STABILITY OF DOUBLE EMULSIONS FOR FOOD APPLICATIONS

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STABILITY OF DOUBLE EMULSIONS FOR FOOD APPLICATIONS

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ABSTRACT

STABILITY OF DOUBLE EMULSIONS FOR FOOD APPLICATIONS

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Double emulsion technology has potential effect on development of diversity and quality of functional foods by decreasing the oil and salt concentration, encapsulating and controlled release of valuable components.

In this study, it was aimed to formulate stable double emulsions to be used for reduction of oil content of mayonnaise samples. W1/O ratios of primary emulsions, stabilized by polyglycerol polyricinoleate, were designed as 2:8, and 4:6, and (W1/O)/W2 ratios of double emulsions were 2:8, and 4:6. Double emulsion ratios, homogenization methods applied to primary emulsion (high speed homogenization, ultrasonic homogenization), and emulsifier types used in W2 phase (sodium caseinate, xanthan gum, lecithin-whey protein concentrate) were used as independent variables. Particle size and distributions, stability, encapsulation efficiency, rheological properties, long term stability and morphological properties of double emulsions were investigated. The most

acceptable double emulsions were integrated into mayonnaise samples and quality parameters were investigated.

Double emulsions prepared by sodium caseinate, and primary emulsions at W1/O ratios of 2:8 and 4:6 and (W1/O)/W2 ratio of 4:6 were found to have the higher stability values, higher apparent viscosity and lower particle size. High speed homogenization applied to primary emulsion affected particle size and viscosity positively, but did not affect stability and encapsulation efficiency of the double emulsions. Mayonnaise sample containing sodium caseinate at a ratio of 4:6 were not different from control mayonnaise in terms of stability and particle size. In addition, by the help of double emulsion, it was possible to reduce oil content of mayonnaise to 36.6%.

Key Words: Mayonnaise, double emulsion, oil content reduction, homogenization method

ÖZ

GIDA UYGULAMALARINDA ÇİFT EMÜLSİYONLARIN KARARLILIĞI

Yıldırım, Merve

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İkili emülsiyon teknolojisi, gıdalardaki yağ ve tuz miktarının azaltılmasında, değerli bileşenlerin kaplanmasında ve bu maddelerin kontrollü salınımıyla fonksiyonel gıdaların çeşitliliğini ve kalitesini arttırmada potansiyel etkiye sahiptir.

Bu çalışmada, mayonezin yağ miktarının azaltılması için kullanılmak üzere, stabil ikili emulsiyon formulasyonlarının oluşturulması amaçlanmıştır. Stabilizatör olarak Polyglycerin Polyrisinoleat (PGPR) ile hazırlanan birincil emülsiyonlardaki su-yağ (S1/Y) oranları (2:8, 4:6) ve ikili emülsiyondaki su-yağ-su ((S1/Y)/S2) oranları (2:8, 4:6) olarak belirlenmiştir. Çalışmada birincil emülsiyona uygulanan homojenizasyon yöntemi (yüksek hızlı homojenizasyon, ultrasonik homogenizasyon) ve S2 fazında kullanılan stabilizatör çeşidi (sodyum kazeinat, ksantan gum, lesitin-peynir altı suyu proteini konsantresi) bağımsız

değişken olarak kullanılmıştır. İkili emülsiyonların ve oluşturulan mayonez örneklerinin parçacık boyutu ve dağılımı, stabiliteleri, reolojik özellikleri, verim, uzun vade stabilitesi ve emülsiyon morfolojisi incelenmiştir. En çok kabul edilen ikili emulsiyon örnekleri mayonez örneklerine eklenilerek, kalite parametreleri araştırılmıştır.

Sodyum kazeinatla ve S1/Y oranları 2:8 ve 4:6 olan birincil emülsiyonlar kullanılarak (S1/Y)/S2 oranı 4:6 olan ikili emulsiyon örneklerinin en yüksek stabilite, en yüksek vizkozite ve en küçük parçacık boyutuna sahip olduğu gözlenmiştir. Birincil emülsiyona uygulanan yüksek hızlı homojenizasyon yönteminin ikili emülsiyonların parçacık boyutu ve vizkozitesini olumlu etkilediği, stabilitesini ve verimini istatistiksel anlamda önemli olarak etkilemediği bulunmuştur. Sodium kazeinatla ve 4:6 oranındaki birincil emulsiyonla hazırlanan mayonez örneklerinin stabilite ve parçacık boyutu açısından kontrol örneklerinden farklı olmadığı bulunmuştur. Ayrıca, ikili emulsiyon yardımıyla mayonezin yağ oranının % 36.6'ya indirilmesi mümkün olmuştur.

Anahtar Kelimeler: Mayonez, ikili emulsiyon, yağ miktarı azaltma, homojenizasyon yöntemi.

To,

My dearly loved ones who remind me, as the ax bites into the wood, to be comforted in the fact that the ache in our heart and the confusion in our soul means that we are still alive, still human, and still open to the beauty of the world, even though we have done nothing to deserve it.

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INTRODUCTION

1.1. Double Emulsions

Multiple emulsions are complicated arrangement of immiscible liquids; in which dispersed phase of a liquid enclose smaller globules of another liquid phase (Garti and Bisperink, 1998). Another description is that outer continuous phase is partitioned from inner dispersed phase with an liquid layer of another phase which is immiscible with other phases (Benichou et al., 2007). This complex interbedded structure of emulsion is known as emulsion of emulsion, or called double emulsion.

The term ‘multiple emulsions’, firstly, was mentioned in 1925 by a petroleum scientist William Seifriz. In his research about phase inversion of petroleum emulsions, he observed compartmentalized structures of emulsions. These intricate morphologies were stabilized by the presence of casein, a food grade protein (Seifriz, 1925). Potential inclusions of both hydrophilic and hydrophobic substances and phases in the same system were utilized in many research areas. Double emulsions are used in pharmaceutical industry, cosmetics, agricultural applications, chemistry as well as food applications.

Double emulsion utilization in pharmaceutical applications is promoted due to properties of controlled release of inner substance and involvement of active

substance inside the double emulsion. They are used as promoter for insulin intake in intestinal tract, (Cole & Whateley, 1997; Engel et al., 1968; Silva-Cunha et al., 1998), as replacer of red blood cell with multiple emulsion of hemoglobin (Zheng, 2009), as detoxification agent for drug overdose (Grossiord & Stambouli, 2007; Hamoudeh et al., 2006) as delivery agent of anticancer agents (Amjadi et al., 2013; Benoy et al., 1972; Higashi et al., 1999), for immobilization of active agents or enzymes (Baccar et al., 2011), for prolonged or sustained release of active substances and drugs (Giri et al., 2013; Gokhale & Jonnalagadda, 2013; Wang et al., 2012), and for encapsulation of vitamin or minerals (Klinkesorn, 2014; O'Regan & Mulvihill, 2010).

Double emulsions have beneficial effect on performance of active agents, controlled transportation of active agents, and production of novel products (Kanouni & Rosano, 2005; Lee et al., 2004; Tal-Figiel, 2007; Vasudevan & Naser, 2002).

As double emulsions provide probable opportunity of targeted delivery, controlled release, and encapsulation of substances with various solubility properties, they are used in various industries such as fuel energy (Lin & Wang, 2003), agriculture (ElShafei et al., 2010), chemical engineering (Pan et al., 2015) and even in lubricants, paints, and separation processes (Aserin, 2008; Sjoblom, 2001).

Although double emulsions constitutionally have a major problem of thermodynamic instability, they offer wide range of possible advantages over simple emulsions (Garti & Aserin, 1996). As many foods such as milk, mayonnaise, dairy spreads, butter, salad dressing, cake batter (Sjoblom, 2001) are emulsion systems, double emulsions become valuable technology to improve food emulsions.

1.1.1. Types of Double Emulsions

There are basically two types of double emulsions; water-oil-water (W/O/W) and oil-water-oil (O/W/O). The former one, water-in-oil-in-water, composed of three phases, where water droplets are dispersed in oil phase and this first emulsion is dispersed in another water phase. Two types of emulsifiers are used for emulsification of this system. Lipophilic emulsifier is employed for inner interface between water and oil, hydrophilic emulsifier is used for maintenance of second interface between oil and water. The second type double emulsion is constructed in the same manner and varying order of liquids and emulsifiers. In oil-water-oil (O/W/O), namely oil-in-water-in-oil, emulsions are composed of water globules, which contains oil phase, are evenly distributed in oil phase. For this type of emulsion arrangement, hydrophilic emulsifier is integrated into inner interface and lipophilic emulsifier is utilized for outer interface between water and oil (Friberg et al., 2003; Garti, 1997a; Muschiolik, 2007).

To summarize, W/O/W emulsions are composed of outer water phase enclosing oil phase which surrounds inner water phase; O/W/O emulsions are formed from outer oil phase enclosing water phase which surrounds inner oil phase. Selection of type of double emulsion is based on characterization of the product that the emulsion will be used in. W/O/W emulsions are more common than O/W/O emulsions, since they have extensive area of utilization (Khan et al., 2006). Yet, double emulsions are continued to be studied by many researchers and scientists and to be applied to many research areas.

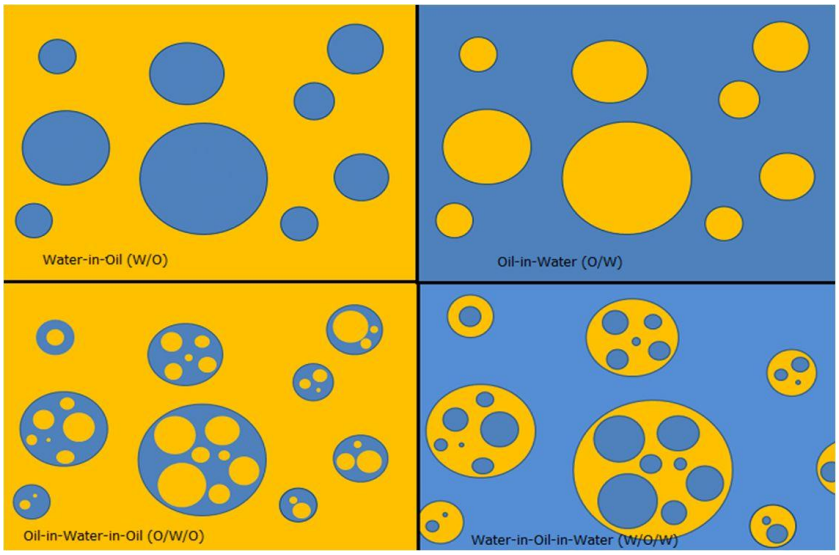

Figure 1.1 Types of emulsions

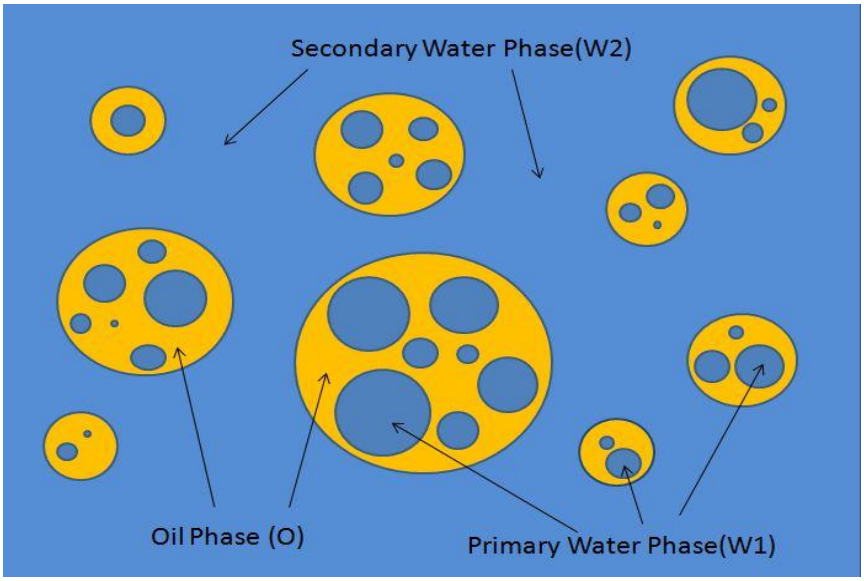

Figure 1.2 Double emulsion of W/O/W

1.2. Double Emulsion in Food Systems

Using double emulsion in food systems is a recent research area. Efficient incorporation of double emulsions into food systems flourish the variety and improve the quality of functional foods by integrating bioactive components, controlling the release of active and sensitive molecules, reducing salt, and fat content of food systems (Garti, 1997b; Sapei et al., 2012).

Potential advantages of double emulsions in food system can be summarized as;

- Encapsulation and controlled release of water-soluble, oil soluble ingredients individually or at the same time,
- Encapsulation and controlled release of minerals, vitamins, antioxidants, flavoring agents or aroma ingredients to mask unwanted flavor, probiotics, micronutrients (sensitive to light, temperature, oxygen, pH)
- Production of functional food with the help of added valuable ingredients,
- Reduction of fat content of food systems,
- Reduction of salt content of food systems (Cofrades et al., 2013; Dickinson, 2010; Jiménez-Colmenero, 2013; Prichapan, 2014).

Despite reasonable advantages of double emulsions, food products with double emulsions are still rare in the market due to instability problems. Scientists try to explore and improve stability of double emulsions and integrate them into products (Benichou et al., 2004; Cofrades et al., 2013).

Cofrades and his colleagues (2013) aimed to reduce fat content of pork meat by means of double emulsions, (W/O/W). They prepared double emulsions by two-step emulsification method. For the first emulsification of W/O, 6% PGPR was introduced in olive oil phase as a lipophilic emulsifier. After pre-homogenization

of first emulsion, it was also homogenized by 2-stage high-pressure homogenizer at 54999/ 6998 kPa which correspond to pressures applied first and second homogenization step, respectively. For the second emulsification step, sodium caseinate solution of 0.5% or whey protein concentrate solution of 6% was employed as a hydrophilic emulsifier. For production of W/O/W emulsion, pre-homogenization was done by blender and 2-stage; high-pressure homogenizer was operated for further homogenization at milder pressures of 14996/ 2999 kPa. Produced double emulsions were analyzed by considering particle size and distribution, stability and microstructures of emulsions. The best resulting double emulsions were incorporated into meat system, and texture, fat & water binding capacities and color of these systems were evaluated. It was concluded that double emulsion incorporation into meat system did not affect the water and fat binding capacity and color of products, significantly (Cofrades, 2013).

Reduction of fat content by double emulsions was not limited to meat systems. Lobato-Calleros et al. (2008) worked on reduction of fat content of fresh cheese by introducing W/O/W emulsion into cheese matrix. The produced W/O/W emulsions were homogenized by rotor stator with two step emulsification method. While PGPR was chosen as lipohilic emulsifier for inner emulsion, low methoxyl pectin, carboxymethylcellulose, and gum Arabic were selected as hydrophilic emulsifiers to stabilize the outer interface. As water (W1) phase was encapsulated into canola oil, fat content of overall cheese product was reduced by inclusion of double emulsions. It was concluded that each hydrophilic emulsifiers affected the parameters differently. While reduced fat cheese with double emulsions containing gum Arabic and low methoxyl pectin resulted in higher hardness values, ones with carboxymethylcellulose appeared to have minimum hardness values. It was suggested that double emulsion constructed by gum Arabic and low methoxyl pectin might be used in order to reduce fat

content for cheese products without significant loss of quality (Lobato-Calleros et al., 2008).

Lobato-Calleros and his colleagues (2009) also applied this technique to other dairy products like low-fat yogurt. According to their inspection, fat content of full fat-stirred yogurt, which was 2.95%, could be reduced to 0.52 and 0.56% when low methoxy pectin (1%) and carboxymethylcellulose (0.5%) was used as stabilizer in double emulsions production, respectively. In this study, double emulsions imbedded into yogurt were prepared with two-step emulsification process. At each step of emulsification, high-shear homogenizer was used at various rotational speeds and time; 5800 rpm for 5 min and 5200 rpm for 10 min for the first and second emulsifying step, respectively. PGPR was used as lipophilic emulsifier, DATEM (diacetyl tartaric acid ester of mono- and diglycerides) was used as hydrophilic emulsifier, and biopolymers of amidated low methoxy pectin and carboxymethylcellulose was served as stabilizing agent in the food system. According to particle size, rheological properties and micro structural analysis, properties of yogurt samples formulated with carboxymethylcellulose were similar to control full-fat yogurt samples (Lobato-Calleros et al., 2009).

The ingredients used in the system influence the stability of double emulsions. Marquez and Wagner (2010) studied the effect of a particular ingredient and its impact on parameter of double emulsion and the resulting food system. They studied the rheological properties of W/O/W emulsions which were formulated by soybean milk and sunflower oil. W/O/W emulsion system was similar to the formulation of whipped dairy cream. The emulsion was composed of 0.5%, 1.0% and 2.0% PGPR as lipophilic emulsifier. For second stage, soybean milk was stabilized by 0.2 % xanthan gum. Ultimate outcome of the study was that calcium addition to inner aqueous phase increased consistency and creamy texture of system (Marquez & Wagner, 2010) .

Another example of double emulsions usage for fat reduction in food system was studied in Mozarella cheese (Xu Yiwen, 2011). The design of double emulsion was based on two-step emulsification of W/O and W/O/W formation with homogenizer at 8000 rpm for 6 min and 7200 rpm for 13 min, respectively. It was aimed to replace butter in milk with double emulsion prepared with corn oil, and eventually reduce the total fat content in the Mozzarella cheese. Lipophilic emulsifier 5% PGPR was used as in oil phase of double emulsions, and 0.1% gellan gum was introduced in inner aqueous phase to increase stability of inner emulsion. In order to identify best hydrophilic emulsifier alternative for the system, gum Arabic, carboxymethylcellulose, low methyl pectin and their blends were investigated. As fat content of cheese decreased by inclusion of double emulsion, nitrogen and moisture content of cheese were elevated. On the other hand, yield of total product was declined. Harder cheese sample was obtained by using double emulsion with low methyl pectin since it underwent cross linking reaction with calcium which was introduced for osmotic balance of double emulsion (Xu Yiwen, 2011).

1.3. Preparation Methods

Preparation of double emulsion is done by mainly three methods: phase inversion method, membrane filtration and two-step emulsification.

1.3.1. Phase Inversion Method

Phase inversion is an emulsification method which refers to instant reverse change of emulsion arrangements, for example W/O emulsion turns into O/W or vice versa. In other words, continuous phase and dispersed phase of an emulsion interchange from inside to outside due to rapid variations in outside phase of the emulsion system. This phenomenon discovered incidentally was starting point of

double emulsion (Seifriz, 1925). Phase inversion is a widely applied technique in emulsions of cosmetic applications (Förster et al., 1994; Miller et al., 2001). It is also a useful technique for double emulsion formation by stabilizing inner emulsion (Hino et al., 2000; Matsumoto, 1986). Phase inversion can be accomplished by modifying particular properties like pH of system, ionic properties, temperature, volume fraction of continuous and dispersed phase or applying force or flow (Preziosi et al., 2013). Matsumoto (1986) suggested a definite procedure for double emulsion production by phase inversion. The study engaged phase inversion due to compositional changes of continuous phase and dispersed phase with systematic W/O/W production. Specified volume of oil phase involving lipophilic emulsifier (Span 80) was mixed with a pin-mixer and water phase including hydrophilic emulsifier was introduced into oil phase with constant volumetric rate while mixing continued. When volume fraction of water phase introduced into lipophilic phase exceeded 70 %, W/O/W emulsions could be observed. A rapid change in volume of dispersed phase contributes to substitution of continuous phase to be water phase containing oil droplets with fixed water particles inside. When further addition of aqueous phase continues and volume fraction of water phase exceeds 0.75, oil layer between the two aqueous phases disrupts and whole system inclined to be water-in oil simple emulsion, as seen in Figure 1.3 Phase inversion. Main advantage of this technique is that fine particulate emulsions can be produced at one step, but the control of phase inversion process is challenging (Garti, 1997b; Matsumoto, 1986).

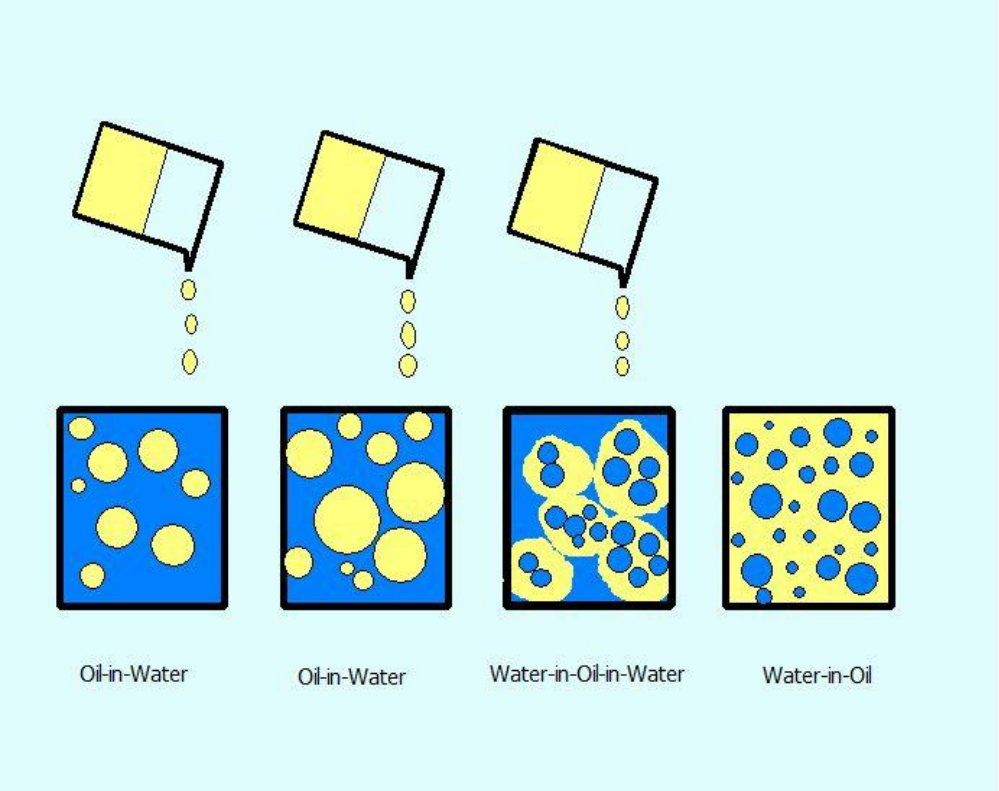

Figure 1.3 Phase inversion

1.3.2. Membrane Filtration

Another novel method to prepare double emulsion is membrane filtration or membrane emulsification. According to type of double emulsion to be produced, inner emulsion, O/W or W/O is produced by means of simple emulsification methods. The produced simple emulsion is termed as dispersed phase of double emulsions. Dispersed phase is allowed to pass through fine pore sized membrane by means of pressure difference, mechanical pressing, while continuous phase of double emulsion with proper emulsifier is flowing over external part of the

membrane (Figure 1.4 Membrane emulsification). The exiting refined droplets of dispersed phase were disengaged from the membrane after they attained critical droplet size for detachment. The overflow of the system becomes double emulsion (Joscelyne & Trägårdh, 2000; Vladisavljevic & Williams, 2005). The membrane emulsification technique is advantageous due to low level of energy input, ease of particle size control, narrower size distribution, and delicate process conditions (Schuch et al., 2014; Vandergraaf et al., 2005). This technique is practiced for production of double emulsion alone as well as combination of other techniques. Shima and his partners (2004) tried to decrease particle size and homogenize distribution of particle size by using membrane filtration after production of coarse W/O/W emulsion produced by two-step emulsification technique (Shima et al., 2004).

