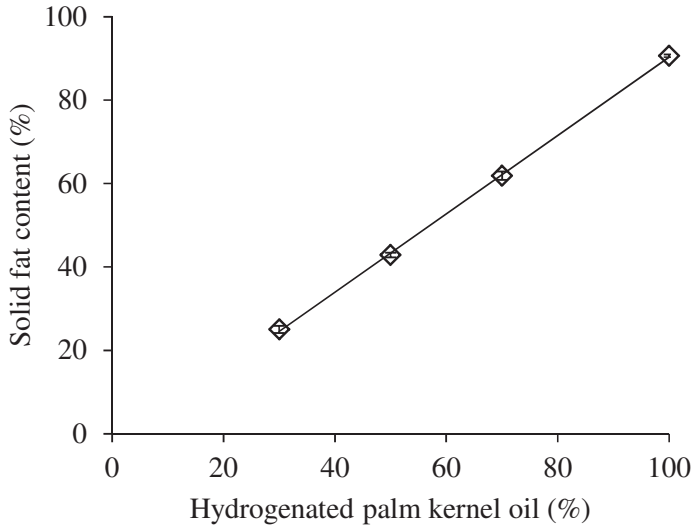


Fig. 4.3. Solid fat content of hydrogenated palm kernel-canola oil blends measured after 24 h at 5 °C (r^2=0.99).

In order to ensure emulsified fat crystallizes to the same extent as the bulk fat, emulsions must be cooled below the crystallization temperature of the bulk fat particularly for small droplets (< 0.2 μm) (Truong, Bansal, Sharma, Palmer, & Bhandari, 2014) since the theoretical number of nucleation sites in each droplet decreases with decreasing droplet size (Skoda & van den Tempel, 1963). Without a nucleation site present, substantial supercooling may be needed in order to spontaneously form a nucleation site (Coupland, 2002).

Depending on the composition of the emulsion, the standard direct and indirect pulsed NMR methods used to measure the SFC of bulk fat may be invalid for measuring the SFC of an emulsion due to the additional signal from the aqueous phase (Gribnau, 1992; van Boekel, 1981). However, some strategies have been developed to work around this problem (Fredrick, et al., 2011; Mutoh, Nakagawa, Noda, Shiinoki, & Matsumura, 2001). In this study, the SFC of the emulsions was also measured following the method

of Fredrick, et al. (2011) (data not shown). In this method, the liquid free induction decay signal from the aqueous phase of the emulsion is subtracted from the liquid signal of the emulsion in order to correct for the contribution of the aqueous phase on the overall signal of the emulsion. The results mirrored Fredrick, et al. (2011) in that the emulsions all approached their equilibrium SFC after 24 h which was near to that of the bulk fat. Therefore, the SFC of the emulsions studied here is expected to be similar to that of the bulk fat after 24 h of storage at 5 °C.

4.4.4 Transmission electron microscopy

In order for fat globules to partially coalesce, attachment must first occur through rupture of the interfacial film, followed by flow of liquid oil between the globules (Walstra, 2003). Fat crystals are thought to aid in film rupture because they tend to adsorb at the fat globule-water interface (Johansson & Bergenstahl, 1995; Johansson, Bergenstahl, & Lundgren, 1995) and may form jagged, irregularly-shaped structures capable of penetrating an interfacial film upon globule collision (Boode & Walstra 1993; Golemanov, Tcholakova, Denkov, & Gurkov, 2006; Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt, 2008). Therefore, the topography of the fat globules under investigation should play a critical role in the attachment and interfacial film rupture process. The interior of the fat globule is also important since the spatial distribution of liquid fat may impact the flow of liquid oil out of the fat globules (Hotrum, Stuart, van Vliet, & van Aken, 2004).

Cryo-TEM micrographs showed that, irrespective of solid fat content and within the size range studied (0.2–3.0 μm), the fat globules were globular shaped with no sharp protruding crystals visible (Fig. 4.4A and C). The micrographs are representative of the highest (100% HPKO) and lowest SFC (30% HPKO) emulsions. First, both emulsions

contained lamellar fat crystals oriented parallel to the interface. Similar fat crystal structures have been imaged by Cryo-TEM in solid lipid nanoparticles (Bunjes, Steiniger, & Richter, 2007) and sodium caseinate-olein nanoemulsions (Truong, et al., 2014; Truong, Morgan, Bansal, Palmer, & Bhandari, 2015). Compared to milk fat fraction nanoemulsions however, the topography of the fat globules studied here contained more protuberances which were more numerous with increasing solid fat content (Fig. 4.4A). The protuberances could form as a result of dislocations in the growing crystal lattice (Truong, Morgan, Bansal, Palmer, & Bhandari, 2015) or through exclusion of low melting point triglycerides from the high melting point triglycerides which crystallize first (Ergun, 2011; Jores, Mehnert, & Mader, 2003). It is not yet clear from this study nor the literature what may cause these protuberances to form. The addition of a high melting point milk fat fraction (stearin-rich fraction) in the work of Truong, Morgan, Bansal, Palmer, & Bhandari (2015) may have led to fewer protuberances compared to the emulsions studied here due to greater similarity in the triglyceride composition. Possibly, greater similarity in triglyceride composition could promote fewer protuberances due to fewer crystal dislocations or greater phase separation of the low, medium and high melting point triglyceride fractions upon crystallization. Both of these mechanisms could perhaps be caused by the formation of more “mixed” crystals found in vegetable and animal fats. Since the topography of the interface ultimately controls the susceptibility to partial coalescence, clarity around the composition and origin of these protuberances is critical to understanding the partial coalescence process.

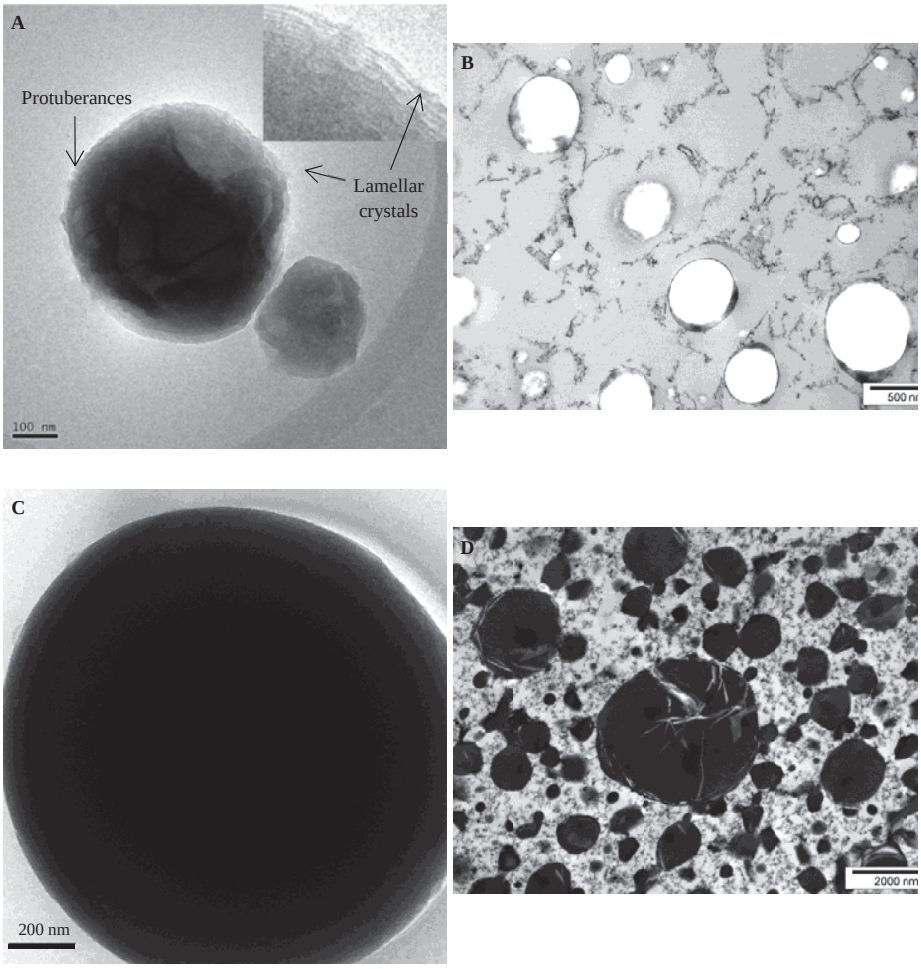


Fig. 4.4. Cryo-TEM (left) and TEM (right) micrographs of fat globules containing blends of hydrogenated palm kernel oil (HPKO) and canola oil from emulsions stabilized by 2% sodium caseinate and Tween 20. A and B) 100% HPKO, 0.5% Tween 20; C and D) 30% HPKO, 1.5% Tween 20.

TEM micrographs similar to those of Liboff, Goff, Haque, Jordan, & Kinsella (1988) and Smith, Goff, & Sun (2004) were also studied (Fig. 4.4B and D). Sample preparation for TEM is significantly more perturbing compared to Cryo-TEM however. For example, the preparation method used here was not suitable for 100% HPKO emulsions. The highly crystalline fat was not fixed during preparation so the globules were

"washed out" by acetone during the dehydration step. Holes in the micrographs where the fat globules resided are clearly visible along with the adsorbed protein layers (Fig. 4.4B). In Fig. 4.4D, the 30% HPKO globules were clearly imaged since the liquid oil was well fixed. The bright areas in several globules have been interpreted to be fat crystals (Liboff, et al., 1988) although their appearance may be altered by the dehydration process since they are not fixed. Bearing this in mind, further interpretation of the crystal properties such as distribution and morphology is not sensible. Likewise, the shape of the globules differs in Fig. 4.4D significantly more so than in Fig. 4.4C indicating that the TEM sample preparation altered the globular structure.

Interestingly, fat crystal lamellae oriented parallel to the interface have been reported elsewhere in both micro and nanoemulsions (Arima, Ueno, Ogawa, & Sato, 2009; Bunjes, et al., 2007; Precht, 1988; Wassell, et al., 2012). According to Bunjes, et al. (2007), the darker bands are the aligned glycerol backbones and the lighter bands are the associated acyl chains. The interior of the globules did not show any lamellar structures which is consistent with the micrographs of Truong, et al. (2014). Presumably, the high degree of curvature near the interior of the globule interferes with lamella formation (Bunjes, et al., 2007).

The lamellar layers located at the interface provide some indication of the spatial distribution of solid and liquid fat within the globule. Within the crystal lattice, it is not expected that the fatty acid composition is the same as the bulk (Sonoda, Takata, Ueno, & Sato, 2006). The higher melting point fractions most likely formed the lamellar layers since they crystallize first and defects in the crystal lattice are not favourable (Sonoda, et al., 2006). Furthermore, Arima, et al. (2009) showed how an additive (sucrose palmitic acid oligoester) increased interfacial heterogeneous nucleation and the degree of lamella orientation parallel to the oil-water interface although the lamellae were also

partly oriented parallel to the interface without the additive. This work supports that crystallization may begin at the interface through interfacial heterogeneous nucleation and promote lamellar orientation parallel to the interface. In the study of Arima et. al. (2009), interfacial heterogeneous nucleation retarded the α to β' crystal transition and as a result improved the alignment of the lamellar planes at the interface. Although the authors did not relate the changes in lamellar orientation at the interface to changes in functionality, it is likely, in the author's opinion, that changes in the lamellar orientation at the interface will affect partial coalescence. Better alignment of the lamellae could hinder the release of liquid oil out of partially crystalline fat globules upon close contact. The role of interfacial heterogeneous nucleation on the emulsions under study here will be explored in Chapter 6.

Beyond the onset of interfacial nucleation and crystallization however, questions remain to be answered around where within the fat globule the lower melting point triglycerides are spatially excluded to after the high and medium melting point triglycerides crystallize. There is some evidence indicating that the liquid oil excluded from the lattice may in part migrate to the periphery of the globule (Ergun, 2011; Jores, Mehnert, & Mader, 2003) rather than wholly to the interior. This is important to note since the periphery SFC and topography of the triglyceride interfacial layer will control the partial coalescence process rather than the bulk properties which are most often measured due to the lack of appropriate measurement techniques for these nanoscale features. Ultimately, the spatial arrangement of liquid oil and other oil soluble components in partially crystalline emulsion particles remains poorly understood (Bunjes, 2011).

4.4.5 Quiescent stability

To determine the susceptibility of the emulsions to partial coalescence under quiescent conditions, emulsions were stored at 5 °C and the changes in thickness were observed by tilting each tube and visually inspecting the sample over time (Table 4.1). All formulations with 0 or 30% HPKO showed no marked change in thickness as did emulsions with low Tween 20 concentration (0 or 0.5%) (results not shown). Overall, the degree of change in thickness increased with increasing SFC and Tween 20 concentration. The change in thickness varied from not apparent to solidified where the firmness was reminiscent of butter. At lower concentrations of SFC and Tween 20, a few days were required before any noticeable changes occurred whereas at higher concentrations of SFC and Tween 20, no flow was observed within a few hours.

After assessing the change in thickness on day 6, the degree of oiling off was measured by heating the emulsions to 65 °C to determine to what extent partial coalescence was associated with the change in thickness during storage. If partial coalescence was prominent, a layer of oil formed on top of the emulsion as a result of full coalescence of partially coalesced globules followed by creaming of large, unstable oil droplets. The degree of oiling off was strongly related to the change in thickness observed prior to heating the emulsions. In the solidified emulsions, virtually all of the fat separated from the aqueous phase indicating the vast majority of the fat globules participated in partial coalescence. Measurable oiling off was also found in moderately and markedly thickened emulsions as well with the exception of the 50% HPKO 3.5% Tween 20 emulsion.

Table 4.1.
Effect of hydrogenated palm kernel oil and Tween 20 concentration on the visible change in emulsion thickness during storage at 5 °C. Oiling off was measured after heating emulsions stored for 6 days.

% HPKO	% Tween 20	Time								% Oiling off
		15 min	4 h	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
50	1.5									-
	2.5									-
	3.5									-
70	1.5									-
	2.5									2
	3.5									10
100	1.5									4
	2.5									37
	3.5									37

No visible change in thickness
Slightly thickened
Moderately thickened
Markedly thickened
Solidified, no flow

Particle size testing was also performed on emulsions which remained fluid enough to disperse in cold water or 1% SDS solution i.e. emulsions which were moderately thickened or less. Emulsions with measurable oiling off contained larger particles not present in the stock emulsions while the changes in the remaining emulsions were negligible ($d_{4,3} \leq 0.83$ μm). This confirms partial coalescence contributed to moderate or marked thickening and solidification. In slightly thickened emulsions, the lack of a change in the particle size distribution indicates the thickening was likely caused by flocculation which was reversible upon dilution for particle size testing. Reversible flocculation is commonly observed in sodium caseinate-stabilized emulsions due to a depletion-driven attractive force between the fat globules (Dickinson & Golding, 1997), which here, increases with increasing Tween 20 concentration (Dickinson, Radford, & Golding, 2003). Confirming the presence of an attractive depletion force, separation of a clear serum phase from the emulsion was observed at room temperature after a few days

of storage and under refrigeration, albeit much more slowly, but only in emulsions with 1.5% Tween 20 or greater. Presumably, the depletion force was higher at 1.5% Tween 20 due to the increased concentration of sodium caseinate in the continuous phase caused by greater displacement from the interface. During the quiescent testing discussed above though, no clear serum separated in any of the tubes under refrigeration. Presumably, tilting the slightly thickened emulsions aided in remixing the phases together. In thicker emulsions, thickening alone was likely sufficient enough to prevent phase separation over the time scale studied here.

The results described above indicate fat globule flocculation likely occurred in all emulsions and increased with time and increasing Tween 20 concentration. Flocculation increases the susceptibility to partial coalescence by increasing the frequency of encounters between fat globules as well as the contact time (Walstra, 2003). Comparison of the adsorbed protein loads (Fig. 4.1) with the quiescent stability results (Table 4.1) reveals that partial coalescence did not occur in mixed sodium caseinate-Tween 20 interfaces (0.5% Tween 20) but did in Tween 20 dominated interfaces. The transition to quiescently unstable behaviour is therefore likely due to the concomitant effects of increased depletion-driven flocculation and interfacial surface area covered by Tween 20. The first factor increases with increasing Tween 20 concentration due to the transfer of sodium caseinate from the interface to the continuous phase and the increase in the number of Tween 20 micelles. The latter factor increases significantly up to 1.5% Tween 20 but much less thereafter. Since no partial coalescence occurred at 0 or 0.5% Tween 20, the adsorbed protein layer likely prevented partial coalescence by providing strong steric and electrostatic forces between flocculated fat globules.

The role of solid fat content, however, is not as clear. As SFC decreased, the degree of thickening and partial coalescence decreased as well. The rate of partial coalescence is

reported to be highest between of 10–50% solid fat content due to the presence of an optimum ratio of liquid oil and crystalline fat (Davies, et al., 2000; Fredrick, et al., 2010; McClements, 2007; Walstra, 2003). At low SFC, the lack of crystalline fat decreases the susceptibility to partial coalescence whereas the lack of liquid oil decreases the susceptibility at high SFC. Ultimately though, this is a generalization since the effect of SFC is dependent on many other factors such as interfacial composition. The thickening behaviour shown in Table 4.1 appears to disagree with the reported effect of SFC on partial coalescence since it was most prevalent at 100% HPKO where SFC was the highest.

Since thickening and partial coalescence increased with increasing SFC, the surface roughness or protrusion distance of fat crystals likely increased with increasing SFC due to the greater number of crystals and the associated density increase (globule shrinkage) (Boode & Walstra, 1993). The rough surface topography of an anhydrous milk fat interface containing either sodium caseinate or Tween 20 was probed using AFM by Thivilliers, et al. (2008). A rough surface feature likely functions as a "pin" which can rupture the interfacial film (Golemanov, et al., 2006) especially in viscous films such as Tween 20. In order to extend beyond stabilizing boundary of the interfacial film, the protuberances only need to extend a few nanometres at most. Although studies generally support that protruding fat crystals rupture the interfacial film, the crystals are rarely directly observed by microscopy for example. Reports of protruding fat crystals are generally limited to comparatively larger micron-size fat globules in emulsions with SDS or other strong fat crystal wetting agents in the continuous phase (Boode & Walstra, 1993). Therefore, liquid oil may not be a necessity for partial coalescence to occur but it may promote a stronger connection between fat globules than perhaps a

hydrophobic interaction between the crystalline fat phases of the high SFC emulsions studied here.

It could also be argued that partial coalescence only occurred in the high SFC emulsions because the SFC was still low or moderate when partial coalescence occurred. From direct pulsed NMR measurements on the 100% HPKO emulsion taken 15 minutes after chilling, the solid fat content was ~60%. Therefore, enough liquid oil was present to facilitate partial coalescence between two partially crystalline globules. Additionally, the presence of supercooled droplets and partially crystalline globules is also likely so partial coalescence driven by interdroplet nucleation may also have taken place. To determine if incomplete crystallization promoted partial coalescence at 100% HPKO, emulsions stabilized by sodium caseinate were separately prepared where the Tween 20 was added after chilling the protein stabilized emulsions for 24 h so that near complete crystallization could occur.

Ultimately though, the quiescent stability was only slightly improved. Quiescently, 1.5–3.5% Tween 20 emulsions prepared in this way were stable for a few days at 5 °C (results not shown) whereas the emulsions in Table 4.1 were only stable for minutes or hours. While tilting the emulsions did not induce thickening, pipetting or slight shaking caused immediate solidification. Heating the solidified emulsions also resulted in significant oiling off. These results indicate that high liquid oil content is not required for aggregation of high SFC fat globules. Rupture of the interfacial film leading to a fat-fat connection between the globules readily occurred even at very low liquid oil content. This aggregation process is likely similar to that of solid lipid nanoparticles (fully crystalline particles) where irreversible aggregation has also been reported (Salminen, et al., 2014). Although the binding of high SFC globules or particles could be through a crystalline fat-fat connection, the mechanism proposed by Ergun (2011) which shows

that liquid oil could be segregated to the fat globule interface also provides an alternative partial coalescence mechanism which should be considered here given the presence of the protuberances in Fig. 4.3A.

In conclusion, 0.5% Tween 20 emulsions were found to be quiescently stable regardless of SFC likely due to a sufficient amount of adsorbed protein which prevented close approach of the fat globules. At ≥ 1.5% Tween 20, partial coalescence may occur due to an increase in the attractive depletion force promoting flocculation and the decrease in repulsive force between fat globules caused by displacement of adsorbed protein. Increasing solid fat content promoted partial coalescence possibly due to an increase in surface roughness caused by fat crystallization or perhaps by some unidentified effects of fat crystallization on the structure of the Tween-rich interfacial film. At ≥ 1.5% Tween 20, liquid oil may facilitate partial coalescence but it is ultimately not required in large proportion relative to crystalline fat.

4.4.6 Stability under shear

The susceptibility to partial coalescence under steady shear was determined by inducing a fluid-semisolid transition under shear at 2000 s^{-1}. The shear viscosity typically remained relatively constant for a period of time followed by a rapid increase (Fig. 4.5). In part 1 of this study (Chapter 3), it was shown how the rapid increase in viscosity was caused by aggregation of the fat globules to such an extent that a jamming transition occurred due to hydrodynamic interactions between the crowded aggregates and globules.

