EFFECT OF THE TYPE OF THE EMULSIFIER ON THE PHYSICAL AND CHEMICAL STABILITY OF OIL-IN-WATER EMULSIONS

by
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EFFECT OF THE TYPE OF THE EMULSIFIER ON THE PHYSICAL AND CHEMICAL STABILITY OF OIL-IN-WATER EMULSIONS

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ABSTRACT

EFFECT OF THE TYPE OF THE EMULSIFIER ON THE PHYSICAL AND CHEMICAL STABILITY OF OIL-IN-WATER EMULSIONS

Emulsions which are immiscible heterogenous systems produced by dispersion of a phase in another phase are widely used in the food industry. It is difficult to produce oil-in-water (O/W) emulsion systems that are stabel both physically and chemically. Oil-in-water emulsions generally consist of three main ingredients: oil, water and emulsifier. Emulsifiers are ingredients that keep the immiscible layers together with the help of theirhydrophobic/hydrophilic nature. Due to food emulsions' complex nature, it is hard to understand the mechanisms involved in emulsification process at oil-water interface. An understanding of physical characteristics with an effect on the production of stable emulsions and their relationship with the emulsions chemical stability is important in order to keep food emulsions stable as a quality indicator from consumer aspect. In this study, physical stability of O/W emulsions produced with 1, 2, 4% emulsifier concentrations and 5, 20, 40 % oil concentrations were improved with 40 % polysaccharide as a texture modifier (DE 12 maltodextrin) in aqueous phase. Emulsions produced by most common food emulsifiers which are soy lecithin (Lec) and citric acid esters of mono and diglicerides (CITREM) as oil based emulsifiers and two protein based emulsifiers, sodium caseinate (SC) and whey protein isolate (WPI) were physically characterized (visual, particle charge, viscosity) in order to understand their pronity to oxidation. Emulsion formulation with 20% oil and 4% CITREM was selected for further studies. 4% 1:1 CITREM-beta cyclodextrin (BCD) and CITREM and 20 % oil-in-water emulsions were produced and stored at 4ºC, 21ºC and 55ºC for a month. BCD was added to emulsion to achieve better stability effect and protective effect against lipid oxidation. They were stored at Suntest (Atlas XLS) for a test cycle equal to exposure of 10 days direct sunlight. Visual observation (CI) of all emulsions were compared; Suntest and 55ºC samples were analyzed chemically. According to their TOTOX and creaming index (CI) values, while BCD addition contributed to physical stability of emulsions, BCD did not result in improvement on oxidative stability because of competition of low molecular weight emulsifier in oil phase (CITREM) and BCD in aqueous phase.

ÖZET

FARKLI EMÜLGATÖRLERİN SU İÇERİSİNDE YAĞ TİPİ EMÜLSİYONLARIN FİZİKSEL VE KİMYASAL STABİLİTESİNE OLAN ETKİSİ

Emülsiyonlar, birbirine karışmayan iki fazın birbiri içerisinde dağılmasıyla oluşmuş homojen görünümlü heterojen sistemlerdir. Genellikle gıda, ilaç ve kozmetik endüstrisinde yaygın olarak kullanılırlar. Yağ, su ve emülgatörden oluşan Y/S tipi emülsiyonların fiziksel ve kimyasal stabilitesini sağlamak zordur. Emülgatörler, bibirine karışmayan iki fazın, hidrofilik ve hidrofobik özellikleri bünyesinde barındırmasıyla karışmasını sağlayan yüzey aktif maddelerdir. Gıda emülsiyonlarının karmaşık yapısından dolayı, genellikle emülsifikasyona dahil olan ve fiziksel / kimyasal stabiliteye etki eden mekanizmaları anlamak zordur. Stabil emülsiyon üretimine etki eden fiziksel faktörleri ve bunların kimyasal faktörlerle olan ilişkisini anlamak, tüketici açısından bir kalite algısı olan ayrılmayan emülsiyonların iç kimyasını geliştirmek için önemlidir. Bu çalışmada, 1, 2, 4% emülgatör ve su içerisinde 5, 20, 40% yağ oranı olan emülsiyonların fiziksel stabilitesi, polisakkarit yapıda bir tekstür modifiye edici eklenmesi ile geliştirilmiştir. Gıda endüstrisinde sıklıkla kullanılan sodyum kazeinat (SC), peynir altı suyu izolatı (WPI) protein bazlı ve soya lesitini (Lec), mono ve digliseritlerin sitrik asit estereri (CITREM) yağ bazlı emülgatörler kullanılak hazırlanan emülsiyonların, oksidasyona en yatkın olanı bulunmak amacıyla fiziksel karaktetistikleri (görsel, parçacık büyüklüğü, parçacık yükü, vizkosite) ölçülmüştür. Ölçüm sonucunda 20% yağ ve 4% CITREM emülsiyon formulasyonu bulunmuştur. 4% 1:1 CITREM-BCD ve CITREM emülsiyonları ve üretilmiş, 4ºC, 21ºC ve 55ºC de 15 gün süre ile depolanmışlardır. BCD, fiziksel ve oksidatif stabiliteyi iyi yönde geliştirici etkisi olması sebebiyle emülsiyonlarda kullanılmıştır. Üretilen emülsiyonlar aynı zamanda Suntest (Atlas XLS) cihazında 24 saatlik test süresince, 10 gün direkt güneş ışığına maruziyetine eşdeğer şekilde tutulmuştur. Tüm sıcaklıktaki emülsiyonlar görsel yönden (CI) ölçüme tabi tutulurken, 55ºC ve Suntest örnekleri oksidasyon testlerine tabi tutulmuştur. CI ve TOTOX değerleri karışaltırıldığında, emülsiyonlara BCD eklenmesinin fiziksel stabiliteye olumlu etkisi bulunmasına karşın, yağ fazında LMWE olan CITREM in su fazındaki BCD ile yarışı sonucu, BCD in kimyasal stabiliteye olumlu etkisi bulunmamıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

μm	micrometer
BCD	Beta cyclodextrin
CD	Cyclodextrin
CITREM	Citric acid esters of mono and diglycerides
DE	Dextrose equivalent
DP	Degree of polymerization
HMWE	High molecular weight emulsifier
Lec	Soy lecithin
MD	Maltodextrin
nm	nanometer
O/W	Oil-in-water
W/O	Water-in-oil
O/W/O	Oil-in-water-in oil
W/O/W	Water-in-oil-in water
LMWE	Low molecular weight emulsifier
pAV	p-Anisidine value
PV	Peroxide value
SC	Sodium caseinate
TOTOX	Total oxidation value
WPI	Whey protein isolate

1. INTRODUCTION

Emulsions which are immiscible heterogenous systems produced by dispersion of a phase in another phase are widely used in the food industry. It is difficult to produce oil-in-water (O/W) emulsion systems that are stable both physically and chemically. Oil-in-water emulsions generally consist of three main ingredients: oil, water and emulsifier. Emulsifiers are ingredients that keep the immiscible layers together with the help of theirhydrophobic/hydrophilic nature. Due to food emulsions' complex nature, it is hard to understand the mechanisms involved in emulsification process at oil-water interface. An understanding of physical characteristics with an effect on the production of stable emulsions and their relationship with the emulsions chemical stability is important in order to keep food emulsions stable as a quality indicator from consumer aspect. In this study, firstly stable model emulsions will be developed by using different stabilizers and afterwards these emulsions will be physically characterized in order to understand the main driver mechanisms causes instability. Moreover, common food emulsions will be modified into developed recipe. The most stable one will be determined according to their measured physical characteristics. After the most physically stable formulation will be selected, it will be used as a blend with BCD in order to improve oxidative stability. Selected formulation and its blend with BCD will be subjected to storage tests at 4, 21 and 55ºC to undertand the heat effect. Also they will be exposed to light. It will be understood that how BCD affects the pysical and oxidative stability at heat and light conditions independently from each other.

1.1. DEFINITIONS

Emulsions are homogenous appearing heterogenous systems which consist of two immiscible phases in the form of one dispersed in another as small spherical droplets. Since they are thermodynamically unstable systems, the interfacial layer between two phases are contributed with necessary surfactant materials to keep their homogenous form and reduce interfacial tension [1-3].

Emulsion systems contribute with better characteristics such as stability, texture, appearance and flavor enhancement to final food, pharmaceutical and cosmetics products. They are

present in sauces, cheeses, meat products, butter, margarine, some spreads, salad dressings and many more products which are major parts of food industry.

In food industry, generally immiscible phases mentioned above are oil and water. Therefore, emulsions are named as oil-in-water (O/W) or water in oil (W/O) depend on which phase is distributed in the other phase. Milk, mayonnaise, ice cream mixes, sauces, whippable toppings, dips, soups, dressings and creamers, cream liqueurs can be classified as O/W emulsions. On the other hand, butter, margarine and fat based spreads can be classified as W/O emulsions. Also various types of multiple emulsions structure can be formed with a similar aspect such as oil-in-water-in-oil (O/W/O), water-in-oil-in-water (W/O/W), oil-in-water-in-water. While droplet part can be named as dispersed/ discontinuous/ internal phase; liquid part can be named as dispersing/ continuous/ external or aqueous phase [2, 4].

While emulsion production process, bulk oil/ water is converted to spherical small droplets dispersed in continuous phase by homogenization. Homogenization is achieved in many ways in food industry such as: mechanical force, pressure application, sound application, colloidal milling and so on [3]. As smaller droplets are desired, larger energy sources are needed [5].

An emulsion system basically consist of three major parts which are dispersed phase, continuous phase and an interfacial agent [2, 4].

Emulsions are divided into two depending on their dispersed phase's droplet diameter. Emulsions are named as macroemulsions if its droplet diameter is between 100 nm – 100 μm; nanoemulsion if its droplet diameter is between 20 – 200 nm; microemulsions if its droplet diameter is between 5 - 100 nm. It was reported that while microemulsions are thermodynamically stable systems, macro and nano emulsions are inherently unstable systems [6-10]. This instability causes structure breakdown over time. Stabilizers as interfacial agents are used to reduce interfacial tension and increase emulsion stability [2, 4]. Stabilizers are divided into two groups depending on their mode of action as emulsifiers and texture modifiers. Emulsifiers are generally adsorbed on the oil droplets' surface and prevent them from aggregation by forming a protective coating around droplets and also reduce the interfacial tension between oil-water interface. Phospholipids, some proteins, solid particles, polysaccharides and small molecule surfactants can be given as examples [2, 11-13]. Texture modifiers like agents modify the structure of continuous phase by increasing its

viscosity and form a gel network. Due to increasing viscosity, emulsion droplets alter the gravitational force named as Brownian motion. They also contribute to the characteristics of final product [2, 14, 15]. Thickeners, gelling agents, polysaccharides such as maltodextrin or some protein molecules are used as texture modifiers.