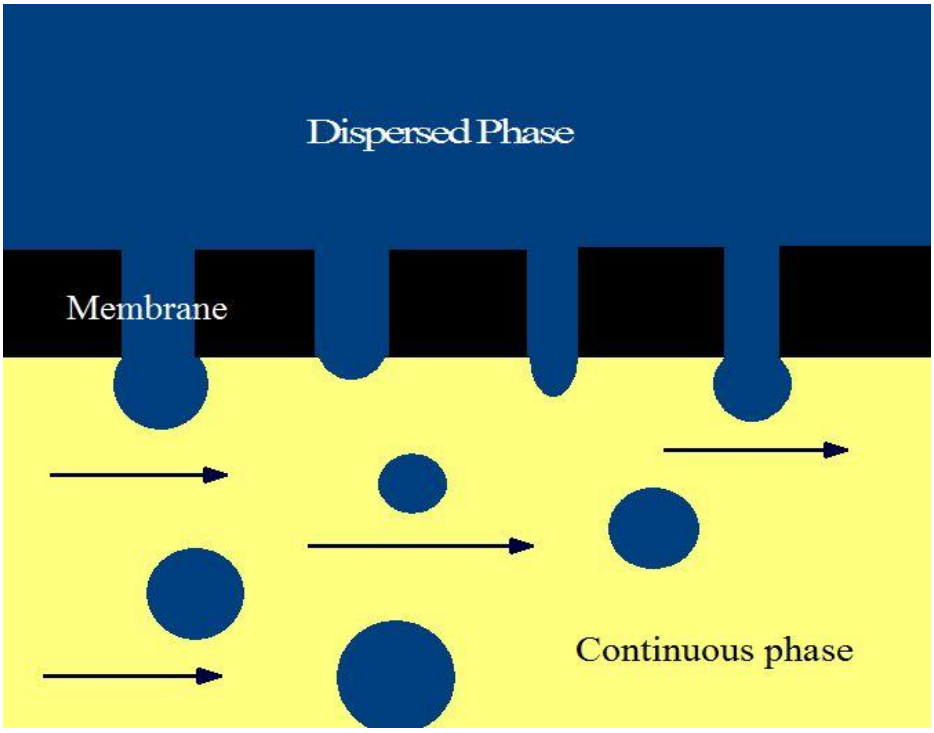

Figure 1.4 Membrane emulsification

1.3.3. Two-Step Emulsification

Two-step emulsification or two-stage emulsification is the most frequently used technique for the production of double emulsions due to its convenience, controllability, reproducibility, and feasibility. As in the other preparation techniques, emulsifiers working oppositely are required for two-step emulsification: hydrophilic and lipophilic emulsifiers. In the first step, inner emulsion of double emulsion is prepared with inclusion of proper emulsifier, by means of strong homogenization and harsh mixing, membrane emulsification or combination of them. At this stage minimum particle size is tried to be reached to produce stable double emulsions. In the second step of emulsification, freshly produced single emulsion is dispersed in proper continuous phase with a soluble emulsifier in it. At this stage of emulsification, homogenization is carried at mild and moderate levels of speed and pressure in order to prevent breakdown of inner emulsion. W1/O/W2 production with two-step emulsification can be summarized as follows;

- Lipophilic emulsifier is introduced into oil phase and completely dissolved. The functions of lipophilic emulsifier are to position at the interface of inner water phase (W1) and oil phase (O) and to stabilize the formed emulsion. Inner water phase (W1) is combined with oil phase and strong homogenization is performed under high shear conditions to make it homogeneous and to achieve fine droplets of water dispersed in continuous oil phase.
- Outer water phase (W2) is prepared with hydrophilic emulsifier to stabilize interface between oil phase and outer water phase. While W2 phase is being mixed with milder shear and speed, freshly formed simple emulsion of W1/O is introduced with drop-wise manner. As mixing

continues, W1/O emulsion is dispersed into W2 phase and W/O/W emulsion is prepared (Figure 1.5).

O/W/O emulsion can also be produced by the same procedure with proper order of emulsifier and arrangement of oil and aqueous phases (Garti, 1997b; Garti & Bisperink, 1998).

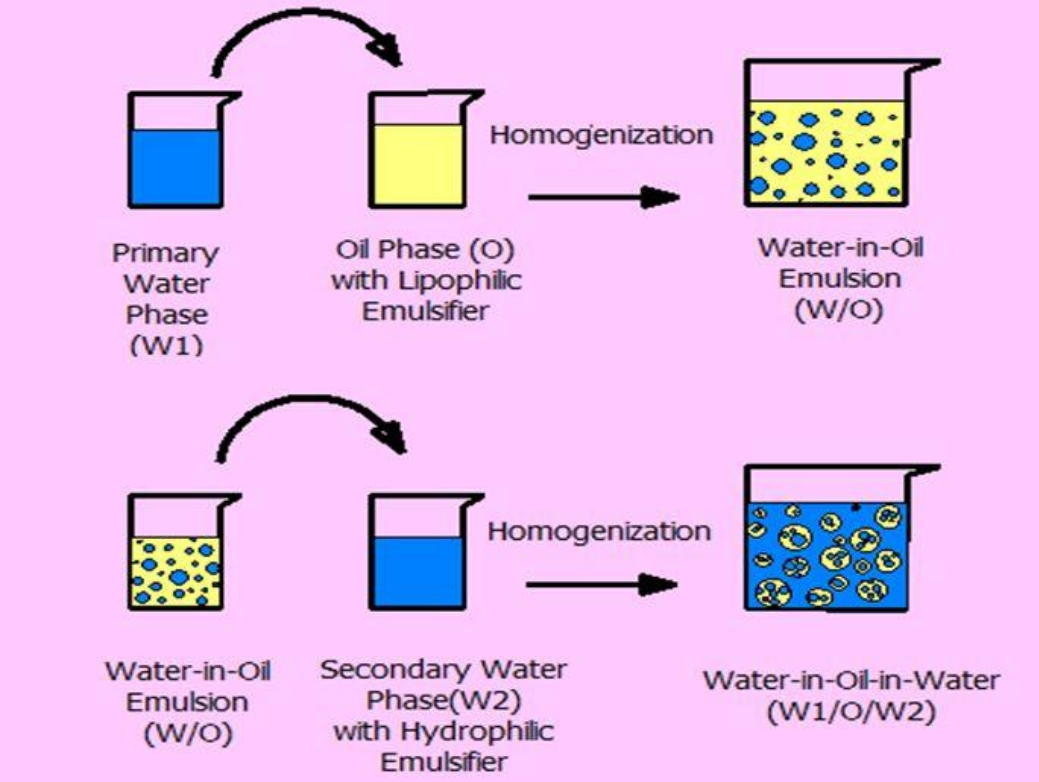

Figure 1.5 Two step emulsification

In various studies, two-step emulsification method is used to prepare double emulsion. Delample et al. (2014) studied the effect of embodied NaCl and D-glucose into inner water phase (W1) on gelation characteristics of double emulsions (Delample et al., 2014). In order to monitor release mechanism of the double emulsions, markers can also be involved into W1 phase of double emulsions (Scherze et al., 2005). According to the study of Fechner et al.(2007), double emulsions were formulated by the addition of 5% gelatin into inner water phase (W1) to obtain stable primary emulsion and eventually stable double emulsion (Fechner et al., 2007). Modifications in two-step emulsification can serve for encapsulation of substantial water soluble agents like carotenoid, vitamin and minerals (Jiménez-Colmenero, 2013).

1.4. Homogenization Methods

Homogenization is defined as the transposition of two or more immiscible liquids into evenly distributed emulsion system (McClements, 2004). Homogenization can be categorized as primary and secondary homogenization. In the presence of proper emulsifier and two immiscible liquids, if coarse particle of one liquid is dispersed into other liquid and formed emulsion, this process is called primary homogenization. Secondary homogenization, on the other hand, is defined as the process that reduces particle size of droplet of current emulsion (Akoh & Min, 2008; McClements, 2004). Without emulsifier, immiscible liquids arrange themselves according to their density, for instance, oil gathers on the top of water separated with minimum contact interface. In order to produce stable colloidal system, emulsifiers are needed to be adsorbed at the interface and to produce a protective layer for coalescence of produced droplets (McClements, 2004). Since homogenization method influences particle size and distribution, stability, rheology, physicochemical properties, appearance, color and production cost of emulsions, homogenization method

must be properly evaluated (McClements, 2004). Many researches focused on the effects of various homogenizers on characteristics of double emulsions (Khalid et al., 2014; Schuch et al., 2014; Vandergraaf et al., 2005).

1.4.1. High Speed Mixer

High speed mixers, namely high-shear mixers or rotor-stator mixers have feature of rotary head that can turn at the speed of 10-50 m/s, eventually they can reach high shear rates of 20,000 to 100,000 s^{-1}. According to the purpose of use, rotating part of the mixer can be in various sizes. This type of mixers deliver high amount of energy by the mixing head at high speeds, thus agitation might be localized around the rotating head (Zhang et al., 2012). The accelerated vertiginous turning of rotating head, up to 3600 rev/min, creates rotational, longitudinal, horizontal, vertical, and radial velocity components that interfere with the interface of water-oil. Existing velocity profiles dispense the water and oil equally throughout the batch processing vessel, result two immiscible liquids to become intermingle, and eventually decrease particle size of produced liquid droplets (McClements, 2004; Zhang et al., 2012). Movement of high-speed mixers is multifunctional processing of dissolving, stirring, blending, dispersing, mixing, emulsifying and de-agglomerating (Kowalski et al., 2011). As process time and rotational speed of high-speed mixer escalate, particle size of the produced droplets is reduced. Furthermore, concentration and characteristics of ingredients, viscosity of solution, power density of mixer, and temperature of solution affect particle size and stability of emulsion. Generally, diameter of particles produced by high-speed homogenizer ranges from 2 to 10 μm (McClements, 2004). According to Bi et al.(2014), high-speed mixer not only decreases the particle size, but also homogenizes the particle size distribution and lower polydispersity index of particle distribution (Bi et al., 2014).

According to research done by Ouzineb et al. (2006), it was concluded that particle size of miniemulsions decreases as time of homogenization increases (Ouzineb et al., 2006). In another study, it was concluded that high speed mixing was more effective than high pressure homogenizer. Stable nanoemulsions produced by high speed homogenizer had limited size distribution and smaller particle size (Scholz & Keck, 2015).

1.4.2. Ultrasonic Homogenization

Ultrasonic homogenization can be defined as homogenization technique that benefit from ultra-sound waves, ranges above 20 kHz, which produce shear and pressure gradients inside a sample and agitate solution as a result of turbulent and cavitational effects. The usage of ultrasound technology as a homogenization technique was reported by Wood and Loomis, in 1927 for the first time (Wood & Loomis, 1927). Since then, it has attracted attention in food and pharmaceutical applications (Merry & Eberth, 1984; Peshkovsky & Bystryak, 2014; Villamiel & de Jong, 2000; Zisu et al., 2011). Although a couple of methods for producing ultrasound has been explored, piezoelectric transducer for laboratory applications and liquid jet generators for industrial usage are the most accepted techniques (McClements, 2004). Condensed ultra-sonic waves are produced by oscillation of piezoelectric crystal induced by high-intensity electric field in piezoelectric transducers (Kaci et al., 2014). For liquid jet generators, oscillation of sharp blades is procured by exit of liquid from a small nozzle, and vibrations of the blade create high-intensity ultra-sound waves.

The main phenomena determining activity of ultrasonic homogenization is the acoustic cavitations (Tang et al., 2013). Under high-intensity acoustic field, formation of vapor cavities was observed due to fluctuations of pressure inside the liquid system. At reduced pressures and ambient temperature dissolved gas nuclei inside the liquid start to expand and collapse after reaching a critical

diameter. Disintegration of vapor bubble cause local increase in temperature and local turbulence due to formation of liquid delocalization at very high speeds (Suslick, 1989). Ultimately, dense shear and pressure gradient, localized turbulence, namely acoustic cavitations, result in decrease size of oil droplets (Kentish et al., 2008; Jafari et al., 2006). During ultrasonic homogenization, temperature increase can be observed, so for heat sensitive emulsion systems temperature should be controlled in a proper way (McClements, 2004).

Various researches are available in the literatures that study the influence of ultrasonic homogenization on emulsion systems and the comparison of ultrasonic homogenization with existing alternative homogenizers. Jafari et al.(2006) evaluated performance of ultrasonication and microfluidization to produce nano-emulsion encapsulating d-limonene. According to their findings, ultrasonication is comfortable to use and easy to clean compared to microfluidization. On the other hand, size distribution of emulsion samples treated with microfluidization is narrower (Jafari et al., 2006). According to research conducted by Tang et al.(2013) which was on nanoemulsion for aspirin production, ultrasonication was found to be more energy-saving compared to microfluidizer for nanoemulsion formation (Tang et al., 2013). Regarding particle size of emulsion and energy efficiency of the process, ultrasonic homogenization is found to be more advantageous compared to mechanical agitation (Abismaïl et al., 1999). Although ultrasonication is widely applied for emulsification and characterization of simple emulsions, its usage for double emulsion formulation is very limited. Tang et al. (2003) studied the effect of gelling and osmotic pressure characteristics of W/O/W emulsions of aspirin. In this study, W/O/W emulsions are produced with the assistance of ultrasonic homogenization, and it was reported that ultrasonic cavitation was efficient for production of stable and uniform multiple emulsions (Tang et al., 2013).

1.4.3. Microfluidization

Microfluidization is a widely employed technique for homogenization of emulsion and reduction of particle size of colloidal dispersions. After invention of microfluidizer and practice for production of emulsions (Washington & Davis, 1988), it is used for primary and secondary homoganizations by many researchers (Olson et al., 2004; Pinnamaneni et al., 2003; Talsma et al., 1989). Microfluidizer is characterized by stable emulsions with mean particle size of 5 μm-50 nm (Fernando et al., 2007). Microfluidizer consist of fluid inlet, air-driven intensifier pump and interaction chamber. Supplied fluid from fluid inlet is driven into interaction chamber by high pressure (up to 270 MPa) produced by intensifier pump. Accelerated steams of emulsions are forced to impinge into interaction chamber through microchannels (McClements, 2004; Tang et al., 2013). High shear resulted by high pressure and cavitation within the interaction chamber helps to produce emulsion with small particles (Jafari et al., 2006).

In literature, it was concluded that increasing pressure and processing time could decrease particle size of the emulsion (Jafari et al., 2006). Microfluidization is comparable to ultrasonication regarding to emulsification efficiency (Jafari et al., 2007; Maa & Hsu, 1999; Jafari et al., 2006; Strawbridge et al., 1995). Major problem of microfluidization is that beyond optimum pressures and number of cycles, re-coalescence of emulsion droplets occur, and eventually cause over-processing (Jafari et al., 2007).

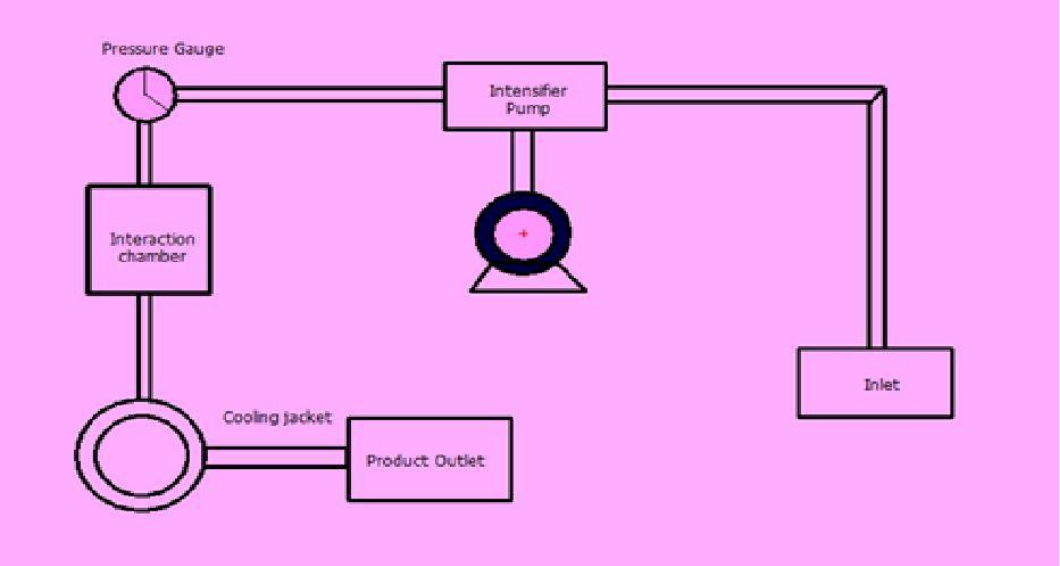

Figure 1.6 Microfluidizer

1.5. Emulsifiers

Emulsifiers, in other words surface active agents, are amphiphilic biopolymers that serve as protector of emulsion droplets from coalescence or aggregation by adsorbtion to the interface of two immiscible liquid (Hasenhuettl & Hartel, 2008; McClements, 2004). Emulsifiers in food systems can also serve as stabilizer, dough strengthener, clouding agent, anti-sticking agent, gloss enhancer, and mouth-feel developer (Hasenhuettl & Hartel, 2008). Emulsifiers have two oppositely serving parts which are hydrophilic portion aligning at the water (hydrophilic phase) and lipophilic portion orienting over oil (lipophilic phase) (Tadros, 2013). There are many emulsifiers that are currently used in

food industry such as mono- and di-glycerides, lecithin, polysorbates, sucrose esters, polyglycerol esters (Chen & Rosenthal, 2015).

Formation of double emulsion requires two different emulsifiers since double emulsion consists of at least two interfaces of water-oil and oil-water. Hydrophobic (lipophilic) emulsifier is needed for stabilization of interface of internal water-in-oil emulsion, whereas hydrophilic emulsifier is required to stabilize outer interface of oil-in-water emulsion. Selections of emulsifier for each interface are determined according to dispersed and continuous phase (Garti & Bisperink, 1998). Since main promoter of stability of emulsion is emulsifier, proper emulsifier selection is crucial for good stability (Benichou et al., 2004).

1.5.1. HLB Value (Hydrophilic/Lipophilic Balance)

HLB value is a quasi-empirical classification technique which provides information about the balance of lipophilic and hydrophilic properties of the emulsifier. The hydrophile-lipophile balance is scaled with number which shows relative affinity of emulsifier for oil or water phase (McClements, 2004). HLB value designates the activity of emulsifier, and determines type of emulsion that emulsifier would be incorporated in (Hasenhuettl & Hartel, 2008). To stabilize and emulsify oil-in-water (O/W) emulsions, hydrophilic emulsifiers with larger HLB values are used. In other words, lipophilic emulsifiers with smaller HLB were benefited to stabilize water-in-oil emulsions (Chen & Rosenthal, 2015). HLB values of emulsifiers are available in the literature, and selection of emulsifier for a specific emulsion can be done accordingly (McClements, 2004).

1.5.2. Lipophilic Emulsifier

Emulsifiers with low HLB value and soluble in oil phase is defined as lipophilic emulsifiers. For W/O/W formation, they are used to stabilize interface of inner emulsion (water-oil interface) (Garti & Aserin, 1996). Polyglycerol polyricinoleate (PGPR), lecithin and Span 80 are the most frequently used lipophilic emulsifiers for double emulsion of W/O/W (Aditya et al., 2015; Scherze et al., 2006; Shima et al., 2004). PGPR has been concluded to be highly competent for stabilizing water-in oil emulsions (Dickinson, 2010; Surh et al., 2007).

In the research conducted by Scherze et al. (2006), the effect of homogenization methods on double emulsions formulated with lecithin and PGPR were compared. It is acquired that w/o emulsion containing PGPR can be produced with simple rotor-stator homogenization (Scherze et al., 2006). In the research done to observe synergistic effects of PGPR and sodium caseinate on stabilization of W/O/W emulsions, it is concluded that increasing concentration of PGPR from 0.5% to 8% (w/v) had no significant effect on particle size of resultant double emulsion (Su et al., 2006).

Lecithin which consists of phospholipids, glycolipids, carbohydrates, triglycerides, sterols and free fatty acids, is naturally formed emulsifier that can be obtained from basically soybean, and egg (Faergemand et al., 2003). Since it is a byproduct of soybean oil manufacture, soybean lecithin is commonly used as emulsifier in food emulsion systems (Stauffer, 2005). Naturally extracted lecithin is soluble in both oil and water due to its both lipophilic and hydrophilic groups. This characteristic enables lecithin to be used as emulsifier of oil-in-water or water-in-oil emulsion. Thus, natural lecithin should be used with blend of proper emulsifier of prepared emulsion (McClements, 2004).

Polyglycerol polyricinoleate (PGPR) is a powerful lipophilic emulsifier produced by esterification of castor oil fatty acid (Márquez et al., 2010). Although it is widely employed as viscosity enhancer for chocolate production, it is also used a lipophilic emulsifier for water-in-oil emulsion formulations (Hasenhuettl & Hartel, 2008). In many researches related to double emulsions, PGPR is used as lipophilic emulsifier due to its high-stability emulsion forming characteristics (Frasch-Melnik et al., 2010; Hattrem et al., 2014; Iqbal et al., 2013; Kaimainen et al., 2015; Lutz et al., 2009; Oppermann et al., 2015; Tabatabaee et al., 2014).

1.5.3. Hydrophilic Emulsifier

Stabilization of outer interface of W/O/W emulsion is enabled with the presence of hydrophilic emulsifier which is incorporated into water phase (W2) before secondary emulsification (Garti, 1997). Hydrophilic emulsifiers are characterized by high HLB value and solubility in water phase (Pal, 2007; Pradhan & Rousseau, 2012). The influence of various hydrophilic emulsifiers on stability of double emulsions is available in the literature (Dalgleish, 2006; Delample et al., 2014; Su et al., 2008; Tabatabaee et al., 2014). Apart from the emulsifiers, protein and polysaccharides like whey protein (Carrillo-Navas et al., 2012), xanthan gum (Knoth et al., 2005), gelatin (Knoth et al., 2005; O'Regan & Mulvihill, 2010), gum Arabic (Desplanques et al., 2012) can also be used instead of hydrophilic emulsifiers for emulsification and stabilization of emulsions.

Sodium caseinate, adjusted acid-coagulated whole casein, is frequently used in food industry as emulsifier, water binding agent, fat binding agent, thickener, and gelation contributor. Since sodium caseinate produces an adsorbed layer of milk protein, emulsions with sodium caseinate are stable against coalescence (Dickinson & Golding, 1997). Sodium caseinate, bovine serum albumin and whey protein isolate are used as hydrophilic emulsifiers to stabilize outer

interface of double emulsion (Garti & Bisperink, 1998). Sodium caseinate is advantageous to be used in double emulsion, since it adsorbs at the oil/water interface and facilitates stabilization. Apart from that, sodium caseinate solution creates gel at moderate concentrations (Delample et al., 2014). Inclusion of low-molecular weight emulsifiers and emulsifiers with protein structure is proven to increase the stability of double emulsion. Nevertheless, to stabilize double emulsions, high concentrations are needed (O'Regan & Mulvihill, 2010).

Whey protein isolate, associate product of cheese production, consists of serum albumin, immunoglobins, peptones, b-lactoglobulin, a-lactoglobulin (Ju et al., 1999). In food industry, whey protein isolate is used as foaming, gelling, emulsifying agent due to its high solubility and amphiphilic structure (Damodaran et al., 2007). There are many researches that whey protein isolate is used as hydrophilic emulsifier for double emulsion production (Benichou et al., 2007; Knoth et al., 2005; Lutz et al., 2009;). Murillo-Martínez et al. (2011) used blends of whey protein isolate and carboxymethylcellulose or low methoxy pectin to evaluate characteristics of double emulsions. It was reported that double emulsion formulated with low methoxy pectin and whey protein isolate blend as hydrophilic emulsifier had smaller particle size of 2.47 μm (Murillo-Martínez et al., 2011).

Xanthan gum is an anionic polysaccharide and it is highly resistant to acid and heat (Chivero et al., 2015). Xanthan gum is widely used agent for emulsion stability, thickening, and rheology enhancement (García-Ochoa et al., 2000). It is used as thickener of aqueous phase in double emulsions. It provides emulsion stability by increasing viscosity of aqueous phase (Benichou et al., 2007; Sun et al., 2007).

1.6. Mayonnaise

Mayonnaise is a thick creamy sauce, oil-in-water emulsion produced with vegetable oil, acidic components (maleic acid, acetic acid and citric acid), emulsifier (naturally occurring egg lecithin), flavoring agents (sweetener, salt, garlic or mustard), inhibitor for unwanted crystals, texture enhancers, and stabilizers (Ma & Barbosa-Cánovas, 1995). Most significant characteristic of mayonnaise is the high oil content of 65-75 % (McClements, 2004).

Since high consumptions of fats related to adverse health problems like cancer, coronary heart disease, obesity, diabetes (Bray & Popkin, 1998; Hu et al., 1997; Hunter et al., 1996; Salmeron et al., 2001), costumers demand reduced-fat food products for healthier food choices (Liu et al., 2007). Nonetheless, fat content of food system is a crucial element for many characteristic of foods like taste, color, shelf life, structure of food, appearance, smell, texture, and composition (Su et al., 2010). Fat reduction or fat removal from a food system may lead to adverse effect on quality parameters. Increasing interest of consumers to reduced-fat products induce the production of products with lower fat content (Ma & Boye, 2012). Food producers and scientists try to provide novel ways to produce lower-fat content and low-calorie foods (Ma & Boye, 2012). Since double emulsion enables to capsulate water particles inside the oil phase, it has potential to reduce oil composition in food systems with same quality and sensory features as whole fat food (Jiménez-Colmenero, 2013). In some attempts for reduction of fat content of mayonnaise, fat mimetic and fat replacers are used (Akoh, 1994). Polysaccharides, gums, carboxymethylcellulose, pectins, fibre and maltodextrose are used as fat replacer as well as thickener and stabilizer for low-fat food systems (Akoh, 1994). Nevertheless, inclusion of fat analogs may result in loss of texture and sensory attributions (Dickinson, 2010). Thus double

emulsion is a highly attractive alternative for fat reduction (Oppermann et al., 2015).

1.7. Objective of The Study

In recent years, increase in diseases related to excess fat consumption give rise to utilization and production of low-fat food products. As obesity, cancer, cardiovascular diseases are proven to be associated with the over-use of fat; awareness of people about healthy food products is increased. Hence, development of functional foods with low-fat or fat- reduced properties become crucial for meeting the consumer demands.

