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Stability of W1/O/W2 Double Emulsion Made With Milk Fat and a Simplified Make Procedure and Its Use in Reduced-Fat Cheese

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STABILITY OF $W_1/O/W_2$ DOUBLE EMULSION MADE WITH MILK FAT AND A SIMPLIFIED MAKE PROCEDURE AND ITS USE IN REDUCED-FAT CHEESE

by

Daniel Bradley Clayton

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ABSTRACT

Stability of $W_1/O/W_2$ Double Emulsion Made With Milk Fat and a Simplified Make Procedure and Its Use in Reduced-Fat Cheese

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Utah State University, 2014

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Double emulsions, such as $W_1/O/W_2$, are dynamic systems with potential applications in many fields. They are water droplets that are dispersed within oil droplets, which in turn are dispersed within a secondary water phase. It is possible to make an oil droplet using this technology that only contains a portion of the overall mass as fat, while the rest is composed of water, allowing for manufacture of reduced-fat items with the same number of oil droplets. This is applicable in cheese where reduced-fat products typically have a rubbery texture due to a lack of fat droplets dispersed within the protein matrix. They are, however, thermodynamically unstable systems by themselves and within food due to two emulsion boundary layers being present and the complexity of food environments.

Reduced-fat cheese was manufactured using $W_1/O/W_2$ double emulsion in place of cream added to the milk at 1.6%, 2.4% and 3.2% oil droplet volume, with cheese made

with O/W_2 added at 1.6% for control reduced-fat cheese and 3.2% for control full-fat cheese. The double emulsion was tested for stability and droplet size prior to use in cheesemaking. Compositional analytics were performed on the cheese, along with confocal imaging of the microstructure. Texture analysis and rheology measurements were taken over 7 months. Though the double emulsion did not completely retain in the cheese during manufacture, similar to improved textural characteristics were measured over time through texture analysis and rheology, in regards to hardness and viscoelasticity, compared to control cheeses. Cheese microstructure also showed differences between control and double emulsion cheese.

A second trial of cheesemaking was carried out with double emulsion containing the soluble fiber inulin at 1% within W_1 and higher shear homogenization steps in attempt to improve double emulsion retention in the final product. The cheeses were made with 3.2% $W_1/O/W_2$ added to milk and 3.2% O/W_2 added to milk as a control. Based on the compositional analytics and confocal imaging, the double emulsion retention in the cheese was similar to the first trial. Confocal images also showed a difference in microstructure between double emulsion cheese and control.

(83 pages)

PUBLIC ABSTRACT

Stability of $W_1/O/W_2$ Double Emulsion Made With Milk Fat and a Simplified Make Procedure and Its Use in Reduced-Fat Cheese

Daniel Clayton

As overweight and obesity numbers continue to climb around the world, consumers continue to search for reduced-fat alternatives to foods they often consume. Given that cheese is naturally high in fat, this is one food that is often targeted for fat reduction. However, as fat plays an important functional role in the texture of cheese by breaking up the continuous protein matrix, reduced-fat products tend to be very chewy and rubbery compared to their full-fat counterparts.

My study aimed at producing a reduced-fat cheese with improved texture compared to other reduced-fat cheese products by incorporating a double emulsion into the cheese in place of cream. The double emulsion consisted of small water droplets dispersed within oil droplets, which in turn were dispersed within a secondary water phase. The oil droplets that would then be incorporated into the cheese could essentially be made up of 40% water droplets and only 60% fat, allowing for a cheese to be designed with the same number of fat droplets as full-fat cheese while having a 40% fat reduction.

In my experiments, I made cheese with varying levels of fat using the double emulsion, along with reduced-fat and full-fat control cheeses that contained oil droplets composed entirely of fat. Though retention of double emulsion in the cheese due to its inherent instability was the key factor, I found that the double emulsion cheeses had similar to improved textural qualities compared to the control cheeses of higher fat.

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I am grateful for my parents in supporting me in all my endeavors in life, including the work put into this thesis. I am particularly appreciative, and wish to thank my wife, Vanessa, for supporting me throughout this process, as well as in all other things. Her support, along with the support of my young daughter, has made it possible for me to finish this thesis, along with find joy in all aspects of my life because of their love.

Daniel Clayton

CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

AMF - Anhydrous milk fat

CO - Canola oil

LSCM - Laser scanning confocal microscopy

O/W_2 - Oil-in-water emulsion in which the water phase was the same as that used in double emulsions

PGPR - Polyglycerol polyricinoleate

W_1 - primary or inner water phase used in a double emulsion

W_2 - secondary water phase or phase in which a W_1/O emulsion is dispersed when making a double emulsion

W_1/O - Water-in-oil emulsion in which the water phase consists of the aqueous phase W_1

$W_1/O/W_2$ - Double emulsion in which a water phase, W_1, is dispersed in oil forming the water-in-oil emulsion that is subsequently dispersed in a secondary water phase, W_2

LITERATURE REVIEW

Introduction

Emulsions can be useful in food applications and are found in many products used on a daily basis. The most common type of emulsions are simple oil-in water emulsions, that contain oil droplets dispersed in a continuous water phase, like with many salad dressings, and water-in-oil emulsions, that contain water droplets dispersed in a continuous oil phase, like with butter. Water-in-oil-in-water ($\mathbf{W_1/O/W_2}$) double emulsions are emulsions of emulsions. They consist of water droplets that are dispersed in oil droplets, which in turn are dispersed in a secondary water phase (see Figure 1). Though there are many different applications and uses for double emulsions both within and without the food realm, they are typically thermodynamically unstable as there are two emulsified boundaries instead of just one as in a standard emulsion, providing for a large obstacle to overcome before they can be used in any practical setting (Muschiolik, 2007). The use of double emulsions can range from pharmaceutical drug delivery, to

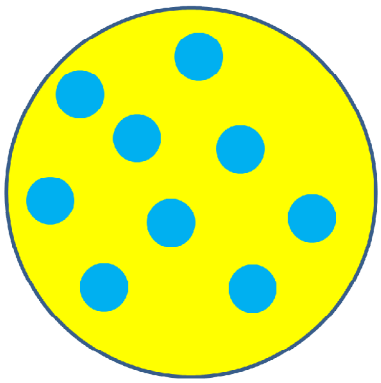

Figure 1. Schematic of a $W_1/O/W_2$ double emulsion, with multiple water droplets held within an oil droplet, which in turn would be dispersed within a secondary water medium.

cosmetics, to food. Within food, they have been shown to have potential as a hydrophilic carrier, a lipophilic carrier, or to aid in fat reduction (McClements et al., 2007; Le Révérend et al., 2010; Giroux et al., 2013). Before double emulsions can become a practical option for regular food use, their instability must first be taken into account, with various approaches to this having already been studied, the practical and promising methods being reviewed here.

Emulsifiers

As there are two interfaces in a $W_1/O/W_2$ double emulsion, two different types of emulsifiers need to be used to create the emulsion. There is the W_1-O interface, where a lipophilic emulsifier is used to create the primary water-in-oil (**W_1/O**) emulsion. There is also the O-W_2 interface, where a hydrophilic emulsifier is used to create the secondary double emulsion.

Lipophilic. The lipophilic emulsifier most commonly used in food double emulsions is polyglycerol polyricinoleate (**PGPR**), which is a synthetic emulsifier. It is a food additive located on the FDA's Generally Recognized As Safe list (Center for Regulatory Services, Inc., 2008). It is good at forming a stable W_1/O primary emulsion, even in a system where only low stress is applied in the fabrication of the emulsion through a rotor stator homogenizer (Sapei et al., 2012). Leal-Calderon et al. (2012) found that with increasing amounts of PGPR from 1% to 6% in the oil phase, with 8% NaCl added to the inner water phase, the average inner water droplet diameter decreased from 7.4 μm to 3.1 μm. However, they also noted that with greater surfactant in the oil phase, destabilization could occur due to interactions with other ingredients, including the

hydrophilic emulsifier. In this case, at a 10% PGPR concentration, interaction with gum Arabic led to instability. There are few other suitable lipophilic emulsifiers for application in a double emulsion system, but work has been done on others such as lecithin and Span 80 (Yan and Pal, 2001; Scherze et al., 2006).

It was found that by adding an enzymatically modified starch in W_1, the PGPR level in a $W_1/O/W_2$ system could be reduced while still maintaining the same encapsulation efficiency (Mun et al., 2011). They found that when 20% of the modified starch was added to a $W_1/O/W_2$ system with 2% PGPR, the encapsulation efficiency after production was greater than a system with 8% PGPR and no starch. A matured gum Arabic used as the hydrophilic emulsifier at 10% in place of 0.5% sodium caseinate was found to reduce the amount of lipophilic PGPR needed down to just 1% in order to maintain an encapsulation efficiency of greater than 90% after one month (Su et al., 2008). PGPR concentration was also able to be reduced from 4% to 2% in a $W_1/O/W_2$ system by adding 0.5% sodium caseinate to W_1 before making the primary W_1/O emulsion (Su et al., 2006). Sodium caseinate in the W_1 allowed for a more stable primary emulsion, which in turn led to a more stable double emulsion. However, PGPR could not be replaced completely in any of these studies.

Hydrophilic. The hydrophilic emulsifier is also important to the stability of the double emulsion system. Proteins, such as whey protein concentrate or isolate, are commonly used as the hydrophilic emulsifier in many studies, and are typically more desirable on a food label for consumers than something such as Tween 20. The key point for hydrophilic emulsifiers is that there are many more options available for use in a

$W_1/O/W_2$ double emulsion system that provide for adequate emulsification by means of natural ingredients compared to lipophilic emulsifier options.

Complexes of emulsifiers with a gum or polysaccharide have been tested to verify if they help improve the stability of double emulsion systems (Lobato-Calleros et al., 2006; O'Regan and Mulvihill, 2010). Use of sodium caseinates as the hydrophilic emulsifier is common, but it was found that conjugating it with maltodextrin and used as the hydrophilic emulsifier, it led to nearly a 50% greater stability in a $W_1/O/W_2$ system, based on encapsulation efficiency (O'Regan and Mulvihill, 2010). Whey protein isolate complexed with xanthan gum as the hydrophilic emulsifier has been found to significantly improve the stability of $W_1/O/W_2$ systems based on droplet size, compared to just whey protein isolate (Benichou et al., 2007). However, there was a threshold found that when more of this emulsifier was added, the stability of the system did not improve anymore. This shows the need to be practical in design of $W_1/O/W_2$ systems, as more is not always better, and perfecting the concentrations of ingredients can make it a more viable option for industrial application where money is an issue.

Other $W_1/O/W_2$ Ingredients

The ingredients in each of the three phases of a double emulsion (W_1, oil and W_2) must also be thought out clearly to create a stable system. Various ingredients can be added to any of the 3 phases of the system to either aid in stability or to be delivered upon consumption. Adding the appropriate ingredients is important to create a stable double emulsion. For double emulsions, encapsulation efficiency, or how much of a certain water soluble substance stays in the inner water phase, is important as well, and can be

viewed as a type of stability for these systems, though not a traditional method of emulsion stability. As these systems are designed to either keep substances within the inner water, or to keep the inner water in place within the oil droplets, it is appropriate to gauge a systems encapsulation efficiency when looking at its efficacy for an application.

For W_1, salt is an important aspect of double emulsion stability. There is evidence that in order to create a stable W_1/O emulsion using PGPR as the lipophilic emulsifier, there must be some salt present in W_1 (Scherze et al., 2006). They tested the difference between emulsions with no salt and emulsions with 0.6% salt in W_1 in order to validate this assumption. This is because sodium chloride in a $W_1/O/W_2$ system containing PGPR appeared to rigidify the interfacial films. The salt may also have the effect of dehydrating the PGPR, allowing the PGPR boundary to become less permeable (Kawashima et al., 1992; Hino et al., 2001). Rosano et al. (1998) found that a small amount of salt in W_1 is necessary for stability of $W_1/O/W_2$ emulsions because of the osmotic pressure it provides in the W_1 droplets. This pressure can be enough to counteract other pressures within emulsions, such as Laplace pressure, which is the pressure difference between the inside and outside of a curved surface, allowing for greater stability. Kawashima et al. (1992) also found that a hypertonic W_1 was needed to create a stable $W_1/O/W_2$ double emulsion. They suggested that a hypertonic W_1 would allow for water transport from W_2 to W_1, which in turn would allow the oil layer to become thicker between W_1 and W_2 as the W_1 expanded and forced the oil out. They tested this with levels of salt in W_1 ranging from 0.06% to 0.59%, and found that with increasing salt in this range, the greater the encapsulation efficiency in W_1.

Though the possible reasons vary, it is clear that some salt is needed for producing stable $W_1/O/W_2$ double emulsions. The concentration of salt needed in W_1 depends on the food environment in which the emulsion will be located and the application of the final product. As mentioned, water is able to transfer from W_2 to W_1 based on osmotic pressure of the system, and this can occur through either a reverse micelle, a spontaneously emulsified droplet, or diffusion of hydrated surfactants through the oil phase (Wen and Papadopoulos, 2000). With this in mind, if the difference in salt concentrations left W_1 higher in salt than W_2, it would be possible that W_1 could overfill with water beyond the capacity of the emulsifier and then break the interfacial barriers, making the system become a simple O/W_2 emulsion. If the salt concentration was higher in W_2, W_1 could transport to the W_2 phase due to osmosis, once again making the system become a simple O/W_2 system.

