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**DEVELOPMENT OF WATER-IN-OIL EMULSIONS
FOR APPLICATION TO MODEL CHOCOLATE PRODUCTS**

A Thesis in

Food Science

by

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ABSTRACT

The sale and consumption of chocolate in warmer climates is limited because of the negative effects of heat on the desirable chocolate characteristics. The addition of a small amount of water to chocolate in a controlled manner provides the potential for increased heat resistance through the formation of an internal sugar skeleton that maintains the structural integrity of chocolate at higher temperatures. Water-in-oil emulsions are one way to deliver this water.

The overall goal of this work was to determine if water-in-oil emulsions produced via a lab-scale cross flow membrane emulsification system are suitable for use in the production of heat resistant chocolate products. In order to accomplish this goal, 30% water-in-soybean oil emulsions were produced with different ingredients in the dispersed or continuous phases as well as under different processing conditions. The droplet size distributions of the resultant emulsions were examined both initially and over time. The results from this investigation were used to produce stable and unstable emulsions which were then added to dispersions of sugar crystals in oil which were then cooled to crystallize the fat and form a model chocolate. Samples prepared in this manner were compared to samples made with the direct addition of unemulsified water in order to determine the impact of different modes of water addition on the formation of a sugar skeleton and melt resistance in the product.

Analysis of emulsion droplet size distributions showed that polyglycerol polyricinoleate (PGPR) was a more effective emulsifier than either soya lecithin or a 50:50 PGPR:lecithin blend at concentrations of 1 to 6 % (w/w; with respect to the continuous phase). At 2% emulsifier, PGPR-stabilized emulsions remained stable over a period of 4 weeks ($d = 22 \mu\text{m}$) while lecithin-stabilized emulsions nearly doubled in droplet size over 6 hours ($d = 43$ to $77 \mu\text{m}$) and blend-stabilized emulsions completely destabilized within 3 hours (initially $d = 68 \mu\text{m}$). Water droplet size decreased with increasing concentration of the emulsifiers used. Emulsion droplet size and size distribution were also significantly affected by the use of different membranes. Decreasing the continuous phase flow rate (95.3 to 64.2 g/sec) resulted in larger droplets ($d = 22$ to $33 \mu\text{m}$) and increasing the dispersed phase flow rate (0.6 to 1.1 g/sec) also resulted in larger droplets ($d =$

22 to 42 μ m). The addition of a gelling agent (2% κ -carrageenan) to the dispersed phase prior to emulsification made it more difficult to produce stable emulsions.

A stable (2% PGPR-stabilized) and unstable (2% lecithin-stabilized) emulsion (30% water-in-soybean oil) were selected from the first portion of this work and 2 g were added to 120 g of a sugar-in-molten confectionery coating fat (CCF) dispersion (50% sucrose) and cooled to produce model chocolate samples. These samples were compared to samples with water added directly and control samples prepared without added water. Microscopy of sugar-in-oil dispersions in the presence of water-in-oil emulsions provided evidence for the formation of sugar aggregates formed by the adsorption of water at the capillaries between hydrophilic surfaces and resultant interparticle capillary forces. The effect of the presence of such structures was examined within the model chocolate samples. A quantitative test was developed in which samples on mesh stages were immersed in hexane. The hexane dissolved the solid fat component and any sugar not incorporated in the sugar skeleton fell through the mesh. Results showed that addition of water in any form to the samples produced a significant sugar skeleton (44 to 47% out of the total 49.2% sucrose in the original sample) while control samples prepared without added water had no skeleton and almost no sugar was retained on the mesh (0.4% out of the total 49.2% sucrose in the original sample). Since the presence of a sugar skeleton alone does not signify heat resistance, a meltability test was also developed. Samples were placed in an oven at 50 °C for 20 minutes and the change in height and the spread area were measured. Samples with water added via emulsions decreased in height by approximately 12% while samples with unemulsified water decreased by 41% and control samples decreased by 60%. Samples with stable emulsions added were able to contain the melted fat better than samples with unstable emulsions added resulting in areas of spread of 10.5 and 14.4 cm² respectively.

This study showed that water-in-oil emulsions produced by cross flow membrane emulsification and added to model chocolate are effective in producing samples with increased heat resistance conferred through the formation of a sugar skeleton.

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Chapter 1

LITERATURE REVIEW

1.1 Emulsions

Emulsions are a type of colloidal system. They are dispersions of one liquid in a second, immiscible liquid, e.g., water and oil. One liquid is distributed in droplets in the other liquid and is known as the dispersed phase. The second liquid into which the first is distributed is known as the continuous phase. Oil and water can be emulsified as either oil-in-water (o/w) or water-in-oil (w/o) emulsions. In addition to these simple emulsions, the dispersed phase can be an emulsion in itself, resulting in a more complex multiple emulsion e.g., water-in-oil-in-water. Multiple emulsions have many important applications, particularly in medicine where studies focus on their function as delivery systems (Aserin, 2008). Other variations of the simple emulsion include biemulsions of two oil types as separate droplets in water (Nielloud and Marti-Mestres, 2000) or emulsions including partially crystalline oil phases. Emulsions tend to be cloudy or white, as the presence of many small droplets scatters the incident light.

Emulsions are used in a wide variety of applications including foods, pharmaceuticals, cosmetics, personal hygiene products, paints, lubricants, polishes, explosives (Pal, 1996). Examples of cosmetic and personal hygiene emulsions include lotions, creams, hair dyes and deodorants (Schramm, 2005) while a common medicinal emulsion is calamine lotion (Moody, 2004). Emulsions are also being explored for their capability as drug carriers for lipophilic drugs (Buszello and Muller, 2000).

Emulsions are frequently used in the food industry, both as products and ingredients. Classic examples of oil-in-water emulsions are salad dressings, sauces, flavored beverages, mayonnaise, milk and cream (Dickinson, 1992). While oil-in-water emulsions are the most common food emulsion, water-in-oil emulsions include important products such as butter and margarine (Dickinson, 1992). They would also have potential application in any fat continuous product.

Food emulsions contain anywhere from less than a percent to more than 75% of dispersed phase (Coupland, 2010). This can be expressed either in terms of a volume percent or a mass percent and is referred to as the dispersed phase volume fraction (ϕ) or the dispersed phase mass fraction (ϕ_m) respectively. The relative amount of dispersed phase influences important emulsion characteristics including appearance, flavor, stability and texture (McClements, 1999).

The droplets in a food emulsion can range in diameter from less than 1 μm to upwards of 100 μm (Dickinson, 1992) and are typically polydisperse in the continuous phase meaning that a range of droplet sizes is present (Coupland, 2010). Since the two liquids are immiscible, the droplets of an emulsion are normally spherical in order to minimize the interfacial area. The sizes of the droplets can be measured with a variety of instrumental techniques (See Section 1.3).

Polydisperse emulsions are also described by plots of particle size distribution data showing the full range of droplet sizes present versus their frequency (%). A log-normal plot is used since most food emulsions are skewed towards larger droplet sizes (McClements, 1999). Again these can be determined based on any of the parameters used in calculating a mean. From the particle size distribution, a standard deviation of the mean can be determined (σ) and is used in order to describe the spread of the distribution. This mean can be calculated a variety of ways based on parameters such as length, area, number, volume, surface- volume or equivalent volume (Coupland, 2010). A mean based on volume fraction ($D_{3,3}$) means that 50% of the total dispersed phase volume is in droplets less than that number. Alternatively, a mean based on number fraction ($D_{0,0}$) means that 50% of the dispersed phase droplets are smaller than that number. A volume fraction mean is skewed more towards larger droplets while a number fraction mean is skewed towards smaller droplets (McClements, 1999).

1.2 Emulsion Stability

Given that the liquids of an emulsion are immiscible, physical stability of a food emulsion is extremely important. Instability can affect appearance, texture, taste and shelf-life of a product.

Emulsion instability arises from the thermodynamic drive to decrease interfacial surface area. It is the kinetic parameters of the system, however, which govern how quickly the emulsion

breaks down and can be used to counter the thermodynamic force. Common mechanisms of emulsion instability include creaming/sedimentation, flocculation, coalescence and Ostwald ripening. Each mechanism, its contributing factors and ways to control it are detailed below.

1.2.1 Creaming/Sedimentation

Creaming and sedimentation are the movement of emulsion droplets based on density differences between the two phases. Creaming occurs in oil-in-water emulsions in which the less dense oil droplets move to the surface, creating a “cream” layer. Conversely, sedimentation occurs in water-in-oil emulsions where the more dense water droplets settle to the bottom. If creaming or sedimentation occurs alone, the droplets can be redispersed in the continuous phase through gentle mixing.

The rate of gravitational separation is usefully understood in terms of Stokes’ law governing the terminal velocity of an isolated droplet (McClements, 1999):

$$v_{\text{Stokes}} = -\frac{2gr^2(\rho_2 - \rho_1)}{2\eta_1} \quad (1.1)$$

where v_{Stokes} is the velocity of the droplet, g is the acceleration due to gravity, r is the radius of the droplet, ρ_1 and ρ_2 are the densities of the continuous phase and dispersed phase respectively and η_1 is the shear viscosity of the continuous phase. If v_{Stokes} is positive the droplet will cream while if it is negative the droplet will sediment.

Based on the variables in the Stokes’ equation, emulsion creaming/sedimentation can be altered and controlled by many factors. Changing the densities of the phases so that they are more similar will drastically decrease the drive for separation. Other ways to decrease creaming/sedimentation include reducing the droplet size, increasing the viscosity of the continuous phase, increasing droplet concentration, preventing flocculation in dilute emulsions, promoting flocculation in concentrated emulsions and increasing the droplet surface charge (McClements, 1999). Stokes’ equation, however, makes some assumptions about the system. The derivation of Stokes’ law assumes one droplet that is a rigid sphere in an infinite and ideal liquid (McClements, 1999; Hiemenz, 1986; Dickinson, 1992) and this is not the case in food

emulsions. A more accurate equation would take into account the fluidity of liquid droplets, the concentration of the emulsion, the polydispersity of the system, the Brownian motion of the system, the degree of fat crystallinity and the interactions between the droplets.

1.2.2 Flocculation

Flocculation, also known as aggregation, occurs due to energetic interactions between droplets. Droplets in an emulsion are constantly in motion and often collide. If the droplets stick to each other without rupture of their surfaces, flocculation has occurred. Flocculation can drastically change the appearance, texture and stability of an emulsion (Walstra, 1993).

As noted earlier, flocculation affects the rate of creaming or sedimentation. Flocculated droplets (flocs) have a larger effective radius which increases the rate of sedimentation/creaming as seen in the Stokes' equation (Coupland, 2010). This is true in more dilute emulsions. In more concentrated emulsions, the flocs provide a network that hinders the movement of other droplets, thus slowing creaming and sedimentation.

Flocculation can be controlled by changing the frequency of the collisions or the strength of the interactions between the droplets. Factors that influence these two requirements for flocculation include the viscosity of the continuous phase, the concentration of the droplets, the droplet size, the relative densities, the charge on the droplet surface, the charge of the continuous phase and the addition of polymers or other colloidal particles to the continuous phase (McClements, 1999).

1.2.3 Coalescence

In contrast to flocculation, coalescence occurs when the interparticle film separating flocculated or otherwise closely associated droplets ruptures and results in the formation of a single larger droplet. In extreme instances this can lead to phase inversion of the system. It also increases the rate of creaming/sedimentation by increasing the droplet diameter. The main ways of controlling coalescence are to decrease the contact between droplets and to increase the strength of the interparticle layers through the use of emulsifiers. The use of emulsifiers is explained in detail in Section 1.2.5.

1.2.4 Ostwald Ripening

Ostwald ripening occurs mainly in water-in-oil emulsions and is the accumulation of material, i.e. water, via mass transfer into larger droplets from smaller droplets. The resulting emulsion has fewer but larger droplets, again increasing the rate of gravitational phase separation. This process is retarded by gelling the continuous phase, decreasing the solubility of the dispersed phase in the continuous phase, reducing interfacial tension and narrowing the droplet size distribution (McClements, 1999).

1.2.5 Surfactants/Emulsifiers

Emulsifiers, typically either small molecule surfactants or proteins, are used mainly to enhance the stability of emulsions and facilitate the formation of small droplets. They may also have the added functions of altering the texture, shelf-life, morphology and rheology of products (Dickinson, 1992; McClements, 1999). Emulsifiers are amphiphilic molecules that have both hydrophilic and hydrophobic portions. These molecules orient themselves at droplet surfaces with the hydrophilic and hydrophobic portions partitioning into the appropriate phase. In doing so, they lower the interfacial tension between the phases, thereby stabilizing the system by decreasing the thermodynamic drive for separation. Their presence also decreases the likelihood of flocculation and coalescence by the formation a protective layer around the droplets (Dickinson, 1992; Walstra, 1993)

There are many different kinds of emulsifiers including polymeric surfactants, fine particles and small-molecule surfactants. Polymeric surfactants can be disordered proteins, globular proteins or some polysaccharides (Coupland, 2010). They are particularly effective at preventing flocculation and coalescence, although their larger size increases the effective droplet diameter and may influence the rate of creaming/sedimentation. Fine particles such as flours, spices and crystals also help to stabilize emulsions (Coupland, 2010).

Small-molecule surfactants have smaller molecular weights than polymeric surfactants and can diffuse rapidly to the surface of droplets (Coupland, 2010). Examples of this type of emulsifier are polysorbates and phospholipids (Dickinson and Woskett, 1989). Small-molecule surfactants are commonly used as food emulsifiers and are typically added to the continuous

phase prior to emulsification. A way of distinguishing between emulsifier types is based on Bancroft's rule which states that the continuous phase is the phase in which the emulsifier is more soluble. Water continuous emulsifiers include sodium dodecyl sulfate and polyglycerol and sucrose esters of fatty acids while common oil continuous emulsifiers (of particular importance to this work) are sorbitan esters, polyglycerol polyricinolate (PGPR) and lecithins (Kato et al., 1996).

PGPR is a potent stabilizer of water-in-oil emulsions made by dehydrating and heating ricinoleic acid followed by esterification with polyglycerol (Stauffer, 2002). PGPR coated water droplets exhibit strong repulsive steric forces which decrease their tendency for flocculation and coalescence (Claesson, Blomberg and Poptoshev, 2004; Knoth, Scherze and Muschiolik, 2005a). It is approved for use in the United States in chocolate at levels up to 0.3 % and in "margarines, low-fat margarines, spreads, creamers and dairy analogs at levels no greater than 1 % by weight" (Palsgaard, 2008). In chocolate, it is used to improve the flow properties while decreasing the amount of cocoa butter needed. Despite its excellent emulsifying capabilities, consumers may be wary of PGPR because of its long, technical name and synthetic nature.

Natural lecithin is a mixture of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid and commonly comes from soybean, rapeseed and egg (Faergemand and Krog, 2003; Knoth, Scherze and Muschiolik, 2005b). It is used as an emulsifier, dispersing agent, wetting agent and viscosity modifier (Dickinson, 1992; Knoth, Scherze and Muschiolik, 2005). Soybean lecithin is most commonly used in the food industry as it is cheaper and easier to obtain. Lecithin can be fractionated or modified enzymatically, chemically or thermally (van Nieuwenhuyzen and Szuhaj, 1998; McClements, 2005). Such treatment can make lecithin more hydrophobic and increase its ability to stabilize water-in-oil emulsions (Weete, Betageri and Griffith, 1994; McClements, 2005). Lecithin is also used in chocolate to decrease viscosity. As a result of its shorter name and natural source, it is generally more accepted by consumers.

1.3 Emulsion Characterization

The inherent thermodynamic instability of emulsions and the effect that this instability can have on product quality make it important to be able to characterize an emulsion initially and

to track changes over time. While characteristics such as dispersed phase volume fraction, droplet crystallinity, rheology and droplet charge can all be measured by certain respective techniques, this section will focus on the measurement of droplet size and distribution (McClements, 1999).

1.3.1 Microscopy

Different types of microscopy can be used to image and characterize emulsions including conventional optical microscopy and confocal scanning laser microscopy. Each technique has certain associated advantages and disadvantages.

Conventional optical microscopy allows emulsion structure to be viewed easily. These microscopes are less expensive than some of their counterparts and are therefore more common. Colored chemical dyes can be added in order to enhance phase contrast and improve image quality (Aguilera and Stanley, 1990; McClements, 1999). Common lipid stains which can assist in this process include Nile red, Nile blue, oil red O and Sudan black B (Aguilera and Stanley, 1990). Fluorescence microscopy is a similar technique that instead uses fluorescent light. Phase contrast is further enhanced in this technique by adding a fluorescing material to the emulsion (Aguilera and Stanley, 1990). Once images are collected in either case, image analysis is performed to determine particle size and distribution.

Problems with optical and fluorescence microscopy arise in the preparation of the sample and in the image analysis. Samples must be dilute enough such that droplets are not overlapping in any images. This can potentially destroy the integrity of the emulsion. Additionally, a cover slip is typically placed over the sample which can distort or destroy the droplets, particularly the larger droplets (Van Dalen, 2002). A slide with a well or culture chamber could be used, but the issue again is overlapping droplets. Resolution is also a problem with these methods because of the large depth of field. Even if a suitable sample preparation method is established, image collection and analysis are extremely time consuming. Enough representative yet random images must be collected to achieve sufficient statistical power and image analysis on each image must either be done manually or with a suitable software program.

Confocal scanning laser microscopy (CSLM), on the other hand, can provide enhanced quality, resolution and three dimensional images (McClements, 1999). The laser beam used in CSLM can be set at various depths to image layers of an emulsion without destroying structure or having to prepare thin slices. This technique is particularly useful for imaging water-in-oil emulsions where the continuous oil phase is partially crystalline such as in butters and margarines (Van lent et al., 2008; Van Dalen, 2002; Blonk and Van Aalst, 1993; Vodovotz et al., 1996). As in optical and fluorescence microscopy, CSLM samples can be stained, particularly with fluorescent dyes (Aguilera and Stanley, 1990). Despite its benefits, sufficient CSLM images still need to be collected and time consuming image analysis techniques are necessary as well.

1.3.2 Light Scattering

Both static and dynamic light scattering techniques can be used to effectively determine particle sizes and distributions of emulsions. Static light scattering is the more common technique for most emulsions while dynamic light scattering is typically used to image samples with very small droplets and narrow distributions (McClements, 1999). The three main methods for static light scattering are based on angular scattering, spectroturbidimetry and reflectance. Mie theory is used to calculate the size of a spherical droplet based on the patterns of scattered light collected by the detectors. Since Mie theory assumes isolated particles, light scattering works best in dilute systems. Concentrated emulsions must be diluted which may affect the emulsion characteristics.

1.3.3 Ultrasonic Spectrometry

Ultrasonic spectroscopy is used in emulsion droplet sizing and functions by relating ultrasonic wave properties to emulsion characteristics, again making use of an appropriate scattering theory. Ultrasonic techniques contrast with the previously mentioned methods in that they can be used with concentrated emulsions and do not require any sample preparation (McClements, 1996; McClements and Coupland, 1996; McClements, 1999; Coupland and McClements, 2001). A negative associated with this technique is that the presence of air bubbles drastically interferes with the signal (McClements, 1996; McClements, 1998).

1.3.4 NMR

Nuclear magnetic resonance spectroscopy (NMR) is used to study the structure, interaction and kinetics of molecules as well as the composition of solutions (Bruker BioSpin, 2011). The basis for this technique is the spin of certain nuclei, including hydrogen nuclei, which causes them to act like small magnets. When an external magnetic field is applied, the nuclei will align parallel the magnetic field in one of two energy states, either spin aligned or spin opposed to the field and precess or rotate around these parallel axes (Carey, 2011). The absorption of a photon of a certain energy enables the nuclei to transition between energy states, or resonate. The frequency of this photon is known as the Larmor frequency. In a simple NMR experiment, a sample is placed in a magnetic field and radio-frequency pulses are applied. After the pulses, the nuclei realign with the magnetic field and the manner in which they do so is analyzed to determine certain desired properties. This type of low-resolution NMR is widely used in the medical, pharmaceutical and food industries and forms the basis of an important method used here.

1.3.4.1 Operation and Theory

The use of NMR for the determination of droplet sizes began in the late 1960's and early 1970's with the application of pulsed magnetic field gradient spin-echo techniques to the study of emulsions (Stejskal and Tanner, 1965; Tanner and Stejskal, 1968; Packer and Rees, 1972). Droplet sizing in this way depends on the restricted diffusion of molecules inside the droplets, as hindered by the droplet walls (Voda and van Duynhoven, 2009). Diffusion is detected over a small period of time during which molecules are able to diffuse across emulsion droplets and encounter the wall barriers (Tanner and Stejskal, 1968; Packer and Rees, 1972).

In order to examine how droplet size measurements are performed, it is first necessary to define a frame of reference from which to examine the movement of the net magnetization vector, M_0 . The frame used in this situation is known as the rotating frame with axes X' and Y' which rotate around the Z axis at the Larmor frequency (Hornak, 1997). This allows for an easier visualization of the movement of the magnetization vectors since vectors moving at the Larmor frequency are stationary in this frame. Vectors moving either faster or slower than the Larmor

frequency are seen to move clockwise or counterclockwise about the Z axis respectively (Figure 1.1B).

In a spin-echo experiment, two radio-frequency pulses are applied to a sample. These pulses are created by passing alternating current through a coil around the X axis at the Larmor frequency which causes rotation of the net magnetization vector, M_0 , which is initially in the Z direction. The first pulse is a 90° pulse which rotates the magnetization into the horizontal X'Y' plane (Figure 1.1A; Hornak, 1997). After this pulse, the free induction decay (FID) signal can be monitored as the magnetization begins to dephase within this plane (Figure 1.1B; Hornak, 1997; Packer and Rees, 1971). A 180° pulse is then applied to the sample at time τ and rotates the magnetization around the X axis by 180° (Figure 1.1C; Hornak, 1997). As a result, the magnetization rephases and an echo signal can be monitored at time 2τ along the Y' axis (Figure 1.1D; Hornak, 1997; Packer and Rees, 1971; Bruker Optik GmbH, 2001).

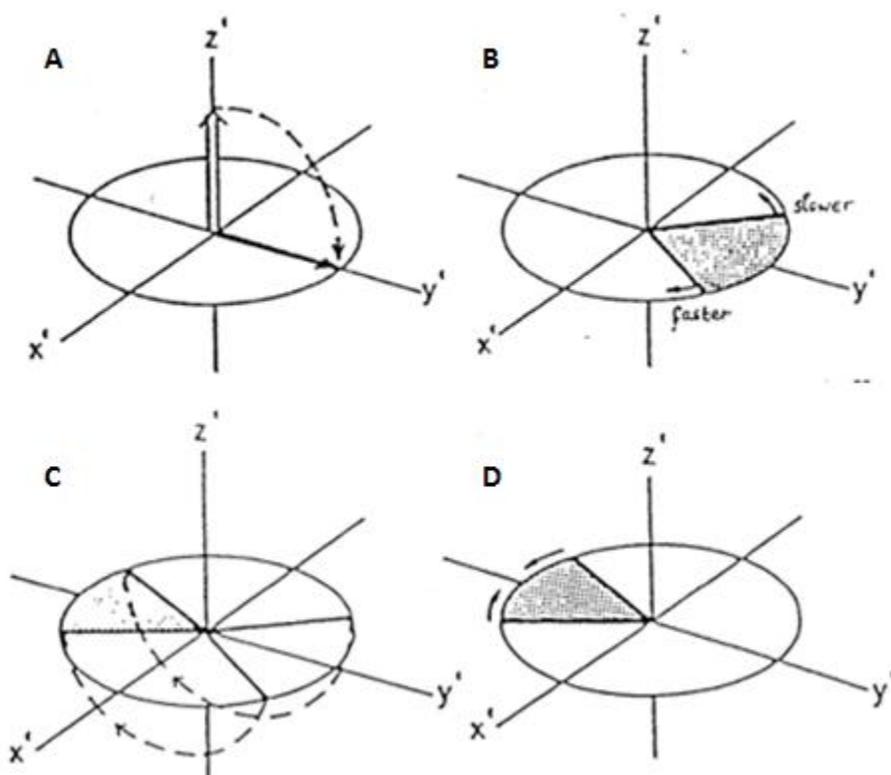


Figure 1.1. Movement and precession of magnetization vectors in the X'Y' plane using the rotating frame after A) 90° pulse; B) subsequent dephasing; C) 180° pulse; D) subsequent rephrasing (adapted from Bruker Optik GmbH, 2001).

When a pulsed magnetic field gradient is added to the spin-echo sequence, two gradient pulses operating around the X' axis are applied that are centered about the 180° pulse. The first pulse occurs at time t_1 after the FID signal but before the 180° pulse and the second occurs after the 180° pulse but before the spin-echo signal. The gradient pulses have a duration of δ , a magnitude of g and are separated by time Δ as seen in Figure 1.2.

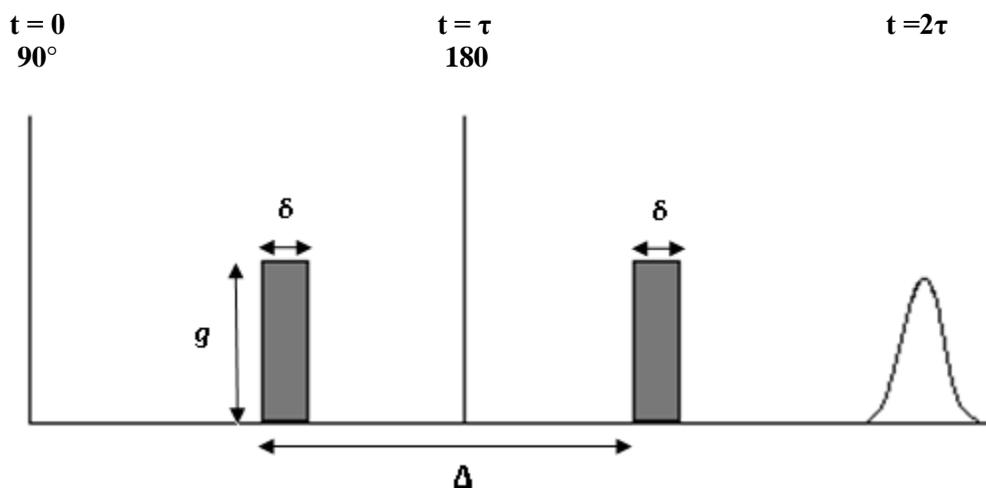


Figure 1.2. Sequence of events in pulsed field spin spin-echo experiment. Shaded regions represent gradient field pulses; δ – pulse width; g – pulse magnitude; Δ – pulse separation.

The first gradient pulse dephases the nuclear spin magnetization. If the pulses are of equal strength and no diffusion occurs, the resultant echo has the same height as when the experiment is performed without the gradient pulses (Bruker Optik GmhB, 2001). If diffusion does occur within the sample, the second pulse can only partially rephase the nuclei and the intensity of the spin-echo signal is decreased (Figure 1.3; Packer and Rees, 1971; Fourel, Guillement and Le Botlan, 1994; Van den Enden et al., 1990; Bruker Optik GmhB, 2001). In general terms, “the first gradient pulse labels the spins according to their position, and the second gradient pulse determines how much they have moved in the interval” (Tanner and Stejskal, 1968). The echo attenuation, R , is the ratio of the echo heights of pulse sequences with and without gradient pulses and can be determined at different values of Δ , the time between pulses. A plot of R vs Δ is a straight line from which the diffusion coefficient can be calculated (Figure 1.4A; Bruker Optik GmhB, 2001).

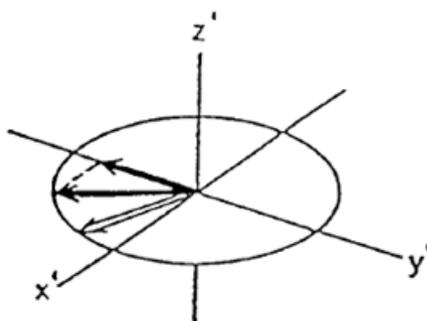


Figure 1.3. Reduced echo height of magnetization vector after pulsed-field spin-echo experiment with diffusion (adapted from Bruker Optik GmbH, 2001).

In a restricted diffusion experiment, the R value reaches a minimum plateau at a certain Δ value for a constant gradient pulse of length δ (Figure 1.4B) because the water molecules can only diffuse so far without encountering a boundary (droplet surface). Similar plateaus are achieved at various R values when the gradient pulse length is altered and Δ is kept constant. This provides a “fingerprint” for the droplet distribution which is then calculated by the software application (Bruker Optik GmbH, 2001).

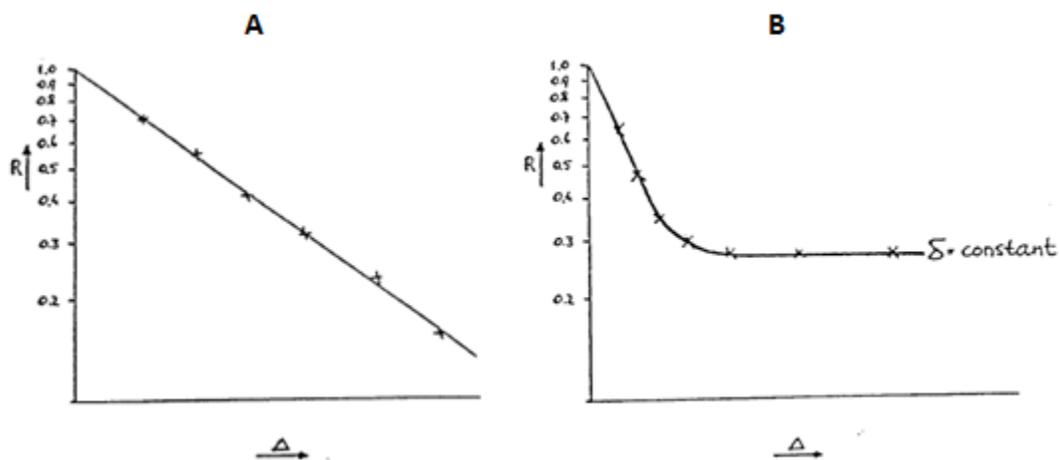


Figure 1.4. Echo attenuation (R) vs. time between gradient pulses (Δ) for A) diffusion and B) restricted diffusion experiments (adapted from Bruker Optik GmbH, 2001).

In order to measure the droplet size of water-in-oil emulsions, the spin-echo experiment with gradient pulses must be modified. The first variation is that the 180° pulse is divided into two 90° pulses in order to have an identifiable echo signal because the Δ used in these experiments is fairly long (Bruker Optik GmbH, 2001). Additionally, something must be done so that the signal from the water protons is not disrupted by the signal from the oil protons (Bruker Optik GmbH, 2001). To accomplish this, the different relaxation rates of oil and water are taken into account. As seen in Figure 1.5, after a 180° pulse the net magnetization of oil protons returns to the original value much faster than water protons and there is a point at time τ_0 that the net magnetization of the oil protons is zero. If the first 90° pulse is applied at time of τ_0 after an initial 180° pulse, the final signal will only be due to the water protons. The value of τ_0 is determined by a measurement on an oil sample without pulses, varying τ_0 until the signal disappears (Bruker Optik GmbH, 2001).

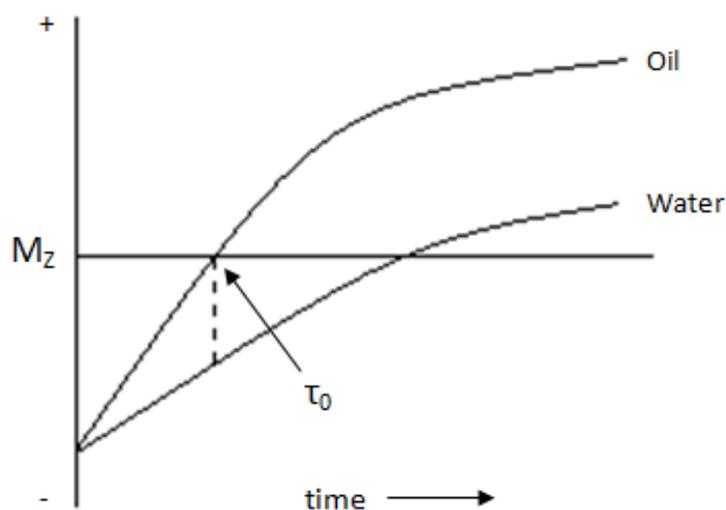


Figure 1.5. Relaxation of oil and water protons after a 180° pulse.