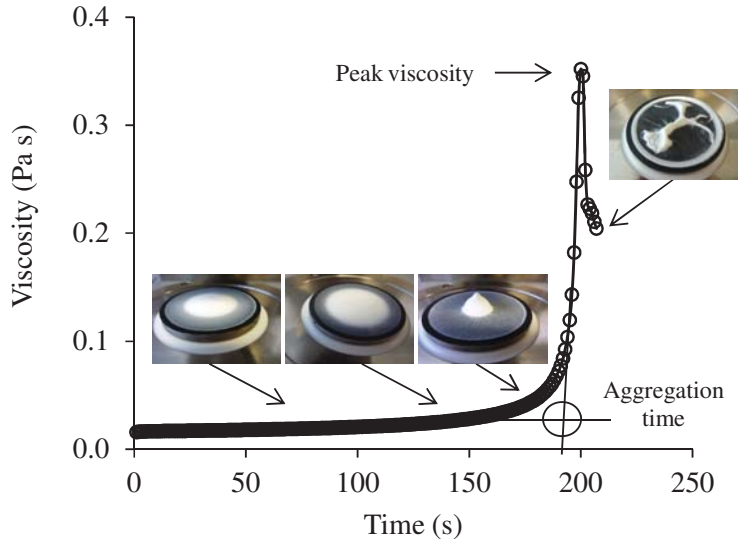


Fig. 4.5. Typical curve of change in viscosity over time at 5 °C during shear at 2000 s^{-1}. Images show the fluid-semisolid transition occurring during shear. The emulsion contained 70% hydrogenated palm kernel oil and 0.5% Tween 20.

4.4.6.1 Aggregation time

Following Guery, et al. (2006), the aggregation time was measured by extrapolating and determining the intersection of the relatively constant viscosity period and the rapid increase in viscosity (Fig. 4.5). Aggregation time decreased significantly over the first 24 h of storage time but changed little thereafter in all cases (Fig. 4.5). The increase in Tween 20 concentration from 0.5 to 1.5% caused a dramatic reduction in aggregation time overall as well as a reversal in the effect of SFC on aggregation time. Emulsions with 0.5% Tween 20 aggregated faster with decreasing SFC whereas at ≥ 1.5% Tween 20, emulsions aggregated faster with increasing SFC. The aggregation time of the 30% HPKO 0.5% Tween 20 emulsion is not shown in Fig.4.6 because it did not display a characteristic jamming-like transition even though aggregation occurred. Assuming the interfacial composition of all of the globules was the same, the lack of a jamming

transition could be explained by a reduced susceptibility to aggregation in the smaller fat globules compared to the larger ones. Walstra (2003) gave several explanations for the increase in susceptibility to partial coalescence with increasing globule size. Large globules are forced together more strongly than smaller globules at equivalent shear stress and additionally the interfacial film area between globules in close contact increases with increasing globule size. So although there was always an inherent difference in fat globule susceptibility to aggregation in all of the emulsions due to polydispersity, the stability of the small globules was much greater relative to the large globules. In the 30% HPKO 0.5% Tween 20 emulsion, due to the specific combination of low SFC and mixed interfacial composition, aggregation in the absence of jamming may be explained by partial coalescence of only the less stable large globules.

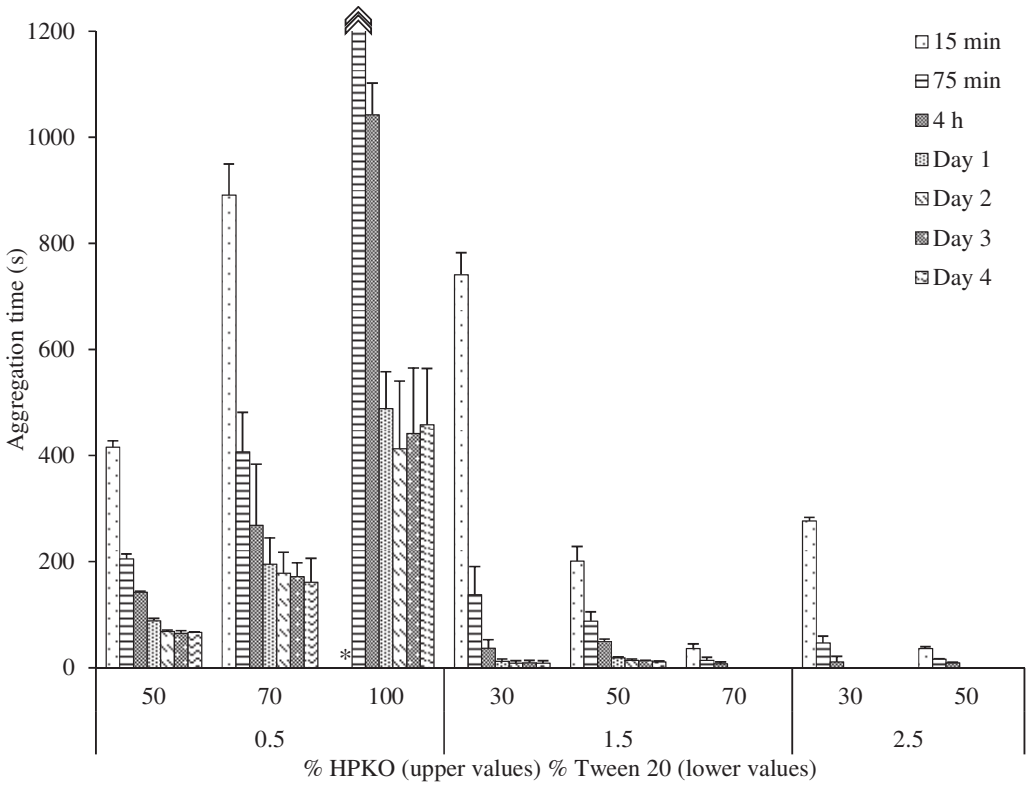


Fig. 4.6. Effect of solid fat content on the shear-induced aggregation time of emulsions with different Tween 20 concentrations and % hydrogenated palm kernel oil in the dispersed phase. Measurements were made at 5 °C. An asterisk indicates aggregation did not occur within 3000 s.

The decrease in aggregation time, observed during the first 24 h of storage time in all samples regardless of % HPKO, was likely related to crystallization of the fat since it takes approximately 24 h for the SFC to approach its equilibrium value (section 3.2) (Fredrick, et al., 2011). Crystallization produces the rough surface features required to rupture the interfacial film and cause partial coalescence (Golemanov, et al., 2006; Walstra, 2003). Fat crystallization is also reported to promote protein displacement due to differences in surface properties between liquid and solid fat (Euston & Mayhill,

2001). This would likewise decrease the aggregation time by allowing for closer globule approach upon collision (Walstra, 2003).

After 24 h of storage, the aggregation time remained relatively unchanged. At 0.5% Tween 20, the aggregation time increased with increasing SFC. This finding qualitatively agrees with the reported effect of SFC on the rate of partial coalescence (McClements, 2005; Walstra, 2003). The authors reported partial coalescence occurs more readily at moderate SFC due to the presence of a sufficient amount of both fat crystals and liquid oil. Here, the fastest aggregation time at 0.5% Tween 20 occurred with 50% HPKO (43% SFC). Based on the reported effect of SFC on the partial coalescence rate, it might be expected that the 30% HPKO emulsion (25% SFC) would aggregate similarly. The 30% HPKO with 0.5% Tween 20 did aggregate under shear but the curve of viscosity versus shearing time did not show the characteristic shape required for the aggregation time to be calculated as in Fig. 4.5. However, considering the aggregation time to be the time required for the emulsion to be expelled from the cone-and-plate gap, both 30 and 50% HPKO emulsions had roughly the same aggregation time.

At 1.5 and 2.5% Tween 20, the aggregation time decreased with increasing SFC. None of the emulsions with 100% HPKO, and only a few with 70% HPKO could be sheared due to either quiescent instability or aggregation when pipetted during transfer to the rheometer. At 2.5% Tween 20, 50% HPKO emulsions were clearly more unstable compared to 30% HPKO, while at 1.5% Tween 20, the emulsions showed similar stability. The stability reflects the quiescent stability results shown in Table 4.1. In both sets of experiments, increasing SFC promoted increased instability. Shear simply accelerated the aggregation process observed quiescently in these emulsions. As shear rate increases, the encounter frequency between fat globules increases as well as the

encounter efficiency due to increased shear stress forcing globules into closer contact (Walstra, 2003). It was expected that high displacement of sodium caseinate (Fig. 4.1) would allow for close contact between the fat globules and in addition increased surface roughness occurred with increasing SFC. Coupled together, these two factors most likely caused the change in the effect of SFC on aggregation time at different levels of adsorbed protein.

4.4.6.2 Extent of fat globule aggregation

The peak shear stress reached during the jamming transition reflects the maximum force applied by the rheometer to maintain the constant shear rate. Since fat globule aggregation was the primary cause for the observed increase in shear stress, the peak shear stress provides an indication of the extent of fat globule aggregation. In Fig. 4.7, the peak shear stress reached during the jamming transition is plotted against shear rate. The peak shear stress increased with increasing shear rate in all emulsions tested. The increase was linear for most emulsions with the exception of 50% HPKO with 0.5 and 1.5% Tween 20 which showed a lower peak shear stress than otherwise expected at high shear rate.

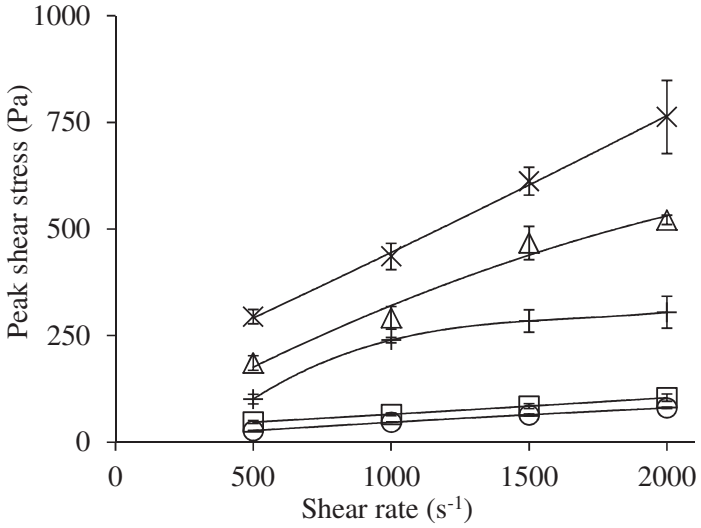


Fig. 4.7. Influence of shear rate on the peak shear stress reached during the jamming transition at 5 °C. Symbols: (□) 100% HPKO, 0.5% Tween 20; (x) 70% HPKO, 0.5% Tween 20; (Δ) 50% HPKO, 0.5% Tween 20; (+) 50% HPKO, 1.5% Tween 20; (○) 30% HPKO, 1.5% Tween 20. Solid lines are a guide for the eye.

Interestingly, the peak shear stress reached during shear depended on both the interfacial composition and solid fat content. The highest peak shear stress values were found at 50 and 70% HPKO whereas 30 and 100% HPKO had the lowest peak shear stress values. At 50% HPKO, 1.5% Tween 20 emulsion had a lower peak shear stress value compared to 0.5% Tween 20 emulsion despite both displaying a jamming transition. Since aggregate breakup or compaction likely occurred during the jamming transition, it can be assumed that the lower peak shear stress was due to the formation of less shear stable aggregates, likely caused by the greater displacement of sodium caseinate at 1.5% Tween 20 compared to 0.5%. As aggregate size increased during shear, redispersion or compaction of an aggregate may have occurred if the cohesive forces could not support the increased hydrodynamic forces (Mühle, 1993). Sodium caseinate likely provided structural support to the aggregates under the increased shear

stress thus hindering aggregate breakup in emulsions with less Tween 20 surface coverage (0.5% Tween 20).

4.4.7 Discussion

Although protein displacement by low molecular weight emulsifiers is well known to increase the susceptibility to partial coalescence, the effect has not been established for different solid fat contents. From the results presented above, the susceptibility to partial coalescence was assessed from both the aggregation time and peak shear stress reached during aggregation (Fig. 4.8). The aggregation time reflects how rapidly the partial coalescence process occurs while the peak shear stress indicates the extent to which partial coalescence occurs. In Fig. 4.8A and B, the data were extrapolated to 0% SFC considering that no partial coalescence was possible without crystalline fat. Both figures interestingly indicated roughly the same trends irrespective of how the susceptibility to partial coalescence was measured for mixed sodium caseinate-Tween 20 films and Tween 20 dominated films. For mixed films, the shape of the curve was parabolic indicating that the fat globules were less susceptible to partial coalescence at both low and high SFC. Thus, the findings agreed well with previously published studies (Davies, Dickinson, & Bee, 2000; Fredrick, Walstra, & Dewettinck, 2010; McClements, 2007; Walstra, 2003). For Tween 20 dominated films however, the fat globules became more susceptible to partial coalescence as SFC increased.

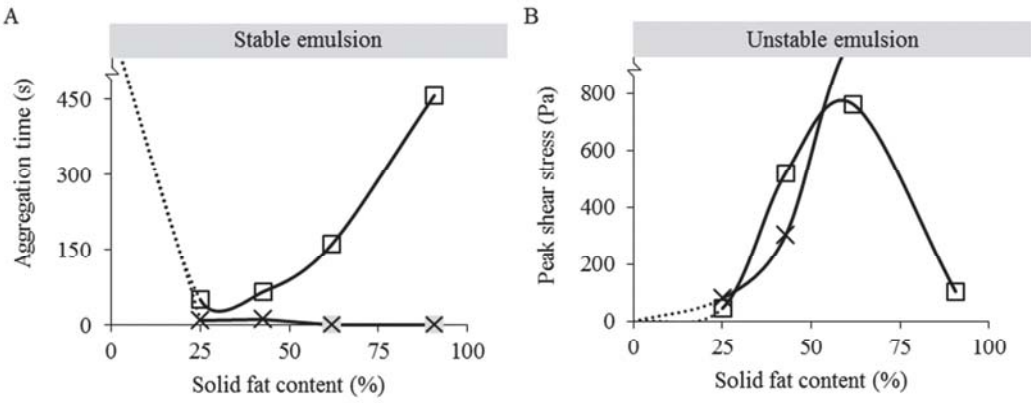


Fig. 4.8. Effect of solid fat content and interfacial film composition on the relative aggregation time (A) and peak shear stress (B) measured under steady shear (2000 s^{-1}) at 5 °C. Dotted line shows data extrapolated to 0% SFC where emulsions cannot undergo partial coalescence. Symbols: (□) Mixed sodium caseinate-Tween 20 interfacial film (0.5% Tween 20); (x) Tween 20 dominated interfacial film (1.5% Tween 20). Filled symbols in (A) indicate the emulsions aggregated immediately when sheared.

Ultimately, liquid oil is required to form the connections between the fat globules studied here. In the past, it was argued interfacial film rupture caused by fat crystals protruding from the interface was one of the primary mechanisms for the release of liquid oil (Walstra 2003). In this study however, there was no evidence from Cryo-TEM of significant fat crystal protrusion beyond the thickness of the interfacial film so crystal protrusion may not have been a primary cause of film rupture in this study. Furthermore, the rate at which liquid oil flowed out of the fat globule was previously thought to depend on the size of the fat crystals since the capillary pressure within the fat globule was affected by pore size (Walstra 2003). However more recently, it was shown by Hotrum, Stuart, van Vliet, & van Aken (2004) that fat crystal size does not restrict the

flow of liquid oil out of fat globules. Hotrum's work also highlighted that the flow of liquid oil out of fat globules depends more on the interfacial film composition i.e. whether the interface is covered with protein or low molecular weight surfactant (Hotrum, Stuart, van Vliet, & van Aken 2003). Considering that Tween 20 interfacial films have poor mechanical properties and provide low steric stabilization relative to sodium caseinate, it was not surprising that Tween 20 dominated emulsions were prone to partial coalescence. However, it was difficult to explain why SFC affected the susceptibility to partial coalescence in this study assuming that fat crystal protrusion was not a primary cause of partial coalescence.

In the author's opinion, one factor worth noting is the dependence of the fat globule deformability on SFC. Under the shear conditions studied here, one would expect that the fat globules become increasingly deformable as SFC decreases depending on the location of the crystals within the globule e.g. adsorbed at the interface (less deformable) or located within the globule (more deformable). At low SFC, the fat globules should be very deformable and during shear undergo a dynamic flow process involving a combination of events including collision, deformation, partial coalescence and aggregate breakup. Such a destructive series of events is expected to increase the partial coalescence rate especially in emulsions where a steric barrier is present such as in the mixed sodium caseinate-Tween 20 interfacial films studied here. Viewed in this light, the parabolic effect of SFC on the susceptibility to partial coalescence may be related to adsorbed protein load and globule deformability (controlled by SFC). In Tween 20 dominated films at high SFC, globule deformability does not play a role since the emulsions are quiescently unstable. This implies collisions between fat globules are highly efficient in causing partial coalescence which is most likely a consequence of the poor mechanical properties of Tween 20. As SFC decreases in Tween 20 dominated

films however, the partial coalescence rate remains relatively high under shear but does not readily occur quiescently. This further implies that the mechanical or barrier properties of the Tween 20 dominated film improve with decreasing SFC. The effect of the mechanical properties of the interfacial film will be indirectly investigated in the following chapters.

Although Tween 20 was the only type of emulsifier studied here, it was expected that emulsifiers with similar film properties and protein displacement potential would show similar behaviour. However, emulsifiers with film properties different to Tween 20, e.g. saturated monoglycerides which crystallize at the interface, may alter these trends since they are reported to modify the fat globule surface roughness independent of SFC (Fredrick, et al., 2013). It can be envisioned that emulsifiers alter fat globule stability by both impacting the surface topography and the availability of liquid oil for securing the connection between partially coalesced globules. Thus, careful selection of emulsifier type and concentration could potentially alter fat globule stability independent of SFC and adsorbed protein load.

4.5 Conclusion

The combined effects of solid fat content (SFC) and interfacial film composition on quiescent and shear-induced partial coalescence were studied in detail. Two different stability regimes were identified. At low protein displacement, fat globules were stable quiescently regardless of SFC but were vulnerable to partial coalescence under shear. The aggregation time decreased with decreasing SFC presumably due to the presence of more liquid oil which aids in fat globule-globule adhesion in the presence of a significant repulsive force from the adsorbed protein. At high protein displacement, emulsions became more unstable both quiescently and under shear with increasing SFC.

The results imply that partial coalescence readily occurs even at low liquid oil content if close contact between the globules is possible. The increased surface roughness of fat globules with increasing SFC may assist with film rupture and fat globule attachment. From Cryo-TEM micrographs, there was no evidence for fat crystal protrusion from the fat globule-water interface beyond a few nanometres at most. These findings support that rapidly cooled emulsions should be generally considered to have rough rather than spikey surfaces structures as commonly suggested in the literature. For partially crystalline food emulsions, these results provide significant insight into how both solid fat content and interfacial film composition can be altered in order to adjust the susceptibility of an emulsion to partial coalescence.

Chapter 5: Effect of Tween and SPAN emulsifiers on the shear stability of hydrogenated palm kernel oil/canola oil-in-water emulsions stabilized by sodium caseinate

5.1 Abstract

The effect of polyoxyethylene sorbitan fatty acid esters (Tween) alone or in combination with sorbitan fatty acid esters (SPAN) on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat, $d_{4,3}$ = ˜0.73 μm) was studied. Low molecular weight emulsifiers like Tweens and SPANS are often used to regulate the susceptibility of partially crystalline oil-in-water emulsions to aggregation and partial coalescence. It was found that emulsion stability increased with increasing chain length of the saturated Tween emulsifiers, while the unsaturated Tween 80 was comparatively much more unstable than the saturated types of Tween. The effect of 1 wt% SPAN (SPAN 20, 40, 60 and 80) in the dispersed phase of emulsions containing different concentrations of Tween 80 (0.2–0.6 wt%) and 1% sodium caseinate was also investigated to determine if the addition of SPAN could reduce the susceptibility to partial coalescence. The emulsions showed sharp stability transitions from stable to unstable over the range of Tween 80 concentrations tested. All SPAN containing emulsions with 0.2 wt% Tween 80 were stable under shear however all emulsions with 0.6 wt% Tween 80 immediately aggregated when shear was applied. At 0.4 wt% Tween 80, the saturated long fatty acid chain length emulsifiers (SPAN 40 and 60) were in general much more stable compared to SPAN 20 and 80 containing emulsions. The possible mechanisms by which these emulsions destabilise are discussed herein.

5.2 Introduction

In the previous chapters, the combined effects of solid fat content and interfacial film composition on the susceptibility of fat globules to quiescent and shear-induced aggregation were investigated. The emulsions studied represented simple model systems in which sodium caseinate stabilized fat globules are rendered more sensitive to aggregation through the addition of Tween 20 which partially displaces protein from the fat globule-water interface and forms Tween-rich domains of condensed monolayers at the interface. Since partially crystalline emulsions stabilized by only Tween 20 are comparatively much more unstable than sodium caseinate stabilized emulsions, the Tween 20 rich domains on the fat globule surface which form when protein is displaced by Tween 20 serve as the most likely site for fat globule-fat globule connections to form upon contact (Walstra, 2003). Although this model food emulsion explains the role of protein displacement on the susceptibility to aggregation quite well, "real" food emulsions such as whip toppings and ice cream commonly have more complex interfaces containing emulsifiers in crystalline or liquid crystalline phases (Goff, 1997; Krog, 1997; Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014). This chapter builds on the previous findings by exploring how the properties of the emulsifiers such as chain length, saturation, melting point and molecular weight affect the susceptibility of partially crystalline oil-in-water emulsions to partial coalescence.