Sapei et al. (2012) found that adding both NaCl at 2%, 4%, 6%, or 8% and gelatin at 3% or 10% into W_1 of a $W_1/O/W_2$ allowed for greater stability in the emulsions compared to those not having both ingredients. Even with a simplified make procedure using a rotor-stator homogenizer at 27,000 rpm for 3 min to make W_1/O and at 10,000 rpm for 2 min to make $W_1/O/W_2$, the emulsions showed no sedimentation after one month. $W_1/O/W_2$ emulsions made with either no NaCl or no gelatin showed far less stability. From this, it is evident that ingredients in $W_1/O/W_2$ phases can have a synergistic effect, such as a gelling agent and an electrolyte, in double emulsion stability. Leal-Calderon et al. (2012) found that through use of different solute concentrations in W_1 and W_2 they could make a $W_1/O/W_2$ double emulsion that would transport W_2 into

W_1 when allowed a small amount of time to equilibrate, allowing for an emulsion with a low amount of fat content. They used 8% NaCl in W_1 and 20% glucose in W_2, but theoretically, there are many other solutes and concentrations that could be used to accomplish the same effect. The $W_1/O/W_2$ system could have as little as 5% overall fat composition while still having up to a 45% globule fraction due to the osmotic swelling. This would be useful in designing low calorie foods, but tests were not shown as to how the larger droplets would hold up to further processing steps that would likely take place in a food environment.

Salt is not the only important ingredient that can be incorporated into W_1. Surh et al. (2007) hypothesized that a W_1 phase containing gelled whey protein would lead to a more stable system, but found that the stability was equivalent to the systems not having the gelled protein when added in at 15%. However, there still is potential for a W_1 ingredient to help stabilize a system by increasing the viscosity of the system or by binding the water tight in the inner phase so it cannot transport out.

The oil phase in double emulsions for food use typically does not have any other ingredients added to it besides the lipophilic emulsifier, though it would have potential to carry any lipophilic bioactive component or essential fatty acids (McClements et al., 2007). The W_2 phase can have a variety of ingredients added to it besides the emulsifier to help with stability or some other function. For example, Leal-Calderon et al. (2012) found that increasing the viscosity of the external water phase with 0.25% to 1.0% xanthan gum led to a smaller average globule diameter, which would help with stability

of an emulsion. Based on this, there is opportunity to further refine the W_2 phase of a double emulsion to get greater stability or other desired attributes.

Emulsification Techniques

Oil and water do not mix on their own, so creating any type of emulsion typically requires some shear force, along with appropriate emulsifying molecules. A double emulsion system can be emulsified in various ways. The goal of the procedure is to get a stable emulsion that retains the inner water droplets within the oil droplets, which creates a unique and challenging situation compared to typical emulsion preparation. Double emulsions are typically manufactured by a two-step emulsification procedure.

The first step of double emulsion preparation involves mixing the W_1 and oil then homogenizing this mixture by a variety of methods. This step can handle greater manufacturing pressure as it is simply making a primary W_1/O emulsion. It has been found that performing this step at temperatures of 40-50˚C instead of room temperature aids in the production by decreasing the viscosity of the system and allowing for smaller droplet formation (Surh et al., 2007).

The second stage of the two-step process involves emulsifying the primary W_1/O emulsion with W_2 to form a $W_1/O/W_2$ emulsion. This step in the processes typically needs to be carried out with lower force/pressure because if the pressure is too high it will drive the inner W_1 out of the oil droplets into W_2, creating a simple O/W_2 emulsion (Garti and Aserin, 1996). Each system will be slightly different in regards to what these pressures will be, but generally, a lower pressure than simple O/W_2 or W_1/O emulsion preparation will be needed.

Emulsification techniques for the various stages differ. Two-stage high-pressure homogenization is often used for each step of the process, with lower pressures being implemented in the second step to avoid releasing W_1 into W_2 (Garti and Bisperink, 1998). This method typically creates a stable double emulsion if the pressures are right, but the emulsion droplets are polydisperse (Surh et al., 2007). Membrane emulsification can also be used in the second step of the process to create double emulsions. Graaf et al. (2005) pointed out in their review of membrane emulsification that the process forms monodisperse products. However, it is time consuming compared to other methods.

Some have found that creating a double emulsion in a more simplified manner, using just a rotor-stator homogenizer, still provides a double emulsion stable enough for practical applications (Sapei et al., 2012). Some have even used only low sheer force of stirring, followed by addition of more W_2 with higher solute levels to allow for water intake into W_1, where no high pressure or rotation speeds were ever used (Leal-Calderon et al., 2012). A rotor stator homogenizer functions by using inertial forces in turbulent flow to disrupt droplets (Scherze et al., 2006). In some food applications, an emulsion does not have to be stable for a month or more, just long enough to incorporate it into the product where it may then be held within the more complex system (Cofrades et al., 2013). Scherze et al. (2006) found that rotor stator homogenization for stable double emulsion preparation was possible when PGPR was the lipophilic emulsifier. This method, they suggested, creates a more practical transition to industrial application by creating an emulsion that is stable enough for the need. Leal-Calderon et al. (2007) also points out that it is of primary importance to fabricate structures that not only fulfill some

functional role in the application, but that are also stable enough to be commercially viable, a condition which would vary case by case.

Utada et al. (2005) devised a method that allowed for fabrication of double emulsions in a 1-step process, in place of the traditional 2-step, that allowed for a more predictable product that was uniform and could be manipulated to change any factor within the system. This was carried out by having an injection tube where W_1 was forced through a small, tapered exit, and upon exiting, a flow of oil would pass and incorporate the W_1 drops into the oil. The now W_1/O droplets were then forced into a tapered opening at the same time W_2 was also forced in, making the W_1/O form into droplets that then became dispersed into W_2, forming a $W_1/O/W_2$. This allowed for a continuous fabrication of emulsion, with parameters being set based on product flows and tapering conditions. Though a process like this is advantageous in creating a stable, precise, double emulsion, the practicality of making a $W_1/O/W_2$ emulsion in this manner is not feasible at this time on a food industrial scale due to time and equipment. Because of this, when it comes to making double emulsions that have potential for industrial application, modification to the traditional 2-step process in a simplistic fashion seems most applicable to industrial settings.

Processing Stability Issues

All emulsions are inherently thermodynamically unstable systems. There are multiple methods that can lead to instability in any emulsion system. One common destabilization method is gravitational separation, which can take place with the droplets creaming either to the top or through sedimentation to the bottom, depending on density

(McClements, 1999). The rate of this separation is proportional to the square of the diameter of the droplets, per Stokes' Law that defines the rate of gravitational sedimentation. Flocculation of droplets into one mass while retaining individual droplet identity, and coalescence of droplets into a mass where individual droplet identity is lost, are also common mechanisms of destabilization for emulsions (McClements, 1999). In double emulsions, this inherent instability is exacerbated by the fact that there are 2 separate emulsion boundaries in 1 system. Several processing factors have to be considered when dealing with stability issues, from emulsifiers used, overall emulsion composition, technique used to emulsify, the temperature of storage, and how long the system will be stored before use. Time is typically a limiting factor because given enough time, any emulsion will destabilize, but many emulsion applications may only need a few hours to a few days of stability to carry out their designed function.

Time is not the only factor to consider in stability of double emulsions in food. Often, food products that could potentially use a double emulsion are subjected to high heat or cold stresses, along with high solute contents creating high osmotic pressure differences, and a $W_1/O/W_2$ double emulsion would have to hold up under these conditions. Mun et al. (2011) found that adding 10% to 20% of an enzymatically modified starch to W_1 of a $W_1/O/W_2$ system gave the double emulsions greater stability against heating and shearing stresses. Use of a mature gum Arabic at 10% concentration as the hydrophilic emulsifier created $W_1/O/W_2$ double emulsions that were stable over a wider pH range than those stabilized with sodium caseinate, showing potential for food products that are quite acidic (Su et al., 2008).

Uses of Double Emulsions in Experimental Food

$W_1/O/W_2$ double emulsions have been used in various food products in attempts to improve texture, reduce fat, or deliver health related compounds. $W_1/O/W_2$ double emulsions offer a great opportunity to reduce the fat content of systems where an O/W_2 emulsion is typically used. McClements et al. (2007) points out their thoughts in a review on lipophilic bioactive compound delivery that a $W_1/O/W_2$ double emulsion is perfect for fat reduction. They believe such a system has the ability to keep the same physicochemical aspects of the system as well as keep the dispersed phase volume fraction the same as usual, while being able to reduce the fat content significantly. Le Révérend et al. (2010) also pointed out when they reviewed options to reduce fat consumption via colloidal methods, the importance $W_1/O/W_2$ systems can have in replacing O/W_2 emulsions because of the high amount of water that can be incorporated in place of oil.

$W_1/O/W_2$ double emulsion was used in pork meat to replace lard with olive oil (Cofrades et al., 2013). They found that they were able to create a double emulsion and incorporate it into pork meat that allowed for a meat product that still had good water and fat binding properties. The emulsion was prepared with 6% PGPR as the lipophilic emulsifier and either 0.5% sodium caseinate or 6% whey protein concentrate as the hydrophilic emulsifier. The preparation method utilized a 2-stage high-pressure homogenization step for both W_1/O and $W_1/O/W_2$ formation, with first and second stage pressures of 55,000/7,000 kPa and 15,000/3,000 kPa, respectively. The double emulsion

was incorporated into the meat system, proving that stability issues in regards to food applications can be overcome and capitalized on.

$W_1/O/W_2$ double emulsions were used to create a whipped foam using vegetable oil in order to have a replacement for whipped dairy cream with no saturated fat (Màrquez and Wagner, 2010). The emulsion was created using a rotor-stator homogenizer for both W_1/O and $W_1/O/W_2$ preparation at 24,000 rpm for 2 min and 1 min respectively. The emulsifiers were 0.5% to 2% PGPR as the lipophilic emulsifier and soybean milk for the hydrophilic emulsifier, with xanthan gum added as a stabilizer. They found that by adding calcium into the internal aqueous phase of the $W_1/O/W_2$ system using sunflower oil as the oil phase and soybean milk as W_2, they were able to get osmotic swelling leading to a creamy texture and higher consistency. This is another example being implemented in food where $W_1/O/W_2$ double emulsions are being formulated to optimize the desired fat composition in a food network.

Lobato-Calleros et al. (2006) made reduced-fat fresh cheese using a double emulsion, with canola oil as the oil phase and various polysaccharides mixed in W_2, and compared viscoelasticity and microstructure to control cheese. A rotor-stator homogenizer was used for both stages of emulsification, PGPR was the lipophilic emulsifier, and esters of monoglycerides and diglycerides were used as the hydrophilic emulsifier. They found that though some of the viscoelasticity results were similar to control cheeses depending on the polysaccharide used in W_2, it was never the same structurally. They later performed sensory analysis on similar cheeses and found that though they differed structurally, on a 1-5 hedonic scale, some of the cheeses received

similar values of overall liking to that of control cheese (Lobato-Calleros et al., 2008). Though they were able to incorporate a double emulsion into the cheese environment, use of canola oil in place of milk fat was bound to have an impairing effect on the final texture of the product, as it is not typically found in natural cheese.

$W_1/O/W_2$ double emulsion was used in place of cream in a cheesemake to deliver vitamin B_{12} (Giroux et al., 2013). They found that they were able to create a cheese using a $W_1/O/W_2$ double emulsion with anhydrous milk fat (**AMF**) as the oil phase through which they could deliver vitamin B_{12} in W_1. The lipophilic emulsifier was 8% PGPR and the hydrophilic emulsifier was either 0.5% sodium caseinate or skim milk. They tried 2 different methods of making the double emulsion, including a valve homogenizer and a rotor-stator, and found that the valve homogenizer led to smaller droplets and greater fat retention in the cheese. Though a rotor-stator is the most simplified method for industrial food application, it appears from this that something would have to be done to the technique or composition in order to have such an emulsion prove stable in a cheese environment.

Wadhwani (2012) experimented with adding $W_1/O/W_2$ double emulsion to cheese as a means to incorporate fiber into the cheese matrix. She used a magnetic stir plate for stage 1 double emulsion production and a rotor-stator homogenizer at 5000 rpm for 1 min for stage 2, 8% PGPR as the hydrophilic emulsifier, and 2% whey protein isolate as the hydrophilic emulsifier. She first attempted to make the $W_1/O/W_2$ emulsion using AMF but found it was not stable when added to 31˚C milk for cheesemaking, as it would quickly crystallize and skim to the top. In order to make a stable $W_1/O/W_2$ double

emulsion for cheese making, she switched to using canola oil, oil not native to cheese, and found that this proved stable enough to incorporate due to its lower melting temperature.

Rogers et al. (2010) experimented with cheese of various fat reduction amounts that ranged from 15% to 91% reduction where the protein/moisture ratio was held constant, and tested the cheese for rheological characteristics. They found that with less fat, the texture quality decreased due to a change in how the cheese broke down. This change in breakdown was caused by less fat droplet interruption of the protein network, showing that the poor texture of reduced and low fat cheese is caused by the lower number of fat droplets in the system. Additional work was done with reduced and low fat cheese held at a constant protein/moisture ratio, and it was found that fat plays the functional role of producing weak points in the cheese matrix that allow for breakdown upon chewing (Rogers et al., 2009). Though double emulsions were not used in this experiment, they show that with less fat, there are fewer breakdowns, so a fat replacer such as a $W_1/O/W_2$ double emulsion would have to fulfill the role of creating weak points in the cheese matrix in order to improve texture.

HYPOTHESIS AND OBJECTIVES

The hypothesis of this study was:

1. A $W_1/O/W_2$ double emulsion using anhydrous milk fat as the oil phase, created by a simplified double emulsion preparation, will be sufficiently stable to be incorporated into reduced-fat cheese.