Thus by comparing the spin-echo signal heights with and without gradient pulses of different pulse lengths and using detailed equations that take into account the diffusion coefficient, the average droplet size and distribution can be calculated (Packer and Rees, 1971; Murday and Cotts, 1968; Tanner and Stejskal, 1968; Voda and van Duynhoven, 2009).

1.3.4.2 Benefits and Comparison

There are many benefits of using pulsed field gradient NMR (pfg NMR) for emulsion droplet sizing compared to other techniques. Experimentation can be performed with a low magnetic field (20 MHz) which means that the necessary spectrometers are relatively low cost (Voda and van Duynhoven, 2009). Additionally, pfg NMR can be used on emulsions with no sample preparation. Emulsions do not need to be diluted and the samples are not damaged by testing (Gabriele et al., 2009; Rousseau and Hodge, 2005; Van lent et al., 2008). The technique can be used on both water-in-oil and oil-in-water emulsions as well as multiple and partially crystalline samples.

Several researchers have performed studies comparing pfg NMR results to those from other particle sizing methods. Fourel, Guillement and Le Botlan (1994) showed that laser diffraction or image analysis were less time consuming techniques for liquid emulsion analysis than NMR. However, when it came to “solid” emulsions, Fourel, Guillement and Le Botlan (1995) found that NMR was a more appropriate method for analysis as compared to image analysis. The group used commercial butters with a range of solid fat contents in their analysis and found that laser diffraction could not be used and image analysis was limited since the samples had to be sliced before measurement. There was sufficient agreement in droplet size distributions between NMR and image analysis and the NMR method exhibited reproducibility.

Two studies compared pfg NMR to confocal scanning laser microscopy for imaging butters or fat spreads. Van Dalen (2002) analyzed fat spreads with fat contents of 40 – 80%. He found that while the two techniques gave comparable results at both 65 and 80% fat content, there were large differences in the measurements of 40% fat spreads. This, however, was ascribed mainly to the shortcomings of CSLM and the only advantage of CSLM over NMR was the collection of visual information about the droplet size distribution. Van lent et al. (2008) also compared CSLM to NMR when analyzing butters. Again, the only shortcoming of NMR was stated to be the lack of visual information. Van lent et al. (2008) also found that NMR was a more accurate method for droplet size distribution analysis, especially in samples with numerous small particles. The method was faster, easier and more reliable than CSLM.

A study by Gabriele et al. (2009) compared NMR and dynamic light scattering in the analysis of dairy emulsions. Researchers found that the main advantage of NMR was the lack of sample preparation that was necessary for light scattering. For light scattering, the dairy emulsion had to be diluted. NMR tests on a diluted sample showed a larger droplet diameter as compared to the results from an undiluted sample. Although a reason for this phenomenon was not stated, Gabriele et al. (2009) acknowledged that sample dilution could change emulsion properties and thus the use of NMR for concentrated emulsion analysis would be favored. The authors noted, however, that NMR cannot be used in studying flocculation, as the aggregated droplets are still intact.

Another restriction of this method is the droplet size that is able to be measured. Large droplets ($>100\ \mu\text{m}$) cannot be accurately measured by the application because the diffusion time before encountering the droplet walls is too large. This limits the use of this technique, especially in long term stability studies, except in cases with very small droplet sizes.

1.4 Methods of Formation

When emulsions are formed from two bulk immiscible phases the interfacial area is increased. This corresponds to an increase in the free energy of the system. As this increase is thermodynamically unfavorable, the formation of emulsions requires the input of work of some type.

Work can be supplied to the system by several different methods in order to form an emulsion. Traditional methods include rotor-stators, high pressure homogenizers, ultrasound homogenizers and microfluidizers. Rotor-stator methods include all forms of mixers and stirrers, colloid mills and toothed disc dispersing machines (Sotoyama et al., 1999; Charcosset, Limayem and Fessi, 2004; Tadros, 2009). In many cases, these techniques are combined, with a coarse pre-mix emulsion produced first by stirring and then emulsified via one of the above methods.

All of these methods are very inefficient as most of the energy input is lost as heat (Gijssbertsen-Abrahamse, van der Padt and Boom, 2004). This heat can damage any labile ingredients in the emulsion. Additionally, the equipment is usually bulky and expensive and controlling droplet size and distribution can be difficult.

Lack of control over the characteristics of the emulsions produced by these methods is another shortcoming. High shear stress is used to break up droplets in these methods. This can lead to coalescence of the dispersed phase if it is not quickly stabilized by emulsifiers (Charcosset, 2009). As a result, it is more difficult to specifically control droplet size and distribution (Sotoyama et al., 1999; Egidi et al., 2008). Inconsistent droplet size can have a drastic effect on the properties of an emulsion, especially in terms of microbiological stability and flavor release in emulsions with a dispersed flavor component (Sotoyama et al., 1999; Charcosset, 2009). All of these issues make scale up from laboratory scale equipment unreliable (Egidi et al., 2008; De Luca et al., 2008).

In contrast to these methods, a more recently developed method, membrane emulsification, which does not involve high shear stress and requires less energy provides a way to control droplet size and distribution (Sotoyama et al., 1999). Membrane emulsification entails the use of microporous membranes and has become more popular in the past 20 years because of these advantages (Charcosset, 2009). This technique will be detailed in the following section (Section 1.5).

1.5 Cross Flow Membrane Emulsification

Membrane emulsification utilizes pressure to push a liquid through a porous membrane (Charcosset, Limayem and Fessi, 2004). Frequently the continuous phase is sheared to aid in droplet detachment although the shear stress is much lower than in other emulsification methods (Egidi et al., 2008). Different types of membrane emulsification methods include pre-mix membrane emulsification and direct membrane emulsification (De Luca et al., 2008). In pre-mix emulsification, a coarse emulsion is passed through a membrane to reduce the droplet size (De Luca et al., 2008). The coarse emulsion is typically produced via traditional emulsification methods (Gutierrez, Rayner and Dejmek, 2009). Vladisavljevic, Shimidzu and Nakashima (2004) explored the use of pre-mix emulsification and argued that the process was commercially unrealistic. This was due to the need for multiple passes through the membrane to achieve a desired droplet size as well as an increased likelihood of fouling (Vladisavljevic, Shimidzu and Nakashima, 2004; Kosvintsev et al., 2005).

In direct membrane emulsification, the dispersed phase is injected into the continuous phase through membrane pores. Droplet detachment typically occurs due to shear stress at the membrane surface that can be produced by a variety of methods. The membrane can be rotated or vibrated in a stationary continuous phase (Zhu and Barrow, 2005; Vladisavljevic and Williams, 2006; Charcosset, 2009). Additionally, agitation of the continuous phase via stirring can cause the shear stress. This is usually done in a set up known as a stirred cell in which a paddle-blade stirrer is moved over the surface of a flat, circular membrane (Stillwell et al., 2007; Egidi et al., 2008). A final method is cross-flow membrane emulsification in which shear stress is produced by recirculating the continuous phase past the membrane surface as seen in Figure 1.6 (Egidi et al., 2008; Charcosset, 2009). Direct cross flow membrane emulsification is used in this work and discussed further below.

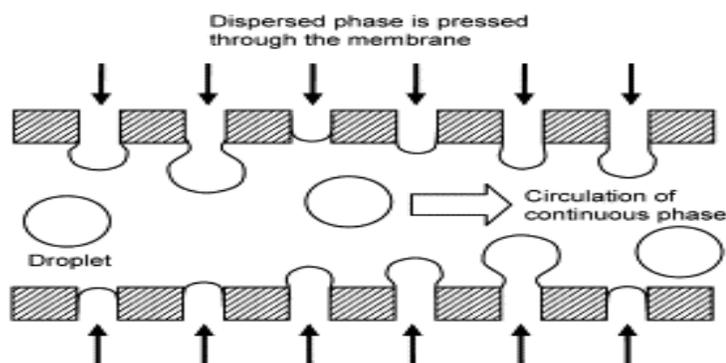


Figure 1.6. Simplified diagram of cross-flow membrane operation (Rayner et al., 2004).

1.5.1 Droplet Formation and Diameter in Cross Flow Membrane Emulsification

1.5.1.1 Theory

Droplet formation in cross-flow membrane emulsification occurs in two steps; droplet growth at the pore and droplet detachment into the flowing continuous phase (Peng and Williams, 1998). The droplet diameter is given by how far droplet growth proceeds before detachment. There are various modeling methods for droplet formation including computational fluid dynamics, surface free-energy minimization, lattice Boltzmann simulations and force or torque balances (Rayner et al., 2004; De Luca et al., 2008). The force or torque balance models are most commonly explored in the literature (De Luca et al., 2004; De Luca et al., 2008; Egidi et al.,

2008). The latter model, torque balance, assumes a rigid spherical droplet. The center of torque is at the attachment point of the droplet to the pore on the side opposite the continuous flow inlet as displayed in point A in Figure 1.7 (De Luca et al., 2008). Detachment occurs when the clockwise torque overcomes the counterclockwise torque at point A.

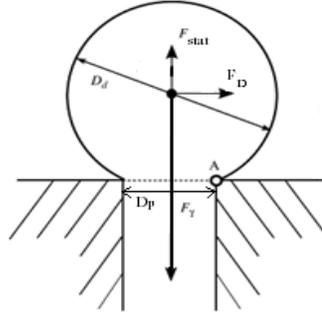


Figure 1.7. Simplified Torque Model- D_d – droplet diameter; D_p – pore diameter; F_{stat} – static pressure force; F_D – cross flow drag force; F_γ – interfacial tension force; A – point of torque (adapted from De Luca et al., 2005).

While multiple forces are acting on the droplet, the main contributors to the torque balance are the torques induced by the static pressure force (F_{stat}), the cross flow drag force (F_D) and the interfacial tension force (F_γ) (Schroder, Behrend and Schubert, 1998). The static pressure force is due to the pressure difference between the inside and outside of the droplet (Egidi et al., 2008). The cross-flow drag force is due to the flow of the continuous phase past the droplet and the interfacial tension force is due to the capillary force of the droplet. The first two forces can be viewed as inducing a detaching torque, pulling the droplet clockwise around point A and away from the pore. The interfacial tension force acts in a counterclockwise direction about point A and essentially holds the droplet onto the pore. Detachment occurs when the detaching torque (static pressure and cross-flow drag) overcomes the attaching torque caused by interfacial tension (Peng and Williams, 1998; De Luca et al., 2008).

The torque balance model can be used to predict how experimental parameters influence the droplet diameter. Thus, by altering these parameters, a more controlled emulsion can be produced with a desired droplet size and narrow size distribution. A narrow distribution is often desired because it decreases Ostwald Ripening and hence increases the stability of the emulsion. In the torque balance model, the cross-flow drag force is given as;

$$F_D = k_\chi 3\pi\mu_c d_d v_c^\infty \approx \frac{3}{2} k_\chi \pi \tau_w d_d^2 \quad (1.2)$$

where k_χ is the wall correction factor ($k_\chi = 1.7$ for a single sphere touching an impermeable wall in the presence of simple shear flow), μ_c is the viscosity of the continuous phase, d_d is the droplet diameter, v_c^∞ is the undisturbed tangential velocity of the continuous phase in the center of the droplet and τ_w is the wall shear stress (De Luca et al., 2004). The interfacial tension force is described as;

$$F_\gamma = \pi d_p \gamma(t) \quad (1.3)$$

where d_p is the diameter of the membrane pore and $\gamma(t)$ is the dynamic interfacial tension as a function of time (De Luca et al., 2004) Lastly, the static pressure force is;

$$F_{stat} = \frac{\gamma(t)}{d_d} \pi d_p^2. \quad (1.4)$$

Various torque balance based models use equations that include some or all of these forces. Oftentimes additional forces such as a dynamic lift force and a buoyancy force are included in the calculation as well, although in most cases these can be considered negligible (De Luca et al., 2008). One model used by De Luca et al. in a 2004 study comparing different torque balance models combines the cross-flow drag force, the interfacial tension force and the static pressure force in order to model the droplet diameter. This relationship is seen in Equation 1.5 below;

$$F_D \frac{d_d}{2} = (F_\gamma - F_{stat}) \frac{d_p}{2}. \quad (1.5)$$

From Equation 1.5 we can see how the different experimental parameters influence resulting droplet diameter. Factors including continuous phase viscosity, continuous phase velocity, pore diameter and interfacial tension are all included in the calculations. These

variables can be altered by selecting appropriate continuous phase oils and surfactants, altering the composition of the dispersed aqueous phase, using membranes with certain characteristics and changing the operational settings of the continuous and dispersed phase pumps (De Luca et al., 2004).

The above parameters can be divided into three areas: the composition of the phases, the membrane characteristics and the processing conditions (Charcosset, 2009). Several previous experiments have been performed to investigate the effect of changing these parameters in a cross flow emulsification system. The vast majority of these investigations have been performed on oil-in-water emulsions. The findings from the literature are summarized in the following subsections, although it is uncertain if all of the determined relationships hold true for water-in-oil emulsions.

1.5.1.2 Composition of Phases

One of the main variations to the phase compositions is the addition of different types and levels of emulsifiers. Emulsifiers can alter the phase viscosity as well as the interfacial tension and these changes directly impact Equation 1.5 and thus the droplet size. Increasing levels of emulsifier have been shown to decrease the droplet size (Schroder, Behrend and Schubert, 1998; Joscelyne and Tragardh, 1999). This is mainly a function of reduced interfacial tension as well as the ability of emulsifiers to protect against droplet coalescence (Schroder, Behrend and Schubert, 1998; Joscelyne and Tragardh, 1999; Yuan et al., 2009; Charcosset, Limayem and Fessi, 2004).

The type of oil used can also influence the droplet size. Different oils have different viscosities and interfacial tensions. The purity of oils also affects the interfacial tension. A study by Gutierrez, Rayner and Dejmek (2009) found that oils free from impurities resulted in narrower size distributions in oil-in-milk emulsions.

In a water-in-oil emulsion, the composition of the water phase can be altered by the addition of gelling agents. This affects the viscosity of the aqueous phase, the aqueous phase flux and perhaps the behavior at the pores. Gelling the dispersed phase after emulsion formation has been shown to increase the stability of the droplets produced (Garti and Benichou, 2004). While

there is no experimental data on how the addition of gelling agents to the dispersed phase affects droplet size, it is likely that the increased viscosity of the aqueous phase results in a decreased aqueous phase flux and therefore produces smaller droplets.

1.5.1.3 Membrane Characteristics

The membranes used in cross flow membrane emulsification can be manufactured from a variety of materials. The most common material is Shirasu-porous-glass (SPG) made from Japanese volcanic ash. Other membrane types include silicon and silicon nitride microsieves, polycarbonate track-etch, ceramic aluminum oxide, α -aluminum and zirconia coated, sacroporous silica glass and polytetrafluoroethylene membranes (Schroder, Behrend and Schubert, 1998; Joscelyne and Tragardh, 1999; Charcosset, 2009; Yuan, Williams and Biggs, 2009; Lepercq-Bost et al., 2010; de los Reyes and Charcosset, 2010). Additionally, membranes are often coated with certain materials or presoaked in the continuous phase to make them either hydrophilic or hydrophobic for oil-in-water and water-in-oil emulsions respectively (Katoh et al., 1996). The type of membrane and its pretreatment have been shown to affect droplet diameter (de los Reyes and Charcosset, 2010) and must therefore be selected depending on desired emulsion characteristics. A membrane coated with the dispersed phase would lead to coalescence at the membrane surface instead of budding of the dispersed phase at the pores.

Another main contributor to emulsion droplet size is the size of the membrane pores. It has been shown that there is a linear relationship between the size of the pores and the size of the resulting droplets (Equation 1.5; Charcosset, Limayem and Fessi, 2004; Gijsbertsen-Abrahamse et al., 2004). It is also necessary that the pore size distribution be narrow (Peng and Williams, 1998). In a study of corn oil or kerosene-in-water emulsions stabilized by sucrose esters and sodium dodecyl sulfate respectively, Katoh et al. (1996) stated that “the dispersion droplet diameter basically depends upon the membrane pore diameter.” The researchers used SPG membrane with pore sizes ranging between 0.57 and 2.34 μm and found a linear relationship between pore size and droplet size.

A final membrane characteristic that affects droplet diameter is porosity which is related to the distance between the pores. According the Peng and Williams (1998) it is preferred that pores are uniformly spaced on the membrane surface and there is enough distance between the

pores such that the droplets do not touch, even during deformation by the continuous phase. If contact between droplets occurs before detachment there is an increased likelihood of coalescence (Peng and Williams, 1998; Gijsbertsen-Abrahamse et al., 2004).

1.5.1.4 Processing Conditions

One of the processing conditions that can be altered in a cross flow membrane emulsification system is the transmembrane pressure. Transmembrane pressure is the pressure difference between the dispersed phase and the continuous phase at the membrane (Lepercq-Bost et al., 2010). This force can be controlled by altering the speed of the continuous and dispersed phase pumps and thus the respective fluxes. There is not, however, a linear relationship between the continuous or dispersed phase flux and their respective pressure, so the pressures must be determined experimentally (Lepercq-Bost et al. 2010). In a study of soybean oil-in-water emulsions produced using alumina membranes, Lepercq-Bost et al. (2010) showed that mean droplet diameter and droplet polydispersity increased with increasing transmembrane pressure. Increasing transmembrane pressure led to more active pores which resulted in increased coalescence at the membrane due to droplets forming in close proximity (Lepercq-Bost et al., 2010).

Another processing condition that can be controlled is the wall shear stress. This parameter is changed by varying the continuous phase pump speed and thus the continuous phase flux past the membrane. Joscelyne and Tragardh (1999) showed that at a constant emulsifier concentration, increased wall shear stress resulted in decreased droplet size. These researchers used a system of oil dispersed in milk via cross-flow emulsification as a possible replacement for mother's milk. They determined that at low shear stresses the droplets coalesced at the membrane surface before detachment by the continuous phase (Joscelyne and Tragardh, 1999). Similar results were also found by Schroder and Schubert (1997) and Katoh et al. (1996), also for oil-in-water emulsions.

1.5.2 Studies of Water-in-Oil Emulsions

While the majority of cross flow membrane emulsification studies have been performed on oil-in-water emulsions, a few have investigated water-in-oil systems. Kandori, Kishi and

Ishikawa (1991a,b) investigated water-in-oil emulsions prepared using hydrophilic SPG membranes. An initial study (Kandori, Kishi and Ishikawa, 1991a) compared the droplet size and distribution obtained from batch (stirred tank) and continuous (cross flow) methods. The emulsions were made with water distributed in toluene with different levels of poly(oxyethylene-oxypropylene) surfactants. The researchers found that there was no difference between the droplet sizes produced by the two different methods. They showed that the droplets produced by either method were fairly monodisperse and the emulsions were stable after one month.

The researchers performed a second study examining the effects of interfacial tension and pore size on droplet diameter (Kandori, Kishi and Ishikawa, 1991b). Although this study did not use the continuous or cross flow method of production, the initial study showed no difference in droplet size, so only the batch method was used. The researchers showed that increasing surfactant concentration and therefore decreasing interfacial tension resulted in decreased droplet size. The size of the droplets was also shown to depend on the membrane pore diameter.

In another study of water-in-oil emulsions, Katoh et al. (1996) prepared emulsions in both corn oil and kerosene with either sorbitan esters or PGPR as emulsifiers. They examined increasing the dispersed phase flux from previously used values to a level that would be acceptable in food manufacturing. The researchers used a hydrophilic membrane that was pre-immersed in oil. In a more in-depth study of the corn oil system, Katoh et al. (1997) found that the droplet diameter was proportional to the pore diameter, droplet size decreased with increasing continuous phase pump speed and the dispersed phase flux could in fact be increased to reasonable levels.

Other parameters of a cross flow membrane emulsification system were examined by de los Reyes and Charcosset (2010) who investigated water-in-oil and ethanol-in-oil emulsions. Preliminary investigation into suitable emulsifiers determined that 5 wt% PGPR in the soybean oil continuous phase produced emulsions that remained stable after approximately one month of storage. The study then looked at the differences between emulsions that were prepared with a membrane wetted with the continuous phase versus with the dispersed phase. The droplet size and polydispersity were monitored as well as the changes in the dispersed phase flux. The researchers found that pre-treatment of the membrane with oil resulted in narrower droplet size distributions and low dispersed phase flux for water-in-oil emulsion.

1.6 Chocolate

Chocolate is a suspension of solids in a continuous phase of semi-crystalline fat. The three main types of chocolate are dark, milk and white. Dark chocolate is a suspension of sugar and non-fat cocoa solids in cocoa butter, while milk chocolate adds milk solids and milk fat to the mix. White chocolate, on the other hand, includes milk solids and milk fat but does not include nonfat cocoa solids. Chocolate can also include emulsifiers such as lecithin and PGPR as well as salt, flavoring or spices. While each country follows its own set of laws and requirements for their chocolate, the United States standard of identity for the three main chocolate types is shown in Table 1.1 (21CFR163, 2010). Note that only cacao fat and milk fat are included in these formulations. The addition of any vegetable derived oils, fats or stearins other than from cacao fat prevents the product from being able to be called a chocolate or chocolate coating (21CFR163, 2010).

Table 1.1. Standards of identity of three main chocolate types based on the US FDA Code of Federal Regulations (21CFR163, 2010)

	Semisweet/Bittersweet Chocolate	Milk Chocolate	White Chocolate
Chocolate Liquor	≥ 35 %	≥ 10 %	0
Sugar	-	-	≤ 55 %
Cacao Fat	-	-	≥ 20 %
Total Milk Solids	≤ 12 %	≥ 12 %	≥ 14 %
Milk Fat	≤ 12 %	≥ 3.39 %	≥ 3.5 %
Emulsifier	≤ 1.0 %	≤ 1.0 %	≤ 1.5 %

1.6.1 Fat

Chocolate is typically 30 to 40% fat, depending on the type and its application (Beckett, 2000; Do et al., 2007). This fat is mostly cocoa butter, although milk fat is a minor component. Cocoa butter is responsible for the “snap, gloss, creamy texture, rich taste and melt-in-the-mouth quality” desired in chocolate (Norton et al., 2009). It is composed mainly of triacylglycerols of three fatty acids: saturated palmitic acid and stearic acid and monounsaturated oleic acid (Afoakwa, Paterson and Fowler, 2007). Cocoa butter can crystallize into a variety of polymorphic forms, each form melting at a different temperature as seen in Table 1.2. Form V has the most desired melting profile for chocolate as it melts between 32 and 34 °C, meaning that

it melts at body temperature in the mouth (Norton et al., 2009; Talbot, 1994). It is also relatively dense which provides the desired physical characteristics and allows the product to pop out of molds efficiently (Haslund, 2010). Polymorphic form V can be produced by tempering the chocolate, as described below in Section 1.7.2.3.

Table 1.2. Respective melting points of the polymorphic forms of cocoa butter^a

Polymorphic Form	Melting Point (°C)
I	16-18
II	21-22
III	25.5
IV	27-29
V	34-35
VI	36

^a adapted from Talbot, 1994

While cocoa butter is the main fat component of chocolate, other fat may be present as well. The milk fat in milk and white chocolate helps to soften the chocolate. It also slows the transition from Form V to Form VI, a higher melting form of cocoa butter (Timms, 1984; Pajin and Jovanovic, 2005). By slowing this transition, the presence of milk fat has been shown to decrease fat bloom, an unsightly whitish growth of fat crystals at the surface which will be discussed in detail in Section 1.8 (Samsudin and Rahim, 1996; Sonwai and Rousseau, 2010).

Depending on the countries' regulations and intended use, other types of vegetable fat can be used in chocolate or compound coatings. These fats can either be cocoa butter equivalents (CBE) which have similar chemical and physical characteristics to those of cocoa butter or cocoa butter replacers (CBR) which are similar to cocoa butter only in their physical properties (Talbot, 1994; Samsudin and Rahim, 1996). Typical CBE blends are produced from mixtures of fractionated fats from palm, illipe and shea and must be tempered like cocoa butter (Talbot, 1994). On the other hand, lauric and non-lauric CBRs produced from palm kernel or coconut oil and palm or soyabean oil respectively do not require tempering as they are non-polymorphic (Talbot, 1994).

1.6.2 Sugar

In accordance with the standard of identity for chocolate, the sweetening component must be a “nutritive carbohydrate sweetener” (21CFR163, 2010). Sucrose from cane or beet is the main sugar component in chocolate, with some lactose contributed by the milk solids in milk and white chocolate (Kruger, 1994). It is used up to approximately 50% in chocolate confectionery products (Kruger, 1994). Although the sugar only truly imparts sweetness to the product, changing the sugar content by 5% or more has a large impact on the flavor (Beckett, 1999). The water content of correctly conditioned sugar is between 0.03 and 0.06%, with sugar crystals readily absorbing water at relative humidities of 65% or above (Kruger, 1994). Water absorption by pure sugar results in hardened lumps as well as possible chemical changes and microbiological issues (Kruger, 1994). Other sugars that can be used in chocolate confections include invert sugar, fructose, glucose and many common sugar alcohols (Kruger, 1994).

1.6.3 Dairy Ingredients

Based on the chocolate standard of identity, milk and white chocolate products must contain one or more optional dairy ingredient (21CFR163, 2010). These ingredients include cream, milkfat, butter, milk, dry whole milk, concentrated milk, evaporated milk, sweetened condensed milk, skim milk, concentrated skim milk, evaporated skim milk, sweetened condensed skim milk, nonfat dry milk, concentrated buttermilk, dried buttermilk and malted milk (21CFR163, 2010). Dairy ingredients have many components that are important to chocolate production including fats, lactose, proteins and minerals (Reimerdes and Mehrens, 1994).

1.6.4 Emulsifiers

Emulsifiers can be added to white chocolate either singly or in combination up to a total amount of 1.5% by weight and to milk and semisweet or bittersweet chocolate up to 1.0 % by weight (21CFR163, 2010). Emulsifiers help to reduce both the viscosity and the yield stress value of chocolate. In doing so, they serve to decrease the amount of cocoa butter necessary for proper flow characteristics which decreases both the fat content and the price as cocoa butter is an expensive ingredient. The most common emulsifier used in chocolate is soya lecithin which has been shown to bind to the surface of sugar particles and thus drastically reduce viscosity

(Johansson and Bergenstahl, 1992; Do et al., 2007). A study by Babin et al. (2005) showed that when lecithin was added at 0.3 % to a suspension of 70% sugar in various oils, approximately 30 to 40% of it was absorbed to the surfaces of the sugar. At higher values, however, lecithin will form micelles and thus increase yield value (Afoakwa, Paterson and Fowler, 2007; Johansson and Bergenstahl, 1992a).

PGPR is another common emulsifier used in chocolate. While PGPR does not have as large of an effect on viscosity, it does considerably reduce the yield value (Rousset, Sellappan and Daoud, 2002). It also binds to solid particles but unlike lecithin, does not form micelles (Rector, 2000). As a result of these benefits, chocolate products made with PGPR can be used very effectively as thin coatings or detailed surface designs (Schantz and Rohm, 2005).

Additionally, lecithin and PGPR can be blended for optimal rheological benefits in chocolate products. Schantz and Rohm (2005) found that a lecithin:PGPR ratio of 30:70 resulted in yield stress minima for both milk and dark chocolate. Viscosity minima were found at a 50:50 ratio for dark chocolate and a 75:25 ratio for milk chocolate (Schantz and Rohm, 2005).

1.7 Chocolate Making Process – Bean to Bar

The manufacturing of chocolate is a complex procedure that can be divided into two smaller processes; producing cocoa liquor, butter solids from beans (Figure 1.8) and turning these products into finished chocolate (Figure 1.9). These processes are detailed in Sections 1.7.1 and 1.7.2 below.

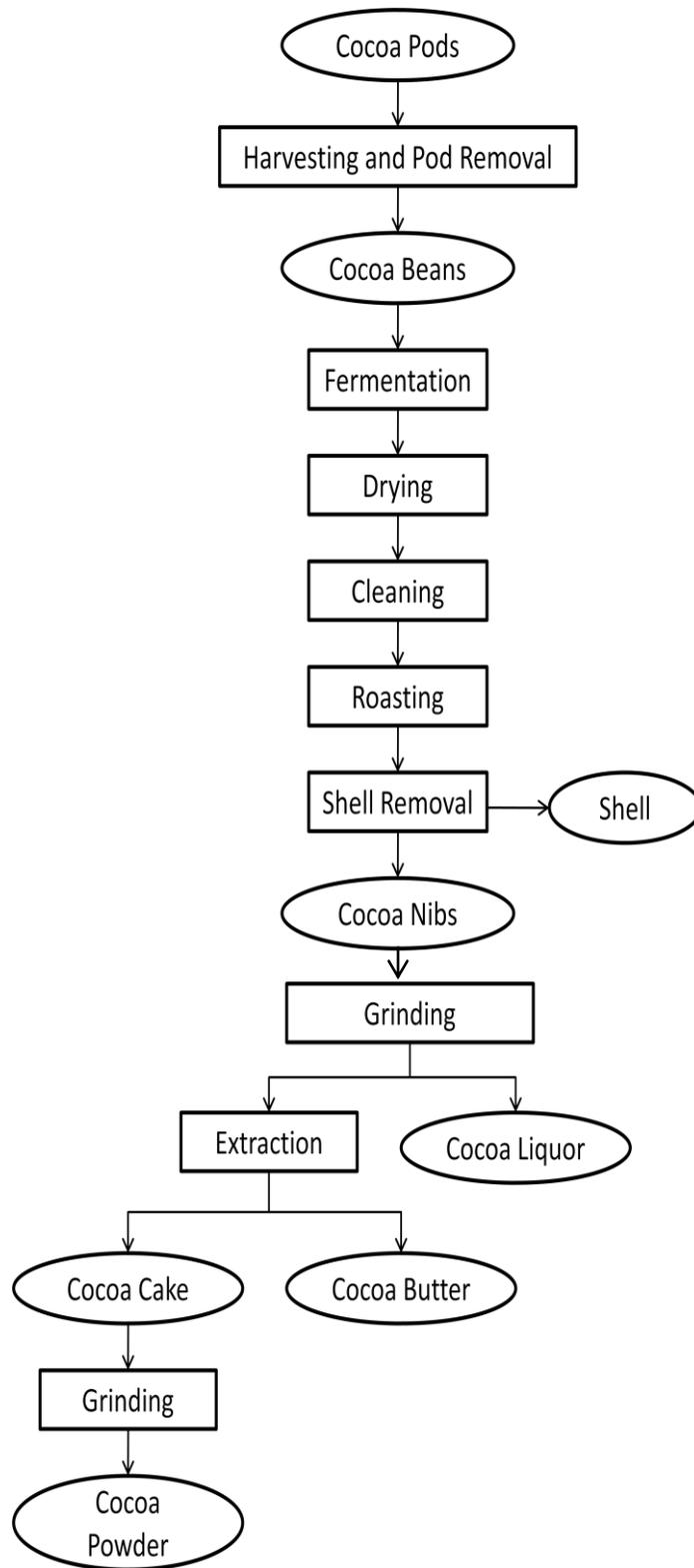


Figure 1.8. Process diagram for cocoa liquor, butter and solids from cocoa pods (Beckett, 1999).