Many strategies have been developed to reduce fat content of food systems such as imitating fat or replacing the fat with another agent. Since fat is an essential component of food systems, fat reduction or replacement of it with a low-calorie component leads to undesirable changes in quality parameters like sensory, texture, and rheological characteristics. Double emulsions are recommended technology for reduction of fat content, encapsulation of solute with distinct solubility, and controlled release of solutes. In contrast to relatively common usage of double emulsions in pharmaceutical industry, they are infrequently used in food industry. Fundamental reason of underutilization of double emulsions is that emulsifiers used in food industry are not effective enough to stabilize double emulsion system and there are pre-determined limits for usage of emulsifiers in food industry. There are limited studies about fat reduction of various food products with double emulsions such as meat, yogurt and fresh cheese. However, there is no research in the literature on reduction of fat content of mayonnaise with this method.

Mayonnaise is chosen to be the sample product in this thesis for incorporation of double emulsions because it is one of the most frequently used sauces in the

world. It is regarded as high-calorie food due to its high fat content of 65-80%. Additionally, since it is an oil-in-water emulsion, incorporation of double emulsion to it would be reasonable.

One of the objectives of this research was to formulate stable double emulsion with different hydrophilic emulsifier types, different W/O/W ratios and different homogenization method, while lipophilic emulsifier is fixed. Inspecting the optimum W/O/W ratio, hydrophilic emulsifier and homogenization method for formulation of stable double emulsion was another objective of this study. Optimum conditions for stable double emulsion are determined according to experimental results of particle size, efficiency, stability and long-term storage. Finally, the most stable double emulsions are utilized to formulate low-fat mayonnaise like sauces.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Sunflower oil, main ingredients in the formulations of double emulsions, was purchased from Komili Temizlik Ürünleri Pazarlama A.Ş. (Topkapı, İstanbul).

Sodium chloride, used in inner water phase (W1) of double emulsion in order to control ionic strength of water phase and monitor its movement into the second water phase (W2), is supplied from Sigma Aldrich Chemical Co. (St. Louis, MO, USA)

Polyglycerol polyricinoleate (PGPR), an efficient lipophilic emulsifier used in oil phase in the first emulsification process, is supplied by ETİ Gıda San. ve Tic. A.Ş (Eskişehir, Turkey).

Soy lecithin, natural emulsifier, was supplied by LIPOID GmbH (Ludwigshafen, Germany).

Casein sodium salt from bovine milk, a hydrophilic emulsifier and xanthan gum from Xanthomonas campestris, a stabilizer used in W2 phase of double emulsion were supplied from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Sodium azide was also supplied from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and it was added to double emulsion as a preservative to prevent microbial contamination during preparation and storage.

Whey Protein Concentrate (WPC) was supplied from Tunçkaya Kimyevi Maddeler (Tuzla, İstanbul).

Grape vinegar and lemon juice used in the preparation of mayonnaise samples were supplied from Kavaklıdere Şarapları A.Ş. (Ankara, Turkey).

Table sugar was supplied from Torku, Konya Şeker San. ve Tic. A.Ş. (Konya, Turkey).

2.2. Methods

2.2.1. Preparation of Double Emulsions

Double emulsions in this study were prepared according to two-step emulsification method.

2.2.1.1. Preparation of Solutions Used in Double Emulsion

The solution of water phases to be used in emulsion processes were prepared a day in advance in order to achieve proper hydration of solutes and uniform homogenization. Distilled water was used in water phases of all emulsions.

For interior water phase (W1) of the double emulsion, 1% (by weight) sodium chloride (NaCl) solution was prepared with distilled water. The solution was stirred with magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany) at 300 rpm for 1 hour for proper homogenization. Then, stock solution was stored at room temperature.

For oil phase (O) of the double emulsion, Polyglycerol polyricinoleate (PGPR) 3% by weight was prepared with sunflower oil. The solution was stirred with magnetic stirrer at 300 rpm for 30 min for proper homogenization, and it was stored in refrigerator for 18 hours.

W2 phase containing 15% sodium caseinate (SC) by weight and 0.05% sodium azide (SA) by weight was premixed at 300 rpm for 60 min by a magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany). Afterwards, it was left in the refrigerator at 4^0C for 18 hours to hydrate the solute completely.

W2 phase containing 1% xanthan gum (XG) by weight and 0.05% sodium azide (SA) by weight was premixed at 5000 rpm for 10 min by high-speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia). Then, it was left in the refrigerator at 4^0C for 18 hours to hydrate the solute completely.

W2 phase containing 4% lecithin (L) and 11% whey protein concentrate (WPC) by weight and 0.05% sodium azide (SA) by weight was premixed at 300 rpm for 60 min by a magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany). Afterwards, it was left in the refrigerator at 4^0C for 18 hours to hydrate the solute completely.

Prior to the formation of double emulsion, the solutions to be included into process were placed in a shaking water bath (GFL 1086, Burgwedel, Germany) at 50^0C and at 80 rpm for 15 min to achieve temperature equivalence with other constituents and proper homogenization.

2.2.1.2. Preparation of W1/O Emulsion

In this study, double emulsions of W1/O/W2 were prepared by referring to the process of two-step emulsification (Cofrades, 2013). In the first step, water phase was emulsified in oil phase containing lipophilic emulsifier with a harsh

homogenization method. In the second step, resulting emulsion (W1/O) of the first step was emulsified in another water phase W2 containing hydrophilic emulsifier with a milder homogenization method, and W1/O/W2 was produced.

Two different W1/O ratios were studied for the first water-in-oil emulsion; 2:8 and 4:6. For 2:8 (W1/O) ratio, the oil phase (O) with 3% PGPR and water phase (W1) prepared previously was heated to 50 ^{0}C in a shaking water bath at 80 rpm for 15 min. The ratio of PGPR used in formulation was determined by preliminary experiments. First emulsion (W1/O) was formulated by drop-wise inclusion of 20 g water phase (W1) into 80 g oil phase containing 3% PGPR by weight; it was homogenized by high-speed homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) at 16000 rpm for 10 min. The speed and time of high speed homogenization process were predetermined according to the findings of preliminary experiments. The same procedure was done for the preparation of the first (W1/O) emulsion of 4:6 by changing oil phase (O) amount to 60 g and water phase (W1) amount to 40 g. The formed first emulsions were stored at room temperature for the double emulsion production.

In order to observe the effect of homogenization technique on characteristics of double emulsions, after high-speed homogenization of water-in-oil (W1/O) emulsion, it was homogenized by Ultrasonic Homogenizer (Sonic Ruptor 400, OMNI International the Homogenizer Company, Georgia, USA) at 120 kHz with 50 % pulse for 10 min. Solid titanium 1" tapped-tip with a length of 12.70 cm and radius of 12.7 mm was attached to the ultrasonic homogenizer. The specifications of ultrasonic homogenization were also predetermined according to the findings of preliminary experiments. An important finding of preliminary experiments was that ultrasonic homogenization increased the temperature of the emulsion. In order to minimize the effects of overheat on first emulsion (W1/O); samples were placed into ice bath kept at 10 ^{0}C during ultrasonic homogenization.

In order to understand whether the produced emulsion was W/O or O/W, emulsions were subjected to a quick test. Two drops of produced emulsion were dripped into water and oil which were placed in different beakers. If emulsion dissolved in oil, it was concluded that outer layer of emulsion is oil and emulsion was water-in-oil. If emulsion dissolved in water, it was concluded that outer layer of emulsion was water phase and emulsion was oil-in-water.

2.2.1.3. Preparation of W1/O/W2 Emulsion

The second step of double emulsions formulation was the emulsification of W1/O emulsion into W2 phase containing hydrophilic emulsifier with a mild homogenization method.

In this study, two water-in-oil (W1/O) to water phase (W2) ratios were studied which were 2:8 and 4:6. As can be seen in Table 1.1, with the chosen ratios of W1/O, four main ratios of double emulsions were studied.

Table 2.1 Oil and water phase ratios in the double emulsion preparation

Abbreviations	Ratio of W1/O primary emulsion	Ratio of (W1/O)/W2 secondary emulsion
2.8.8	2:8	2:8
2.8.6	2:8	4:6
4.6.8	4:6	2:8
4.6.6	4:6	4:6

For (W1/O/W2) emulsion with formulation of 2.8.8, 120 g of second water phase (W2) prepared with sodium caseinate (SC) was placed into mixing bowl of food processor (Arçelik K-1190 Robolio, 700 W , Arçelik Inc. Istanbul, Turkey). 30 g of the first emulsion at a ratio of 2:8 (W1/O) was introduced into mixing bowl in drop-wise manner while processor was working at the first stage of the rotary function. The homogenization in mixing bowl of food processor was continued for 10 min. The parameters including time of process and stage of rotary function were decided according to the outcomes of preliminary experiments. For production of other ratios of double emulsion, the amount corresponding to the ratio of first emulsions and amount of second water phase (W2) were aligned to reach total emulsion amount to 150 g in the mixing bowl.

One of the independent variables of this study is the type of hydrophilic emulsifier included into W2 phase. For each of W2 solution, parameters of second homogenization were determined according to preliminary experiments and characteristic properties of solution. Second emulsification process with W2 solution containing xanthan gum was carried out in mixing bowl of food processor for 10 min with maximum rotational speed. Double emulsions containing W2 solutions with lecithin and whey protein concentrate were prepared by mixing at the fifth stage of mixing bowl of food processor for 10 min.

Finally, produced double emulsions were immediately placed in refrigerator at 4ºC in beakers closed with plastic films for further studies and tests. By this way, flocculation, disruption or coalescence of emulsions was minimized. Each experiment was performed in duplicate.

2.2.2. Preparation of Mayonnaise with Double emulsions

After the determination of the ratio of phases and process conditions of double emulsions having the best quality parameters, mayonnaise samples were prepared.

As water phase of the mayonnaise, W2 phase of the double emulsions were prepared with xanthan gum, sodium caseinate and whey-lecithin with decided concentrations. Then measured amount of W2 solutions were placed into mixing bowl of food processor and mixed for 1 min at pre-determined mixing rates of corresponding W2 phase for homogenization. Water-in-oil (W1/O) emulsions with ratio of 2.8 and 4.6 were introduced into W2 solution, drop wise. Amount of W2 solution and water-in-oil primary emulsion was determined by preliminary experiments and this ratio was different for each W2 solution. The ratio of primary emulsion included into W2 phase was determined according to critical fraction of dispersed phase of corresponding W2 solution. For 15% sodium caseinate solution, 1% xanthan gum, and for mixture of 4% lecithin and 11% whey protein concentrate, critical fraction of dispersed phase was determined as 61%, 64% and 76%, respectively. And mayonnaise samples were prepared accordingly.

Additionally, 2% vinegar, 3% lemon juice, 2 % salt, 2% sugar were included into W2 phase for flavor. Control samples were prepared by adding oil into W2 while mixing continued. Double emulsified mayonnaise samples were prepared by adding primary emulsion with ratios of 2:8 and 4:6. Prepared mayonnaise was taken into a beaker for further analysis and placed into refrigerator to inhibit degradation.

2.2.3. Analysis of Double Emulsions and Mayonnaise

2.2.3.1. Particle Size Analysis

Particle sizes and distributions of the oil phase inside the double emulsion and mayonnaise samples were determined by laser diffraction particle size analyzer by Mastersizer 3000 (Malvern Instruments, Worcestershire, UK). The particle size analysis was based on Mie Scattering Theory. The measurement parameters were determined according to emulsion structure. Since water phase (W2) of double emulsion was continuous phase, optical properties of dispersant were chosen accordingly with refractive index of 1.33. Considering dispersed phase was oily phase in double emulsion, the optical properties of dispersed (W1/O) phase were chosen accordingly with refractive index of 1.464 and globule absorbance of 0.01. During measurement of the particle size, obscuration range was between 8% and 20% and rotary function of cell was settled to 1600 rpm to prevent reduction of emulsion particle size during measurement. Measurements were done in duplicate for each sample.

The mean particle size of oil globules inside the double emulsion was expressed as the Sauter mean diameter, abbreviated as D(3,2) and calculated with the following equation (McClements, 2005):

$$D_{32}=\frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$ **Equation 2.1**

where, d_i denotes the diameter of particles and n_i denotes the number of related particles per unit volume of total particles.

The polydispersity of size distribution, span of oil globules, was other important parameter for characterization of distribution width of particles in emulsion (McClement, 2005).

Span was enumerated with the following formulation:

$$Span = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \qquad \textbf{Equation 2.2}$$

where, $d_{0.9}$, $d_{0.5}$, and $d_{0.1}$ were diameter size of particles that are inside the range of 90%, 50% and 10% of cumulative sample particles.

During particle size analysis, span and D (3, 2) were calculated instantly via the software of supplied by Malvern with the particle size analyzer.

2.2.3.2. Encapsulation Efficiency

In order to state the efficiency of emulsification, amount of NaCl encapsulated into W2 phase was determined by conductivity measurements. Since W1 phase was prepared with 1% NaCl by weight, it was hypothesized that measurable NaCl amount after double emulsion production was non capsulated NaCl. The ratio of retained amount of NaCl to total amount of NaCl incorporated into W1 phase represents the encapsulation efficiency (EE) of double emulsion. It was formulated as follows (Sapei et al., 2012);

$$\boldsymbol{EE}(\%) = \mathbf{100} - \boldsymbol{FR}(\%) \qquad \textbf{Equation 2.3}$$

$$\boldsymbol{FR}(\%) = \frac{\boldsymbol{M_t}}{\boldsymbol{M_\infty}} \times \mathbf{100} \qquad \textbf{Equation 2.4}$$

where, FR(%) is the percentage of NaCl released to W2 phase just after production of double emulsion, M_t was the amount of not-capsulated NaCl in W2 phase, and M_∞ was the amount of NaCl (g) incorporated in W1 phase. The amount of NaCl inside the W2 phase was determined by conductivity meter (InoLab, Cond7110, WTW, Oberbayern, Germany). For the amount of NaCl inside a solution, a calibration curve, Table B.1, was drawn relating percent amount of NaCl and corresponding conductivity of the solution.

To measure the conductivity, 15 g of double emulsion was taken into 25 ml beaker and probe of conductivity meter was dipped into emulsion completely. The conductivity values were converted into percent NaCl by the help of the calibration curve. Each experiment was repeated twice.

In order to eliminate the effect of hydrophilic emulsifier on the conductivity of the double emulsion, conductivity of second water phase solutions of xanthan gum, sodium caseinate and lecithin-whey protein concentrate were measured before emulsion formation. And acquired conductivity values were subtracted from the measured conductivity values of the double emulsions before encapsulation efficiency calculations.

2.2.3.3. Stability

The stability of double emulsions and mayonnaise samples was determined by centrifugation of double emulsion and finding weight of supernatant part. Newly formed 7 g of double emulsion (M_0) was taken into 10 ml centrifuge tubes, which were hermetically sealed with plastic cabs. Two replicates of each sample were placed in centrifuge (Hettich Mikro 200/200R, Sigma Laborzentrifugen GmbH, Germany) for 15 min at 5000 rpm and at 20ºC. Supernatant part (M_1) of sample was weighted carefully and stability of emulsion was calculated as ratio of separated part to initial weight of emulsion;

$$Emulsion\ Stability(\%) = \frac{M_1}{M_0} \times 100 \qquad \textbf{Equation 2.5}$$

2.2.3.4. Optical Imaging

In order to observe the morphological properties of double emulsions, double emulsions were inspected under light microscope. Double emulsions were diluted with corresponding W2 phase in order to observe globules clearly. The

diluted solutions were mounted evenly onto glass microscope slide as a very thin layer and slide was placed into inverted light microscope (PrimoVert, Zeiss, Jena, Germany). Images were extracted by microscopic camera (Sony CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan) and analyzed by software named TopView. The images of double emulsions were taken with four magnification levels; 4×, 10×, 20×, and 40×.

2.2.3.5. Rheological Measurements

Rheological measurements of double emulsions and mayonnaise were done by a rheometer equipped with cone and plate geometry (Kinexus, Malvern Instruments, Ltd, Worcestershire, UK). Approximately 2 g of samples of double emulsions were positioned into measuring area as ordered by the software of rheometer. After structure alignments and temperature equalization, measurement is done by revolving cone with cone angle of 4º, diameter of 40 mm, and 0.001 mm of gap. All rheological measurements were carried out at 25ºC. Apparent viscosities versus shear rate values were measured while shear rate was increased from 0.1 to 100 s^{-1}. Test on rheological measurement was carried out by duplicate, 3 hours after the preparation of emulsions.

2.2.3.6. Long Term Stability

Long term stability of the double emulsions was measured by monitoring gravitational separation of the emulsion samples placed in two different environments with different temperatures. Two glass tubes with 12 g of samples were labeled. One of them was placed into refrigerator at 4°C and the other one was kept at room temperature of 20°C. Gravitational separation was monitored and separated part of water phase inside the tubes was measured for a defined time intervals.

2.2.3.7. Color

Color measurements were done for mayonnaise samples. It is determined by CR-5 Color Reader Bench-top colorimeter (Konica Minolta, Japan). The measurements were done in terms of CIE L*, a*, b* color scale. L*, a*, b* was corresponds to whiteness/darkness, redness/greenness, and yellowness/blueness, respectively. All samples were measured with two replications. Total color change ΔE was calculated by following equation, $BaCl_2$ was taken as reference for calculations;

$$\Delta E^* = [(L_o^* - L^*)^2+(a_o^* - a^*)^2+(b_o^* - b^*)^2]^{1/2} \quad \textbf{Equation 2.6}$$

2.2.3.8. Statistical Analysis

The gathered results were analyzed by analysis of variance (ANOVA) to determine if there is a significant difference between the results or not ($p \leq 0.05$). If significant difference was present between the samples, Tukey's Test was applied with the significance level of 5% on Minitab (Version 16.2.0.0, Minitab Inc., Coventry, United Kingdom).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Double Emulsion

The effects of emulsifier type, W/O/W ratio, and homogenization method on particle size, rheological properties, stability, encapsulation efficiency, optical imaging, and long term stability of double emulsions were studied.

3.1.1. Particle Size of Double Emulsions

Particle size of emulsions is the most important parameter for emulsion technology since it affects appearance, stability, texture, shelf life and flavor of emulsion (McClements, 2004). As a result, particle size and distribution must be monitored thoroughly. In this study, relationship between particle size and other parameters were observed as well.

In literature, it was reported that particle size distributions of double emulsions could be observed as monomodal (Cofrades et al., 2013; Mun et al., 2010) and bimodal (Regan & Mulvihill, 2009; Sapei et al., 2012) distributions. Although it is a widely studied parameter, there is not a consensus about the distribution characteristics of particle size of double emulsions. Hemar et al. (2010) and Su et al.(2006) reported that double emulsions prepared with PGPR as lipophilic emulsifier had bimodal particle size distribution (Hemar et al., 2010; J. Su et al.,

2006). On the other hand, Cofrades et al. (2013) reported that double emulsions prepared with PGPR as lipophilic emulsifier, sodium caseinate and lecithin-whey protein concentrate as hydrophilic emulsifier exhibited monomodal droplet size distribution (Cofrades et al., 2013). As can be seen in Figure 3.1, particle size distribution of double emulsions prepared in this study had both bimodal and monomodal characteristics. Double emulsion prepared with sodium caseinate was observed to have monomodal size distribution. However, samples prepared with lecithin-whey protein concentrate and xanthan gum had bimodal size distributions. Size distribution characteristics can be affected by homogenization method, duration of homogenization and interaction between emulsifiers. Bou et al. (2014) also summarized that differences in droplet size distribution were dependent on homogenization qualifications, composition of double emulsion, viscosity of ingredients as well as concentration and type of emulsion (Bou et al., 2014).

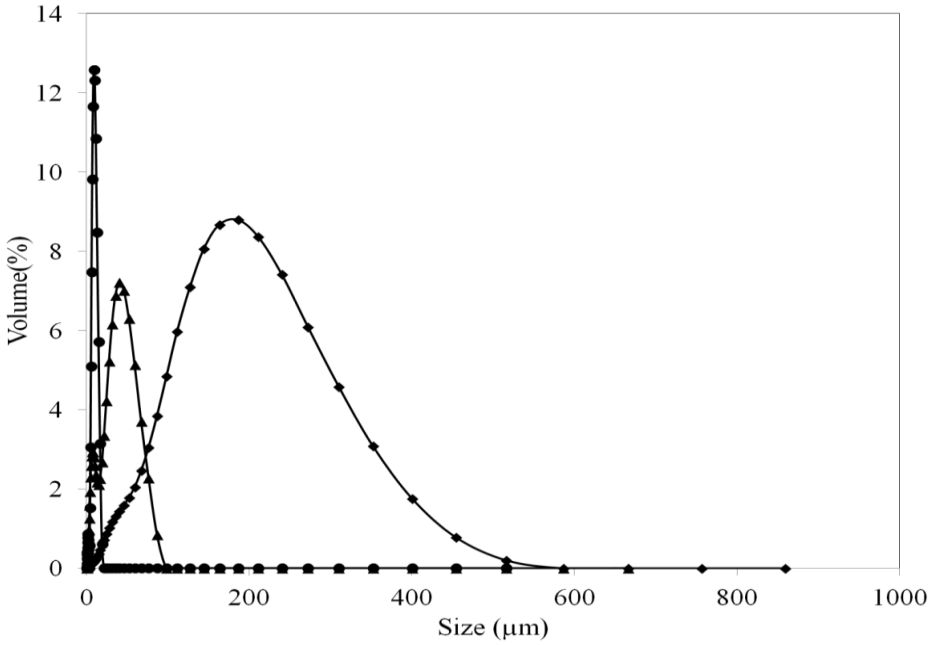

Figure 3.1 Particle size distribution graph of double emulsions having formulation of 2.8.8 prepared with high speed homogenization and formulated with sodium caseinate: (●), xanthan gum: (♦), lecithin-whey protein concentrate: (▲)

Span values of the particle size measurements were also determined in the experiments. Average span (polydispersity of particle size) values were found to be 1.012, 1.783, and 1.299 for samples with sodium caseinate, xanthan gum and lecithin-whey protein concentrate, respectively. Smaller span values indicated monomodal size distribution and lower polydispersity.

In order to analyze the particle size of double emulsions, Sauter mean diameters D(3,2) of double emulsions were compared.

Sauter mean diameters of double emulsions prepared with different hydrophilic emulsifier of sodium caseinate, xanthan gum, and lecithin-whey protein concentrate were found to be different (Figure 3.2- Figure 3.4). Sauter mean diameter ranged between 3-9 μm, 50-300 μm, and 10-20 μm for double emulsions prepared with sodium caseinate, xanthan gum and lecithin-whey protein concentrate, respectively. Average particle sizes of the samples were 5.8, 178.5, and 11.8 μm for sodium caseinate, xanthan gum, and lecithin-whey protein concentrate, respectively. According to statistical analysis, it was determined that Sauter mean diameter of double emulsion prepared with xanthan gum was larger and significantly different than that of double emulsion prepared with sodium caseinate and lecithin-whey protein concentrate (Table A.1). Xanthan gum is an anionic thickener that is widely used in food industry. According to recent research, it was proven that xanthan gum was not adsorbed at the oil-water interfaces. By the inclusion of xanthan gum, double emulsions were stabilized by increasing the viscosity of second aqueous phase (W2) (Seddari et al., 2013). During mixing and homogenization, droplets of primary emulsion were incorporated into dense network of continuous phase and stabilization was achieved by viscous structure. However, highly viscous structure of continuous phase inhibited formation of fine droplets of primary emulsion even at high shear rates during homogenization. Another reason of larger particle size of double emulsions with xanthan gum was possibly due to the development of thicker layer around the emulsion droplets. High molecular weight and high concentration of biopolymers form thick gel around the droplets regardless of droplet size. According to the study conducted by Saedity et al. (2014), SEM images revealed that high biopolymer content resulted in more spherical shaped particles whereas low concentration of biopolymers resulted in random shapes (Saeidy et al., 2014). This phenomenon was linked to the increase of coacervates and sedimentation of coating material on oil droplets (Tamjidi et al., 2013).