The objectives of this study were:

1. Determine stability parameters of a simple $W_1/O/W_2$ double emulsion containing anhydrous milk fat, as a function of time and temperatures relevant to cheese making application, along with incorporation into cheese.
2. Use a gelling agent in the inner water phase, along with higher shear for the emulsification process, to improve stability when adding a $W_1/O/W_2$ double emulsion to cheese.

MATERIALS AND METHODS

Materials

Anhydrous milk fat was obtained from Kraft, USA (Northfield, IL) and from Grassland Dairy Products, Inc. (Greenwood, WI). Polyglycerol polyricinoleate (**PGPR**) was obtained from Palsgaard Industri de Mexico (St. Louis, MO). Whey protein concentrate (**WPC**) was obtained from Glanbia Nutrionals Inc. (Fitchburg, WI). Canola oil (**CO**) was obtained from Great Value (Bentonville, AR). Nile Red was obtained from Sigma (St. Louis, MO) and fluorescein isothiocyanate (**FITC**) was obtained from Invitrogen (Eugene, OR).

Milk was obtained from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan) and was skimmed, bringing it down to a minimal fat content of 0.3%. The milk was then pasteurized at 74°C for 16 s and stored at 4°C until needed. *Lactococcus lactis* was used as the primary starter (DVS 850, Chr. Hansen Inc., Milwaukee, WI). Annatto color was added to cheese milk from DSM Foods Specialty Inc. (Parsippany, NJ). Double-strength chymosin was obtained from Chr. Hansen Inc. (ChyMax, Milwaukee, WI). Inulin (Fructafit IQ) was obtained from Sensus America Inc. (Lawrenceville, NJ).

Methods

Emulsion Preparation. Emulsions were prepared for Objective 1, as described in Figure 2, at 50°C by adding W_1 containing 0.4% salt (wt/wt) to AMF with 8% (wt/wt) PGPR dropwise while on a stir plate in a 40:60 (water:AMF) ratio to create a primary

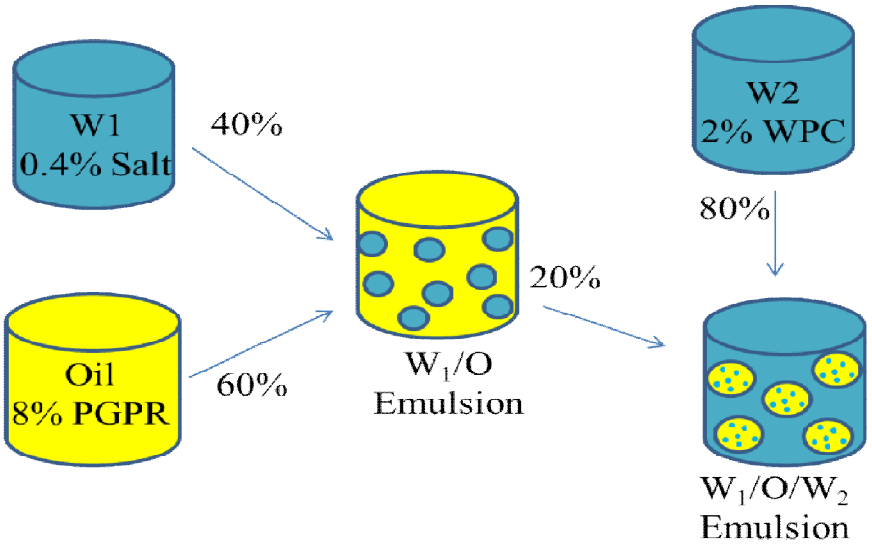

Figure 2. Schematic of $W_1/O/W_2$ double emulsion preparation steps, including creation of a simple W_1/O emulsion, which in turn is emulsified into a secondary water medium.

W_1/O emulsion. W_1/O emulsion was then added into a 2% (wt/wt) WPC in a 20:80 (W_1/O:WPC) ratio on a stir plate. This mixture was then mixed using a bench top homogenizer (Omni General Laboratory, Omni International, Kennesaw, GA) at 5,000 rpm for 1 min to create a $W_1/O/W_2$ double emulsion. The O/W_2 emulsions for the control samples were made by adding pure AMF to 2% WPC and homogenizing with a bench top homogenizer at 5,000 rpm for 1 min.

For Objective 2, $W_1/O/W_2$ was made by adding W_1 with 0.5% (wt/wt) NaCl and 1.0% (wt/wt) inulin added to a 8% (wt/wt) PGPR solution in AMF dropwise on a stir plate, followed by mixing using a bench top homogenizer at 10,000 rpm for 5 min. This W_1/O was then added dropwise into a 2% (wt/wt) WPC solution on a stir plate, and then mixed using a bench top homogenizer at 5,000 rpm for 2 min. O/W_2 emulsion used for

control cheese samples was made by adding pure AMF to 2% WPC, followed by mixing with a bench top homogenizer at 5,000 rpm for 2 min.

Emulsion Stability. To measure temperature stability, 5 to 7 mL of AMF $W_1/O/W_2$ double emulsion was placed in flat-bottomed test tubes and held at temperatures of 30, 35, 40, and 50°C for 3 h, with CO double emulsions as a control. Backscattering along the length of the tubes was measured using a vertical scan macroscopic analyzer (TurbiScan MA2000; Sci Tec Inc., Sandyhook, CT) every 15 min for 3 h. Changes over time in the thickness of a serum layer were then determined as an indicator of instability.

Emulsion Droplet Size Distribution. Emulsions were tested for droplet size range in a LS Beckman Coulter droplet size analyzer (LS 230, Coulter Corporation, Miami, FL) that uses a laser to measure the droplets. Measurements were done in triplicate, using freshly prepared emulsion with each replicate. Each emulsion replicate was measured a minimum of two times on the droplet size analyzer. This measurement gives the volume and number fraction of the water-in-oil droplets in the double emulsion system.

Emulsion Droplet Microstructure. Emulsion samples were imaged using laser scanning confocal microscopy (**LSCM**) (LSM 710, Carl Zeiss Microscopy LLC, Thornwood, NY) equipped with a KR/AR laser to excite the dye used. Emulsions viewed using confocal microscopy were prepared with 0.2% Nile Red dispersed in the oil phase so as to clearly show which phase of the emulsion was oil and which phases were water. The Nile Red dye was excited with a laser at 568 nm. At least 3 images of each emulsion were taken.

Cheesemaking. Open vats were filled with 15 or 16 kg milk and preheated to 50°C. It was then cooled to 31°C at which point it was inoculated with a *Lactococcus lactis* starter culture and allowed to ripen for 30 min before addition of rennet. Calcium chloride was added during ripening at a rate of 10.0 mL/100 kg, as well as annatto color at a rate of 8.1 mL/100 kg. Right before the rennet was added, fresh emulsion (less than 1 h after preparation), either O/W_2 for control cheeses or $W_1/O/W_2$ for experimental cheeses, was added to the milk at the appropriate listed concentrations and stirred manually to disperse it. Double-strength chymosin was diluted 20-fold with chlorine-free water and was then added at a rate of 10.0 mL/100 kg and the milk was allowed to stand for 30 min. The curd was cut with 1.6 cm wire knives when a firm coagulum was achieved, then allowed to rest for 5 min. At this time, stirring and heating began until the temperature reached 38°C after 32 min. It was then held at 38°C until the pH reached 6.30, at which time the whey was drained. The cheese was formed into one slab and cut into two pieces. It was then Cheddared by flipping the pieces every 10 min and stacking two-high at pH 5.95. When the curd reached pH 5.40, it was milled then salted at a rate of 30g salt/kg curd over three applications 10 min apart. It was then hooped and pressed at 207 kPa for 15 to 30 min, followed by 414 kPa until the cheese had been in the hoops for a total of 4 h for Objective 1 or 2 h for Objective 2. The cheese was then unhooped, vacuum-sealed, and stored at 6°C until tested at various time points.

Cheese Composition. Measurements of pH, moisture, salt, and fat were performed after 1 mo of storage. The pH was measured by stomaching 20 g of cheese with 10 g water for 1 min at 260 rpm, after which a reading on a glass pH electrode was

taken. Moisture was measured in triplicate using a microwave oven (CEM Corp., Indian Trail, NC) by weight loss. Salt was measured by stomaching grated cheese with water for 4 min at 260 rpm, after which the solution was filtered and salt content was measured using a chloride analyzer (Model 926; Corning Scientific, Medfield, MA). Fat was determined in duplicate through a modified Babcock method (Richardson, 1985).

Cheese Microstructure. Confocal microscopy was carried out on all cheese samples using LSCM equipped with a Kr/AR laser to excite the dyes. Cheese samples were allowed to come to room temperature and then sliced down to about 1 cm^2 and about 1-mm thick. A 0.2% Nile Red dye dispersed in acetone was prepared to aid in visualization of the fat phase of the cheese. A 0.2% FITC dye dispersed in acetone was prepared to aid in visualization of the protein phase. Once the cheese sample was on the slide, two drops of each dye was placed on the surface and allowed to penetrate the cheese for a minimum of 5 min prior to visualization. The microscope slide containing the cheese sample was then placed, inverted, on the confocal microscope. No cover slip was used as the cheese was adhesive enough to the slide and cover slip application often deformed the surface of the cheese. Lasers at 568 nm and 488 nm were used to excite the Nile Red and FITC, respectively. Either a single plane was imaged or a 30 to 40 mm z-stack was captured and then a composite image obtained using the Maximum Projection processing function.

Cheese Rheology. Cheese samples were prepared in a cylindrical shape with a 40 mm diameter and 1-2 mm thickness and dynamic oscillation tests were performed. An AR-G2 TA Instruments Rheometer (TA Instruments, New Castle, DE, USA) was used to

evaluate the linear viscoelastic region of the cheese in regards to the elastic modulus and loss modulus. A 40 mm flat metal geometry was used. The bearing mode was set to stiff, and a 1 min equilibration time was in place when the cheese was first placed on the temperature-controlled plate, which was set at 25°C. A strain sweep test was carried out on the cheese and the angular frequency remained at 1 Hz throughout the testing.

Cheese Texture Analysis. Texture profile analysis of the cheese was performed in triplicate at 6, 19 and 32 wk of age on a Texture Analyzer TA.XT *plus* (Stable Micro Systems, Godalming, Surrey, UK). The attributes for this objective chosen to be of interest were hardness, adhesiveness, springiness, and cohesiveness, as described by Bourne (1968). Hardness is the peak force exerted by the cheese on the initial compression. Adhesiveness is the downward force exerted by the cheese during the retraction from the initial compression, measured by the area of work after the first compression that has negative g-force. Springiness is length of compression from the second depression divided by the length of compression from the first depression. Cohesiveness is the area of work during the second compression divided by the area of work of the first compression. The cheese samples were made using a cylindrical cheese borer with a diameter of 1.6 cm, and were cut down to 2 cm in length. A 2-bite, 25% compression test was carried out in order to calculate hardness, adhesiveness, cohesiveness, and springiness.

Experimental Design

Objective 1. $W_1/O/W_2$ double emulsions for Objective 1 were freshly prepared for each test and cheesemake. They were tested for stability for 3 h, droplet size distribution,

and imaged via confocal microscopy. Batches of cheese were made in triplicate and included 2 controls, which used O/W_2 emulsions in place of cream. The controls included a reduced-fat cheese with O/W_2 emulsion added at 1.6% volume (**RF CON**) and a full-fat control with O/W_2 emulsion added at 3.2% volume (**FF CON**). The 3 experimental emulsion cheeses had varying levels of added $W_1/O/W_2$ emulsion, including 1.6% added emulsion (**WOW 16**), 2.4% added emulsion (**WOW 24**), and 3.2% added emulsion (**WOW 32**). The added emulsion percentages are in respect to the oil droplet or W_1/O droplet volumes, as the aim was to get these droplets incorporated into the cheese matrix, and the W_2 phase was used simply to deliver these droplets into the milk system. The experiment was carried out in a randomized block design, with days being the blocks.

The fat levels were designed so that FF CON would represent a standard cheddar and WOW 32 would theoretically have the same number of droplets, but 40% less fat due to the $W_1/O/W_2$ double emulsion droplets only being 60% fat and 40% water. The same idea was in place for RF CON and WOW 16, where RF CON was designed to be a 50% reduced-fat cheese, and WOW 16 was to have the same number of fat droplets in the protein matrix, but 40% less fat. WOW 24 was designed to be a point in the middle of WOW 32 and WOW 16, and to have the same overall fat content as RF CON but would have an increased number of emulsion droplets.

The cheese was tested for composition, and texture analysis was carried out at 6, 19, and 32 wk of age. Rheology was carried out on the cheese at 19 and 32 wk of age. The cheese was also imaged through LSCM at about 1 mo of age.

Objective 2. After initial results from Objective 1, a second $W_1/O/W_2$ double emulsion cheesemake was planned, with the aim being to help retain a greater amount of $W_1/O/W_2$ double emulsion in order to get an even greater positive effect through its incorporation. The W_1 composition for this objective was changed to include 1.0% (wt/wt) inulin and 0.5% (wt/wt) NaCl in water, compared to just 0.4% (wt/wt) NaCl in water for Objective 1 double emulsions. A higher shear and longer time homogenization step was added in making the primary W_1/O emulsion and the length of time for the $W_1/O/W_2$ emulsion homogenization step was increased. The emulsions for Objective 2 were also tested for droplet size measurement and distribution, along with being imaged through LSCM.

Two different batches of cheese were made in triplicate in a randomized block design by day. One batch was a FF CON control cheese made with an O/W_2 emulsion, and the other was a $W_1/O/W_2$ double emulsion cheese, WOW 32. Both were added at 3.2% in respect to the volume of the oil or W_1/O droplets, as this was the fraction aimed at being incorporated into the cheese, while the W_2 was simply a means to deliver the droplets without interacting with the casein. The double emulsion in the cheese was added at a concentration aimed to give the same number of fat pockets as the control with a 40% fat reduction. The cheese was tested for composition and was imaged through LSCM.