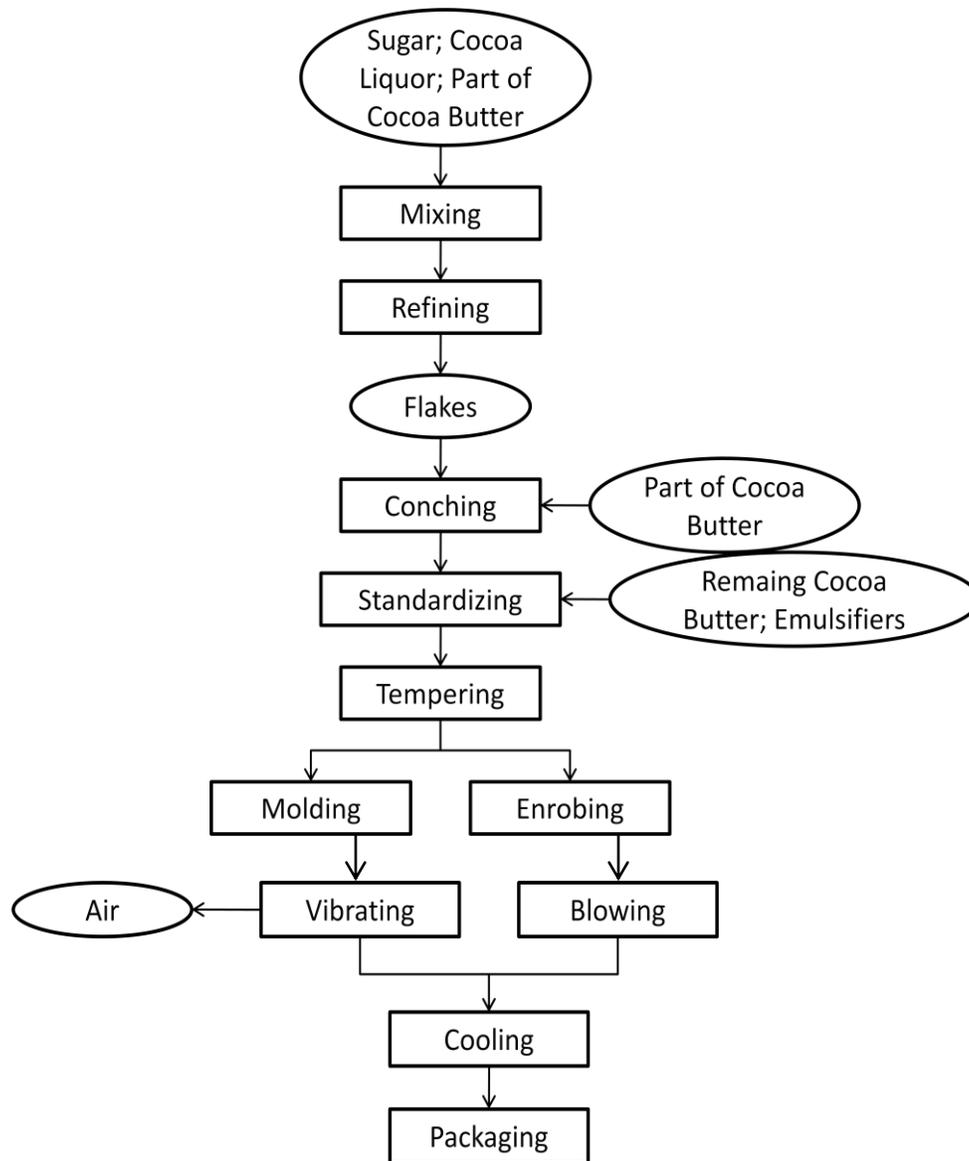


Figure 1.9. Process flow diagram for finished chocolate (modified from Beckett, 1999).

1.7.1 Beans to Cocoa Liquor, Butter and Solids

Cocoa beans are the seeds inside pods of the *Theobroma cacao* tree. There are three main varieties, Forastero, Criollo and Trinitario, and the differences between the varieties and their growing environment have a large impact on finished flavor. Forastero is typically grown in the Amazon and West Africa and accounts for over 70% of chocolate produced (Adriaenssens,

2010). Criollo accounts for 5 to 8% of chocolate products and is grown in Central America and Asia. Trinitario, a hybrid of the previous two varieties from Trinidad and Grenada, makes up around 20 percent of chocolate (Adriaenssens, 2010).

Ripe cocoa pods are harvested from the trees and cut open so that the beans inside can be removed. The beans are then spread out and allowed to ferment, typically enclosed in banana leaves (Hancock, 1994; Adriaenssens, 2010). This processing step allows for the development of color and flavor in the bean and lasts approximately 5 to 7 days (Fowler, 1999). The beans are then spread out and dried, typically by the sun or by artificial drying in wetter regions. Once dry, the beans are filled into large sacks and transported where necessary.

Once the beans reach a processing facility, they are sorted and cleaned and then roasted. Roasting helps to further develop the bean flavor, with flavor differences coming from variations in time, temperature and water content (Adriaenssens, 2010). While it is the beans that are typically roasted, the next few steps can be switched around such that the nib or the cocoa mass is roasted instead (Hancock, 1994). These methods each have associated advantages and disadvantages and can be performed by a variety of machines.

A process called winnowing takes place in which the shell of the bean is broken and the cocoa bean kernel or nib is separated. The nib is then ground into cocoa mass or liquor which is a mixture of cocoa solids and cocoa butter. The grinding process also serves to reduce the particle size of the cocoa solids and is done by different types of grinding mills (Hancock, 1994). This cocoa mass can then be treated and used in chocolate making or pressed to separate the cocoa butter from the cocoa solids or powder.

1.7.2 Finished Chocolate

Chocolate liquor, cocoa butter and cocoa powder produced in the above method are now ready to be used in the manufacture of chocolate products. Again, depending on the type of chocolate, varying quantities of these ingredients are used.

1.7.2.1 Refining

In the next step in chocolate production, the appropriate cocoa components are mixed with sugar and milk ingredients (for milk or white chocolate). Refining is a grinding process that can either be done dry or wet with a fraction of the cocoa butter added (8 to 24% fat) (Beckett 1999, 2000). The process is typically done with a two or five roll refiner. The refining step is extremely important because the particle size produced here directly impacts the mouthfeel and rheological properties of the finished chocolate (Afoakwa, Paterson and Fowler, 2007). Particles that are greater than 35 μm can be perceived by the tongue, feel gritty or sandy and are therefore undesirable to the consumer (Jackson, 1994). Chocolate in the US and Britain are refined to between 20 and 30 μm while other European chocolate is refined to 15 to 22 μm (Jackson, 1994). The downside of small particle sizes is that it means an increase in surface area that must be coated by fat or emulsifiers to have the same viscosity and yield value. Some researchers have found, however, that a bimodal particle size distribution results in less fat needed and comparable rheological properties (Beckett, 1999; Do et al., 2007).

1.7.2.2 Conching

The next step in chocolate production is called conching. Conching is a mixing and shearing process that has two important impacts on the product; it changes the viscosity and texture from a dry crumb to a liquid and it develops the final flavor attributes of the chocolate (Ley, 1994; Afoakwa, Paterson and Fowler, 2007). Conching is done by a variety of conche types including longitudinal, rotary or round and continuous conches (Ley, 1994). The process can be performed at different times and temperatures, depending on the desired characteristics of the final product. It normally occurs at temperatures over 50 °C over a period of a few hours to up to a day (Beckett, 2000).

During the conching process, the chocolate mass undergoes mixing to evenly disperse all of the ingredients, drying to reduce the moisture content from approximately 1.6% to between 0.6 and 0.8% and shearing to break up agglomerates and distribute the fat (Ley, 1994; Muntener, 2010). The three phases of conching are the dry, pasty and liquid phases (Ley, 1994; Afoakwa, Paterson and Fowler, 2007). In the first phase, the dry crumb produced from refining is mixed and sheared under heating. Since the moisture content is the highest at this stage, temperatures

should be increased slowly and kept below 60 °C to prevent grit formation (Ley, 1994; Muntener, 2010). It is during this stage that a majority of the moisture evaporates and takes with it many undesirable volatile components (Ley, 1994).

The second phase of conching, the pasty phase, serves to further develop the chocolate flavor, remove additional moisture and homogenize the mass (Ley, 1994). Agglomerates are broken up and the fat is distributed (Muntener, 2010). Since only free fat or fat not coating any particles, is responsible for the flow properties, it is important to release as much fat as possible (Muntener, 2010). The final phase involves intense stirring and shearing (Ley, 1994). At this point emulsifiers and additional cocoa butter are added to achieve the desired flow properties (Ley, 1994; Afoakwa, Paterson and Fowler, 2007; Muntener, 2010).

1.7.2.3 Tempering

As stated previously, cocoa butter is polymorphic and can crystallize into any of six forms. Form V, a β form, is desired in chocolate products for appropriate physical characteristics. Tempering can be done either by batch or continuous methods and by hand or by machine. The four main steps in tempering involve complete melting, cooling to crystallization, crystallization and removal of unstable crystals (Talbot, 1994). In the first step, the chocolate is heated to approximately 50 °C. This melts all the possible crystal polymorphs and removes any crystal memory. The mass is then cooled to approximately 32 °C while mixing to induce crystal formation. Crystallization continues at 27 °C and then the mass is heated again to 29-31 °C to melt out any of the unstable crystals that may have formed (Talbot, 1994). Once the chocolate is properly tempered, it can be poured into molds for finished products or bulk blocks or it can be used for enrobing. It is packaged, wrapped and stored to protect the product from moisture, insects, dust, handling and flavor absorption (Beckett, 1999).

1.8 Heat Resistant Chocolate

When normal chocolate is exposed to temperatures above the melting point of the fat, the product will become soft, lose its snap and stick to fingers and the wrapper. The impacts on the product tend to be worse for products with a chocolate confectionery coating (Kempf and Downey, 1956; Schubiger and Rostagno, 1965; Mandralis and Weitsenecker, 1996; Best et al.,

2005). Additionally, when melted chocolate is cooled again the fat has now lost its temper and can crystallize into unstable polymorphic forms. This causes a phenomenon known as chocolate bloom. Bloom is the appearance of whitish grey crystals larger than 5 μm on the surface that tend to look like mold and is seen unfavorably by consumers. Studies have shown that it is an evolution to the larger Form VI crystals that accounts for bloom (Hodge and Rousseau, 2002; Rousseau and Sonwai, 2008; Sonwai and Rousseau, 2010).

As a result of the many negatives associated with chocolate in warm environments, chocolate consumption in warm, tropics regions and during warmer seasons in some countries is drastically reduced (Ogunwolu and Jayeola, 2006). Transport and storage must be temperature controlled and in some regions, only stores with refrigeration ability can stock chocolate (Ogunwolu and Jayeola, 2006). This is also an issue for United States troops abroad in warmer climates where chocolate is carried as a quick source of calories. In 1937 The Hershey Chocolate Corporation came out with a heat resistant D Ration chocolate bar that was supplied to troops in World War II, although it did not have great flavor (Aaseng, 2005; D'Antonio, 2006). A different version, the Tropical Bar, was introduced in 1945 and later improved upon and used in both Korea and Vietnam. During the Gulf War the Desert Bar, which could withstand up to 60 °C, was produced and shipped by Hershey's (Aaseng, 2005; D'Antonio, 2006). These bars, however, do not have the same flavor or texture as traditional chocolate and thus the development of a heat resistant chocolate with the same sensory attributes is still to be desired.

1.8.1 Definition

There is no standard definition for "heat resistant chocolate" but it is referred to in the patent literature as heat resistant, thermostable, tropicalized, thermally robust and shape sustaining (O'Rourke, 1959; Giddey and Dove, 1984; Kealey and Quan, 1992; Alander, Warnheim and Luhti, 1996; Best et al., 2005). Inventors of heat resistant products have different qualifying characteristics for their products. One factor important to most inventors is shape retention at some specified elevated temperature. Some inventors merely state that shape retention must occur above room temperature, at elevated temperatures or at temperatures like in summer or tropical countries (Kempf and Downey, 1956; Schubiger and Rostagno, 1965; Kincs, 1993; Davila and Finkel, 2005). Alander, Warnheim and Luhti (1996) on the other hand require shape retention at 35 to 40 °C. Yet others require 40 °C (Takemori, Tsurumi and Takagi, 1993;

Best et al., 2005; Simburger, 2009) or even 50 °C (Beckett, 1995) to classify the product as heat-resistant. Jeffery, Glynn and Khan (1977) necessitate the highest level of heat resistance at 65.55 °C.

Other important factors listed by inventors of heat resistant chocolates include not being soft, not being sticky to the touch or to the wrapper, not having increase likelihood of fat bloom and not having significant sensory differences. These characteristics are listed in Table 1.3 with their corresponding inventors. When referring to sensory differences, most inventors report in the patent literature that the same “finished” flavor is still present and the texture, mouthfeel and appearance are not changed, however these claims are typically not supported by sensory data.

Table 1.3. Desired characteristics of heat resistant chocolate as stated by inventors of such products

Characteristic	Patents
Not Soft	Giddey and Dove, 1991; Kincs, 1992; Mandralis and Weitzenecker, 1996
Not Sticky to the Touch	Giddey and Dove, 1991; Takemori, Tsurumi and Takagi, 1992; Davila and Finkel, 2005; Simburger, 2009
Not Stick to Wrapper	Schubiger and Rostagno, 1965; Beckett, 1995; Best et al., 2005; Davila and Finkel, 2005
No Sensory Differences	Kempf and Downey, 1956; Schubiger and Rostagno, 1965; Giddey and Dove, 1984, 1991; Kealey and Quan, 1992; Kincs, 1992; Takemori, Tsurumi and Takagi, 1992; Best et al., 2005; Davila and Finkel, 2005
No Increase in Bloom	Giddey and Dove, 1984,1991; Kincs, 1992; Best et al., 2005

As a result of this wide array of desired heat resistant characteristics and qualifying temperatures, Dicolla (2009) proposed three different levels of heat resistance at which the chocolate is not sticky to the touch, retains its shape and is acceptable to a sensory panel. The levels are mild heat resistance from 26.7 to 32.2 °C, intermediate heat resistance from 32.3 to 37.8 °C and extreme heat resistance from 37.9 °C and higher (Dicolla, 2009).

1.8.2 Measurement of Heat Resistance

The variety of characteristics required by researchers and inventors for heat resistant chocolate leads to various ways for testing and examining products. Many placed the chocolate in ovens set between 35 to 50 °C for set periods of time ranging from a few minutes to a couple of hours and monitored shape retention (Takemori, Tsurumi and Takagi, 1992; Beckett, 1995; Alander, Warnheim and Luhti, 1996; Mandralis and Weitzenecker, 1996). Giddey and Dove (1984, 1991) took this one step further and left their chocolates in an oven at 50 °C overnight.

Best et al. (2005) describe a more quantitative method for examining shape retention upon heating. Samples of their chocolate were molded into bars of set dimensions. These bars were weighed and their width was measured at ten evenly spaced points along their length. The bars were placed into an oven at 40 °C for 1 hour after which they were dropped 18 inches onto a solid, flat laboratory bench. The bars were allowed to cool and then the width was remeasured at the ten points. Equation 1.6 was then used to calculate the shape retention index (SRI) of the sample.

$$SRI = 100\left(1 - \frac{d_2^s - d_1^s}{d_2^c - d_1^c} \cdot \frac{w^c}{w^s}\right) \quad (1.6)$$

In this equation, d_1 and d_2 are the average width before and after the test procedure, w is the weight of the bar and the superscripts are the test sample (s) and the control sample (c). This equation results in a zero if there is no significant difference between the test and control samples and a 100 if there is complete shape retention. A SRI value of 80% was acceptable while 90% and above was preferred (Best et al., 2007).

In addition to these shape retention observations and measurements, some inventors quantitated the softening of their products through penetration testing. Giddey and Dove (1984) placed samples in an oven for 1 to 2 hours at 40 °C or 2 hours at 37 °C (Giddey and Dove, 1991) and sample were then subjected to piercing with a vertical needle using progressive force. The force required to penetrate 3 mm into the sample was recorded. Results showed that 6 g of force was used for control chocolates while the tropicalized chocolate made via their method required

58 g of force (Giddey and Dove, 1984). Simburger (2009) also used penetration testing, measuring the force of samples stored at 50 °C for 2 hours with a Stevens texture analyzer.

In another interesting heat resistance test, Giddey and Dove (1984) packed their chocolate in the trunk of a car and left it there for 15 days during the summer. The samples were then inspected for gloss, shine and bloom.

Other instrumental techniques are used to examine heat resistant chocolates including a Gallenkamp melting point apparatus to measure melting points (Ogunwolu and Jayeola, 2006) and a Brabender thermorheograph to monitor viscosity changes (Best et al., 2007). Many inventors also employed sensory evaluation techniques to check for differences in mouthfeel (Giddey and Dove, 1984, 1991; Takemori, Tsurumi and Takagi, 1992).

Schubiger and Rostagno (1965) tested their heat resistant chocolate by immersing samples in ether for several hours. Their theory for heat resistance was based on the formation of a sugar edifice that will be described in detail in Section 1.8.3.3. Ether is a fat solvent capable of extracting the fat from chocolate. Since the fat of typical chocolate is what confers structure, samples of normal chocolate would collapse completely upon extraction by ether. A chocolate sample that possesses a sugar edifice that gives it structure would not collapse upon immersion in ether. This test can also show the extent to which heat resistance permeates the chocolate as well as its homogeneity (Schubiger and Rostagno, 1965).

1.8.3 Methods of Manufacture of Heat Resistant Chocolate

While there are many techniques for developing heat resistance, the majority can be divided into three mechanistic categories. These categories include increasing the melting point of the continuous phase fats, increasing the viscosity of the continuous phase and forming a sugar skeleton.

1.8.3.1 High Melting Fats

The first and perhaps simplest method of producing heat resistant chocolate is by using fats other than cocoa butter that have higher melting points. Cocoa butter softens at 28 °C,

melting completely by 34 °C. Higher melting fats can either be added as a total replacement for cocoa butter or as a partial replacement. The solid fat creates a fat crystal lattice that traps the melted cocoa butter. Illipe and fractionated shea butters are commonly used for this purpose (Jeyarani and Reddy, 1999). Other vegetable fats, particularly modified fats, have also been used and decrease the cost of the product by at least partially replacing expensive cocoa butter. Jeyarani and Reddy (1999) investigated blends of mahua and kokum fats for use as cocoa butter extenders. They found that by fractionating and blending the fats, they could create a suitable cocoa butter extender for use along with cocoa butter in heat resistant chocolate.

One disadvantage of this approach is that the use of these fats as a replacement for cocoa butter in chocolate is restricted in many countries (Best et al., 2005). Additionally, the use of higher melting fats typically results in a waxy mouthfeel that is unfavorable to consumers. Products with these fats lack the smooth melting characteristics of a cocoa butter containing product (Takemori, Tsurumi and Takagi, 1992; Best et al., 2005). Consumers still want a product to melt well in their mouth but just not in the package on in their hand.

1.8.3.2 Increased Viscosity

Another approach to heat resistant chocolate is by increasing the viscosity of the continuous phase. By doing so, melted cocoa butter will not be able to flow from the original product shape. This was the theory behind Hershey's D Ration chocolate bar which had added oat flour (Hershey Community Archives, 2011). Ogunwolu and Jayeola (2006) made heat resistant chocolate products with increased viscosity as the proposed mechanism by incorporating either cornstarch or gelatin. The addition of 10 % of cornstarch or gelatin increased the melting point to 50 °C and 45 °C respectively. The hypothesis was that this occurred due to an increase in the viscosity of the fat due to the thickening/stabilizing properties of these ingredients. While most sensory characteristics were not rated significantly different than traditional chocolate, there were significant differences in the sweetness of the cornstarch product and the taste and smoothness of the gelatin product.

The common disadvantage of heat resistant chocolate made by the above mechanism is that the chocolate products do not have the same mouthfeel as traditional chocolate. These products may contain aggregates of solid particles or have a different melting profile. The

methods also include detailed extra steps in the chocolate making process and make it necessary to purchase and install new equipment. Additionally, Ogunwolu and Jayeola (2006) noted that the percent moisture of their chocolate with added cornstarch was significantly higher than that of the control or gelatin chocolate. This indicates that the product may have developed heat resistance through a different mechanism instead of through an increase in continuous phase viscosity. This mechanism is due to the addition of water to chocolate and is described in the following section.

1.8.3.3 Sugar Skeleton

The final method for the development of heat resistance involves the formation of a continuous sugar skeleton within the chocolate structure. The theory to explain this is that the water is absorbed onto the sugar particles, creating a syrup layer around them (Chevalley, 1994) and changing their interactions within the dispersion (Johansson and Bergenstahl, 1992b). Details of the theory are expanded in Section 1.10 below. However, if the sugar molecules can be welded together to form an internal edifice, then even if the cocoa butter melts, the product will be able to maintain its form. Thus it would be the sugar skeleton that gives the chocolate its structure instead of the crystallized cocoa butter.

In order to visualize the sugar skeleton, the procedure described by Schubiger and Rostagno (1965) can be used in which the product is immersed in ether. If no sugar structure exists, the sugar will collapse away with the extracted fat. In the other hand, if there is a sugar skeleton, the product will hardly look different without the fat.

Schubiger and Rostagno (1965) proposed a heat resistant chocolate by mixing amorphous sugar made by one of three proposed methods with a standard conched chocolate mass. The product can be tempered and molded and is subjected to a thermal treatment at 25 °C for 20 to 30 days. This heat treatment releases moisture from the amorphous sugar allowing for the formation of a sugar skeleton within the structure as long as the right amount of fat has been added such that it does not coat the sugar particles.

A similar approach by many inventors is the addition of a polyol. The method of addition varies, but eventually the polyol serves to release water in a controlled manner. If not

added correctly, the polyol can cause immediate and drastic increases in viscosity to the point that it is impossible to work with the product. Davila and Finkel (2005) add a polyol directly to molten chocolate but in order to overcome previous disadvantages to this method, the mass undergoes mixing for an extended period of time. By doing so, the sample goes from being highly viscous upon addition back to a viscosity typical of chocolate products as the water becomes evenly distributed. This product can then be tempered and molded or used as a coating.

Other workers avoid direct polyol addition, instead opting to add it in various protected forms. Kincs (1992) proposed adding a polyol-in-oil emulsion using mixing and sometimes an additional homogenization technique. This emulsion was then added to a melted confectionery coating formula and used as a coating with increased heat resistance and bloom retardation. Beckett (1995) used a similar method, but further encapsulated a polyol, usually glycerol, by spray cooling a homogenized polyol-in-oil emulsion. This was then added to melted, conched chocolate before, during or after tempering and then molded into bars.

Mandralis and Weitzenecker (1996) propose a polyol gel or polyol and water gel that was either frozen, ground and mixed with cocoa powder or emulsified in cocoa butter to form beads. The gels were made using a variety of techniques and gelling agents. The gel particulates were then added to a melted chocolate mass with mixing. A total polyol content of between 0.2 and 60 % existed in the final product which was then allowed to harden for a period of days. The polyol was believed to diffuse slowly out of the gel and eventually result in the formation of a sugar skeleton. It was also stated that this invention may be used for making low cost or low calorie chocolate because of the addition of polyol containing water and can also be used to add aromas or flavors.

Kempf and Downey (1956) added a water and emulsifier (favorably lecithin) “emulsion” to a conched chocolate mass. The water and emulsifier emulsion was sprayed over the mass while it was under agitation. Heat resistance was said to be conferred to the product because the emulsifier enables the water to be absorbed at the surface of the milk solids causing them to swell and create a supporting structure within the composition. While this was the explanation of Kempf and Downey (1956) it is more likely the formation of a sugar skeleton that is the true mechanism in this case.

O'Rourke (1959) combined the use of polyols with the addition of humectants and protein that were added to the typical chocolate ingredients during refining. The chocolate mass is processed as normal and can be molded or used for enrobing. Once the final product is made, it is subjected to a humid environment, either at environmental conditions or an artificial environment if the environmental humidity is not high enough. The humectant in the product absorbs moisture and creates an internal structure along with the moisture from the polyol.

In addition to adding water by the above methods, water can also be added in the form of a foam. The foam made by Giddey and Dove (1991) can be of a variety of compositions, including different foamers, viscosity enhancers and thickeners. The foam is stirred into a chocolate mass and the product is molded and cooled. The foam must be strong enough to withstand the mixing process and able to evenly disperse the water throughout the chocolate mass. It is preferred that 0.1 to 5 % water be in the final composition. Upon cooling the foam degasses and the water binds the sugar particles into a skeleton.

As in the products with increased continuous phase viscosity, the trouble with adding amorphous sugar, polyols, humectants or foam to chocolate is the potential for formation of large aggregates. In order to prevent this, inventors propose methods of adding water that is stabilized in smaller amounts. Similar to the use of the foam by Giddey and Dove (1991), this allows the water to be added without causing large viscosity increases or the development of large particulates. One way to do this is through the formation of a water-in-oil emulsion or microemulsion formed through various technologies. The use of water-in-oil emulsions in chocolate products is further detailed in Section 1.9 below.

1.9 Water-in-Oil Emulsions in Chocolate

Typical chocolate has a water activity of less than 0.5 and is thus not prone to spoilage (Lenovich, 1986). However, when water is added directly to melted chocolate, it causes the melt to quickly seize up with a drastic increase in viscosity. In addition to increased viscosity, water in chocolate also reduces snapability (Norton et al., 2009) and causes it to be subject to microbiological instability. Current research however focuses on the addition of water droplets into a fat continuous phase resulting in an emulsion that is used in chocolate formulation. The

use of emulsions provides a controlled method of water addition so that the negative aspects typically associated with water addition can be overcome.

A product patented by Simburger (2009) involves a chocolate mass that is between 1.8 and 7% water. The inventor discusses the addition of water-in-oil emulsions as a preferred method to accomplish this goal. These water-in-oil emulsions are produced with a high speed mixer in the presence of emulsifiers resulting in an average droplet size of around 10 μm . These emulsions are then added to a standard chocolate mass to the desired water content. The chocolate product received a microwave heat treatment before, during or after cooling which serves “to induce the formation of a secondary microstructure,” creating a sugar skeleton within the sample through the stimulated release of water.

Jeffery, Glynn and Khan (1977) patented an oil-in-water emulsion made with a high speed mixer in which chocolate ingredients were emulsified in water. This emulsion was then evaporated to approximately 10% moisture content and further dried under gasification. This produced an expanded product with less than 5% moisture that was claimed to be temperature-resistant.

In a similar application, microemulsions can also be used to add water in even smaller droplet sizes. Since the water droplets are so small, the sugar lattice is able to be constructed uniformly and without the formation of large aggregates in theory. Alander, Warnheim and Luhti (1996) use microemulsions that are produced spontaneously in the presence of an emulsifier. Kealey and Quan (1992) make microemulsions through the utilization of reverse micelle technology. Using this method, a hydrated lecithin is produced that is comprised preferably of 80 to 85% water. The emulsion can then be added to tempered chocolate in amounts ranging from 1 to 3%.

The water-in-oil emulsion does not necessarily need to be added in liquid form. Giddey and Dove (1984) made emulsions with a final water content of 30 to 80% in droplets between 0.1 to 100 μm that can either be used as is or solidified and powdered for later use. The liquid or powdered emulsion is then added to a conched chocolate mass under agitation to a total water content of 1 to 4% by weight. The product must immediately be molded into bars or used as a confectionery coating. A tropicalized chocolate product is thus produced that can withstand

storage at 40 to 50 °C. The heat resistance of the product is highest after two weeks of storage at 15 to 20 °C or one to two days at 27 to 28 °C.

Other water-in-oil emulsions for application to chocolate also involve polyols or sweeteners. Takemori, Tsurumi and Takagi (1992) made a water-in-oil emulsion with water soluble sugars and sugar alcohols in the aqueous phase distributed in a fat phase with emulsifier. The emulsion was produced by mixing in fats and oils that had a SFI not more than 20 at 20 °C and not more than 10 at 30 °C. The emulsions produced were 10 to 50% water and were then mixed with a standard chocolate mass to a total water concentration of 4 to 15%. The product was molded and it was determined that heat resistance was greatest after 20 days of storage at 18 °C after which the product retained its shape even when heated to 50 °C.

Similarly, Best et al. (2005) make a “tropicalizing agent” which can then be used in chocolate confections. The agent was a water-in-oil emulsion in which the water phase included sugars and/or polyols and a gelling agent. Once emulsified, the temperature was reduced such that gelled beads formed in the continuous fat phase. This could be incorporated into a chocolate mass that could then be molded or used for enrobing. Once solidified, the release of the gel was initiated by a variety of different processes. The gel was able to diffuse through the chocolate mass and form adhesion points between existing sugar crystals.

In a third example, Traitler, Windhab and Wolf (2000) made water-in-oil emulsions first by stirring water and an emulsifier in which water may contain a polyol to improve microbial stability. It can also contain other water soluble ingredients such as flavoring agents, preservatives or vitamins. The rough emulsion was homogenized by a colloid mill to microdroplets approximately 2 µm in diameter. This emulsion was stable for at least 1 hour. A molten chocolate mass was added to the emulsion with slight to moderate stirring. The chocolate composition was tempered and poured into molds. The final mass contains anywhere from 1 to 40% water by mass. This was done for economical reasons, to create a heat resistant chocolate, to make a low calorie chocolate, to incorporate water into a chocolate with neutral flavor and for the possibilities of adding nutrients or bioactive components to chocolate.

Not unlike the product made by Traitler, Windhab and Wolf (2000), water-in-oil emulsions can also be produced for use in chocolate for reasons other than heat resistance.

Rosenthal et al. (1966) created an emulsion of 8 to 62.5% water-in-oil by stirring or by homogenization. The resulting emulsion is best used as a chocolate dip-coating for water-containing foods, particularly ice cream and is a lower fat and lower cost product. Schlup and Lioutas (1995) patented water-containing chocolate tablets with up to 16% water in which an aqueous mixture of water, sweetener and milk solids are added to cocoa solids coated in cocoa butter in the presence of an emulsifier. Beckett et al. (2007) propose a low-calorie/ low fat milk chocolate produced by adding a water-in-oil emulsion produced by stirring which to a conched dark chocolate under agitation such that there exists 1 to 30% water. A milk powder suspension is then made and incorporated into the water-containing dark chocolate to a final preferred water content of 10 to 15% by weight. Rey et al. (2008) also made a low fat confection by mixing an aqueous phase that can include sugars, preservatives, structuring agents and flavoring agents with a fat phase using a hand mixer. Cocoa powder, cocoa liquor and or milk ingredients can then be added to create a low fat confection that is at least 60% water.

Water-in-oil emulsions can also be utilized in making reduced fat and reduced calorie cocoa butters. These emulsions are made in order to study their properties and eventually apply them to chocolate products. Vastenavond (2006) created a reduced fat, reduced calorie cocoa butter by creating a water-in-oil emulsion with membrane emulsification. The aqueous phase is a mixture of a pectin solution and a gum, sugar and calcium solution. A membrane tube was moved linearly with an electronically induced vibrator to generate shear at the membrane surface. The aqueous phase was then dispersed in a continuous phase of melted cocoa butter, cocoa butter hardener and PGPR. The emulsion can be up to 50 to 90% aqueous phase and is meant to be used in chocolate formulations for a reduced calorie product.

Similarly, Norton et al. (2009) made emulsions with cocoa butter as the continuous phase with a high shear mixer and on a bench scale margarine line. The margarine line was able to produce stable emulsions with 1% sugar in the aqueous phase and using 2% lecithin or 1% PGPR as an emulsifier. It was shown that lecithin produces emulsions with a narrower size distribution, however at 10 and 20% water, 100% of the PGPR stabilized droplets were smaller than 100 μm . After five weeks of storage, the cocoa butter had changed to form V, producing a melting curve in DSC similar to that of correctly tempered chocolate. Emulsions made on the margarine line contained 20% water and 1% PGPR with a range of sugar concentrations. NMR was performed on the emulsions and showed that the droplet size was approximately 1 μm . By controlling the

temperatures used in this process, a correctly tempered chocolate product was produced, as evident in DSC results. The goal of the work was to investigate water-in-cocoa butter emulsions for later application to chocolate products with lower fat contents but still having the same sensory attributes.

Whether water-in-oil emulsions are produced for the development of heat resistance or in order to product lower calorie/fat confections, it is important to understand the interaction of all ingredients present. Water can be very destructive to chocolate products if not added in a controlled manner. When uncontrolled, it can ruin the product through drastic viscosity increases, the formation of large agglomerates which negatively impact the sensory attributes or by corrupting the microbial stability. While water-in-oil emulsions have been shown to be a good form of control, further investigation must be done into their formation, stability and ingredient interactions.

The above methods show that the formation of a heat resistant chocolate is possible through the controlled addition of water. In order to properly understand the mechanism responsible for this heat resistance it is necessary to examine the interactions of sugar particles in oil dispersions and the influences of water and emulsifiers. The following section provides the theoretical background for the formation of a continuous sugar skeleton in chocolate.

1.10. Theory of Sugar Skeleton Formation

Figure 1.10A shows a dispersion of particles in oil. The particles behave much like the droplets of emulsions described in Section 1.2 above, with some of the same modes of instability. The rheology of the suspension is a function solely of the viscous forces acting and depends on the volume fraction of the particles (Dickinson, 1992). However, if a network forms between the particles the viscosity will increase, something which Wildemuth and Williams (1985) refer to as the “liquid microstructure.” They state that this microstructure “will be the primary determinant of the rheology” and can lead to the gelation of the dispersion. The gel led particle network can keep the continuous phase liquid contained, thereby increasing the viscosity. A dispersion of particles in oil in which a network has developed is shown in Figure 1.10B.