1.2. EMULSIFIER AGENTS

In emulsion formulation development, the most important factor is choosing the proper emulsifier according to desired final product. A food emulsifier should :

- Be surface active in order to reduce surface tension at oil-water interface by creating a membrane contributing electrostatic and structural interactions between droplets
- Be adsorbed on the oil droplets to protect them from coalescence
- Increase the viscosity of emulsion
- Have a hydrophilic head and a hydrophobic tail to be adsorbed on oil and make bonds between o-w interface
- Not be toxic, proper to human use [16]

Emulsifiers are classified as low molecular weight emulsifiers (LMWE) and high molecular weight emulsifiers (HMWE) and solid particles. Natural sourced polar lipids and generally synthetic emulsifiers are small and surface active LMWEs including a hydrophilic head and a hydrophobic tail. They have got generally 10-20 carbon backbone with 1 or more hydracarbon chains. While mono- and diglycerides (monoacyl and diacyl glycerols), sucrose esters, Tweens (polyoxyethylene sorbitan esters), citric, lactic, and acetic acid esters of mono- and diglycerides, polysorbates Spans (sorbitan esters) and lecithin as a polar lipid are LMWEs, amphiphilic proteins are HMWEs [17, 18]. In some studies, silica was used as solid particles with the aim of developing physical chemistry of aqueous phase [17]. Pichot et.al,2009 [19], investigated the O/W stabilized emulsion both food grade emulsions with presence of monolein as surfactant and hydrophilic silica as colloidal particles and their absence. It was reported that silica contribute the emulsions' physical stability in a positive way.

In food emulsion formulation development researches in a wide range from beverages to mayonnaise focuses on food emulsifiers and polysaccharides.

Although there are many studies present in literature which use aqueous phase modifying agents such as food hydrocolloids and buffers, in present study, maltodextrin was used as a polysaccharide texture modifier, as it will be explained in results and discussion section [20, 21].

In present study, maltodextrin as texture modifier, two protein based and two oil based common food emulsifiers which are whey protein isolate (WPI), sodium caseina (SC), soy lecithin (Lec), citric acid esters of mono and diglycerides (CITREM) and beta-cyclodextrin (BCD) O/W emulsions were used.

1.2.1. Maltodextrins as Texture Modifiers

Maltodextrins (MDs) are products of starch hydrolysis. They are produced from low conversion of starch into hydrolyzates with two stage conversion as acid treatment and bacterial conversion respectively. MDs are defined as starch hydrolysis products with dextrose equivalent less than 20. They are widely used in flavour industry as material carrier of spray dried flavours, fruit concentrates, seasonings, synthetic sweeteners, flavour enhancers and so on. For instance there are some examples for their use as wall materials for capsules [22]. They are used with comperably advantaged characteristics such as cold water solubility, low or no sweet taste contribution to final product, well water holding ability and non hygroscobicity [23]. Reducing power of starch derived oligosaccharides are named as hydrolyzate of D-glucose on dry matter hydrolyzate. They are inverse values with degree of polimerization (DP) of anhydro glucose units. While starch hydrolization, D-glucose polymers joined by a-(1,4) and a-(1,6) linkages are resulted from amylose and amylopectin degredation. Also, maltodextrins' DE values vary according to their reducing power. For example DE 16 MD has 16% reducing power of water phase. This value changes between 3-20 % for MDs [24]. Commercial MDs are obtained from generally natural sources such as; corn, rice and potato starches. Since these sources show different chemical content, also their MDs have different characheristics according to their DE value and obtained source such as viscosity, solubility, freezing temperature and so on [25, 26] . For example Udomrati et.al. (2011) [27] investigated the effects of DE 9, 12 and 16 tapioca MDs with different concentrations on the stability of oil-in-water emulsions. They found that the stability of O/W emulsions are highly related with different DE values and concentrations. Also,

Turchiuli et.al.(2013) studied on DE 12 and 21 MD and found that DE 12 MD was efficient than DE 21 MD in fine emulsion production. Furthermore, Wang et.al (2008) [26] investigated different sourced DE 10 MDs in order to understand their physicochemical properties and relation between their chemical structure. They reported that their emulsions showed different characteristics such as water sorption, freezing temperature, greater retrogradation tendency and viscosity related with their amylose-amylopectin percentages.

In the content of this study, DE 12 MD was used as texture modifying agent in order to increase emulsions physical stability. When MDs are used as stabilizers, additional emulsifiers are required to produce physically stable emulsion systems [28].

There are some studies that use MD with food emulsifiers for the production of stable emulsions. Tween 80 which is a water soluble, non ionic emulsifier was used in many studies investigating the physical structure improvement of MDs. It was used with the aims of investigating rheology of corn oil-in-water emulsions with 0-35 % DE 10-36 MDs [29]; understanding rheological property and stability improvement with DE 9, 12 and 16 MDs in aqueous phase [27]); observing the effect of DE 5, 10, 15, 20 of tapioca MDs on emulsion stability [25]. Also, soy protein isolate – MD conjugate was used in order to investigate physical property changes during freze thaw process [30]. As a protein based emulsifier WPI was used at presence of MD DE 15 to understand freezing temperature influence of MD concentration on O/W emulsion stability [31].

1.2.2. Milk Proteins as Food Emulsifiers

Milk can be considered as one of the richest protein sources. Milk protein has two major groups as whey protein and caseins. While typical bovine milk contains 3.5% protein (w/w), its content is 80:20 casein and whey protein, respectively. Casein is formed from α1-, α2-, β-, and κ-caseins and whey protein is formed from α-lactalbumin, β-lactoglobulin, serum albumin, and immunoglobulin. They are both used in many aims such as gelling, foaming and emulsification [32].

When protein molecules are in water to form O/W emulsions, they are located in oil-water interface themselves by bonding with oil with their hydrophobic tails and with water with their hydrophilic head groups. Due to this location of molecules, they form a viscoelastic

film structure around oil droplets (Figure 1.1) [33, 34]. This film layer protects oil droplets and reduces interfacial tension from effects which may destabilize emulsions and behave like an energy barrier [35].

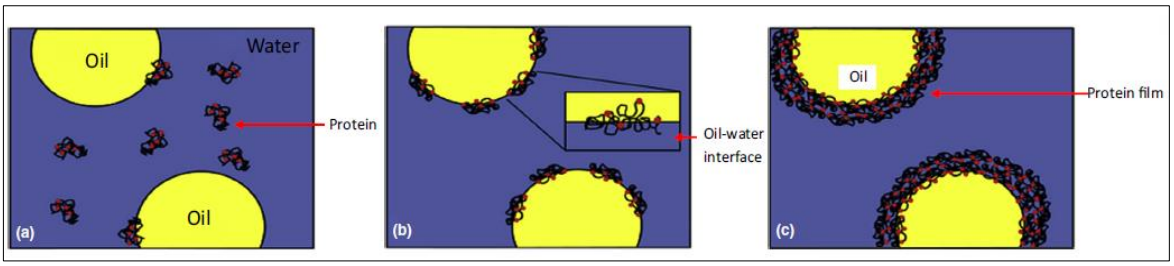

Figure 1.1. Shematic description of proteins at O/W emulsions between oil-water interface [33]

Moreover, different proteins' final products shows different characteristics (e.g. as water-oil holding capacity, gelation, foaming or emulsification etc.) depending on their functionality.

In this study, whey protein isolate (WPI) and sodium caseinate (SC) were used as protein based emulsifiers. Both WPI and SC are surface active proteins, their mode of action can be seen in figure 1.1.

Whey is the transqulent part yielded from cheese manufacturing process. When this vitamin and mineral rich part is purified, major parts of bovine milk which are whey proteins (20%) and casein (80%) are obtained (fig. 1.2). Whey is seperated into 3 forms with different techniques as whey protein powder, whey protein concentrate and whey protein isolate [33]. Also, they are classified as HMWEs.

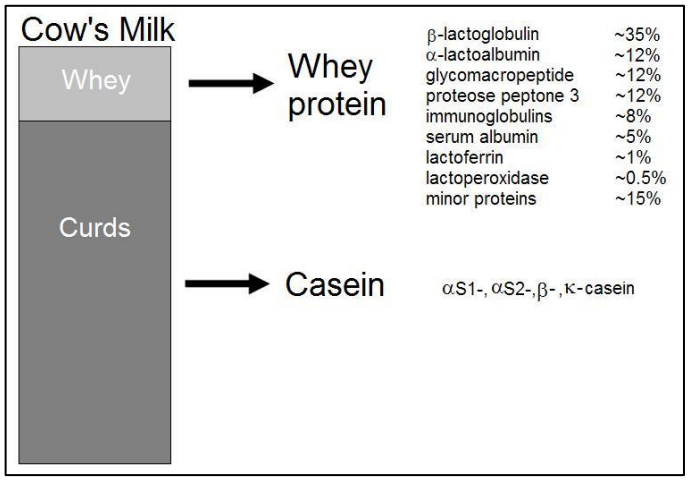

Figure 1.2. Chemical composition of whey

1.2.2.1. Whey Protein Isolate

Whey protein isolate (WPI) as a commonly used food emulsifier in food industry is the purest form of whey. It contains 90% or more protein. It is a globular protein with the ability of adsorbing at oil-water surface in order to form monolayer protect emulsion from mechanical destabilizations [33, 36].

1.2.2.2. Sodium Caseinate

Sodium caseinate (SC)is another commonly used emulsifier in food industrywhich is derived from the resting casein part after whey protein is removed from whey. It is obtained by acid treatment of casein. This process leads to the loss of most of calcium phosphate groups which keeps casein micelles together. Due to amphiphilic structure of α_{s1}-casein and β-casein parts, SC shows good emulsifying activity in O/W emulsions [37]. It is capable of dispersing in water and highly soluble in water. Its interfacial activity is higher than whey proteins (β-globullins) [38].

1.2.3. Oil Based Molecules as Food Emulsifiers

1.2.3.1. Lecithin

Commonly used type of lecithin is soy lecithin which is consist of many phospholipids. It can be produced both synthetic ways and natural sources such as soy. Since its chemical structure (figure 1.3.) includes hydrophilic and lipophylic parts, it is capable of reducing interfacial tension between oil-water interface. It also melts at 60 ºC. In the case of its excessive addition, it does not change the viscosity of products, however consequences with undesirable sensory properties [39].