Statistical Analysis

A randomized block design was used to study the effect of $W_1/O/W_2$ double emulsions in cheese, for both Objective 1 and Objective 2. The block was the day

experimental cheeses were made within one of the three days the experiments were carried out. Statistics based on the composition of the cheese was carried out using Proc GLM on Statistical Analysis Software (SAS Institute, Inc., Cary, NC). For statistical analysis of the droplet size of the double emulsions, PROC TTEST was used, and PROC GLM was used for analysis of the stability testing, based on a 2 by 4 factorial design. For statistical analysis of the TPA and rheology results, a split plot design was carried out, with the day of the cheesemake as the whole plot unit and emulsion type as the whole plot factor. At the whole plot level, a randomized complete block design was put in place, with weeks of aging as the split plot factor. Proc GLIMMIX was utilized to carry out statistical comparisons of the split plot design.

RESULTS AND DISCUSSION

Objective 1

$W_1/O/W_2$ Emulsion Droplet Microstructure. $W_1/O/W_2$ double emulsions are dynamic and unstable systems. In order to verify that the preparation method for $W_1/O/W_2$ double emulsions worked in creating the system, LSCM was carried out on samples. Representative images of AMF $W_1/O/W_2$ double emulsions are displayed in Figure 3, along with $W_1/O/W_2$ double emulsions made with CO as a control, in Figure 4. In the images, the white pixels are from fluorescence from Nile Red and represents location of lipid material.

The images clearly show large suspended oil droplets, with additional non-oil droplets within each oil droplet. These pictures validate the assumption that the procedure used did create $W_1/O/W_2$ double emulsion droplets. In the images, the oil droplets and the water-in-oil droplets range in size considerably, showing a high degree of polydispersity. This polydispersity is due to the preparation method used, where only low shear was applied in making the W_1/O and the subsequent $W_1/O/W_2$ emulsion. High shear was not used in the preparation method as it was found that with increased shearing came increased breakage of the double emulsion droplets, leading to a simple O/W_2 emulsion system as the inner W_1 droplets were forced out of the oil. It is possible to create smaller, monodisperse double emulsion droplets through use of membrane emulsification (Graaf et al., 2005). However, in order to use a more practical method for larger scale industrial application, the two-step simplified process was used.

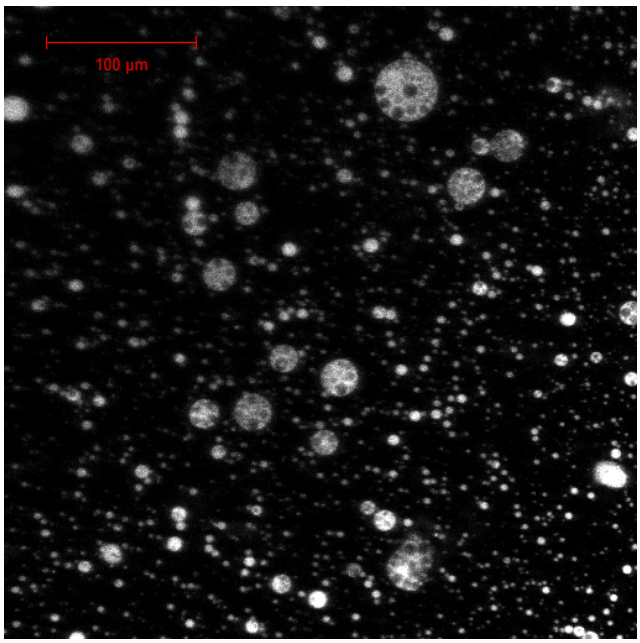

Figure 3. Laser scanning confocal micrograph of milkfat $W_1/O/W_2$ double emulsion used for cheese manufacture in Objective 1 approximately 1 h after production. White corresponds to fluorescence from Nile Red in the presence of lipid.

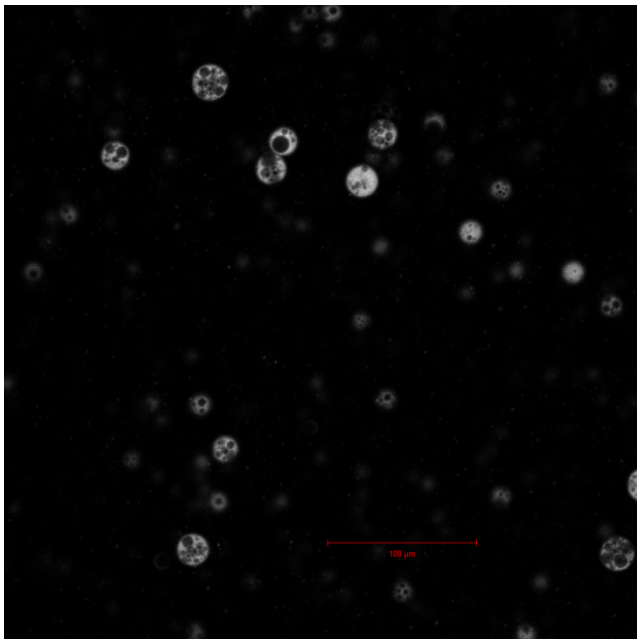

Figure 4. Laser scanning confocal micrograph of canola oil $W_1/O/W_2$ double emulsion used for a control in Objective 1 approximately 1 h after production. White corresponds to fluorescence from Nile Red in the presence of lipid.

$W_1/O/W_2$ *Emulsion Stability.* The stability of the $W_1/O/W_2$ double emulsion, as measured by thickness of a serum layer, showed that temperature had a significant effect on the time it took the emulsions to destabilize. Figure 5 shows the stability over a 3 h window of $W_1/O/W_2$ double emulsions made with AMF stored at 30, 35, 40, and 50°C. Thickness of the serum layer increases with emulsion droplets coalescing and/or creaming causing the solution to clear at the bottom of the tubes, indicating a loss of emulsion stability. The larger the serum layer, the greater the loss of stability in the system. The difficulty of incorporating a $W_1/O/W_2$ double emulsion into cheese with an oil phase composed of AMF is that it is prone to crystallization leading to destabilization, as the crystallization range of milk fat includes the range from 30 to 35˚C. As Cheddar and other similar cheesemakes are often carried out within this temperature range, it was possible that the entire system could be destabilized due to temperature. To see what effect oil type had on emulsion stability, $W_1/O/W_2$ double emulsions with CO as the oil phase in place of AMF were also carried out as a control over 3 h at holding temperatures of 30, 35, 40, and 50˚C, and those results are contained in Figure 6. See Appendix A for specific statistical results.

$W_1/O/W_2$ double emulsions with an oil phase composed of AMF were slightly less stable (higher serum layer thickness) after 3 h than $W_1/O/W_2$ double emulsions with an oil phase composed of CO, when comparing emulsions held at the same temperatures over that time frame. After 3 h, the AMF $W_1/O/W_2$ emulsions held at 30, 35, 40 and 50˚C had serum layer thickness (mean ± standard deviation) of 4.1± 0.3, 4.5 ± 0.4, 4.6 ± 0.3,

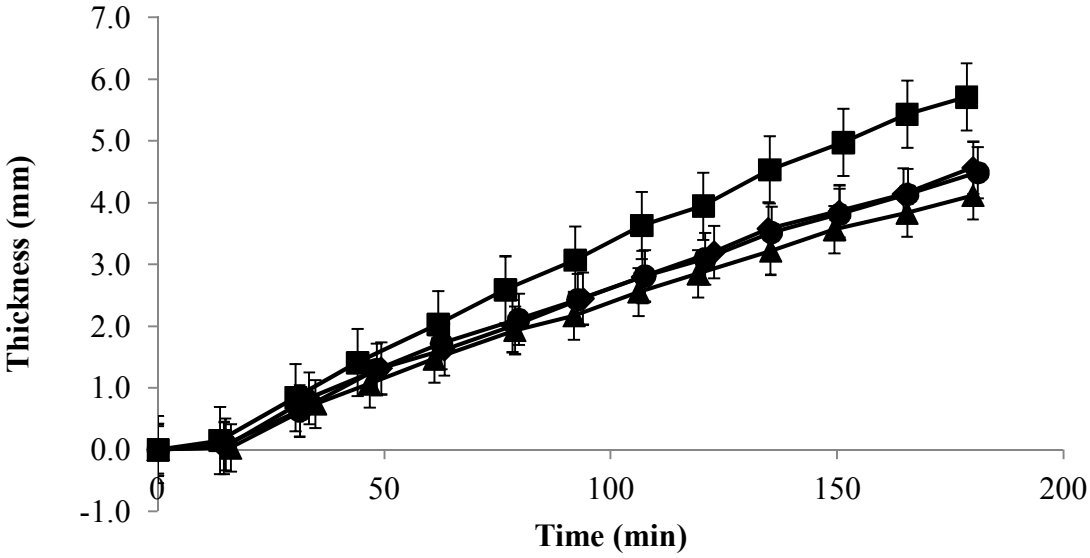

Figure 5. Instability of anhydrous milk fat $W_1/O/W_2$ double emulsion measured as increase in serum layer thickness over time at 30˚C (▲), 35˚C (●), 40˚C (♦), and 50˚C (■). Error bars represent standard error.

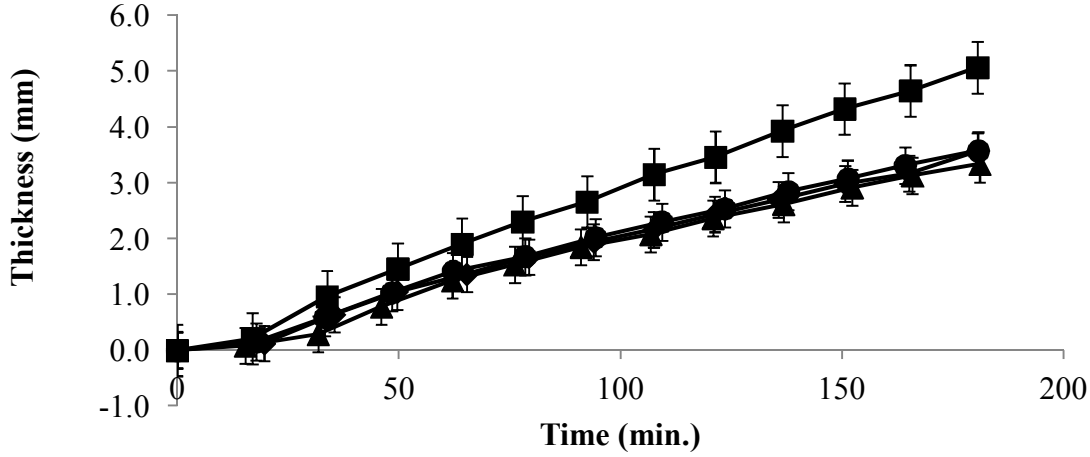

Figure 6. Instability of canola oil $W_1/O/W_2$ double emulsion measured as increase in serum layer thickness over time at 30˚C (▲), 35˚C (●), 40˚C (♦), and 50˚C (■). Error bars represent standard error.

and 5.7 ± 0.4 mm, respectively. The similar $W_1/O/W_2$ emulsions made using CO were slightly more stable, with serum layer thickness (mean ± standard deviation) of 3.3 ± 0.3, 3.6 ± 0.2, 3.6 ± 0.4, and 5.1 ± 0.4 mm, respectively. There was a significant difference in stability based on temperature, with 50˚C storage temperature resulting in greater instability than the other holding temperatures. The CO samples were also significantly more stable than the AMF samples. The interaction term of temperature*oil was not significant.

The complete crystallization range of AMF is very wide, ranging from about 10 to 35˚C, which encompasses a high, low and middle melting fractions, though the majority melts around 30 to 35˚C (Ronholt et al., 2013). The $W_1/O/W_2$ emulsions composed of AMF held at 30 to 35˚C, which falls within this range, actually showed the most stability, with no visual signs of crystallization after 3 h. Crystallization of the AMF could potentially lead to destabilization of the emulsion (Wadhwani, 2012). Crystallization is a dynamic process that depends on both thermodynamics and kinetics. The $W_1/O/W_2$ emulsions with AMF held at 30˚C were thermodynamically unstable as they were held below the crystallization temperature of AMF. However, bulk fat has been found to crystallize at higher temperatures than the same fat emulsified (Vanapalli et al., 2002). This is because bulk fat crystallizes through a heterogeneous mechanism in the presence of impurities such as dust, while emulsified fat crystallizes via homogenous nucleation, thus occurring at lower temperatures than the bulk (McClements et al., 1993).

It was hypothesized before the experiment that an AMF $W_1/O/W_2$ emulsion would be more stable at 50˚C due to no crystallization occurring, even if it were to only

occur at a slow rate for the lower holding temperatures. As this was not the case, the lower stability in both the AMF and CO $W_1/O/W_2$ emulsion systems held at 50˚C may arise from there being more energy in the system by means of a higher holding temperature, leading to more double emulsion droplets coalescing and creaming within the system.