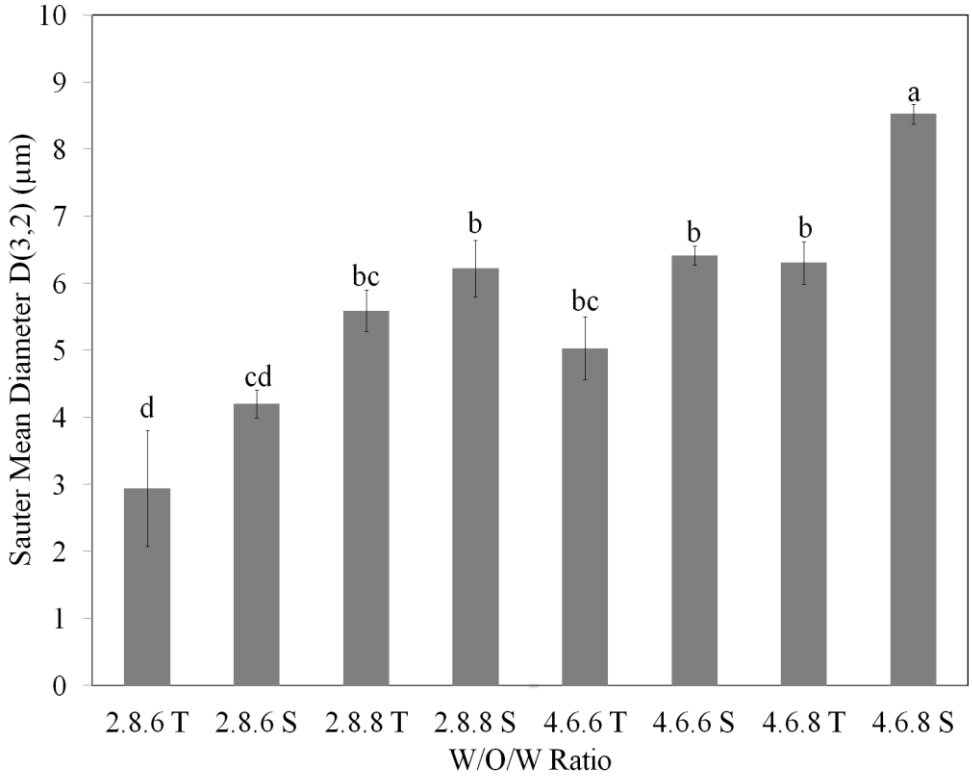

Figure 3.2 Sauter mean diameter of different double emulsion formulations containing sodium caseinate; T: High speed homogenization, S: Ultrasonic homogenization. Bars with different letters represent significant difference ($p \leq 0.05$).

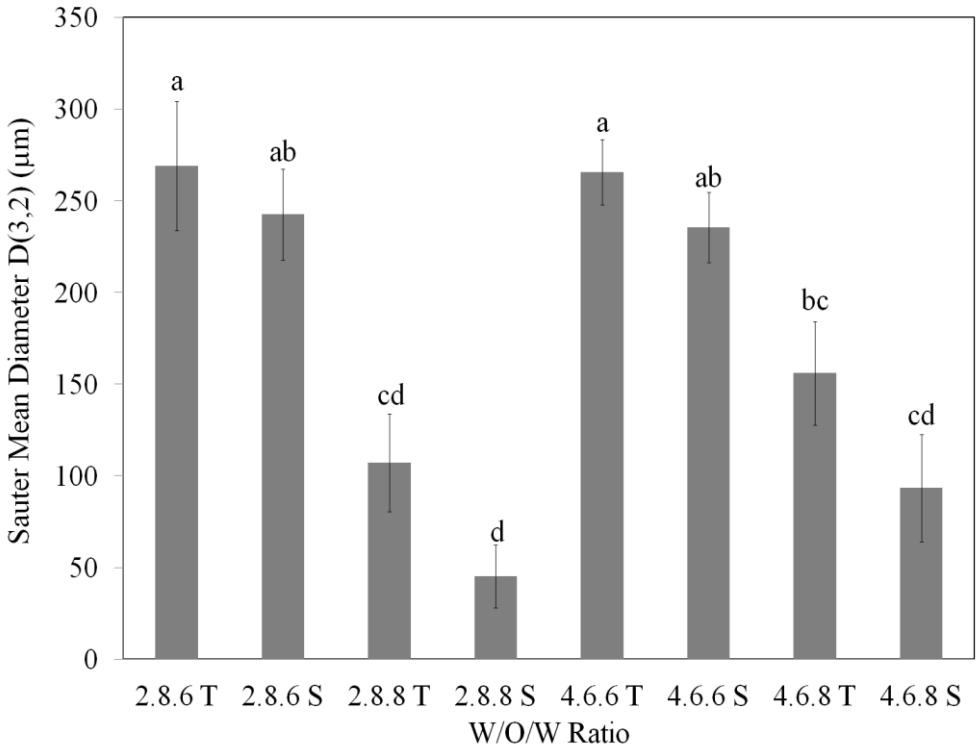

Figure 3.3 Sauter mean diameter of different double emulsion formulations containing xanthan gum; T: High speed homogenization, S: Ultrasonic homogenization. Bars with different letters represent significant difference ($p \leq 0.05$).

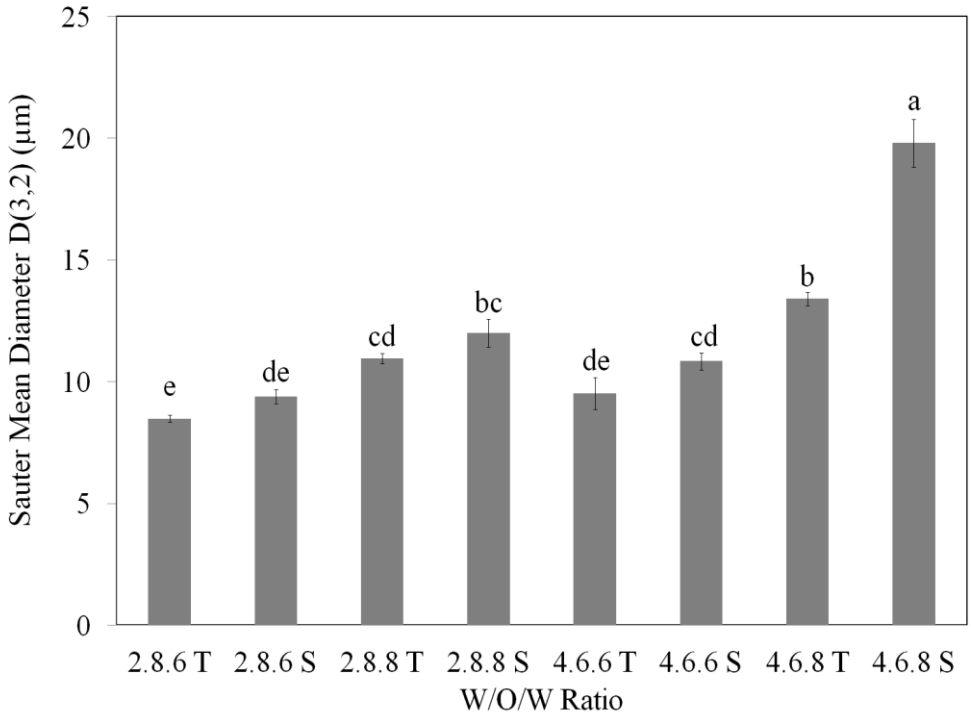

Figure 3.4 Sauter mean diameter of different double emulsion formulations containing lecithin-whey protein concentrate; T: High speed homogenization, S: Ultrasonic homogenization. Bars with different letters represent significant difference ($p \leq 0.05$).

Since Sauter mean diameter of samples with xanthan gum was extremely larger compared to that of samples with lecithin-whey protein concentrate and sodium caseinate, statistical analysis was done by excluding samples with xanthan gum for statistical reliability. Average particle size of double emulsions prepared with lecithin-whey protein concentrate was significantly different than that of samples

with sodium caseinate (Table A.1). Cofrades et al. (2013) reported similar results for sodium caseinate and whey protein concentrate. The difference between sodium caseinate and lecithin-whey protein concentrate was attributed to structural differences of these proteins which made caseins more competent for coverage of water-oil interface (Cofrades et al., 2013).

Phase ratio of double emulsion is another parameter that manipulates particle size of double emulsions. For double emulsions formulated with sodium caseinate and lecithin-whey protein concentrate, Sauter mean diameters of double emulsion formulations of 2.8.6 and 4.6.6 were lower than double emulsion formulations of 2.8.8 and 4.6.8. When these findings were evaluated in terms of total oil-like-phase (primary emulsion) composition of the double emulsions, it can be concluded that as oil-like-phase composition of double emulsion increased, particle size of the droplets decreased. This results can be supported by findings of Raymundo et al (2002). Increase in oil content and protein composition decreased Sauter mean diameter of low-fat mayonnaise-type simple emulsions (Raymundo et al., 2002). Since primary emulsion in double emulsion behaves like oil phase in simple emulsions, it can be a reasonable explanation in this study too.

However, this outcome contradicts with the conclusions of Saeidy et al.(2014) about the effect of oil content on particle size. It was argued that high oil content inhibited the activity of stirrer to dissipate oil droplets and led to larger droplets (Saeidy et al., 2014). This discussion is valid for xanthan containing emulsions. For samples containing xanthan gum, it was observed that formulations of 2.8.6 and 4.6.6 had higher droplet size compared to samples with 2.8.8 and 4.6.8 formulations (Table A.3). According to the observations in the experiments, xanthan gum solutions used as stabilizer were already viscous as compared to the second aqeous phase solutions before second homogenization and dispersion of the oil could be interfered due to the viscosity of solution. On the other hand,

viscosity of double emulsion with sodium caseinate and lecithin-whey protein concentrate increased during second homogenization. Therefore, for samples containing sodium caseinate and lecithin-whey protein discussion of under-homogenisation or inhibition of dispersion of primary emulsion were irrelevant.

Broader inspection revealed that Sauter mean diameter of double emulsion at the same ratio of primary emulsion to second aqeous phase–in other words couples of 2.8.6-4.6.6 and 2.8.8-4.6.8 had similar droplet size. Regardless of content of inner primary emulsion, samples with same ratio of (W1/O) to second aqeous phase W2 had similar characteristics of particle size. According to a study about rheological properties of double emulsions, it was found that W/O/W emulsion were similar to a simple O/W emulsion prepared by the same volume fraction of dispersed phase, but lower oil content (De Cindio & Cacace, 1995). Combined with our findings, ratio of dispersed primary emulsion to continous phase (W2) was concluded to be more determinative than composition of primary emulsion.

Garti et al.(1996) suggested that one of the strategies to produce stable double emulsion was to reduce particle size of primary emulsion as much as possible (Garti & Aserin, 1996). For this reason, harsh conditions for homogenization of primary emulsion were chosen for many researchers. Harsh conditions were ensured by long time of homogenization, high speed-shear homogenization, extra homogenization step like ultrasound, membrane emulsification, and microfluidization (Frasch-Melnik et al., 2010; Oppermann et al., 2015; Vandergraaf et al., 2005). In this study, two different homogenization techniques were applied to primary emulsion in order to decrease particle size of primary emulsion, which were high speed homogenization and ultrasonic homogenization followed by high speed homogenization abbreviated by T and S in the graphs, respectively. According to statistical analysis, homogenization method affected the Sauter mean diameter of the double emulsion. Applying ultrasonic homogenization to primary emulsion followed by high speed

homogenization resulted in higher Sauter mean diameter than double emulsions prepared with only high speed homogenization for samples containing sodium caseinate and lecithin-whey protein concentrate. For samples containing xanthan gum, statistical analysis indicated that ultrasound decreased Sauter mean diameter of double emulsion significantly. This result was also supported by many studies. It was concluded that high energy input by means of ultrasonication or high mechanical agitation resulted in smaller droplet size for emulsion systems (Chandrapala et al., 2012; Schuch et al., 2014) This is an acceptable statement when particle size of emulsion is in the range of simple emulsion systems like the samples of xanthan gum. However, when the particle size of emulsion is already in micron size like the samples of sodium caseinate and lecithin-whey protein concentrate, droplet characteristics is affected by over-processing phenomenon. This phenomenon was explained as increase in droplet size of sunflower oil emulsions as energy input increased (Desrumaux & Marcand, 2002). The same result was observed in the ultrasonic study applied to flaxseed oil-in-water emulsions by Kentish et al. (2008). It was stated that larger sized droplets were obtained in emulsions when energy power levels were higher. As a conclusion, it could be suggested that for efficient homogenization with ultrasonication, optimization must be done according to system to be studied (Kentish et al., 2008). This phenomenon could be explained by poor functionality of emulsifiers, and the increase in the possibility of random collision of produced droplets in the presence of high energy input. Increase in collision rate and Brownian motion gave rise to coalescence of droplet and droplet breakage (Jafari et al., 2006; Marquez & Wagner, 2010). For ultrasonic homogenization, over-processing occurs not only by coalescence of droplets but also by formation of cloud of cavitational bubbles (Chandrapala et al., 2012).

3.1.2. Rheological Properties of Double Emulsions

The effects of different hydrophilic emulsifiers, homogenization methods and W/O/W ratios on rheological properties of double emulsions were studied. All the samples of double emulsions behaved as non-Newtonian fluid. Since their apparent viscosities decreased as shear stress increased, their behavior was concluded to be shear thinning behavior (Figure 3.5-Figure 3.7). Similar results were recorded in the literature (Carrillo et al., 2015; Zinoviadou et al., 2012). Garti et al. (1998) also stated that rheological measurements of double emulsions showed shear-thinning behavior (Garti & Bisperink, 1998). This behavior was attributed to structural deformation of network that was formed in equilibrium state. As shear stress was applied, the structure of the double emulsion was interfered by elongation in the direction of deformation and rupture of network occurred. Increase in shear stress caused deformation of droplets of primary emulsion. All these mechanisms led to decrease in viscosity as shear stress increased (Carrillo-Navas et al., 2012).

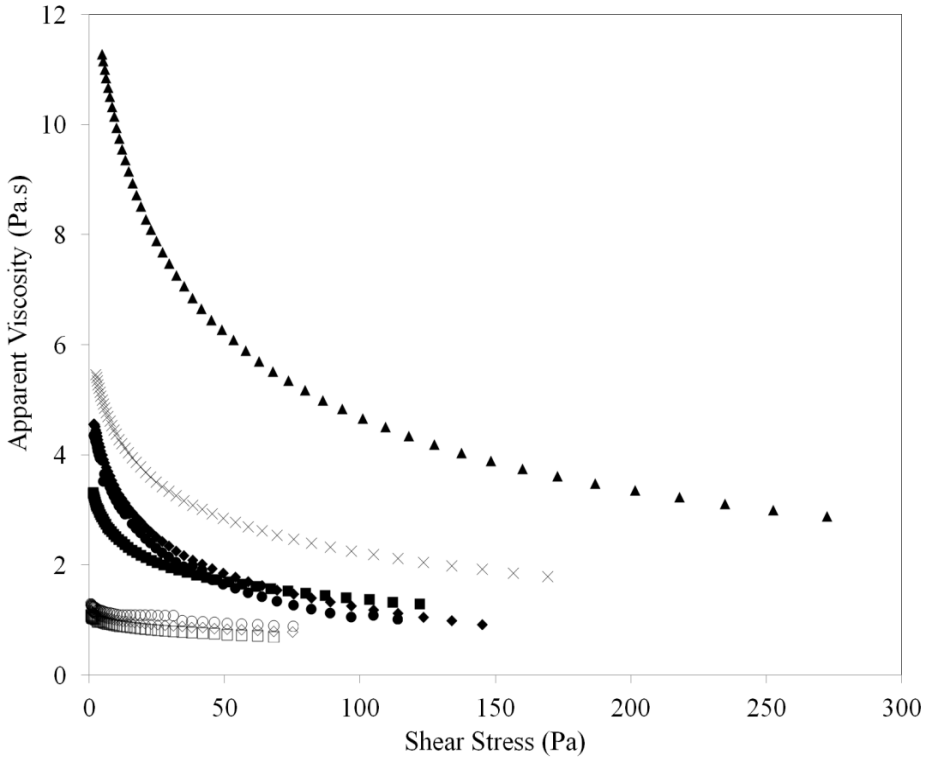

Figure 3.5 Apparent viscosity of double emulsions containing sodium caseinate at different W/O/W ratios; (♦): 4.6.8 T, (■): 4.6.8 S, (▲): 4.6.6 T, (x):4.6.6 S, (◊): 2.8.6 T, (●): 2.8.6 S, (○): 2.8.8 T, (□): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

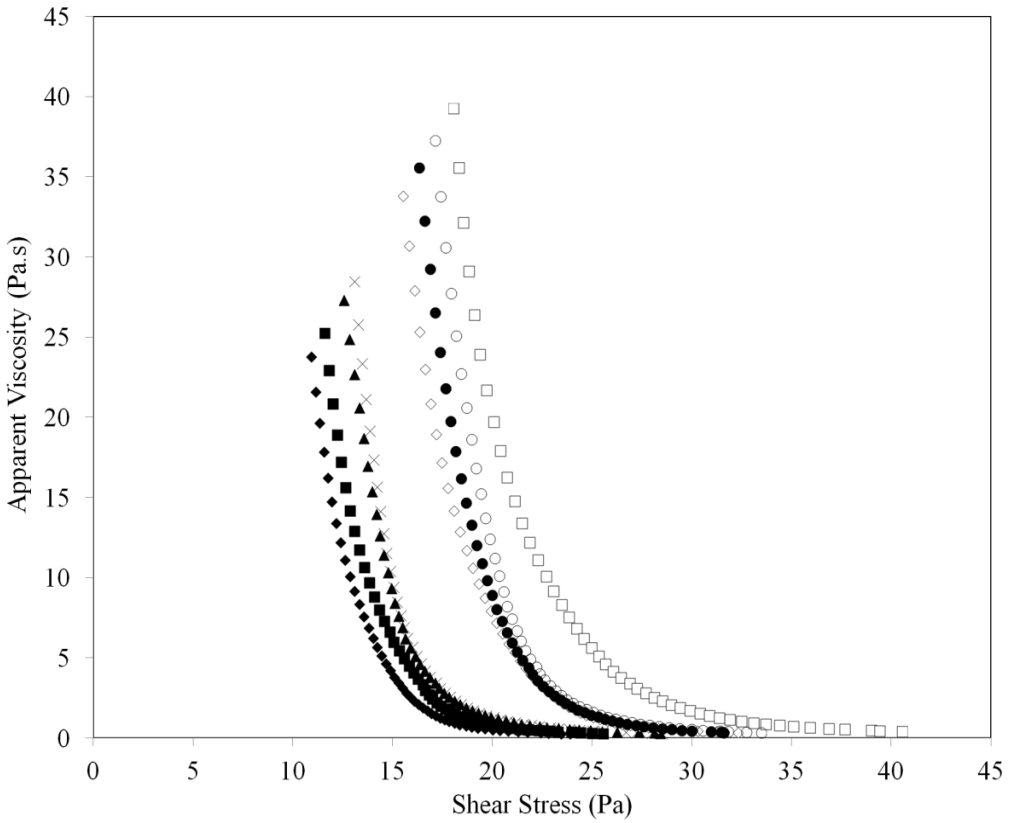

Figure 3.6 Apparent viscosity of double emulsions containing xanthan gum at different W/O/W ratios; (◊): 4.6.8 T, (●): 4.6.8 S, (□): 4.6.6 T, (○):4.6.6 S, (▲): 2.8.6 T, (x): 2.8.6 S, (♦): 2.8.8 T, (■): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

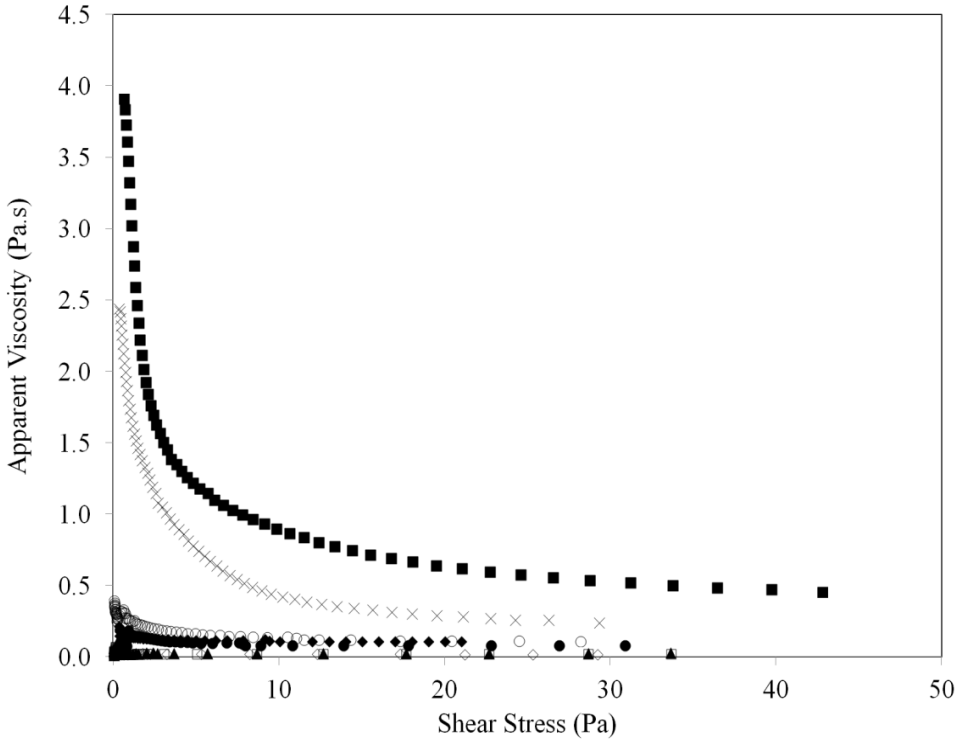

Figure 3.7 Apparent viscosity of double emulsions containing lecithin-whey protein concentrate at different W/O/W ratios; (▲): 4.6.8 T, (◊): 4.6.8 S, (x): 4.6.6 T, (○):4.6.6 S, (●): 2.8.6 T, (■): 2.8.6 S, (♦): 2.8.8 T, (□): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

In the literature, rheological properties are explained by many models like Carreaua, Oswalt-de Waele or power law (Carrillo-Navas et al., 2012; Hernández-Marín et al., 2013). The flow properties of double emulsion were fitted to different models, but the model that best described the experimental data was chosen to be power law with high coefficient of determination (r^2 > 0.97). Power law is described as (Sahin & Sumnu, 2006);

$$\eta = K\gamma^{n-1}$$

where, n is flow behaviour index (dimensionless); K is consistency index with (Pa s^n); γ is shear rate (s^{-1}) and η is apparent viscosity (Pa.s). And for each double emulsion, K and n values are determined and given in Table 3.1.

Table 3.1 Power Law coefficients of double emulsions containing sodium caseinate (SC), xanthan gum (XG) and lecithin-whey protein concentrate (LWPC).

Emulsion Formulation	Emulsifier type	Homogenization method	K (Pa s^n)	n	R^2
2.8.6	SC	High Speed	5.943	0.8542	0.9997
2.8.6	SC	Ultrasound	2.635	0.8261	0.9930
2.8.8	SC	High Speed	0.8963	0.9217	0.9979
2.8.8	SC	Ultrasound	0.789	0.9422	0.9989
4.6.6	SC	High Speed	9.687	0.7596	0.9988
4.6.6	SC	Ultrasound	8.983	0.7906	0.9969
4.6.8	SC	High Speed	0.6054	0.9437	0.9980
4.6.8	SC	Ultrasound	0.9627	0.9574	0.9999

Table 3.1 (continued)

2.8.6	XG	High Speed	13.81	0.1443	0.9964
2.8.6	XG	Ultrasound	13.26	0.1374	0.9947
2.8.8	XG	High Speed	12.83	0.1324	0.9974
2.8.8	XG	Ultrasound	12.88	0.1377	0.9975
4.6.6	XG	High Speed	19.16	0.1527	0.9973
4.6.6	XG	Ultrasound	18.05	0.1131	0.9967
4.6.8	XG	High Speed	15.68	0.1541	0.9943
4.6.8	XG	Ultrasound	18.65	0.1232	0.9978
2.8.6	LWPC	High Speed	0.2886	0.8096	0.9984
2.8.6	LWPC	Ultrasound	0.2499	0.8142	0.9980
2.8.8	LWPC	High Speed	0.0279	0.9217	0.9779
2.8.8	LWPC	Ultrasound	0.0229	0.8783	0.9803
4.6.6	LWPC	High Speed	0.4079	0.7073	0.9985
4.6.6	LWPC	Ultrasound	0.3627	0.8100	0.9985
4.6.8	LWPC	High Speed	0.0332	0.8889	0.9805
4.6.8	LWPC	Ultrasound	0.0359	0.8867	0.9824

Initial apparent viscosities of double emulsions characterized by hydrophilic emulsifier used in the double emulsion formulations were different from each

other. As can be seen in Figure 3.5-3.7, initial apparent viscosities of double emulsions containing sodium caseinate and lecithin-whey protein concentrate were lower than that of samples containing xanthan gum. Ranges of initial viscosities of double emulsions were 12-1.05, 25-45 and 4-0.04 (Pa.s) for sodium caseinate, xanthan gum and lecithin-whey protein concentrate, respectively. Thickening property of xanthan gum might be the reason for higher initial viscosity of the double emulsions.

In order to evaluate apparent viscosities of the double emulsions further, apparent viscosity values of each double emulsion at constant shear stress (25 Pa) were extracted from each data set, and shown in Figure 3.8.

According to statistical analysis, average viscosity values of the double emulsions prepared with sodium caseinate, xanthan gum, and lecithin-whey protein concentrate are determined as 2.6, 1.2 and 0.2 Pa.s, respectively. Although initial viscosity interval of double emulsions with xanthan gum was higher than double emulsions prepared with sodium caseinate and lecithin-whey protein concentrate, its viscosity at 25 Pa was lower than the samples with sodium caseinate (Figure 3.8). It might be attributed to the vulnerability of larger oil droplets of emulsions to break-up. As particle size of the emulsion increased, as can be seen in Figure 3.2-4, it might be more prone to deformation due to increasing shear stress. This fact was also discussed in previous study as viscosity of the emulsions including fine droplets would be higher than that of emulsions containing coarse droplets of double emulsion (Pal, 2011).