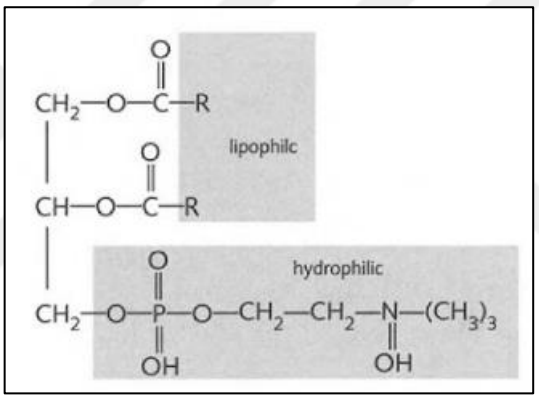

Figure 1.3. Chemical structure of lecithin [39]

1.2.3.2. Citrem

Citrem is the esterification products of monoglycerides with citric acid (12-20 % citric acid (w/w) in final product). IT is formed when the saturated monoglycerides turns to α-like crystals. It melts between 55-50 ºC. It is very hydrophilic and gives texture to margarine and beverage emulsions in industry. It can disperse in hot water, insoluble in cold water and can be soluble in edible oils and fats. When the temperature is higher than its melting point, it can form stable emulsions by forming strong molecular interaction between participating polar groups (figure 1.4).

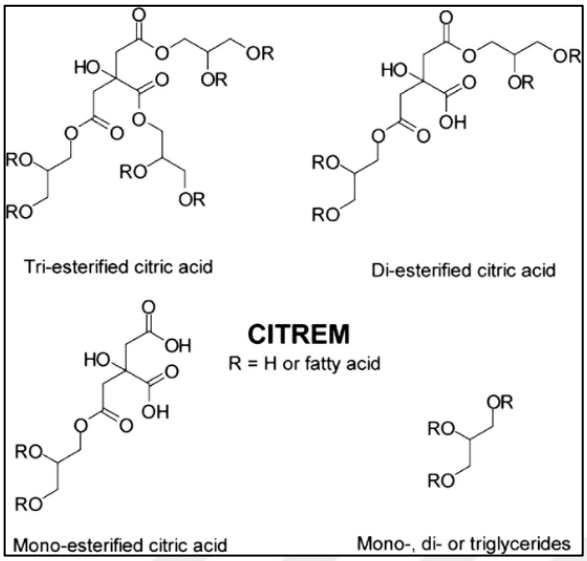

Figure 1.4. CITREM chemical structure [40]

1.3. EMULSION CHARACTERISTICS AND STABILITY

Physicochemical, functional and sensory properties of systems are influenced strongly from emulsion matrix properties and droplet characteristics. In production of emulsions, morphology, rheology, size and charge of droplets are distinctive factors.

All emulsions which are formed by two immiscible liquids have tendency to kinetic instability. They eventually seperate if enought time is given to its physical observation.. Emulsions can be named as stable as soon as their psycochemical properties are not changed during a period of time. Different droplet characteristics and compositional materials are directly related with stability term [41]. Rheological behaviour, size, charge, droplet-droplet interactions are important characteristics.

Macroemulsions have high tendency to physical instability. Instability mechanism is generally pushed by several physical factors due to complex nature of food emulsions. There are many molecules tat can be present at interface (figure 1.5) such as non ionic surfactants, ionic surfactants, amphiphilic biopolymers, solid particles as emulsifiers and there may be a part of unadsorbed emulsifiers between oil droplets in aqueous phase. Also there are various physical conditions such as pH, acidity, viscosity, gravity, concentration of oil or water may have main roles on instability mechanisms. Droplet concentration, droplet charge, droplet

size are generally affected by these factors and lead to emulsions becoming unstable. It is generally hard to understand dominant factor that causes instability [3].

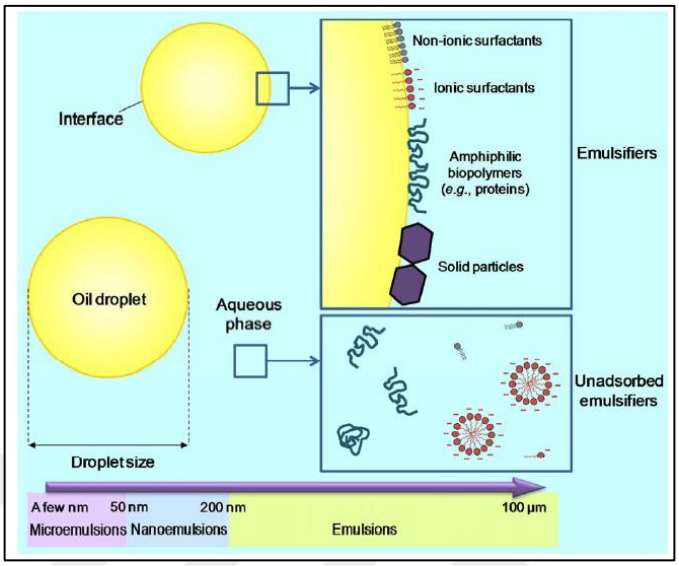

Figure 1.5. Schematic description of possible emulsion ingredients [17]

1.3.1. Physical Instability Mechanisms of Emulsions

During storage of emulsions, gravitational seperation of emulsions occurs. There are a few common modes of actions as phase inversion, creaming, sedimentation, flocculation, coalescence and Ostwald ripening (figure 1.6.).

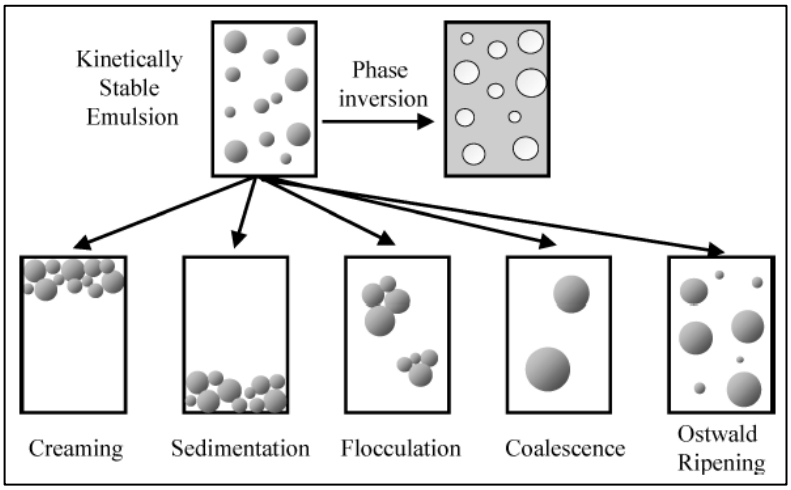

Figure 1.6. Most common instability mechanisms for food emulsions [2]

Phase inversion happens spontaneously by inversion of dispersed phase into continuous phase or vice versa. This mechanism requires a very low energy level and it is pushed by some external parameter such as salinity, using a co surfactant, concentration of oil and water, temperature etc [42]. **Creaming** as gravitational seperation occurs because of the density difference between upward and downward phases; low density particles (oils for O/W emulsion systems) start to move through surface. However its inverse case ; **sedimentation** can happen when the high density dispersed phase moves downwards in surrounding liquid. Another emulsion breaking mechanism occurs when two or more oil droplets stick together to form an aggregare; they flocculate (**flocculation**). Even they stick together, they do not lose their individual form. In addition, **coalescence** occurs when two or more oil droplets come together to form a bigger oil droplet. **Ostwald ripening** is the spontaneous phase change pushed by coalescenced larger oil droplets with aqueous phase. Its main reason is to mass transport of dispersed phase through continuous phase [2].

1.3.2. Emulsion Stability Testing Methods

In order to understand the mechanisms of emulsions stability,some methods are applied. Visual and morphological observation of emulsion and droplet characterics are investigated.

The most important / critical droplet characteristics are droplet concentration, size, charge, interaction between droplets and rheology of emulsion [5].

1.3.2.1. Visual observation

One of most important parameters about final product quality is its appearance. Since in visual observation instability can be observed by naked eyes without requiring any special laboratory equipment or device, it is the cheapest method of measuring it [5, 43]. Instability is generally caused by creaming or sedimentation mechanisms which were explained before.

Stability is expressed in creaming rate determination. There are many methods for measuring stability such as emulsion stability index [44]; stability rating [45]; emulsifying activity test [46].

Creaming is measured generally by keeping the emulsions in cylindrical containers for a period of time without motion and measuring the migration of oil from downwards to surface of O/W emulsions due to gravitational force [5]. This test gives an interpretation about stability of emulsions systems [47]. In order to achieve precise observations, some analytical instruments are required. In this study, creaming was expressed with creaming index (CI).

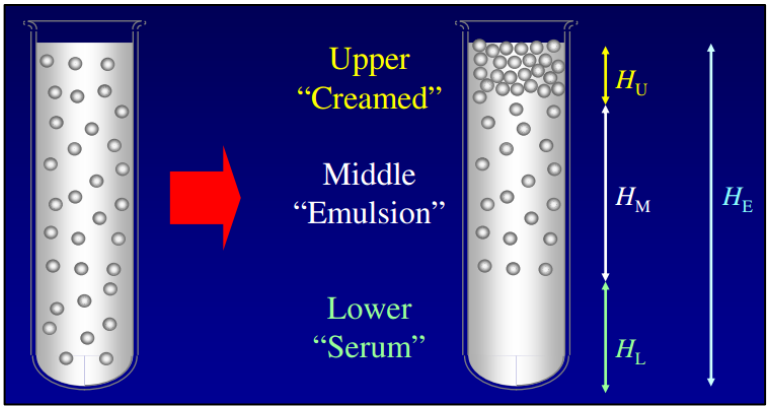

Figure 1.7. Creaming mechanism of O/W emulsions [3]

Another easy observation method is microscobic observation. This method provides information about morphology of emulsions that can not be obtained by naked eyes. Also,

dimension and distribution of emulsion droplets which may cause instabilities can be obtained by this method. There is a droplet size constraint about this method. Emulsion droplets should be under 100 μm for an efficient observation [5, 48]. Several types of microscobes may be used such as electron, confocal fluorecent, atomic force and optical in order to understand mechanisms from different aspects and in detail. However, there are some reasons of microscobic droplet measurement for not being an advantageous method such as sample dilution or slide spread being very time consuming (which also causes to breaking of original structure of emulsion); material consuming (every sample requires a freshly opened slide); subjective results because of unstandardized official methods.

1.3.2.2. Droplet characteristics

1.3.2.2.1. Droplet concentration

Droplet concentration can be described as oil droplets per unit volume of emulsion. According to final product, concentration changes. For example, mayonnaise includes 50% of oil droplets, soft drinks and beverages generally includes 0.1% oil droplets per unit volume. Droplet concentration contributes to stability, flavour, texture, release characteristics of emulsions.

1.3.2.2.2. Particle size

Droplet size is one of the main characteristics which gives information about emulsification efficiency of an emulsion system. It directly affects its rheology and sensory attributes [1]. Emulsions are named as monodisperse (if droplets are uniformly distributed at the same size) and polydisperse (if various size of droplets are present in emulsion) [2]. Also, if an emulsion is polydisperse, its particle size is expressed as droplet size distribution and calculated by average droplet size (Z_{AVG}). With the help of this method, undesirable droplet interactions can be predicted during a period of time with a precise data, as average droplet size increases over time, it indicates that oil droplets are coming together in emulsion [5]. However, it is still hard to decide the instability mechanism of droplets whether it is coalescence, flocculation etc.