It was an objective to see if an AMF $W_1/O/W_2$ double emulsion could be stable from 30 to 35˚C long enough to be incorporated into milk and converted to cheese before the system became too unstable and did not retain within the cheese protein matrix. Though the AMF $W_1/O/W_2$ was slightly less stable than the CO $W_1/O/W_2$ at the 30 to 35˚C storage temperature, the AMF $W_1/O/W_2$ appeared to be stable enough to meet the requirements of being incorporated into cheese. It was believed that 3 h into a cheesemake, the potential to lose the $W_1/O/W_2$ double emulsion due to destabilization would be greatly reduced due to the milks conversion into a solid mass and the draining of the whey from the curds. Also at this point, the protein matrix should already be disrupted by the increased number of droplets, so the $W_1/O/W_2$ would have fulfilled its function. Though by classic definition of an emulsion being stable if its peak thickness increases less than 1 mm per day, and our system increased at 4.1 to 4.5 mm in 3 h, for this application and the fact that the emulsion would always be added to milk within an hour of production, it seemed stable enough. It was believed that an AMF $W_1/O/W_2$ double emulsion with this composition and make procedure could be added to milk and retained in the cheese.

$W_1/O/W_2$ Emulsion Droplet Size Analysis. The D_{32} means (± standard deviation), which are the surface area mean, were 3.09 ± 0.16 μm and 2.60 ± 0.06 μm for AMF and CO compositions, respectively. The D_{43} means (± standard deviation), which are the volume mean diameter, were 7.00 ± 0.54 μm and 6.51 ± 0.75 μm for AMF and CO compositions, respectively. Stability of emulsions depends, in part, on the size of the emulsion droplets in the system, as droplet size is a determining factor of creaming rate. Since $W_1/O/W_2$ double emulsions are larger in most cases than simple emulsions because they are droplets within droplets, this is a reason why double emulsions are inherently more unstable than single emulsions (Benichou et al., 2004). The D_{43} measured for the AMF samples were not significantly different from the CO samples, based on T-test results, but D_{32} of AMF double emulsion was statistically larger than the CO emulsion. However, the difference in D_{32} values was still relatively small, and with D_{43} values being equal, it was deemed that an AMF double emulsion had potential for a cheese application. See Appendix B for specific statistical results.

Cheese Composition. Emulsion cheese composition values are contained in Table 1. The composition between cheese varied significantly, as was expected, due to the difference in emulsion type and amount added to each vat. Salt and pH had no difference between samples. Fat was significantly different between many of the samples, but not as much as was expected. The WOW 16 cheese had less fat added than WOW 24, which had less fat added than WOW 32, but WOW 24 was not significantly different from either in fat content. The moisture content between all three double emulsion cheese

samples was not significantly different, but they were all significantly higher than RF CON and FF CON. See Appendix C for specific statistical results.

In this experiment, the greatest impact on moisture content is the water contained within the $W_1/O/W_2$ droplets, because even though with decreased fat, moisture contents typically rise, the WOW cheeses and their comparable controls would have had the same number of fat droplets leading to similar serum pockets. The $W_1/O/W_2$ cheeses were expected to have higher moisture contents compared to the control cheese because each $W_1/O/W_2$ fat droplet was 40% aqueous solution and 60% oil. Because of this, the most relevant composition change in regards to stability of the double emulsions in the cheese was the moisture content and its variation. The moisture levels proved to be significantly different. FF CON did have a typical moisture value for full-fat cheese and RF CON did have a typical moisture value for reduced-fat cheese that was not preacidified. With increased moisture content in WOW samples, it does appear that at least some of the

Table 1. Mean (± standard deviation) composition of double and single emulsion ($W_1/O/W_2$ or O/W_2) Objective 1 cheeses.

	Objective 1 Cheeses[1]					
(%)	**WOW 16**	**WOW 24**	**WOW 32**	**RF CON**	**FF CON**	**P-Value**
Moisture	46.0 ± 0.9[a]	45.3 ± 1.5[a]	45.0 ± 1.1[a]	40.4 ± 1.4[b]	36.1 ± 0.6[c]	<0.001
Fat	11.2 ± 2.4[d]	12.4 ± 1.7[c,d]	17.0 ± 4.1[c,b]	21.5 ± 1.1[b]	31.4 ± 0.4[a]	<0.001
pH	5.30 ± 0.03	5.32 ± 0.04	5.30 ± 0.02	5.26 ± 0.03	5.33 ± 0.09	0.474
Salt	1.40 ± 0.08	1.45 ± 0.10	1.48 ± 0.09	1.44 ± 0.19	1.48 ± 0.19	0.917

[1]WOW 16 = 1.6% double emulsion added for cream; WOW 24=2.4% double emulsion added for cream; WOW32=3.2% double emulsion added for cream; RF CON=1.6% O/W_2 emulsion added for cream; FF CON=3.2% O/W_2 emulsion added for cream
[a-d]Means within a row with the same superscript letter were not significantly different

double emulsion in the cheese was retained, but whether that was in a stable emulsion form or destabilized emulsions creating more serum pockets is not clear from this data.

Fat retention was clearly not as efficient among all the cheeses, based on similar fat results among samples with different amounts of fat added. Based on WOW 16 and WOW 32 designed to have 40% less fat than RF CON and FF CON, respectively, and WOW 24 to have a value right in the middle, it was calculated that WOW 16, WOW 24 and WOW 32 would have 11.2%, 15.0%, and 18.8% fat, compared to the actual fat values of the controls. All double emulsion samples were lower in fat than would be predicted, especially WOW 24, which in the original plan was to have the same amount of fat as RF CON but more droplets. This indicates that there was some issue with fat retention in the system, due to either the stability or size of the emulsion or how the cheese was made. However, FF CON and RF CON may not have had typical fat retention either, for these same reasons.

With all other aspects in the cheese composition being equal, WOW 32 would have higher moisture than FF CON, to account for its 40% aqueous phase per fat droplet. It was not expected for moisture to be higher due to increased serum pockets caused by fat reduction though because the same number of fat droplets should be dispersed throughout the cheese protein matrix, leaving equal spacing for free water in both cheeses. The same idea for moisture percentages applies to WOW 16 and RF CON. While the expected increase in moisture was not achieved, neither were the expected fat percentages, explaining part of the disparity between expected and actual values. While the moisture values are not as high as expected between WOW 32 and FF CON, as well

as WOW 16 and RF CON, they are still significantly higher. As higher moisture percentage was retained, it appears there was also some retention of $W_1/O/W_2$ double emulsion in the cheese protein matrix.

One hundred percent retention of double emulsion in cheese does not seem probable given that only about 90% of native milk fat in a typical cheese make is retained. Maximizing the retention in order to see differences in texture is what is important. Based upon the composition it appears that though not perfect, the double emulsion, or the components of it if it reverted into a simple O/W_2 emulsion, was retained within the cheese matrix, to an extent, and thus had the potential to have an effect on the texture.

Cheese Rheology. Rheology was carried out on Objective 1 cheese at 19 and 32 wk of age. G' or storage modulus, which corresponds to elasticity, and G'' or loss modulus, which corresponds to viscosity, values were calculated from the linear viscoelastic region of the cheese when frequency was kept constant at 1 Hz. This allowed us to compare how the viscoelasticity compared between cheeses and from 19 wk to 32 wk of storage. Figures 7 and 8 show how G' and G'' changed over time in respect to all five samples. See Appendix D for specific statistical analysis.

For both G' and G'' the factors emulsion type and aging time showed significant difference between the samples, but not the interaction term emulsion type*aging time. For G', which deals with the elasticity of the cheese, WOW 32 averaged the lowest value over time at 70.3 kPa, while WOW 16, WOW 24, FF CON, and RF CON had increasing values of 95.7, 105, 107, and 143 kPa respectively. From 19 wk to 32 wk, the overall

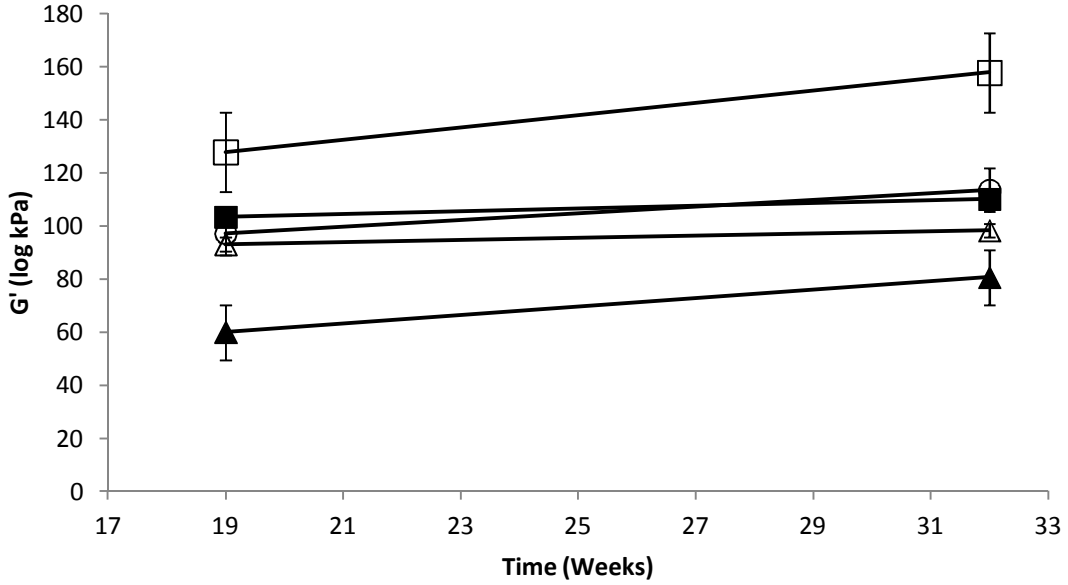

Figure 7. G' values of Objective 1 cheeses measured at 19 and 32 wk in their linear viscoelastic regions at a constant frequency of 1 Hz. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

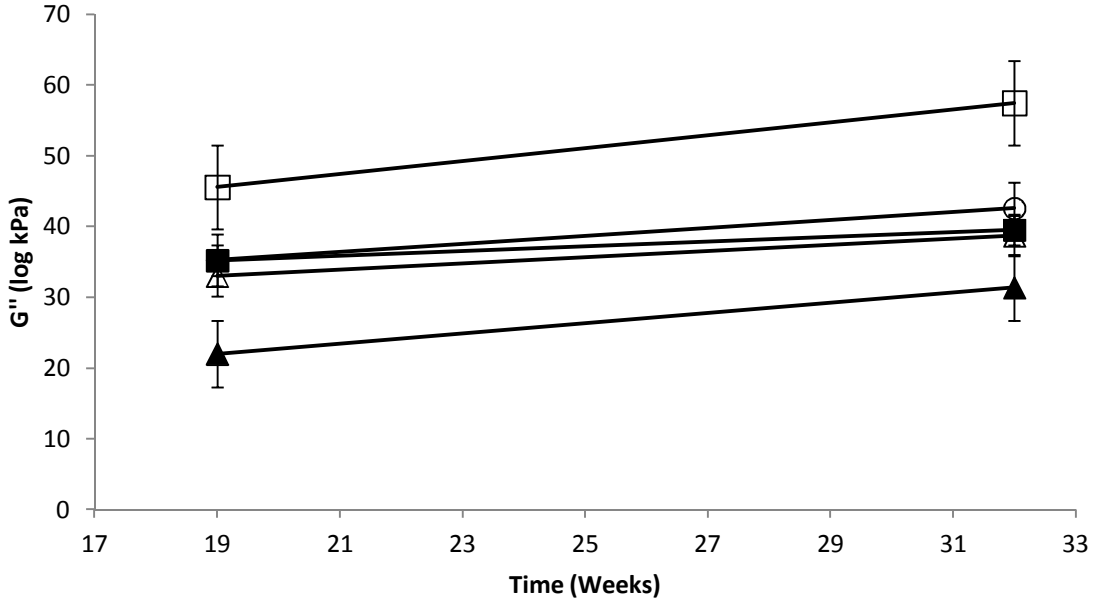

Figure 8. G'' values of Objective 1 cheeses measured at 19 and 32 wk in their linear viscoelastic regions at a constant frequency of 1 Hz. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

values for all emulsion types increased from 96.3 kPa to 112.1 kPa, showing an increase in the elasticity of the cheese.

For G'', which deals with the viscosity of the cheese, WOW 32 averaged the lowest value over time at 26.7 kPa while WOW 16, FF CON, WOW 24, and RF CON averaged 35.9, 37.4, 38.9, and 51.5 kPa, respectively. From wk 19 to 32, G'' increased from 34.2 to 41.9 kPa. With the $W_1/O/W_2$ double emulsion cheeses being lower in both G' and G'' values in nearly every case compared to FF CON and RF CON, there was less overall stress response from the test, showing a softer texture. From this, it is clear either way that the double emulsion had a positive effect on the cheese. The RF CON had the highest values for both G' and G'', while FF CON and the double emulsion cheeses had lower values. In comparing the double emulsion cheese values to the control cheese values, it can be seen that G' and G'' values near that of FF CON would be desirable for improved textures, as standard reduced-fat cheese, such as RF CON, is known for its poor texture compared to full-fat cheese, such as FF CON. It is clear from the results that the use of $W_1/O/W_2$ double emulsion in cheese clearly did have an effect on the final product leading to a lower storage and loss modulus, compared to the appropriate control.

Cheese Texture Analysis. Texture analysis was carried out on Objective 1 cheeses 3 times over 32 wk in order to monitor how the cheese changed over time and how the $W_1/O/W_2$ double emulsion cheeses compared to the controls. The results of the texture analysis are found in Figures 9-12. See Appendix E for specific statistical analysis.