Low molecular weight food grade emulsifiers are typically composed of a hydrophilic headgroup e.g. sorbitan, glycerol or a sugar, and a hydrophobic fatty acid tail group containing 1–2 fatty acids (Krog, 1997). Tween and SPAN emulsifiers both contain the same base structure of a single fatty acid tail bonded to a sorbitan headgroup. The only difference between them is that Tween emulsifiers are ethoxylated to increase their

solubility in water. However, the much larger ethoxylated headgroup hinders the association of the fatty acid tails between molecules both in solution and at interfaces (Boyd, Parkinson, & Sherman, 1972; Lu & Rhodes, 2000). For example, bulk Tween 40 is liquid at room temperature but SPAN 40 is a solid owing to the difference in the size of the headgroup (Table 5.1). Ethoxylation of the sorbitan headgroup also increases the hydrophilic-lipophilic balance (HLB) which renders Tween emulsifiers soluble in water compared to its oil soluble, low HLB counterpart SPAN. In general, the emulsifiers approach the oil-water interface from their respective phase and compete for the interface. Alone, Tween emulsifiers are excellent stabilizers of oil-in-water emulsions despite their high HLB value (Boyd, et al., 1972). The fatty acid tails penetrate partly into the oil droplet whilst the hydrophilic headgroup remains hydrated by the aqueous phase. SPAN emulsifiers on the other hand typically must be used in conjunction with a high HLB emulsifier like Tween in order to form an oil-in-water emulsion. SPANS and Tweens are often used in conjunction to form stable oil-in-water emulsions since the ideal HLB range for stabilization is between approximately 8–10 which lies between the HLB values of both SPAN and Tween (Berton, Genot, Guibert, & Ropers, 2012; Boyd, et al., 1972; Gullapalli & Sheth, 1999; Lu, et al., 2000; Pilpel & Rabbani, 1988).

Table 5.1.
Properties of Tween and SPAN emulsifiers*

Emulsifier	Primary fatty acid	HLB	Approximate molecular weight (g/mol)	Form	Melting point range (°C)
Tween 20	C12:0	16.7	1228	Viscous liquid	N/A
Tween 40	C16:0	15.6	1277	Viscous liquid	N/A
Tween 60	C18:0	14.9	1309	Semi-solid	N/A
Tween 80	C18:1	15.0	1310	Viscous liquid	N/A
SPAN 20	C12:0	8.6	346	Viscous liquid	N/A
SPAN 40	C16:0	6.7	403	Solid	45–47
SPAN 60	C18:0	4.7	431	Solid	54–57
SPAN 80	C18:1	4.3	429	Viscous liquid	N/A

N/A – not applicable
*Data collected from the ingredient manufacturer

At air and oil-water interfaces, combinations of Tween and SPAN also pack more efficiently compared to Tween alone leading to the formation of more robust and densely packed interfacial layers (Pilpel, et al., 1988). Each of the three polyoxyethylene chains on the Tween headgroup extend into the aqueous phase from the interface approximately 1.4 nm and together form a relatively large hydrophilic headgroup together with the sorbitan group which consequently hinders the association of the fatty acid tails of neighbouring Tween molecules extended into the oil phase. The fatty acid tail of SPAN however can fit amongst the Tween fatty acid tails due to the smaller size of the SPAN sorbitan headgroup (Lu, et al., 2000). SPAN 40 and 60 crystallize below 45 °C and alter the interfacial film structure considerably as a result. Pilpel and Rabbani (1987) studied 20–60 wt% sunflower oil-in-water emulsions stabilized by blends of SPAN 40 and Tween 40 up to 10 wt%. Depending on the blend ratio, liquid crystalline interfaces formed in sunflower oil-in-water emulsions after cooling the emulsions to room temperature. A liquid crystalline interface was only formed between molar ratios of SPAN and Tween from 3:1 to 7:1. The optimal 5:1 molar ratio or 6:4 weight ratio produced the highest interfacial viscosity as well the best

emulsion stability over time. The optimal 5:1 molar ratio agrees with the ideal theoretical packing arrangement. Pilpel, et al. (1988) calculated that the most efficient packing arrangement contained alternating sequences of 5 SPAN molecules and 1 Tween molecule due the large volume occupied by the hydrophilic Tween headgroup and relatively small volume occupied by the SPAN headgroup.

In partially crystalline oil-in-water emulsions, more complexity in the interfacial film is introduced when the oil phase is partially crystalline. In the bulk, fat crystals form and grow on nucleation sites which are commonly foreign matter such as dust particles. In oil-in-water emulsions however, the finite foreign matter becomes distributed throughout the dispersed oil droplets and thus most droplets will not contain foreign matter. The lack of foreign matter means emulsions must generally be cooled to much lower temperatures below the melting point of bulk fat (supercooling) before crystallization begins (Coupland, 2002). Often times, the emulsifiers may serve as nucleation sites through a process called interfacial heterogeneous nucleation where the degree of supercooling necessary to induce crystallization is reduced. Although few studies have investigated how interfacial heterogeneous nucleation affects the stability of partially crystalline oil-in-water emulsions, it may result in smaller fat crystals and an increase in parallel orientation of the crystals to the interface (Arima, Ueno, Ogawa, & Sato, 2009).

Cooling from the melt, several phase transitions could occur depending on the crystallization or more generally phase transition temperature of the emulsifier, fat and the complexed interfacial layer containing a blend of emulsifiers, oil and water. In addition, if the crystallization temperature of the emulsifier is greater than the crystallization temperature of the fat, the emulsifier crystals may serve as nucleation sites for the fat interfacial heterogeneous nucleation and consequently alter the structure

of the fat crystals nearest to the interface and potentially throughout the entire fat globule (Coupland, 2002; Skoda & van den Tempel, 1963). Additionally, both emulsifier and fat crystallization at the oil-water interface will hinder penetration of Tween fatty acid tails into the oil phase. This will certainly alter the packing density and arrangement and possibly the degree of complexation between emulsifiers such as SPAN and Tween. On a hydrophobic solid surface where the fatty acid tails cannot penetrate, Tween 20 and 80 may adopt a flat conformation at the interface compared to Tween 40 and 60 which adsorb perpendicularly and hence form thicker, more densely packed interfacial layers (Graca, Bongaerts, Stokes, & Granick, 2007).

This chapter builds on the previous work by examining the effect of Tween emulsifier chain length on emulsion stability as well as more complex interfacial films containing combinations of Tween 80 and different SPAN emulsifiers. The purpose of this work was to provide insight on how interfacial film structures generated by the emulsifiers and fat crystals alter both the resistance of the film to rupture, displacement of adsorbed protein and the partial coalescence process.

5.3 Material and methods

5.3.1 Ingredients

The ingredients used in this chapter were previously described in Section 3.3.1 with the exception of SPAN emulsifiers. SPAN 20 and SPAN 40 were purchased from Sigma-Aldrich (St. Louis, MO). SPAN 60 and SPAN 80 were supplied by Rikevita (Malaysia) SDN. BHD.

5.3.2 Emulsion preparation

Sodium caseinate stabilized stock emulsions containing a blend of 70 wt% hydrogenated palm kernel oil (HPKO) and 30 wt% canola oil were first prepared and stored overnight at room temperature. The fat blend was prepared by mixing the melted fats at 65 °C. The solid fat content of the fat blend at 5 °C was 61.9 ± 0.2% as measured by the direct pulsed NMR method. In formulations containing SPAN, 1 wt% of the emulsifier was blended in the fat phase. Coarse emulsions were prepared from sodium caseinate solution (5 wt%), fat, sodium azide and Milli-Q water heated to 65 °C in a water bath by high shear mixing (Ultraturrax T25, Janke & Kunkel GMBH & Co. KG) for 1 min at 13,500 rpm. The emulsions were then reheated to 65 °C before homogenization (3 passes) using a pre-heated, two-stage homogenizer (Panda, Niro Soavi, Italy) operated between 180–210 bar. The stock emulsions contained 1.5 wt% sodium caseinate, 52.5 wt% fat and 0.02 wt% sodium azide.

Working emulsions (25 g) containing the final formulation were prepared by diluting stock emulsions with 5 wt% Tween emulsifier solution and Milli-Q water at room temperature. The Tween solutions were prepared in 30 mM imidazole buffer (pH 6.8) by heating the solution to 65 °C on a stir plate and cooling overnight under gentle stirring. After combining the stock emulsion and Tween solution, each working emulsion was then placed in a 65 °C water bath for 30 min in order to melt any fat crystals that may have formed before cooling the emulsions in ice-water to approximately 5 °C. All formulations contained 1 wt% sodium caseinate, 35 wt% fat and 0.02 wt% sodium azide. All emulsions were tested after at least 24 h of storage at 5 °C in order to allow enough time for the fat phase to fully crystalline. Emulsions

prepared with SPAN contained 1 wt% of the emulsifier in the fat phase. All the emulsions studied were prepared and tested in duplicate.

The emulsions in this chapter and the following were prepared with 1 wt% sodium caseinate rather than 2 wt% used previously. The purpose was to reduce depletion flocculation driven attraction between globules by having less non-adsorbed sodium caseinate.

5.3.3 Particle size analysis

The particle size testing method was previously described in Section 3.3.3.

5.3.4 Shear stability

Emulsion stability under shear was measured according to the method described in Section 3.3.5 with modifications. The aggregation time and peak shear stress of emulsions under shear was measured at a constant shear rate (2000 s^{-1}) after the emulsions were placed in cold storage at 5 °C for at least 24 h.

5.3.5 Adsorbed protein load

The adsorbed protein load was measured following the procedure described in Section 3.3.4.

5.4 Results

5.4.1 Adsorbed protein load

Partially crystalline oil-in-water emulsions with low adsorbed protein content were prepared by diluting stock emulsions containing 1.5% sodium caseinate ($d_{4,3}$ = ~0.73

μm) with Tween emulsifiers (Tween 20, 40, 60 and 80 abbreviated as T20, T40, T60 and T80). Regardless of Tween type and concentration (0.8 or 1.6 wt%), the addition of Tween reduced the adsorbed protein load approximately 4-fold from 2.0 to 0.5 mg m^{-2} (Fig. 5.1). The emulsions containing blends of Tween emulsifiers contained 0.4 wt% of each emulsifier. An emulsion containing 1.6 wt% T80 was not tested due to rapid destabilisation soon after chilling.

The similar displacement behaviour by different Tween emulsifiers is consistent with Woodward, et al. (2009) who compared the displacement of T20 and T60 from an air/water interface. The authors found both emulsifiers displaced sodium caseinate at a similar surface pressure despite T60 having slightly less interfacial activity. The authors concluded that protein displacement was more controlled by the structure of the protein film rather than the structure of the emulsifier. Furthermore, the authors showed that the residual adsorbed protein structure after Tween percolation was dominated by κ-casein. Hence, the results indicate that 0.8–1.6 wt% Tween regardless of type is sufficient to displace most of the sodium caseinate film but not all due to the strong affinity of the residual κ-casein dominated film for the interface. This residual protein film likely plays a critical role in maintaining the quiescent stability of these emulsions by providing steric stabilization.

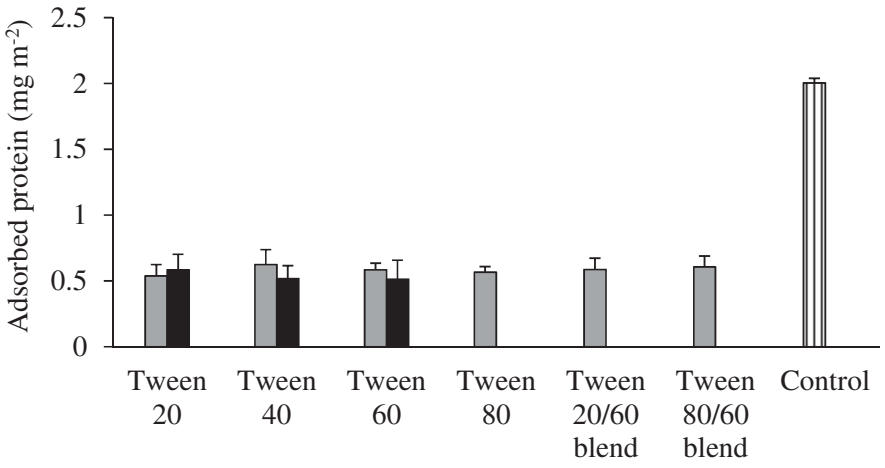


Fig. 5.1. Effect of Tween type and concentration on the adsorbed protein content of partially crystalline oil-in-water emulsions stabilized by 1 wt% sodium caseinate. Colour scheme: (Grey) 0.8 wt% Tween; (Black) 1.6 wt% Tween; (Striped) 1 wt% sodium caseinate only. The emulsions containing blends of Tween emulsifiers contained 0.4 wt% of each type of Tween.

5.4.2 Effect of Tween type and concentration on emulsion stability

The emulsions described in the previous section were subjected to steady shear (2000 s^{-1}) to investigate the effect of Tween type and concentration on the susceptibility of the emulsions to aggregation. The two concentrations of Tween were selected for the purpose of studying interfacial films containing predominantly Tween emulsifier. The PSD of the emulsions ($d_{4,3}$ = ~0.73 µm) were virtually identical to the emulsions containing sodium caseinate and Tween 20 described in Chapter 4 (Fig. 4.1). Emulsions containing the unsaturated emulsifier (T80) were the most unstable. T80 emulsions (0.8 wt%) immediately aggregated when shear was applied (data not shown). The sheared emulsion formed a thick paste similar in texture to fat continuous butter or spread. Fat globule aggregation also coincided with partial expulsion of the emulsion from the cone

and plate gap. On the other hand, the emulsions containing saturated Tween emulsifiers (T20, T40 and T60) were stable against aggregation for at least several hundred seconds of shearing time (Fig. 5.2). The fat globules in emulsions with T20 aggregated to a large extent as indicated by the abrupt and large increase in viscosity. Aggregation led to the formation of a paste similar to the T80 emulsion which indicates that prolific fat globule aggregation occurred in both emulsions. The emulsions containing Tween 40 however were much more stable to aggregation. The viscosity only increased slightly compared to the initial viscosity and the aggregation process was arrested since no further increase in viscosity occurred with continued shearing time. The emulsion containing Tween 60 (T60) was stable under shear and was not found to aggregate even after 1000 s of shearing time.

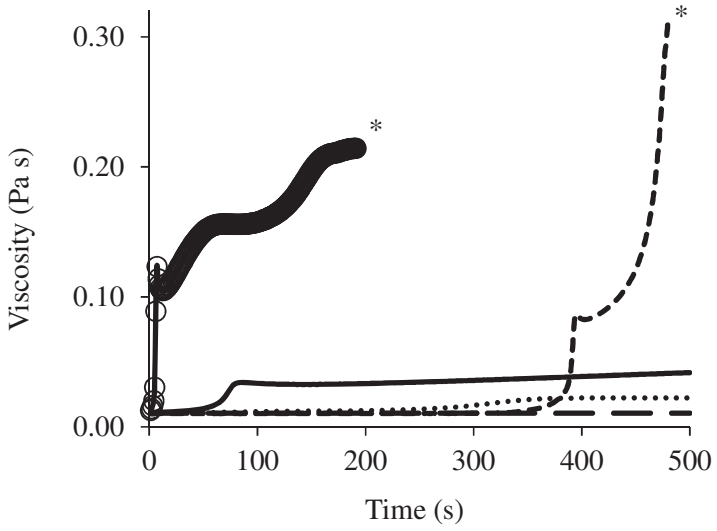


Fig. 5.2. Effect of Tween type (0.8 wt%) and 50/50 blends of Tween emulsifiers on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat, 1 wt% sodium caseinate) measured under stead shear (2000 s^{-1}) at 5 °C. An asterisk indicates the point at which the emulsion was expelled from the gap. Lines and symbols: (short dash) Tween 20; (dotted) Tween 40; (long dash) Tween 60; (solid line) Tween 20/Tween 60; (○) Tween 80/Tween 60.

Emulsions containing 50/50 blends of Tween emulsifiers were also prepared and sheared. The blending of T60 with either T80 or T20 resulted in less stable emulsions compared to the emulsion prepared with 0.8 wt% T60 (Fig. 5.2). Interestingly though, the shear stability of the emulsion with a T20/T60 blend was more similar to the T60 containing emulsion whereas the emulsion with a T80/T60 blend was more similar to the emulsion with 0.8 wt% T80. The results imply both emulsifiers were present at the interface in the blends but T80 has a greater impact on stability compared to T20 in the blends with T60.

Emulsions were also prepared with 1.6 wt% of each saturated Tween emulsifier. Each of the emulsions aggregated rapidly (within a few seconds) but expulsion of the fat globules from the gap did not coincide with aggregation (Fig. 5.3). The T20 containing emulsion aggregated to form a semisolid paste but expulsion from the gap took approximately 60 s whereas the T40 emulsion took approximately 150 s before aggregates were expelled from the gap. The T60 containing emulsion also aggregated but like the 0.8 wt% T40 emulsion and T20/T60 blend emulsion in Fig. 5.2, aggregation was arrested after an initial rise in viscosity. Here, no thick paste was formed nor were aggregates expelled from the gap. It was noted that slip certainly took place once the emulsion became semisolid and this caused a slower increase in viscosity after the initial rapid increase. During this time, large fat globules formed and were ultimately expelled from the gap once their size was sufficient to span the gap between the cone and plate. This process of shear-induced aggregation is likely somewhat chaotic in nature but it was found that the curves from semisolid formation up to aggregate expulsion were reproducible. Since they were reproducible, the aggregation behaviour was most likely dependent on the emulsifier type and less on chaotic events associated with slip, inhomogeneous flow or aggregate migration, for example.

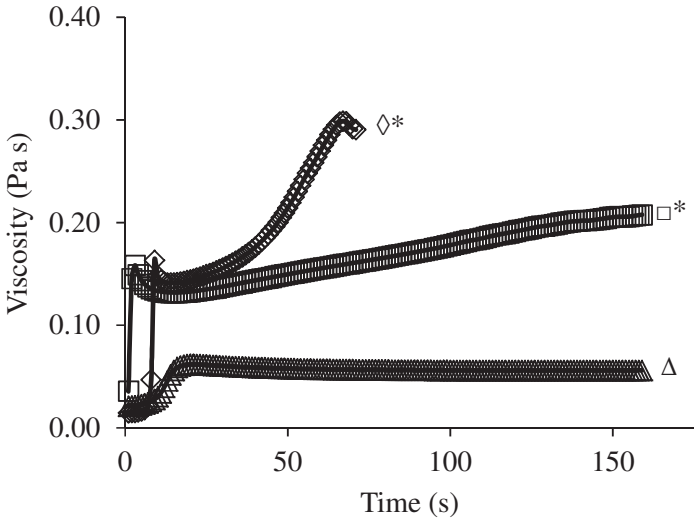


Fig. 5.3. Effect of Tween type (1.6 wt%) on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat, 1 wt% sodium caseinate) measured under steady shear (2000 s^{-1}) at 5 °C. An asterisk indicates the point at which the emulsion was expelled from the gap. Symbols are added at the end of some curves for clarity. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60.

As part of the study described above, aggregation times and peak shear stresses were calculated from the curves of viscosity versus time (Table 5.2). The results indicated that the unsaturated emulsifier Tween 80 decreased emulsion stability to a much greater extent than the saturated emulsifiers. An emulsion with 1.6 wt% T80 was not tested since the emulsion was already unstable at 0.8 wt%. For the saturated emulsifiers, the peak shear stress decreased with increasing fatty acid chain length at both Tween concentrations (0.8 and 1.6 wt%) implying that longer chain fatty acids better prevent fat globule aggregation compared to shorter chain lengths. This trend held true for the extent of aggregation (measured by the peak shear stress) but only partly for the aggregation time. Although T80 had the fastest aggregation time (unstable to shear) and T60 the slowest at both Tween concentrations, T20 and T40 did not follow the chain

length trend. Given that these emulsions were very unstable to shear at 2000 s^{-1}, a less intensive shear treatment may be more appropriate to test the susceptibility to aggregation when destabilisation occurs rapidly.

Table 5.2.
Effect of Tween type and concentration on the shear stability of 35 wt% partially crystalline oil-in-water emulsions measured at 5 °C under constant shear rate (2000 s^{-1}).

Emulsifier	Concentration (wt%)	Aggregation time (s)	Peak shear stress (Pa)
Tween 20	0.8	364.8 ± 20.4	178.2 ± 4.8
Tween 40	0.8	293.1 ± 43.7	47.0 ± 2.4
Tween 60	0.8	Stable	Stable
Tween 80	0.8	Unstable	Unstable
Tween 80/Tween 60	0.4/0.4	5.6 ± 1.7	265.0 ± 18.3
Tween 20/Tween 60	0.4/0.4	61.0 ± 6.2	72.1 ± 3.6
Tween 20	1.6	5.8 ± 1.9	359.6 ± 33.3
Tween 40	1.6	1.5 ± 0.5	329.2 ± 12.7
Tween 60	1.6	10.8 ± 1.3	131.4 ± 7.8
Control	0	Stable	Stable

Cells contain means ± 1 standard deviation. The control contained only sodium caseinate.