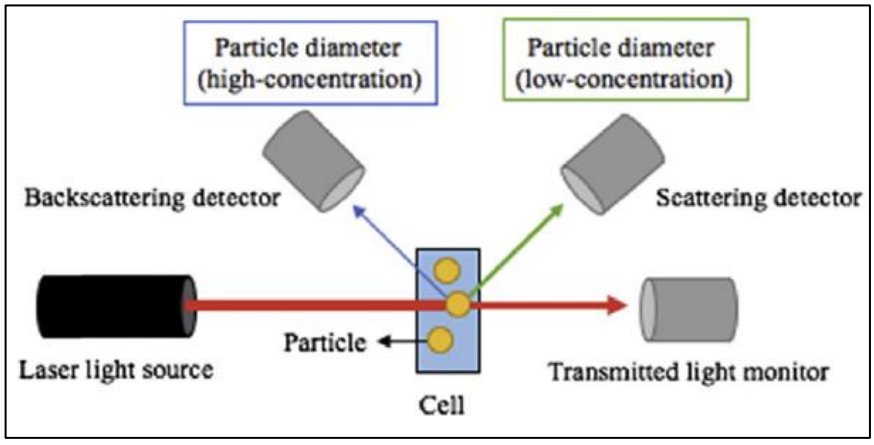

Figure 1.8. Schematic mechanism of light scattering technique

Common droplet size distribution measurement techniques are light scattering, electrical pulse counting and ultrasonic spectrometry. DLS (dynamic light scattering method)is the most commonly used droplet characterization technique. In DLS method, light scattering resulted from Brownian motion is measured. The fluctuations of laser light to all directions are observed during measurement (figure 1.8). While smaller particles moves faster with fast fluctiations, slow particles (larger droplets) fluctuations consequence with big fluctuations [49].

1.3.2.2.3. Droplet charge

Electrical charge of droplet also affects the stability mechanism and psycochemical behavior of emulsion. On droplet charge characteristics, the pH of medium and charges of other materials in emulsion have significant effects. After emulsions are formed with emulsifier adsorption at droplet water surface, they are charged with their hydrophilic parts. This part generally determines the charge of the emulsion droplets. Also, droplets are charged with same sign of charges and this makes them unable to attach to each other emulsion droplets. An electrical double layer occurs at oil-water interface. In addition, because of the charge and its consequent electrical layer around droplets, droplets are not able to come together. This situation lasts until the repulsive force between them is altered. If magnitude of this force is high, emulsion droplets keep their stable form. However, if it is low, stability time gets shorter [1, 3].

Electrochemical properties are determined by microelectrophoretic techniques. According to this technique, an electric field is applied on diluted sample. Direction and velocity of particles are determined by moving droplets in applied electric field [3, 21]. The sign and velocity is also determined according to droplets' size and its polarity in water. If the emulsifier's hydrophilic tail is charged negative in water, it moves to interact with the positive site of electric field [21]. Zeta potential is a term which gives the velocity of oil droplets in applied electric field. Parallel to this information, as droplets are getting smaller, zeta potential gets higher. In addition, there is an energy barrier present which should be altered in order to prevent particles collide which is +/- 30 mV suitable threshold accepted for producing more stable emulsions [50].

Carefull determination of emulsifier types and other materials contribute to the oil-water interface influence the electrical characteristics. Droplet charge of nonionic surfactants (such as Tween and Spans) have tendency to have lower charge compared to anionic surfactants (such as Lec and CITREM) charge negative.polysaccharides charges negative (pectin, starch etc.) and while proteins charged negative above their isoelectric point, they charged positive under isoelectric point (WPI, SC) [10].

1.3.2.2.4. Rheology

As viscosity of a system increases, its tendency to coalescence also increases. Rheological properties as flow behaviour which are directly related to final product quality is measured by shear and compression devices such as viscometer or rheometer. According to food viscosity, some manufacturing parameters changes such as mixing efficiency, pumping power consumption and so on. Rheological property of emulsions is affected by some factors which are viscosity and chemical composition of continuous phase which are electrolyde concentration or pH value, droplet characteristics (concentration and size), internal viscosity of them [51]. The viscosity of materials are measured by applying force on them. The material responses as stress named as shear stress which is expressed as measure of deformation as a function of time. Measures of responses to forces are shown with the help of rheograms [50]. Emulsion systems behaves Newtonian at low oil concentrations. However, emulsion systems start to behave non Newtonian as oil droplet concentration increases.

1.4. OXIDATIVE DETERIORATION IN EMULSIONS

The main quality problem in food emulsions is lipid oxidation. Lipid oxidation is explained as “chemical changes that resulted from the interaction of lipids with reactive oxygen species”. In order to form emulsions, oil droplets are converted into a form as dispersing in a continuous medium or vice versa. Therefore, emulsified oils behave different than bulk oils in terms of oxidation process because according to their physical change, their exposure changes to stress conditions which may cause deterioration [52]. While some emulsion products require an amount of oxidation consequences with sensorial properties to final

products such as gaining some cheeses good smell and taste [52, 53] , for most products it causes undesirable changes with potentially toxic substances with off-flavours (rancidity). Oxidative stability can be expressed as oxidative rancidity or oxidative deterioration [41].

Table 1.1. Factors may affect oxidative deterioration in an O/W emulsion

Chr	**Property**	**Factors**
Lipid Phase	Composition	Degree of unsaturation
		Prooxidant impurities
		Inherent antioxidants
		Added antioxidants
	Physical state – solid fat and crystal properties	Solubility, partioning and diffusion antioxidants and prooxidants
	Physical properties	Rheology determined diffusion of antioxidants and prooxidants
		Polarity determines partition coefficients
Aqueous phase	Composition – pH, ionic strenght, solutes	Prooxidant impurities
		Inherent antioxidants
		Added antioxidants
		Micelles may alter location of antioxidants and prooxidants
		Reducing agents that can redox cycleprooxidant metals
	Physical state – ice crystal structure and location	Solubility, partitioning and diffusion of antioxidants and prooxidants
	Physical properties	Rheology determines diffusion and antioxadants and prooxidants
Interfacial phase	Composition	Anti-/prooxidant activity
		Impurities (hydroperoxides)
	Thickness	Steric hindrance of interaction btw water- and oil soluble components
	Charge	Electrostatic attraction/repulsion of antioxidants and prooxidants
	Permeability	Diffusion of antioxidants and prooxidants in lipid andaqueous phase
Structural Org.	Emulsion	Droplet concentration
		Droplet size distribution (surface area and light scattering)
	Spray Dried Powder	Porosity
		Exposed lipid levels
		Emulsion droplet characteristics
	Hydrogel properties	Hydrogel composition, structure and properties

Factors affecting oxidative deterioration in O/W emulsions can be seen in Figure 1.9. [17, 54]. Composition, physical state of oil and physical properties of oil phase and aqueous phase; composition, thickness, charge and permability of interfacial phase; emulsion's structural organization are the factors affecting oxidative stability.

Size distribution of oil droplets, oil droplet concentration, physical state of emulsion droplets, interfacial characteristics and range and magnitude of droplet droplet interactions significantly influence lipid oxidation in food emulsion products [52]. Also droplet characteristics which are droplet concentration, particle size and particle charge are related with oxidative stability [10].

1.4.1. Mechanism of Lipid Oxidation

Lipid oxidation occurs in three steps radical chain reactions which are named as initiation, propagation and termination (equation 1.1, 1.2, 1.3, 1.4, 1.5, 1.6)

Initiation:

$$LH \xrightarrow{initiator\ (heat\ or\ light)} L\cdot + H\cdot \tag{1.1}$$

Propagation:

$$L\cdot + O_2 \longrightarrow LOO\cdot \tag{1.2}$$

$$LOO\cdot + LH \longrightarrow LOOH + L\cdot \tag{1.3}$$

Termination:

$$2LOO\cdot \tag{1.4}$$

$$LOO\cdot + L\cdot \tag{1.5}$$

$$L\cdot + L\cdot \tag{1.6}$$

In initiation phase, radical species are occured because of a stress factor such as heat or light. While propagation phase as the chain part of reactions, reactive free radicals react with stable molecules in order to create new free radicals. Free radicals continue to create new free radicals. In termination, free radicals in medium react with each other and they form non radicals.

Mechanisms and influencing factors of lipid oxidation shoul be evaluated in detail in order to prevent or slow down its formation during manufacturing or storing processes. There are many analytical techniques to monitor lipid oxidation in bulk oils and fats. In addition, a number of techniques which generally requires extraction oil from system is present to monitor oxidative changes in emulsion systems. While some techniques measures the concentration of oxygen, lipid, antioxidant and hydroperoxide losses, some of them measure conjugated diene or hydroperoxide formation and also some of methods measures alcohol, aldehyde, ketone and hydrocarbone by product formations at the end of a distinct time [52].

Food manufacturers should carefully monitor these changes in order to reduce the oxidative deterioration. These changes explained below are directly related with composition of emulsions. In order to understand which parameter is highly related with lipid oxidation, physical characteristics and environmental conditions should be examined well.

1.4.2. Methods for Measuring Oxidative Deterioration

Oxidation types occuring in lipids are autoxidation, thermal oxidation, enzymatic oxidation, photo-oxidation in different conditions . In this study, thermal oxidation and photo oxidation were examined.

1.4.2.1. Measurement of primary oxidation products

1.4.2.1.1. Peroxide value (PV)

As lipids are oxidized, hydroperoxide formation as primary products and volatile and nonvolative secondary oxidation products result from primary product breaking down.

Peroxide value (PV) indicates the hydroperoxide formation rate during oxidative changes at beginning stages of oxidation. PV value is assumed as the common quality indicator of oil

and fat involved systems during food manufacturing and storing. There are a number of methods generally used to determine PV value. Most commons are spectrophotometric ferric ion complex measurement, iodmetric titration and infrared spectroscopy [55, 56]. Iodimetric titration method gives the peroxide value by recuding ROOH with KI and resulting with I_2 formation with titration. Ferric ion complex method gives peroxide value by reducing ROOH with Fe^{+2} and measues the formation of Fe^{+3} in resulted solution. Results are obtained spectrophotometry at 500-510 nm from the red complex of SCN^- or at 560 nm from blue-purple complex with xylenol orange. FTIR (Fourier Transform Infrared) is another spectrophotometric method gives the PV by reducing ROOH with TPP (triphenylphosphine) at 542 cm^{-1} TPOO (triphenylphosphine oxide). Chemuluminescence also gives PV of oils from reaction of luminol and heme catalyst by measuring the emission of oxidized luminol. Also PV can be measured by GS-MS (Gas Chromotography-Mass Spectroscopy) method by reducing ROOH to ROH with ROH measurement. On the other hand, UV spectrometry is a method for measuring conjugated dienes and trienes by estimating number of them at 230-234 nm generally [56].