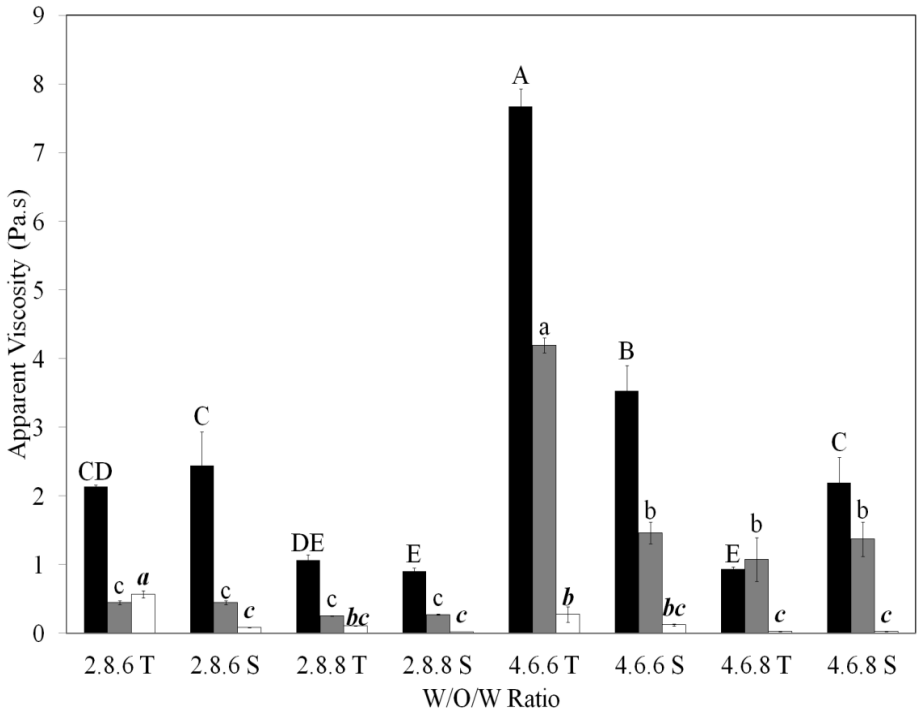

Figure 3.8 Apparent viscosities (corresponding to 25 Pa) of double emulsions at different W/O/W formulations containing sodium caseinate: (■), xanthan gum: (■), lecithin-whey protein concentrate: (□), and T: High speed homogenization, S: Ultrasonic homogenization. Capital, italic and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$)

The relationship between particle size and viscosity of the double emulsion are complicated if the viscosity of the outer aqueous phase is higher. According to a recent study, highly concentrated gelatin solution of the aqueous phase required

increased rates of shear stress during homogenization. High shear stress that was applied for proper homogenization of primary emulsion and droplet formation led to rupture of existing primary emulsion droplets and caused release of active agent (Muguet et al., 1999).

Observations in this study showed that as inclusion of primary emulsion proceeded during second homogenization, the viscosity of the emulsion prepared increased. In other words, as mass fraction of the dispersed phase increased, viscosity of the double emulsion increased. Supportive empirical results were observed from Figure 3.8. Samples with formulation of 2.8.6 and 4.6.6 with higher ratio of internal dispersed phase had higher viscosity values of 2.9 and 1.0 Pa.s, respectively. It can be explained by an analogous well-known knowledge, up to critical volume fraction of dispersed phase, viscosity of the simple emulsion increases because of formation of closely packed thick system (McClements, 2004). This phenomenon was also observed in double emulsion formulations, namely catastrophic inversion. According to Fernando et al. (2007), when the internal droplet fraction of double emulsions exceeds the critical value, double emulsion turns into simple emulsion immediately (Fernando et al., 2007). According to another study, critical dispersed phase ratio was described as the point where “too much” internal phase was included into continuous phase of the emulsion, immediate coalescence between droplets occurred and dispersed phase became continuous phase (Tyrode et al., 2005). Preliminary experiments showed that each emulsifier at a certain concentration had its specific catastrophic inversion limit and up to that limit viscosity of the emulsion increased. Fernando et al. (2007) also stated that this inversion was related to C_h (initial concentration of the hydrophilic surfactant in the external water phase) and CMC (critical micellar concentration) which was another property about the emulsifier concentration (Fernando et al., 2007).

This phenomena was also explained a formulation by Krieger-Doughert (Schuch et al., 2013), spherical particles is related to volume fraction value of the particles (Schuch et al., 2013).

$$\eta = \eta_0 \left(\frac{\phi}{\phi_c}\right)^{-2} \qquad \textbf{Equation 3.1}$$

where η is the relative viscosity of the emulsion prepared, ϕ is the volume fraction of the particles, and ϕ_c is a critical packing parameter, η_0 is relative initial viscosity of the continuous phase.

As volume fraction of particles increases, viscosity of the emulsion increases up to critical packing parameter at which particles become firmly packed together and the emulsion acquired solid-like characteristics where particles are not influenced by attractive and repulsive interactions. As equation implies, after reaching ϕ_c, viscosity of emulsion decreases immediately.

3.1.3. Stability of Double Emulsions

Stability is the main desired factor of double emulsion studies. Many methods were developed for measurement of double emulsions stability like heat exposure, ageing, backscattering intensity measurement and centrifuge (Desplanques et al., 2012; Zinoviadou et al., 2012). In this study, stability of produced double emulsions was quantified by separated residual of centrifugation due to its convenience and promptness. Stability of double emulsions was demonstrated with percentage of separation compared to initial amount of double emulsion subjected to centrifuge.

Average of stability values of double emulsions produced by sodium caseinate, xanthan gum and lecithin-whey protein concentrate were found to be 76.4, 42.3 and 34.0 %, respectively. The results became reasonable when the parameters

that affected the stability of emulsion like droplet size and rheology of produced double emulsion were evaluated.

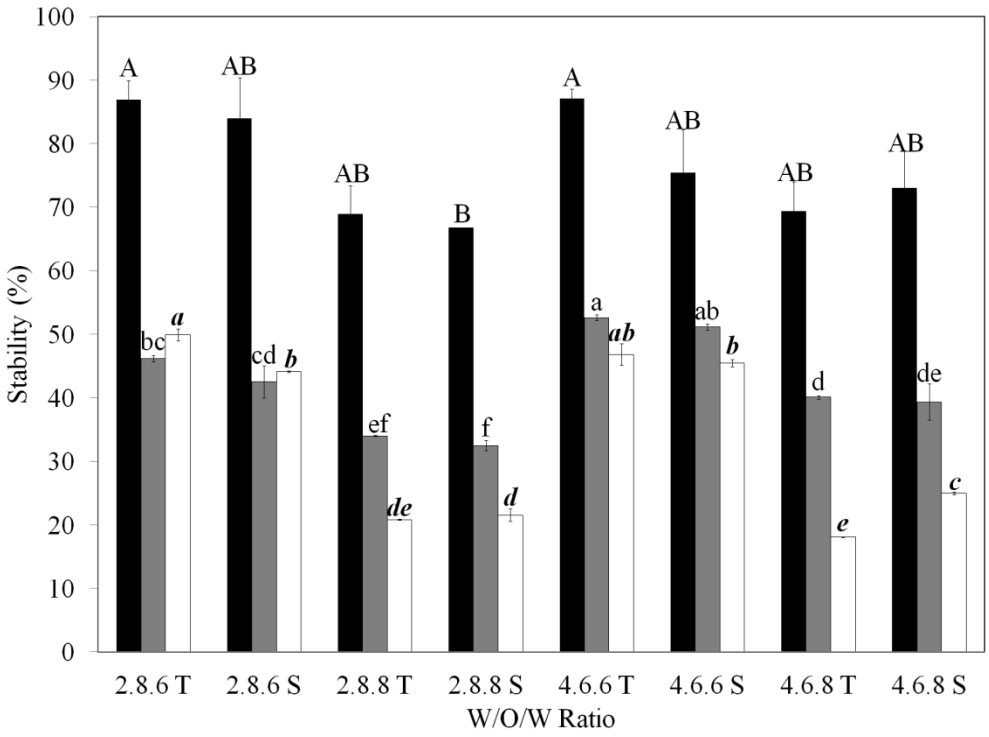

Figure 3.9 Stability values of double emulsions at different ratios of W/O/W formulations containing sodium caseinate: (■), xanthan gum: (■), lecithin-whey protein concentrate: (□), and T: High speed homogenization, S: Ultrasonic homogenization. Capital, italic and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$)

As can be interpreted from apparent viscosity values at constant shear stress (25 Pa), viscosity of double emulsions with sodium caseinate, xanthan gum and lecithin-whey protein concentrate are 2.6, 1.2 and 0.2 Pa.s, respectively (Figure 3. 8). Higher apparent viscosity of emulsion increased the stability of emulsion as proven by experimental results (Figure 3.9). Stability of double emulsions are interfered by numerous physicochemical mechanisms like gravitational separation, flocculation, coalescence, phase inversion, Ostwald ripening, aggregation, coagulation, and diffusion through middle phase of double emulsion (Dickinson, 2010; Hattrem et al., 2014; McClements, 2004; Pawlik et al., 2010; Tadros et al., 2004). Gravitational separation is one of the main problems concerning stability of double emulsions, which can be explained as partition of disintegrated phases of the double emulsion due to densities of the phases.

According to Stoke's Law as viscosity of fluid increases, speed of droplet decreases and speed of a droplet is directly proportional to square of droplet radius (Geankoplis, 2003). Therefore, it can be concluded that droplets with smaller particle size move at low speed due to gravitational forces and reduction of droplet size decelerates gravitational separation. This theoretical knowledge was proven to be practically true by many researchers (Cofrades et al., 2013; Garti, 1997b; Okochi & Nakano, 2000). As a result, it could be concluded that double emulsions with smaller droplet size were more stable than double emulsions with larger droplet size. Samples with sodium caseinate which was observed to have minimum Sauter mean diameter were the most stable samples among other double emulsion samples (Figure 3.2 and Figure 3.9). This can be explained by the combined effect of minimum droplet size and large viscosity values of the double emulsions containing sodium caseinate.

Droplet size of double emulsions prepared with lecithin-whey protein concentrate was smaller than that of samples with xanthan gum. Therefore, it

was expected that double emulsions with lecithin-whey protein concentrate would be more stable than samples with xanthan gum. This unexpected result could be explained by characteristic feature of xanthan gum. As it can be observed viscosity values of the samples in Figure 3.8, double emulsions containing lecithin-whey protein concentrate had the smallest viscosity values as compared to double emulsions containing xanthan gum. Xanthan gum is commonly used thickening agent in food industry, and it is also benefited by double emulsion formulations (Benichou et al., 2004; Delample et al., 2014). According to the study of Ye et al. (2004), polysaccharides like xanthan gum addition increases stability of emulsion by enhancing viscosity of continuous phase. At higher levels of xanthan gum, stability increases as coalescence decreases due to viscosity of emulsion (Ye et al., 2004). In another study about the double emulsions, it was stated that the inclusion of cellulose nanofibril, high molecular weight polysaccharide, into secondary aqueous phase increased viscosity. Increased viscosity of emulsion accompanied by small droplet size increased stability of emulsion (Carrillo et al., 2015).

As stability values were evaluated with respect to oil-like-phase content of the double emulsion, it can interpreted that as dispersed phase content of double emulsions increased, the stability of the double emulsion increased. Samples with 2.8.6 and 4.6.6 formulations had higher stability values as compared to samples with 2.8.8 and 4.6.8 formulations (Table A.7). As it was explained in rheological properties, up to a critical dispersed phase ratio, apparent viscosity of the solution increased and this increased the stability of the emulsion.

3.1.4. Encapsulation Efficiency of Double Emulsions

In the literature, encapsulation efficiency of W1/O/W2 emulsions was determined by conductivity method, DSC method or photometric observation of marker agent after centrifugation (Pawlik et al., 2010; Schuch et al., 2014). In

this study, conductivity of second aqueous phase (W2) was measured by immersing the conductivity probe into double emulsion after preparation. It was aimed to detect conductivity change with respect to non capsulated NaCl ions present in the second aqueous phase W2. Since indicator agent of NaCl was incorporated only into the first aqueous phase W1, NaCl composition corresponding conductivity value was determined by a calibration curve (Table B.1) Encapsulation efficiency was calculated as percentage of encapsulated NaCl.

Encapsulation efficiency is an important parameter concerning double emulsions used for encapsulation of active agent. Although it is frequently mentioned in the literature, it is a rarely studied property.

According to Schuch et al. (2013 & 2014), encapsulation efficiency of double emulsions is correlated with their droplet size. The conclusion revealed that larger double emulsion droplets, higher the encapsulation efficiency was (Schuch et al., 2013; Schuch et al., 2014). This trend was observed for each hydrophilic emulsifier type as well as each ratio of the double emulsions, as seen in Figure 3.2-4, and Figure 3.10. Average droplet size of double emulsions with sodium caseinate, xanthan gum, lecithin-whey protein concentrate were 5.7, 178.5 and 11.8 μm, respectively. Corresponding encapsulation efficiency values were 94.1 % for sodium caseinate, 97 % for xanthan gum, and 96.1 % for lecithin-whey protein concentrate. Regardless of emulsifier type, ratios of double emulsion with the smallest particle size had the minimum encapsulation efficiency (Table 3.2).

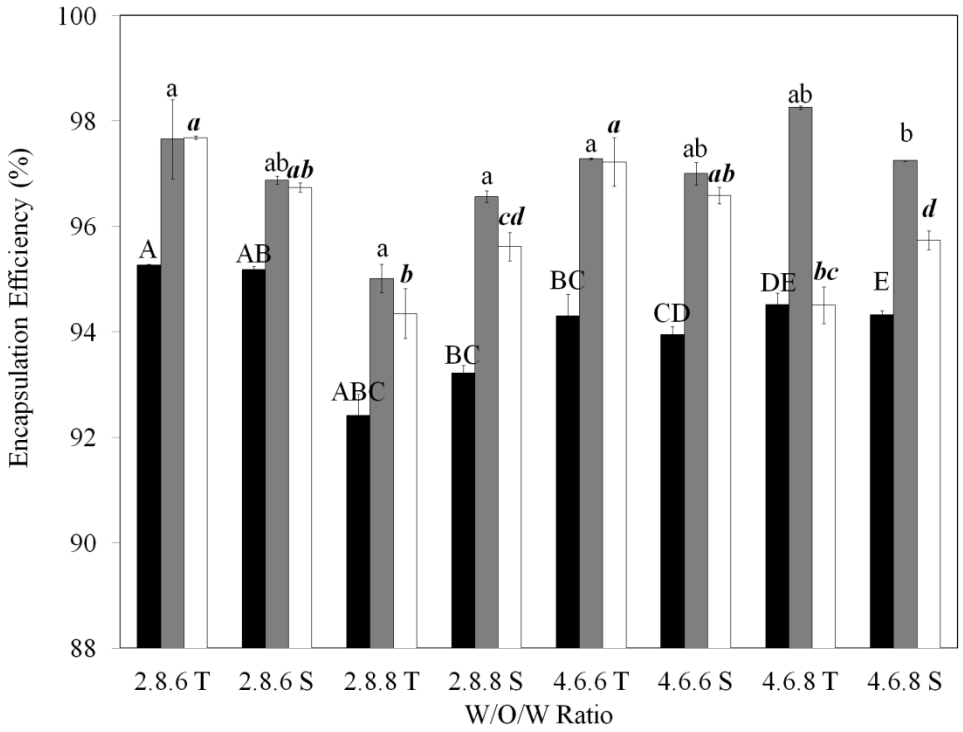

Figure 3.10 Encapsulation efficiency of double emulsions at different ratios of W/O/W formulations containing sodium caseinate: (■), xanthan gum: (■), lecithin-whey protein concentrate: (□), and T: High speed homogenization, S: Ultrasonic homogenization. Capital, italic and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$).

Table 3.2 Average droplet size and average encapsulation efficiencies of corresponding double emulsions.

Emulsion formulation	Droplet Size(μm)	Encapsulation Efficiency (%)
2.8.6	90.4	96.6
4.6.6	89.6	96.1
4.6.8	50.1	95.8
2.8.8	31.2	94.5

As a conclusion, for the production of double emulsions with high encapsulation efficiency, larger particle size should be achieved. This conclusion could be explained by the total area of the interface between O/W2 which became larger as particle size of the oil droplet decreased. For smaller oil droplets, possibility of contact of inner aqueous phase (W2) with the interface of oil-water was higher as compared to larger droplet size (Schuch et al., 2013).

Encapsulation efficiency was also affected from the characteristics of the emulsifier used as hydrophilic emulsifier. Double emulsions containing xanthan gum resulted in the highest encapsulation efficiency due to both bigger droplet size and characteristics of xanthan gum (Figure 3.10 & Table A.1). Gelling property of xanthan gum increased the encapsulation efficiency of double emulsion, as well. Gel formation was found to inhibit the release of caffeine (hydrophilic agent) encapsulated in the inner aqueous phase and to increase encapsulation efficiency (Dickinson, 2010).

3.1.5. Long Term Stability of Double Emulsions

Incorporation of double emulsions in food systems is difficult due to their inheriting thermodynamic instability and rapid coalescence (Garti & Aserin, 1996). For industrial use, or further studies double emulsions are required to stay stable in long term. Long term stable emulsions are specified as "approaching thermodynamic stability" (Izquierdo et al., 2002). In this study, long term stability of double emulsions is measured by gravitational separation of water phase. After preparation, double emulsions were placed into tubes with a known mass and separated water phase was measured. Measurements were done at different time intervals determined by preliminary experiments for each hydrophilic emulsifier type.

Long term stability of double emulsions prepared with sodium caseinate was measured for each week up to 10^{th} week at which maximum phase separation was observed. Samples exposed to 4°C was proven to be more stable compared to samples kept at 20°C. Since high temperatures led to elevated collision of droplets and rapid ripening of interfaces, it was an expected result. Apart from that, elevated temperature accelerated the denaturation of emulsifiers used for double emulsion formation (Iqbal et al., 2013).

Long term stability of double emulsion is mainly affected by rheological properties of the emulsion. As it was explained before, apparent viscosity of double emulsion decreased the velocity of the particle, so gravitational separation was inhibited. Samples with 4.6.6 and 2.8.6 formulations having maximum viscosity values and relatively smaller particle size were stable in long term as it was expected (Figure 3.1 & Table A.1). Samples with 2.8.6, 4.6.6 formulations were observed to have similar gravitational separation values (Figure 3.11-14).

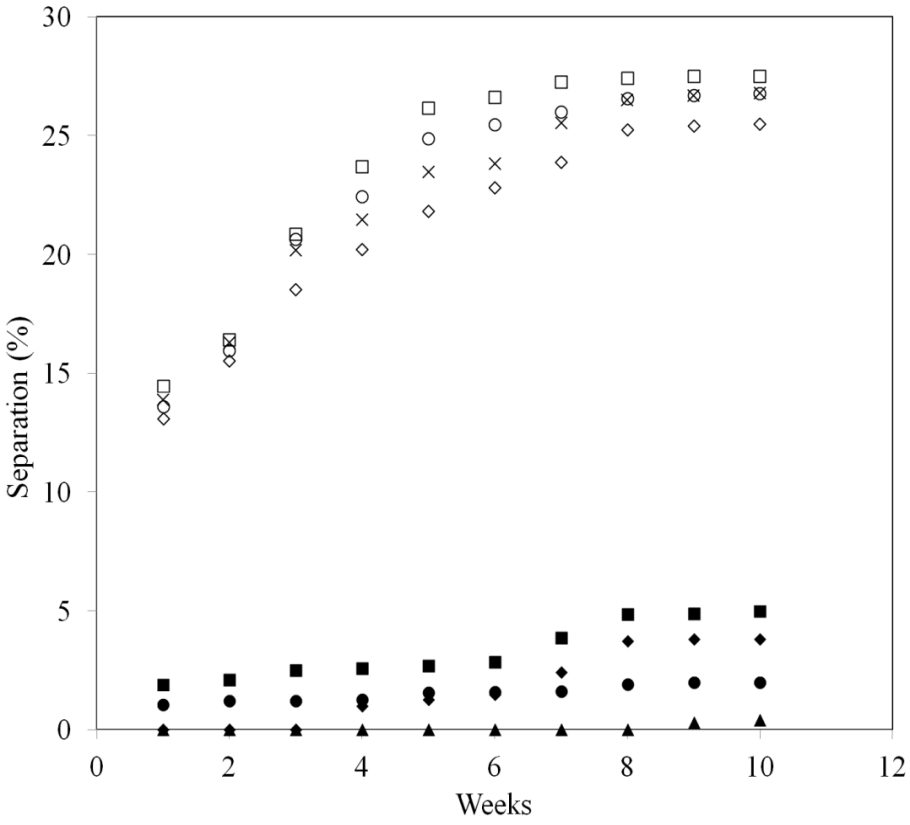

Figure 3.11 Percent separation due to gravitational separation of double emulsion at 4°C containing sodium caseinate at different W/O/W ratios; (◊): 4.6.8 T, (○): 4.6.8 S, (▲): 4.6.6 T, (●):4.6.6 S, (♦): 2.8.6 T, (■): 2.8.6 S, (□): 2.8.8 T, (x): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

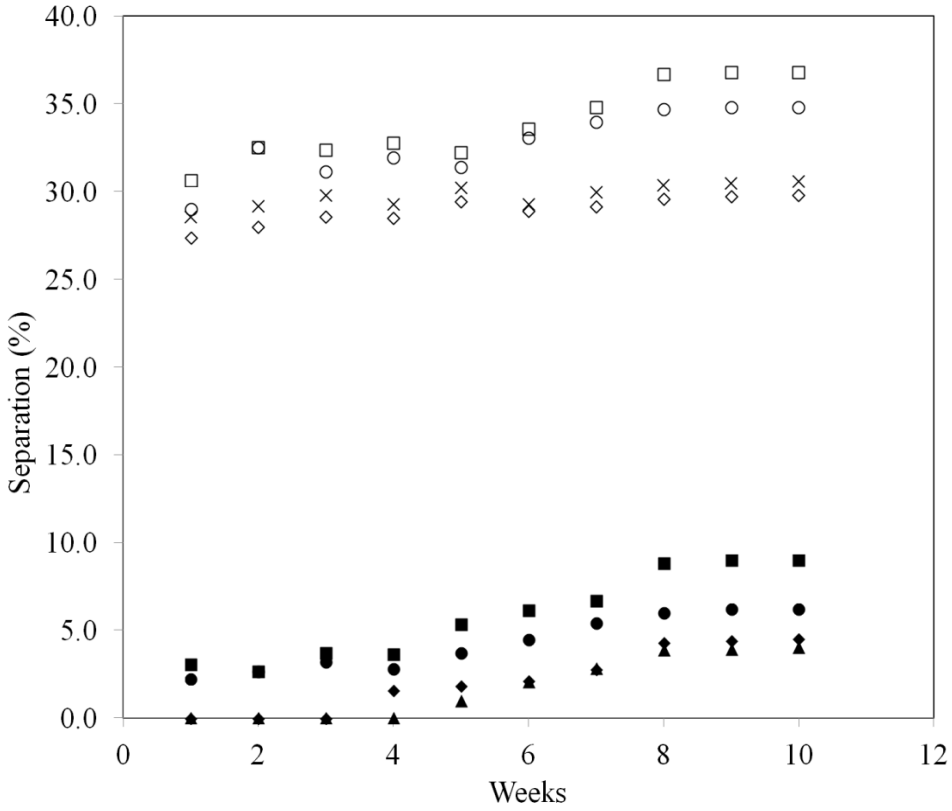

Figure 3.12 Percent separation due to gravitational separation of double emulsionat 20°C containing sodium caseinate at different W/O/W ratios; (◊): 4.6.8 T, (○): 4.6.8 S, (▲): 4.6.6 T, (●):4.6.6 S, (♦): 2.8.6 T, (■): 2.8.6 S, (□): 2.8.8 T, (x): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

Samples containing lecithin-whey protein concentrate were observed to be separated more rapidly as compared to samples containing sodium caseinate and they were separated up to 45%. This result is similar to stability of double emulsions containing corresponding emulsifiers. For double emulsions containing lecithin-whey protein concentrate, 2.8.8 and 4.6.8 formulations were

observed to separate up to 30-45, whereas 4.6.6 and 2.8.6 formulations were separated up to 15-25 %. As it was observed for the samples containing sodium caseinate, samples of 4.6.8 and 2.8.8 formulations were more prone to gravitational separation than samples with 2.8.6 and 4.6.6 formulations. Explanations for temperature was valid for both samples with sodium caseinate and lecithin-whey protein concentrate.