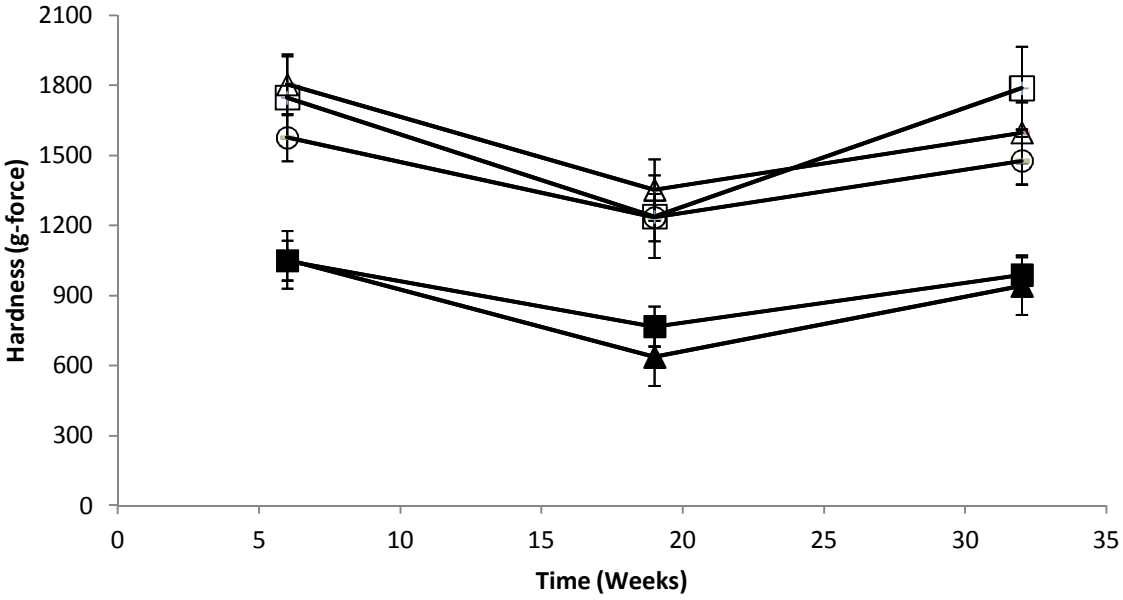

Figure 9. Hardness values of Objective 1 cheeses over time measured at 6, 19, and 32 wk on a texture analyzer through a 25% compression 2-bite test. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

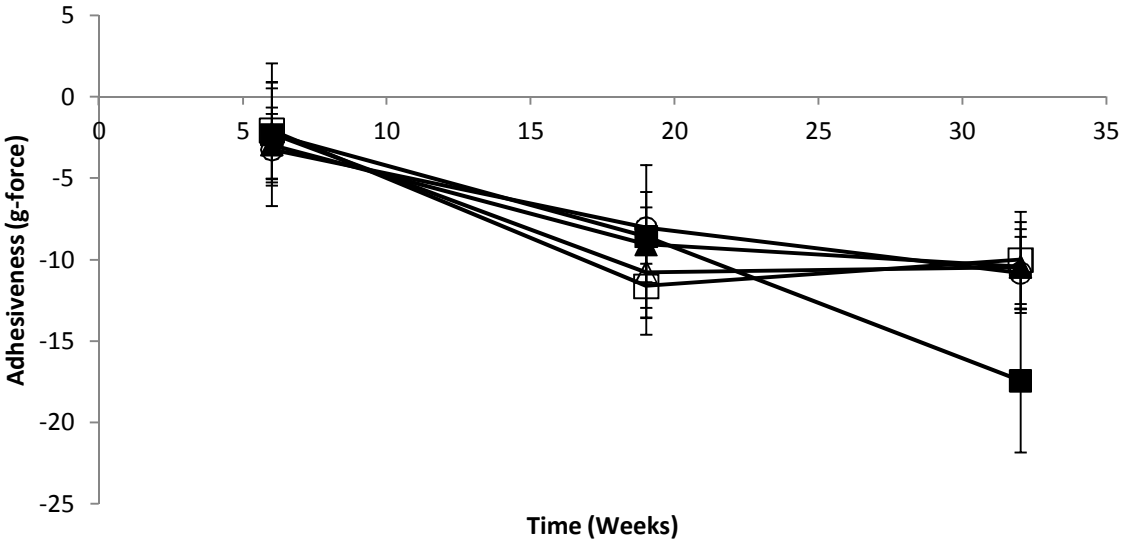

Figure 10. Adhesiveness values of Objective 1 cheeses over time measured at 6, 19, and 32 wk on a texture analyzer through a 25% compression 2-bite test. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

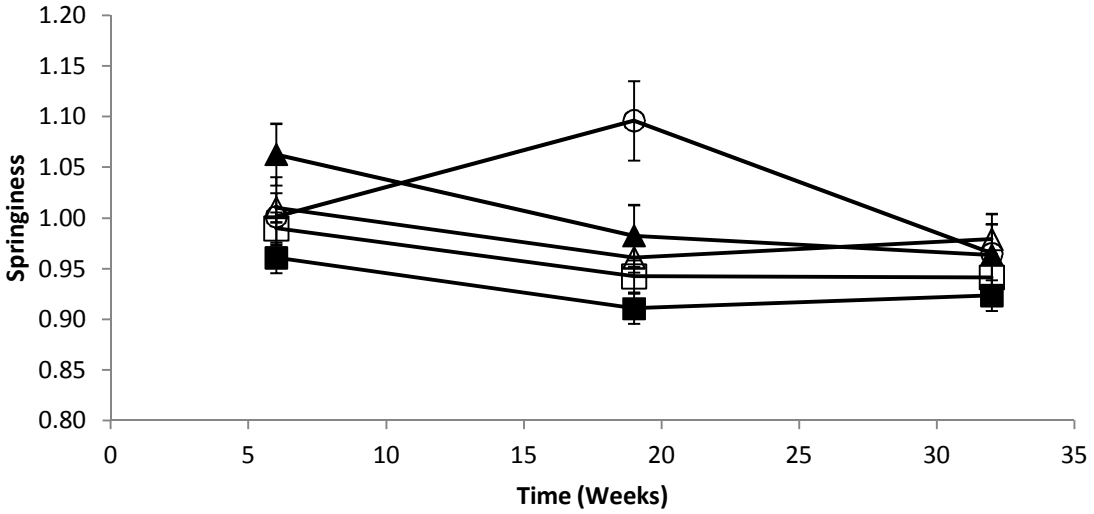

Figure 11. Springiness values of Objective 1 cheeses over time measured at 6, 19, and 32 wk on a texture analyzer through a 25% compression 2-bite test. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

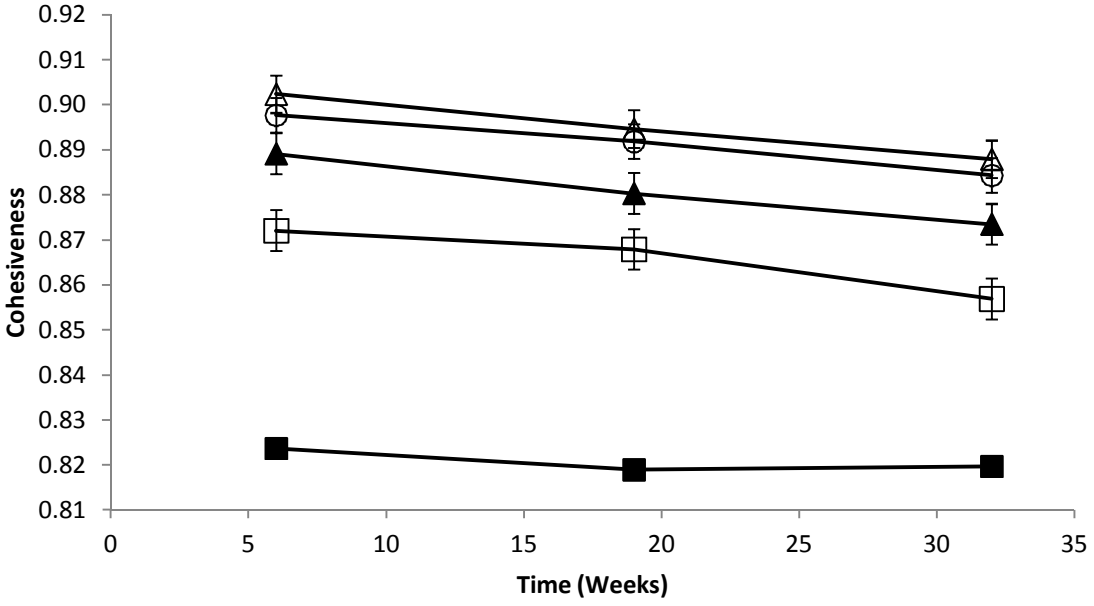

Figure 12. Cohesiveness values of Objective 1 cheeses over time measured at 6, 19, and 32 wk on a texture analyzer through a 25% compression 2-bite test. WOW 16 (Δ); WOW 24 (○); WOW 32 (▲); RF CON (□); FF CON (■). Error bars represent standard error.

Texture profile analysis is a 2-bite test aimed at measuring various texture attributes in a sample based on how much force the sample exerts upon being stressed and how much deformation takes place. There are several attributes that can be measured with TPA, but not all apply to every type of sample. Hardness is a good indicator when it comes to low- and reduced-fat cheese texture, compared to controls, as increased hardness is often a defect in the product (Banks et al., 1989). The hardness results for Objective 1 cheese showed statistical difference for both the factors aging time and cheese type, but not for the interaction of these two terms. The lack of a significant interaction term means that the cheese samples change in hardness the same way over time, even though they are significantly different from each other. For this case, though the cheese have different hardness values, they all decreased in hardness between 6 to 19 wk, but then increased in hardness from 19 to 32 wk of storage.

The post hoc test for cheese type, in regards to hardness, showed that over time FF CON and WOW 32 were both statistically lower in hardness than WOW 16, WOW 24, and RF CON, but there was no difference between the two. There was also no difference between WOW 16, WOW 24, or RF CON. Though WOW 32 had significantly less fat added to the milk, and subsequently less fat in the cheese, it still had the same hardness as a full-fat cheese control. This is likely due to its design to have the same number of droplets interrupting the protein matrix, creating weak spots to keep the hardness the same. Even though the fat content was lowered in WOW 32, there was no increase in hardness, compared to FF CON. The same applies for WOW 16 and WOW 24 compared to RF CON. For the double emulsion cheese not to have increased in

hardness even though it had less fat indicates that there were potentially equal interruptions to the protein matrix between double emulsion cheeses and controls.

However, one downside of hardness measurements with cheese is that more than just particle numbers and distribution can affect them. Increased moisture within the cheese, even when there is reduced fat, can help to lower the hardness values of cheese. The double emulsion cheeses did have more moisture than their appropriate control, so it is possible that the similar hardness values arise due to the increased moisture, and not due to equal protein interruptions within the cheese matrix. However, the increased moisture could also be due to the double emulsions being present, whether in a stable form or through destabilizing, but in so doing creating additional small serum pockets within the protein matrix, helping the texture that way. What is clear though is that a cheese made with double emulsion and less fat than a control, had the same hardness values as the control, which does show some promise for the use of double emulsions, even if the mode of action is not clear.

For the measurements of adhesiveness, only time had a significant effect on differentiating between samples, not emulsion type, and the trend was that samples at 19 and 32 wk were significantly more adhesives than at 6 wk. For springiness, there was no significant difference due to emulsion type or time between all the samples. For cohesiveness, emulsion type, month and emulsion type*month all were significant factors. The general trends were that all three double emulsion cheeses had significantly higher cohesive values than RF CON and FF CON, with RF CON having significantly higher cohesive values than FF CON. In addition, WOW 16 had significantly higher

cohesive values than WOW 32. Cohesiveness values decreased over storage time for all samples. As cohesiveness measures how the cheese withstands a second compression compared to the first, it can be seen that both the controls broke down more from the first compression than any of the double emulsion cheeses, and the cheese was more prone to breakdown as it aged, which would be expected.

Cheese Microstructure. Confocal microscopy results for the Objective 1 cheeses displayed a varying degree of protein and fat concentrations from sample to sample, as would be expected based on the design, along with varying fat configuration and distributions within each respective casein matrix. Representative images from each of the 5 types of emulsion cheeses can be found in Figures 13-17.

A cheese matrix can be looked at as a continuous protein network interspersed with fat globules. In the LSCM images, the cyan pixels represent fluorescence from FITC bound to proteins and magenta pixels represent fluorescence from Nile Red in lipid environments. Black pixels in the images represent lack of fluorescence meaning that no lipid or protein is present in that region. These would represent serum pockets in the cheese matrix while there is the possibility that some protein dense portions of the cheese were not penetrated by FITC.