In this study, PV value will be measured with Shanta and Decker's spectrophotometric ferric ion complex method which gives ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions and calculated as below.

$$PV = \frac{(A_s - A_b)\ x\ m}{55.84\ x\ m_0\ x\ 2} \tag{1.7}$$

where A_s is he absorbance of the sample; A_b absorbance of the blanks; m is the slope of the calibration curve which is 41.52 for IDF method; m_0 is the mass of oil sample in grams [57].

1.4.2.2. Measurement of Secondary Oxidation Products

1.4.2.2.1. P-Anisidine Value

Secondary Oxidation products are volatile and nonvolative products resulting from break down of primary oxidation products. Aldehydes, ketones, alcohols, epoxy compounds, volatile organics are some examples. Some methods are used to determine secondary oxidation products such as TBA (Thiobarburitic Acid), p-Anisidine, Carbonyls, OSI (Oxidative Stability Index) (rancimat) and GC (gas chromotograpgy). TBA is a method that measures mainly malonealdehydes by spectrophotometric techique. p-Anisidine method gives aldehydes and alkenal formation at 350 nm with AOCS standard method. Carbonyls method measures the total carbonyls or specific carbonyl compound appared during breaking down. Spectrophotometry and HPLC (High Pressure Liquis Chromotography) is used in carbonyl determination. In addition, OSI method gives the volatile organic acid value by monitoring changes in conductivity rapid. Gas Chromotography gives the colative carbonyls and hydrocarbons by direct headspace rapid analysis [56]. In this study, secondary oxidation products will be determined with p-Anisidine method with AOCS Cd-1890 and calculated as below:

$$PAnV = (25x(1.2As - Ab)/m) \quad (1.8)$$

where A_s is the final absorbance of oil solution reacted with p-anisidine, A_b is the absorbance of oil solution and m is the mass of test portion (AOCS(Cd-10-90)).

In this study, in order to determine primary and secondary oxidation products; ferric ion complexes method and p-Anisidine method will be used respectively.

1.4.2.2.2. TOTOX Value

TOTOX is a number of total oxidation resulted from pOV and pAn values. During lipid oxidation, firstly pOV products as hydroperoxides are forming in oxidized structure. Then, as hydroperoxides decompose, pOV rises and pAn increases. Therefore, TOTOX value gives the both hydroperoxides and its breakdown products and provides an approximate value of progressive oxidation values. While PV gives the oxidation at initial period of oxidation, p-

Anidisine shows oxidation at later stages. In order to have a better estimation about oxidative deterioration, TOTOX value is calculated as:

$$TOTOX\ V = 2PV + pAnV \quad (8)$$

Where the PV is peroxide value and pAnV is the p-Anisidine value for deteriorated oil samples [56, 58].

1.4.3. Strategies for Reduction of Oxidative Deterioration

As it was mentioned before, oxidative deterioration of samples are strongly influenced from environmental conditions and physical characteristics of emulsion ststems. Also, oxidative deteriorations' mode of action is different in emulsion systems than bulk oils. In order to prevent food products from oxidative deterioration or to slow it down, environmental conditions, emulsifier type and its relation with droplet characteristics on it have started to investigated.

Haahr and Jacobsen (2008) [59] investigated how oxidative stability of 10% n-3 enriched oil (high susceptibility to oxidation) in water emulsions prepared with real food emulsifiers; Tween 80, Citrem, SC and Lec affected by metal chelation by EDTA and pH effect. They studied on potential effects of different emulsifier types formulations' metal chelation ability, free radical scavenging activity and protective effect around oil droplets from hydroperoxide formation.

In addition, [60] investigated the oxidative stability of 40% fish oil-in-water mayonnaises enriched with 4, 10 and 14% fish oil; prepared with egg yolk and milk protein based emulsifiers and stored at 2 and 20º C. They tried to understand how physical factors such as droplet size and viscosity were related with oxidative stability and how physical environment such as iron added medium influenced it. Suprisingly they found out that milk protein emulsifiers oxidized faster than egg yolk contained ones even iron amount is higher than the others. Therefore they suggested that the lipid droplet size and thickness of double layer around the oil particles are not parameters that influence oxidation. Several factors such as other ingredients in medium, ingredient quality, antioxidant and prooxidant material presence and viscosity also has important effect on it when food emulsions are considered.

Moreover, Nielsen et.al (2013) [21] suggested emulsifier type and physical conditions affect the phyical and oxidative stability of emulsions. They conducted a research by using four different types of emulsifiers which are WPI, SC, Lec and milk phospholipids and 5% fish oil in their oil-in-water emulsion formulations at different pHs and both without iron and iron addition conditions. Viscosity, droplet size, zeta potential, primary and secondary oxidation products were evaluated. They found that SC emulsions are more oxidatively stable compared to others.

Kiokias et.al. (2006) [61] studied on 10 – 40 % cottonseed, sunflower, corn and olive kernel oils in water emulsions with 0.5 – 2 % Tween, SC and WPI concentrations. They suggested that several compositional parameters are present against lipid oxidation such as lipid phase and protein content. For this reason, they achieved oxidative stability measurement for 20 days at 40º and 60ºC. They prepared 20% oil concentration, 2% SC emulsions with four different types of oil and understood that the highest oxidation rates are in sunflower oil emulsions. Therefore, they chose the most vulnerable sunflower oil for further experiments in order to improve oxidative stability. Sunflower oil-in-water emulsions were prepared and subjected to oxidation test at 60ºC prepared with 100, 75, 50, 25, 0 % SC + 0, 25, 50, 75, 100 % Tween blends seperately and respectively. They found that as protein concentration increases in emulsion, oxidative stability increases at the same time.

Osborn et.al. (2004) [62] conducted a study on parameters that affect the lipid oxidation in structured lipids. Emulsions were prepared with WPI and sucrose fatty acid esters and 10-30 % oil concentrations. They achieved the oxidation at 50ºC and measured peroxide values, p-Anisidine values and calculated TOTOX value as well.They found out that as oil concentration increases, TOTOX value also increases. Emulsions' droplet size belonged to three different pressure mixing emulsions did not significantly affect the peroxide values.

Fomuso et.al. (2002) [63] compared lecithin, WPI, mono and di glycerols and sucrose fatty acid esters prepared with high pressure homogenization. They concerned that as oil droplets gets smaller, oil tend to be prone to oxidation depending on increasing surface area resulted from particle sizes. Droplet size, 48 days creaming stability and oxidative stability as an indicator of physical stability was observed. They have reported maximum creaming was observed for 0.25 % and 1% lecithin concentrations with 15 and 4.7%. Moreover, oxidative difference behaviour was resulted from surface charge of oil droplets such as anionic, catioic and nonionic surfactants depending on emulsifier type. In addition, high emulsifier concentration lead to high oxidative stability.

Ries et.al., (2010) [64] studied the effect of basic characteristics of milk protein based O/W emulsions (SC & WPI) on oxidative deterioration. Also, he examined the relation of droplet size, protein type and protein size and unadsorbed protein percentage on it. Oxidation measurements were achieved with lipid hydroperoxide measurements and hexanal formation in headspace analyse. They found out that when droplets smaller in emulsion systemsare

smaller, oxidation is increasing. Hydophilic groups of milk proteins show antioxidative activity. Ries et.al. conducted a research by supporting this idea with SC' better antioxidant acitvity than WPI. Also, it was reported that as protein concentration decreases, oxidative stability increases. When they examined the unadsorbed protein percentage at O/W interface by replacing the aqueous phase with protein solution, they found that this parameter also affected the oxidative stability by lowering it.

1.5. CYCLODEXTRINS

Cyclodextrins (CDs) which are cyclic maltooligosaccharides composed of glucose units linked by alpha (1-4) glucosidic bonds used as multifunctional food ingredients used in food, pharmaceutical, chemical, cosmetics, textile and agricultural industries. CDs derivatives alpha, beta and gamma are produced enzymatically with starch modification with CDs transferanz (CDTaz). They are complexes coming together with intermolecular bondings with two or more ions or coornination compounds named supramolecules. CDs are the most important supramolecules because they are biological sourced ones [65].

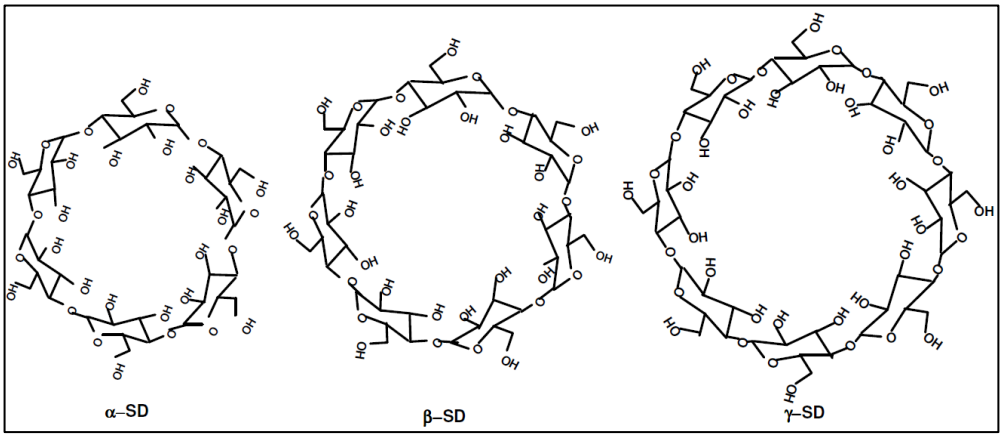

Figure 1.9. Chemical structure of alpha, beta and gamma CDs [66]

They are inexpensive and show multifunctional properties such as: oxidative protection against light and heat of active ingredients, undesirable sensory properties elimination and technological advantages by stabilizing the formulations for a long time related with product shelf life [65, 66]. Chemical structures of CDs molecules can be seen on figure 1.9, 1.10. It was reported that the internal cavity diameter of molecules in the order of alpha < beta <

gamma CDs [68]. They can be considered as empty capsules and can be fulfilled with different materials such as flavours, essential oils, antioxidants and behaves like hosts in order to form inclusion complexes. They form this inclusion complexes by behaving as a host to hydrophobic materials with their hydrophobic cavity.