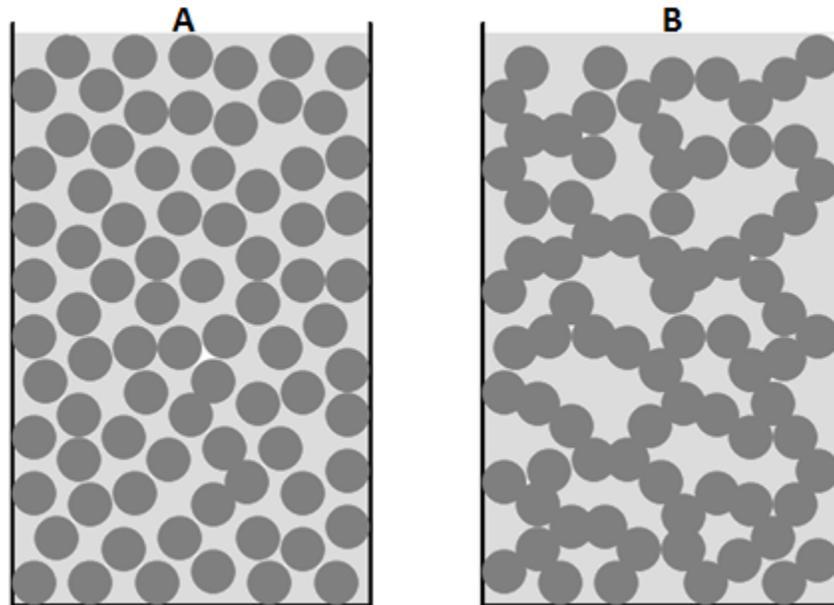


Figure 1.10. Sugar-in-oil dispersions A) without and B) with network formation.

In order to form a network within a dispersion, the particles must stick together (Figure 1.10) and the net attractive force between the particles must be greater than any mechanical shear. There are many colloidal and surface forces that may be important in dispersions and can give rise to interparticle attraction including:

- Electrostatic forces due to the interaction of charged material that is adsorbed on the surfaces of particles (Coupland, 2010)
- Steric forces between adsorbed layers in close contact (Israelachvili, 1992; Walstra, 1996)
- Hydrophobic forces between hydrophobic particles in the presence of water (Babin, 2005)
- Hydrogen bonding forces between adsorbed material of adjacent particles (Israelachvili, 1996)
- Van der Waals forces due to dipoles and induced dipoles of particles and adsorbed material (Hiemenz and Rajagopalan, 1997)

For hydrophilic particles in a hydrophobic media (e.g., sugar in air or oil), electrostatic forces are insignificant, steric forces are too short range and hydrophobic forces are not present. Although hydrogen bonding forces would be relatively strong, the particles must first come into

close contact. While van der Waals forces are fairly long range attractive forces and may cause slight attraction of dry particles, these forces alone are not enough to cause the formation of a strong sugar skeleton. Consequently, dry powders suspended either in air or in oil are usually reasonably free-flowing if the volume fraction does not approach close packing.

However, when a small amount of water is present in the system, the interactions between particles change. Cracks in and gaps between particles create regions of lower vapor pressure which allow water vapor to condense in these regions, even below the dew point, in a process called capillary condensation (Billings, Bronlund and Paterson, 2005). This occurs because the concave shape at these regions lowers the chemical potential of the liquid phase as seen by the shaded regions in Figure 1.11.

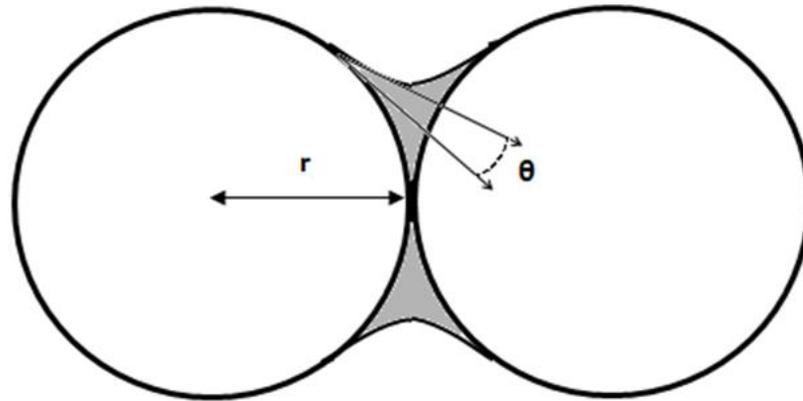


Figure 1.11. Illustration of capillaries created by two neighboring particles. Shading indicates regions of water adsorption. Θ – wetting angle; r – particle radius.

The adsorption of water leads to the formation of water bridges between particles (Billings, Bronlund and Paterson, 2005). The result is strong adhesive forces due to the interfacial tension of the curved region of liquid (Johansson and Bergenstahl, 1992; Butt, 2011). The forces are known as capillary forces with force (F_c) given by the equation:

$$F_c = 2\pi r \Gamma \cos \theta \quad (1.7)$$

where r is the particle radius, Γ is the surface tension and θ is the wetting angle as shown in Figure 1.7 (Koos and Willenbacher, 2011).

The effect of capillary forces described above can be seen in the work of Scoville and Peleg (1981) in which a model system of uniform glass beads was used to investigate the influence of water on the bulk properties of powders. The workers showed that water bridges alone caused significant changes in the bulk properties and the effect was most significant at smaller particle sizes. At larger sizes, the attractive forces from the water bridges were not strong enough to support a physical structure of such heavy particles.

While Scoville and Peleg (1981) were not able to directly measure the magnitude of the forces acting, Claesson et al. (1997) used a force balance to investigate the interaction between mica surfaces at different water activities in the presence of a liquid triglyceride. The workers showed that water preferentially adsorbed on the surfaces, as seen through the low contact angle of water on mica in the oil. When the water activity was increased above 0.90, a strong attractive force was observed between the surfaces indicating the formation of water capillaries. In a related study, Billings, Bronlund and Paterson (2005) measured the strength of the liquid bridges formed in sucrose at different water activities and determined that significant liquid bridging occurs at a water activity of 0.77. This water activity corresponds with the inflection point in the moisture sorption isotherm of sucrose which can be used to visualize moisture adsorption.

The forces which result from water adsorption by particles also affect the bulk properties of the dispersion. Whereas dry powders were relatively free-flowing, powders that are wet become aggregated. This aggregation tends to increase viscosity or lead to gelation of the dispersion. This behavior can be seen through rheological examination of wet dispersions. In one such study, Moreyra and Peleg (1981) performed rheological tests on food powders including sucrose and showed that moisture on the surface of the particles was the main contributor to the physical changes observed in the powders. The physical changes of sucrose again mirrored the pattern of the moisture sorption isotherm.

Johansson and Bergenstahl (1992b) also examined the rheological parameters of sucrose, this time in soybean oil systems. The workers utilized a network model originally proposed by van den Tempel (1961) to explain their results. This model “assumes that the attractive forces between the particles dominate particle-particle interactions. The particles flocculate and arrange themselves into a three-dimensional network of chains. Each chain consists of a linear array of particles attached to each other.” Therefore, as long as this “structure” is not significantly

disrupted, this model can be used in conjunction with a relationship between yield stress and energy of interaction (Gillespie, 1960) to determine interaction forces and energies from rheological data. Johansson and Bergenstahl (1992b) used this as a basis for their analysis and determined that van der Waals forces were too weak to be responsible for the interactions seen in their system. They proposed that these forces influence the formation of a primary network but then water bridges are formed between particles and result in a secondary network with higher energy interactions and stronger adhesive forces.

Johansson and Bergenstahl (1992a,b) also investigated the particle interactions in oils through sedimentation experiments. The basis of the method is that the formation of agglomerates through particle attraction affects particle sedimentation behavior (Tiller and Khatib, 1984; Johansson and Bergenstahl, 1992a). Conversely, repulsive forces between particles allows them to pass each other more easily when sedimenting and results in a more dense and compact sediment (Johansson and Bergenstahl, 1992a). In experiments reported by Johansson and Bergenstahl (1992a,b,c) soybean oil was used as a representative food oil in sucrose crystal sedimentation studies. The workers investigated the influence of water on sedimentation behavior and showed that the sediment volume increased with water addition up to 1 % water content (Johansson and Bergenstahl, 1992c). These results correspond linearly to those in the rheological studies. They argued that increased sediment volume was due to increased attractive forces, specifically water bridges.

Through the research described above, it can be seen that the addition of water to particles results in increased adhesion and development of a network through the formation of water bridges. If this network is able to develop to a sufficient extent, then heat resistance could be conferred to a chocolate product. Food materials, however, are more complex than non-adsorbent model powders and the adsorption of water onto sugar particles causes more than just the formation of water bridges. Water also alters the physical properties of the surface of various food particles by softening and local plasticization (Moreyra and Peleg, 1981). This action contributes to changes in both the individual and bulk characteristics of food powders and is also a factor in the development of agglomerated or “caked” products (Scoville and Peleg, 1981). However, if the amount of water added is too large, the sugar particles dissolve and the resultant water-in-oil emulsion will be unstable (Johansson and Bergenstahl, 1992c).

If water is added to a suspension of partially soluble particles (e.g., sugar particles) and the suspension is subsequently dried out, the dried sugar may form a very strong glass or crystal bridge holding the particles together. Billings, Bronlund and Paterson (2005) demonstrated the formation of solid bridges when moisture was added to sucrose suspensions to form capillary bonds then dried. In this case, solid bridges resulting from the recrystallization of dissolved sugar were two to three times stronger than liquid bridges and strongly contributed to caking of sugars. These strong solid bridges provide yet another force capable of supporting a sugar skeleton and help to explain how heat resistant chocolate products maintain structure even after exposure to heat which could evaporate a portion of the liquid water bridges.

It is important to note that the emulsifiers commonly used in chocolate, lecithin and PGPR, are capable of altering the sugar particle interactions in oil as well. Emulsifiers adsorb to the surfaces of sugar particles dispersed in oil in order to decrease the unfavorable sugar-oil interactions. This adsorption can compete with the adsorption of water to the interface and thus change the interactions between particles in the presence of water (Ziegler, Garbolino and Coupland, 2003). Lecithin has been shown to adsorb to sugar surfaces in multilayers or aggregates which increases the repulsive forces between particles, decreases adhesive forces, stabilizes the dispersion and results in a denser, more packed sediment (Johansson and Bergenstahl, 1992a; Ziegler, Garbolino and Coupland, 2003). PGPR, on the other hand, tends to adsorb in “a loosely packed monolayer” (Ziegler, Garbolino and Coupland, 2003).

Ziegler, Garbolino and Coupland (2003) showed including 0.05% lecithin or 0.1% PGPR significantly reduced the size of sucrose aggregates in oil. While emulsifiers can affect the sugar particle interactions, at $a_w > 0.75$ enough water may be adsorbed to the sugar surface to form water bridges and promote adhesion (Ziegler, Garbolino and Coupland, 2003). Johansson and Bergenstahl (1992c) also show that high levels of phosphatidylcholine (a major component of lecithin) decreased sucrose in soybean oil sediment volume, suggesting that it weakened the adhesive forces between sugar particles. With the addition of up to 1% moisture, however, the sediment volume was still higher than the dry volume of sugar crystals. Thus while emulsifiers may alter the interaction of particles in oil in the presence of water, it is still safe to assume that sufficient water bridges can be formed to promote the formation of an internal sugar structure that can provide heat resistance to chocolate products, especially at low emulsifier concentrations.

1.11. Water in Chocolate – Summary

Now that the theory for sugar skeleton formation in chocolate products has been detailed, it is important to revisit the addition of water to chocolate. Added water typically causes a drastic increase in the viscosity of a sugar in oil dispersion. This disrupts many processes used in traditional chocolate manufacture as the product does not flow or mold as easily. To prevent this, water must be added in a controlled form that does not immediately react with the sugar particles. A decreased reaction time would allow the water to become more evenly dispersed within the sugar in oil dispersion. Mixing in this case must also be done in a way that does not significantly affect the interaction between the water and the sugar particles. Once dispersed, the water can then react with the sugar particles. The interactions discussed above suggest a mechanism by which the reaction of water with sugar particles would impact the formation of a sugar skeleton and therefore confer heat resistance to a chocolate product.

Chapter 2

STATEMENT OF THE PROBLEM

Water-in-oil emulsions are used in many common food applications, either as products or ingredients in oil continuous systems. Traditional emulsification methods require considerable inputs of energy, use bulky and expensive equipment and produce substantial amounts of heat which can damage ingredients. A relatively new technique for making emulsions, cross flow membrane emulsification, has been shown to remedy many of these problems. In order to apply this technique to foods, it is first important to fully understand the operation in a small, lab-scale system. The addition of emulsions produced by this method to an oil continuous food such as chocolate can then be explored for the possibility of enhanced heat resistance.

Many studies and patents have reported the addition of water to chocolate for the development of heat resistance. The proposed mechanism for this is the formation of a sugar skeleton due to capillary forces between sugar particles upon addition of water. Water is typically added in some sort of bound form and several patents report the use of water-in-oil emulsions. However, there was no work to date on the use of cross flow membrane emulsification for producing such emulsions. In order to optimize the heat resistance of chocolate products it is necessary to understand how water-in-oil emulsions produced by this method interact with sugar particles and develop the sugar skeleton within a sugar-in-oil dispersion.

The overall goal of this work is to determine if suitable water-in-oil emulsions can be produced via a lab-scale cross flow membrane emulsification system for use in the production of heat resistant model chocolate products. Two objectives were defined in order to reach this goal:

1. The first objective is to explore the effects of changing ingredients (type and concentration of emulsifiers in the continuous phase and κ -carrageenan in the dispersed phase) and operational parameters (different membranes and continuous and dispersed phase flow rates) on the droplet size and distribution of water-in-oil emulsions produced with the cross flow membrane emulsification system.

Conditions to produce both stable and unstable emulsions will be identified, with stable emulsions maintaining a consistent droplet size over time.

2. The second objective is to investigate the interactions of water-in-oil emulsions with sugar-in-oil dispersions and determine if heat resistant model chocolate can be produced through the incorporation of emulsions made with the cross flow membrane emulsification system. In this study, a method for producing a model chocolate will be developed and chocolates made with and without added water with water added either directly or as a stable or unstable emulsion. Quantitative testing procedures will be developed to evaluate sugar skeleton formation and heat resistance because of a lack of such defined procedures in the literature.

Chapter 3

MANUFACTURE OF WATER-IN-OIL EMULSIONS BY CROSS FLOW MEMBRANE EMULSIFICATION: EFFECT OF PROCESSING PARAMETERS AND FORMULATION

3.1. Introduction

Emulsions are dispersions of one liquid as droplets in a second, immiscible liquid. They are frequently used in the food industry, both as products and ingredients. Classic food examples of oil-in-water emulsions are salad dressings, sauces, flavored beverages, mayonnaise, milk and cream (Dickinson, 1992). While oil-in-water emulsions are the most common food emulsion type, water-in-oil food emulsions include important products such as butter and margarine (Dickinson, 1992). They also have potential application in any fat continuous product such as chocolate. The following study will focus on this type of emulsion.

Water-in-oil emulsions are inherently unstable due to the immiscible phases present and instability can drastically impact product characteristics and shelf-life. Instability arises due to the thermodynamic drive to decrease interfacial surface area and can occur via a variety of mechanisms including sedimentation, flocculation, coalescence and Ostwald ripening. The rate of sedimentation of water-in-oil emulsions is understood in terms of Stokes' law governing the terminal velocity of an isolated droplet which takes into account the radius of the droplet, the densities of the phases and the shear viscosity of the continuous phase (McClements, 1999). Flocculation or aggregation is the sticking together of droplets without rupture of their interparticle films while coalescence occurs when the interparticle film ruptures resulting in a single, larger droplet. Ostwald ripening is the mass transfer of water from smaller droplets to larger droplets also causing them to increase in size. All of these methods of instability result in larger effective droplet sizes, thus increasing the rate of sedimentation. These instability mechanisms, however, can be minimized through the alteration of phase compositions and the use of effective production techniques.

Traditional methods of emulsion production include the use of rotor-stator mixers, high pressure homogenizers, ultrasound homogenizers and microfluidizers. Rotor-stator methods include all forms of mixers and stirrers, colloid mills and toothed disc dispersing machines

(Sotoyama et al., 1999; Charcosset, Limayem and Fessi, 2004; Tadros, 2009). All of these methods require large energy inputs, the majority of which is lost by heat which can damage any labile ingredients (Gijssbertsen-Abrahamse, van der Padt and Boom, 2004). Equipment is also usually bulky and expensive and controlling droplet size and distribution can be difficult. In contrast to these methods, a more recent method, membrane emulsification using microporous membranes, does not involve high shear stress, requires less energy and provides an easy way to control droplet size and distribution (Sotoyama et al., 1999). Membrane emulsification has become more popular in the past 20 years because of these advantages (Charcosset, 2009).

Membrane emulsification utilizes pressure to push a liquid dispersed phase through a porous membrane (Charcosset, Limayem and Fessi, 2004). Frequently the continuous phase is sheared to aid in droplet detachment (Egidi et al., 2008). This can be done by a variety of methods including recirculation of the continuous phase in a process known as cross flow membrane emulsification as seen in Figure 3.1.

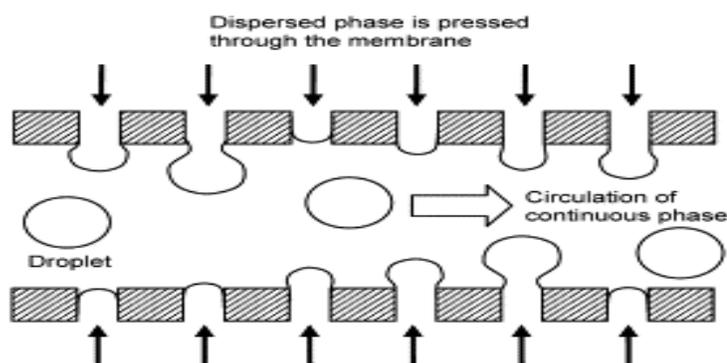


Figure 3.1. Simplified diagram of cross flow membrane operation (Rayner et al., 2004)

Droplet formation in this system occurs in two steps; droplet growth at the pore and droplet detachment into the flowing continuous phase (Peng and Williams, 1998). The droplet diameter is given by how far droplet growth proceeds before detachment and can be modeled through a variety of techniques including a torque balance model (De Luca et al., 2004; De Luca et al., 2008; Egidi et al., 2008). While multiple forces are acting on a droplet, the main contributors to the torque balance are the torques induced by the static pressure force, the cross flow drag force and the interfacial tension force (Schroder, Behrend and Schubert, 1998). The

continuous phase viscosity, continuous phase velocity, pore diameter and interfacial tension are factors included in the calculations of these forces. These variables can be altered by selecting appropriate continuous phase oils and emulsifiers, altering the composition of the dispersed aqueous phase (such as by adding a gelling agent), using membranes with certain characteristics and changing the operational settings of the continuous and dispersed phase pumps (De Luca et al., 2004). While these variables have been explored for water-in-oil emulsions made by traditional methods and oil-in-water emulsions made by cross flow membrane emulsification, there is limited literature available exploring these variables in water-in-oil cross flow membrane formed emulsions.

The following study used a lab-scale cross flow membrane emulsification system to produce 30% water-in-oil (w/w) emulsions for potential application in food systems. This concentration was chosen because 30% water-in-oil emulsions are easily measured by pulsed field gradient NMR, which was used to determine droplet size and distribution. This measurement technique was selected from the available sizing techniques because it does not require any sample preparation, does not damage the samples and works even with concentrated emulsions (Gabriele et al., 2009; Rousseau and Hodge, 2005; Van lent et al., 2008). Some of the accessible factors involved in determining droplet size described above were explored by altering the type and concentration of emulsifier, membrane pore size and shape, continuous and dispersed phase flow rates and dispersed phase composition. Data collected from these experiments will help to determine the operational and compositional parameters required to produce stable emulsions with the lab-scale cross flow membrane emulsification system. Based on the factors in Stokes' law, a "stable" emulsion in this experiment was defined as consisting of small droplets with a narrow droplet size distribution that does not significantly change over the time scale of the experimentation.

It was hypothesized that increased emulsifier concentration, higher continuous phase flow rate, lower dispersed phase flow rate and the presence of a gelling agent in the dispersed phase will result in a more stable emulsion. Additionally, it was hypothesized that there would be a difference in the emulsification behavior of the emulsifiers used; polyglycerol polyricinoleate (PGPR), soy lecithin and a 50:50 PGPR:lecithin blend and that the use of different membranes would affect droplet size and distribution.

3.2 Materials and Methods

3.2.1 Materials

Soybean oil (100% pure, food grade) was obtained from a local supercenter and used without further purification. Grindsted® PGPR and Carrageenan CL220 were donated by Danisco (Brabrand, Denmark) and Yelkin® TS soy lecithin was donated by ADM (Decatur, IL). The phospholipid composition of the lecithin (acetone-insoluble matter: 62.0%) specified by the manufacturer was 15% PC, 13% PE, 9% PI and 4% PA. Both emulsifiers were used without further purification. Deionized water was used in all trials.

3.2.2 Methods

3.2.2.1 Cross Flow Membrane Emulsification

General. A lab-scale cross flow membrane emulsification unit and four cylindrical membranes were supplied by Micropore Technologies, Inc. (Loughborough, UK). The membranes were hydrophobic, facilitating the formation of water-in-oil emulsions. The dimensions of the membranes and pores as well as approximate number of pores are shown in Table 3.1. A representative membrane (Membrane I) and detailed microscope images of the membranes are shown in Figure 3.2.

Table 3.1. Membrane and pore dimensions and number of pores

Membrane	Pore Type	Pore Width (μm)	Pore Length (μm)	Membrane External Diameter (mm)	Membrane Length (mm)	Estimated Number of Pores
I	Slotted	40	450	15	106	1715
II	Slotted	8	420	15	109	1717
III	Slotted	8	420	15	124	1954
IV	Circular	20	20	15	53	2464

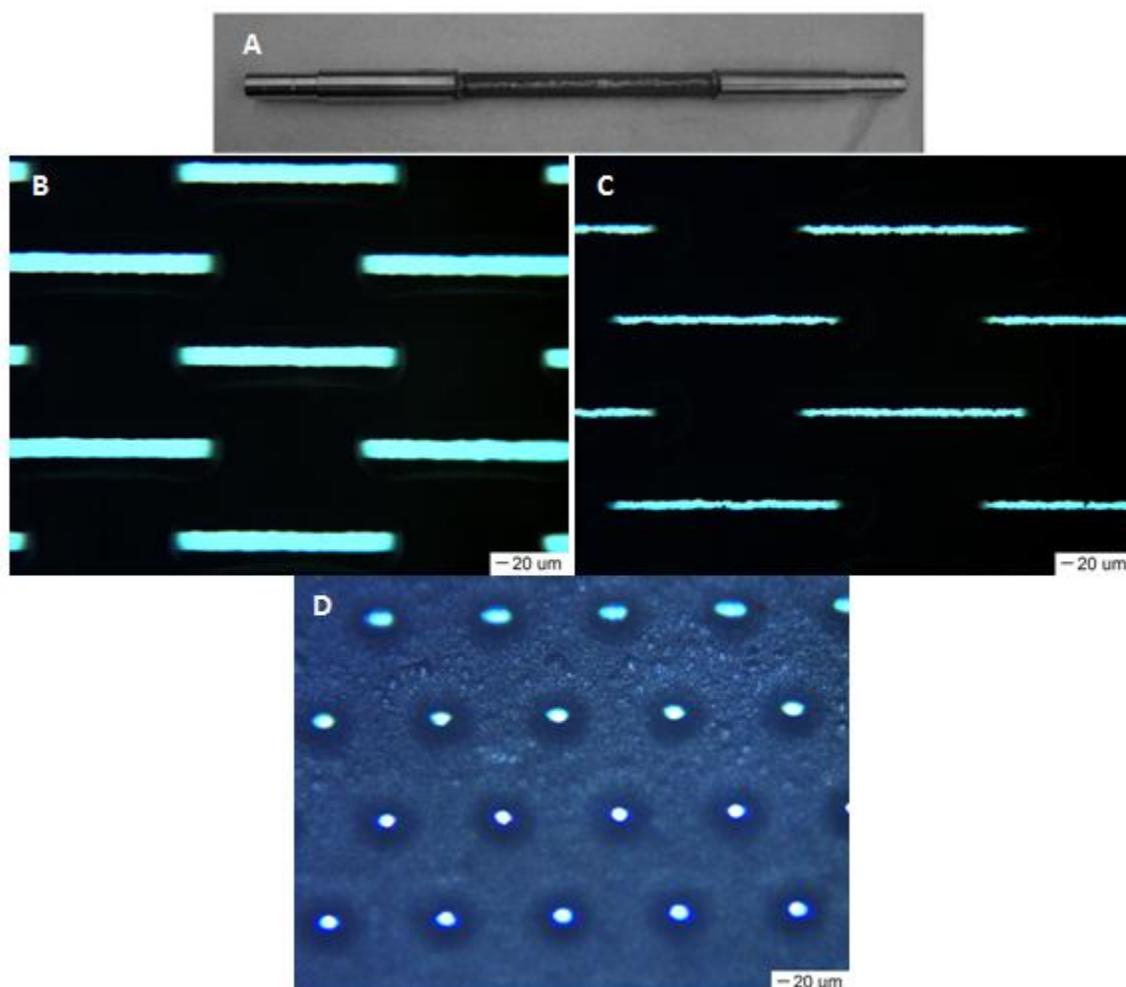


Figure 3.2. A) Photograph of Membrane I and microscope images of B) Membrane I; C) Membranes II and III; D) Membrane IV
Scale bar in B,C,D 20 μm (Micropore Technologies, Inc).

The basic emulsification method included a dispersed water phase injected through a membrane into a circulating oil phase. The dispersed phase flow rate was controlled by the settings on a peristaltic pump while the continuous phase flow rate was similarly controlled by changing the revolutions per minute (rpm) of a second, larger peristaltic pump. Setup of the emulsification system is shown in Figure 3.3.

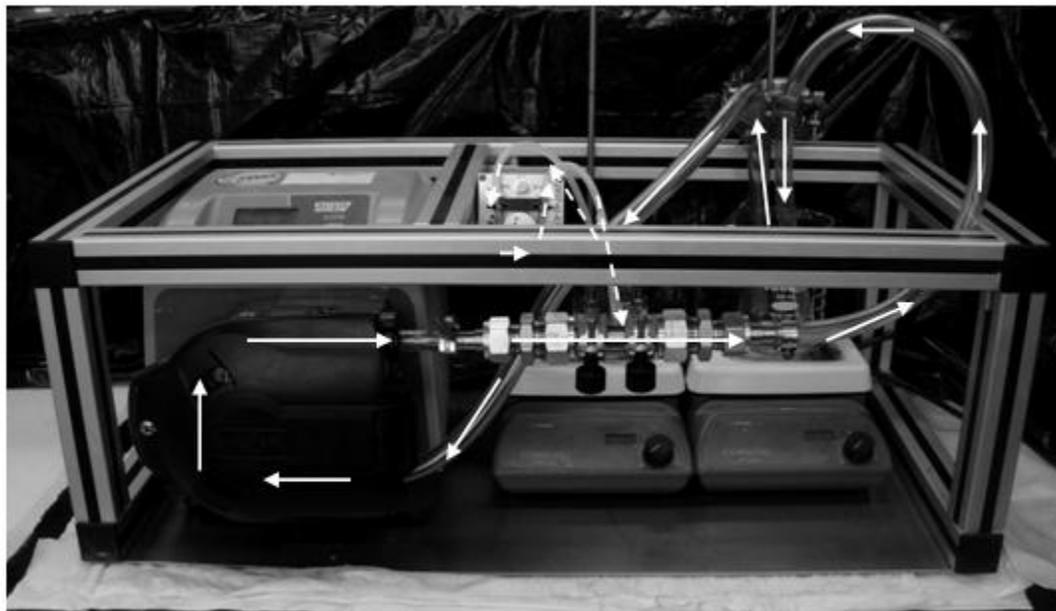


Figure 3.3. Photograph of cross flow membrane emulsification system. Arrows show flow of continuous phase (solid) and dispersed phase (dashed).

The following is the general method for the production of emulsions. The continuous phase (400 g) of soybean oil and the appropriate emulsifiers was obtained and stirred until all emulsifier was dispersed, as determined visually. The dispersed phase (176.4 g) of distilled water was obtained as it was previously determined that 5 g water remained in the dispersed phase tubing after injection. Thus the total amount of aqueous phase injected into the oil was 171.4 g, resulting in a 30% (w/w) water-in-oil emulsion. Unless otherwise specified, the dispersed phase pump was set to a setting of 4 and the continuous phase pump was set to 150 rpm, referred to hereafter as “standard pump conditions”. The water was injected into the continuous phase using Membrane I (unless otherwise specified) and two samples were taken for particle size determination. Each composition was prepared a minimum of three times. Samples were also poured into test tubes immediately after production and stored quiescently at room temperature to monitor behavior over time.

Determining Flow Rates and Pressures. The mass of soybean oil (continuous phase) pumped through the system per unit time was measured for different speed settings of the large peristaltic pump. The dispersed phase flow rate was similarly determined as the mass of water

per unit time injected into soybean oil at different speed settings of the small peristaltic pump. All flow rate determinations were performed in triplicate. Pressure in the system before and after the membrane at different large pump settings was determined using a DP25B-S Strain Meter/Controller (Omega Engineering, Inc.; Stamford, CT) equipped with a pressure transducer.

The continuous phase flow rate of soybean oil through the system at pump speeds of 100 and 150 rpm was determined to be 64.2 g/sec and 95.3 g/sec respectively. The dispersed phase flow rate at pump settings of 4 and 7 was 0.6 g/sec and 1.1 g/sec respectively. The flow rate through each pore of the four membranes was calculated at a flow rate of 0.6 g/sec (Table 3.2).

Table 3.2. Flow rate through one pore of each membrane at dispersed phase flow rate of 0.6 g/sec

Membrane	Flow Rate Through Pore (g*pore ⁻¹ *sec ⁻¹)
I	3.5 x 10 ⁻⁴
II	3.5 x 10 ⁻⁴
III	3.1 x 10 ⁻⁴
IV	2.4 x 10 ⁻⁴

The pressures in the continuous phase circuit before and after a membrane at both continuous phase pump speeds is shown in Table 3.3. Because of the nature of the peristaltic pump, both low and high pressure values are listed.

Table 3.3. Pressures in continuous phase circuit before and after a membrane at two continuous phase pump speeds.

Pump Speed (rpm)	Pressure Before Membrane (kPa)	Pressure After Membrane (kPa)
100	0.068 – 0.075	0.057 – 0.067
150	0.091 – 0.107	0.083 – 0.093

Emulsifiers. The three emulsifiers in this study were PGPR, lecithin and a 50:50 PGPR:lecithin blend. The emulsifiers were studied at concentrations of 0.5, 1, 2, 4 and 6 % with respect to the continuous phase (w/w). Based on preliminary data, emulsions with a 2%

concentration of emulsifier was chosen to be tracked at various time points over a 24 hour time period. Emulsions produced with 1, 2 and 4% PGPR were also tracked over a period of 4 weeks.

Carrageenan. In order to determine the effect of adding carrageenan to the dispersed phase of emulsions, a control emulsion was prepared as a reference. This control emulsion was 30% water-in-oil stabilized with 2% lecithin which was previously determined to be unstable at 25 °C. This was done to be able to isolate any stability change due to carrageenan. Carrageenan containing emulsions were produced with 2% κ -carrageenan in the dispersed phase, which was shown to produce a solid gel at 25 °C. The emulsions were produced under standard pump conditions as defined previously, however the temperatures of the phases were changed. The continuous phase was heated to and maintained at between 60 and 65 °C. The dispersed phase was heated to and maintained at between 70 and 75 °C and injected into the continuous phase at this temperature. For carrageenan containing emulsions, κ -carrageenan was fully dissolved in the heated water with stirring prior to injection. Once the dispersed phase was injected, two samples were taken and placed in screw cap test tubes. A thermometer was placed in one test tube and both were put in a freezer at -22 °C. Samples were continually observed until the thermometer read 25 °C after which they were removed from the freezer. A cap was added to the tube without the thermometer. This tube was inverted five times to re-suspend the droplets and two samples were taken for droplet size determination. Emulsions both with and without carrageenan were made in triplicate.