By blending the most stable emulsifier (T60) with T20 or T80, it was confirmed that T60 generally promoted greater emulsion stability. In the T80/T60 blend, a measurable aggregation time was found compared to the 0.8 wt% T80 emulsion which aggregated immediately upon shearing. Peak shear stress values however could not be compared since expulsion of the T80 emulsion was virtually immediate upon shearing. In the T20/T60 blend, the aggregation time (61.0 s) was less compared to the 0.8 wt% T20 emulsion (364.8 s). Although the decreased aggregation time implied the T20/T60 emulsion blend was less stable than the T20 emulsion, the emulsion blend never aggregated during shear to a similarly high extent as the T20 emulsion. The peak shear

stress decreased dramatically to 72.1 Pa from 178.2 Pa in the 0.8 wt% T20 emulsion due to the T20/T60 blend never being expelled from the gap during the shearing time applied here (1000 s). The possible mechanisms by which Tween 60 promotes emulsion stability may include promoting the formation of a densely packed interfacial film and/or altering fat crystallization adjacent to the interfacial film (Arima, Ueno, Ogawa, & Sato, 2009; Coupland, 2002; Pilpel, et al., 1988; Skoda & van den Tempel, 1963).

To better understand the impact of Tween type and concentration on the shear stability, emulsions of similar particle size were prepared without sodium caseinate. Interestingly, the shear stability of these emulsions was similar in trend to the emulsions prepared with 1.6 wt % Tween and 1 wt% sodium caseinate both in terms of the aggregation time (Table 5.3) and the peak shear stress (Table 5.4). The results indicate the interfacial film of the 1.6 wt% Tween emulsions with sodium caseinate were similar in terms of barrier properties to the emulsions prepared without sodium caseinate. Hence, the results provide evidence that protein displacement controls the susceptibility of the emulsions to partial coalescence in large part, but differences exist in terms of the effect of the type of Tween which cannot be explained by differences in their effect on adsorbed protein content. One explanation may be that longer chain length Tween emulsifiers penetrate further into the fat globule than shorter chain lengths (Boyd, et al., 1972). Greater penetration depth may interfere with liquid oil release (Hotrum, et al., 2003) and/or promote the formation of more uniform, close packed interfacial film (Pilpel, et al., 1988) thus improving shear stability.

Table 5.3.
Effect of saturated Tween type and concentration on the aggregation time of 35 wt% partially crystalline oil-in-water emulsions at 5 °C prepared with and without 1 wt% sodium caseinate under constant shear rate (2000 s^{-1}).

Emulsifier	Aggregation time (s)		
	1% NaCas		0% NaCas
	0.8% Tween	1.6% Tween	0.8% Tween
Tween 20	364.8 ± 20.4	5.8 ± 1.9	13.3 ± 1.7
Tween 40	293.1 ± 43.7	1.5 ± 0.5	9.4 ± 0.7
Tween 60	Stable	10.8 ± 1.3	13.8 ± 0.3

Cells contain means ± 1 standard deviation

Table 5.4.
Effect of saturated Tween type and concentration on the peak shear stress of 35 wt% partially crystalline oil-in-water emulsions at 5 °C prepared with and without 1 wt% sodium caseinate under constant shear rate (2000 s^{-1}).

Emulsifier	Peak shear stress (Pa)		
	1% NaCas		0% NaCas
	0.8% Tween	1.6% Tween	0.8% Tween
Tween 20	178.2 ± 4.8	359.6 ± 33.3	544.7 ± 192.2
Tween 40	47.0 ± 2.4	329.2 ± 12.7	263.3 ± 26.4
Tween 60	Stable	131.4 ± 7.8	166.7 ± 3.8

Cells contain means ± 1 standard deviation

5.4.3 Effect of Tween 80 and SPAN emulsifiers on emulsion stability

Emulsions containing 1 wt% sodium caseinate and 70% HPKO fat blend were prepared with 1 wt% SPAN in the fat phase and different concentrations of Tween 80 (0.2–0.6 wt%). Over this narrow range of T80 concentration, the emulsions transitioned from shear stable to very unstable (Table 5.5). None of the emulsions containing 0.2 wt% T80 underwent any shear-induced aggregation when sheared at 2000 s^{-1} for up to 1000 s whereas at 0.6 wt% T80 or above, all of the emulsions aggregated immediately when

shear was applied. At the intermediate T80 concentration studied (0.4 wt%), a range of stabilities were found, but unfortunately were not generally reproducible (Table 5.5). Both repetitions of the SPAN 80 containing emulsions for example aggregated under shear but the aggregation times differed by over 400 s. In the SPAN 20 and 40 emulsions, each repetition differed in even broader terms in that one repetition aggregated whilst the other either aggregated immediately or did not aggregate at all. Only in the emulsions with SPAN 60 were the results reproducible but no aggregation occurred in these emulsions.

Table 5.5.
Effect of Tween 80 concentration and SPAN type (1 wt%) on the shear stability of 1 wt% sodium caseinate stabilized 35 wt% partially crystalline oil-in-water emulsions at 5 °C under constant shear rate (2000 s^{-1}).

Emulsifier	0.2% Tween 80		0.4% Tween 80		0.6% Tween 80	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
SPAN 20	S	S	U	A	U	U
SPAN 40	S	S	A	S	U	U
SPAN 60	S	S	S	S	U	U
SPAN 80	S	S	A	A	U	U

Letters: S – Shear stable, no aggregation; A – Shear sensitive, aggregated under shear; U – Shear unstable, aggregated immediately when sheared.

In Fig. 5.4, the aggregation behaviour of one set (first repetition) of emulsions is shown. The relative increase in viscosity provides an indicator of the extent of aggregation. It is evident that the emulsions with SPAN 20 and 80 both aggregated to a much greater degree compared to SPAN 40. Most interestingly, the stability trend of the saturated SPANS (SPAN 20, 40 and 60) mirrors that of the emulsions prepared with only saturated Tween emulsifiers (Section 5.4.3). In both sets of emulsions, the emulsifiers

containing primarily stearic acid (SPAN 60 and T60) were the most stable followed by the emulsifiers containing palmitic acid (SPAN 40 and Tween 40) and lauric acid (SPAN 20 and T20) respectively. Based on the findings that T80 produced more unstable emulsions compared to T20, it was expected that SPAN 20 would also produce more stable emulsions compared to SPAN 80 when used in conjunction with T80. However, the stability trend differed somewhat since the combination of SPAN 20 and T80 was more unstable than the combination of SPAN 80 and T80 in both repetitions.

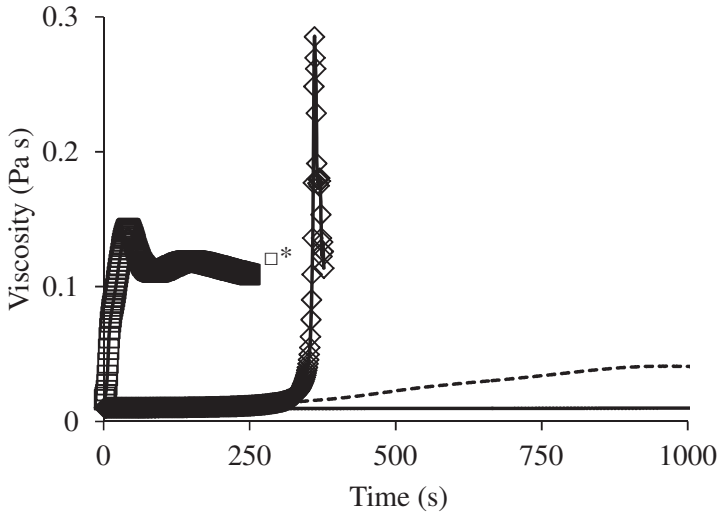


Fig. 5.4. Effect of 0.4 wt% Tween 80 and different types of SPAN on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat) measured at 5 °C and stabilized by 1 wt% sodium caseinate under constant shear (2000 s^{-1}). Symbols: (□) SPAN 20; (- -) SPAN 40; (—) SPAN 60; (◊) SPAN 80.

The poor repeatability of the repetitions could have been due to a variety of reasons including simple sample preparation error. However, the particle size distributions after 24 h of storage at 5 °C were measured and the results indicated that the emulsions which aggregated immediately when sheared had already undergone destabilisation

quiescently during storage (Table 5.6). The shear stable formulations containing 0.2 wt% Tween 80 had very similar mean particle sizes compared to the emulsions prepared without Tween 80 indicating that no destabilisation occurred during storage. On the other hand, all of the emulsions which were shear unstable i.e. emulsions prepared with 0.6 wt% Tween 80, were found to have an increased mean particle size. With 0.4 wt% Tween 80, the emulsions containing SPAN 20 and 40 both had slightly increased mean particle sizes indicating some instability had occurred. It is also worth noting at this point that quiescent destabilisation was not found in any of the emulsions containing 1.6 wt% Tween 20, 40 or 60 only (Section 5.4.2), despite the emulsions being very sensitive to shear. Therefore, the poor repeatability of the shear stability in the emulsions containing SPAN was likely due in large part to greater quiescent instability. Emulsions which are quiescently unstable are more vulnerable to perturbations like small changes in temperature and agitation which could here have occurred during chilled storage or transfer to the rheometer by pipette.

Table 5.6.
Effect of Tween 80 concentration on the volume weighted mean particle diameter ($d_{4,3}$) of emulsions containing different types of SPAN (1 wt%) stored quiescently at 5 °C for 24 h.

Tween 80 concentration (wt%)	SPAN 20	SPAN 40	SPAN 60	SPAN 80
0	0.76 ± 0.05	0.71 ± 0.03	0.71 ± 0.02	0.72 ± 0.04
0.2	0.76 ± 0.05	0.71 ± 0.03	0.70 ± 0.03	0.72 ± 0.03
0.4	5.62 ± 6.87	1.28 ± 0.83	0.73 ± 0.05	0.74 ± 0.07
0.6	16.65 ± 4.73	13.22 ± 0.32	5.09 ± 3.31	18.35 ± 1.97

Cells contain means ± 1 standard deviation.

Quiescent instability is ultimately highly dependent on the extent of protein displacement by the emulsifiers. Adsorbed protein load measurements also indicated substantial variability between repetitions of the SPAN 20 and 40 containing emulsions (Fig. 5.5) which mirrors the variability in the particle size data (Table 5.6). The protein load results suggest the emulsions will be quiescently stable as long as the surface load remains > ~1.0 mg m^{-2}. These results help to explain in part why one repetition of the 0.4 wt% SPAN 20 containing emulsion was unstable and the other shear sensitive (Table 5.5) but they do not explain why for example both repetitions of SPAN 80 containing emulsions (0.4 wt% T80) aggregated under shear whilst both SPAN 60 containing emulsions did not aggregate because both emulsions had nearly identical protein surface loads.

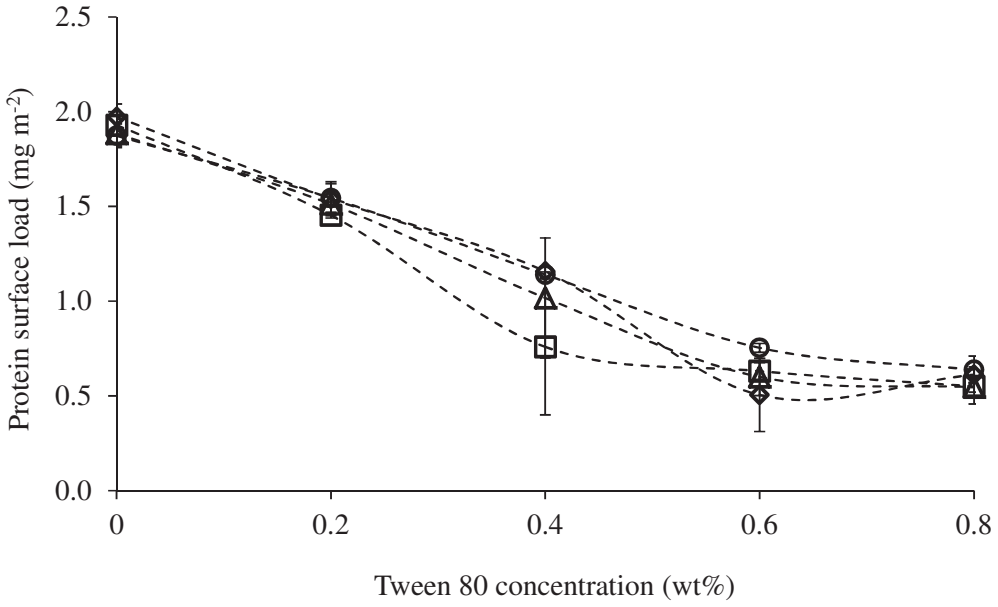


Fig. 5.5. Effect of Tween 80 on the concentration of protein adsorbed at the fat globule-water interface in emulsions with different types of SPAN emulsifier (1 wt% in fat phase). Dashed lines are a guide for the eye. Symbols: (□) SPAN 20; (Δ) SPAN 40; (○) SPAN 60; (◊) SPAN 80.

5.5 Discussion

In general, there is little discussion in the literature on how the properties of interfacial films effect shear-induced aggregation in partially crystalline oil-in-water emulsions. Rather than focus on the interfacial film properties, researchers have typically explained their results in terms of how the potential (usually not measured directly) size and protrusion distance of fat crystals at the oil-water interface alters the stability of partially crystalline oil-in-water emulsions (Rousseau, 2000). Studies on liquid oil-in-water emulsions can be utilized however as a basis for predicting how these films would function in a partially crystalline fat globule emulsion. In liquid oil droplets, there is general agreement that more densely packed and thicker interfacial films better prevent

droplet coalescence compared to loosely packed or disordered thin films. When droplets collide, it is envisioned that densely packed interfaces partly absorb the energy of the collision and either resist fracture or yield at a greater energy threshold (Bos & van Vliet, 2001). Disorganized interfacial films may more easily fracture or yield, thus allowing for greater droplet deformation and opportunity for film rupture leading to droplet coalescence. For completeness, it is worth noting that the lateral mobility and rate of adsorption of low molecular weight emulsifiers to "empty" sites on the oil-water interface formed in response to deformation are also of great importance in terms of the emulsifier's ability to stabilise liquid oil droplets (Walstra 2003). However for partial coalescence, the mechanical properties of the interfacial layers are likely of greater importance due to the rough fat globule surface and low deformability of high SFC partially crystalline fat globules compared to liquid oil droplets.

In partially crystalline oil-in-water emulsions, certainly the deformation of the fat globule upon collision is limited by the internal crystal structure of the droplet. Therefore the dissipation of the globule collision forces would be less and greater stress would be placed on the interfacial film as a result. Furthermore, local surface roughness caused by the crystalline fat likely would have a "pin" effect where the local pressure on the tip of a protruding fat crystal or rough surface site would be much greater and thus lead to a higher likelihood of interfacial film rupture (Golemanov, Tcholakova, Denkov, & Gurkov, 2006). In this way, viscous or yielding films which flow under stress are expected to be poor stabilisers whereas elastic films should provide better stability due to their inhibitory effect on film rupture.

However, there are further complications due to the solid fat content of the fat globule, fat crystal properties and the polymorphic nature of some emulsifiers. A simple but

unrealistic model for the interfacial layers of fat globules is one where individual layers of fat crystals, oil soluble emulsifier and water soluble emulsifier exist at the interface. In reality, the crystalline triglycerides, oil soluble emulsifiers and water soluble emulsifiers interlink to form interfacial layers ranging in thickness from approximately 5–10 nm (Pilpel, et al., 1988). These layers may also contain liquid oil and/or water (lamellar gel phase) depending on the types of emulsifiers present and hence be thicker and exhibit unique mechanical properties (Westerbeek & Prins, 1991). The extent of complexity of the interface in "real" food emulsions is usually not known because techniques to dynamically measure these interfacial film structures *in situ* either do not exist or are in their infancy. Scanning microbeam small angle X-ray diffraction for example is a relatively new technique which can measure the polymorphic crystal structure and arrangement at the interface (Arima, Ueno, Ogawa, & Sato, 2009).

A more in depth understanding of the true nature of partially crystalline interfacial layers would greatly enhance the understanding of how partial coalescence may occur. For example, Hotrum, Stuart, van Vliet, & van Aken (2003) studied how different protein films covering oil droplets fracture at an air-water interface and how liquid oil flows following fracture to cover the interface. Relevant to partial coalescence, the authors discussed how the pore size within a fat crystal network may prevent liquid oil from flowing out of the droplets by capillary action. They calculated that if all of the pores were less than 4 nm the capillary force should be great enough to hinder flow out of a fat globule (Hotrum, Stuart, van Vliet, & van Aken, 2004). Although the authors did not attempt to produce an emulsion with these small pore sizes in the interfacial film (only protein films were studied), it seems plausible that densely packed interfacial films composed of low molecular weight emulsifiers would have less than 4 nm between molecules. Furthermore, emulsifiers which themselves crystallise or form

liquid crystalline phases should certainly provide a barrier to liquid oil release. Interfacial heterogeneous nucleation may also be a possible avenue to produce small, more ordered crystals at an oil-water interface.

The ability of emulsifiers to pack into dense orderly structures depends in large part on the relative size and shape of the hydrophilic headgroup and lipophilic tailgroup. These influence the so-called shape compatibility, the extent to which the tailgroups associate and the extent to which the headgroups repel each other (Lu, et al., 2000). Pilpel, et al. (1987) studied the rheology and microstructure of 10–70 wt% sunflower oil-in-water emulsions stabilized by different ratios of SPAN 40 and Tween 40 at emulsifier concentrations from 1–10 wt%. The authors examined the flow behaviour of the emulsions as well as hysteresis in the flow curves in an effort to capture the effect of emulsifier concentration and SPAN/Tween ratio on the flow behaviour. Hysteresis was first found at 3 wt% emulsifier and the hysteresis loop area increased with increasing emulsifier concentration. Pronounced pseudoplastic behaviour was exhibited 5 wt% emulsifier and above. The hysteresis loop area in emulsions with SPAN/Tween ratios of 3/7, 4/6, 6/4 and 7/3 were studied at each emulsifier concentration. The transition from ratios of 4/6 to 6/4 was associated with approximately a 4-fold increase in hysteresis loop area. This 4-fold increase was consistent across emulsifier concentration from 4–10 wt%. Notably, the change in hysteresis loop area from 3/7 to 4/6 or 6/4 to 7/3 was negligible across the same emulsifier concentration range (4–10 wt%). From electron micrographs, it could be deduced that the more viscoelastic flow behaviour at high SPAN/Tween ratios was due to the formation of lamellar liquid crystals. Although the authors supported that the liquid crystals may have formed in the oil and aqueous phases, no liquid crystals were present above 50 wt% oil which indicates the concentration of hydrophobic SPAN 40 needs to exceed a critical concentration in the

oil phase in order to form crystals. In a separate study, Pilpel, et al. (1988) also investigated the stability of the emulsions and found that the best stability was exhibited for an emulsifier weight ratio of 6/4 or 5:1 molar ratio. The authors supported the best stability was found in this ratio due to the packing arrangement of the molecules. The large hydrophilic headgroup of Tween 40 located on the aqueous phase side of the interface hinders close packing of the fatty acid tail groups. Between the Tween molecules, 5 SPAN molecules may fit well due the smaller hydrophilic head group of SPAN and thus create an optimally packed interfacial layer for stability.

In this study, it is reasonable to assume that differences in packing arrangements between the emulsifiers were present and these differences altered the susceptibility to partial coalescence. Furthermore, crystallization of SPAN 40 and 60 is possible given their high melting points and this could in turn alter the stability as well. The formation of liquid crystalline interfacial layers however was unlikely given the much lower total emulsifier concentration used here ($\leq$ 1.6 wt%) compared to Pilpel, et al. (1988) ($\geq$ 5 wt%). Further research is needed to characterize the possibly complex interfacial behaviour of these partially crystalline oil-in-water emulsions containing Tween 80 and different SPAN emulsifiers. Possibly SPAN 40 and SPAN 60 produced more stable emulsions at 0.4 wt% due to their crystallization at the oil-water interface and better packing compared to SPAN 20 and SPAN 80, however Tween 40 and Tween 60 also formed more stable emulsions compared to Tween 20 and Tween 80 despite not undergoing a phase transition. This finding points to a more general mechanism governing the stability of these emulsions unrelated to crystallization of the emulsifier but a consequence of the chain length of the fatty acid tail group and the presence of unsaturated bonds. In the following chapter, the effect of Tween chain length and

saturation will be studied in more detail to better understand the role of these properties on both fat crystallization, protein displacement and interfacial film properties.

5.6 Conclusion

Longer chain length saturated Tween emulsifiers like Tween 40 and Tween 60 better prevent shear-induced aggregation compared to shorter chain length (Tween 20) and unsaturated (Tween 80) Tween types. A more complex stability relationship was found for combinations of Tween 80 and different types of SPAN. The transition of these SPAN containing emulsions from stable to unstable occurred over a narrow range of Tween 80 (0.2–0.6 wt%). Emulsions with saturated long chain length SPAN 40 or 60 were more stable compared to emulsions with unsaturated SPAN 80 or saturated short chain SPAN 20. Notably, crystallisation of the SPAN emulsifier did not appear to significantly improve emulsion stability. This study supports that in general saturated long chain length fatty acid emulsifiers better prevent partially crystalline emulsion destabilisation but the mechanism(s) by which they promote stability are not clear from this work.