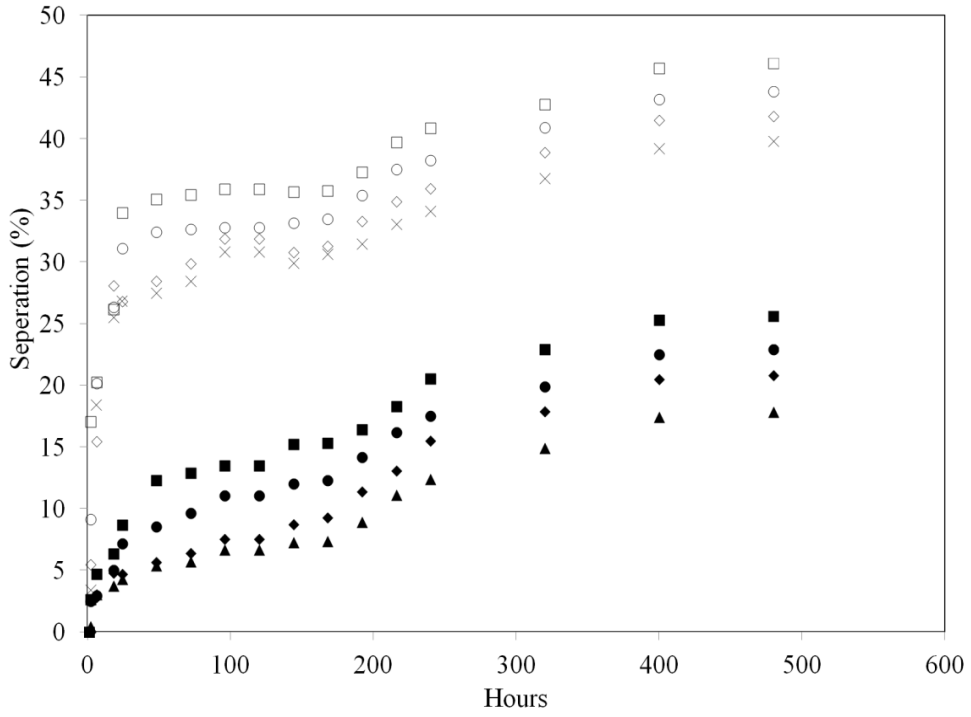

Figure 3.13 Percent separation due to gravitational separation of double emulsionat 4°C containing lecithin-whey protein concentrate at different W/O/W ratios; (◊): 4.6.8 T, (○): 4.6.8 S, (▲): 4.6.6 T, (●):4.6.6 S, (♦): 2.8.6 T, (■): 2.8.6 S, (□): 2.8.8 T, (x): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

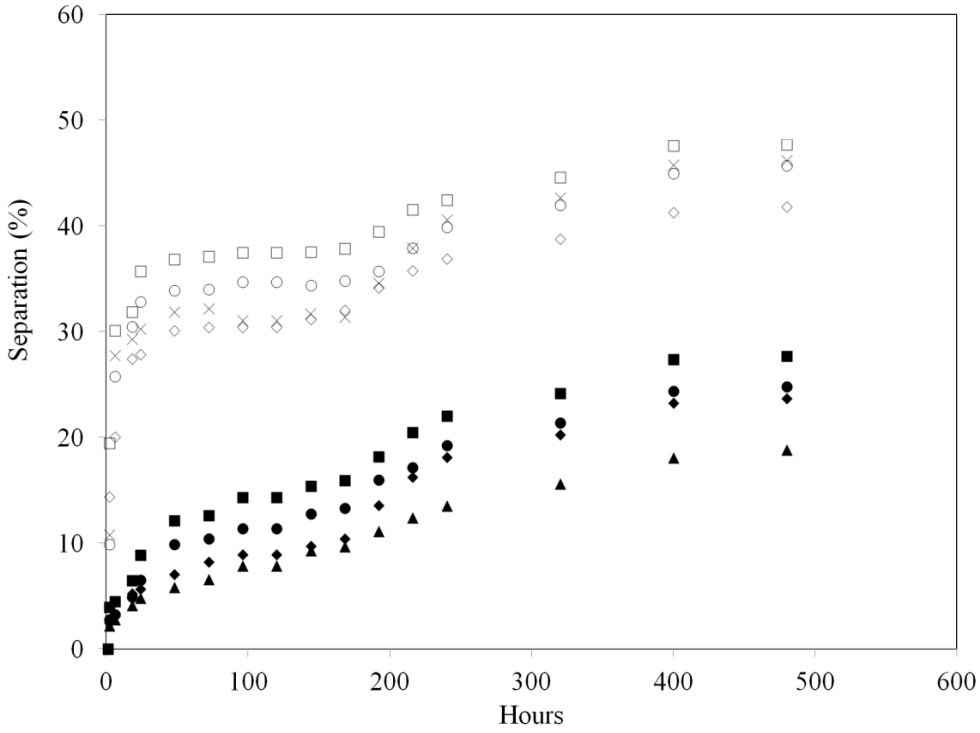

Figure 3.14 Percent separation due to gravitational separation of double emulsionat 20°C containing lecithin-whey protein concentrate at different W/O/W ratios; (◊): 4.6.8 T, (○): 4.6.8 S, (▲): 4.6.6 T, (●):4.6.6 S, (♦): 2.8.6 T, (■): 2.8.6 S, (□): 2.8.8 T, (x): 2.8.8 S, and T: High speed homogenization, S: Ultrasonic homogenization.

Although separation of double emulsions with sodium caseinate and lecithin-whey protein concentrate could be observed and measured, it was not be possible to obtain quantitative data for xanthan containing samples. Samples with xanthan gum went through a phase inversion, cluster of oil droplets aggregated and coalescence occurred. Settlements of fragmented gelled xanthan gum did not allow a visible separation of the emulsion.

3.1.6. Optical Imaging of Double Emulsion

Optical images of the double emulsions were obtained by inverted light microscopy. For different homogenization methods and emulsifier types, morphological properties of double emulsions were observed (Figure 3.15-Figure 3.17).

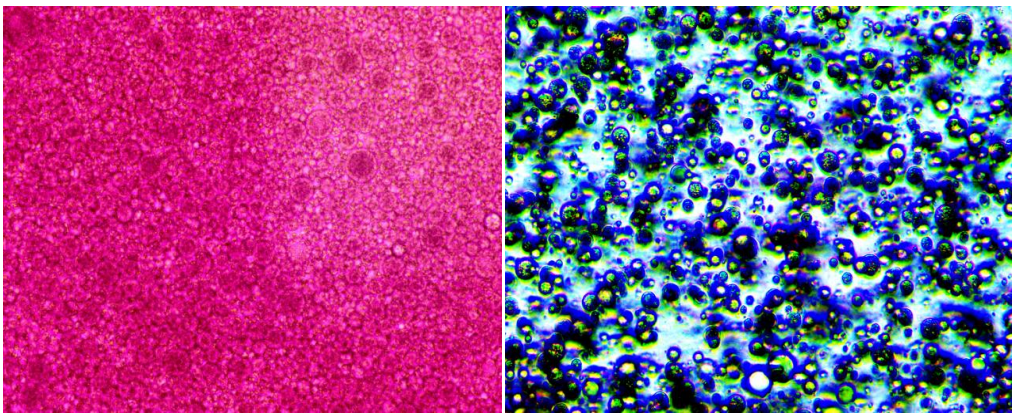

Figure 3.15 Optical images of double emulsion containing sodium caseinate with 2.8.8 formulation prepared by ultrasonic homogenizer (left image), and high speed homogenizer (right image) and at magnification factor of x40.

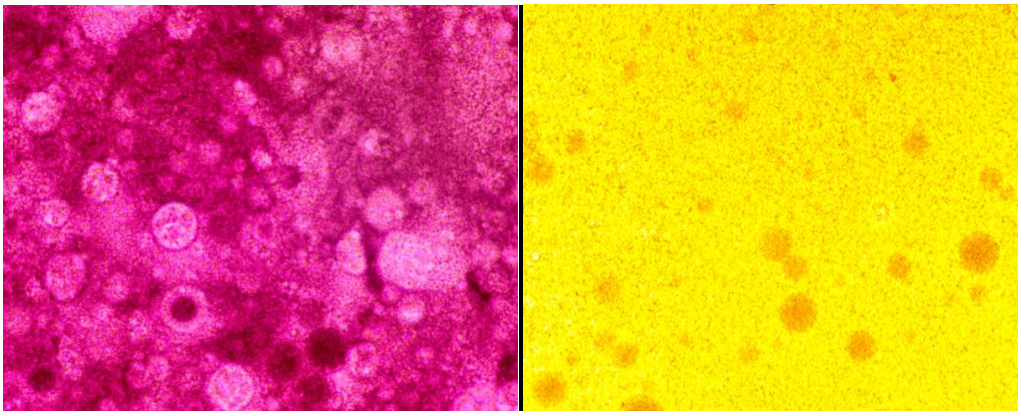

Figure 3.16 Optical images of double emulsion containing lecithin-whey protein concentrate with 2.8.8 formulation prepared by ultrasonic homogenizer (left image), and high speed homogenizer (right image) and at magnification factor of x40.

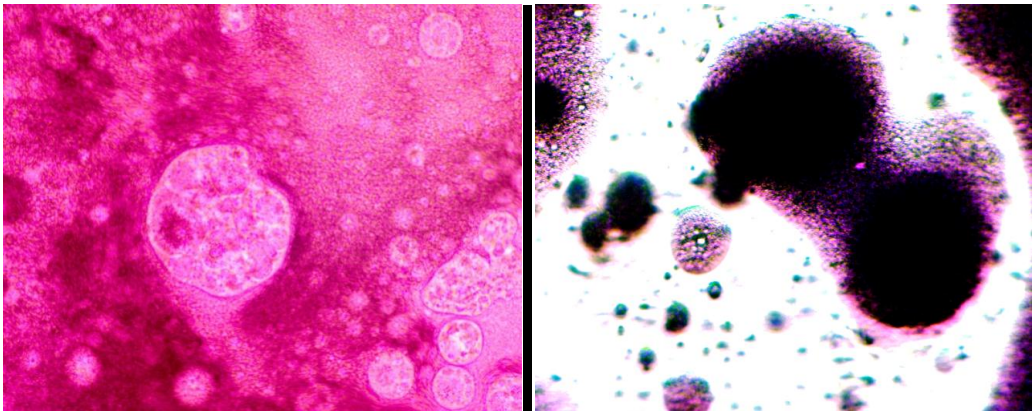

Figure 3.17 Optical images of double emulsion containing xanthan gum with 2.8.8 formulation prepared by ultrasonic homogenizer (left image), and high speed homogenizer (right image) and at magnification factor of x40.

Observations of microscopic evaluations revealed that a sample containing xanthan gum had capillary vein structures between the primary emulsion droplets. Due to larger particle size, samples containing xanthan gum was prone to phase inversion. It was observed that fragments of samples containing xanthan gum turned into simple emulsions of W/O during rheological measurements.

As it can be interpreted from the images, particle sizes of samples determined by particle size analyzer were similar to particle size observed by microscopy. In other words, the largest Sauter mean diameter in particle size analysis and the largest droplets in images were observed in emulsions containing xanthan gum (Figure 3.17). Another significant point is that monomodal behavior of particle size of sodium caseinate was observed by the optical image of the samples. Particle size of the droplets were similar to each other and size distribution was concentrated around one average. On the other hand, particle size distribution measurements revealed that xanthan gum and lecithin-whey protein concentrate showed bimodal distribution. As it can be identified from optical images, samples with lecithin and xanthan gum had particles with varying sizes, leading to bimodal distribution of the particle size.

3.2. Mayonnaise Production by Using Double Emulsions

Mayonnaise, characteristically, is a food material with high fat content up to 70-80%. According to conclusions driven from the results of the double emulsion investigation, production of mayonnaise with different emulsifiers could vary due to their critical packaging parameter for corresponding concentration of the emulsifier. Control sample was produced for each type of the emulsifier with different oil amounts determined by preliminary experiments. Mayonnaise formulations were designed by using optimum conditions obtained by double

emulsion experiments. Primary emulsions with ratios of 2.8 and 4.6 were prepared by high speed homogenization because high speed homogenization led to smaller particle size, higher apparent viscosity with lower energy input. Controlled mayonnaise samples were prepared by incorporation of oil phase into water phase.

3.2.1. Particle Size of Mayonnaise

Mayonnaise is, mainly, an oil-in-water emulsion. Characteristics of mayonnaise and stability are associated with droplet size and distribution of the oil droplets. When particle size of oil droplet dispersed in water phase decreases; stability of mayonnaise increases (Mattia et al., 2015) Thus, particle size and distribution of mayonnaise samples are important parameters to evaluate quality. It was observed that emulsifier type was a determinant property of particle size characteristics. As can be seen in Figure 3.18, it was observed that each hydrophilic emulsifier type resulted in a different pattern of size distribution. Samples with sodium caseinate have monomodal size distribution with span values varying between 1.033-1.120. Xanthan gum and lecithin-whey protein concentrate led to bimodal size distribution with span values changing between 1.733-1.920 and 1.384-1.550, respectively. This results were consistent with the study done about double emulsions prepared with xanthan gum for encapsulation of betalain (Kaimainen et al., 2015). This result was also similar to the results found in double emulsion preparation part of this study (Figure 3.1).

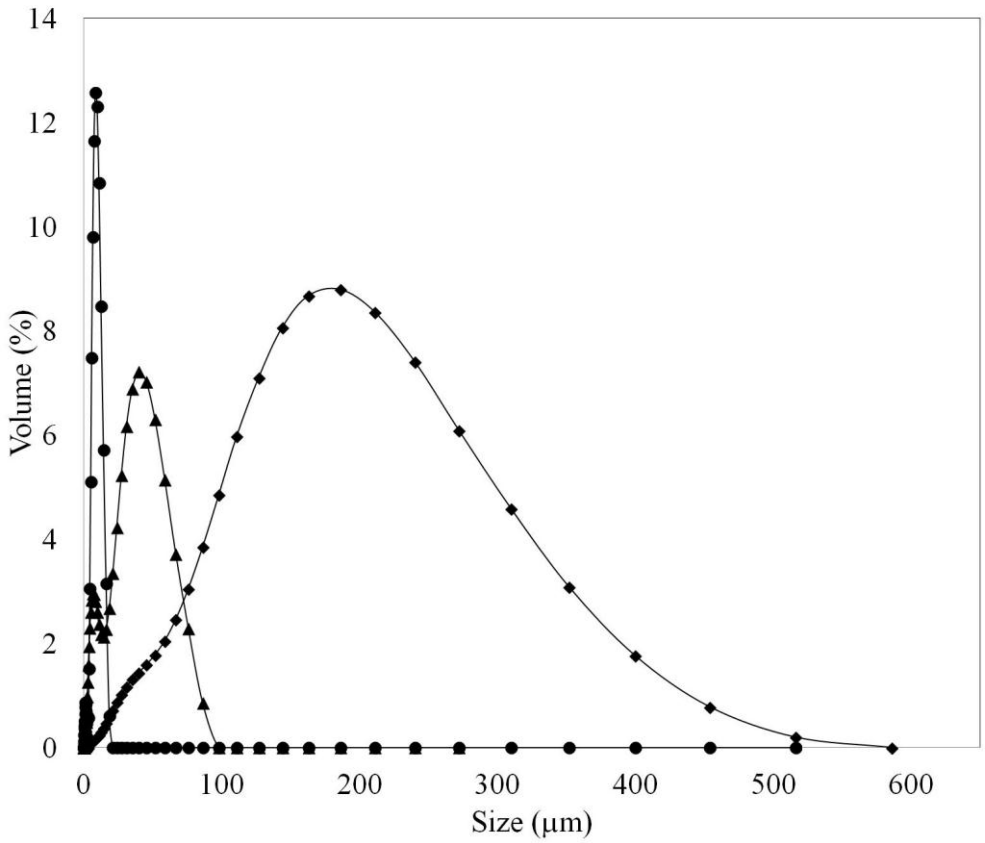

Figure 3.18 Particle size distribution graph of double emulsions having ratio of 2.8.8 prepared with high speed homogenization and formulated with sodium caseinate: (●), xanthan gum: (♦), lecithin-whey protein concentrate: (▲)

In the double emulsion results, it was concluded that regardless of composition of the inner water phase, the same ratio of second emulsion affected particle size similarly (Figure 3.2-Figure 3.4). For mayonnaises containing sodium caseinate and lecithin-whey protein concentrate, the same discussion is valid (Figure 3.19). However, for mayonnaise production with xanthan gum, the changes in particle size results are observed for different ratios of primary emulsion.

Samples with 4.6 primary emulsion resulted in higher particle size compared to 2.8 primary emulsions. Sauter mean diameters of xanthan containing mayonnaise were larger than lecithin-whey protein concentrate and sodium caseinate containing ones (Table 3.3). Similar sequence in terms of particle sizes was observed in double emulsions (Figure 3.2- Figure 3.4). This result was attributed to dense network structure of xanthan gum that inhibited the formation of smaller particle size and development of thicker layer of around emulsion droplet. Especially, particle sizes of double emulsified mayonnaise samples containing sodium caseinate and lecithin-whey protein concentrate were similar to control mayonnaise samples.

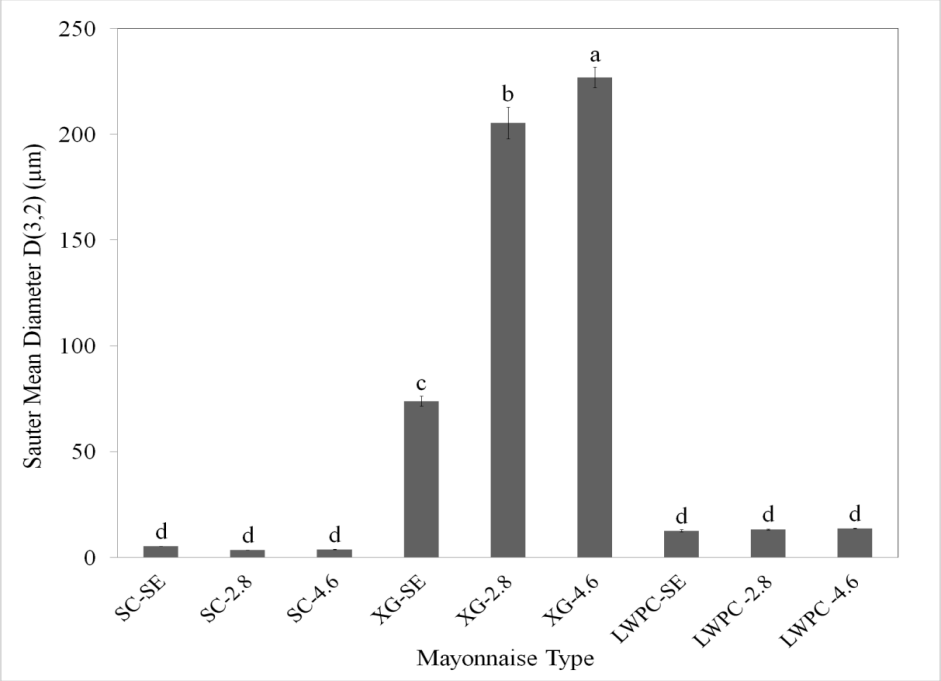

Figure 3.19 Sauter mean diamater values of control mayonnaise (SE): simple emulsion, mayonnaises with different ratios of primary emulsion, (W/O): 2:8 and 4:6, containing SC: sodium caseinate, XG: xanthan gum, LWPC: lecithin-whey protein concentrates. Bars with different letters represent significant difference ($p \leq 0.05$).

Table 3.3 Sauter mean diameter and span values of corresponding mayonnaise samples

Mayonnaise Sample	Sauter mean diameter (μm)	Span
SC-SE	5.29	1.120
SC-2.8	3.49	1.033
SC-4.6	3.76	1.110
XG-SE	73.84	1.733
XG-2.8	205.42	1.850
XG-4.6	226.96	1.920
LWPC-SE	12.58	1.384
LWPC-2.8	13.29	1.533
LWPC-4.6	13.72	1.733

3.2.2. Rheological Properties of Mayonnaise

The rheology of mayonnaise is an important parameter because rheological properties are affected by the formulation of mayonnaise, and process conditions (Peressini et al., 1998). Its influential effect on consumer choice makes rheological properties of mayonnaise crucial. There are numerous studies

investigating rheological properties of mayonnaise samples (Kishk & Elsheshetawy, 2013; Laca et al., 2010; Li et al., 2014).

As can be seen in Figure 3.20-3.22, the increase in shear stress decreased apparent viscosity of mayonnaise. In the study, investigations of rheological properties showed that flow behavior of mayonnaise samples with simple oil phase and primary emulsions could be described by power law model and Herschel Bulkley model with R^2 = 0.9908 and 0.9992, respectively (Table 3.4 & 3.5). In the literature, there are researches describing the flow behavior of mayonnaise by power law and Herschel Bulkley model, as well (Laca et al., 2010; Liu et al., 2007; Ma & Boye, 2012). Type of emulsifier affected the rheological properties differently. For sodium caseinate, inclusion of primary emulsion was found to increase consistency coefficient (K). It could be attributed to decreased particle size of the mayonnaise samples produced, since reduced particle size were known to improve rheological properties of the mayonnaise sample.

Since the results of rheological investigation differed for each emulsifier type, apparent viscosity at a constant shear stress could not be used for comparison. Therefore, rheological properties were compared using consistency coefficients of mentioned flow models. As it was mentioned before, particle size and apparent viscosity were inversely related. The increase in particle size led to weaker rheological properties. Mayonnaise samples with sodium caseinate had minimum average particle size of 4.18 and had the highest consistency coefficient values of 349.38 and 216.07 for Herschel Bulkley model and power law model, respectively. It was well observed that as particle size of mayonnaise decreased, apparent viscosity of the mayonnaise sample increased. In other words, larger particle size led to weaker rheological properties and lower consistency coefficient (Table 3.4 -Table 3.5)

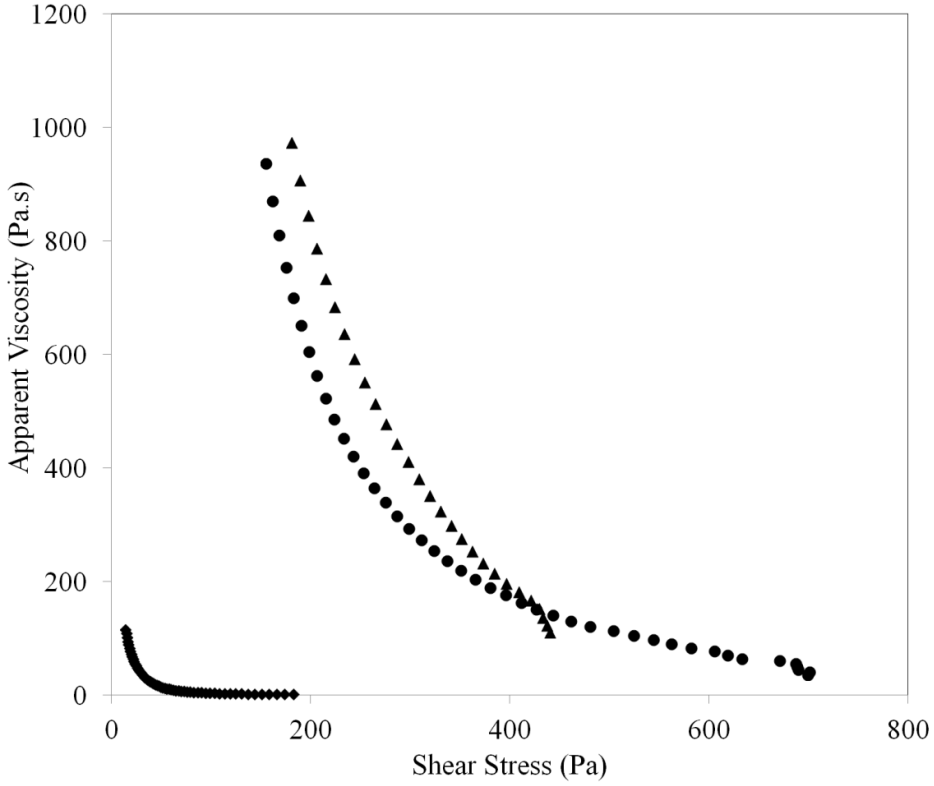

Figure 3.20 Apparent viscosity of control mayonnaise sample and mayonnaise prepared with sodium caseinate at different ratios; (♦): simple emulsion, (■): W/O ratio of 2:8, (▲): W/O ratio of 4:6.