3.2.2.2 NMR Analysis

Emulsion droplet size was measured by pulsed field gradient NMR (pfg NMR) analysis (Minispec mq20, Bruker, The Woodlands, TX) with a “Water Droplet Size Application V1.1 Rev 2” program for water-in-oil emulsions. Diffusion coefficients were measured with the “Diffusio Application.” The diffusion coefficients for pure water and gelled water with 2% carrageenan at 25 °C were 2.299×10^{-5} and 1.706×10^{-5} cm²/sec respectively. A constant gradient strength γ of 2 T m⁻¹ was used with gradient pulse widths (δ) varied automatically by the application between 0.05 and 5 ms. Eight gradient pulse widths were used with a gradient pulse separation (Δ) of 210 ms. The application was calibrated at 5 °C and measurements were performed at 25 °C with the appropriate changes in diffusion coefficients. Each measurement took between 12 to 30 minutes.

Emulsions were tested in duplicate, with measurements taken within the same hour considered to be at the same time point.

The NMR droplet size application assumes that the distributions are log-normal. The application provides values for the geometric mean of the volume weighted distribution which will be referred to in this text as D3_3 or droplet size and the standard deviation of the log-normal distribution which will be referred to as sigma. Figure 3.4 shows sample distributions (D3_3 = 22.13 μm , sigma = 0.985) indicating these values with D3_3 shown on the cumulative distribution and sigma indicated on the relative frequency distribution. Sigma provides a way to characterize the width of the distribution. Since sigma is calculated from the logarithmic distribution, its true unit is $\ln(\mu\text{m})$, however this unit is not used when reporting sigma values as it does not have any practical meaning and will also not be used when reporting sigma in this study.

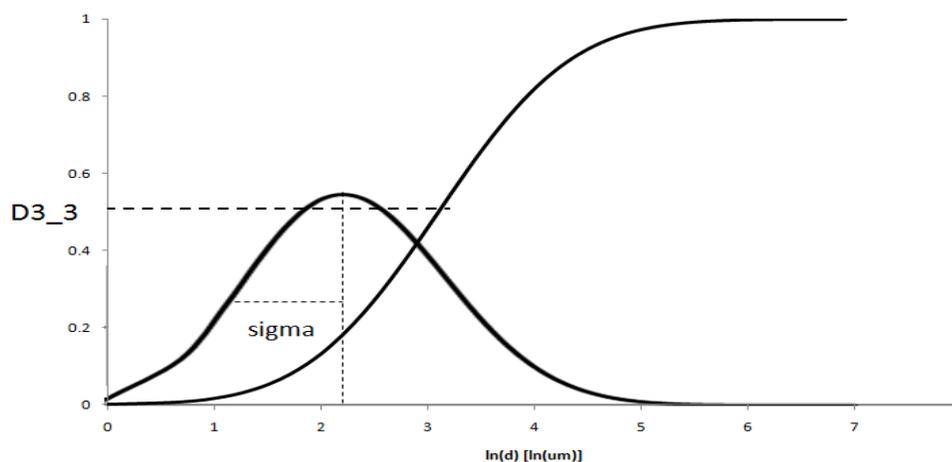


Figure 3.4. Sample log-normal cumulative and relative frequency distributions with D3_3 = 22.13 μm and sigma = 0.985.

It is important to note that the maximum droplet size that can be reported by the software is 100 μm since the time for diffusion is so long in droplets above this size that the effect of restriction cannot be determined accurately. Thus emulsions with this value as an output have an average droplet size at or above 100 μm . The means reported in the following figures exclude any measurements of 100 μm and are identified with an asterisk followed (*). These measurements were also excluded from the calculation of standard deviation of the mean. Thus any measurement indicated with an asterisk is a low estimate of the actual value.

3.2.2.3 Microscopy

Optical microscopy was performed using an Olympus BX41 microscope (Hitech Instruments, Inc., Edgemont, PA) equipped with SPOT Advanced Version 4.0.9 imaging software (Diagnostic Instruments, Inc) on samples prepared by the above methods. One drop of the sample was placed on a glass microscope slide and covered by a cover slip. Analysis was performed at room temperature.

3.2.2.4 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc.; La Jolla, CA) for analysis of variance (ANOVA) and Tukey's post-test and Minitab Student Release 14 (Minitab Inc.; State College, PA) for 2-Sample t-tests with significance at $\alpha = 0.05$. Probability plots of the droplet size (D3_3) and sigma were performed by Minitab Student Release 14 (Minitab Inc.; State College, PA) and all values were greater than 0.05, indicating normal distributions.

3.3 Results and Discussion

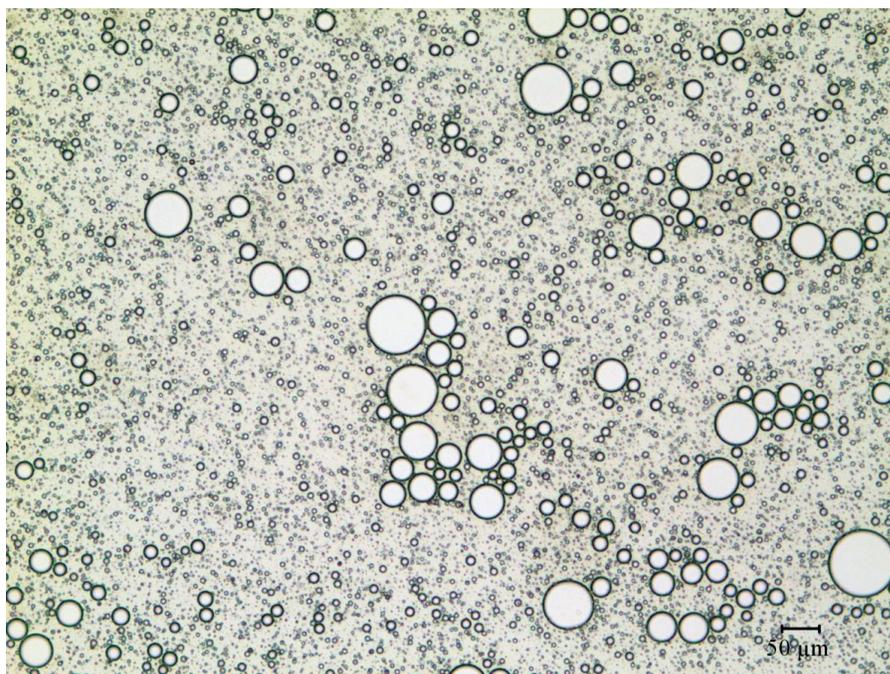
3.3.1 Emulsion Formation

Water-in-oil emulsions (30% w/w) were produced via cross flow membrane emulsification using PGPR, lecithin and a 50:50 PGPR:lecithin blend as emulsifiers under standard pump conditions with membrane A. Images of the emulsions stabilized with 2% of the respective emulsifiers immediately after production are shown in Figure 3.5A-C.

Droplets stabilized by PGPR were perfectly spherical and highly polydisperse with many fine droplets and a few large ones with diameters up to approximately 90 μm (Figure 3.5A). The larger droplets appeared to be clustered together. This may be due to flocculation or alternatively may be due to the constrictive effects of the cover slip causing larger particles to move to regions with more space.

The optical micrographs of lecithin-stabilized emulsions were different from PGPR-stabilized emulsions (Figure 3.5B). Again, the droplets were highly polydisperse but in this case the largest particles ($> 100 \mu\text{m}$) were often non-spherical. The droplets were highly aggregated with large gaps between groups of droplets. The aggregates were also seen on the microscope slide without magnification. The blend-stabilized emulsions were also highly polydisperse and contained a mixture of spherical and non-spherical droplets (Figure 3.5C). The droplet sizes were typically larger than those seen in PGPR- or lecithin-stabilized emulsions. Some droplets were also seen to coalesce during observation.

A



B

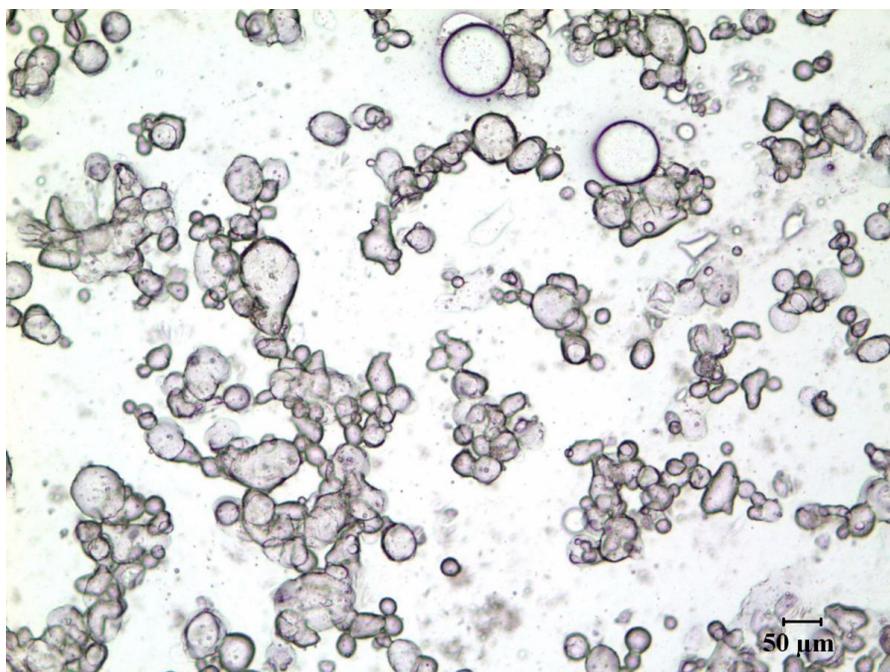


Figure 3.5. Optical microscope image of 30% water-in-oil emulsion produced under standard conditions stabilized by A) 2% PGPR; B) 2% lecithin; C) 2% 50:50 PGPR:lecithin blend. Scale bars 50 μm .

C

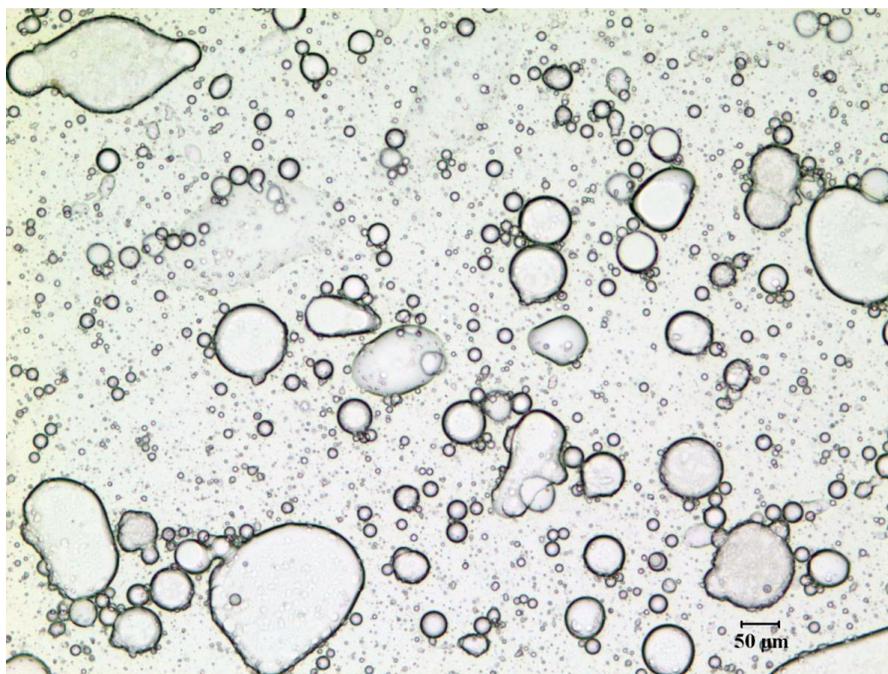


Figure 3.5. (Continued)

The influence of emulsifier concentration on the resulting initial droplet diameter and distribution of these emulsions was investigated via NMR analysis. The droplet diameters (D_{3-3}) and the sigma values are shown in Figure 3.6A and B. Overall, droplet diameter is shown to decrease with increasing emulsifier concentration (Figure 3.6A). The PGPR-stabilized droplets are significantly smaller than the lecithin and blend stabilized droplets at a concentration of 1%. The PGPR-stabilized droplets also show a large initial decrease in droplet diameter from 0.5 to 1% PGPR. The lecithin-stabilized droplets are significantly smaller than the blend droplets from 1 to 4%. At 6% emulsifier concentration, the droplet diameters of the lecithin and blend emulsions are not statistically different.

Sigma values (the standard deviation of the droplet size distribution) display a similar decrease with increasing emulsifier concentration for all emulsifiers (Figure 3.6B). In this case, the sigma values of lecithin-stabilized emulsions are significantly smaller than those of blend-stabilized emulsions at all concentrations and PGPR-stabilized emulsions at concentrations between 1 and 4%. The sigma of PGPR- and blend-stabilized emulsions are not significantly

different at 0.5% but PGPR-stabilized emulsions have smaller sigma values that are significantly different from those of blend-stabilized emulsions at all other concentrations. The difference between the sigma values of PGPR- and lecithin-stabilized was also demonstrated in work by Knoth, Scherze and Muschiolik (2005a) in which lecithin-stabilized emulsions had a narrower size distribution than PGPR-stabilized emulsions.

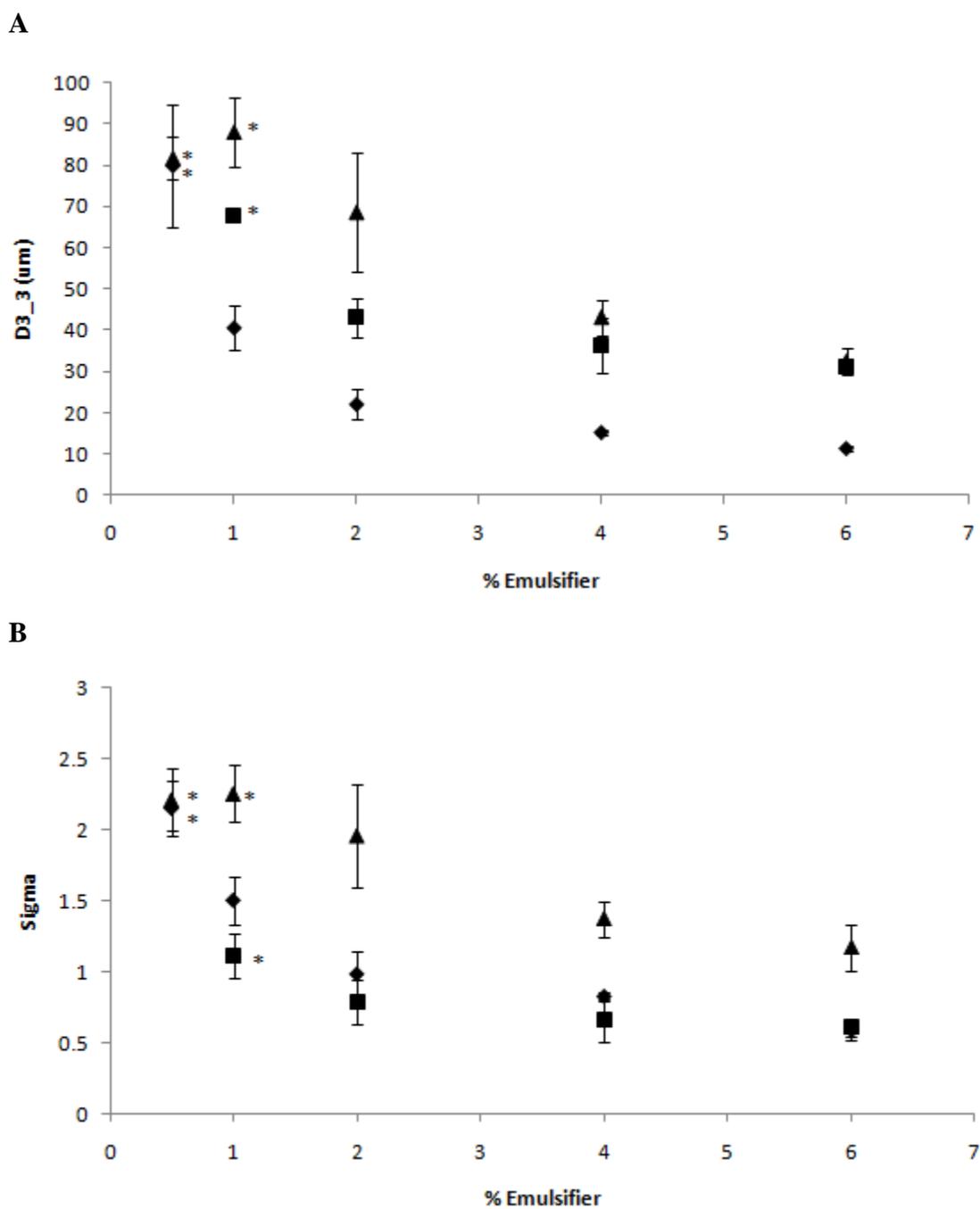


Figure 3.6. Average initial A) droplet size and B) sigma value of 30 % w/o emulsions produced with increasing concentrations of PGPR (diamonds), lecithin (squares) and 50:50 blend of PGPR:lecithin (triangles). Concentration is of soybean oil continuous phase. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation. Asterisks indicate that measurements of 100 μm were removed from the calculation of the mean. No point is shown for 0.5% lecithin-stabilized emulsions as all measurements were 100 μm.

It appears that the droplet size of blend-stabilized emulsions is governed by the amount of lecithin present. This behavior is illustrated in Figure 3.7 which compares the droplet sizes of lecithin-stabilized and blend-stabilized emulsions based on the lecithin concentration of the continuous phases at 1 and 2%. At these levels the droplet sizes of the blend-stabilized emulsion are not statistically different than those of the lecithin-stabilized emulsion while they are statistically different from the PGPR-stabilized emulsions (not shown). This behavior is difficult to explain but is possibly due to interactions between the two emulsifiers of the blend. I will return to this point after considering additional data (Section 3.3.3).

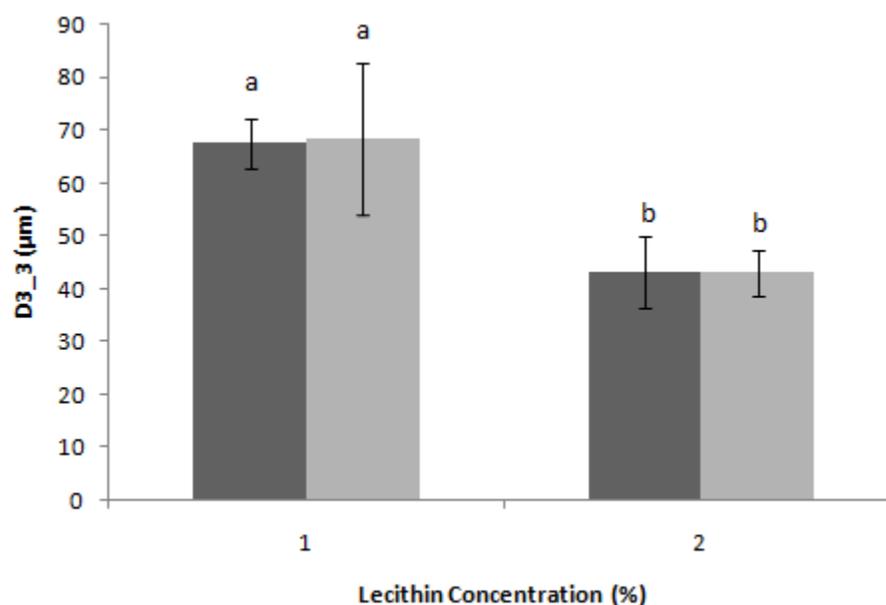


Figure 3.7. Average initial droplet diameter vs lecithin concentration of 30 % w/o emulsions produced with lecithin (dark bars) and 50:50 blend of PGPR:lecithin (light bars). Concentration is of soybean oil continuous phase. The 1% and 2% lecithin concentrations in the PGPR:lecithin blend also contained 1% and 2% PGPR respectively. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$.

3.3.2 Emulsion Stability

Just because a particular emulsifier is able to form fine droplets is no indication that those droplets will be stable. Microscopy of lecithin- and blend-stabilized emulsions immediately after formation already showed evidence of flocculation. To elucidate the effect of emulsifier on emulsion stability, the size distributions of emulsions prepared with a fixed amount of emulsifier

(2%) were monitored over a period of 24 hours (Figure 3.8A and B). Again, the means reported exclude any measurements of 100 μm and are identified with an asterisk followed (*). These measurements were also excluded from the calculation of standard deviation of the mean.

PGPR-stabilized emulsions remained at a consistent droplet size of approximately 21.4 μm over the 24 hour period with a consistent sigma value of approximately 1, indicating a stable emulsion. The lecithin-stabilized emulsion droplet size increased from 43.1 to 77 μm in a six hour time-span after which there was no further change. This indicates that there was sufficient lecithin present to stabilize the interfacial area of this size of droplets. This behavior was mirrored in the sigma value which increased from 0.8 to 1.4 during this time period, becoming significantly different than the sigma value of PGPR-stabilized emulsions after 24 hours. The blend-stabilized emulsions, on the other hand, rapidly destabilized with a majority of samples furnishing measurements of 100 μm after two to three hours.

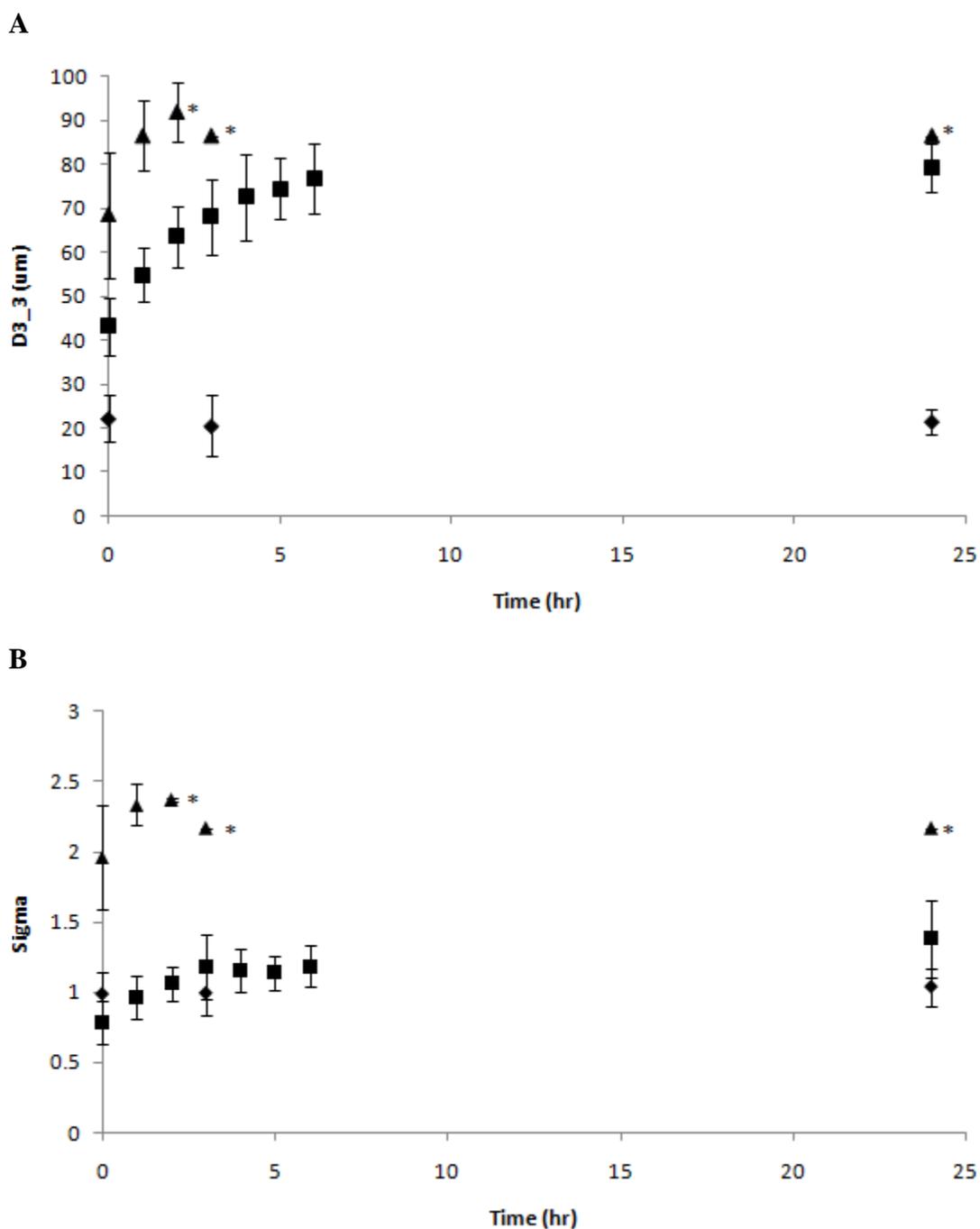


Figure 3.8. Average A) droplet diameter and B) sigma value of 30 % water-in-oil emulsions produced with 2 % of emulsifiers PGPR (diamonds), lecithin (squares) and 50:50 blend of PGPR and lecithin (triangles) in soybean oil continuous phase. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation. Asterisks indicate that measurements of 100 μm were removed from the calculation of the mean.

The stability of the emulsions over longer times-scales was determined by visual observation after quiescent storage in test tubes (Figure 3.9). After a week of storage, emulsion appearance did not noticeably change. PGPR-stabilized emulsions sedimented into three distinct layers: a small top layer of clear oil, a middle layer that was white and cloudy and a bottom layer that was brighter white and cloudy. In contrast, lecithin-stabilized emulsions sedimented into two layers: the large top layer was clear oil while the bottom layer was cloudy and appeared to be a collection of aggregated droplets. The blend-stabilized emulsions were different from the previous two emulsions. Four layers were apparent with a small top clear layer similar to PGPR-stabilized emulsions, followed by a larger slightly cloudy layer. This was followed by a very small white layer that had an aggregated structure reminiscent of the lecithin stabilized emulsions. A bottom layer consisted of a clear water phase. Again, this distinctive behavior of blend-stabilized emulsions supports the hypothesis that some interaction between the PGPR and the lecithin is taking place, resulting in droplet coalescence and phase separation. This behavior will be discussed further in Section 3.3.3.

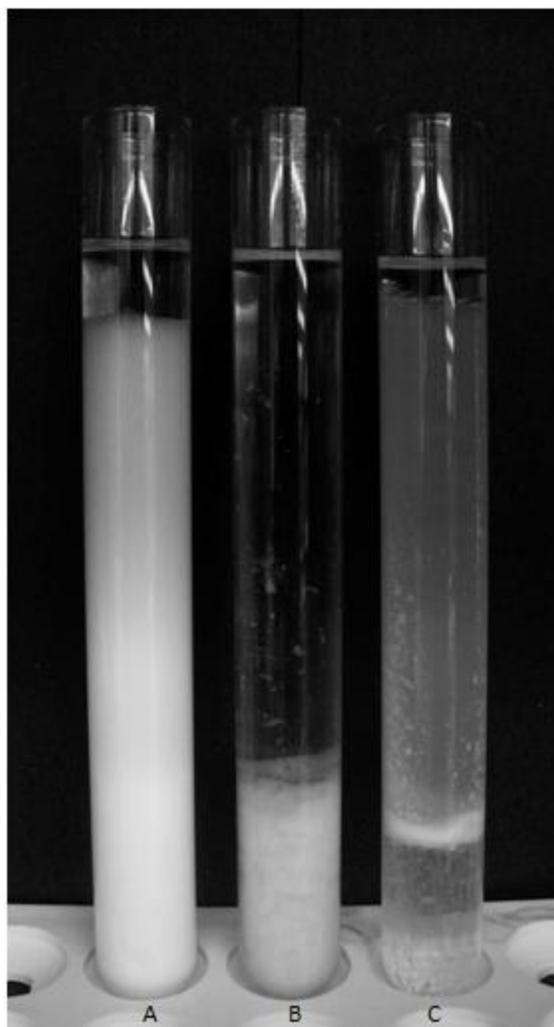


Figure 3.9. Photograph of emulsions in test tubes produced by the standard method with A) 2% PGPR in oil phase; B) 2% lecithin in oil phase; C) 2% 50:50 PGPR:lecithin blend in oil phase. Emulsions produced between 2 to 4 weeks prior to photograph.

As seen in Figures 3.8 and 3.9, PGPR-stabilized emulsions were stable over a longer period of time than the other emulsions. To further investigate the stability of PGPR-stabilized emulsions, the droplet size distributions of emulsions stabilized with 1, 2 and 4% PGPR were monitored over a period of four weeks and shown in Figure 3.10A and B. Clearly the lecithin and blend covered droplets could not be studied over this timescale.

While higher levels of PGPR resulted in smaller droplet sizes and sigma values, all the emulsions showed no statistical difference in droplet size between time 0 and 4 week

measurements and only the emulsions stabilized with 4% PGPR showed a significant increase in sigma value (0.83 to 1.06 at week 4).

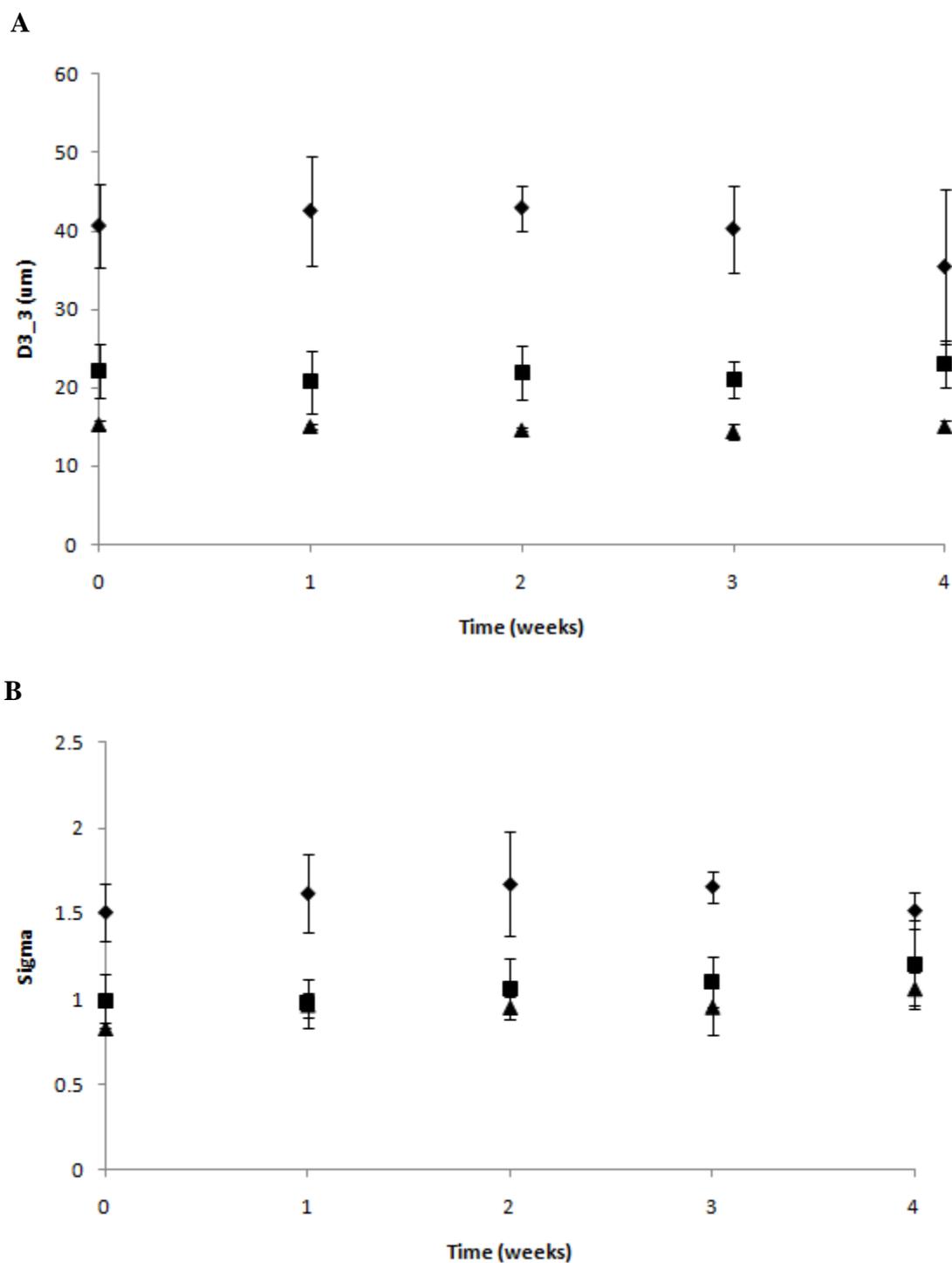


Figure 3.10. Average A) droplet diameter and B) sigma value of 30% w/o emulsions produced with 1% (diamonds), 2% (squares) and 4% (triangles) PGPR in soybean oil continuous phase over 4 weeks. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation.