Chapter 6: Effect of Tween emulsifiers on the shear stability of hydrogenated palm kernel oil/canola oil-in-water emulsions stabilized by sodium caseinate

6.1 Abstract

Having established in Chapter 5 that saturated long chain length Tween emulsifiers reduce the susceptibility to partial coalescence better than short chain, further research was conducted to identify how Tween emulsifiers alter emulsion stability. The effect of protein, emulsifier and fat interactions on the susceptibility of 35 wt% partially crystalline oil-in-water emulsions to shear-induced aggregation was systematically investigated. The effects of Tween 20, 40, 60 and 80 (0.8–1.6 wt%) on the stability of emulsions prepared with and without 1 wt% sodium caseinate were studied. Generally, 1 wt% sodium caseinate emulsions with the unsaturated emulsifier Tween 80 were the most unstable followed by the saturated emulsifiers Tween 20, 40 and 60 in order of increasing fatty acid chain length. Long chain saturated Tween emulsifiers (Tween 40 and 60) improved emulsion stability regardless of whether sodium caseinate was present indicating that alone these Tweens form more robust interfacial films compared to the shorter chain length Tween 20 and the unsaturated Tween 80. The Tween type dependent effect on supercooling and fat crystallization caused by interfacial heterogeneous nucleation was also investigated using pulsed NMR. With sodium caseinate, the degree of supercooling decreased and the crystallization rate diminished with increasing saturated fatty acid chain length but only negligible changes were found without sodium caseinate. These findings indicate that long chain saturated Tweens improve emulsion stability by forming robust interfaces but with sodium caseinate also improve stability through interfacial heterogeneous nucleation. These novel findings provide guidance on how combinations of proteins and emulsifiers can be used to

modify and control the stability of partially crystalline oil-in-water emulsions through their combined effects on fat crystallization and interfacial film properties.

6.2 Introduction

The relative importance of the factors affecting the susceptibility of partially crystalline fat globules to partial coalescence is important to the food industry. It is inherently difficult to link emulsion stability with a single destabilisation mechanism due to the many interactions between the ingredients in typical partially crystalline oil-in-water food emulsions. For example, emulsifiers alter the interaction potential between fat globules by both complexing and competitively adsorbing with proteins at the oil-water interface (Bos, Nylander, Arnebrant, & Clark, 1997). Emulsifiers also alter the mechanical properties of the interfacial film by adopting different molecular arrangements such as liquid, liquid crystalline or crystalline phases (Krog, 1997). Additionally, the emulsifiers may alter fat crystallization near the interface by serving as nucleation sites for fat crystals through interfacial heterogeneous nucleation (Krog, 1997; Skoda & van den Tempel, 1963).

In this chapter, the role of several Tween emulsifiers (polyoxyethylene sorbitan fatty acid esters) on the shear stability of partially crystalline oil-in-water emulsions prepared both with and without 1 wt% sodium caseinate was examined. Three types of saturated Tween (Tween 20 [laurate], 40 [palmitate] and 60 [stearate]) and 1 unsaturated Tween (Tween 80 [oleate]) were studied. These emulsifiers all contain a hydrophilic polyethoxylated sorbitan ester head group and only differ in the fatty acid tail group and range in HLB from 14.9–16.7 (Table 5.1). Although the stability of partially crystalline oil-in-water emulsions containing Tween emulsifiers has been studied before (Ariyaprakai, Limpachoti, & Pradipasena, 2013; Palanuwech & Coupland, 2003;

Tangsuphoom & Coupland, 2009; Thanasukarn, Pongsawatmanit, & McClements, 2006; Truong, Bansal, Sharma, Palmer, & Bhandari, 2014), the authors are not aware of a comparative study of the effects of Tween emulsifiers on partial coalescence. In general, the susceptibility of partially crystalline oil-in-water emulsions stabilized by sodium caseinate to partial coalescence increases with increasing Tween concentration due to protein displacement (Thivilliers-Arvis, Laurichesse, Schmitt, & Leal-Calderon, 2010). Tween emulsifiers displace adsorbed protein from the oil-water interface according to an orogenic mechanism where the emulsifier adsorbs within the continuous protein film at low emulsifier-to-protein molar ratios (Woodward, Gunning, Mackie, Wilde, & Morris, 2009). Above a critical concentration of Tween such as used in this study, the majority of the protein but not all is displaced (Woodward, Gunning, Mackie, Wilde, & Morris, 2009). The Tween-rich domains within the protein film may then serve as sites for partial coalescence due to the poor stabilizing ability of Tween compared to protein (Dalgleish, 2006; Walstra, 2003).

However there are several examples of studies where stable emulsions were produced despite containing low amounts of adsorbed protein at the oil-water interface (Davies, Dickinson, & Bee, 2001; Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014; Pelan, Watts, Campbell, & Lips, 1997). In these studies, the emulsions contained a combination of proteins and emulsifiers including different types of monoglycerides. Generally, the authors concluded that emulsion stability at low protein load depended on the type of monoglyceride due to its effect on fat crystallization and/or the properties of the interfacial film. For example, Munk, et al. (2014) studied 25 wt% hydrogenated palm kernel oil-in-water emulsions containing sodium caseinate, sugar, sorbitol and monoglycerides. At high monoglyceride concentration (0.5–1.0 wt%), emulsion texture depended on the type of monoglyceride added. Saturated monoglyceride (GMS), lactic

acid ester of monoglyceride (LACTEM) and unsaturated monoglyceride (GMU) resulted in liquid, semisolid or solid textures respectively. The authors proposed the differences in emulsion stability were caused by changes in the interfacial film properties rather than specifically adsorbed protein content. Specifically, GMS may have caused Pickering stabilization due to direct crystallization at the interface, LACTEM may have formed an α-crystalline gel at the interface and GMU may have promoted crystal protrusion from the interface leading to greater instability.

Unlike monoglycerides, Tween emulsifiers do not undergo any temperature dependent phase transitions (Gulseren & Coupland, 2008). Therefore, Tween emulsifiers are reported to destabilize partially crystalline oil-in-water emulsions by either simply displacing protein from the interface or modifying the fat crystal properties (Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt, 2008). Thivilliers, et al. (2008) used AFM to show how Tween 20 (5 wt%) increased the surface roughness of anhydrous milk fat globules compared to sodium caseinate stabilized globules. Crystal protrusion from the interface or simply greater surface roughness is expected to increase the susceptibility of fat globules to partial coalescence through an increase in the ease of rupture of the stabilizing interfacial film when the globules come into close contact (Golemanov, Tcholakova, Denkov, & Gurkov, 2006; Walstra, 2003). In addition to altering fat globule surface roughness, the chain length of low molecular weight emulsifiers including Tween have been shown to alter the degree of supercooling in hydrocarbon and triglyceride emulsions through promotion of interfacial heterogeneous nucleation (Golemanov, et al., 2006; McClements, Dungan, German, Simoneau, & Kinsella, 1993; Skoda, et al., 1963; Truong, et al., 2014). Golemanov, et al. (2006) provided clear evidence that the stability of paraffin-in-water emulsions increased with increasing fatty acid chain length but the mechanism by which interfacial heterogeneous

nucleation promoted stability was not investigated. Arima, Ueno, Ogawa, & Sato (2009) however showed that a sucrose ester enhanced interfacial heterogeneous nucleation of a Tween 80 stabilized emulsion. The authors found interfacial heterogeneous nucleation enhanced parallel orientation of the fat crystal lamella at the oil-water interface. Presumably, parallel fat crystal orientation would render the emulsions less susceptible to partial coalescence by creating a smoother fat globule surface with fewer protruding crystal sites capable of rupturing the interfacial film of another fat globule upon collision.

In this chapter, the stability of partially crystalline oil-in-water emulsions containing very low adsorbed protein content that exhibit drastically different stability dependent on the type of Tween emulsifier present were examined. Emulsions with low protein loads were studied in order to minimize the effect of differences in protein displacement between different Tween emulsifiers at equivalent concentration and in addition to ensure that the emulsions destabilized during shear over a similar time scale. Since Tween 20, 40, 60 and 80 do not themselves undergo any phase transitions at the oil-water interface over the temperature range studied here and are of very similar molecular structure, these emulsifiers are an excellent group for investigating the specific mechanisms by which proteins, emulsifiers and fat interact and alter the stability of partially crystalline oil-in-water emulsions.

6.3 Materials and methods

6.3.1 Ingredients

The ingredients used in this chapter were previously described in Section 3.3.1.

6.3.2 Emulsion preparation

Emulsions were prepared according to the method described in Section 3.3.2 with modifications. A stock, protein stabilized oil-in-water emulsion was first prepared for each emulsion. Sodium caseinate (5 wt%) was dispersed by gentle stirring at room temperature overnight. The dispersed phase of the sodium caseinate stabilized stock emulsion contained a blend of 70% HPKO and 30% canola oil prepared by gentle stirring of the melted fats. The solid fat content of the fat blend at 5 °C was 61.9 ± 0.2 as measured by the direct pulsed NMR method. The stock emulsions ($d_{3,2}$ = 0.59 ± 0.04 μm) contained 1.5 wt% sodium caseinate, 52.5 wt% fat and 0.02 wt% sodium azide. Each stock emulsion was stored overnight at room temperature before use.

Working emulsions (25 g) were prepared in 50 ml tubes by diluting the stock emulsion with 5 wt% Tween solutions and water at room temperature. The Tween solutions were prepared in 30 mM imidazole buffer (pH 6.8) by heating the solution to 65 °C on a stir plate and cooling overnight under gentle stirring. After mixing the working emulsions, the samples were heated in a 65 °C water bath for 30 min to melt any fat crystals that may have formed. The tubes were then transferred to an ice-water bath for 15 min to reduce the temperature to approximately 5 °C before storing them at 5 °C. Each working emulsion contained 1 wt% sodium caseinate and 35 wt% fat (pH 6.8) and was tested after at least 24 h of storage at 5 °C. All the emulsions studied were prepared and tested at least in duplicate.

In one experiment, the working emulsions were prepared using stock emulsion and Tween solution stored previously at 5 °C for 24 h. In order to study how the adsorption of Tween onto a pre-crystallized globule impacted emulsion stability, no heat treatment was applied.

6.3.3 Shear stability and flow behaviour

The critical shear stress causing fat globule aggregation was determined using a shear stress sweep (1–60 Pa at 1 Pa s^{-1}) in a stress-controlled rheometer (MCR 301 Anton Paar GmbH, Germany) with a truncated cone-and-plate geometry (50 mm diameter, 2° angle, 47 μm gap). The critical shear stress was determined from the viscosity profile as the intersection point of the extrapolated rapid rise in viscosity and the baseline viscosity. The shear stability of some emulsions was also determined under constant shear rate (2000 s^{-1}) as described in Section 3.3.5. In a separate set of experiments, the flow behaviour at 5 °C was determined using sequential increasing and decreasing shear rate sweeps from 0.1–200 s^{-1}. In all studies, the temperature was maintained at 5 °C by a Peltier temperature controlled plate and hood. The cone and pipettes were pre-chilled before use to help maintain the emulsion temperature at 5 °C.

6.3.4 Free fat content

The free fat content of emulsions subjected to only chilling (5 °C) and chilling followed by shearing was measured using a modified version of the dyed oil method described by Palanuwech, Potineni, Roberts, and Coupland (2003). In this method, an aliquot of dyed liquid oil is added to an emulsion and mixed thoroughly. Any free oil mixes with the dyed oil and dilutes the dye concentration. After centrifugation to collect the dyed oil, the change in absorbance before and after mixing with the emulsion can be used to determine the concentration of free oil transferred from the emulsion to the dyed oil.

In this study, the dyed oil method was adapted for measuring the free fat released from ~1.2 ml of emulsion sheared in a cone and plate geometry. After shearing, the cone was left in place and the emulsion was quickly heated to 65 °C on the Peltier plate in order

to melt the fat. The cone was then promptly raised and 720 µl of dyed molten fat (0.0015 wt% Oil Red O in 70% HPKO fat blend) was pipetted and mixed with the emulsion using the pipette tip. The mixture was then pipetted off the plate into a 2 ml microcentrifuge tube and stored at 65 °C until the remaining emulsions were sheared and collected. Each tube was then vortex mixed for 30 s before centrifugation at 15,000 x *g* for 10 min. The tubes were then stored in a 65 °C oven during the transfer of the melted dyed fat to 96 well microplates. Three 200 µl aliquots of each sample were transferred to the microplate. Sealed microplates were held at 65 °C for 30 min then immediately measured for absorbance at 517 nm in a microplate reader (Multiskan Go, Thermo Fisher Scientific, USA). At room temperature, the separated dyed oil generally turned turbid indicating the presence of crystalline fat. Therefore, the absorbance was measured above the melting point of the fat so that no crystals would interfere with the measurement by scattering light.

To measure the free fat content of unsheared emulsions, 1.3 ml of working emulsion was transferred to a 2 ml microcentrifuge tube, heated to 65 °C for 15 min then stored at 5 °C for 24 h. Next, 670 µl of molten dyed fat was added before transferring the tubes to a 65 °C water bath for 15 min. The tubes were then vortex mixed, centrifuged and analysed as described above.

The free fat content of each set of emulsions was determined using a standard curve generated from a series of known concentrations of dyed fat diluted with undyed fat. Each concentration was measured in triplicate. A separate standard curve was produced for each microplate.

From the author's experience with this method, a systematic error in the dyed fat absorbance after mixing the dyed fat with the emulsions was identified. The absorbance

increased after mixing the dyed fat with a sodium caseinate stabilized emulsion (unchilled and unsheared) which should not contain any free fat. The increase in absorbance indicates the dyed fat increased in dye concentration however this was not possible. Therefore, some sort of contamination likely caused the systematic error but the source was not identified. Nevertheless, the systematic error was eliminated by normalizing the data by subtracting the free fat content of the sodium caseinate stabilized emulsion (~-2% free fat) from all free fat content measurements. In this way, the free fat content measurements essentially compare all of the emulsions against an emulsion expected to not contain any free fat.

6.3.5 Solid fat content

The solid fat content (SFC) of emulsions was measured by low resolution pulsed NMR using The Minispec MQ20 (Bruker Optics, GmbH, Rheinstetten, Germany). SFC was determined by the standard direct method using a 90° pulse, 2 s recycle delay time and the standard algorithm supplied by Bruker. NMR tubes were filled to a height of 25 mm with emulsion and placed in a 65 °C heating block for 30 min. The tubes were then transferred to the temperature controlled NMR probe maintained at 5 °C. Scans were performed every 60 s for 1 h. Each emulsion was measured in duplicate although the variation between duplicate samples was negligible. The cooling rate was measured offline using a thin wire thermocouple positioned in the centre of the sample. The SFC of bulk fat was measured according to the method described in Section 4.3.4.

6.4 Results and discussion

6.4.1 Emulsion stability under shear

Partially crystalline oil-in-water emulsions stabilized by 1 wt% sodium caseinate and 0.8, 1.2 or 1.6 wt% Tween emulsifier were found to have a very broad susceptibility to shear-induced aggregation over this range of Tween concentration. In order to study the shear stability, more intensive shear was required to destabilize 0.8 wt% Tween emulsions compared to the less shear stable emulsions containing 1.2 or 1.6 wt% Tween. Emulsions with 0.8 wt% Tween were subjected to a constant shear rate (2000 s^{-1}) and were found to range from completely unstable to stable depending on the type of added Tween (Fig. 6.1A). Emulsions with the unsaturated emulsifier T80 aggregated immediately when sheared and were consequently expelled from the gap thereafter due to large scale fat globule aggregation. Likewise, T20 emulsions aggregated and were expelled from the gap but only after approximately 330 s of shearing time. T40 emulsions also aggregated but the process was arrested as indicated by the small plateau in viscosity after 550 s of shear. T60 emulsions did not aggregate even after shearing up to 3000 s. Shear-induced fat globule aggregation or the lack thereof was also verified by measuring the change in particle distribution by laser light scattering. In summary, the shear stability trend for different types of Tween at 0.8 wt% was T60 > T40 > T20 > T80.

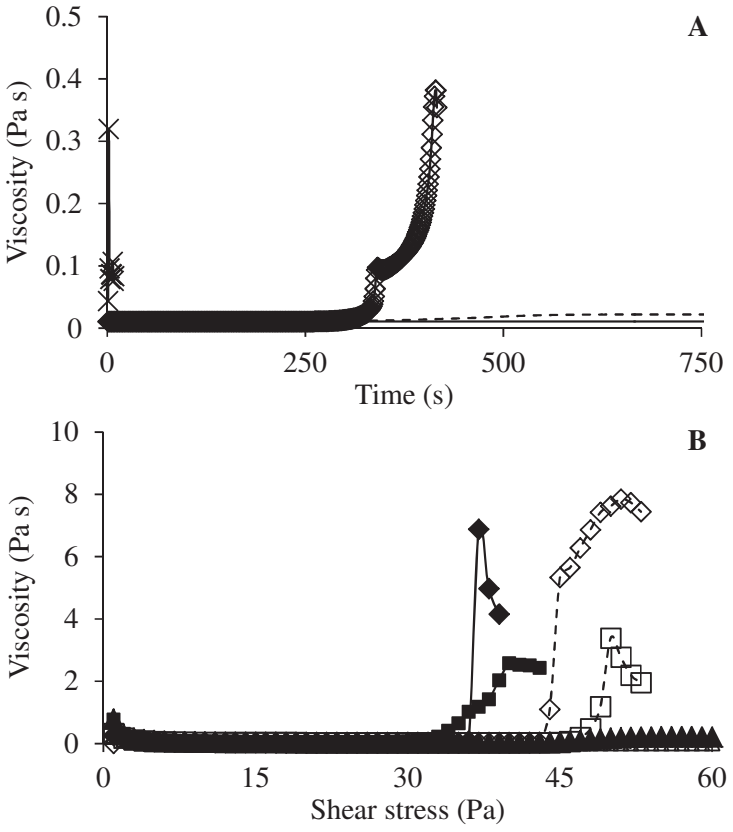


Fig. 6.1. Effect of Tween type and concentration on the shear stability of partially crystalline oil-in-water emulsions (35 wt% fat) at 5 °C stabilized by 1 wt% sodium caseinate. Typical plots showing the response to shear are shown. (A) Relatively more shear stable emulsions containing 0.8 wt% Tween were sheared over time at 2000 s^{-1}. Symbols: (◊) Tween 20; (- -) Tween 40; (—) Tween 60; (X) Tween 80. (B) Less shear stable emulsions containing 1.2 wt % (open symbols) or 1.6 wt% (closed symbols) Tween were subjected to a shear stress sweep (1 Pa s^{-1}). Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60.

Under constant shear rate, fat globules in shear sensitive emulsions continuously collide with one another and form aggregates which grow in size over time (Fuller, Considine, Golding, Matia-Merino, & MacGibbon, 2015). The kinetics of this aggregation process

depends on the susceptibility of the fat globules to aggregation (Walstra, 2003). Once the clusters grow in size and number sufficient to form a crowded environment, a rapid increase in viscosity may occur due to the transition from fluid to semisolid (Fuller, Considine, Golding, Matia-Merino, & MacGibbon, 2015).

At higher concentrations of Tween emulsifier, the constant shear rate test used above was also capable of differentiating the emulsions in terms of shear stability; however, aggregation occurred within a few seconds for the saturated Tween emulsifiers so a less shear intensive method was utilized in order to destabilize the emulsions over a longer time scale. Furthermore, emulsions with T80 were found to be very vulnerable to aggregation quiescently at higher Tween concentrations so these emulsions were not studied further under shear. Emulsions with each type of saturated Tween emulsifier (T20, T40 and T60) were subjected to a shear stress sweep from 1–60 Pa at 1 Pa s^{-1}. Under an increasing shear stress, the fat globules are forced together more strongly (Walstra, 2003). In theory, this should result in the sudden onset of aggregation once a critical shear stress is reached (Davies, Dickinson, & Bee, 2000). For example, the 0.8 wt% T80 emulsion in Fig. 6.1A aggregated immediately because the shear stress at 2000 s^{-1} was greater than the critical value required to force the highly shear sensitive fat globules into close contact.

The critical shear stress causing fat globule aggregation was dependent on both emulsifier concentration and type (Fig. 6.1B). At 0.8 wt%, no aggregation was found regardless of Tween type (data not shown). At 1.2 wt%, T20 emulsions aggregated at the lowest shear stress compared to T40 emulsions. T60 emulsions on the other hand remained stable up to 60 Pa. At 1.6 wt%, all of the emulsions aggregated however the

T60 emulsions only slightly thickened indicating the extent of aggregation was much less compared to T20 and T40.