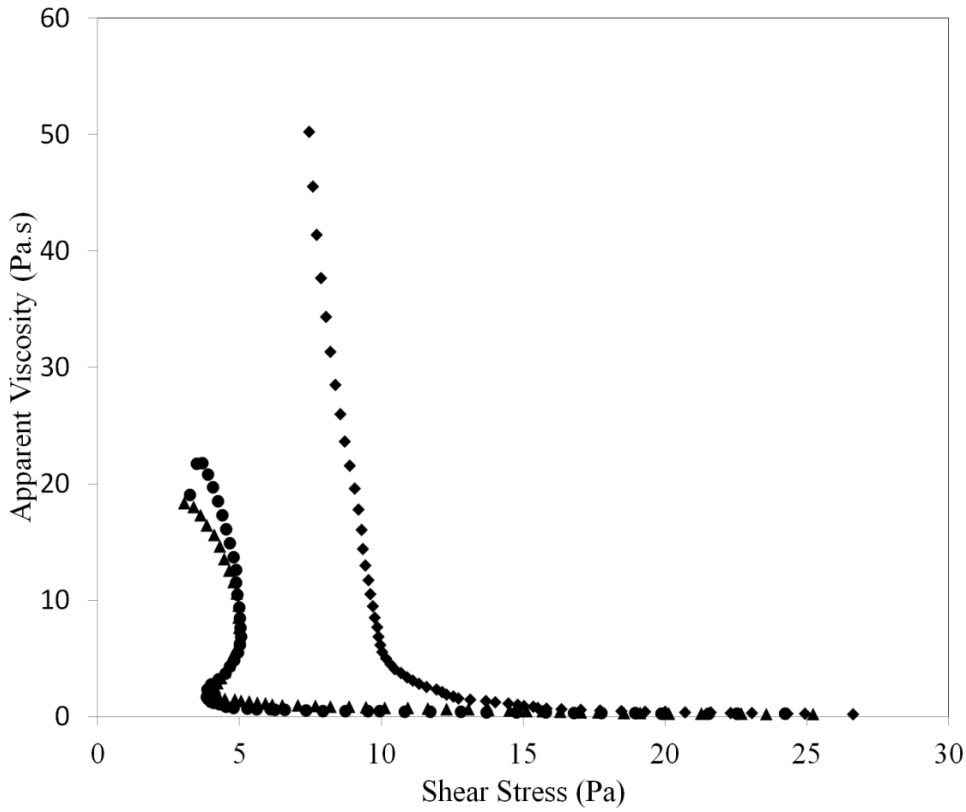

Figure 3.21 Apparent viscosity of control mayonnaise sample and mayonnaise prepared with xanthan gum at different ratios; (♦): simple emulsion, (■): W/O ratio of 2:8, (▲): W/O ratio of 4:6.

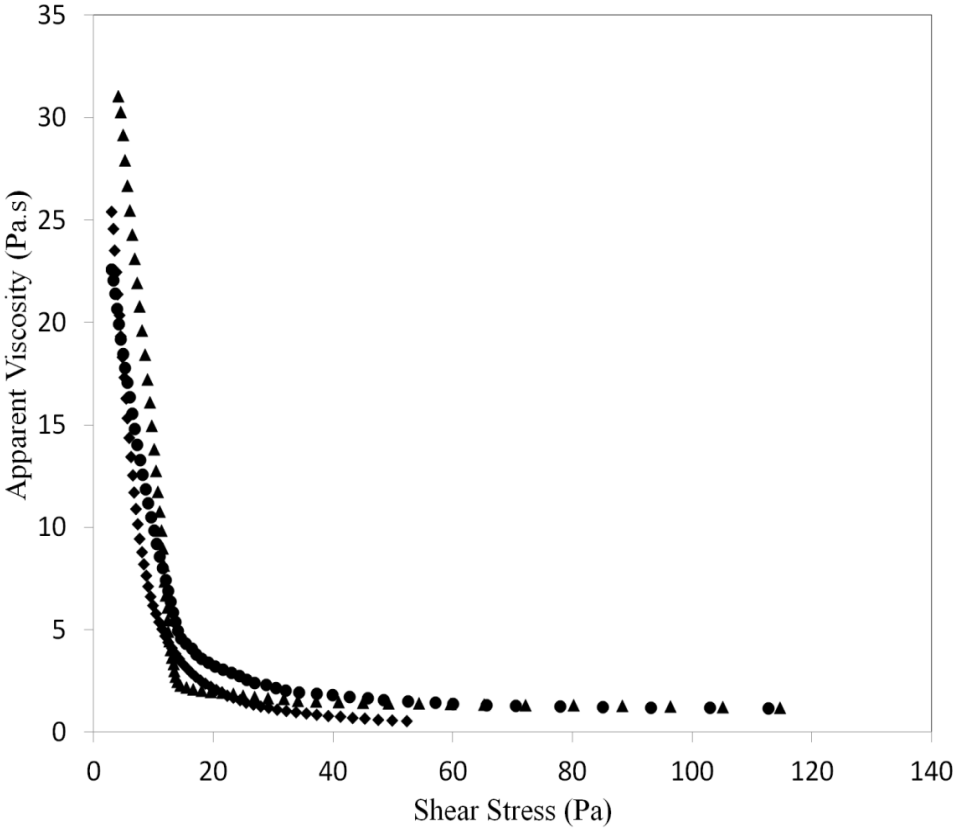

Figure 3.22 Apparent viscosity of control mayonnaise sample and mayonnaise prepared with lecithin-whey protein concentrate at different ratios; (♦): simple emulsion, (■): W/O ratio of 2:8, (▲): W/O ratio of 4:6.

Table 3.4 Hershely- Bulkey Model coefficients of mayonnaise samples prepared with different emulsifier types and ratios of primary emulsion.

Mayonnaise Sample	Yield Stress(Pa)	K	n	R^2
SC-SE	5.164	26.15	0.4179	0.9996
SC-2.8	-53.85	669.0	0.2999	0.9997
SC-4.6	-342.7	353.0	0.1451	0.9997
XG-SE	7.249	28.20	0.4795	0.9978
XG-2.8	-8.052	5.566	0.3855	0.9997
XG-4.6	-29.78	2.095	0.1397	0.9992
LWPC-SE	0.4825	7.669	0.4182	0.999
LWPC-2.8	3.535	5.616	0.6054	0.9986
LWPC-4.6	8.638	1.179	0.843	0.9992

Table 3.5 Power Law Model coefficients of mayonnaise samples prepared with different emulsifier types and ratios of primary emulsion.

Mayonnaise Sample	K	n	R^2
SC-SE	31.72	0.3747	0.9995
SC-2.8	321.2	0.3545	0.999
SC-4.6	295.3	0.3290	0.9972
XG-SE	9.472	0.1759	0.9758
XG-2.8	2.892	0.6153	0.998
XG-4.6	1.525	0.4869	0.9922
LWPC-SE	9.940	0.4051	0.995
LWPC-2.8	9.526	0. 4341	0.9971
LWPC-4.6	8.155	0.3699	0.9638

3.2.3. Stability of Mayonnaise

Stability of mayonnaise samples are determined by the particle size and rheological properties of the mayonnaise samples. Since each emulsifier type had different capacity for incorporation of oil phase or primary emulsion, comparison of stability with respect to emulsifier type was unfair. Thus stability of samples was compared for each emulsifier type, individually.

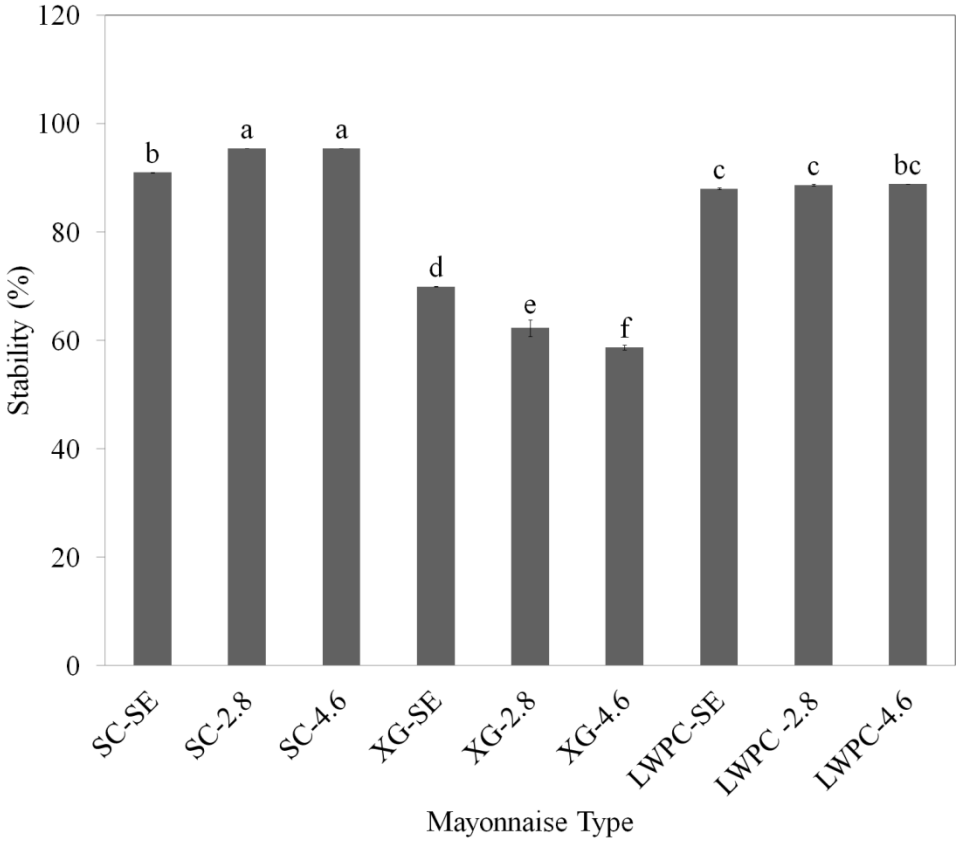

Figure 3.23 Stability of control mayonnaise (SE): simple emulsion, and mayonnaise prepared with (SC): sodium caseinate, (XG): xanthan gum, (LWPC): lecithin-whey protein concentrate, and different W/O ratios of 2:8 and 4:6. Bars with different letters represent significant difference ($p \leq 0.05$).

Reduced droplet size increases the stability of the double emulsions (Carrillo et al., 2015). This behavior was detectable in samples with sodium caseinate (Figure 3.23), control samples having maximum droplet size of 5.285 μm had lower stability as compared to mayonnaise prepared with double emulsion. This trend was valid for lecithin-whey protein concentrate. In other words, increased droplet size decreased stability of emulsion due to lowered movement speed and

gravitational separation. Additionally, it was reported that reduced particle size of emulsion increased the viscosity of the double emulsion. Increased viscosities of double emulsion decreased the movement of droplet and inhibited coalescence, sedimentation and other instabilities within the emulsion. Combined effect of reduced particle size and increased viscosity of emulsion increased the stability of the mayonnaise samples. This effect was observed in the various emulsion studies related with cellulose nanofibrils (Carrillo et al., 2015), gum arabic and xanthan gum (Zhang & Liu, 2011). This trend was also observed in the formulation of double emulsion part of this study. The highest stability values were obtained in samples containing sodium caseinate with minimum particle size and highest viscosity values (Figure 3.2 & Figure 3.8). Since it is obtained similar stability values with control mayonnaise samples, mayonnaise production with double emulsion could be suggested.

3.2.4. Color of Mayonnaise

Inspection on the color of the mayonnaise samples revealed that usage of primary emulsion instead of oil did not affect the color of mayonnaise sample produced. Statistically, mayonnaise samples prepared by double emulsion had same E* values with their counterparts produced by simple emulsification (Table 3.6).

Color measurements of mayonnaise samples displayed that hydrophilic emulsifier type affected the color of the mayonnaise. Since color of the resultant products was related to the ingredients that are used in them, different characteristics of emulsifier led to variances in color of mayonnaise samples. Sodium caseinate was fairly white originally, thus accompanied by the highest L* (lightness) values. Lecithin, characteristically, had dark yellow color and b* (yellowness) values were affected by the lecithin used in resultant mayonnaise samples.

Table 3.6 Average L*, a*, b* and ΔE values of mayonnaise samples

Mayonnaise sample	L*	a*	b*	ΔE*
SC-SE	72.47	-0.22	18.54	26.93
SC-2.8	71.98	-0.34	19.23	27.67
SC-4.6	71.85	-0.29	20.12	28.19
XG-SE	60.12	-0.2	12.75	37.20
XG-2.8	61.3	-0.19	9.5	35.67
XG-4.6	62.4	-0.22	8.12	34.51
LWPC-SE	68.12	-0.11	25.06	33.87
LWPC-2.8	70.22	-0.15	25.04	32.09
LWPC-4.6	70.32	-0.18	25.1	32.05

3.2.5. Oil Content Reduction in Mayonnaise

The major aim of the thesis was to reduce the fat content of mayonnaise by using double emulsions. The inclusion of primary phase into mayonnaise samples decreased total oil content of mayonnaise samples compared to initial fat content. Table 3.7 showed the oil content of control mayonnaise samples and double emulsified mayonnaise samples with primary emulsion with ratio of 2:8 and 4:6. The incorporation of primary emulsion with ratio of 2:8 decreased the

oil content of mayonnaise samples by 20% compared to initial fat content of mayonnaise regardless of hydrophilic emulsifier type. Primary emulsion with ratio of 4:6 decreased the oil content of mayonnaise samples by 40% compared to initial fat content. Mayonnaise sample prepared by sodium caseinate with primary emulsion at ratio of 4:6 had similar stability, particle size and viscosity values with control mayonnaise. Moreover, no color difference was observed between double emulsified mayonnaise samples and control one. In addition, this formulation had an oil content of 36.6 %. Thus, it was possible to reduce oil content of mayonnaise by using double emulsions without losing any quality.

Table 3.7 Oil content of the mayonnaise samples

Mayonnaise Type	Control Sample (%)	Mayonnaise Samples Prepared with Primary Emulsion with Ratio of 2:8 (%)	Mayonnaise Samples Prepared with Primary Emulsion with Ratio of 4:6 (%)
Sodium Caseinate	61	48.8	36.6
Xanthan Gum	64	51.2	38.4
Lecithin-Whey Protein Concentrate	76	60.8	45.6

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In order to obtain the best double emulsion formulation for usage in food products, particle size, stability, encapsulation efficiency, long term stability, and rheological property analyses were conducted for samples prepared with different W/O/W ratio, emulsifier types and homogenization methods. Then, by using the double emulsion formulation with higher stability, higher apparent viscosity and lower particle size, double emulsified mayonnaise samples were prepared by inclusion of primary emulsion at two different W/O ratios.

Regardless of the ratio of primary emulsion, samples with the same ratio of (W1/O)/W2 had similar characteristics of particle size. This similarity was valid for other characteristics of double emulsions like stability, viscosity, separation, encapsulation efficiency, rheology.

Double emulsions prepared with 4.6.6 and 2.8.6 formulations had the highest stability, encapsulation efficiency, viscosity and lowest particle size values for samples containing sodium caseinate and lecithin-whey protein concentrate. Also increasing dispersed phase ratio up to critical dispersed phase fraction had an increasing effect on rheological properties. Considering long term stability measurements, the most stable double emulsion were sample containing sodium caseinate and 4.6.6 formulation.

In particle size analysis, it was observed that each hydrophilic emulsifier type affected particle size differently. Double emulsion containing sodium caseinate had the smallest particle size while the ones containing xanthan gum had the largest particle size.

Encapsulation efficiency of the double emulsions was correlated with droplet size of the double emulsions. As droplet size increased, encapsulation efficiency increased. Samples with xanthan gum had the largest droplet size and the highest encapsulation efficiency.

High speed homogenization was efficient as compared to ultrasonic homogenization according to particle size and rheological properties.

Considering particle size, stability and rheological measurements, double emulsified mayonnaise samples prepared with sodium caseinate and primary emulsion ratio of 2:8 and 4:6 were the best formulation. These formulations were similar to control mayonnaise in terms of particle size, stability and color. It was possible to reduce oil content of mayonnaise to 36.6% by using double emulsion without affecting quality adversely. This study provides insight that double emulsions can be used to reduce oil content of different food emulsions.

For future study, inclusion of double emulsions into different food materials and release mechanism of the double emulsions could be studied. Furthermore, it could be recommended that hydrophilic emulsifiers could be used in combination with stabilizers or with other hydrophilic emulsifiers for more stable emulsion formulations.

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APPENDIX A

STATISTICAL ANALYSIS

Table A.1 Three way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by homogenization process of UltraTurrax and Ultrasonic homogenization; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

General Linear Model: Particle Size versus Ratio; Emulsifier; Process

```
Factor      Type   Levels  Values
Ratio       fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Emulsifier  fixed       3  l+wp; sc; xg
Process     fixed       2  S; T


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source              DF  Seq SS  Adj SS  Adj MS       F      P
Ratio                3   30525   30525   10175   50,46  0,000
Emulsifier           2  301557  301557  150778  747,73  0,000
Process              1    2295    2295    2295   11,38  0,002
Ratio*Emulsifier     6   67650   67650   11275   55,91  0,000
Ratio*Process        3     336     336     112    0,56  0,648
Emulsifier*Process   2    5939    5939    2970   14,73  0,000
Error               30    6049    6049     202
Total               47  414351


S = 14,2002   R-Sq = 98,54%   R-Sq(adj) = 97,71%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
 33   244,000  275,533   8,696   -31,533     -2,81 R
```

Table A.1 (Continued)

```
 40    72,700   99,941   8,696   -27,241     -2,43 R
 42   260,000  235,967   8,696    24,033      2,14 R
 43   126,000  101,982   8,696    24,018      2,14 R
 47   176,000  149,409   8,696    26,591      2,37 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio   N  Mean  Grouping
2.8.6  12  89,4  A
4.6.6  12  88,8  A
4.6.8  12  49,6     B
2.8.8  12  31,2        C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier   N   Mean  Grouping
xg          16  176,8  A
l+wp        16   11,8     B
sc          16    5,7     B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
T        24  71,7  A
S        24  57,8     B

Means that do not share a letter are significantly different.
```

Table A.2 Three way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by homogenization process of UltraTurrax and Ultrasound; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate and lecithin-whey protein concentrate.

General Linear Model: Particle Size versus Ratio; Emulsifier; Process

```
Factor      Type   Levels  Values
Ratio       fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Emulsifier  fixed       2  l+wp; sc
Process     fixed       2  S; T


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS   Adj MS       F      P
Ratio        3  139,839  139,839   46,613   22,37  0,000
Emulsifier   1  302,273  302,273  302,273  145,09  0,000
Process      1   28,823   28,823   28,823   13,84  0,001
Error       26   54,165   54,165    2,083
Total       31  525,100


S = 1,44336   R-Sq = 89,68%   R-Sq(adj) = 87,70%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
 24   19,1000  16,0300  0,6250    3,0700      2,36 R
 32   20,5000  16,0300  0,6250    4,4700      3,44 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio  N  Mean  Grouping
4.6.8  8  12,0  A
2.8.8  8   8,7    B
4.6.6  8   8,0    B C
2.8.6  8   6,3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier   N  Mean  Grouping
l+wp        16  11,8  A
sc          16   5,7    B
```

Table A.2 (Continued)

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
S        16   9,7  A
T        16   7,8    B

Means that do not share a letter are significantly different.
```

Table A.3 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by homogenization process of UltraTurrax and Ultrasound; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of xanthan gum

General Linear Model: Particle Size_1 versus Ratio_1; Process_1

```
Factor     Type   Levels  Values
Ratio_1    fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Process_1  fixed       2  S; T


Analysis of Variance for Particle Size_1, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
Ratio_1     3   98009   98009   32670  56,51  0,000
Process_1   1    8204    8204    8204  14,19  0,003
Error      11    6359    6359     578
Total      15  112572


S = 24,0444   R-Sq = 94,35%   R-Sq(adj) = 92,30%


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio_1  N   Mean  Grouping
2.8.6    4  255,7  A
4.6.6    4  250,5  A
4.6.8    4  124,7    B
2.8.8    4   76,2    B

Means that do not share a letter are significantly different.
```

Table A.3 (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Process_1  N   Mean  Grouping
T          8  199,4  A
S          8  154,1    B

Means that do not share a letter are significantly different.
```

Table A.4 One way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate

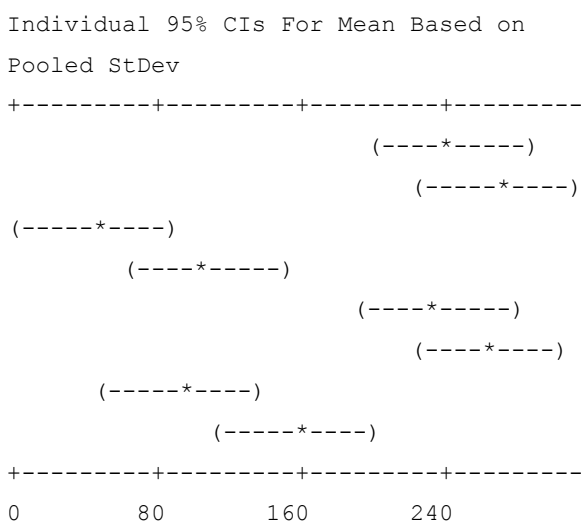
One-way ANOVA: Particle Size versus Ratio

```
Source  DF      SS     MS      F      P
Ratio    7  38,863  5,552  31,12  0,000
Error    8   1,427  0,178
Total   15  40,291

S = 0,4224   R-Sq = 96,46%   R-Sq(adj) = 93,36%

Grouping Information Using Tukey Method
Ratio    N    Mean  Grouping
4.6.8 S  2  8,5250  A
4.6.6 S  2  6,4100    B
4.6.8 T  2  6,3050    B
2.8.8 S  2  6,2200    B
2.8.8 T  2  5,5900    B C
4.6.6 T  2  5,0300    B C
2.8.6 S  2  4,2000      C D
2.8.6 T  2  2,9400        D

Means that do not share a letter are significantly different.
```

Table A.5 One way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of xanthan gum

One-way ANOVA: Particle Size versus Ratio

```
Source  DF      SS     MS      F      P
Ratio    7  107380  15340  23,64  0,000
Error    8    5192    649
Total   15  112572

S = 25,48   R-Sq = 95,39%   R-Sq(adj) = 91,35%
```




```
Pooled StDev = 25,48
```

```
Grouping Information Using Tukey Method

Ratio    N    Mean  Grouping
2.8.6 T  2  269,00  A
4.6.6 T  2  265,50  A
2.8.6 S  2  242,50  A B
4.6.6 S  2  235,50  A B
```

Table A.5 (Continued)

```
4.6.8 T  2  156,00    B C
2.8.8 T  2  107,20      C D
4.6.8 S  2   93,35      C D
2.8.8 S  2   45,20        D

Means that do not share a letter are significantly different.
```

Table A.6 One way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of lecithin-whey protein concentrate.