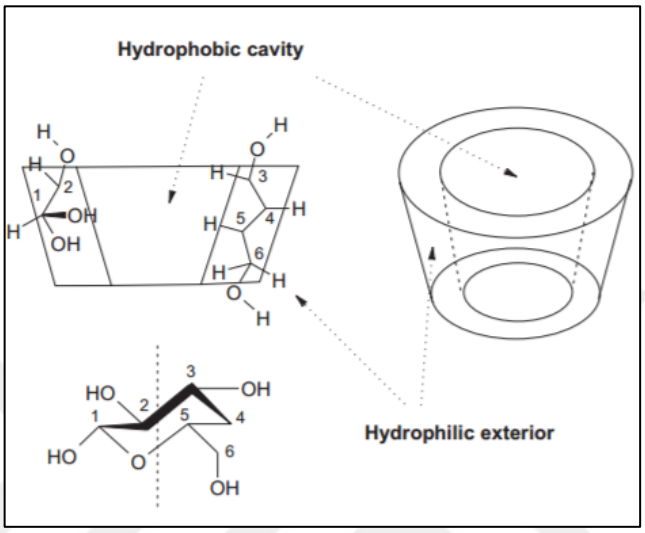

Figure 1.10. Structure of CDs [10]

Alpha and gamma CDs needs to be purified before using them therefore, generally beta BCD is used in industry. It was also reported that most strongly adsorbed CD type is beta > gamma >> alpha respectively [67].

CDs are widely used in pharmauceutic, cosmetic and food industries. In food industry CDs have a wide range in application. Their main action areas are: Prevention from oxidation of lipophylic food materials such as flavours, essential oils etc by effect of air, light or heat treatment, increasing solubility of vitamins and food colorant, masking off flavours and tastes [68].

1.5.1. CDs as Emulsifiers

The first study was achieved by Shimada et.al (1992a) [69], they investigated the capacity of α and β-CD stability. CDs lowered the interfacial tension by forming inclusion complex with fatty acids in triglycerides. They achieved that while triglyceride stayed in aqueous part, other two fatty acids stayed in oil part. Also it was reported that, according to size of fat chain, it can form inclusion complex with more than one CD.

In other study of Shimada et.al, 1992b [69] , oxidative stability was improved with xanthan gum addition to system as a polysaccharide. While BCD was used as emulsifier, there were no supportive information about emulsion physical and oxidative stability about BCD.

Inoue et.al. (2008) [67] has firstly reported a study which can be used as a fundamental guide prior to CD studies. In that paper, n-alkane in water emulsions were stabilized by alpha, gamma and β-CDs. Also it was reported that at low concentrations, although they show some surface activities, they could not form stable emulsions. In contrasy, at high concentrations, they could form solid stable emulsions since CDs precipitated at O/W interface. At the end of study, BCD was found as the most stable emulsifier by supplying the better stability effect.

On the other hand, there are a few studies uses CDs and various emulsifier blends and evaluates lipid oxidation. Moon Lee et.al (2013) [70] has investigated the oxidative stability and retardation of odor of gamma CDs and SC mixtures on fish oil inclusion complexes. Stability test was conducted at 55 C for 5 days. As a result, the lowest peroxide, p-Anisidine values, conjugated diene formation and odor intensity was observed for 80% gamma CDs + 20% SC formulations.

Wang et.al (2014) [23] conducted a study on BCD and soy lecithin inclusion complexes. Here, lecithin is an oil based emulsifier which has similarities with present study. Inclusion complexes and control groups were exposed to 40 and 80 C for 7 days. As a result, they reported that the BCD-Lec inclusion complexes have better physicochemical properties may contribute the quality of some food products in food industry such as milk powder, bread as well as in cosmetics and medicine industry.

Moreover, there are few studies examines the physical stability of individually BCD and its blends emulsions.

Mathapa and Paunov (2013) [71] evaluated the size and shape of alpha and beta CDs based oil inclusion complexes formed microcrystalls. Similarly emulsions were prepared with 0.01-0.8 oil fractions by using Ultraturrax. They reported the remarkable stability against coalescence was achieved at 0.5 and 0.6 oil fractions.

Cheong et al. (2016) [72] evaluated the kenaf seed oil-in-water emulsions with the help of the synergistic effect of SC + Tween 20 and BCD and pickering property of inclusion complexes. They measured the droplet size and zeta potential of these emulsions. They found the optimum mixture to produce physically stable emulsions with high viscosity, high zeta potential and high creaming stability. Optimum formulation was reported as 57.9% (w/w) SC, 27.6% Tween 20 and 14.5% (w/w) BCD.

Shim et.al. (2003) [73] examined the changes in functional properties of cholesterol removed whipping cream by BCD addition. They tried to improve foam stability in physical stability means. Although foam stability achievement was expected, coalescence were observed and achievement could not be observed. They suggested that the stirring time or shear sould have been reduced.

In this study, 1, 2, 4% emulsifier concentrations and 5, 20, 40 % oil concentrations in water emulsions will be produced and improvement strategies will be discussed and the most logical improvement will be discussed and applied on formulations. In addition, emulsions with improved conditions will be prepared with most common food emulsifiers which are soy lecithin (Lec) and citric acid esters of mono and diglicerides (CITREM) as oil based emulsifiers and two protein based emulsifiers, sodium caseinate (SC) and whey protein isolate (WPI) emulsions will be physically characterized (visual, particle charge, particle charge, viscosity) in order to understand their physical properties and their relation with oxidation as a quality indicator. The formulation which is highly prone to oxidation since its physical character will be determined. The emulsifier alone and its 1:1 blend with BCD will be produced and stored at 4º C, 21º C and 55º C for a month. They also will be stored at Suntest (Atlas XLS) for a test cycle equal to exposure of 10 days direct sunlight to understand effect of BCD against light conditions. Visual observation (CI) of all emulsions will be compared; Suntest and 55ºC samples will be analyzed chemically. As a result, the properties that influence oxidative stability will be examined in detail and an emulsifier blend with BCD will be produced to understand its effect on physical and chemical properties.

2. MATERIALS AND METHODS

2.1. MATERIALS

Lacprodan DI-9224 which is a functional whey protein isolate for protein fortification of clinical, sports and nutrition products and also suitable for pasteurization and UHT processes was kindly donated by Arla Food Ingredients (Viby J., Denmark). Its protein (Nx6.38) as is content is minimum 88%, protein (Nx6,38) d.nm is min. 92%, lactose content is max 0.2%, fat is max. 0.2%, ash is max 4.5%, moisture is max 6.0% and also rich in minerals (0,5% Na, 0.2% P, 0,05% Cl, 1.3% K, 0.1% Ca.). In addition, it is reported that Lacprodan DI-9224 was produced according to relevant EU regulations, for food and food ingredients and or FAO/QHO Codex Alimentarus, when relevant and non-GMO. Miprodan 30 Sodium Caseinate which is the spray dried pure milk protein produced from skimmed fresh pasteurized milk made by acid precipitation of the casein, direct neutralization and spray drying was also kindly donated by Arla Food Ingredients (Viby J., Denmark). Its protein (Nx6,38) d.m is min 93.5% and (Nx38) as is is min 88%. Also, lactose content is mix 0.3%, fat content is max 1.5%, ash is max. 4.0% and moisture is max. 6.0% . Palsgaard CITREM 3307 (Palsgaardvej, Denmark) citric acid esters of mono and diglycerides of fatty acids (E472c) sourced from vegetable fat was kindly donated by Teknaroma/İstanbul. Liquid, Non GMO Soy Lecithin with 0.27% moisture content, 25.80 mg KOH/gm acid value and 0.10 meq O2/kg peroxide value was obtained from Shankar Soya Concepts, India. In addition, Glucidex IT 12 Maltodextrin (Roquette/France) with dextrose equivalent (DE) 12 was kindly donated by Barentz Food and Chemistry Trade Co. Ltd/İstanbul. Glucidex IT 12 is generally used as texturizer, powder carrier or fermentable substrate and also it is suitable for ice cream, confectionary, soups,beverages and flavourings as applications. Sunflower oil (Yudum) were purchased from a local market. Sodium azide (Reagentplus, >=99.5%) was obtained from Sigma-Aldrich. Chloroform, Methanol (>=99.93%), Sodium Sulphate(99%-100.5%), Barium Chloride Dihydrate Crystalline, Iron(II) Sulfate Heptahydrate (reagentplus>=99.0%), Ammonium Thiocyanate (>=97.5%), 2,2,4 Trimethylpentane (isooctane) and Hexane were obtained from Sigma Aldrich.