Interestingly, the critical shear stress causing aggregation followed the same trend in terms of Tween type at 1.2 wt% Tween (Table 6.1) compared to the aggregation time at 0.8 wt% (Fig. 6.1A). This shear stability trend (T60 > T40 > T20) indicates that the shear stability increases with increasing fatty acid chain length. The saturated Tween emulsifiers T60, T40 and T20 contain primarily stearic acid (C18:0), palmitic acid (C16:0) and lauric acid (C12:0) respectively. However, this trend changed at 1.6 wt% Tween to T60 > T20 > T40. Also note, the same trend was found for aggregation time in Chapter 5 (Table 5.2) when 1.6 wt% emulsions were sheared at 2000 s^{-1}. To better understand this process, 0.8 wt% Tween emulsions ($d_{3,2}$ = 0.64 ± 0.01 μm) without sodium caseinate were prepared and studied. Without sodium caseinate, the shear stability trend mirrored that of the 1.6 wt% Tween emulsions. The similarity in the trend of sodium caseinate-free emulsions and 1.6 wt% Tween emulsions containing sodium caseinate indicates both emulsions contained similar interfacial film structures. At 1.2 wt% Tween or less, there appears to be a Tween type dependent interaction with sodium caseinate leading to altered stability compared to emulsions prepared without sodium caseinate.

Table 6.1.
Effect of Tween type and concentration on the critical destabilizing shear stress (Pa) of 35 wt% partially crystalline oil-in-water emulsions measured at 5 °C during a shear stress sweep (1–60 Pa at 1 Pa s^{-1}).

	1% Sodium caseinate			0% Sodium caseinate
Emulsifier	0.8% Tween	1.2% Tween	1.6% Tween	0.8% Tween
Tween 20	Stable	43.5 ± 0.5	35.0 ± 1.0	36.3 ± 1.7
Tween 40	Stable	47.0 ± 2.0	28.7 ± 1.8	34.5 ± 0.5
Tween 60	Stable	Stable	43.8 ± 0.4	39.0 ± 0.0

Means ± standard deviation of two replicates

The shear stress sweep data also indicated that the extent of aggregation measured by the peak shear viscosity differed depending on Tween type (Table 6.2). There was a clear trend indicating that increasing fatty acid chain length promoted lower peak shear viscosities. Notably, this trend also held for the emulsions prepared without sodium caseinate indicating that longer chain saturated Tween emulsifiers better protect against fat globule aggregation once aggregation is initiated by shear.

Table 6.2.
Effect of Tween type and concentration on the peak viscosity (Pa s) of 35 wt% partially crystalline oil-in-water emulsions measured at 5 °C during a shear stress sweep (1–60 Pa at 1 Pa s^{-1}).

	1% Sodium caseinate			0% Sodium caseinate
Emulsifier	0.8% Tween	1.2% Tween	1.6% Tween	0.8% Tween
Tween 20	Stable	6.14 ± 1.32	9.10 ± 2.85	9.50 ± 1.43
Tween 40	Stable	1.69 ± 1.58	3.32 ± 1.03	4.60 ± 0.20
Tween 60	Stable	Stable	0.25 ± 0.04	3.60 ± 0.13

Means ± standard deviation of two replicates

According to Graca, Bongaerts, Stokes, & Granick (2007), Tween emulsifiers form interfacial films ranging from approximately 2–3 nm in thickness depending on the conformation of the emulsifier at the interface. Although T80 and T20 differ in terms of

chain length, they form interfacial films of ~2 nm regardless of concentration. Graca, et al. (2007) proposed this was caused by the T80 fatty acid tail adopting a flatter, parallel orientation at the interface compared to the saturated Tween emulsifiers which adopt a perpendicular orientation and form thicker, more densely packed films as a result. These differences may be less important for liquid oil emulsions since the fatty acid tails protrude into the oil phase. But when crystallized, the crystalline fat globule surface may act as a barrier to the lipophilic tails leading to adsorption and packing arrangements more similar to those found on hard surfaces. Therefore, the findings which show that on their own saturated longer chain lengths promote better shear stability may in part be explained by the Tween type dependent differences in film thickness, packing arrangement and film density.

Collectively, the results above indicate that longer saturated fatty acid chain lengths promote better emulsion stability in the presence of sodium caseinate in terms of when the onset of aggregation occurs and the extent of aggregation thereafter. However, the trend does not hold for 1.6 wt% Tween emulsions containing sodium caseinate or in sodium caseinate-free emulsions. Presumably, with increasing Tween concentration and dependent on Tween type, subtle changes in the interfacial film structure occurred which were not detected by simply measuring the adsorbed protein load using the depletion method. Certainly, relative surface coverage on its own could not be correlated to changes in emulsion stability, since according to protein load measurements the concentration of residual protein at the interface was independent of emulsifier type or concentration (within the studied range). However, these subtle changes in interfacial film properties have yet to be determined as providing an explanation as to why such large differences in shear stability were found.

6.4.1.1 Free fat content

In order to better explain the Tween type dependence on shear stability, the free fat content was measured after shearing the same emulsions described above using a shear stress sweep. When partially crystalline fat globules aggregate through partial coalescence, liquid oil is thought to flow between and connect fat globules together (Walstra, 2003). Under shear, the simultaneous processes of partial coalescence and aggregate break up lead to the liberation of liquid oil and perhaps crystalline fat from the clusters of partially coalesced globules. After heating above the melting point of the crystalline fat, the "free fat" coalesces and may visibly phase separate through a process commonly called oiling off. Several methods have been developed to quantify free or extractable fat (Evers, 2004; Palanuwech, Potineni, et al., 2003; Pelan, et al., 1997; Vanapalli, Palanuwech, & Coupland, 2002). A modified dyed oil extraction method based on the method of Palanuwech, Potineni, et al. (2003) was developed for the purpose of measuring the free fat content of small volumes of emulsions (~1.2 ml) sheared using a cone-and-plate geometry. As controls for the shearing effect, the free fat content of unsheared but previously chilled (24 h at 5 °C) emulsions were also measured. The free fat content measurements were normalized against the free fat content of an emulsion not expected to have any free fat i.e. a 1 wt% sodium caseinate emulsion neither sheared nor chilled.

The unsheared emulsions with T40 and T60 generally contained less than 2% free fat irrespective of Tween concentration (Fig. 6.2A). The free fat content of unsheared emulsions with T20 however increased with increasing Tween concentration from < 1% at 0.8 wt% T20 to 6.1% at 1.6 wt% T20. From the author's experience using this method, measurement errors of 1–2% are typical so the high amount of free fat

encountered in the 1.6 wt% T20 emulsion was surprising given that the emulsion was not sheared when it contained crystalline fat. It is worth noting that only negligible changes in the particle size distribution of the T20 emulsions before and after chilling were found so the apparent increase in free fat could not be explained by fat globule aggregation. Based on the author's experiences with these emulsions, it is reasonable that the unsheared T20 emulsions contained free fat but there is not an adequate explanation for the origin of its release during chilling.

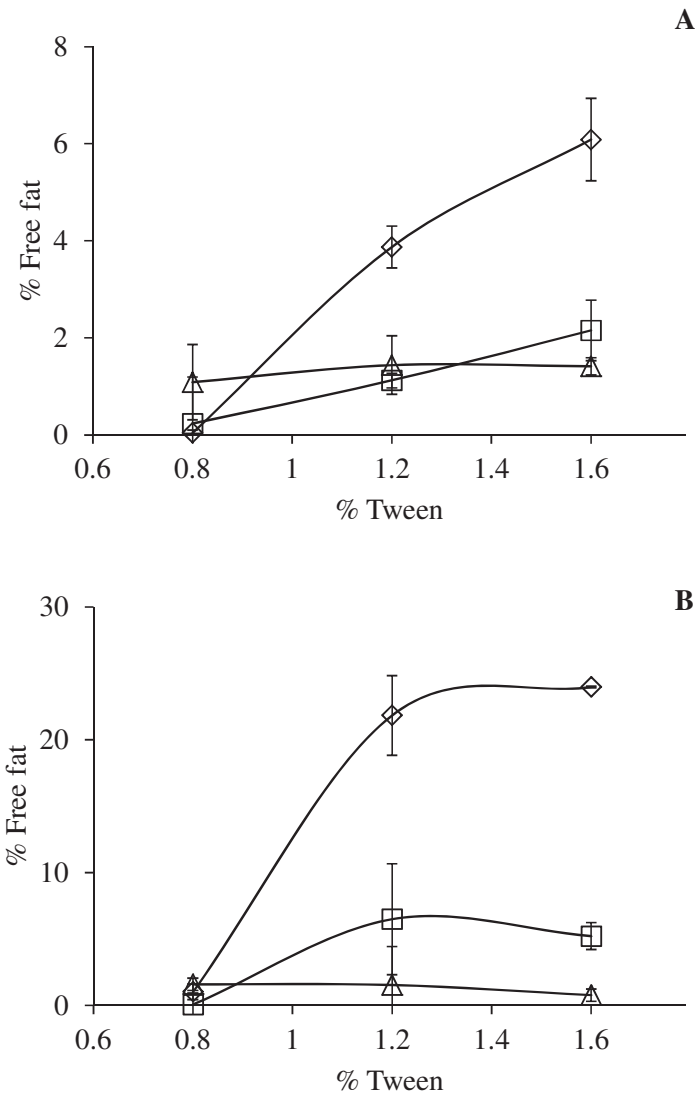


Fig. 6.2. Free fat content in 35 wt% partially crystalline oil-in-water emulsions stabilized by 1 wt% sodium caseinate and Tween emulsifier either (A) unsheared or (B) sheared at 5 °C. Solid lines are a guide for the eye. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60.

After shearing the emulsions containing 0.8 wt% Tween, the free fat content was measured but no increase over the unsheared controls was found irrespective of Tween type (Fig. 6.2B). At higher concentrations of Tween however, very large increases in

free fat were found which increased nonlinearly with Tween concentration. These results agree well with the theory that free fat is released as a consequence of partial coalescence and aggregate break up during shear (Precht, 1988). By comparing Table 6.2 and Fig. 6.2B, there was a clear correlation between the extent of aggregation indicated by the peak viscosity and free fat content (Fig. 6.3). The T20 emulsions had both the highest peak viscosity and free fat content followed by T40 and T60 emulsions. Intuitively, the measurements should correlate with one another since they both depend on fat globule aggregation. The 1.6 wt% T60 emulsion thickened during the shear stress ramp indicating some aggregation had occurred however no increase in free fat was found compared to the unsheared control. Aggregation was also confirmed by measuring the particle size distribution by laser light scattering after dispersing the sheared emulsion in 1% SDS solution. Here a 25-fold mean increase in the $d_{4,3}$ value was found. Overall, the good agreement between the free fat measurements and the peak viscosity data support that the extent of fat globule aggregation is Tween type dependent.

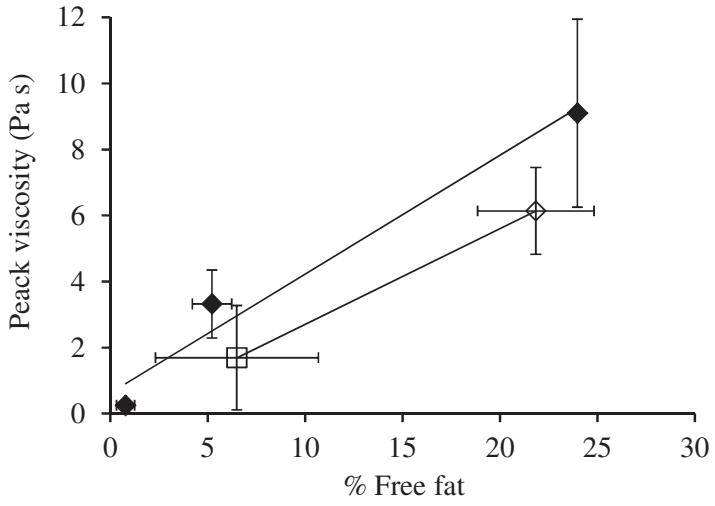


Fig. 6.3. Relationship between free fat content and peak viscosity of 35 wt% partially crystalline oil-in-water emulsions stabilized by 1 wt% sodium caseinate and 1.2 wt% (open symbols) or 1.6 wt% (closed symbols) Tween emulsifier. Solid lines are a guide for the eye. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60.

6.4.2 Degree of supercooling and crystallization profile

In many studies on emulsions containing crystalline triglyceride or hydrocarbons in the dispersed phase, it has often been reported that emulsifiers alter the onset crystallization temperature depending on the saturated fatty acid chain length (Golemanov, et al., 2006; McClements, et al., 1993; Skoda, et al., 1963). Long saturated fatty acid chain lengths generally decrease the degree of supercooling required for fat crystallization compared to short chain emulsifiers and especially unsaturated emulsifiers. Long chain fatty acids promote interfacial heterogeneous nucleation by serving as nucleation sites for crystal growth. Unsaturated emulsifiers however do not promote interfacial heterogeneous nucleation since the fatty acid tails are poor templates for crystal growth (Douaire, et al.,

2014). In this study, the onset crystallization temperature and the crystallization profile were measured by monitoring the change in SFC over time using pulsed NMR. The SFC was monitored after the NMR tubes containing the emulsions were transferred from a 65 °C heating block to the NMR measurement cell held at 5 °C.

Both the degree of supercooling and crystallization profile were dependent on Tween type in emulsions containing 1 wt% sodium caseinate at 0.8 wt% Tween (Fig. 6.4A). Consistent with other studies, the degree of supercooling decreased with increasing saturated fatty acid chain length (Fig. 6.5). Interestingly, this trend was not present in sodium caseinate-free emulsions (Fig. 6.4B). Although the degree of supercooling in T20 and T80 emulsions was similar both with and without sodium caseinate, the saturated emulsifiers T40 and T60 were apparently ineffective in lowering the degree of supercooling and thus promoting interfacial heterogeneous nucleation to the same extent as when present at the interface in combination with sodium caseinate (Fig. 6.5).

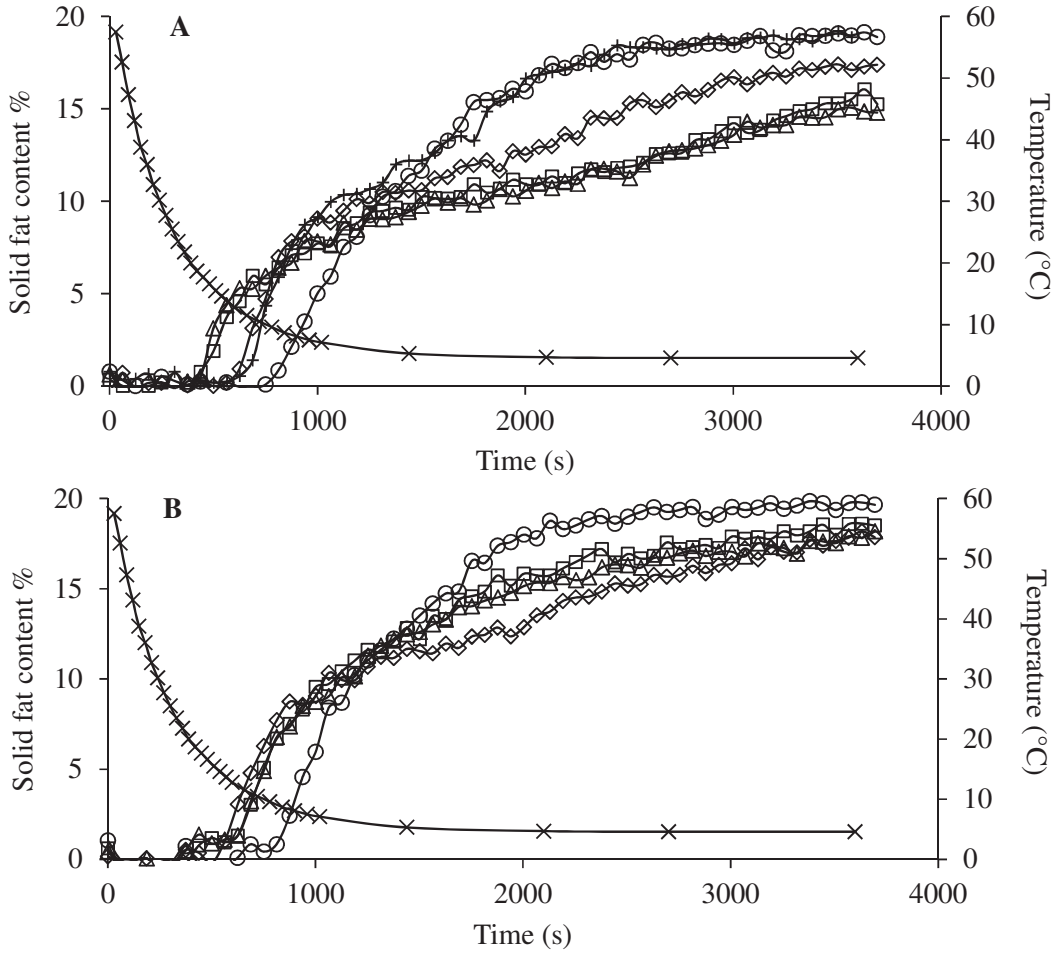


Fig. 6.4. Effect of Tween type and sodium caseinate addition on the crystallization behaviour of partially crystalline oil-in-water emulsions (35 wt% fat) containing (A) 1% sodium caseinate and 0.8% Tween or (B) 0.8% Tween. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60; (○) Tween 80; (+) 1% sodium caseinate only; (x) Emulsion temperature.

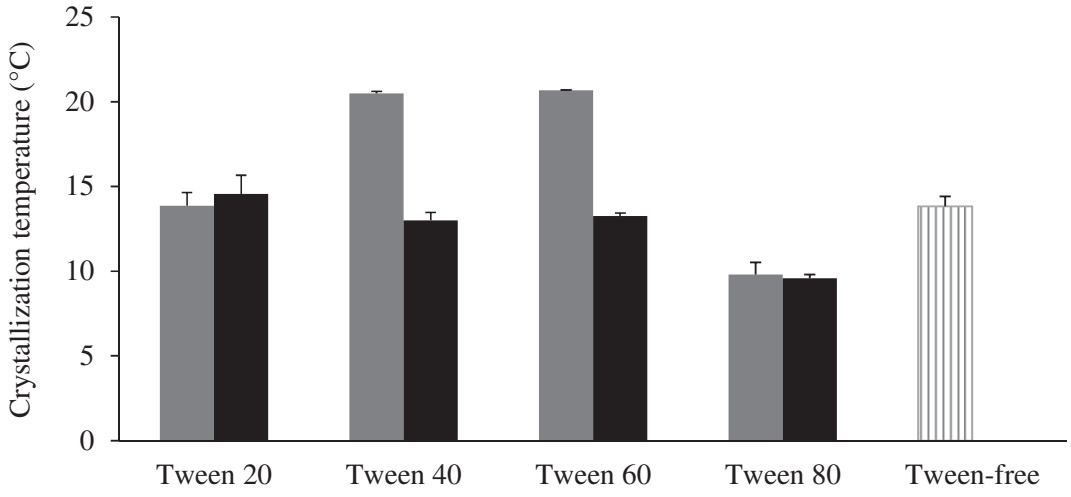


Fig. 6.5. Effect of Tween type and sodium caseinate addition on the onset crystallization temperature of partially crystalline oil-in-water emulsions containing 35 wt% fat. Colour scheme: (grey) 1% sodium caseinate and 0.8% Tween; (black) 0.8% Tween; (striped) 1% sodium caseinate.

Studies often suggest that interfacial nucleation requires similarity between the fatty acid tails of the emulsifier and the fatty acids of the dispersed phase (Sato & Ueno, 2011). The primary fatty acids contained in the blend of hydrogenated palm kernel oil and canola oil used here were lauric (28.5%), oleic (25.3%), stearic (11.5%), palmitic (9.6%) and myristic (9.6%). Since T20 contains primarily lauric acid and the fat was primarily lauric-based, the lack of an effect on interfacial heterogeneous nucleation suggests that the fatty acid tails of T20 may not protrude into the fat phase to the extent needed to serve as a template for fat crystallization (Smith, Bhaggan, Talbot, & van Malssen, 2011). T40 and T60 which contain primarily palmitic and stearic acids respectively may on the other hand protrude far enough into the fat phase to promote nucleation. Furthermore, since the dispersed phase remained unchanged in this study, either the protein itself or trace surface active impurities must interact with T40 and T60

to promote interfacial heterogeneous nucleation. Further research beyond the scope of this study is required to elucidate the unique interaction which may be occurring.

Triglycerides tend to first crystallize in the α-crystal form then rapidly convert to the more stable crystal form which, in the case of hydrogenated palm kernel oil is β' (Siew, 2001). In several articles, Sato's group reported hydrophobic sucrose esters improved the stability of palm kernel oil-in-water emulsions through a reduction in large crystals associated with β'-crystal formation. The reduction of large crystals may be due to a combination of effects caused by interfacial heterogeneous nucleation, decrease in the crystallization rate and slowing of the α- to β'-crystal transition (Arima, Ueji, Ueno, Ogawa, & Sato, 2007; Arima, et al., 2009; Awad & Sato, 2002). Although a similar association between interfacial heterogeneous nucleation, decreased crystallization rate and increased emulsion stability was found in this study, it was only achieved with hydrophilic T40 and T60 emulsifiers in combination with sodium caseinate. Therefore, there must be a unique interaction between the saturated long chain Tween emulsifiers and sodium caseinate proteins or trace surface active impurities. This interaction is supported by Arima, et al. (2007) who reported a combination of hydrophobic and hydrophilic sucrose esters improved emulsion stability better than hydrophobic sucrose ester alone. Notably, the hydrophilic sucrose ester alone was ineffective in increasing the emulsion stability. Therefore in this study, hydrophobic proteins or trace impurities may work in conjunction with T40 and T60 to promote emulsion stability through a similar combination of effects involving higher crystallization temperature and/or altered polymorphic crystal transitions.