3.3.3 Discussion of Emulsion Formation and Stability

The results for initial droplet distribution support the hypothesis that increasing emulsifier concentration results in reduced droplet diameters and distributions (Figure 3.6). This is evident from the torque balance model for predicting droplet diameter as interfacial tension is one of the main factors in Equation 1.5. Emulsifiers lower the interfacial tension by adsorbing onto the surface of droplets as they develop at the membrane (Charcosset, Limayem and Fessi, 2004; Charcosset, 2009). The addition of more surfactant lowers the surface tension even further. Additionally, higher levels of emulsifier are able to better stabilize existing droplets leading to decreased coalescence during recirculation of the continuous phase during emulsion production (Charcosset, Limayem and Fessi, 2004; Charcosset, 2009). Furthermore, the decrease in droplet diameter with increasing emulsifier concentration would be expected to level off when the amount of emulsifier able to be adsorbed at the droplet surface reached a maximum and Figure 3.6A shows the beginning of this behavior with smaller changes in droplet diameter occurring between 4 and 6% in all systems.

The data on both emulsion formation and stability (Figures 3.5, 3.6 and 3.8) show that the type of emulsifier affects droplet size distribution. Schroder, Behrend and Schubert (1998) also found this to be true for water-continuous emulsifiers used in stabilizing oil-in-water emulsions produced by membrane emulsification. The smaller droplet sizes and increased stability of emulsions stabilized by equivalent concentrations of PGPR show that this emulsifier is more effective at stabilizing water-in-oil emulsions. There are two explanations for this behavior. Firstly, lower interfacial tensions are expected to generate smaller sizes (Schroder, Behrend and Schubert, 1998). Indeed surface tension is present in the equations of two of the forces included in the model for predicting droplet diameter (Equations 1.3 and 1.4; De Luca et al., 2004). A study by Knoth, Scherze and Muschiolik (2005a) reported the interfacial pressures at the water-sunflower oil interface with 2.5% PGPR and lecithin as 23.09 and 16.41 mN/m respectively. Thus PGPR solutions have a lower interfacial tension and can be expected to produce smaller emulsion droplets. However, the emulsifiers and oil used in the study by Knoth, Scherze and Muschiolik (2005a) are not identical to those used in this study. Despite variables in the composition of these components, the general trends observed are expected to hold for this study as well.

A second explanation for the smaller droplet sizes and increased stability of PGPR stabilized emulsions is the stabilizing steric effects of PGPR-coated surfaces. PGPR is typically larger than lecithin (≈ 1050 D vs 750 D; Ziegler, Garbolino and Coupland, 2003; Palsgaard, 2008) and has a polymeric structure consisting of loops and tails. This structure allows PGPR to provide a strong steric barrier around emulsion droplets, thereby preventing flocculation and decreasing the likelihood of coalescence. Dedinaite and Campbell (2000) measured the steric repulsion between emulsifier-coated hydrophilic mica surfaces in triolein and showed that PGPR was more efficient at providing a strongly repulsive barrier than lecithin. It is possible that droplets formed at the membrane surface in this study detached with similar droplet diameters, but then PGPR-stabilized emulsions were able to prevent droplet coalescence through steric stabilization.

The amount of PGPR shown to produce stable emulsions in this study is lower than that reported in previous work. Benichou, Aserin and Garti (2001) investigated the stability of PGPR-stabilized water-in-oil emulsions and found that stable emulsions could only be produced at high emulsifier concentrations ($\geq 10\%$). Knoth, Scherze and Muschiolik (2005a) also found that at least 4% PGPR was needed for stable emulsions. These concentrations are greater than those determined to produce stable emulsions in this study (1%), however this can be attributed to the sizes of the droplets produced. Both Benichou, Aserin and Garti (2001) and Knoth, Scherze and Muschiolik (2005a) studied droplets less than 10 μm in diameter while the droplets in this study averaged 40 μm in diameter (at 1% PGPR). The amount of PGPR per m^2 of droplet surface area in these two studies was approximately three times less than that in the present work.

Blend-stabilized emulsions consisted of larger droplets with greater sigma values and were less stable than PGPR- or lecithin-stabilized emulsions (Figures 3.6 and 3.8). It should be noted that chunks of lecithin were apparent when the continuous phase of blend-stabilized emulsions was initially produced. Continued stirring was necessary to dissolve the emulsifiers, which eventually went into solution, as determined visually. In the case of PGPR or lecithin alone, only moderate stirring was needed. This suggests that PGPR and lecithin interact in some way in solution that may modify their individual abilities to stabilize emulsion droplets when present together. Dedinaite and Campbell (2000) observed similar turbid behavior in anhydrous mixtures of phospholipid and PGPR in triolein. The workers also observed the interaction between mica plates in the presence of these emulsifiers and interpret these findings as PGPR

adsorbing onto phospholipid aggregates (Dedinaite and Campbell, 2000). The interaction of PGPR and lecithin, particularly in the bulk continuous phase, may mean that less emulsifier is available to stabilize emulsion droplets.

3.3.4 Effect of Membranes on Emulsion Formation

The influence of changing the membrane characteristics on droplet size and distribution of emulsions was investigated by producing and testing 2% PGPR-stabilized emulsions made at standard pump conditions (Figure 3.11). The properties of the four membranes used are summarized in Table 3.1 and the flow rate through one pore of each membrane is summarized in Table 3.2.

Membrane IV produced emulsions with larger diameters than membranes I and II but similar to membrane III. The droplets produced by membranes I, II and III were not statistically different from one another. Various other studies and existing theory suggest that pore size should be proportional to droplet size at set operating conditions (Equation 1.5; Charcosset, Limayem and Fessi, 2004; De Luca et al., 2004; Gijsbertsen-Abrahamse, van der Padt and Boom, 2004; Charcosset, 2009). However membrane I had larger pore size than II and III yet produced similarly-sized droplets.

One hypothesis for this discrepancy is that because the flow rate of the dispersed phase through the pores of membranes I-III was similar, the membranes were able to produce similar sized droplets before detachment from the membrane (Table 3.2). An alternative hypothesis is that the membranes with smaller pores initially produced smaller droplets, however the amount of emulsifier present was not sufficient to stabilize these droplets. The smaller droplets coalesced either close to the membrane or during the circulation of the continuous phase, resulting in stabilized droplets approximately the same diameter as those produced by membrane I. In a study on vegetable oil-in-milk emulsions, Joscelyne and Tragardh (1999) also found that there was no dependence of droplet size on membrane pore size at a 2% emulsifier concentration.

It is hard to directly compare the size of the pores in membrane IV with the others as although the area of each pore was smaller ($314 \mu\text{m}^2$ versus 18000 and $3360 \mu\text{m}^2$ for membranes I and II/III respectively), they were circular rather than slotted. Kobayashi et al. (2002) found

that circular pores produced much larger soybean oil-in-water droplets than oblong pores. This is consistent with the data which shows that the average droplet diameter of emulsions produced by membrane IV was significantly larger than the average droplet diameters of emulsions produced by membranes I or II. Again, it is possible that membrane IV produced small droplets initially, but the concentration of emulsifier was not sufficient to stabilize the small droplets which coalesced to a larger droplet size prior to measurement.

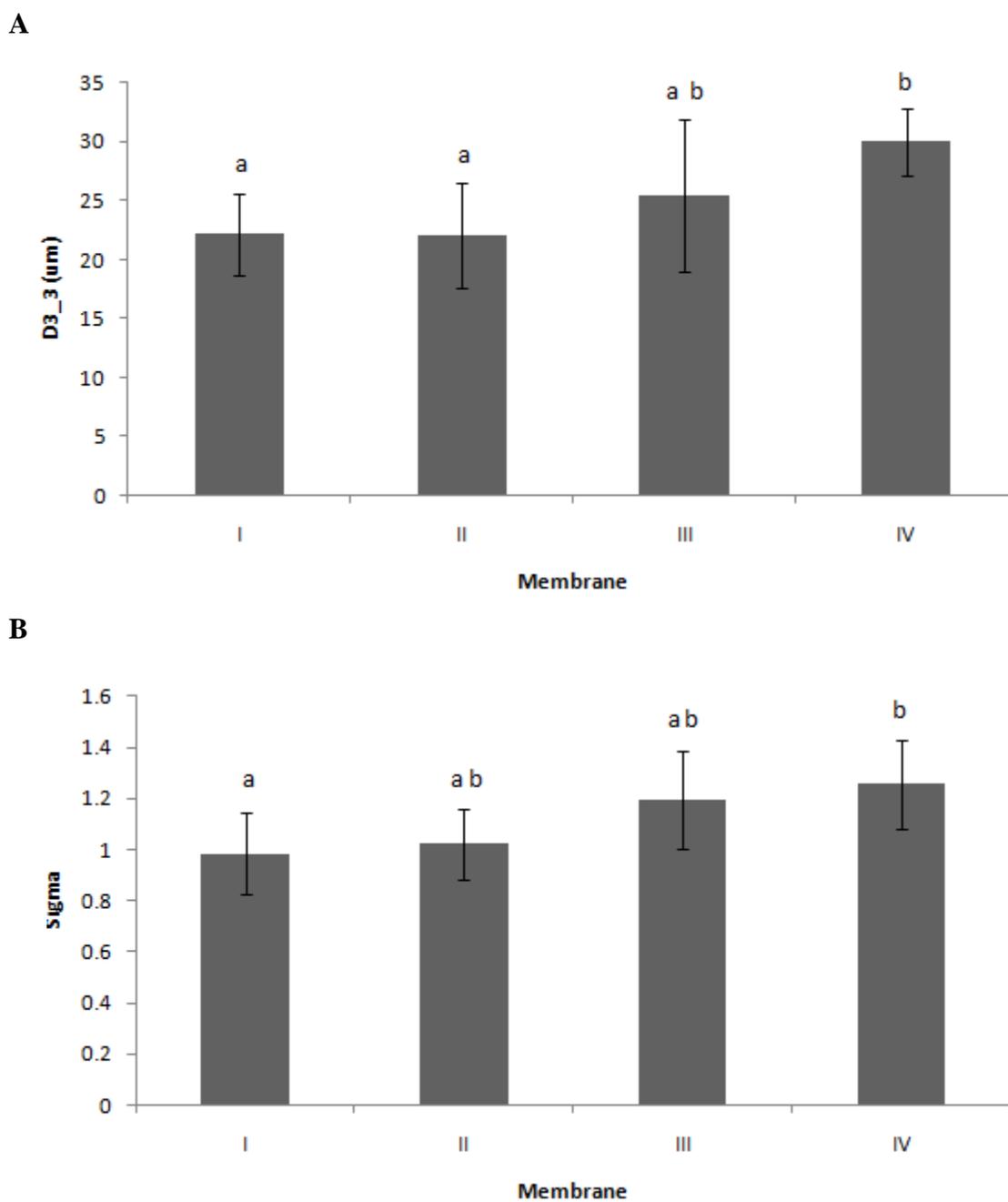


Figure 3.11. Average initial A) droplet diameter and B) sigma value of 30% w/o emulsions produced by membranes I-IV with 2% PGPR in soybean oil continuous phase. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$.

3.3.5 Effect of Altered Flow Rates on Emulsion Formation

The influence of continuous phase flow rate and dispersed phase flow rate were investigated by changing the respective pump speeds. Decreasing the continuous phase flow rate from 95.3 g/sec to 64.2 g/sec significantly increased both the droplet diameter and the sigma value (Figure 3.12A and B). The continuous phase velocity is a main contributor to the shear stress at the membrane surface which causes droplets to detach (Equation 1.2). Various studies have shown that increasing the continuous phase velocity or subsequent shear stress decreases the droplet diameter; however this work was in studies of oil-in-water emulsions (Kato et al., 1996; Joscelyne and Tragardh, 1999).

Increasing the dispersed phase flow rate from 0.6 g/sec to 1.1 g/sec also significantly increased the droplet diameter, however it did not significantly alter the sigma value (Figure 3.12A and B). Again, this is consistent with the literature which showed that when the dispersed phase flow rate is increased the droplets increase more in volume and therefore size prior to detachment (Peng and Williams, 1998). While dispersed phase flow rate is not directly involved in the calculation of droplet diameter, it is possible that it influences the dynamic interfacial tension of the droplets (Equations 1.2-1.5). It is more difficult for surfactant to stabilize the rapidly expanding interface of quickly growing droplets and therefore these droplets would have higher dynamic interfacial tension. Additionally, increased dispersed phase flow rate increases the likelihood of coalescence at the membrane which results in larger droplet diameters (Charcosset, Limayem and Fessi, 2004).

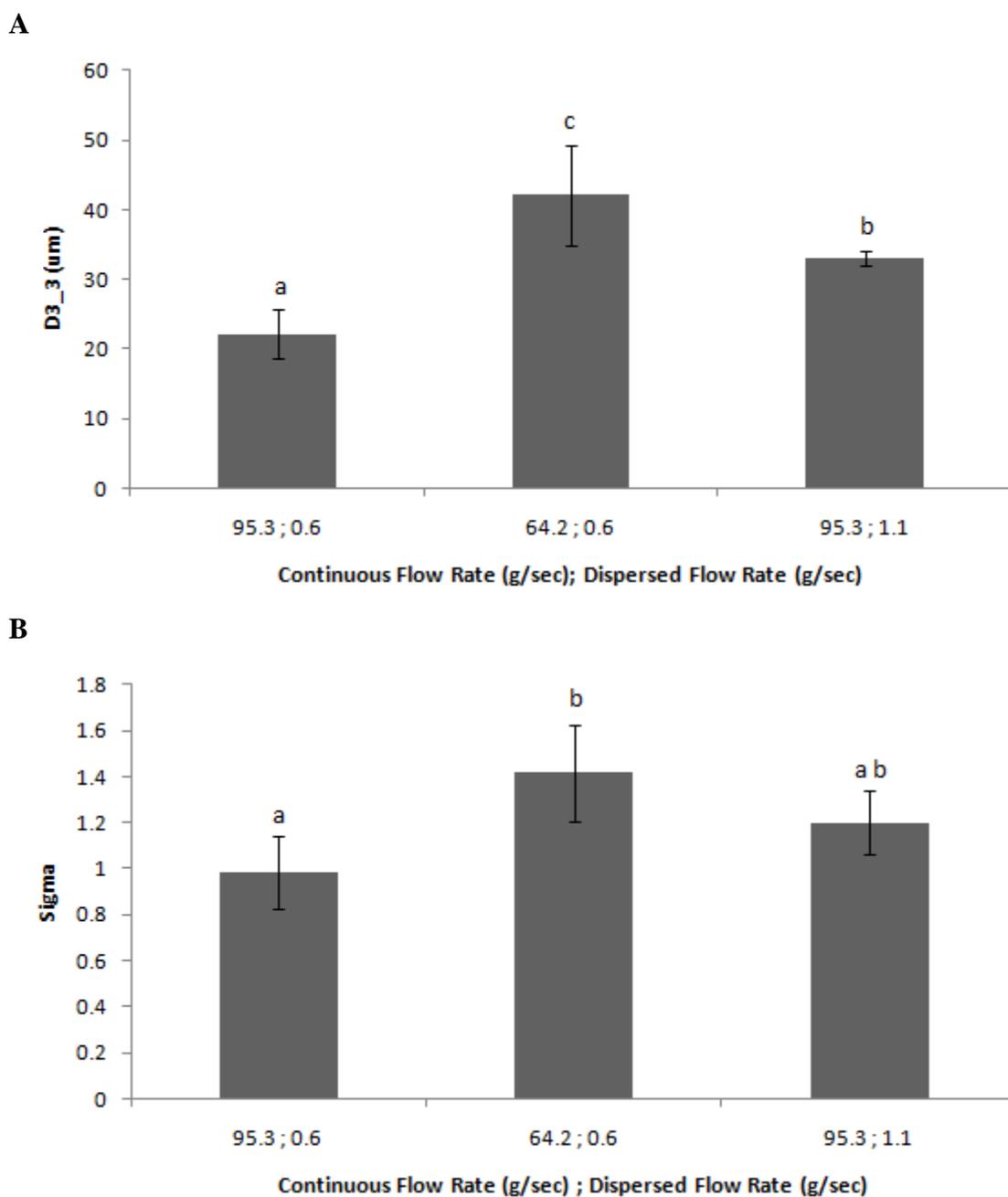


Figure 3.12. Average initial A) droplet diameter and B) sigma value of 30% w/o emulsions produced at different operational conditions with 2% PGPR in soybean oil continuous phase. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$.

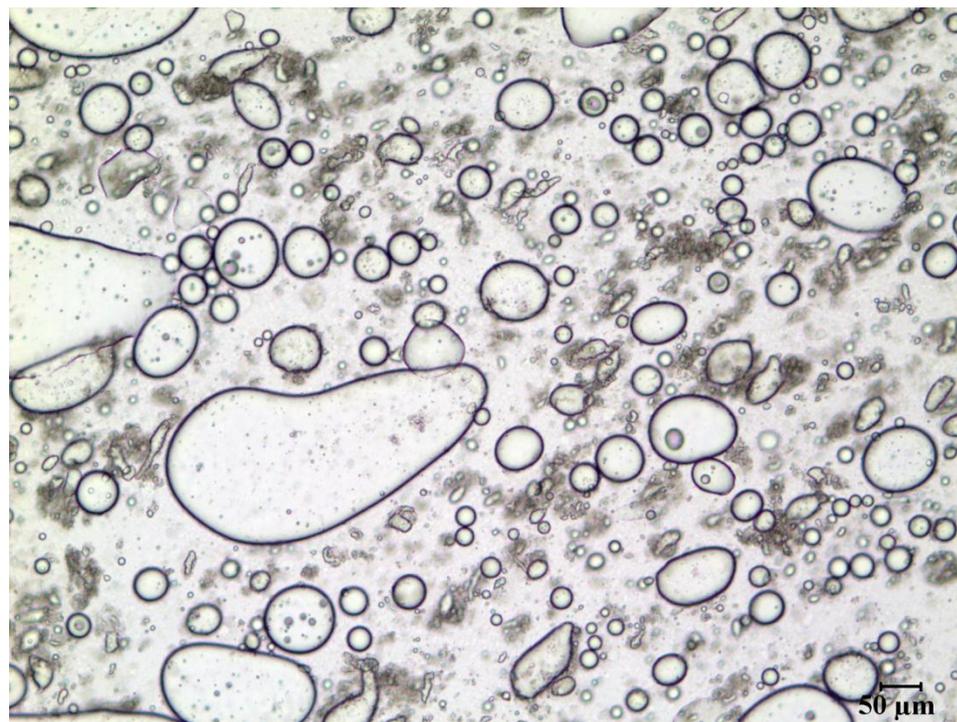
3.3.6 Effect of Gelled Dispersed Phase on Emulsion Formation

The influence of a gelling agent on emulsion characteristics was examined by making emulsions with and without carrageenan in the dispersed phase. The emulsions were stabilized with 2% lecithin as these were previously shown to be unstable. An unstable emulsion was used because I hypothesized that the addition of carrageenan to the dispersed phase would cause emulsion droplets to gel upon cooling, therefore preventing coalescence and increasing the stability. Emulsions were made under heated conditions so that the dispersed phase would not gel until after emulsification. The control emulsions were produced under heated conditions as well to eliminate confounding variables. Preliminary investigation showed that a 2% solution of carrageenan in water produced a solid gel when cooled from 70 to 25 °C so this concentration was used in the dispersed phase.

The control emulsion sedimented extremely rapidly and the sediment appeared highly aggregated, similar to previous observations of lecithin-stabilized emulsions. The sediment was re-suspended by inverting test tube before NMR sampling. The emulsion prepared with carrageenan added to the dispersed phase sedimented equally as rapidly, however upon cooling, large aggregates of solidified droplets had formed making re-suspension difficult. Optical microscope images of these two emulsions show marked differences. The control emulsion (Figure 3.13A) consisted of many round and oblong droplets that were slightly flocculated, but reasonable well dispersed. Larger droplets are seen in these emulsions compared to the lecithin-stabilized emulsions produced earlier at room temperature conditions (Figure 3.5B). This is likely due to the decreased viscosity of the continuous phase due to increased temperature. This decrease in viscosity also caused faster sedimentation of the droplets which were then able to coalesce into the larger droplets because of the increased droplet density in the bottom of the test tube.

The emulsions with carrageenan (Figure 3.13B) showed few single droplets, with the majority of the dispersed phase coalesced into large gelled chunks. Air bubbles were also noticeable, trapped within the gel matrix.

A



B

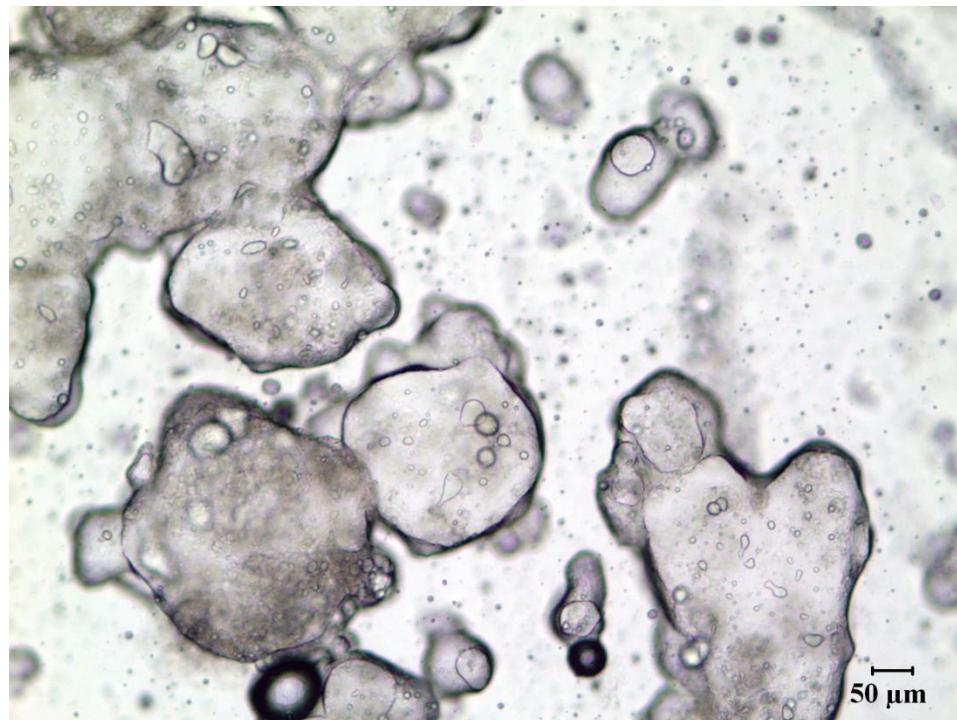


Figure 3.13. Optical microscope image after cooling of lecithin-stabilized emulsion A) without and B) with 2% carrageenan in dispersed aqueous phase produced by standard pump settings and under heated conditions. Scale bar 50 μm.

NMR analysis of these emulsions mirrored the visual observations. Both the average droplet diameter and the sigma value of the carrageenan containing emulsion were significantly higher than those of the control (Figure 3.14A and B). It should be noted that four of the six measurements reported for the carrageenan containing emulsions were of the maximum droplet size indicating that the true value was anywhere at or above 100 μm . These values have been removed from the means and are marked with an asterisk (*).

These results are contrary to the hypothesis that gelled droplets would be more stable against coalescence and hence result in a more stable emulsion with smaller droplet size and sigma value. There are three proposed explanations for this discrepancy. Firstly, it is possible that the increased viscosity of the dispersed phase with added polymer sufficiently decreased the flow of the dispersed phase through the membrane to result in larger droplets. This relationship of viscosity and dispersed phase flux has been discussed in the literature (Charcosset, Limayem and Fessi, 2004). The second explanation is that the presence of carrageenan in the dispersed phase increased the density of the droplets. Based on Stokes' law, this caused the droplets to sediment faster and they were able to coalesce in the sediment volume before the emulsion was cooled to the point that gelling occurred. While this is possible, the change in density due to carrageenan would be very small and most likely would not significantly affect droplet sedimentation velocity. Lastly, the presence of carrageenan may have caused any flocculated gelled droplets to stick together with stronger forces whereas the flocculated control droplets were able to be re-dispersed upon inversion of the test tube. Overall, this experiment shows that unless processing conditions are otherwise controlled, the addition of carrageenan to the dispersed phase makes the production of a stable emulsion more difficult.

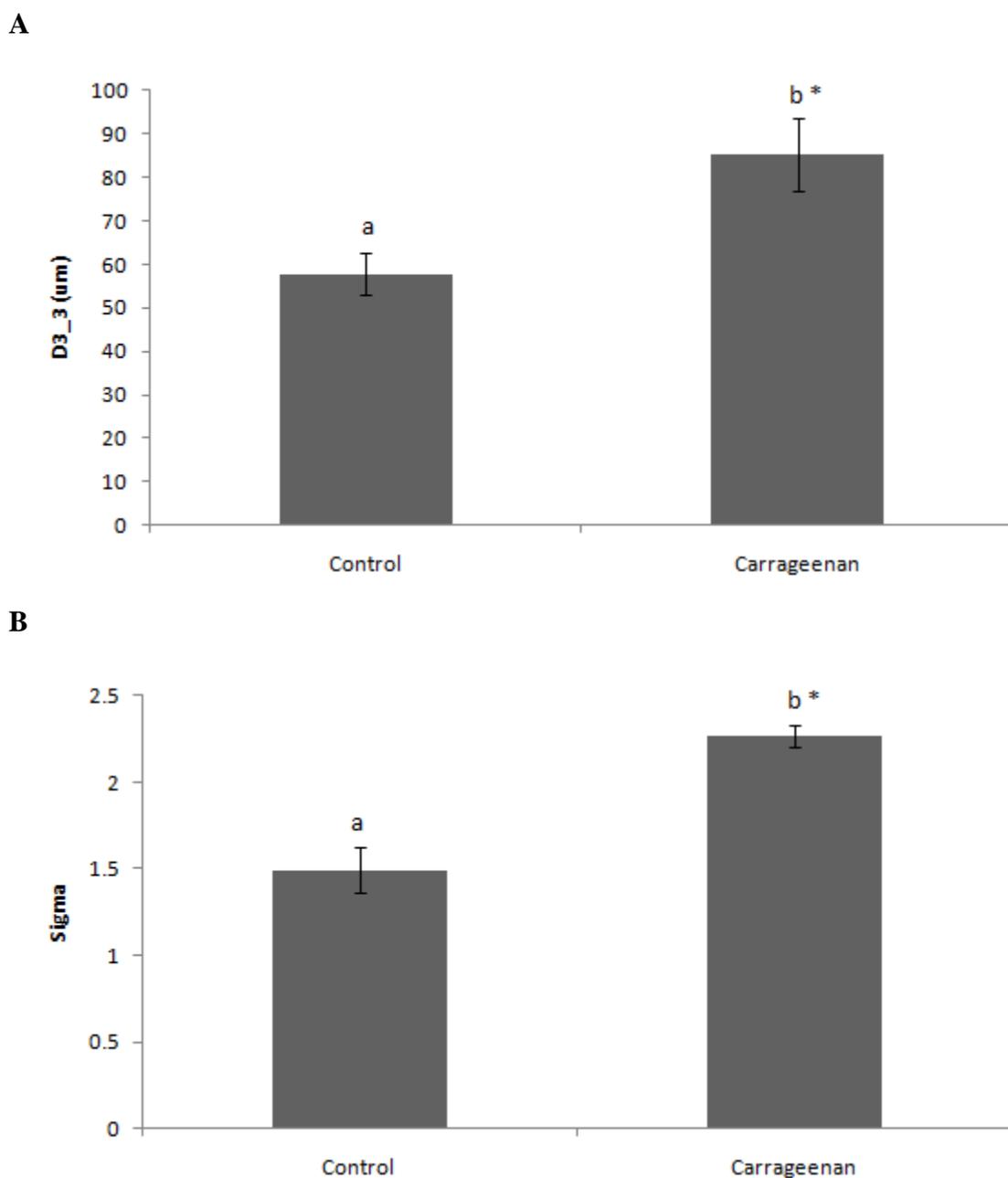


Figure 3.14. Average initial A) droplet diameter and B) sigma value of 30% w/o emulsions with and without carrageenan in dispersed phase. Control – 2% lecithin in continuous phase, produced under hot conditions; Carrageenan – 2% lecithin in continuous phase, 2% carrageenan in dispersed phase, produced under hot conditions. Samples were cooled to 25 C in freezer at – 22 °C. Analyzed by pfg NMR at 25 °C. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$. Asterisks indicate that measurements of 100 µm were removed from the calculation of the mean.

3.4. Conclusions

The aim of this study was to examine the effect of changing ingredients and processing parameters on water-in-oil emulsions produced by a cross flow membrane emulsification system. Droplet size and distribution of emulsions consisting of 30% water in soybean oil were analyzed via NMR to provide a measure of initial emulsion formation and stability. In general, it was shown that increasing concentration of emulsifier decreased average droplet diameter and the sigma value (a measure of the width of the distribution). PGPR-stabilized emulsions resulted in the smallest droplets of the three emulsifier systems studied and these droplets did not significantly increase in size over time at concentrations ranging from 1 to 4%. On the other hand, initial measurements of emulsions stabilized by lecithin or a 50:50 PGPR:lecithin blend showed larger mean droplet sizes. Emulsions stabilized by 2% lecithin doubled in droplet diameter over 6 hours before stabilizing at this value and emulsions stabilized by a 2% blend destabilized beyond measurement after 3 hours.

Membrane pore size did not have a significant effect on the droplet diameter and sigma value except between membranes I and IV, however, differences in membrane length and the flow rates through the pores may have resulted in confounding variables. The circular pores of membrane IV resulted in larger droplets that were significantly different from those of membranes I, however no conclusion can be drawn about the effect of pore size as other variables are present. The only conclusion to be drawn from the work with different membranes was that changing the membrane can change the droplet size and distribution of the resulting emulsion. To accurately determine the effect of pore shape and size all other variables need to be kept constant.

Using a stable PGPR-stabilized emulsion, the droplet size and sigma value were shown to increase by either decreasing the continuous phase flow rate or by increasing the dispersed phase flow rate.

The addition of a gelling agent, κ -carrageenan, to the dispersed phase resulted in significantly larger droplet sizes and sigma values demonstrating that it is more difficult to produce a stable emulsion under these conditions.

Overall, numerous conditions for producing both stable and unstable water-in-oil emulsions with a cross flow membrane emulsification technique were described giving some flexibility in the implementation of this type of system in oil continuous food production.

Chapter 4

EFFECT OF MODE OF WATER ADDITION ON THE INTERACTIONS BETWEEN SUGAR PARTICLES DISPERSED IN LIPID

4.1. Introduction

Chocolate is a food enjoyed by many worldwide but its consumption is lower in warm, tropical regions and during warmer seasons in otherwise cool countries (Ogunwolu and Jayeola, 2006). This is due to softening of chocolate at higher temperatures leading to loss of snap and causing the product to stick to fingers and the wrapper. Companies and inventors have developed many different formulations for heat resistant chocolate products (Kempf and Downey, 1956; O'Rourke, 1959; Schubiger and Rostagno, 1965; Jeffery, Glynn and Khan 1977; Giddey and Dove, 1984, 1991; Kealey and Quan, 1992; Kincaid, 1992; Takemori, Tsurumi and Takagi, 1993; Beckett, 1995; Alander, Warnheim and Luhti, 1996; Mandralis and Weitzenecker, 1996; Jeyarani and Reddy, 1999; Traitler, Windhab and Wolf, 2000; Aaseng, 2005; Best et al., 2005; Davila and Finkel, 2005; D'Antonio, 2006; Ogunwolu and Jayeola, 2006; Simburger, 2009).