It was expected that a similar number of fat droplets would be interspersed throughout the cheese matrix in both the WOW16 and RF CON samples. They were designed to have the same number of fat droplets while WOW 16 was to have a 40% fat reduction due to each of its fat droplets only being 60% fat and 40% aqueous phase. In visually comparing the images of these two samples, there appears to be a greater

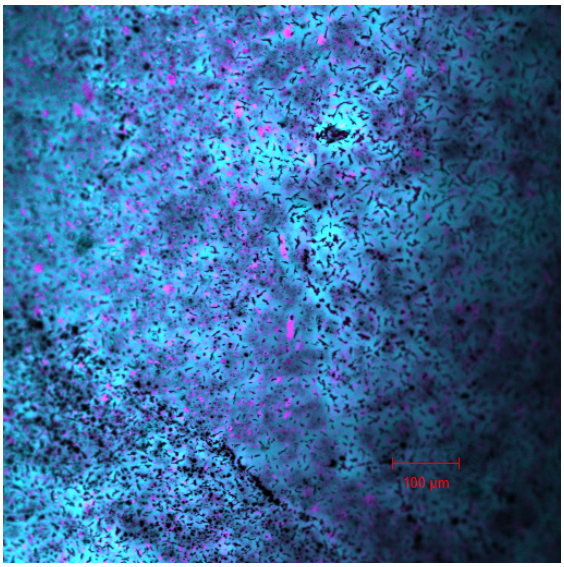

Figure 13. Laser scanning confocal micrograph of cheese made with 1.6% $W_1/O/W_2$ double emulsion from Objective 1 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

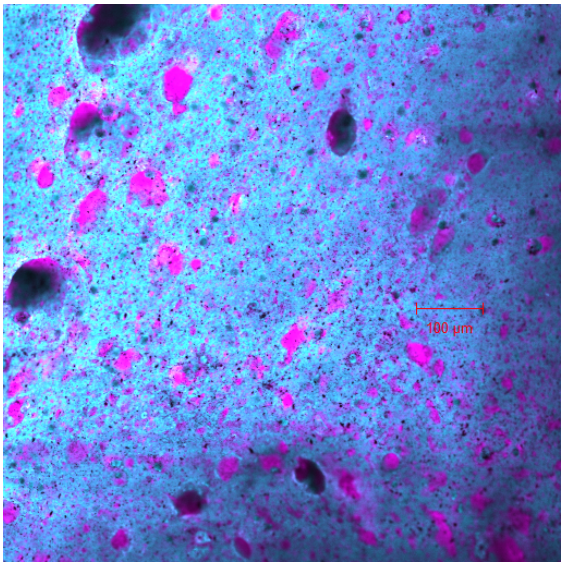

Figure 14. Laser scanning confocal micrograph of cheese made with 2.4% $W_1/O/W_2$ double emulsion from Objective 1 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

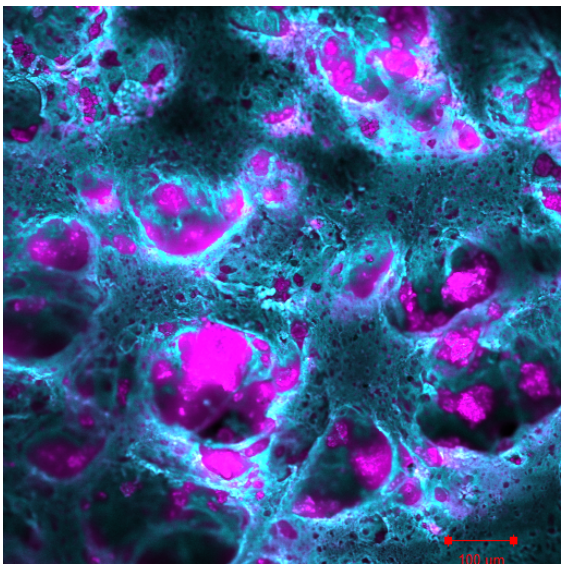

Figure 15. Laser scanning confocal micrograph of cheese made with 3.2% $W_1/O/W_2$ double emulsion from Objective 1 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

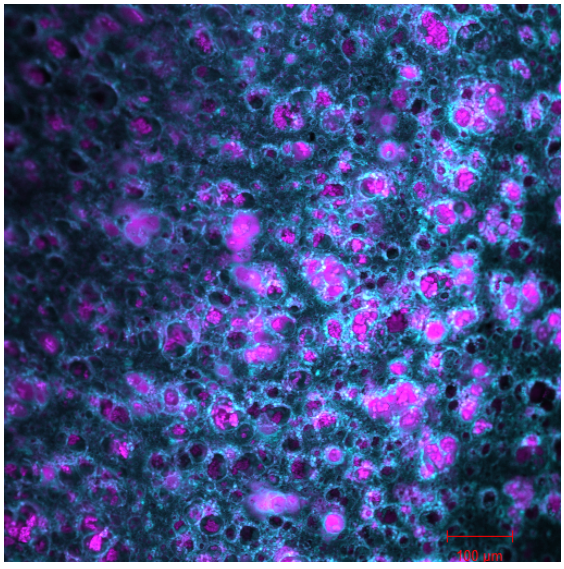

Figure 16. Laser scanning confocal micrograph of cheese made with 1.6% O/W_2 emulsion from Objective 1 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

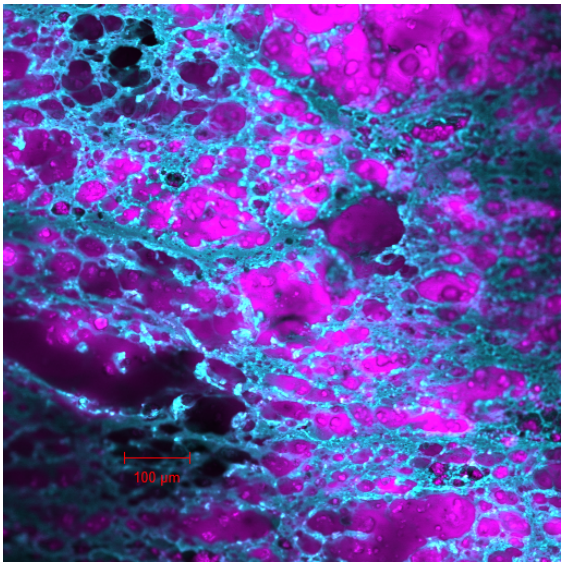

Figure 17. Laser scanning confocal micrograph of cheese made with 3.2% O/W_2 emulsion from Objective 1 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

proportion of cyan in WOW 16. The magenta regions in the RF CON sample appear to be more coalesced into fewer and larger regions. This would indicate that the fat in RF CON coalesced more than in WOW 16, leading to fewer individual droplets, and larger droplets overall.

It was also expected that WOW 32 and FF CON would have the same number of fat droplets for the same reason WOW 16 and RF CON would. However, in both WOW 32 and FF CON samples there are few, if any, distinct fat globules held within the protein matrix, but rather, the fat is all coalesced together while still interdispersing the protein network. As both of these samples had more fat than any of the other samples, it is not surprising that the fat has coalesced to this extent.

On visual inspection of the magenta and cyan regions in the WOW 32 and FF CON samples, it does appear there were more magenta pixels in the FF CON images. From the cheese composition data, WOW 32 had 17% fat and FF CON had 31% fat, so it is not surprising to see more magenta in the sample with more fat. However, as mentioned previously, it was expected to visualize the same number of fat droplets in the two samples. However, the LSCM images do not show obvious visual water dispersed in the fat in WOW 32, or any of the other $W_1/O/W_2$ samples, though it is not clear how this would show up in these images.

In the images of the $W_1/O/W_2$ emulsions (Figs 3 and 4), the aqueous W_1 portion of the double emulsions appeared within the fat droplets as spherical black areas devoid of any fluorescence. This was not seen when the double emulsion cheese samples were imaged, suggesting that the $W_1/O/W_2$ double emulsions destabilized and reverted to a simple O/W_2 emulsion, or there was not sufficient resolution using the method used to image the cheese to allow for the imaging of the W_1 droplets.

If the $W_1/O/W_2$ emulsions had reverted into simple O/W_2 emulsions, it would be expected that cheese made with $W_1/O/W_2$ emulsions would then act like a cheese with the respective fat reduction based on how much $W_1/O/W_2$ was added. It has been shown that typically with increased fat reduction, there is an increased hardness of the cheese (Banks et al., 1989). This was not the case with the cheeses made with $W_1/O/W_2$ emulsions during these experiments. Rather, they had similar hardness based upon the number of emulsion droplets used to make the cheese. That is, WOW 32 cheese had

comparable hardness to FF CON cheese, and likewise, WOW 16 cheese compared to RF CON, based on texture analysis results previously discussed.

TPA hardness is based on several other factors than fat amount, including moisture levels, pH, and distribution of the various components in cheese, but the cheesemakes were similar to eliminate many sources of error so that texture differences could be attributed more to the double emulsion and its affect than on other factors. Though WOW 16 did have higher moisture than RF CON, 46.0% compared to 40.4%, if this water was still within fat droplets it would be invisible to the protein network in creating a less hard texture. If the water was not in fat droplets, it is still possible that when the double emulsions destabilized, some of the water that was released remained in the protein network creating larger serum pockets, helping to keep the hardness the same as the control. In either circumstance, the double emulsion could theoretically play a contributing factor in allowing the hardness of the double emulsion cheese with less fat to be similar to the controls with more fat. WOW 32 was also higher in moisture than FF CON, 44.9% compared to 36.1%, respectively.

Whether the higher moisture in the double emulsion cheese was entrapped in the cheese by remaining in a stable $W_1/O/W_2$ double emulsion system or incorporation through larger serum pockets when the double emulsion destabilized is not clear based on the LSCM images. During the cheesemaking, when the cheese came out of the press, RF CON and FF CON were always larger in volume compared to the double emulsion cheese, while if the double emulsion had remained completely stable in the cheese, it would have been expected for the blocks of control and sample cheeses to be the same

size. This may point to the emulsion having destabilized and some of it being lost in the whey and some left behind as larger serum pockets in the cheese, but that is not conclusive based on the confocal images.

Objective 2

Due to lower than expected fat contents of the WOW cheeses in Objective 1, an experiment of $W_1/O/W_2$ double emulsion cheesemakes was designed and carried out as Objective 2. The main difference between Objective 1 and Objective 2 was that the emulsions were prepared with greater shear in Objective 2 in an attempt to deliver a smaller droplet size, and a small amount of inulin was added into W_1 of the $W_1/O/W_2$ double emulsions. Objective 2 consisted of manufacture of FF CON and WOW 32 cheeses. These were expected to have similar number of emulsion droplets added to the milk and represent cheese made from full-fat milk and a cheese with 40% fat reduction. An examination of their chemical composition and microstructure would then enable verification of stability of the $W_1/O/W_2$ emulsion within the cheese system.

$W_1/O/W_2$ Emulsion Droplet Microstructure. Images using LSCM were taken to verify the new $W_1/O/W_2$ emulsion preparation method worked in creating a double emulsion and a representative image is shown in Figure 18. In the image, the larger droplets are the oil droplets, and the texture within these oil droplets is the W_1 droplets, which appears as brighter white, possibly due to smaller W_1 droplets allowing for more light reflection to shine through from the fat behind the W_1 droplets. The oil droplets are dispersed in a continuous W_2 phase. The $W_1/O/W_2$ double emulsion was different from the emulsion produced by the method used in Objective 1with smaller W_1 droplets within

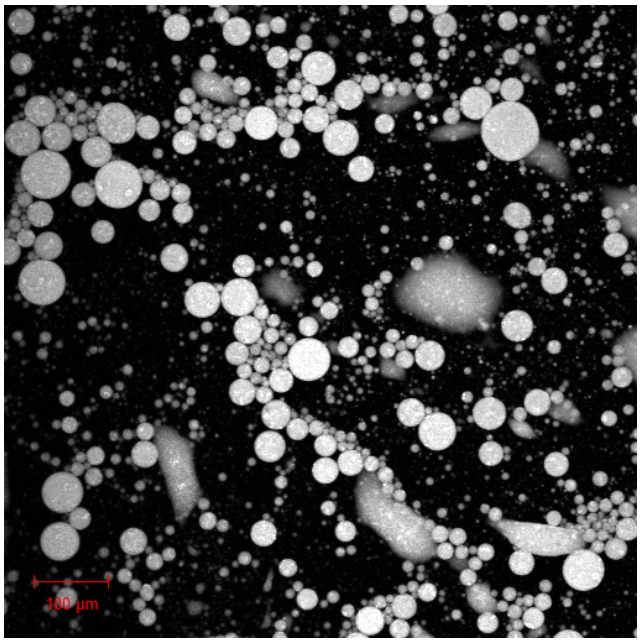

Figure 18. Laser scanning confocal micrograph image of milk fat $W_1/O/W_2$ double emulsion approximately 1 h after production with Nile Red used as the excitable dye to visualize the fat, as represented by the large white spheres in image, which was used for production of Objective 2 cheese. The inner water droplets within fat droplets are whiter than the oil droplets because due to their small size, the fluorescence from the dye in the fat behind and between the water droplets still manages to reflect and shine through.

the oil droplets. The W_1 droplets are smaller because a higher shear rate and longer shear time were used in the homogenization of the first step of the process. These smaller W_1 droplets are only noticeable by the appearance of bright white texture within the oil droplets because they are so small.

$W_1/O/W_2$ Emulsion Droplet Size Analysis. Droplet size analysis was carried out on the $W_1/O/W_2$ double emulsion formulation used in Objective 2, as the procedure and ingredients had changed slightly compared to Objective 1 $W_1/O/W_2$ emulsion. The D_{32} means (± standard deviation) was 9.85 ± 0.56 µm and the D_{43} was 21.94 ± 3.08 µm for Objective 2 AMF $W_1/O/W_2$ double emulsions, while the D_{32} and D_{43} for the AMF $W_1/O/W_2$ double emulsion used in Objective 1 were 3.09 ± 0.16 and 7.00 ± 0.54µm,

respectively. Both the D_{32} and D_{43} measured values for Objective 2 emulsion were significantly greater than those for Objective 1. See Appendix F for specific statistical analysis.

Objective 2 $W_1/O/W_2$ emulsion had 1% inulin and 0.5% NaCl added to W_1. From the confocal image of the Objective 2 double emulsion, it appears that the W_1 droplets are much smaller than those in Objective 1, but droplet size analysis shows that the oil droplets are much larger in Objective 2. Because of the smaller W_1 droplets, it was deemed that this emulsion could be used for the cheesemake.

Cheese Composition. Cheese composition data for Objective 2 is contained in Table 2. Salt values between WOW 32 and FF CON were not significantly different, and though pH was significantly different, this difference was marginal and both were within the normal range for Cheddar cheese. See Appendix G for specific statistical analysis.