Another interesting finding from the pulsed NMR study was the relationship between the onset crystallization temperature and the crystallization rate. Compared to bulk fat

and T80 emulsions (both with and without sodium caseinate), the saturated Tween emulsifiers especially in the presence of sodium caseinate altered the change in SFC over time (Fig. 6.6). Interfacial heterogeneous nucleation appeared to arrest the rapid increase in SFC which occurred during the first 3–7 minutes after crystallization began. Consequently, the crystallization rate was greatly diminished compared to both the emulsion prepared with only 1 wt% sodium caseinate and the T80 emulsion (Fig. 6.4A). For example, the T40 and T60 containing emulsions with sodium caseinate had 15% SFC after 1 h whereas the control (1% sodium caseinate only) and T80 emulsions had 15% SFC after only 30 min. After the initial rapid increase in SFC, the differences in the crystallization rates may be due in part to interdroplet heterogeneous nucleation caused by a crystalline fat globule colliding with a supercooled globule (Hindle, Povey, & Smith, 2000; McClements, et al., 1993). Hindle, et al. (2000) reported that 1 wt% sodium caseinate only stabilized cocoa butter emulsions crystallized at a slower rate than 0.8% v/v T20 only stabilized emulsions due to a greater number of effective droplet collisions in the T20 emulsions caused by the poorer barrier properties of T20 compared to sodium caseinate. In this study, it was found that the 1 wt% sodium caseinate emulsion crystallized more rapidly than the 1 wt% sodium caseinate and 0.8 wt% T20 emulsion despite having an onset crystallization temperature virtually the same as in Hindle's study. However, the results do not necessarily disagree with Hindle's findings since interdroplet heterogeneous nucleation depends on both the barrier properties of the interfacial film and the degree of crystal protrusion from the fat globule. Shown in Fig. 6.7, there was a reasonable correlation ($r^2 = 0.80$) between the onset crystallization temperature and the SFC after 2000 s of holding time. Assuming that interfacial heterogeneous nucleation leads to less crystal protrusion from the interface, and thus a reduction in successful collisions causing interdroplet nucleation,

then higher onset crystallization temperatures may have lowered the crystallization rates due to effects on the surface roughness rather than the barrier properties of the film. Alternatively, the higher onset crystallization temperature may have altered the dominate crystal type (α or β') initially formed in the emulsion upon crystallization. Emulsifiers, especially those with long chain length, have been reported to change the crystallization rate simply by promoting crystallization in a different crystal polymorph (Awad & Sato, 2002). The work of Awad & Sato (2002) showed how interfacial heterogeneous nucleation in palm kernel oil-in-water emulsions changed the initial crystal type formed from α to β′ type. Crystallisation first into β′-crystals may slow fat crystallisation compared to first crystallising in the α-type. Demixing of α-crystals has been reported to provide additional nucleation sites for β′-crystals to form thus resulting in more rapid crystallisation (Janssen & MacGibbon, 2007).

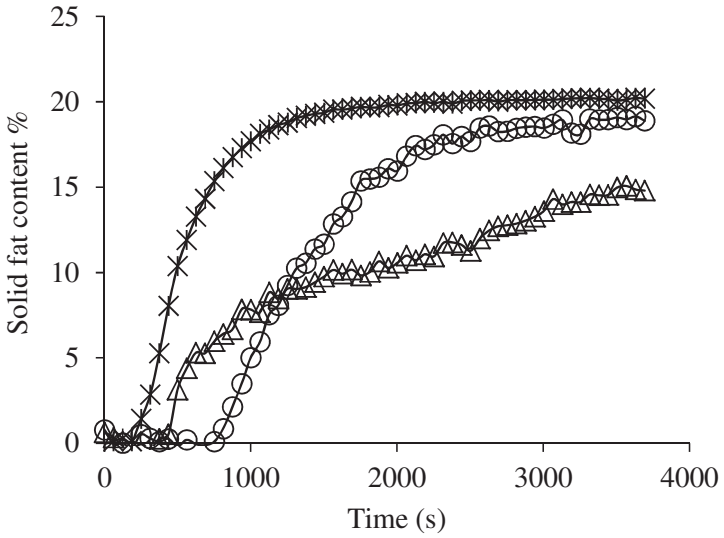


Fig. 6.6. Comparison between the crystallization profiles of bulk fat and partially crystalline oil-in-water emulsions (35 wt% fat) containing 1% sodium caseinate and 0.8 wt% saturated or unsaturated Tween emulsifier. The measured bulk fat solid fat content values were multiplied by 0.35 (fat content of the emulsions) for ease of comparison with the emulsions. Symbols: (x) Bulk fat (70% hydrogenated palm kernel oil/30% canola oil); (Δ) Tween 60 - saturated; (○) Tween 80 - unsaturated.

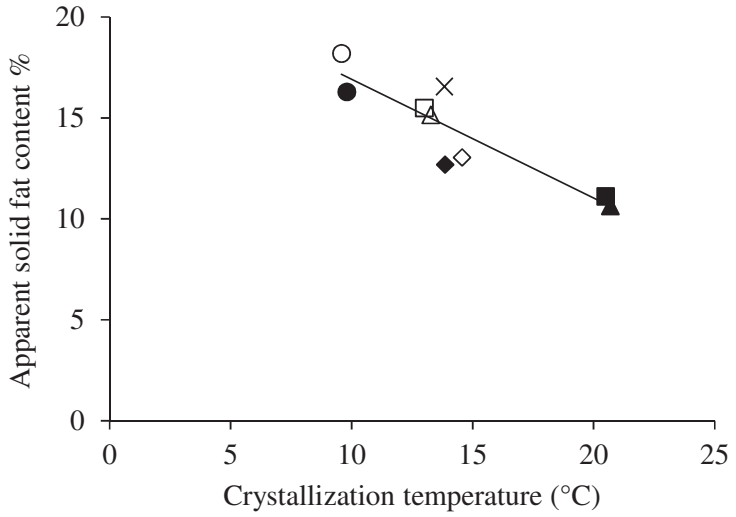


Fig. 6.7. Correlation between the onset crystallization temperature and SFC of emulsions after 2000 s of holding time at 5 °C. The solid line is a linear regression line of best fit (r^2 = 0.80). Filled symbols indicate emulsions contained 1 wt% sodium caseinate and 0.8 wt% Tween whereas empty symbols indicate emulsions contained only Tween. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60; (○) Tween 80; (X) 1% sodium caseinate only.

6.4.3 Effect of Tween addition pre– and post–fat crystallization on flow behaviour and stability

In the following section, emulsions were prepared with Tween emulsifier added before (as studied throughout this manuscript) and after fat crystallization. The purpose was to investigate if emulsion stability changes when Tween emulsifiers adsorb to the fat globule interface after fat crystallization rather than before. If emulsion stability differs between preparation methods, then the penetration of the emulsifier tailgroup into liquid oil must be influencing emulsion stability.

All of the emulsions with Tween added before fat crystallization were subjected to sequential increasing and decreasing shear rate sweeps (Fig. 6.8) in order to examine the flow behaviour and identify any hysteresis. The maximum shear rate (200 s^{-1}) was selected so that the shear stress did not exceed the minimum critical destabilizing shear stress identified in Table 6.1. However, no hysteresis was found in any of the emulsions tested. For this reason, only the increasing shear rate sweep data are shown in Fig. 6.8 with the exception of one emulsion. In Fig. 6.8B, the apparent hysteresis, indicated by the arrows, was actually caused by fat globule aggregation which could be visibly observed. It was clear from these results that the addition of Tween prior to fat crystallization led to a transition from a Newtonian sodium caseinate stabilized emulsion to a more viscous, shear thinning emulsion with increasing Tween concentration (Fig. 6.8). The lack of hysteresis coupled with shear thinning behaviour indicates the increase in viscosity with Tween concentration was caused by the formation of a crowded environment rather than the formation of a network linked by irreversible chemical bonds. The reversibility of the viscosity with decreasing shear rate supports that physical interactions between the components of the emulsion contributed substantially to the viscosity. The addition of Tween solution to a sodium caseinate stabilized emulsion is not sufficient to cause the increase in viscosity since the Tween micelles are very small and do not contribute significantly to viscosity at the low concentrations used here.

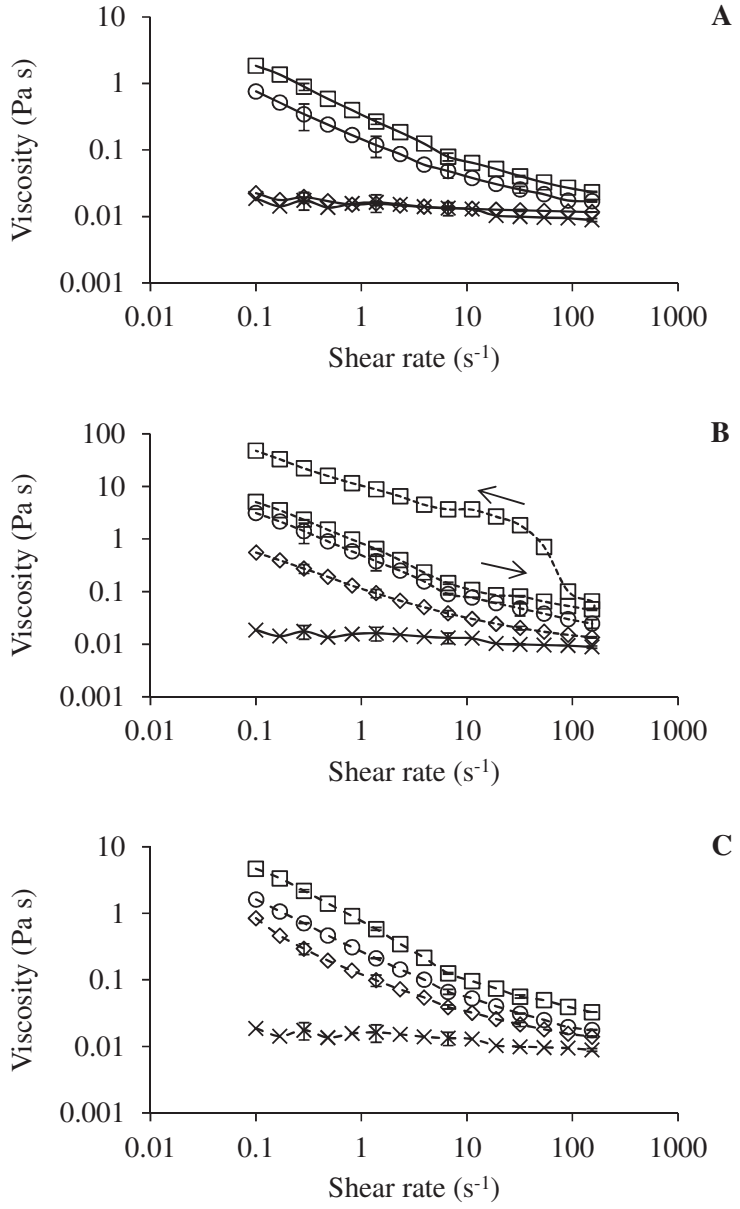


Fig. 6.8. Shear rate sweep at 5 °C of 35 wt% partially crystalline oil-in-water emulsions containing 1 wt% sodium caseinate and Tween 20 (A), Tween 40 (B) or Tween 60 (C) added after fat crystallization. Solid lines are a guide for the eye. Arrows indicate the direction of the shear rate sweep. Symbols indicate Tween concentration: (x) 0 wt%; (◊) 0.8 wt%; (○) 1.2 wt%; (□) 1.6 wt%.

To better understand these findings, emulsions were also prepared with 1.6 wt% Tween added after fat crystallization. This was achieved by adding chilled Tween solution to the 1.5 wt% sodium caseinate stabilized stock emulsion stored for 24 h at 5 °C. These emulsions were tested for flow behaviour and shear stability over 1 week of storage at 5 °C after Tween addition.

After mixing the Tween solutions and sodium caseinate stabilized emulsions, slight shear thinning behaviour was observed and the viscosity was not substantially influenced by the type of Tween emulsifier (Fig. 6.9A). After 4 (Fig. 6.9B) and 7 (Fig. 6.9C) days of chilled storage however, the viscosity of the emulsions with T80 and T20 increased whilst the viscosity of the T40 emulsion only increased slightly and the T60 emulsion remained unchanged.

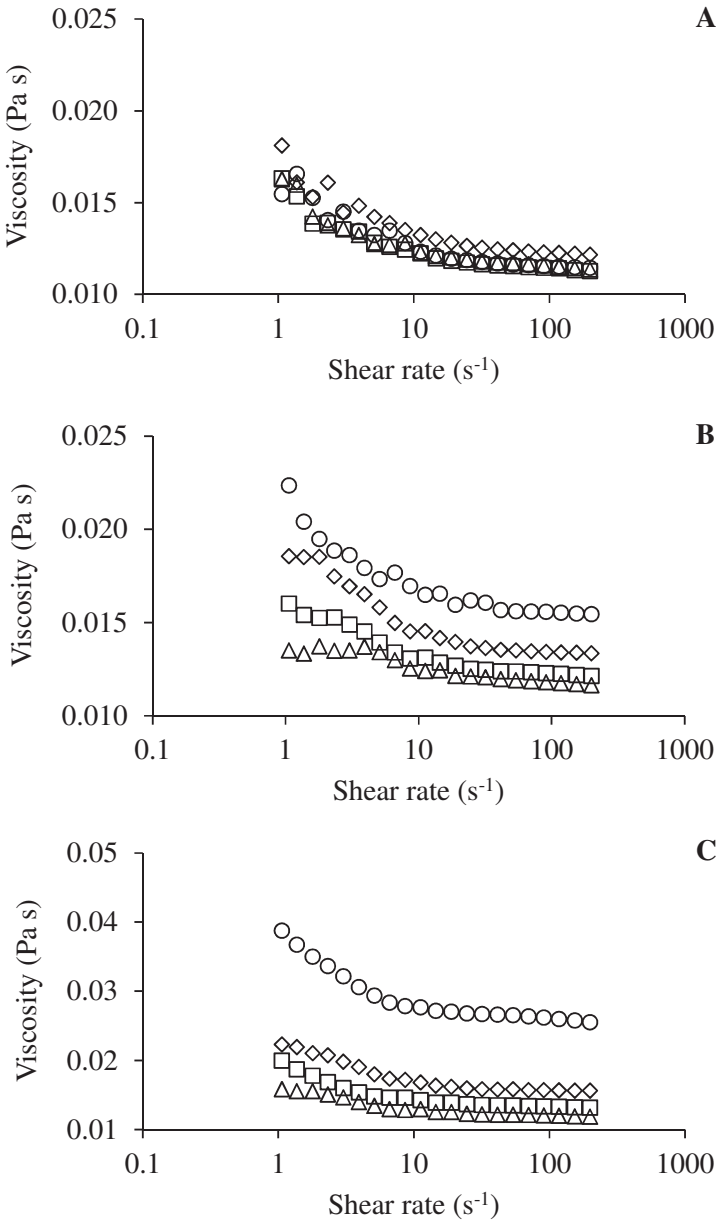


Fig. 6.9. Shear rate sweep of 35 wt% partially crystalline oil-in-water emulsions containing 1 wt% sodium caseinate and different types of Tween emulsifier (1.6 wt%). Both the Tween emulsifiers and sodium caseinate stabilized emulsions were chilled at 5 °C for 24 h prior to mixing. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60; (○) Tween 80. Symbols: (A) Day 1; (B) Day 4; (C) Day 7.

In Fig. 6.10, the shear viscosity of the emulsions with Tween added after fat crystallization and aged for 7 days is shown along with 1.6 wt% Tween emulsions prepared with Tween prior to fat crystallization. When Tween was added after fat crystallization, the emulsions remained largely Newtonian after 7 days of aging compared to the emulsions containing Tween added prior to fat crystallization. There was approximately a 1–2 orders of magnitude higher viscosity at low shear rates ($< 1\ s^{-1}$) when Tween was added prior to fat crystallization rather than after. Note that the differences in scale between Fig. 6.9 and 6.10 make the slightly shear thinning emulsions in Fig. 6.9 appear Newtonian in Fig. 6.10. Also, note that T80 was not added prior to fat crystallization because the emulsions were found previously to be quiescently unstable at the concentrations studied here.

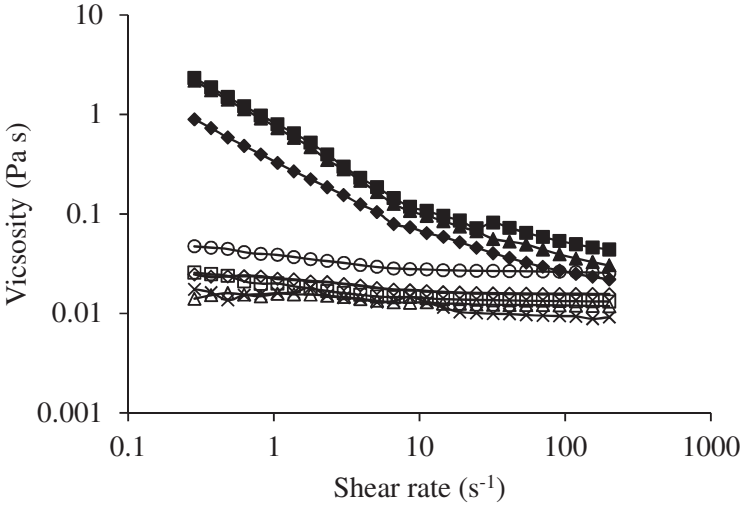


Fig. 6.10. Shear rate sweep of 35 wt% partially crystalline oil-in-water emulsions containing 1 wt% sodium caseinate and different types of Tween emulsifier (1.6 wt%) at 5 °C. Emulsions were stored for 7 days at 5 °C prior to testing. Closed symbols indicate the Tween emulsifier was added prior to fat crystallization. Open symbols indicate Tween was added to a sodium caseinate stabilized emulsion chilled at 5 °C for 24 h prior. Symbols: (◊) Tween 20; (□) Tween 40; (Δ) Tween 60; (○) Tween 80; (x) 1 wt% sodium caseinate only (no Tween).

The increased viscosity of the T80 and T20 containing emulsions was also associated with decreased shear stability (Fig. 6.11). Over 7 days of storage, the T80 containing emulsion transitioned from relatively shear stable to unstable indicated by immediate aggregation when sheared at 2000 s^{-1}. The T20 containing emulsions aggregated approximately 5-fold sooner after 7 days of storage. Notably though, neither the T40 or T60 emulsions aggregated after 3000 s of shearing time even after 7 days of chilled storage (data not shown). These results indicate that by adding Tween after fat crystallization the shear stability can be dramatically increased in the case of T40 and

T60. However, T20 and T80 containing emulsions quickly evolve into shear sensitive emulsions.

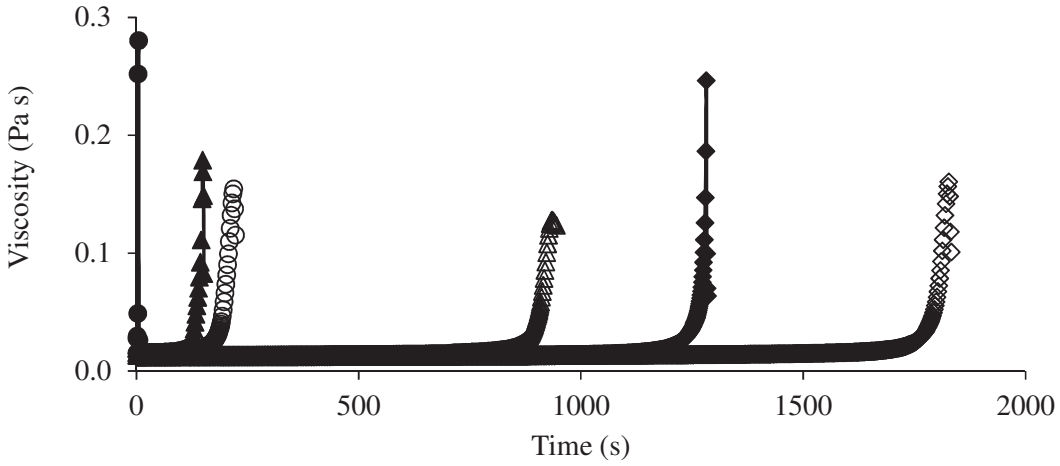


Fig. 6.11. Change in shear stability over time of 35 wt% partially crystalline oil-in-water emulsions under steady shear (2000 s^{-1}) at 5 °C stabilized by either 1.6 wt% Tween 20 (open symbols) or Tween 80 (closed symbols) and 1 wt% sodium caseinate. The Tween emulsifiers were added to sodium caseinate stabilized emulsions chilled prior at 5 °C for 24 h. Symbols: (◊) Day 1; (Δ) Day 4; (○) Day 7.

6.5 Discussion

In this chapter, the effect of different saturated and unsaturated Tween emulsifiers on the shear stability of partially crystalline oil-in-water emulsions was investigated. Throughout this study, it was generally found that the saturated Tween emulsifiers (T20, T40 and T60) provided better shear stability compared to unsaturated T80. For the saturated Tween emulsifiers, shear stability generally increased with increasing fatty acid chain length i.e. T60 was the most stable followed by T40 and T20. To explain these results, the changes in fat crystallization and flow behaviour caused by different

types and concentrations of Tween emulsifiers both with and without sodium caseinate present were investigated.