Proposed methods for making heat resistant chocolate are based on three functional mechanisms; addition of higher melting fats, increasing the viscosity of the continuous phase and the development of a sugar skeleton. The last named of these is perhaps the most promising method, is widely used in the patent literature and will be the focus of this work (Kempf and Downey, 1956; O'Rourke, 1959; Schubiger and Rostagno, 1965; Jeffery, Glynn and Khan 1977; Giddey and Dove, 1984, 1991; Kealey and Quan, 1992; Kincaid, 1992; Takemori, Tsurumi and Takagi, 1993; Beckett, 1995; Alander, Warnheim and Luhti, 1996; Mandralis and Weitzenecker, 1996; Traitler, Windhab and Wolf, 2000; Best et al., 2005; Davila and Finkel, 2005; Simburger, 2009).

The development of an internal sugar skeleton is accomplished through the addition of a small amount of water in some form to the system. Adding water results in increased adhesion

between sugar particles and the development of a network through the formation of water bridges due to capillary condensation. Water bridging has previously been demonstrated between particles in air (Moreyra and Peleg, 1981; Scoville and Peleg, 1981; Billings, Bronlund and Paterson, 2005) as well as particles in oil dispersions (Johansson and Bergenstahl 1992b,c; Claesson et al., 1997; Dedinaite et al., 1997; Dedinaite et al., 1998; Dedinaite and Campbell, 2000).

When liquid water is added directly to a chocolate product, it reacts immediately with the sugar particles causing the product to seize up quickly with a drastic increase in viscosity and results in a product with reduced snap (Norton et al., 2009). For these reasons, water that is added to chocolate in order to increase heat resistance is typically added in a controlled manner such as amorphous sugar or polyols (O'Rourke, 1959; Schubiger and Rostagno, 1965; Kincs, 1992; Beckett, 1995; Mandralis and Weitzenecker, 1996; Davila and Finkel, 2005), foams (Giddey and Dove, 1991) or water-in-oil emulsions/microemulsions (Schubiger and Rostagno, 1965; Jeffery, Glynn and Khan 1977; Giddey and Dove, 1984, 1991; Kealey and Quan, 1992; Takemori, Tsurumi and Takagi, 1993; Alander, Warnheim and Luhti, 1996; Traitler, Windhab and Wolf, 2000; Best et al., 2005; Simburger, 2009). The water must be in small amounts and in a stable enough form to allow complete mixing before reaction with the sugar particles. This would allow for the development of an even and comprehensive sugar skeleton. While some success has come from the methods stated previously, most of the workers still note drastic viscosity increases and the formation of large agglomerates which negatively impact the sensory attributes. Most methods also require additional equipment which is hard to integrate into existing chocolate production lines or changes to processing conditions. While water-in-oil emulsions have been shown to be a good form of controlled water addition to chocolate, further investigation must be done into their formation, stability and interactions with chocolate ingredients.

There is no standard, accepted test for melt resistance in chocolate (Dicolla, 2009). Desired characteristics of heat resistance chocolate include shape retention at elevated temperatures, not being soft, not being sticky to the touch or to the wrapper, not having increased likelihood of fat bloom and having no significant sensory differences (Kempf and Downey, 1956; Schubiger and Rostagno, 1965; Jeffery, Glynn and Khan 1977; Giddey and Dove, 1984, 1991; Kealey and Quan, 1992; Kincs, 1992; Takemori, Tsurumi and Takagi, 1993; Beckett, 1995;

Alander, Warnheim and Luhti, 1996; Mandralis and Weitzenecker, 1996; Best et al., 2005; Davila and Finkel, 2005; Simburger, 2009). Inventors of heat resistant products require shape retention at temperatures range from 35 to 65.55 °C in order to call their products heat resistant (Kempf and Downey, 1956; Schubiger and Rostagno, 1965; Jeffery, Glynn and Khan, 1977; Kincs, 1993; Takemori, Tsurumi and Takagi, 1993; Beckett, 1995; Alander, Warnheim and Luhti, 1996; Best et al., 2005; Davila and Finkel, 2005; Simburger, 2009). Measurement techniques used by these workers include observing behavior after heating in an oven, penetration testing, melting point measurements, viscosity measurements and sensory evaluation techniques, however there is no standard method used by all.

A technique described by Best et al. (2005) involves the measurement of shape retention upon heating. The width of chocolate bars was measured at ten evenly spaced points along their length. The bars were then placed into an oven at 40 °C for 1 hour after which they were dropped 18 inches onto a solid, flat laboratory bench. They were allowed to cool and the width was remeasured at the ten points. Changes in the measurements were used to calculate a parameter related to shape change. Another test performed on heat resistant chocolate is a method described by Schubiger and Rostagno (1965) used to reveal any sugar skeleton that may have developed within the sample. This procedure involves immersion in ether which removes the fat from a chocolate product. However these workers do not describe any quantification steps for the residual material.

To the best of our knowledge, no heat resistant chocolate products have been proposed using water-in-oil emulsions manufactured by cross flow membrane emulsification. Earlier I showed that emulsions with controlled droplet sizes and distributions can be produced by this method using the common chocolate emulsifiers soy lecithin and polyglycerol polyricinoleate (PGPR). The control, reproducibility, low operational cost and potentially feasible integration into existing chocolate production lines make this a promising method for the controlled addition of water to chocolate and the subsequent development of heat resistance.

The goal of the following experiment was twofold. The first was to investigate the interaction of sugar-in-oil dispersions and water-in-oil emulsions, particularly those produced by cross flow membrane emulsification. Microscopy was used to visualize the interactions and the effect of stable (2% PGPR stabilized) and unstable (2% lecithin stabilized) water-in-oil emulsions

on a chocolate model system was compared to a control of unemulsified water. The second goal was to develop and use quantitative techniques to demonstrate any resultant melt resistance. Real chocolate contains sugar and cocoa butter and can also contain cocoa solids non-fat and milk solids depending upon the type of chocolate. Rather than work directly with real chocolate, I used a model system of sugar dispersed in a non-polymorphic confectionery coating fat (CCF), which will be referred to hereafter as model chocolate. This was done for two reasons. Since it has been shown that the behavior of chocolate dispersions in the presence of water is controlled largely by the sugar rather than the cocoa particles, I used only sugar particles in my model system (Ziegler, Garbolino and Coupland, 2003). Additionally, a confectionery coating fat was used because while it has the same melting properties as cocoa butter, it does not crystallize into different polymorphic forms so there was no need for tempering.

I hypothesize that the water of water-in-oil emulsions forms water bridges between sugar particles as a result of capillary condensation, thus creating a sugar skeleton within the model chocolate and providing heat resistance. This would be seen when either PGPR or lecithin stabilized emulsions were added to the model chocolate system. I also hypothesize that the addition of a more stable emulsion (PGPR stabilized) would result in increased heat resistance compared to addition of an unstable emulsion (lecithin stabilized) or unemulsified water because it would be better able to disperse throughout the molten model chocolate before reacting with the sugar, leading to the formation of a more comprehensive skeleton.

4.2 Materials and Methods

4.2.1 Materials

Soybean oil (100% pure, food grade) was obtained from a local supercenter and used without further purification. Grindsted® PGPR and Carrageenan CL220 were donated by Danisco (Brabrand, Denmark) and Yelkin® TS soy lecithin was donated by ADM (Decatur, IL). The phospholipid composition of the lecithin (acetone-insoluble matter: 62.0%) specified by the manufacturer was 15% PC, 13% PE, 9% PI and 4% PA. Both emulsifiers were used without further purification. Deionized water was used in all experiments. Bakers Special fine granulated sugar was donated by Domino Specialty Ingredients (West Palm Beach, FL) and was used in confocal microscopy studies. Pure powdered sugar was obtained from a local grocery store for

use in chocolate model making. Both sugars were stored in vacuum desiccators until use. Confectionery coating fat (CCF, palm kernel oil, hydrogenated palm oil and citric acid as a preservative) was donated by Cargill Dressings, Sauces and Oil (Charlotte, NC). The hexanes used was ACS Grade supplied by VWR International, LLC (Radnor, PA).

Chocolate model molds were purchased from Kerekes Bakery and Restaurant Equipment (BakeDeco.com, Brooklyn, NY). Molds were made of clear polycarbonate plastic and consisted of 36 cylindrical cavities 3/16 inches in diameter and 1-1/8 inches in height. Polystyrene petri dishes used in melting work (100 x 15 mm) were from VWR International, LLC (Radnor, PA). Materials used in hexane immersion studies included Bright Aluminum Screening (New York Wire, Mt. Wolf, PA), which was purchased from a local hardware store, and 24 gauge beading wire (Horizon Group USA, Inc., Warren, NJ), which was purchased from a local supercenter.

4.2.2 Methods

4.2.2.1 Emulsions

Optical Microscopy. A lab-scale cross flow membrane emulsification system was supplied by Micropore Technologies, Inc. (Loughborough, UK) and used to make the emulsions used in optical microscopy. A 3% solution of PGPR in soybean oil was obtained (11.4 and 368.6 g respectively). Deionized water was injected through a membrane with 40 μm wide slotted pores (External diameter- 15 μm ; Length – 106 μm ; Slotted pores – 40 x 450 μm) to result in a 3.85% (w/w) water-in-oil emulsion. The system consisted of a large peristaltic pump to circulate the continuous phase which was set at 150 rpm (flow rate of 95.3 g/sec) and a small peristaltic pump for the dispersed phase which was set at 4 (flow rate of 0.6 g/sec).

Confocal Microscopy. A water-in-oil emulsion was prepared by adding water dropwise to stirred oil. An Erlenmeyer flask was filled with a 3% solution of PGPR in soybean oil (1.425 g and 46.075 g respectively). A stir bar was added and the solution was stirred without heating. Deionized water (2.5 g) spiked with fluorescein isothiocyanate (FITC) was dripped into the stirring solution using a pipet over a period of 1.5 minutes. Once the water was added, the emulsion was stirred for a further 5 minutes.

Model Chocolate Making. The cross flow membrane emulsification system was used to produce all emulsions for use in model chocolate making. A 2% solution of emulsifier (either PGPR or lecithin) and soybean oil was obtained (8 g and 392 g respectively). Deionized water was injected through the same membrane used previously to result in a 30% (w/w) water-in-oil emulsion. The large and small peristaltic pump settings were also the same as those used previously.

4.2.2.2 Optical Microscopy

Optical microscopy was performed using an Olympus BX41 microscope (Hitech Instruments, Inc., Edgemont, PA) equipped with SPOT Advanced Version 4.0.9 imaging software (Diagnostic Instruments, Inc). Fine granulated sugar crystals were placed on a microscope slide in a single layer. A drop of soybean oil was added to the sugar and covered with a cover slip. The slide was placed under the microscope and a drop of emulsion was applied next to the cover slip. The cover slip was gently agitated six times back and forth and images were captured.

4.2.2.3 Confocal Microscopy

Fluorescence imaging was performed with a Olympus FV1000 Laser Scanning Confocal Microscope (Olympus America Inc., Melville, NY) equipped with FV10-ASW version 2.1 software. A Blue Argon (488 nm) laser was used and the same procedure was used as for optical microscopy with the exception of using the fluorescein containing emulsion. All confocal microscopy was done at the Cytometry Facility at the Huck Institutes of the Life Sciences, Penn State University. This facility is funded, in part, under a grant with the Pennsylvania Department of Health using Tobacco Settlement Funds. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

4.2.2.4 Model Chocolate Making

CCF (60 g) was weighed into a 250 mL beaker and placed in a forced convection oven. Powdered sugar (60 g) was added to the CCF once it reached 70° C. The sample was mixed with a metal spatula 50 times clockwise followed by 50 times counterclockwise. This was repeated twice for a total of 200 turns and the beaker was returned to the oven. Once the sample reached

68° C, 2 g of an additional liquid was added. This additional liquid was either A) 2 g soybean oil, B) 0.6 g of water and 1.4 g soybean oil, C) 2 g of a 30% water-in-oil emulsion stabilized with 2% PGPR in the continuous phase or D) 2 g of a 30% water-in-oil emulsion stabilized with 2% lecithin in the continuous phase. In all cases where water was added, the amount used corresponded to 1% of the mass of sugar. The emulsions used were produced by the cross flow membrane emulsification system as described in Section 4.3.2.1. The mixture was stirred 50 times clockwise and 50 times counterclockwise and immediately poured into the 10 cylinders of a chocolate mold. The mold was tapped gently against the table to remove air bubbles. Samples were allowed to harden at room temperature for 3 hours. Molds were then placed in a freezer at -22 °C for 20 minutes and then tapped upside-down on a counter until they demolded. Samples were allowed to equilibrate to room temperature for 2 hours before testing. All samples were prepared in triplicate.

4.2.2.5 Hexane Immersion

Fat was extracted from the molded samples by immersing them in hexane. The hexane dissolved the fat leaving the sugar from the samples. Small pieces of sugar would fall through the supporting mesh but cross-linked sugar was retained and could be weighed.

Squares of aluminum screening were cut to approximately 5.75 x 5.75 cm to create a stage for the chocolate. Approximately 1 cm of each corner was folded upwards. Beading wire was attached to each corner of the stage. A sample stage in an empty beaker is shown in Figure 4.1.

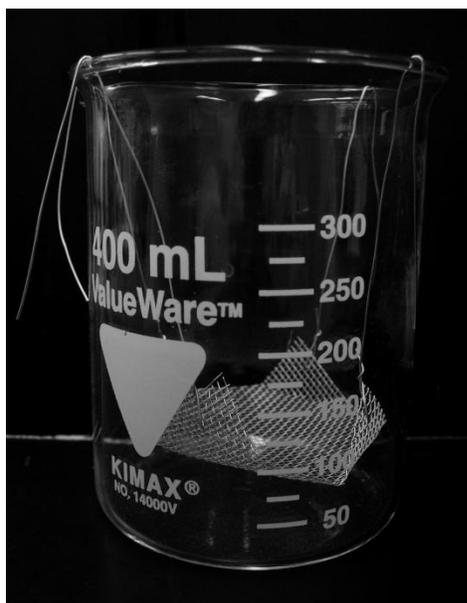


Figure 4.1. Sample empty wire mesh stage for hexane immersion in an empty beaker.

The mass of the stage and wires was recorded. A sample was placed in the center of the stage and the total mass was recorded. A 400 mL beaker was placed in a fume hood and filled to 375 mL with hexane. The stage containing the model chocolate was lowered slowly into the hexane until the bottom of the stage was at the 75 mL level and the wire was bent over the top of the beaker to secure the stage. The beaker was covered and allowed to sit in the fume hood for 72 hours and room temperature. After this period, the stage was gently lifted out of the hexane, tapped three times against the side of the beaker and placed in an empty beaker to dry for 24 hours. The final mass of the sample was then recorded. Three samples from each model chocolate batch were tested for a total of 9 samples per added liquid type.

4.2.2.6 Meltability

Meltability was determined using a test based on Arnott and modified Schrieber tests traditionally used with cheeses (Arnott, Morris and Combs, 1957; Muthukumarappan, Wang and Gunasekaran, 1999). In this method the changes in dimensions of a sample of molded sample was measured before and after heating to a temperature such that the solid fat melted then cooled back to room temperature.

A sample was placed in the middle of an open petri dish and a digital caliper was used to measure the height at four points equally spaced around the perimeter. The height of the petri dish was also recorded. Three dishes of the each model chocolate batch were placed in the center of a forced convection oven set at 50 °C for 20 minutes. Dishes were removed from the oven and allowed to cool at room temperature for 24 hours. The height of the sample was again measured at four evenly spaced points around the perimeter.

Top view photographs of samples next to a ruler were taken and the spread of each sample was determined via image analysis in Adobe Photoshop CS4. The measurement scale for each photograph was set using the photographed ruler. The Quick Selection Tool was used to highlight the sample spread area and the measurement of the area was recorded.

4.2.2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc.; La Jolla, CA) for analysis of variance (ANOVA) and Tukey's post-test with significance at $\alpha = 0.05$.

4.3 Results and Discussion

4.3.1 Interaction of Sugar Particles in Oil in the Presence of Water

The interactions of water-in-oil emulsions with sugar particles dispersed in oil were visualized using both optical and confocal microscopy. Figure 4.2A shows an optical microscope image of sugar particles in soybean oil and Figure 4.2B shows similar sugar particles in soybean oil in the presence of an emulsion that was produced by cross flow membrane emulsification. The concave shape indicative of capillary condensation can be seen in the corners between the two sugar particles in Figure 4.2B causing the particles to stick together. This was not observed in images without the presence of water.

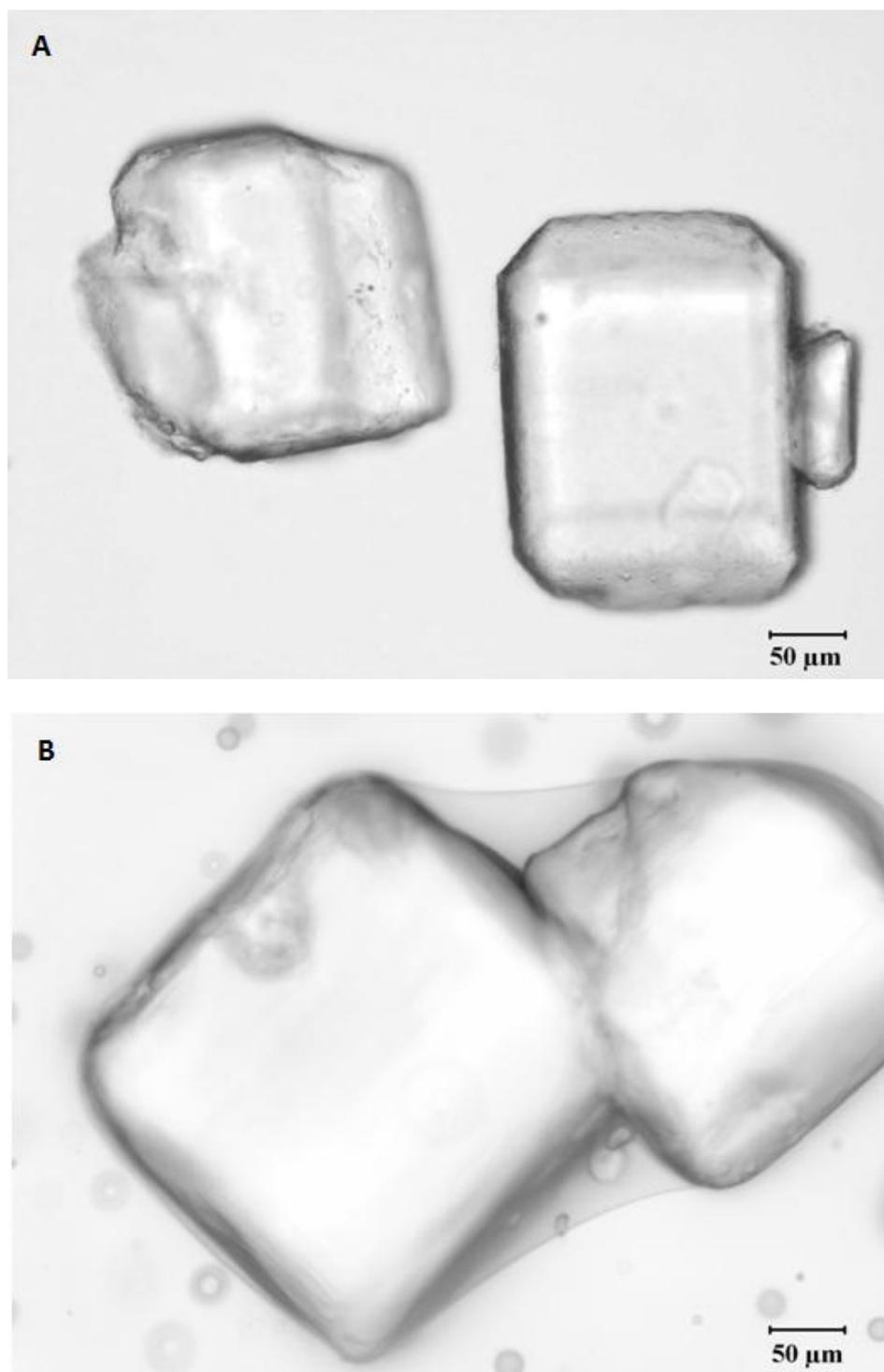


Figure 4.2. A) Optical microscope image of fine granulated sugar crystals in soybean oil and B) in the presence of a 3.85% w/o emulsion stabilized with 3% PGPR and produced by cross flow membrane emulsification. Scale bars equal 50 μm.

Fluorescence microscopy was used to confirm that the concave region seen in Figure 4.2B was in fact due to moisture adsorption, fluorescence microscopy was utilized. A 5% water-in-oil emulsion with 3% PGPR was prepared using an aqueous phase spiked with fluorescein. Cross flow membrane emulsification was not used in this case as the volumes required by this method were too large. Instead, the fluorescein-spiked emulsion was produced by adding water dropwise to stirred oil.

In the fluorescence microscopy images, green fluorescing water was seen in water droplets, surrounding the sugar particles and particularly between adjacent particles (Figure 4.3). This image supports a model of the water droplets destabilizing in the presence of sugar crystals. The de-emulsified water adsorbs at the sugar surfaces where it accumulates in capillaries between crystals to minimize its surface curvature and therefore provides an attractive force between the crystals. Simultaneously some sugar can be expected to dissolve in the water (not visualized in this work) although this will only have a secondary affect on the strength of the bond formed provided the amount of water remains relatively low. In the chocolate model systems described in this study, the amount of water added to the system was 1% with respect to the mass of the sugar. At this level, a relatively small amount of the sugar, approximately 0.8%, is expected to dissolve. This will hardly change the crystal size and therefore not significantly affect the strength of the capillary force between crystals via changes in particle size (Equation 1.7). Dissolution of sucrose, however, does result in a modest increase in surface tension (~2% for 100% sucrose at 20 °C, Docoslis, Giese and van Oss, 2000) which is another factor in the calculation of capillary force. The result would be a proportionally increased capillary force. Additionally, it is possible that the saturated sugar syrup between crystals increases their cohesion because of the increased viscosity of the liquid. Finally, if the system is dried, the sugar syrup will either form a glass or recrystallize, physically linking the crystals into a sintered block. These “solid bridges” formed between particles are up to three times stronger than the liquid bridges and provide yet another force to hold the particles together (Billings, Bronlund and Paterson, 2005). Overall, these observations and the resultant forces provide a mechanism for sugar skeleton formation when water-in-oil emulsions are added to a chocolate model system.

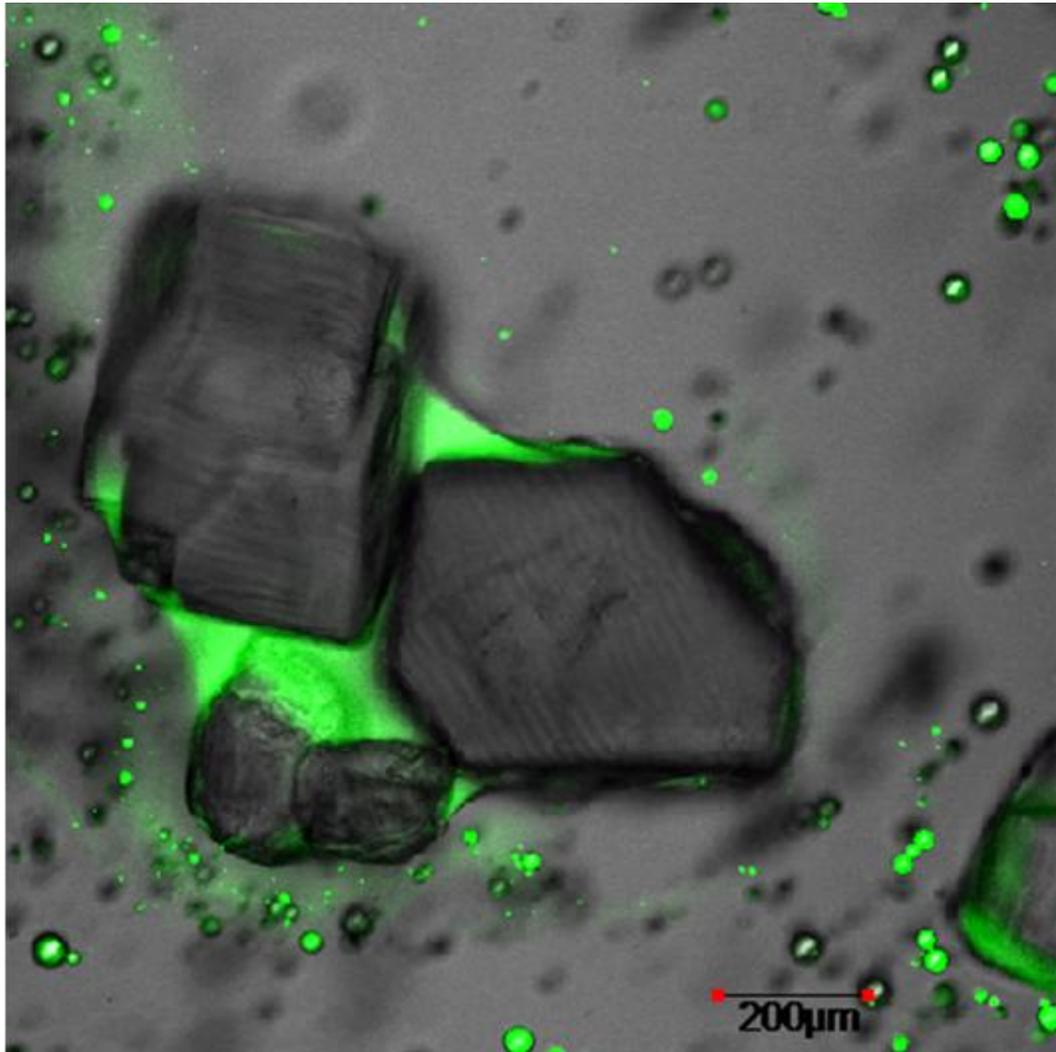


Figure 4.3. Confocal microscope image of fine granulated sugar in soybean oil in the presence of a 5% w/o emulsion stabilized with 3% PGPR in the continuous oil phase. Dispersed water phase was spiked with fluorescein and appears green in the image. Scale bar equals 200 μm .

4.3.2 Chocolate Model Systems in the Presence of Water

Model chocolate was successfully made using the procedure described in Section 4.2.2.4. Water was added to 50% powdered sugar in CCF mixtures in different forms (i.e., directly as liquid water, via a stable emulsion or via an unstable emulsion) and the effect on sugar skeleton formation was determined. When water in any form was added to the molten model chocolate, the samples became more viscous and harder to pour into molds. Some small sugar aggregates

were apparent and more air bubbles developed that needed to be removed upon molding. This observation is consistent with sugar aggregates forming very quickly after water addition. After demolding however, the samples were visually indistinguishable. Viscosity characterization of a similar system is shown in Appendix A.

4.3.2.1 Hexane Immersion

Model chocolate samples were subjected to fat extraction via hexane immersion in order to examine the remaining sugar skeleton. The proportion of the residual mass that did not pass through a wire mesh was used as a measure of skeleton formation. Figure 4.4 shows the average percent mass remaining from the four model chocolate types produced in this study. If no sugar fell through the mesh, the percent remaining would equal 49.2%, the total percent of sugar in the sample. This is indicated in the figure by the dotted line.

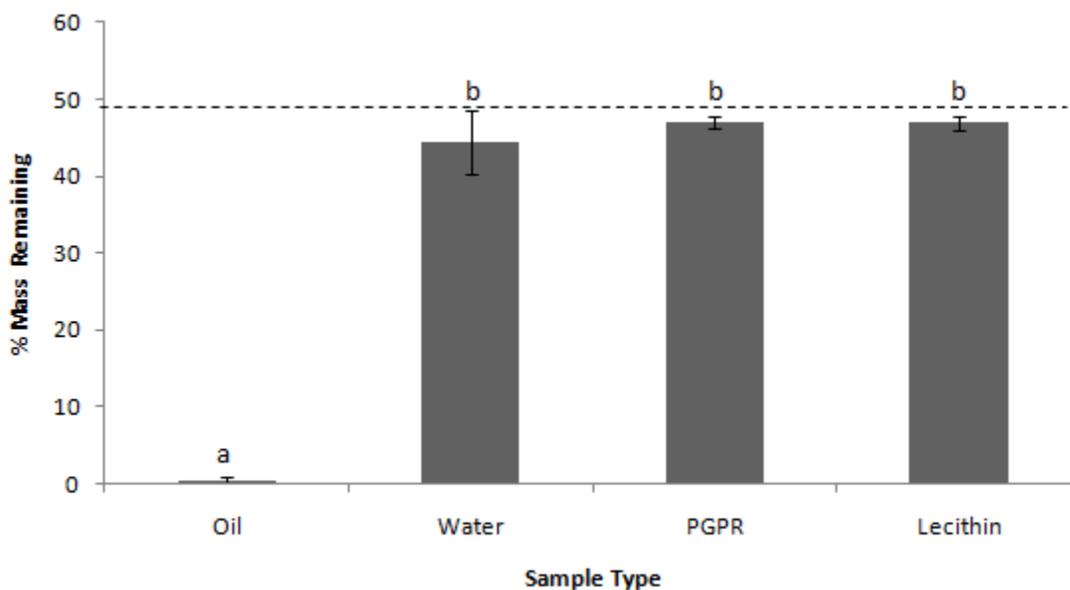


Figure 4.4. Percent mass of model chocolate remaining after hexane immersion for 3 days. Sample type indicates the type of liquid added to the sample; Oil – 2g soybean oil; Water – 0.6 g water, 1.4 g soybean oil; PGPR – 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; Lecithin – 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$. Dotted line indicates the percent sugar in the original sample.

The results show no statistically significant difference in percent mass remaining between sample made with the addition of water in any form, either directly or as an emulsion. The percent mass remaining of these samples ranged from 44 to 47% indicating almost all of the sugar was bound in a continuous skeleton and did not pass through the mesh. On the other hand, the model chocolate with only oil added showed significant loss of mass following solvent extraction with an average of only 0.36% sugar remaining. This average was significantly different ($\alpha = 0.05$) from the other samples.

Examples of the model chocolate samples after hexane immersion are shown in Figure 4.5. Visually, the sugar skeletons from the three model chocolate types with water added in some form appeared very similar. The sugar skeletons had heights and widths similar to those of the sample before fat extraction but appeared pock-marked where sugar aggregates had fallen from the surface. The model chocolate with only oil added appeared drastically different with only a few small sugar aggregates present on the mesh that were too large to fall through.

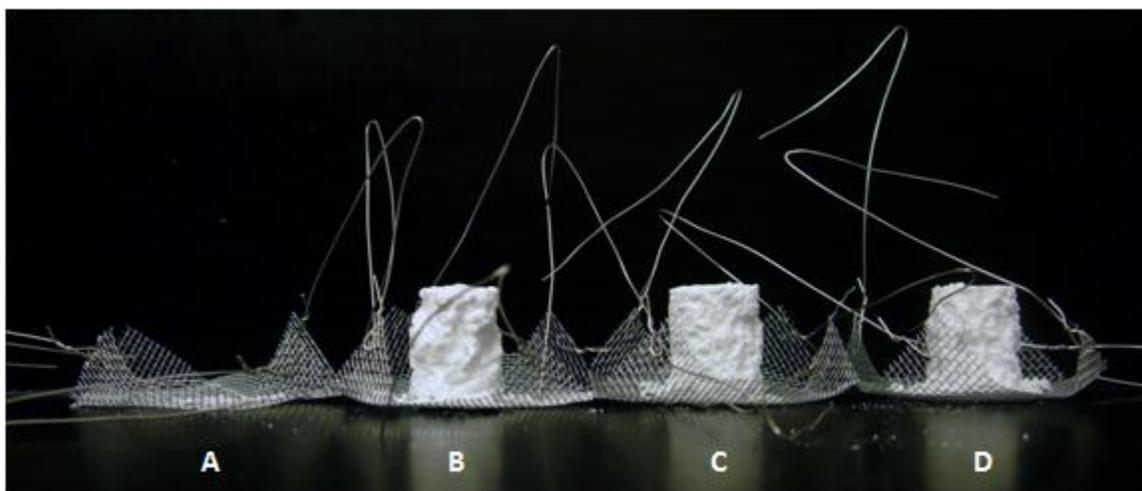


Figure 4.5. Photograph of model chocolate samples after fat extraction via hexane immersion. Samples made with different liquid added A) 2g soybean oil; B) 0.6 g water, 1.4 g soybean oil; C) 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; or D) 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification.