One-way ANOVA: Particle Size versus Ratio

```
Source  DF       SS      MS      F      P
Ratio    7  180,460  25,780  99,28  0,000
Error    8    2,077   0,260
Total   15  182,537

S = 0,5096   R-Sq = 98,86%   R-Sq(adj) = 97,87%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level    N    Mean  StDev  --------+---------+---------+---------+-
2.8.6 S  2   9,395  0,290    (--*-)
2.8.6 T  2   8,480  0,141  (-*--)
2.8.8 S  2  12,000  0,566            (-*--)
2.8.8 T  2  10,950  0,212          (-*--)
4.6.6 S  2  10,850  0,354          (-*-)
4.6.6 T  2   9,520  0,651      (-*--)
```

Table A.6 (Continued)

```
4.6.8 S  2  19,800  0,990                                  (--*-)
4.6.8 T  2  13,400  0,283               (-*--)
                          --------+---------+---------+---------+-
                               10,5      14,0      17,5      21,0

Pooled StDev = 0,510


Grouping Information Using Tukey Method

Ratio    N    Mean  Grouping
4.6.8 S  2  19,800  A
4.6.8 T  2  13,400    B
2.8.8 S  2  12,000    B C
2.8.8 T  2  10,950      C D
4.6.6 S  2  10,850      C D
4.6.6 T  2   9,520        D E
2.8.6 S  2   9,395        D E
2.8.6 T  2   8,480          E

Means that do not share a letter are significantly different.
```

Table A.7 Three way ANOVA and Tukey's Comparison Test for rheological measurement of double emulsions prepared by homogenization process of UltraTurrax and Ultrasound; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

General Linear Model: Rheology versus Ratio; Emulsifier; Process

```
Factor      Type   Levels  Values
Ratio       fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Emulsifier  fixed       3  l+wp; sc; x
```

Table A.7 (Continued)

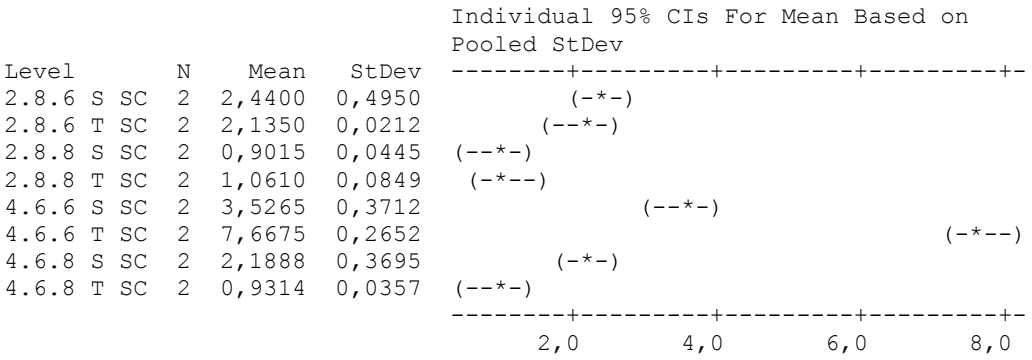
```
Process     fixed       2  S; T


Analysis of Variance for Rheology, using Adjusted SS for Tests

Source              DF    Seq SS   Adj SS   Adj MS      F      P
Ratio                3   41,2061  41,2061  13,7354  42,89  0,000
Emulsifier           2   48,5256  48,5256  24,2628  75,76  0,000
Process              1    2,8802   2,8802   2,8802   8,99  0,005
Ratio*Emulsifier     6   26,5079  26,5079   4,4180  13,79  0,000
Ratio*Process        3   14,3920  14,3920   4,7973  14,98  0,000
Emulsifier*Process   2    0,5842   0,5842   0,2921   0,91  0,413
Error               30    9,6082   9,6082   0,3203
Total               47  143,7042


S = 0,565926   R-Sq = 93,31%   R-Sq(adj) = 89,53%


Unusual Observations for Rheology

Obs  Rheology       Fit   SE Fit  Residual  St Resid
  6   3,26400   4,32899  0,34656  -1,06499     -2,38 R
 13   7,85500   6,86501  0,34656   0,98999      2,21 R
 37   0,19550   1,21501  0,34656  -1,01951     -2,28 R
 38   0,13290  -0,81786  0,34656   0,95076      2,13 R
 46   0,11090  -0,81786  0,34656   0,92876      2,08 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier   N  Mean  Grouping
sc          16   2,6  A
x           16   1,2     B
l+wp        16   0,2        C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio   N  Mean  Grouping
4.6.6  12   2,9  A
2.8.6  12   1,0     B
4.6.8  12   0,9     B
2.8.8  12   0,4     B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
```

```
T        24   1,6  A
S        24   1,1    B

Means that do not share a letter are significantly different.
```

Table A.8 One way ANOVA and Tukey's Comparison Test for rheological measurement of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate

One-way ANOVA: Rheology versus Ratio

```
Source  DF       SS      MS       F      P
Ratio    7  69,9722  9,9960  133,15  0,000
Error    8   0,6006  0,0751
Total   15  70,5728

S = 0,2740   R-Sq = 99,15%   R-Sq(adj) = 98,40%
```




```
Pooled StDev = 0,2740
```

```
Grouping Information Using Tukey Method

Ratio       N    Mean  Grouping
4.6.6 T SC  2  7,6675  A
4.6.6 S SC  2  3,5265    B
2.8.6 S SC  2  2,4400      C
4.6.8 S SC  2  2,1888      C
2.8.6 T SC  2  2,1350      C D
2.8.8 T SC  2  1,0610        D E
4.6.8 T SC  2  0,9314          E
```

Table A.8 (Continued)

```
2.8.8 S SC  2  0,9015          E

Means that do not share a letter are significantly different.
```

Table A.9 One way ANOVA and Tukey's Comparison Test for rheological measurement of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of xanthan gum

One-way ANOVA: Rheology versus Ratio

```
Source  DF       SS      MS       F      P
Ratio    7  23,9052  3,4150  137,95  0,000
Error    8   0,1980  0,0248
Total   15  24,1033

S = 0,1573   R-Sq = 99,18%   R-Sq(adj) = 98,46%


Level       N    Mean   StDev
2.8.6 S XG  2  0,4473  0,0293
2.8.6 T XG  2  0,4454  0,0281
2.8.8 S XG  2  0,2712  0,0069
2.8.8 T XG  2  0,2534  0,0062
4.6.6 S XG  2  1,4611  0,1539
4.6.6 T XG  2  4,1910  0,1075
4.6.8 S XG  2  1,3690  0,2475
4.6.8 T XG  2  1,0741  0,3159

             Individual 95% CIs For Mean Based on Pooled StDev
Level         +---------+---------+---------+---------
2.8.6 S XG      (-*-)
2.8.6 T XG      (-*-)
2.8.8 S XG    (-*-)
2.8.8 T XG    (-*-)
4.6.6 S XG              (-*-)
4.6.6 T XG                                      (-*-)
4.6.8 S XG            (-*--)
4.6.8 T XG          (-*-)
              +---------+---------+---------+---------
             0,0       1,2       2,4       3,6

Pooled StDev = 0,1573


Grouping Information Using Tukey Method
```

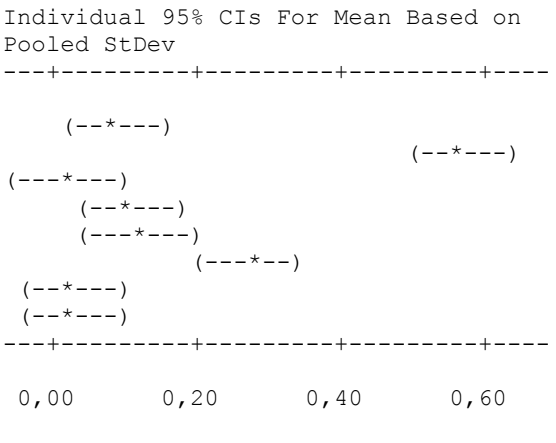
Table A.9 (Continued)

```
Ratio       N    Mean  Grouping
4.6.6 T XG  2  4,1910  A
4.6.6 S XG  2  1,4611    B
4.6.8 S XG  2  1,3690    B
4.6.8 T XG  2  1,0741    B
2.8.6 S XG  2  0,4473      C
2.8.6 T XG  2  0,4454      C
2.8.8 S XG  2  0,2712      C
2.8.8 T XG  2  0,2534      C

Means that do not share a letter are significantly different.
```

Table A.10 One way ANOVA and Tukey's Comparison Test for rheological measurement of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of lecithin-whey protein concentrate.

One-way ANOVA: Rheology versus Ratio

```
Source  DF       SS       MS      F      P
Ratio    7  0,48653  0,06950  34,69  0,000
Error    8  0,01603  0,00200
Total   15  0,50256

S = 0,04476   R-Sq = 96,81%   R-Sq(adj) = 94,02%
```




```
Pooled StDev = 0,04476
```

Table A.10 (Continued)

```
Grouping Information Using Tukey Method

Ratio        N     Mean  Grouping
2.8.6 T LWP  2  0,56635  A
4.6.6 T LWP  2  0,27525    B
4.6.6 S LWP  2  0,12190    B C
2.8.8 T LWP  2  0,10700    B C
2.8.6 S LWP  2  0,08432      C
4.6.8 T LWP  2  0,02813      C
4.6.8 S LWP  2  0,02343      C
2.8.8 S LWP  2  0,02153      C

Means that do not share a letter are significantly different.
```

Table A.11 Three way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared by homogenization process of UltraTurrax and Ultrasound; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

General Linear Model: Stability versus Ratio; Emulsifier; Process

```
Factor      Type   Levels  Values
Ratio       fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Emulsifier  fixed       3  l+wp; sc; x
Process     fixed       2  S; T


Analysis of Variance for Stability, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS  Adj MS       F      P
Ratio        3   3498,3   3498,3  1166,1   46,06  0,000
Emulsifier   2  16193,5  16193,5  8096,7  319,80  0,000
Process      1     33,1     33,1    33,1    1,31  0,260
Error       41   1038,0   1038,0    25,3
Total       47  20763,0


S = 5,03173   R-Sq = 95,00%   R-Sq(adj) = 94,27%


Unusual Observations for Stability

Obs  Stability      Fit  SE Fit  Residual  St Resid
```

Table A.11 (Continued)

```
 14    70,6731  84,4583  1,9215  -13,7852     -2,96 R
 39    18,0365  28,0462  1,9215  -10,0096     -2,15 R
 47    18,1640  28,0462  1,9215   -9,8822     -2,13 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio   N  Mean  Grouping
4.6.6  12  59,8  A
2.8.6  12  58,9  A
4.6.8  12  44,1    B
2.8.8  12  40,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier   N  Mean  Grouping
sc          16  76,4  A
x           16  42,3    B
l+wp        16  34,0      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
T        24  51,7  A
S        24  50,1  A

Means that do not share a letter are significantly different.
```

Table A.12 One way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate.

One-way ANOVA: Stability versus Ratio SC

```
Source    DF      SS     MS     F      P
Ratio SC   7   985,0  140,7  6,39  0,009
Error      8   176,1   22,0
Total     15  1161,1
```

Table A.12 (Continued)

```
S = 4,692   R-Sq = 84,83%   R-Sq(adj) = 71,56%

                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level        N    Mean  StDev  -+---------+---------+---------+--------
2.8.6 S SC   2  83,976  6,458                   (-------*-------)
2.8.6 T SC   2  86,907  3,064                      (-------*-------)
2.8.8 S SC   2  66,772  0,000  (-------*------)
2.8.8 T SC   2  68,896  4,506    (-------*-------)
4.6.6 S SC   2  75,440  6,741           (------*-------)
4.6.6 T SC   2  87,052  1,587                      (-------*-------)
4.6.8 S SC   2  73,011  5,933        (-------*-------)
4.6.8 T SC   2  69,342  4,641     (------*-------)
                                -+---------+---------+---------+--------
                                60         70        80        90

Pooled StDev = 4,692


Grouping Information Using Tukey Method

Ratio SC     N    Mean  Grouping
4.6.6 T SC   2  87,052  A
2.8.6 T SC   2  86,907  A
2.8.6 S SC   2  83,976  A B
4.6.6 S SC   2  75,440  A B
4.6.8 S SC   2  73,011  A B
4.6.8 T SC   2  69,342  A B
2.8.8 T SC   2  68,896  A B
2.8.8 S SC   2  66,772    B

Means that do not share a letter are significantly different.
```

Table A.13 One way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of xanthan gum

One-way ANOVA: Stability_1 versus Ratio_XG

```
Source     DF      SS      MS      F      P
Ratio_XG    7  757,18  108,17  54,85  0,000
Error       8   15,78    1,97
Total      15  772,95
```

Table A.13 (Continued)

```
S = 1,404   R-Sq = 97,96%   R-Sq(adj) = 96,17%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  -------+---------+---------+---------+--
2.8.6 S XG  2  42,497  2,534                (---*--)
2.8.6 T XG  2  46,132  0,488                      (--*--)
2.8.8 S XG  2  32,498  0,777  (--*---)
2.8.8 T XG  2  34,009  0,120    (---*--)
4.6.6 S XG  2  51,190  0,492                             (--*--)
4.6.6 T XG  2  52,618  0,447                               (--*--)
4.6.8 S XG  2  39,354  2,827            (--*--)
4.6.8 T XG  2  40,089  0,248             (--*---)
                              -------+---------+---------+---------+--
                                  35,0      42,0      49,0      56,0

Pooled StDev = 1,404


Grouping Information Using Tukey Method

Ratio_XG    N    Mean  Grouping
4.6.6 T XG  2  52,618  A
4.6.6 S XG  2  51,190  A B
2.8.6 T XG  2  46,132    B C
2.8.6 S XG  2  42,497      C D
4.6.8 T XG  2  40,089        D
4.6.8 S XG  2  39,354        D E
2.8.8 T XG  2  34,009          E F
2.8.8 S XG  2  32,498            F

Means that do not share a letter are significantly different.
```

Table A.14 One way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of lecithin-whey protein concentrate

One-way ANOVA: Stability_2 versus Ratio_LWP

```
Source     DF        SS       MS       F      P
Ratio_LWP   7  2630,401  375,772  602,97  0,000
Error       8     4,986    0,623
Total      15  2635,387

S = 0,7894   R-Sq = 99,81%   R-Sq(adj) = 99,65%
```

Table A.14 (Continued)

```
                                 Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level          N    Mean  StDev  ---+---------+---------+---------+------
2.8.6 S LWP    2  44,127  0,124                            (*)
2.8.6 T LWP    2  49,934  0,916                                   (*)
2.8.8 S LWP    2  21,549  0,987     (-*)
2.8.8 T LWP    2  20,819  0,075     (*)
4.6.6 S LWP    2  45,433  0,582                             (*-)
4.6.6 T LWP    2  46,838  1,660                               (*)
4.6.8 S LWP    2  24,963  0,222          (*)
4.6.8 T LWP    2  18,100  0,090  (*)
                                 ---+---------+---------+---------+------
                                   20        30        40        50

Pooled StDev = 0,789


Grouping Information Using Tukey Method

Ratio_LWP      N    Mean  Grouping
2.8.6 T LWP    2  49,934  A
4.6.6 T LWP    2  46,838  A B
4.6.6 S LWP    2  45,433    B
2.8.6 S LWP    2  44,127    B
4.6.8 S LWP    2  24,963      C
2.8.8 S LWP    2  21,549        D
2.8.8 T LWP    2  20,819        D E
4.6.8 T LWP    2  18,100          E

Means that do not share a letter are significantly different.
```

Table A.15 Three way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double dmulsions prepared by homogenization process of UltraTurrax and Ultrasound; ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

General Linear Model: Encapsulation ef versus Ratio; Emulsifier; Process

```
Factor      Type   Levels  Values
```

Table A.15 (Continued)

```
Ratio       fixed       4  2.8.6; 2.8.8; 4.6.6; 4.6.8
Emulsifier  fixed       3  l+wp; sc; x
Process     fixed       2  S; T


Analysis of Variance for Encapsulation efficiency, using Adjusted SS for
Tests

Source      DF   Seq SS  Adj SS  Adj MS      F      P
Ratio        3   27,048  27,048   9,016  17,58  0,000
Emulsifier   2   67,198  67,198  33,599  65,53  0,000
Process      1    0,028   0,028   0,028   0,06  0,816
Error       41   21,023  21,023   0,513
Total       47  115,297


S = 0,716063   R-Sq = 81,77%   R-Sq(adj) = 79,10%


Unusual Observations for Encapsulation efficiency

     Encapsulation
Obs     efficiency      Fit  SE Fit  Residual  St Resid
 39        94,1690  96,0669  0,2735   -1,8978     -2,87 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Ratio   N  Mean  Grouping
2.8.6  12  96,6  A
4.6.6  12  96,1  A B
4.6.8  12  95,8    B
2.8.8  12  94,5      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier   N  Mean  Grouping
x           16  97,0  A
l+wp        16  96,1    B
sc          16  94,1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
S        24  95,8  A
T        24  95,7  A
Means that do not share a letter are significantly different.
```

Table A.16 One way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of sodium caseinate

One-way ANOVA: Particle Size versus Ratio

```
Source  DF       SS      MS      F      P
Ratio    7  12,8560  1,8366  33,09  0,000
Error    8   0,4440  0,0555
Total   15  13,3000

S = 0,2356   R-Sq = 96,66%   R-Sq(adj) = 93,74%


                              Individual 95% CIs For Mean Based on
Pooled StDev
Level       N    Mean  StDev     +---------+---------+---------+--------
-
2.8.6 S SC  2  95,185  0,060                                  (---*---)
2.8.6 T SC  2  95,267  0,018                                   (---*---)
2.8.8 S SC  2  94,320  0,085                         (---*---)
2.8.8 T SC  2  94,520  0,212                           (---*---)
4.6.6 S SC  2  93,944  0,166                      (--*---)
4.6.6 T SC  2  94,300  0,418                         (---*---)
4.6.8 S SC  2  92,413  0,405     (---*---)
4.6.8 T SC  2  93,221  0,146              (---*---)
                                 +---------+---------+---------+--------
-
                              92,0      93,0      94,0      95,0

Pooled StDev = 0,236


Grouping Information Using Tukey Method

Ratio       N     Mean  Grouping
2.8.6 T SC  2  95,2665  A
2.8.6 S SC  2  95,1845  A B
2.8.8 T SC  2  94,5200  A B C
2.8.8 S SC  2  94,3200    B C
4.6.6 T SC  2  94,3005    B C
4.6.6 S SC  2  93,9437      C D
4.6.8 T SC  2  93,2207        D E
4.6.8 S SC  2  92,4131          E

Means that do not share a letter are significantly different.
```

Table A.17 One way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of xanthan gum

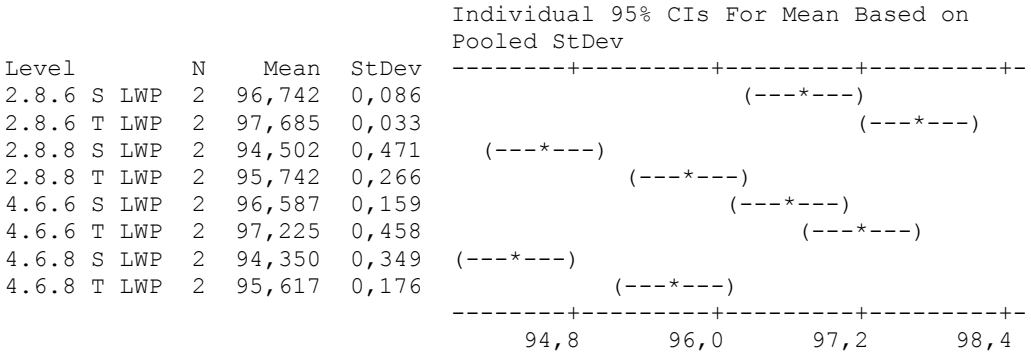
One-way ANOVA: Particle Size versus Ratio

```
Source  DF      SS     MS     F      P
C5       7  12,625  1,804  6,08  0,011
Error    8   2,375  0,297
Total   15  14,999

S = 0,5448   R-Sq = 84,17%   R-Sq(adj) = 70,32%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  ---+---------+---------+---------+------
2.8.6 S XG  2  96,883  0,080               (-----*-----)
2.8.6 T XG  2  97,657  0,750                     (-----*-----)
2.8.8 S XG  2  98,258  0,033                         (-----*-----)
2.8.8 T XG  2  97,249  0,013                   (-----*-----)
4.6.6 S XG  2  97,002  0,216                  (-----*-----)
4.6.6 T XG  2  97,284  0,017                    (-----*----)
4.6.8 S XG  2  95,012  0,269  (-----*-----)
4.6.8 T XG  2  96,566  1,298             (-----*-----)
                              ---+---------+---------+---------+------
                              94,5      96,0      97,5      99,0

Pooled StDev = 0,545


Grouping Information Using Tukey Method

C5          N     Mean  Grouping
2.8.8 S XG  2  98,2582  A
2.8.6 T XG  2  97,6573  A
4.6.6 T XG  2  97,2840  A
2.8.8 T XG  2  97,2488  A
4.6.6 S XG  2  97,0023  A B
2.8.6 S XG  2  96,8826  A B
4.6.8 T XG  2  96,5657  A B
4.6.8 S XG  2  95,0117    B

Means that do not share a letter are significantly different.
```

Table A.18 One way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double dmulsions prepared by ratios of 2.8.6, 2.8.8, 4.6.6 and 4.6.8; hydrophilic emulsifiers of lecithin-whey protein concentrate

One-way ANOVA: Particle Size versus Ratio

```
Source  DF       SS      MS      F      P
C9       7  20,7816  2,9688  34,47  0,000
Error    8   0,6890  0,0861
Total   15  21,4706

S = 0,2935   R-Sq = 96,79%   R-Sq(adj) = 93,98%
```




```
Pooled StDev = 0,293
```

```
Grouping Information Using Tukey Method

C9           N     Mean  Grouping
2.8.6 T LWP  2  97,6854  A
4.6.6 T LWP  2  97,2254  A
2.8.6 S LWP  2  96,7418  A B
4.6.6 S LWP  2  96,5869  A B
2.8.8 T LWP  2  95,7418    B
4.6.8 T LWP  2  95,6174    B C
2.8.8 S LWP  2  94,5023      C D
4.6.8 S LWP  2  94,3498        D

Means that do not share a letter are significantly different.
```

Table A.19 One way ANOVA and Tukey's Comparison Test for particle size of mayonnaise samples prepared by simple emulsion, and primary emulsions with ratios of 2.8 and 4.6: hydrophilic emulsifiers; sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

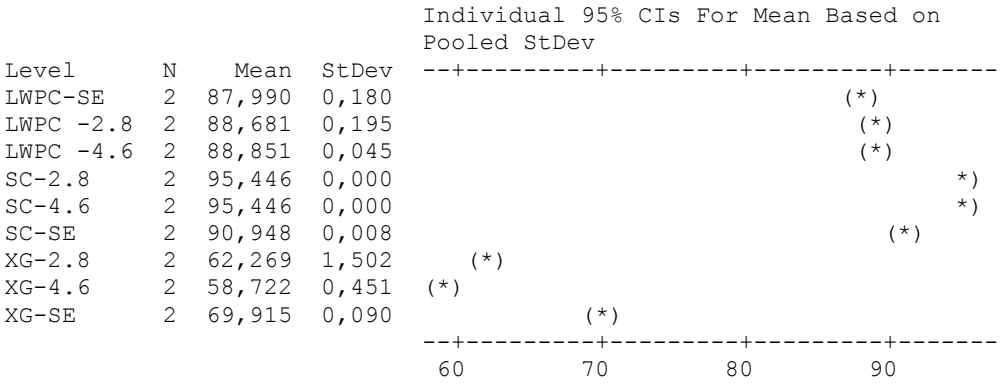
One-way ANOVA: Particle size versus type of mayonnaise

```
Source  DF        SS       MS        F      P
C1       8  130191,7  16274,0  1694,88  0,000
Error    9      86,4      9,6
Total   17  130278,1

S = 3,099   R-Sq = 99,93%   R-Sq(adj) = 99,87%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level      N    Mean  StDev  +---------+---------+---------+---------
LWPC-SE    2   12,57   0,60   (*)
LWPC -2.8  2   13,29   0,24   (*)
LWPC -4.6  2   13,72   0,10   (*)
SC-2.8     2    3,50   0,04  (*
SC-4.6     2    3,76   0,06  (*
SC-SE      2    5,29   0,05  (*)
XG-2.8     2  205,41   7,52                                  (*)
XG-4.6     2  226,95   4,87                                      (*)
XG-SE      2   73,84   2,40             (*)
                           +---------+---------+---------+---------
                           0        60       120       180

Pooled StDev = 3,10


Grouping Information Using Tukey Method

C1         N    Mean  Grouping
XG-4.6     2  226,95  A
XG-2.8     2  205,41    B
XG-SE      2   73,84      C
LWPC -4.6  2   13,72        D
LWPC -2.8  2   13,29        D
LWPC-SE    2   12,57        D
SC-SE      2    5,29        D
SC-4.6     2    3,76        D
SC-2.8     2    3,50        D

Means that do not share a letter are significantly different.
```

Table A.20 One way ANOVA and Tukey's Comparison Test for stability of mayonnaise samples prepared by simple emulsion, and primary emulsions with ratios of 2.8 and 4.6: hydrophilic emulsifiers; sodium caseinate, xanthan gum and lecithin-whey protein concentrate.

One-way ANOVA: Stability versus mayonnaise type

```
Source  DF        SS       MS        F      P
C1       8  3292,647  411,581  1458,64  0,000
Error    9     2,540    0,282
Total   17  3295,187

S = 0,5312   R-Sq = 99,92%   R-Sq(adj) = 99,85%
```




```
Pooled StDev = 0,531
```

```
Grouping Information Using Tukey Method

C1         N    Mean  Grouping
SC-4.6     2  95,446  A
SC-2.8     2  95,446  A
SC-SE      2  90,948    B
LWPC -4.6  2  88,851    B C
LWPC -2.8  2  88,681      C
LWPC-SE    2  87,990      C
XG-SE      2  69,915        D
XG-2.8     2  62,269          E
XG-4.6     2  58,722            F

Means that do not share a letter are significantly different.
```

APPENDIX B

CALIBRATION CURVE

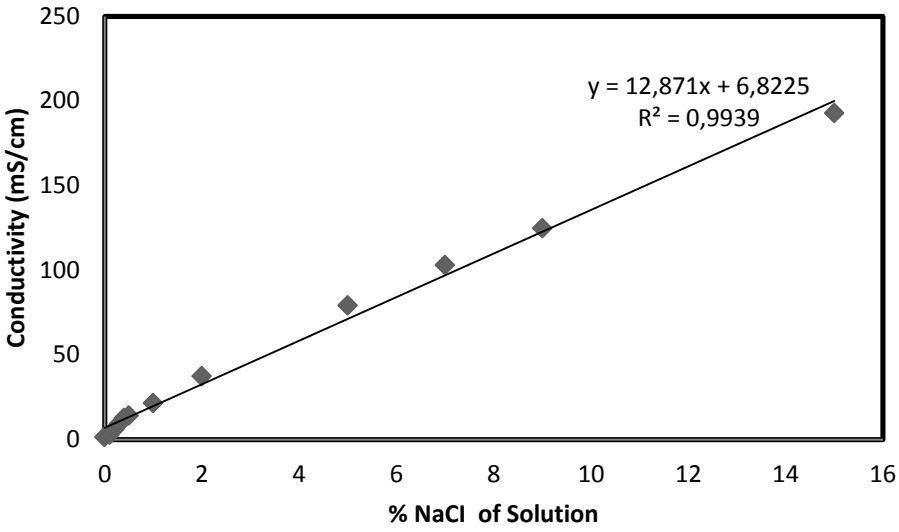


Figure B.1 Calibration curve of conductivity measurements corresponding % NaCl solution.