It was most interesting to observe that the extent of fat globule aggregation followed a trend where it decreased with increasing saturated fatty acid chain length both in the presence and absence of sodium caseinate (Table 6.2). The critical destabilizing shear stress however did not follow this trend. Here, T40 produced the most unstable emulsions followed by T20 and T60 (Table 6.1). The same difference in trends was also found in Chapter 5 (Table 5.2) when comparing aggregation time data with peak shear stress. To reconcile these differences, it is important to note that the measurements indicate different measurements of stability. The peak viscosity is related to the extent of fat globule aggregation i.e. the number and shape of the fat globule clusters, as well as the stability of the clusters under an increasing shear stress. The end point of the peak viscosity is determined by the drop in viscosity after the rapid rise and hence the peak viscosity indicates when the clusters are no longer stable and large scale break up occurs. The critical destabilizing shear stress however is related to the interfacial film properties since they effect partial coalescence and cluster formation under an increasing shear stress. Based on these considerations, the discrepancy found between the shear stability measurements both in this chapter and in Chapter 5 is not unreasonable since they measure different stages in the aggregation process.

Furthermore, it was also found that the free fat content measured after the shear rate sweep followed the same trend as the peak viscosity. The free fat measurement technique utilized here measures the amount of de-emulsified fat which clumps together as the result of fat globule cluster break up, which occurs at the peak of the viscosity rise. Since the two measurements followed the same trend, it can be inferred that fat

globule clusters in T60 containing emulsions are more vulnerable to cluster break up since they reach a lower peak viscosity compared to T40 and T20. As a consequence, virtually no free fat was released from the fat globule clusters upon break up in T60 containing emulsions (Fig. 6.2B). Possibly, T60 better prevents the release of liquid oil, which connects the fat globules together in clusters, leading to weaker clusters that break up before a highly jammed state can form.

By studying the onset crystallization temperature and change in SFC over time, it was shown that T40 and T60 promoted interfacial heterogeneous nucleation and slowed the rate of crystallization over time. Comparing these data with the shear stability, there is a correlation between onset crystallization temperature and shear stability in emulsions containing a combination of sodium caseinate and Tween emulsifiers (Fig. 6.8). However, saturated Tween emulsifiers were found to have no effect on the crystallization temperature when used without sodium caseinate but the shear stability in the absence of sodium caseinate was found to be dependent on the type of Tween. Therefore, interfacial heterogeneous nucleation may alter shear stability but it is likely not the primary mechanism by which Tween emulsifiers affect shear stability.

The addition of Tween emulsifiers was also shown to promote shear thinning behaviour and generally increase the low shear rate viscosity (Fig. 6.9). Interestingly, no hysteresis was found during the decreasing shear rate sweep indicating that the interactions between ingredients causing the change in rheology upon Tween addition were reversible. Depletion flocculation is a common occurrence in sodium caseinate and Tween containing emulsions due to the difference in size between the fat globules and caseinate submicelles (Dickinson, Radford, & Golding, 2003). Depletion flocculation may lead to an increase in viscosity under conditions leading to the formation of a

crowded environment and thus is a possible contributing mechanism to the increase in viscosity upon Tween addition.

An additional mechanism by which the viscosity may increase is network formation caused by displacement of protein from the oil-water interface (Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014). The authors studied the effects of GMS (saturated monoglyceride) and GMU (unsaturated monoglyceride) emulsifiers on the stability of 25 wt% HPKO-in-water food emulsions containing sodium caseinate (0.6 wt%), sucrose (10 wt%) and sorbitol (1 wt%). The authors studied protein displacement as a function of emulsifier concentration using a conventional depletion method (similar to this study) and additionally by Front-Face Fluorescence spectroscopy which does not involve any phase separation. The authors reported significant protein displacement when both GMS and GMU were included in the emulsions. However, no change in viscosity occurred with increasing GMS concentration (0.0–1.0 wt%) whereas emulsions with GMU became semi-solid with as little as 0.2 wt%. The formation of a semi-solid structure was found to be caused by network formation due to some unknown but detectable interactions between displaced protein and the fat globule interface. These types of interactions were not found in emulsions containing GMS. GMS containing emulsions were proposed to not form a network because of crystallization of GMS at the oil-water interface.

Comparing this study to Munk, Larsen, et al. (2014), there is a striking similarity in the stability of the Tween containing emulsions studied here with the GMU emulsions. In both cases, viscosity increased with increasing emulsifier concentration and shear stability decreased as a result. Assuming GMS crystallization prevented network formation and the corresponding viscosity increase, it would make sense that network

formation occurred for all the Tween emulsifiers studied since they like GMU do not undergo phase transitions at low temperatures. Furthermore if crystallization of GMS prevents network formation by hindering interaction between displaced protein and the fat globule interface, then the mechanism may be related to the formation of a dense, closely packed interfacial film. Due to the unsaturated bond in T80, it would be expected to have the least uniform interfacial film and hence have similar behaviour to GMU. Indeed, T80 containing emulsions were the most shear sensitive emulsions studied here.

The addition of Tween emulsifiers post-fat crystallization provided evidence which further differentiates these emulsifiers. T80 containing emulsions and to a lesser extent T20 emulsions increased in viscosity over time and became more shear sensitive whereas the emulsions with T40 and T60 were shear stable and had only a slight increase in viscosity (Section 6.4.3). Assuming the viscosity increase and shear sensitivity increase was caused by protein displacement, it can be inferred that T80 and T20 promoted protein displacement at 5 °C but T40 and T60 did not. The adsorbed protein measurement used throughout this research was not attempted since the method requires heating the emulsions to 40 °C where the interfacial activity of T40 and T60 would be restored. Unfortunately, the authors are not aware of any studied comparing the oil-water interfacial activity of Tween emulsifiers below ambient temperature (< 20 °C) so no insight can be gleaned from published studies. Therefore, further research is necessary in order to verify that the interfacial affinity of saturated Tween emulsifiers decreases at low temperature with increasing chain length. Perhaps, the long fatty acid chains allow for stronger self-association of the micelles at 5 °C leading to a reduction in the “free” Tween concentration in the aqueous phase. Alternatively, the number of micelles (aggregation number) may increase with decreasing temperature thereby

depleting the concentration of “free” Tween. If in fact T40 and T60 have reduced interfacial affinity at 5 °C, then it provides further evidence for why emulsions with the emulsifiers and sodium caseinate were more shear stable. However, it does not explain why shear stability increased with saturated fatty acid chain length when no protein was present in the emulsion.

Tween emulsifiers adsorbed to an oil-water interface in the absence of sodium caseinate should theoretically have differences in packing arrangement caused by differences in chain length and the presence or absence of unsaturated bonds (Graca, et al., 2007). How this interfacial structure impacts the stability of the emulsions under study is however unclear. If the partially crystalline fat globule surface is considered a hard surface in which the fatty acid tails cannot penetrate, then evidence is available from the literature that indicates the unsaturated bond of T80 may promote disorder in the interfacial film by adopting a horizontal orientation next to the surface (Graca, et al., 2007). The straight chain saturated Tween types may form more uniform films where the molecules adopt a side-by-side orientation and the uniformity may increase with chain length (Graca, et al., 2007). In reality, too little is known about the interfacial films of partially crystalline fat globules to refer to them as hard surfaces but the tendency for high SFC emulsions to rapidly destabilize when the interfacial film is dominated by Tween 20 (Section 4.4.7) supports that the interfacial film properties of Tween emulsifiers change dramatically with SFC.

6.6 Conclusion

The effect of Tween fatty acid chain length and saturation on shear stability, fat crystallization and flow behaviour in partially crystalline oil-in-water emulsions prepared with and without sodium caseinate has been described. Generally, shear

stability increased with increasing fatty acid chain length of the saturated Tween emulsifiers both with and without the presence of sodium caseinate. Tween 40 and 60 altered the crystallization rate and the onset crystallization temperature in the presence of sodium caseinate but not without. This study could not pinpoint a particular property of Tween 60 which enables it to improve shear stability better than the other Tween emulsifiers other than possible differences in the arrangement of the molecules in the interfacial film driven by the longer chain length. Further research is required to understand the properties of Tween interfacial films in partially crystalline oil-in-water emulsions.

Chapter 7: Summary and recommendations for future work

7.1 Summary of Chapters

In Chapter 3, the usefulness of high shear rate induced aggregation for the purpose of determining the susceptibility of partially crystalline oil-in-water emulsions to shear-induced aggregation was first established. By varying interfacial composition and solid fat content, a variety of aggregation behaviours were described. The extent of displacement of sodium caseinate by Tween 20 from the fat globule-water interface caused considerable changes in the stability of partially coalesced aggregates. Moderate protein displacement led to the formation of relatively stable aggregates whereas high displacement led to the formation of more unstable aggregates under shear. In addition, interfacial film composition was found to regulate the dependency of aggregation time on shear rate while the solid fat content played a minor role. These findings help bring clarity to the respective roles of adsorbed proteins and low molecular weight emulsifiers along with solid fat content on partial coalescence in a model system designed to minimize the effects of secondary factors commonly found in “real” food emulsions.

In Chapter 4, the combined effects of solid fat content and interfacial composition on quiescent and shear-induced partial coalescence were studied. Two different stability regimes were identified. At low protein displacement, fat globules were stable quiescently regardless of SFC but were vulnerable to partial coalescence under shear. The aggregation time decreased with decreasing SFC presumably due to the presence of more liquid oil which aids in fat globule-globule adhesion in the presence of a significant repulsive force from the adsorbed protein. At high protein displacement, emulsions became more unstable both quiescently and under shear with increasing SFC.

The results implied that partial coalescence or aggregation readily occurs even at low liquid oil content if close contact between the globules is possible. From Cryo-TEM micrographs, there was no evidence for fat crystal protrusion from the fat globule-water interface beyond a few nanometres at most. The findings support that rapidly cooled emulsions should be generally considered to have rough rather than spikey surface structures as commonly suggested in the literature.

The findings from Chapters 3 and 4 helped to answer several research questions posed at the beginning of this research. The findings confirmed that adsorbed protein load primarily controlled the stability of the partially crystalline oil-in-water emulsions studied while SFC had a secondary effect which could not be explained simply by the solid:liquid ratio. It also showed that the susceptibility to partial coalescence was much less impacted by interfacial film composition when the fat globules contained low to medium SFC (˜25–50%). At high SFC however, interfacial film composition had a very large effect possibly caused by a combination of factors such as rearrangement of the adsorbed Tween emulsifiers leading to a weaker film, rougher fat globule surface and lower deformability of the fat globules.

In Chapter 5, long chain length saturated Tween emulsifiers like Tween 40 and Tween 60 were found to better prevent shear-induced aggregation compared to shorter chain length (Tween 20) and unsaturated (Tween 80) Tween types. A more complex stability relationship was found for combinations of Tween 80 and different types of SPAN. The transition of these emulsions from stable to unstable occurred over a narrow range of Tween 80 (0.2–0.6 wt%). This study supported the hypothesis that in general longer chain length fatty acid emulsifiers better prevent partially crystalline emulsion destabilisation. Since Tween emulsifiers do not undergo phase transitions to the author's knowledge, they can be ruled out as a major mechanism for improved stability.

In general, it may be that saturated, long chain length emulsifiers improve shear stability by simply packing more densely at oil-water interfaces or penetrating deeper into the fat globules.

The results from Chapter 5 did not indicate that the blending of low and high melting point emulsifiers could improve the stability of the emulsions studied here and hence helps to answer one of the key research questions. The addition of SPAN emulsifiers did not improve the stability of emulsions containing different concentrations of Tween 80. Possibly, the unsaturated bond in Tween 80 prevents interaction between the fat soluble SPAN emulsifiers and Tween 80. Perhaps if a saturated Tween type had been used instead of Tween 80, interactions between the saturated SPANs and Tweens could have occurred leading to better stability compared to emulsions stabilized by Tween alone.

In Chapter 6, the effect of Tween fatty acid chain length and emulsifier saturation on shear stability, fat crystallization and flow behaviour in partially crystalline oil-in-water emulsions prepared with and without sodium caseinate was described. Tween 80 was generally found to produce the most unstable emulsions followed by Tween 20, Tween 40 and Tween 60. This trend was found both in the presence and absence of sodium caseinate. Although Tween emulsifiers altered the crystallization rate and the onset crystallization temperature, the effects were only found in the presence of sodium caseinate. Therefore, some other factor related to differences in molecular structure must be the primary cause for the enhanced stability of long chain saturated Tween emulsifiers. These results confirm that for saturated emulsifiers the fatty acid chain length plays a major role in governing the susceptibility to partial coalescence. Furthermore, the results imply that phase transitions which often occur in long chain length fatty acid emulsifiers are not required for the increase in stability to occur.

7.2 Overall conclusion: Structural rearrangement of the interfacial film occurs in response to fat crystallization

In Chapter 4, SFC was found to have a large effect on the quiescent and shear stability of emulsions containing T20 dominated interfaces. In Chapter 6, the shear stability of emulsions depended on the type of Tween even when the emulsions contained no sodium caseinate. Considering there are no obvious differences in the solubility of the different Tween emulsifiers studied here nor their ability to displace adsorbed protein, structural differences in Tween films may explain why emulsions with different types of Tween had different stabilities under shear.

In Figure 7.1, high SFC fat globules are depicted as having Tween 20 molecules adsorbed to a hard surface (A & B) whereas at low SFC the fatty acid tails of Tween 20 are embedded in liquid oil (C & D). Tween 20 and other high HLB emulsifiers are envisioned to adsorb to a liquid oil interface with the hydrophobic tail embedded in liquid oil. As SFC increases upon cooling, liquid oil may remain in contact with the fatty acid tails but will ultimately become depleted as the fat crystallises. With crystallization, there is also an increase in density of approximately 10% when the liquid oil crystallises leading to shrinkage of the globule which could perhaps expose crystalline fat to the aqueous phase rather than be covered with a layer of liquid oil. Furthermore, Tween 20 adsorption like other water soluble high HLB emulsifiers is reversible so a constant exchange of molecules occurs at the interface (Bos & van Vliet, 2001). Therefore, high HLB emulsifiers like Tween 20 should encounter a more solid surface upon re-adsorption after fat crystallization. The anchoring of the fatty acid tails in liquid oil will certainly affect the mechanical properties of the interfacial film by limiting the mobility of the adsorbed molecules more so than if they were loosely

interacting with a hard crystalline fat surface. This hypothesis helps explain why Tween 20 dominated emulsions underwent rapid partial coalescence upon cooling quiescently in Chapter 4. Although the emulsions became more unstable with increasing SFC, 30 and 50% HPKO emulsions were relatively much more stable compared to 70 and 100% HPKO respectively. From the point of view of this research, the change in mechanical properties of the interfacial film due to structural rearrangement better explains why the stability decreased with increasing SFC than an increase in the number of protruding fat crystals or protrusion distance with increasing SFC.

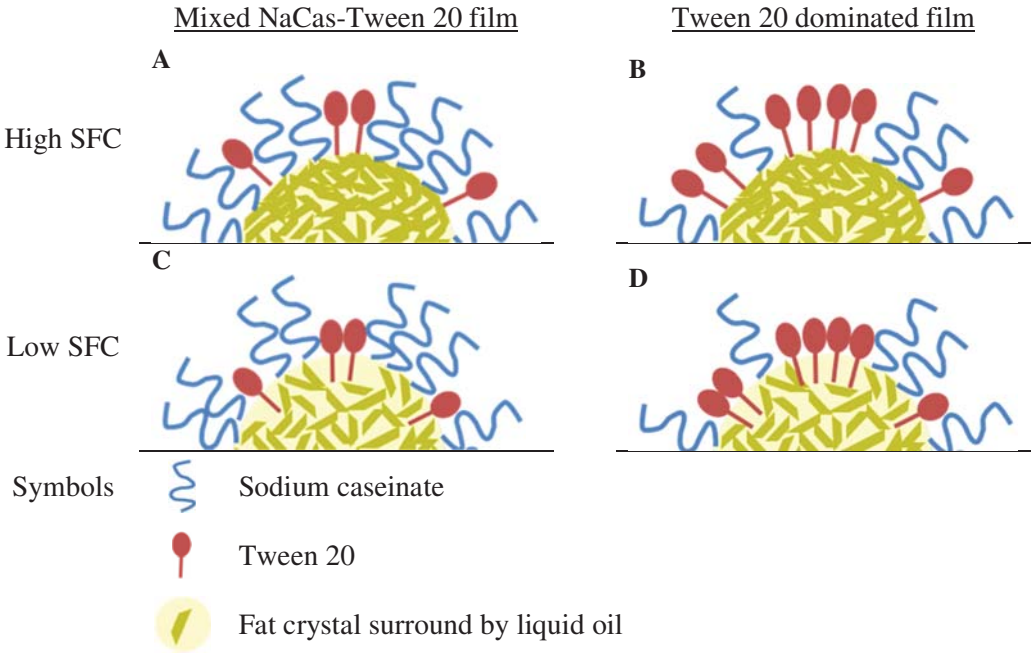


Fig. 7.1. Schematic diagram showing potential structural changes of two interfacial films in response to high and low solid fat content.

In mixed sodium caseinate-Tween 20 films studied in Chapter 4, no quiescent partial coalescence occurred at any SFC presumably due to the strong steric repulsion from sodium caseinate. Under shear however, there was a parabolic response of the susceptibility to partial coalescence at different SFC (Fig. 4.8). Assuming enough

sodium caseinate was present to prevent close approach quiescently, it must be considered that the aggregation process under shear is dynamic in order to explain the parabolic response. Under shear, a constant series of globule collisions, attachment and breakup processes occur under shear. In order for the fat globules to attach together, the repulsive force of sodium caseinate must be overcome and the interfacial film must be ruptured. In the case of globules with different SFC, it must be considered that the low SFC globules are deformable but the SFC within is not. The authors envision the deformable nature of low to medium SFC globules aids partial coalescence by increasing the likelihood of emulsifier rich hot spots coming in contact. This may be caused by an increase in contact time or surface area in contact between deformed fat globules. The lack of deformation in high SFC fat globules may impede hot spot collisions by inhibiting their contact.

The same process of structural rearrangement of the interfacial film described above for Tween 20 should also apply for the other types of Tween emulsifiers. However, differences in the film structure must occur in response to fatty acid chain length since the depth of penetration into a fat globule increases with chain length. In this way, it may be envisioned that longer chain length emulsifiers like Tween 60 are more firmly embedded into the surface of partially crystalline fat globules and thereby better inhibit partial coalescence. By being better embedded or adsorbed onto the fat globule surface, long chain length saturated Tween emulsifiers appear to inhibit partial coalescence by altering the release of liquid oil from the fat globule after film rupture. The inhibition of liquid oil release may explain why the aggregation time was generally longer for long chain length saturated Tween emulsifiers as well as why free fat content and peak shear stress were strongly reduced in their presence.

7.3 Recommendations for future work

7.3.1 Adsorbed protein load

Measuring the adsorbed protein load of partially crystalline oil-in-water emulsions which contain relatively low amounts of adsorbed protein using a method which relies on centrifugation and phase separation of the emulsion must be done with caution (Munk, Larsen, van den Berg, Knudsen, & Andersen, 2014). Factors such as the centrifugation temperature and partial coalescence during centrifugation will surely affect the adsorbed protein load. Furthermore, weak network formation between the fat globules, proteins and potentially stabilizers may lead to separation of a serum phase that does not contain a representative sample of the actual serum phase meaning unadsorbed protein may be trapped in the upper layer as a consequence of weak network formation. Following Munk, Larsen, van den Berg, Knudsen, & Andersen (2014), the front-face fluorescence technique should be investigated further as an alternative technique to characterize the interfacial film composition. In addition, adsorbed protein load should ideally be measured at the temperature of interest, e.g. 5 °C in this study, in order to rule out any temperature dependent differences in adsorbed protein load due to differences in the affinity of the emulsifier for the interface.

7.3.2 Fat globule surface topography

Researchers have attempted to measure how rough the surfaces of fat globules are or rather how far fat crystals protrude from the globule interface. Certainly for small globules around 1 μm in diameter there appear to be no reports showing a suitable way to accomplish this with conventional microscopy techniques. However, it may be that this will become possible with further optimisation of sample preparation techniques for

Cryo-TEM. AFM has also been shown to provide information about the surface roughness and may provide insight as researchers improve the technique further (Thivilliers, Laurichesse, Saadaoui, Leal-Calderon, & Schmitt, 2008). The ability to characterize the topography of the fat globule-water interface will provide a significant step change in the ability to understand and explain how different factors affect the stability of partially crystalline oil-in-water emulsions.

7.3.3 Low molecular weight emulsifier film properties

Partially crystalline oil-in-water emulsions containing Tween 60 were surprisingly stable in that a clear reason for this stability appears absent from literature. Very little information is available on the mechanical properties of Tween emulsifiers, likely because the films are very weak due to a lack of lateral interactions between adsorbed molecules. A possible way to quantify the interfacial film properties *in situ* is using AFM (Balasuriya, Ong, Gras, & Dagastine, 2012). The authors used AFM to directly measure Young's modulus and apparent interfacial film thickness in natural and processed milk fat globules. This technique appears to be the most applicable for measuring the properties of adsorbed Tween films in partially crystalline oil-in-water emulsions given the ability to perform the measurements on actual fat globules rather than planar films.

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