Both moisture and fat were significantly different. WOW 32 cheese was expected to have 40% less fat than FF CON, assuming the same fat retention for each sample, while the actual fat reduction was 29% (and only 20% when considered on a dry basis). Likewise it was expected that if the $W_1/O/W_2$ double emulsion droplets remained intact there would be an increase in moisture content of the WOW 32 cheese compared to FF CON. There was only a 20% increase in moisture (and only 5% increase when considered on a moisture on a fat free basis) which indicates that a significant amount of the W_1 phase in the $W_1/O/W_2$ double emulsions was lost from the emulsion droplets during cheesemaking. Such destabilization of the $W_1/O/W_2$ double emulsion droplets would reduce the number of individual droplets present in the cheese. This would in turn

Table 2. Mean composition of double and single emulsion ($W_1/O/W_2$ or O/W_2) Objective 2 cheeses.

(%)	Objective 2 Cheeses[1]		
	WOW 32	**FF CON**	**P-value**
Moisture	42.4 ± 1.0^{a}	35.4 ± 0.5^{b}	0.004
Fat	22.8 ± 0.3^{b}	32.3 ± 0.4^{a}	<0.001
pH	5.30 ± 0.03^{b}	5.37 ± 0.04^{a}	0.002
Salt	1.75 ± 0.13	1.73 ± 0.09	0.803

[1]WOW32 = 3.2% double emulsion added for cream; FF CON = 3.2% O/W emulsion added for cream

[a-b]Means within a row with the same superscript letter were not significantly different

have a detrimental effect on being able to retain moisture entrapped in the cheese protein matrix. With less entrapped moisture in the cheese matrix, there is a lower yield of cheese. Then when fat is measured as a percentage of the total mass of cheese a higher percent of fat is obtained with a lower moisture cheese.

The 42.4% moisture content of the WOW 32 cheese made in Objective 2 was slightly lower than the 44.9% moisture content of the WOW 32 cheese made in Objective 1. Therefore, there was not any apparent improvement in $W_1/O/W_2$ double emulsion stability during cheesemaking as a consequence of adding 1% inulin to the W_1 phase or using the higher shear to decrease the size of the W_1 phase droplets.

Cheese Microstructure. Images using LSCM of the 2 Objective 2 cheeses were similar to those of Objective 1, where cyan pixels represent protein and magenta pixels represent fat. It was not clear from the LSCM images if there was still water in the fat or if the $W_1/O/W_2$ had reverted to an O/W_2 single phase emulsion. In visually comparing the images, there does appear comparable amount of protein and fat in each sample and, different from Objective 1 images, the fat droplets have retained more individuality rather than a majority of the fat coalescing. This could be due to the revised emulsification

techniques used for Objective 2. A lesser degree of coalescence in Objective 2 cheese microstructure images, compared to Objective 1 images for the same cheeses, shows that the addition of inulin and increased homogenization had an effect on microstructure. The WOW 32 and FF CON images also appear much more similar to each other in Objective 2 than they did in Objective 1. Representative images from Objective 2 cheeses can be found in Figures 19 and 20.

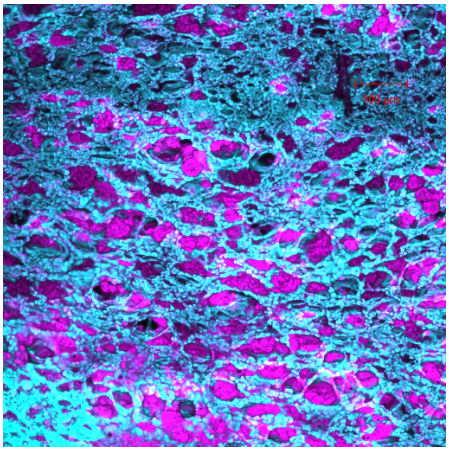

Figure 19. Composite laser scanning confocal micrograph based on a 30 mm z-stack of images of cheese containing 3.2% $W_1/O/W_2$ double emulsion added to milk from Objective 2 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, white areas represent areas in which both protein and lipid were present in the z-dimension, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum.

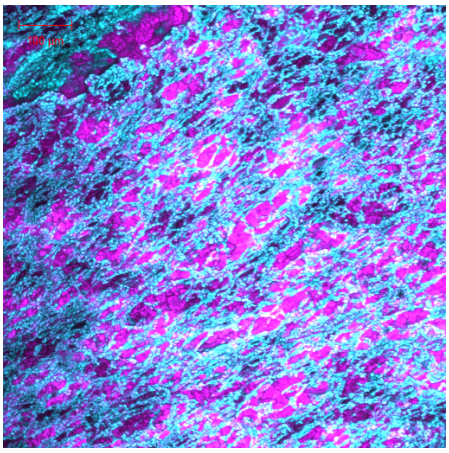

Figure 20. Composite laser scanning confocal micrograph based on a 30 mm z-stack of images of full-fat control cheese from Objective 2 after 6 wk storage. Magenta corresponds with fluorescence from Nile Red in the presence of lipid material, cyan corresponds with fluorescence from fluorescein isothiocyanate associated with protein, white areas represent areas in which both protein and lipid were present in the z-dimension, and black areas were indicative of regions devoid of both lipid and protein and assumed to be serum

CONCLUSION

A sufficiently stable $W_1/O/W_2$ double emulsion containing AMF as the oil phase was made such that it could be added to milk for use in cheese production. Such emulsions had similar stability to $W_1/O/W_2$ double emulsion containing vegetable oil as the oil phase. Stability was similar at 30°C to 40°C, so there was no need to change the cheesemaking parameters. Using higher shear during the emulsion make procedure produced smaller inner aqueous droplets, and doing so with 1% inulin appeared to be better for the system. This work with double emulsion fabrication showed potential for a practical and simplified method of production of double emulsions containing milk fat for use in reduced-fat cheese.

Cheese made using a $W_1/O/W_2$ double emulsion had similar to slightly improved textural qualities compared to cheese made with a simple O/W_2 emulsion, based on the G' and G'' viscoelasticity measurements, along with TPA hardness. However, in these experiments the retention and stability of the fat droplets as a $W_1/O/W_2$ double emulsion was not as high as expected. Though cheese was made successfully when double emulsion was added in place of cream, the retention appeared to be less than expected, but positive texture results give promise for continued research for improvement of reduced-fat cheese texture through double emulsion application.

I have shown that a $W_1/O/W_2$ double emulsion based on milkfat can be prepared that remains sufficiently stable to be added into milk and incorporated into curd upon renneting of the milk. Further research on emulsion preparation techniques to achieve greater $W_1/O/W_2$ double emulsion stability and retention during curd handling and cheese

manufacture is needed. Based on this research, likely next steps could include fabrication of smaller double emulsion droplets, changing the inner aqueous phase to have additional inulin or another gelling agent added, discovering at what point the double emulsion destabilizes, and further studying texture during aging with these additional changes. If the $W_1/O/W_2$ double emulsion integrity can be maintained then even greater textural improvements than were seen in these experiments could be achieved.

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APPENDICES

Appendix A: Objective 1 Emulsion Stability Analysis

The GLM Procedure
Dependent Variable: PeakThickness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	15.44318333	2.20616905	18.14	<.0001
Error	16	1.94640000	0.12165000		
Corrected Total	23	17.38958333			

R-Square	Coeff Var	Root MSE	deltabs Mean
0.888071	8.160271	0.348784	4.274167

Source	DF	Type III SS	Mean Square	F Value	Pr > F
fat	1	4.87801667	4.87801667	40.10	<.0001
temperature	3	10.38808333	3.46269444	28.46	<.0001
fat*temperature	3	0.17708333	0.05902778	0.49	0.6973

Appendix B: Objective 1 Droplet Size Analysis

The TTTest Procedure
Variable: D_{32}

oil	N	Mean	Std Dev	Std Err	Minimum	Maximum
1	3	3.0895	0.1567	0.0905	2.9770	3.2685
2	3	2.6040	0.0594	0.0343	2.5370	2.6500
Diff (1-2)		0.4855	0.1185	0.0968		

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	5.02	0.0074
Satterthwaite	Unequal	2.5622	5.02	0.0220

TTest Procedure
Variable: D_{43}

oil	N	Mean	Std Dev	Std Err	Minimum	Maximum
1	3	7.0023	0.5388	0.3111	6.5040	7.5740
2	3	6.5070	0.7473	0.4315	5.6720	7.1130
Diff (1-2)		0.4953	0.6514	0.5319		

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	0.93	0.4044
Satterthwaite	Unequal	3.6368	0.93	0.4093

Appendix C: Objective 1 Cheese Proximate Analysis

The GLM Procedure
Dependent Variable: acidity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01318667	0.00219778	1.17	0.4076
Error	8	0.01505333	0.00188167		
Corrected Total	14	0.02824000			

R-Square	Coeff Var	Root MSE	acidity Mean
0.466950	0.818148	0.043378	5.302000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.00588000	0.00294000	1.56	0.2674
cheese	4	0.00730667	0.00182667	0.97	0.4739

Dependent Variable: moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	214.3561467	35.7260244	23.16	0.0001
Error	8	12.3402267	1.5425283		
Corrected Total	14	226.6963733			

R-Square	Coeff Var	Root MSE	moisture Mean
0.945565	2.919480	1.241986	42.54133

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.8845733	0.4422867	0.29	0.7581
cheese	4	213.4715733	53.3678933	34.60	<.0001

Dependent Variable: salt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.09734667	0.01622444	1.17	0.4068
Error	8	0.11094667	0.01386833		
Corrected Total	14	0.20829333			

R-Square	Coeff Var	Root MSE	salt Mean
0.467354	8.117915	0.117764	1.450667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.08485333	0.04242667	3.06	0.1031
cheese	4	0.01249333	0.00312333	0.23	0.9168

Dependent Variable: fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	829.2000000	138.2000000	35.29	<.0001
Error	8	31.3250000	3.9156250		
Corrected Total	14	860.5250000			

R-Square	Coeff Var	Root MSE	fat Mean
0.963598	10.58179	1.978794	18.70000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	23.1750000	11.5875000	2.96	0.1091
cheese	4	806.0250000	201.5062500	51.46	<.0001

Appendix D: Objective 1 Cheese Rheology Analysis

The Glimmix Procedure
Response Variable: gprime

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	12.31	0.0017
month	1	10	9.26	0.0124
emultype*month	4	10	0.78	0.5646

Response Variable: gdoubleprime

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	12.53	0.0016
month	1	10	19.12	0.0014
emultype*month	4	10	0.58	0.6870

Appendix E: Objective 1 Cheese TPA Analysis

The Glimmix Procedure

Response Variable: hardness

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	15.39	0.0008
week	2	20	40.42	<.0001
emultype*month	8	20	1.06	0.4301

Response Variable: adhesiveness

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	0.27	0.8883
month	2	20	15.21	<.0001
emultype*month	8	20	0.67	0.7096

Response Variable: springiness

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	2.44	0.1312
month	2	20	2.14	0.1441
emultype*month	8	20	1.05	0.4346

Response Variable: cohesiveness

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
emultype	4	8	231.34	<.0001
month	2	20	30.38	<.0001
emultype*month	8	20	2.56	0.0420

Appendix F: Objective 2 Droplet Size Analysis

The TTEST Procedure
Variable: d32

oil	N	Mean	Std Dev	Std Err	Minimum	Maximum
1	3	3.0895	0.1567	0.0905	2.9770	3.2685
3	3	9.8503	0.5587	0.3226	9.2070	10.2140
Diff (1-2)		-6.7608	0.4103	0.3350		

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	-20.18	<.0001
Satterthwaite	Unequal	2.3128	-20.18	0.0012

The TTEST Procedure
Variable: d43

oil	N	Mean	Std Dev	Std Err	Minimum	Maximum
1	3	7.0023	0.5388	0.3111	6.5040	7.5740
3	3	21.9417	3.0838	1.7804	18.3950	23.9900
Diff (1-2)		-14.9393	2.2136	1.8074		

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	4	-8.27	0.0012
Satterthwaite	Unequal	2.122	-8.27	0.0120

Appendix G: Objective 2 Cheese Proximate Analysis

The GLM Procedure
Dependent Variable: acidity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.01091667	0.00363889	218.33	0.0046
Error	2	0.00003333	0.00001667		
Corrected Total	5	0.01095000			

R-Square	Coeff Var	Root MSE	acidity Mean
0.996956	0.076523	0.004082	5.335000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.00490000	0.00245000	147.00	0.0068
cheese	1	0.00601667	0.00601667	361.00	0.0028

Dependent Variable: moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	74.84411667	24.94803889	91.31	0.0109
Error	2	0.54643333	0.27321667		
Corrected Total	5	75.39055000			

R-Square	Coeff Var	Root MSE	moisture Mean
0.992752	1.344569	0.522701	38.87500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	1.83330000	0.91665000	3.36	0.2296
cheese	1	73.01081667	73.01081667	267.23	0.0037

Dependent Variable: salt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.02186667	0.00728889	0.55	0.6963
Error	2	0.02653333	0.01326667		
Corrected Total	5	0.04840000			

R-Square	Coeff Var	Root MSE	salt Mean
0.451791	6.619599	0.115181	1.740000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.02080000	0.01040000	0.78	0.5606
cheese	1	0.00106667	0.00106667	0.08	0.8034

Dependent Variable: fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	165.5783333	55.1927778	441.54	0.0023
Error	2	0.2500000	0.1250000		
Corrected Total	5	165.8283333			

R-Square	Coeff Var	Root MSE	fat Mean
0.998492	1.261940	0.353553	28.01667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
repl	2	0.2033333	0.1016667	0.81	0.5515
cheese	1	165.3750000	165.3750000	1323.00	0.0008