2.2. METHODS

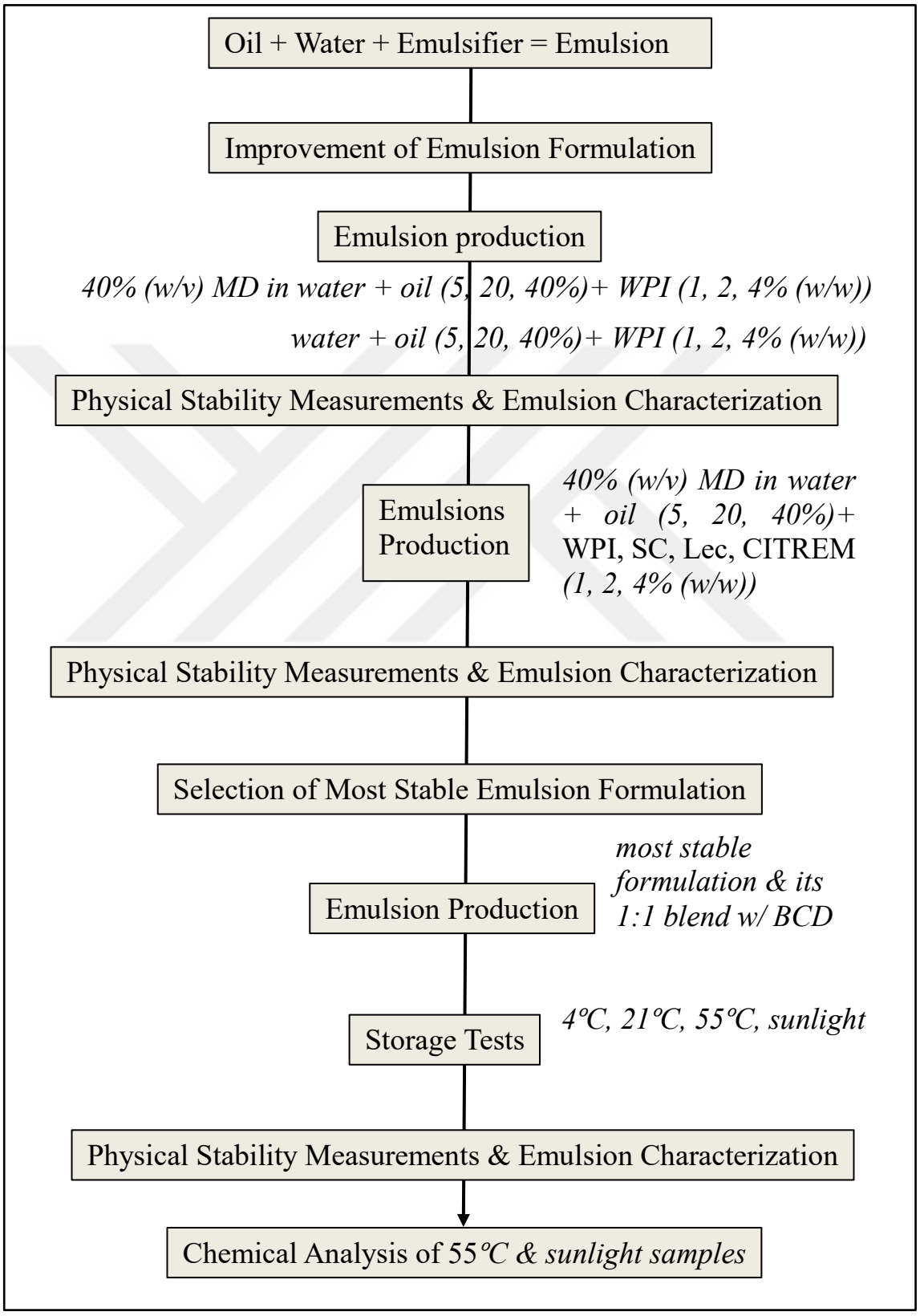

Figure 2.1. Flow chart of emulsion production

Firstly, regarding to pre-studies; BCD and WP emulsions' stability was investigated and BCD emulsions were investigated. Emulsions were stored at 55 C for 11 days. At the end of this time interval, while cracks and oil leakage was observed from appared bubbles, there was no physical change at BCD emulsions. Also, oxidative stability of individual whey protein and BCD emulsions were investigated. It was found that weaknesses of the experimental set-up like the impurity of whey protein sample, limitations to the peroxide value determination method, time constraint and other limitations required more accurate methods. Therefore, BCD was selected as emulsifying agent for trials in order to select proper materials to investigate.

2.2.1. Emulsion Preparation

Oil-in-water emulsions were prepared by using four different emulsifiers which are sodium caseinate (SC) and whey protein isolate (WPI) as protein based emulsifiers; soy lecithin (Lec) and citric acid esters of mono and di glycerides (CITREM) as oil based emulsifiers. Model emulsions were designed as 5, 20, 40 % of oil (wt/wt) and 1, 2, 4% of emulsifier (wt/wt) compositions. Emulsifiers were dissolved in the proper fractions of emulsions before homogenization; while sodium caseinate and whey protein isolate were dissolved in aqueous part of emulsion, soy lecithin and citric acid esters of mono and diglycerides were dissolved in oil part.

At first, emulsions were prepared without maltodextrin (MD). Since physical stability challenges were occurred, MD DE 12 was started to use as stabilizer in order to handle the stability issue. The emulsions were prepared in 5 minutes as a two stage process: 4 minutes homogenizaton at 15,000 rpm and 1 minute fast homogenization at 20,000 rpm. The aqueous phase was prepared with 40% (w/v) MD by stirring them for half an hour for aeration and hydration until it becomes a clear solution. Homogenizations were achieved by adding the oil part slowly into 40% (v/v) MD in water aqueous phase at 15,000 rpm with UltraTurrax (IKA, Germany) at proper temperatures for emulsifiers. For water based emulsions, oil was added at the end of first minute. For stability experiment, sodium azide (0.05 wt%) were added to bottles during homogenization to inhibit microbial spoilage.

Final emulsions were distributed into 40 ml glass tubes prior to physical analysis. Emulsions were prepared and analyzed in the first 6 hours from prepatation. All measurements were conducted triplicate.

Table 2.2. Formulation trials and their achievement status

Action	Status
Comparison pH 5 Sodium Phosphate buffer and water aqueous phase	
➢ 25% oil (v/v) + 1.5 % (w/v) BCD in pure water	✗
➢ 25% oil (v/v) + 10 % (w/v) BCD in pure water	✗
➢ 25% oil (v/v) + 10 % (w/v) BCD in pH 5 buffer	✗
➢ 10% oil (v/v) + 10 % (w/v) BCD in pure water	✗
➢ 10% oil (v/v) + 10 % (w/v) BCD in pH 5 buffer	✗
Addition of a thickening agent to formulation for 24 h stability at least	
➢ 0.45% (wt/wt) addition to aqueous phase	✓
➢ Mixing 0.45 % (wt/wt) XG solution with final emulsion	✗
Decision of 1:1 mixture of an emulsion and BCD.	
Involving another emulsifier to experiment plan	
➢ 5% oil (wt/wt) + 0.5 SC (wt/wt) + pure water at 45 C	✗
➢ 5% (wt/wt) oil + 0.5% (wt/wt) WPI + pure water	✗
➢ 5% (wt/wt) oil + 2% (wt/wt) WPI + pure water	✗
Removing XG from system	
Combinations of 1, 2, 4% (wt/ wt) WPI + 5, 20, 40 % emulsions preparation	
Stabilizer addition to system	
MD type investigation and selection of DE 12 MD	
Producing one day stable emulsions with addition of maltodextrin	✓
Combinations of 1, 2, 4% (wt/ wt) WPI + 5, 20, 40 % emulsions preparation in the presence of 40% (w/v) MD	

2.2.2. Physical Characterization of Emulsions

All formulations were subjected to visual observation, particle size analysis and rheology measurements.

2.2.2.1. Visual Observation

2.2.2.1.1. Storage Tests

After homogenization, emulsions were transferred into 40 ml glass tubes with a lid and stored at room temperature for 24 hours. The creaming index (CI) was measured at 1 h, 6 h and 24 hours time intervals. CI is expressed as :

$$CI(\%) = \left(\frac{HL}{HE}\right) x100 \tag{2.1}$$

where the HE is height of emulsion and the HL is the turbid lower layer of emulsion [72].

2.2.2.2. Particle Size Analysis

Droplet size measurements were achieved in the first 1 hour after preparation. It was achieved with Malvern Zetasizer ZS Nano Series (Worcestershire, UK). The emulsion was dispersed in water in order to avoid multiscattering effect (PDI should not be equal to 1, should be close to 0). Also, during measurement, water (RI:1.33) was used as dispersant, sunflower oil droplet was selected as sample and RI was set to 0,001 at 25C.

2.2.2.3. Surface Charge Analysis

Zeta potential measurements were performed in the first 1 hour from preparation, was measured right after the droplet size with the Zetasizer ZS nano (Malvern Instruments, Worcestershire, UK) series by using a capillary DTS 1070C cuvette. Emulsions were diluted with pure water in order to measure the velocity of oil droplets between two electrodes that create electric field. Results were extracted from software.

2.2.2.4. Rheology

Viscosity measurements were done in the first 1 hours after preparation. Viscosity was measured by using Kinexus pro rheometer (Malvern Instruments, Worcestershire, UK) with rotational (cub & bob) probe which is used for fluids and also fluid like matters. Measurements were achieved at room temperature and each measurement lasted for 7 minutes. Viscosity behaviour on alternative flow curve graphic were drawed respect to shear rate with 30 data points. Results were obtained from r Space for Kinexus software.

2.2.3. Chemical Analysis of Emulsions

At the first part of experiment, the most physically stable emulsifier type with optimum ratio of oil and emulsifier ratio were determined as 4% CITREM with 20 % oil (w/w) concentration. In order to monitor oxidative deterioration of this formulation with and without BCD as 4% CITREM and 1:1 CITREM : BCD emulsions were prepared and seales into 40 ml glass tubes. Their physical stability was measured by visual observation at 4 C, 21 C and 55 C. Particle size, droplet charge and viscosity parameters were measured as explained above. Additionally, storage tests below were conducted.

2.2.3.1. Storage Tests

In order to monitor thermal changes, samples stored for 15 days at 55ºC oven. Also, in order to monitor the light effect on oxidative deterioration, emulsions were stored in SunTest XLS (Atlas) (fig. 2.3) for a test cycle which is equal to exposition of direct sunlight for 10 days. Primary and secondary oxidation which gives the chemical characteristics of oxidized oil were measured.

Figure 2.3. Atlas Suntest XLS

2.2.3.2. Primary Oxidation Determination of Oil

2.2.3.2.1. Peroxide Value

Prior to analysis, an amount of emulsions was mixed with 2:1 chloroform-methanol mixture, vortexed for 1 minute and centrifuged at 15 000 rpm (Sigma 3-30 K Centrifuge). Chloroform phase including analyte was extracted from resulted phase separation. ≤0.1-0.3 g (0.3 ml chloroform-fat soln.) oil was analyzed according to Shanta & Decker's [57] modified spectrophotometric method for food lipids. To prepare iron(II)chloride solution 0.4 g barium chloride dehydrate was dissolved in 50 ml water. This solution was added slowly and with constant stirring to an iron –(II) sulfate solution prepared as dissolving 0.5 g FeSO4.7H2O in 50 ml water. 2 ml of 10N hydrochloric acid was added to resulting solution. The barium sulfate precipitate was filtered off to give a clear iron(II) solution, which was stored in a dark bottle and kept in the dark. To prepare ammonium thiocyenade solution, 30 g ammonium thiocyenade was dissolved in water, and the volume was made up to 100 ml. To determine PV, the sample 0.01-0.30 g was mixed in a tube with 9.8 ml chloroform:methanol (7:3(v/v)) on a vortex mixer for 2-4 s. Ammonium thiocyenade solution (50µl) was added, and the sample was mixed on a vortex mixer for 2-4 s. After 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm against blank solution by using a spectrophotometer. The entire process should be completed in 10 minutes. All the

measurements were achieved and calculation were done by assuming all oxidized oil in emulsion was transferred through chloroform layer at phase separation interface.

PV is calculated as :

$$PV = \frac{(A_s - A_b)\ x\ m}{55.84\ x\ m_0\ x\ 2} \quad (2.2)$$

where A_s is the absorbance of the sample; A_b absorbance of the blanks; m is the slope of the calibration curve which is 41.52 for IDF method; m_0 is the mass of oil sample in grams [57].

2.2.3.3. Secondary Oxidation Determination of Oil

2.2.3.3.1. p-Anisidine Value

Prior to analysis, an amount of oil was dissolved in 2:1 hexane-methanol. It was vortexed for 1 minute and centrifuged at 15 000 rpm for 5 minute for two times. The aldehyde measurement during the breakdown of hydroperoxides were determined by AOCS (Cd-10-90) method by using a spectrophotometer. P-anisidine solution were prepared with 0.25 g p-anisidine and 100% anhydrous acetic acid. 0.5 g samples were diluted to 25 ml 2,2,4 Trimethylpentane. Absorbance of solution were measured at 350 nm (Thermo Scientific UV-Vis-Evolution 220) against blank. Extracted hexane layer was transferred into 10 ml volumetric flask and completed with iso-octane. Measurements were achieved with reduced volumes. 1 ml p-Anisidine solution was poured on 5 ml oil solution; after 10 minutes, absorbance were measured and recorded. All the measurements were achieved and calculation were done by assuming all oxidized oil in emulsion was transferred through hexane layer at phase separation interface.