The results of the hexane immersion study indicate that substantial sugar skeletons formed when water of some type was added to the system. This provides support for possible heat resistance of these samples, which is investigated further below. I had hypothesized that water added as an emulsion would result in a larger percent mass remaining when compared to water added directly. I had also hypothesized that the addition of a stable emulsion (i.e., PGPR-stabilized) would result in a larger percent mass remaining than the addition of an unstable emulsion (i.e., lecithin-stabilized). The theory behind these hypotheses was that water added as an emulsion would be able to disperse throughout the model chocolate better before adsorbing to sugar particles resulting in a more uniform skeleton and that the difference would be more apparent for a more stable emulsion. These hypotheses were not supported by the data in this study.

One possible reason for this discrepancy is that the method of incorporation was too vigorous. The added liquid was stirred into the model chocolate by hand 50 times clockwise and 50 times counterclockwise. It is possible that this allowed for an even incorporation of the directly added water or caused significant breakdown of the emulsions.

A second possible reason is that the emulsions, regardless of stability on their own, became highly unstable when added to such a highly concentrated sugar dispersion. If this were the case, the same results would be seen even if the emulsions were incorporated in a different manner. Thus to elucidate the cause of the similar sugar skeleton results for the samples with added water different methods of incorporation should be studied.

Finally, the empirical test selected may not have been sufficiently sensitive to the differences between water-containing samples. A slightly more aggressive treatment of the fat-free sugar skeletons might have disrupted weaker structures and revealed differences not seen in the method used. It should also be stressed that the presence of a sugar skeleton that is not in itself proof that the skeleton is able to confer useful heat resistance.

4.3.2.2 Meltability

To test heat resistance, model chocolate samples were placed in an oven at 50 °C for 20 minutes and the percent height difference and area of spread were determined. The theory of this

method is that the sugar skeleton of a heat resistant chocolate produced by the addition of water provides solid-like structure to the chocolate that prevents any change in shape even when the fat has melted. Therefore, a model chocolate with increased heat resistance should display a small percent height difference and a small spread area.

The images in Figure 4.6 and 4.7 show differences in both height and spread area after heat exposure respectively for representatives of each type of model chocolate. The control samples with only oil added melted down to a small round, smooth mound with a spread that was typically to one side of the dish. This indicates that the sample fell sideways during heating instead of collapsing down on itself. The samples with water added directly also appeared to have fallen to one side. These samples were not smooth like the oil only samples but instead appeared clumpy with protruding chunks of sugar. The samples with emulsions added did not fall sideways. They appeared similar to unheated samples with some sagging evident at the base of most samples where the weight of the sample caused some expansion.

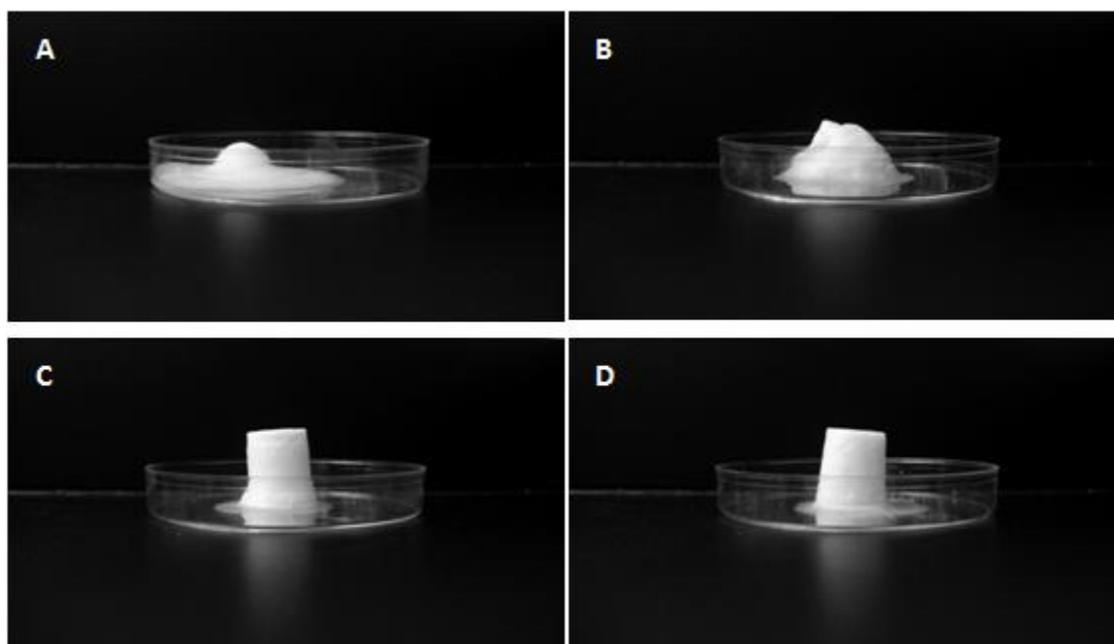


Figure 4.6. Photograph of side view of model chocolate samples after heating in oven at 50 °C for 20 minutes. Samples made with different liquid added A) 2g soybean oil; B) 0.6 g water, 1.4 g soybean oil; C) 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; or D) 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification.

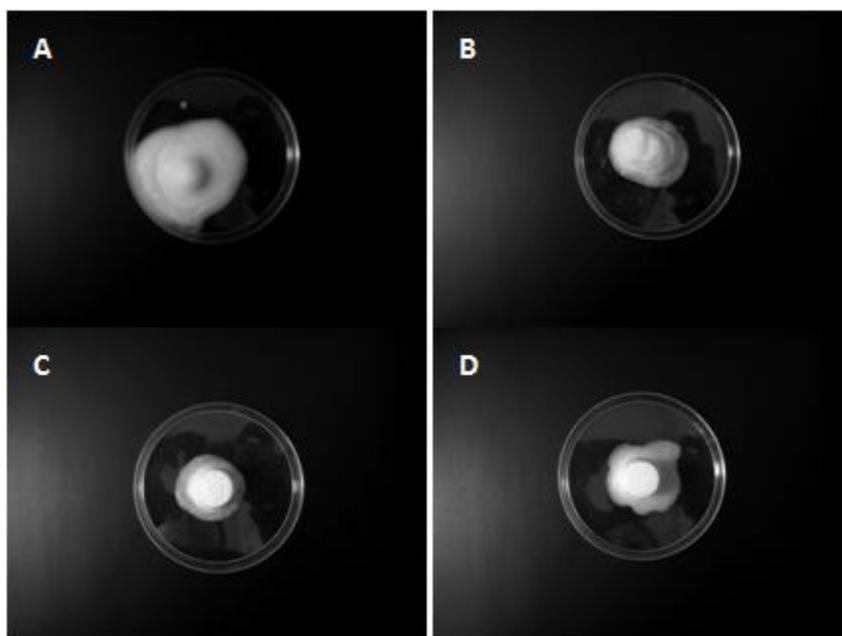


Figure 4.7. Photograph of top view of model chocolate samples after heating in oven at 50 °C for 20 minutes. Samples made with different liquid added A) 2g soybean oil; B) 0.6 g water, 1.4 g soybean oil; C) 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; or D) 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification.

The average percent height difference and average spread area for the four model chocolate types are shown in Figure 4.8 and Figure 4.9 respectively. The samples with only oil added resulted in the largest percent height difference than the other three types of chocolate at approximately 60%, a value significantly different from all others. The directly added water sample had the second highest percent difference in height of approximately 40%. This value was significantly different from the samples with water added via emulsion. The samples with added emulsion stabilized by PGPR and lecithin resulted in percent height differences of 11.8 and 11.5% respectively, values which were not significantly different from each other.

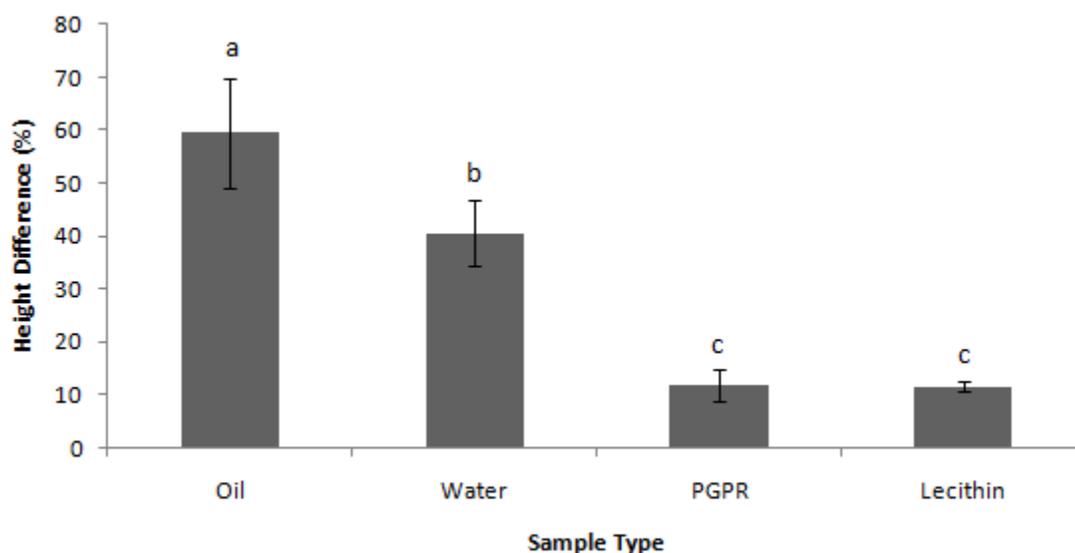


Figure 4.8. Percent height difference of samples after heating in oven at 50 °C for 20 minutes. Sample type indicates the type of liquid added to the sample; Oil – 2g soybean oil; Water – 0.6 g water, 1.4 g soybean oil; PGPR – 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; Lecithin – 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$.

In terms of the area of the spread seen in Figure 4.7, the oil only sample had the largest value at approximately 26 cm². Again this value was significantly different from all other samples. The direct water and lecithin-stabilized emulsion samples had the second and third highest spread areas at approximately 15 and 14 cm² respectively. While these values were significantly different from that of the PGPR-stabilized emulsion samples, they were not significantly different from each other. The PGPR-stabilized emulsion samples had the smallest spread area at approximately 11 cm².

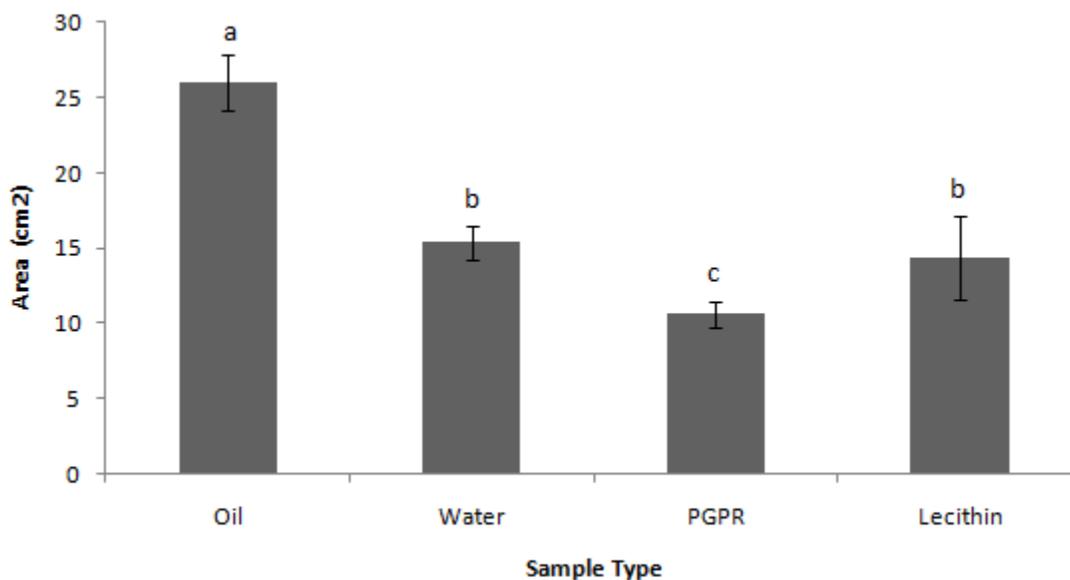


Figure 4.9. Area of sample spread after heating in oven at 50 °C for 20 minutes. . Sample type indicates the type of liquid added to the sample; Oil – 2g soybean oil; Water – 0.6 g water, 1.4 g soybean oil; PGPR – 2g of 30% water-in-soybean oil emulsion stabilized with 2% PGPR; Lecithin – 2g of 30% water-in-soybean oil emulsion stabilized with 2% lecithin. Emulsions produced via cross flow membrane emulsification. Error bars indicate standard deviation and letters indicate significant difference at $p = 0.05$.

In contrast to the hexane immersion work, the melt test displayed significant differences between samples with water added directly versus samples with water added via an emulsion. This was seen especially in the height difference of the samples. Even though a similar sugar skeleton seemed to be formed as seen via hexane immersion (Figures 4.4 and 4.5), the model chocolate with water added directly did not hold up to heat as well as the model chocolates with added emulsions. This provides evidence for the production of a more uniform and comprehensive skeleton when emulsions were used to deliver the water. Evenly distributing the water through the model chocolate through use of an emulsion may have resulted in a more complete sugar particle network that was stronger as a result. This increased strength may have enabled the model chocolates with added emulsion to retain much of their height upon heating whereas the model chocolates with water added directly sagged and often fell over sideways. The additional strength was important for height retention because of the increased force on the skeleton due to the gravitational force of the melted CCF. In comparison, in the hexane immersion work the CCF was dissolved away from the skeleton and there were no additional

gravitational forces. Thus the sugar skeletons of samples with water added directly were able to maintain structure when the CCF was extracted but not when it was melted.

The type of emulsion used to deliver the water also had an effect on the spread area. The model chocolate made with a stable PGPR-stabilized emulsion was able to contain the melted fat better than the model chocolate made with a less stable lecithin-stabilized emulsion, resulting in a smaller area. In fact, the spread in the samples prepared with lecithin-stabilized emulsion was not significantly different than the spread in the samples with water added directly. Low spread is consistent with an effective, extensive and strong sugar particle network. The more stable emulsion was able to distribute more evenly within the melted model chocolate before reaction with sugar particles.

For all of these measurements there could be a secondary effect due to the adsorption of surfactants in the emulsions on the surfaces of sugar crystals. Lecithin has been shown to adsorb to sugar particles and decrease the adhesive forces between them, resulting in significant differences in rheology, sedimentation behavior and aggregate size in sugar in oil dispersions (Johansson and Bergenstahl, 1992a,b,c; Ziegler, Garbolino and Coupland, 2003). Lecithin may also adsorb to the water covering sugar particles, again decreasing the adhesive forces. Decreased adhesive forces could result in a weaker sugar skeleton. PGPR on the other hand, was shown to have little effect on sedimentation behavior or rheology and only significantly affected aggregate size at concentrations of at least 0.1% (Ziegler, Garbolino and Coupland, 2003). The concentrations of lecithin investigated by these workers was higher than the concentration of lecithin in this study so it is therefore unclear whether this low concentration of lecithin had a significant effect on the formation of a sugar skeleton that contributed to the differences seen in the spread area of model chocolates with different types of emulsions added.

4.4. Conclusions

The first aim of this study was to examine the effect of water-in-oil emulsions on sugar-in-oil dispersions, particularly in the context of emulsions made by cross flow membrane emulsification and a chocolate model system. Images from both optical and fluorescence microscopy showed that the water from water-in-oil emulsions adsorbs on and between sugar particles via capillary condensation. This supports the proposed mechanism behind the formation

of a sugar skeleton in chocolate with added emulsions. In order for this mechanism to work effectively it is first necessary for the water to be intimately mixed with the sugar-in-oil dispersion prior to adsorption. Emulsions are able to facilitate this even distribution of water throughout a chocolate model system, after which it adsorbs on and between sugar particles resulting in the formation of water bridges. These water bridges provide the strong adhesive forces necessary for a sugar skeleton and the development of heat resistance.

An additional aim of this study was to develop quantitative methods to detect and analyze heat resistance in model chocolate. The first test took an existing method of visualizing the sugar skeleton of heat resistant chocolate and modified it in order to better measure the comprehensiveness and quality of the skeleton. This test showed that it could accurately detect the existence of a sugar edifice, although there was no significant difference in the percent sugar remaining between model chocolates made with water added directly or as an emulsion. This was perhaps due to the empirical nature of the test and altering the pore size of the mesh or the aggressiveness of the shaking technique after removal from hexane could alter the results.

Additionally, the presence of a sugar skeleton was not in itself an indication of the level of heat resistance. Heat resistance was demonstrated via a melt test. The melt test took aspects of existing heat resistance testing on chocolate and combined it with tests typically used to test melting behavior of cheeses. By measuring the height difference and area of spread, the melt test was able to show the ability of the sugar skeleton to hold up against heat and to contain the melted fat. This test showed significant differences between model chocolate with water added directly and model chocolates with water added via an emulsion in terms of height difference. It also showed significant differences between model chocolates with a stable versus unstable emulsion added in the area of spread measurement, with samples with a stable emulsion able to contain more of the melted fat. It should be noted that because model chocolate was used in this study, these results have limited applicability to the quality of real chocolate. This study also did not measure chocolate stickiness upon heating which is another attribute of concern in heat resistant chocolate.

This experiment showed that model chocolate with increased heat resistance can be made by the addition of water-in-oil emulsions produced by cross flow membrane emulsification. The model chocolate can be characterized in terms of the sugar skeleton and behavior under heated

conditions. While these tests show the increased heat resistance of the products made in this experiment, this heat resistance cannot be accurately compared to that of other products unless the same tests are performed on those products. These test methods do, however, help to provide a standardized method of testing heat resistance that could be used in the future.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The overall goal of this work was to determine if water-in-oil emulsions produced via a lab-scale cross flow membrane emulsification system are suitable for use in the production of heat resistant model chocolate products. In order to accomplish this goal, 30% water-in-oil emulsions were produced with different ingredients in the dispersed or continuous phases as well as under different processing conditions. The droplet size distributions of the resultant emulsions were examined both initially and over time. The results from this investigation were used to produce stable (2% PGPR-stabilized) and unstable (2% lecithin-stabilized) emulsions which were then added to dispersions of sugar crystals in oil which were then cooled to crystallize the fat and form a model chocolate. Samples prepared in this manner were compared to samples made with the direct addition of unemulsified water in order to determine the impact of different modes of water addition on the formation of a sugar skeleton and melt resistant in the product.

Results from the two objectives that were defined relating to this goal as well as their impacts and outstanding questions are summarized below.

The first objective (Chapter 3):

The first objective of this study was to determine the effects of changing ingredients and operational parameters on water-in-oil emulsions produced by cross flow membrane emulsification. Average droplet size and sigma value were shown to decrease in emulsions with increasing concentrations of the emulsifiers PGPR, lecithin or a 50:50 PGPR:lecithin blend. PGPR-stabilized emulsions were stable over time with consistent droplet sizes and distributions for both short and long term measurements. Lecithin-stabilized emulsions doubled in droplet size over a six hour period and blend-stabilized emulsions were completely unstable within three hours. Emulsion droplet size and distribution were also significantly affected by the use of different membranes, different continuous phase flow rates and different dispersed phase flow rates. The addition of a gelling agent to the dispersed phase prior to emulsification made it more difficult to produce stable emulsions.

The results of this study could be used for industrial application of similar cross flow emulsification systems. The general trends can be extrapolated to scaled-up systems and can be used to tailor an emulsion for a specific application. One such application is to chocolate, an oil-continuous system, and this application will be examined in the discussion of the second goal of this work. One outstanding question from this part of the work has to do with the addition of a gelling agent to the dispersed phase. I had hypothesized that this would increase the stability of emulsions because the gelling agent would protect the droplets from coalescence. While the opposite behavior was observed in this study, it is possible that the method of emulsion production or the choice of gelling agent may have influenced this result. It would therefore be of interest to further examine effects of the addition of a gelling agent to the dispersed phase under different conditions.

In this study, pfg-NMR was used for the measurement of droplet diameter and sigma (the standard deviation of the log-normal distribution, i.e., a measure of the width of the distribution). This technique had many advantages as emulsion samples were able to be measured immediately after production without any sample preparation or dilution. However, there were a few negatives associated with using this technique for this project. Firstly, the droplet sizing application requires 10 to 30 minutes to make accurate measurements. Droplet size may change significantly during this time period, especially in the case of unstable emulsions. This also made measurement in duplication difficult, as the second sample was measured up to 30 minutes after the first. Thus the droplet sizes reported in this work may not be an accurate reflection of the droplet initially produced from the membrane. Additionally, the maximum droplet size that was able to be measured by the pfg-NMR was 100 μm . Beyond this point the application cannot distinguish the effects of restricted diffusion in such large droplets. A few of the emulsions produced in this study furnished measurements of 100 μm and could therefore not be included in calculations of the average droplet diameter or sigma value. For these reasons, pfg-NMR may be better suited for use with stable emulsions consisting of small droplets. It is likely, however, that the downsides did not significantly affect the overall conclusions in this study.

The second objective (Chapter 4):

The second objective was to investigate the interactions between water-in-oil emulsions and sugar-in-oil dispersions. Optical and fluorescence microscopy of sugar-in-oil dispersions with added emulsions showed the formation of water bridges between sugar particles due to

capillary condensation, confirming the mechanism leading to formation of a sugar skeleton. In order to develop a sufficient sugar skeleton for heat resistance it is necessary for the water droplets to be intimately mixed within the sugar dispersion before reaction with sugar. Water-in-oil emulsions have the potential for such controlled delivery of water and were therefore added to a chocolate model system in order to investigate the effect on the formation of a sugar skeleton as well as determine any resultant heat resistance. A method was developed for making model chocolate and samples with water added via stable or unstable emulsions were compared to samples with water added directly and control samples prepared without added water. The different formulations used in this study were chosen so that the amount of emulsifier (0.0328%) was well below the values restricted by law (1 to 1.5% total). This means that additional emulsifier could be added to the molten model chocolate for traditional purposes. The oil added to the system was also not at a level that should cause problems in traditional chocolate manufacture, however the use of other fats could be investigated as well.

Two quantitative tests were developed in order to aid in this investigation. The first test involved immersion in hexane to extract fat from model chocolate samples which were placed on wire mesh stages. Any sugar not incorporated in the sugar skeleton would fall through the holes in the mesh. After hexane immersion, all samples with water added showed considerable sugar skeletons (44 to 47% out of the 49.2% total sucrose in the original sample) with no significant difference in the weight of the skeletons between treatments whereas the control model chocolate did not have any skeleton. This procedure is, however, fairly empirical in nature with results dependent upon the size of the mesh and how vigorously it is shaken upon removal from hexane. Additionally, the height of the sugar skeleton in the hexane immersion study could not be accurately measured with this method because the mesh stand was not flat when placed on a table top. This excluded accurate measurement by a ruler and the skeletons were too delicate to be measured by calipers. In the future, similar mesh stages could be constructed with the mesh pulled flat across the opening of a short, open cylinder such as a section of PVC piping. This would still allow for exposure of hexane to all model chocolate surfaces and the ability for sugar to fall through the mesh, but would also enable the height of the sample to be accurately recorded. Regardless, a similar procedure could be instituted as common practice for the testing of other heat resistant chocolates in which heat resistance is conferred through a sugar skeleton. This would provide the industry with a method of comparison between products as long as the aforementioned variables were kept constant.

The second test involved heating the model chocolate samples in an oven at 50 °C for 20 minutes. The change in height and the area of spread were determined as measurements of product deformation. Samples with water added via emulsions did not decrease in height as much as samples with water added directly or the control samples. The area of spread showed a difference between samples with stable or unstable emulsions added, with samples with stable emulsions added able to contain more of the melted fat. This test is also somewhat empirical in nature in terms of the times and temperatures that were selected. Different times and temperatures could be investigated to examine the degree of heat resistance or when differences between products become significant. Similar to the hexane immersion method, this method could provide a way to compare different heat resistant chocolate products if standard conditions were chosen. Having such defined tests for these products would be invaluable for product developers so that the characteristics of different formulations could be fully understood and the heat resistance of chocolate products could be optimized.

While the different formulations in this study resulted in products with different characteristics, the method used shows only one way of incorporating emulsions into a model chocolate system. Other methods of incorporation should be investigated in order to optimize the ability of emulsions to evenly distribute water to a sugar-in-oil dispersion. Suggested methods include stirring the emulsion into the model chocolate less vigorously and adding the emulsion in smaller portions over time. It would also be helpful to investigate the characteristic timescale of droplet reaction with sugar particles as well as the timescale of mixing. A second approach would be to make the water droplets less reactive with sugar particles. Approaches to this might include alternative surfactant systems or, more promisingly, using partially-dried gels as the dispersed phase rather than free water. Additionally, because only a model system was used in this study, it would be important to see how the results from this study are affected by the addition of other ingredients commonly used in chocolate products, particularly cocoa particles and milk solids. The use of cocoa butter in place of CCF could also alter the results, as a particular polymorphic form is desired in chocolate products.

The work pertaining to this second goal has important impacts for the chocolate and confectionery industries. The results show a promising application of water-in-oil emulsions produced via cross flow membrane emulsification to chocolate products for the development of

heat resistance. Such products include solid, molded chocolates as well as chocolate or confectionery coatings. This would expand the sale of chocolate to warmer regions of the world and allow for more people to be able to consume chocolate worldwide. It may also be possible to use this emulsification technique in reduced calorie applications if the aqueous phase is altered such that it does not react with the sugar particles.

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APPENDIX A

The rheology of 30% sugar-in-oil dispersions was investigated with a Haake Fisons RV20 Rotovisco Rheometer (Karlsruhe, Germany). Bakers Special fine granulated sugar (3.5g; Domino Specialty Ingredients; West Palm Beach, FL) was added to soybean oil (8.169g) in a standard rheometer cup and stirred. A cylindrical bob with a spiral groove was used. Initial shear stress was measured at an instrumental setting of 9D. Measurements were taken every 15 seconds for 2 minutes. The cup was then removed and 1 mL of liquid was added (oil, unemulsified oil and water or water-in-oil emulsion). The cup was reattached and measurements were recorded as before. The amount of water used was 1% with respect to the sugar (w/w). The emulsion was a 3.85% water-in-soybean oil emulsion stabilized with 3% PGPR in the continuous phase. Shear stress was standardized against the initial value of the sugar in oil dispersion.

The shear stress with added water (unemulsified or as an emulsion) was shown to be less than when no water was added (Figure A.1). This contradicts what was expected as I hypothesized that the addition of water would lead to the formation of aggregates and increase the viscosity (or shear stress). When water was added to the system in any form, large aggregates formed and were forced over the top of the cup. This meant that they were no longer influencing the shear stress which likely resulted in the decrease seen in the data. Additionally, because of the issue with the methodology, the data was not reproducible. Replication of the experiment with added emulsion (Trials A-C) resulted in different trends in standardized shear stress (Figure A.2). Different methodologies were attempted but it was concluded that there was no way to accurately measure what was occurring within the system upon addition of water.

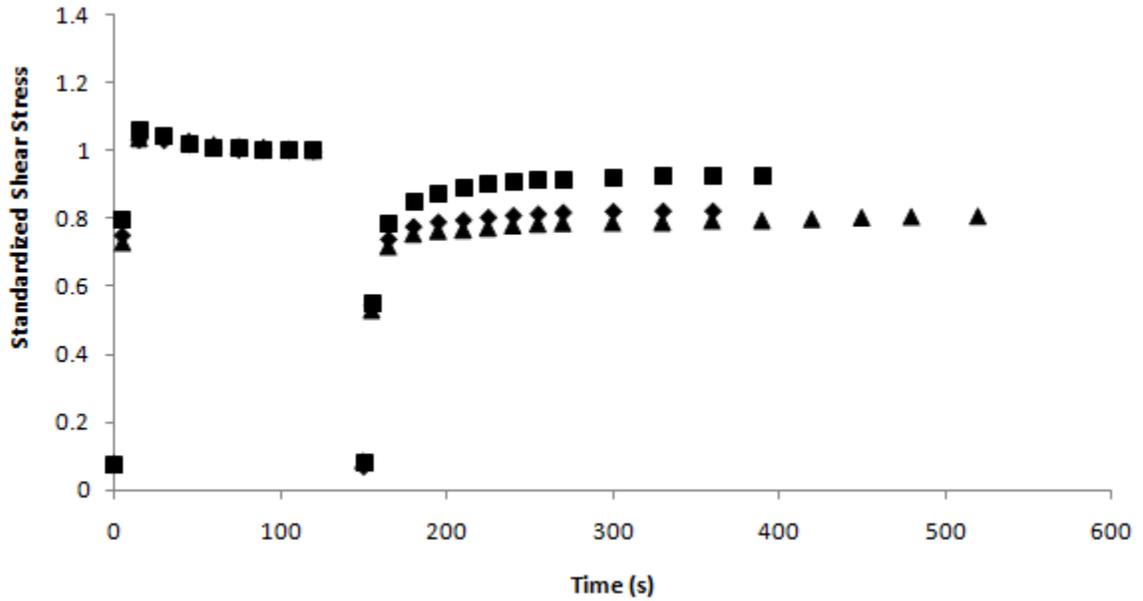


Figure A.1. Standardized shear stress vs time for stopped cup addition of oil (diamond), oil and unemulsified water (square) and emulsion (triangle).

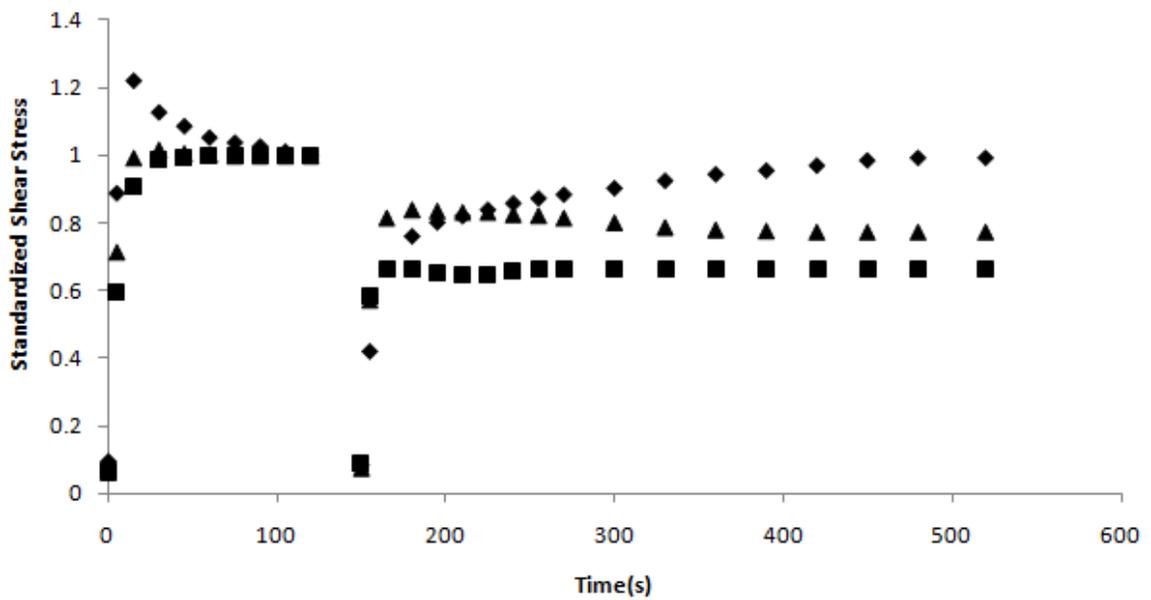


Figure A.2. Standardized shear stress vs time for stopped cup emulsion addition; Trial A (diamond); Trial B (square); Trial C (triangle).