According the formula below, p-Anisidine values (PAnV) were determined:

$$PAnV = (\frac{25x(1.2As - Ab)}{m}) \quad (2.3)$$

where As is the final absorbance of oil solution reacted with p-anisidine, Ab is the absorbance of oil solution and m is the mass of test portion.

2.2.3.3. TOTOX Value

TOTOX is a number of total oxidation resulted from pOV and pAn values. TOTOX value gives the both hydroperoxides and its breakdown products. Also it provides an approximate value of progressive oxidation values.

TOTOX value is calculated as:

$$TOTOX\ V = 2PV + pAnV \tag{2.4}$$

where the PV is peroxide value and pAnV is the p-Anisidine value for deteriorated oil samples [56, 58].

3. RESULTS AND DISCUSSION

3.1. FORMULATION OF MODEL EMULSIONS

Emulsions are thermodinamically unstable systems where two immiscible liquids are dispersed within each other as forms of small droplets. Food emulsion systems are important in food manufacturing because they have the role of contributing to the characteristic texture of foods, as well as serving as ingredients in many products such as sauces, cheeses, and meat products. Basically, emulsion structure is composed of an aqueous phase, an oily phase and an emulsifying agent. Choosing proper major materials to accomplish the food emulsion formulations is essential in order to keep them physically stable and to investigate the science behind it. Also physically stable emulsions influence consumer perception from quality aspect. They have tendency of retardation of sedimentation or creaming which result from density difference between droplets, its surrounding phase and it is also dependent on droplet size and density difference of emulsion. There are various trials conducted for choosing proper formulation raw materials of emulsions. .

Water and pH 5 sodium phosphate buffer solution were compared to determine aqueous phase of emulsions as it is seen in figure 3.1 and 3.2. Dickinson (2003) [20] has reported the principal factors affecting oil-in-water emulsion stability and mentioned about the nature of continuous aqueous phase and nature of dispersed oil phase. According to criteria, ionic environment and solubility of oil in continuous phase influences the emulsion stability.

For first trial, 25% oil (v/v) was slowly added into 1.5% BCD (w/v) in water. Than, as second trial, 25% oil (v/v) was slowly added into 10% BCD (w/v) in water. For third trial, 10% oil (v/v) was slowly added into 10% BCD (w/v) in water. After 24 hours, while separation was observed for water aqueous phase and for both oil concentrations (fig.3.1) , pH 5 buffer solution aqueous phase emulsions did not separate as much as pure water used emulsions. Both for 25% and 10% oil concentrations, seperation was observed at same emulsifier ratio (fig.3.2).

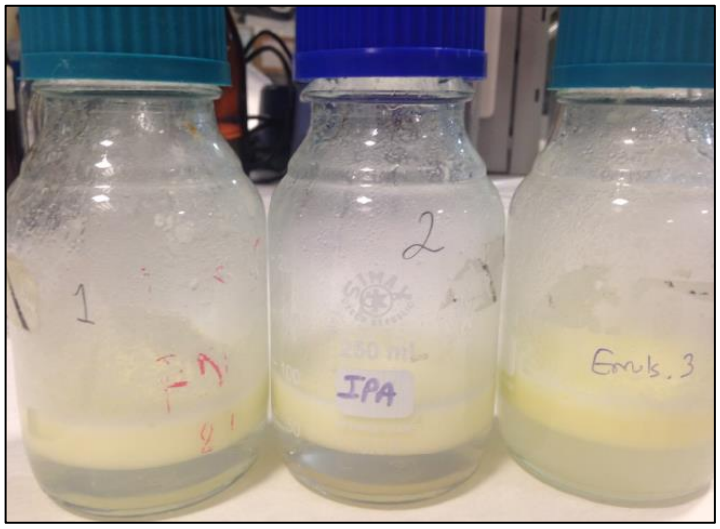

Figure 3.1. First three trials with BCD and water aqueous phase

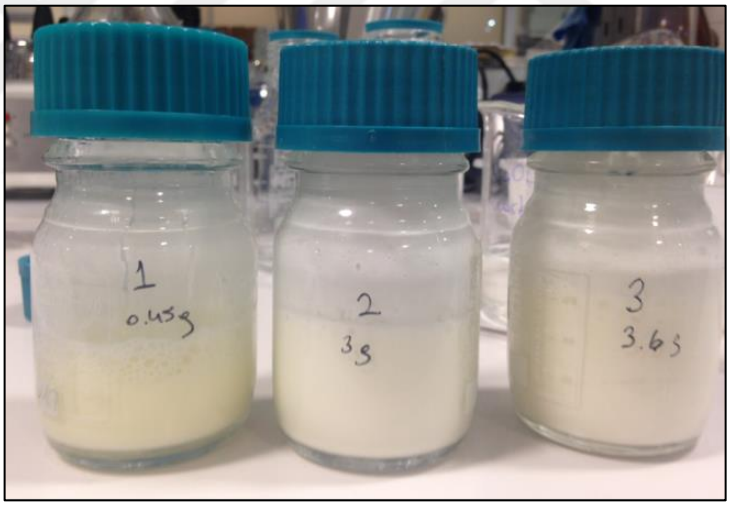

Figure 3.2. First three trials with buffer solution aqueous phase

Since 24 hours stability has not been achieved yet, a thickening agent was decided adding to formulation. Thickening agents, mainly polysaccharides are used to increase the viscosity of continuous phase [36, 74, 75]. Xanthan gum which is an anionic polysaccharide, widely used in food industry, was added to less separated emulsions in the ratio of 0.45% (w/v) of buffer used emulsions both in form of solution and adding to aqueous phase. As it is seen in fig.3.2, separation was seen for first trial. There was no significant difference observed for second and third trials.

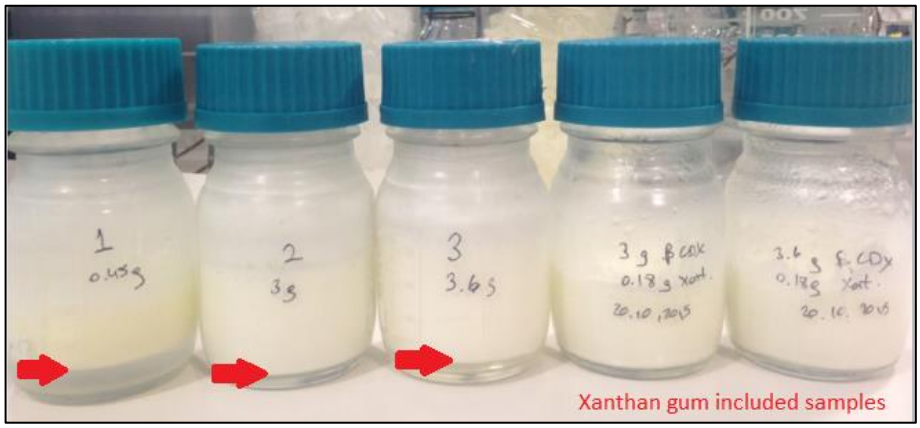

Figure 3.3. Comparison of xanthan gum included samples and no added samples

It was understood that using buffer solution is preferable over water phase on its own. Also, using 0,45% (w/w) xanthan gum addition should be needed for keeping emulsions stable for 24 hours. Even 0.45% (w/w) xanthan gum and pH 5 buffer was needed for preparing BCD in different ratios and oil phase contained 1 day stable emulsions, the concentration of buffer solution was very high. It was not possible to handle with that much raw material for preparing buffer in experimental scale. Also in industrial scale, that would not be feasible. Therefore, using buffer as aqueous phase was not realistic. As a result, pure water was assumed as aqueous phase for model emulsion formulation.

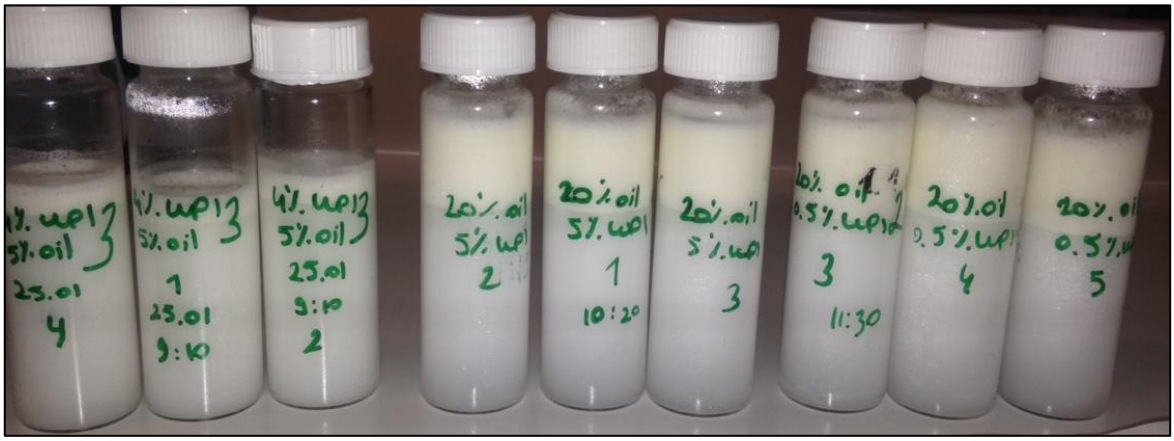

Figure 3.4. Creaming status of various WPI and oil concentrations samples

At this stage, major inputs of emulsions were determined as BCD, sunflower oil, pure water and xanthan gum.

Moreover, according to Turkish Food Codex, BCD can not be found in ready to use products, diluted by consumer as final product, more than 1g/1kg dosage. As a result, it was decided to try other emulsifiers and their mixture with BCD in emulsion system [93].

It was thought that the separation issue may be sourced from emulsifier type. McClements explained that, emulsifier type is a very important issue. It affects the long term stability and overall performance of emulsions. In food industry, certain proteins, phospholipids, polysachharides, solid particles and small molecule surfactants are widely used for stabilizing emulsion systems [2]. Milk proteins which are caseins and whey proteins were tried to use as individual emulsifier. For this reason, SC and WPI were became involved to formulation seperately.

On the other hand, size of droplets is another major parameter on emulsion stability since it may cause gravitational separation, flocculation and coalescence [3]. For this reason, SC and WPI reformulation was planned. In practice, food emulsions are composed of various droplet sizes. As it can be seen in figure 3.5, this situation makes emulsions name polydisperse emulsions [2]. In droplet size measurements, multiple scattering is a critical term which determines the upper limit for sample concentration for larger particles. In addition, electrical potential between particles is another term that determines the stability. Zeta potential which is a potential between particle surface and dispersed liquid changes dependent on the distance from the particle surface is measured in order to determine whether an emulsion will remain stable or not. Also, if the zeta potential is higher than +30 mV and lower than -30 mV, this emulsion is considered as physically stable.

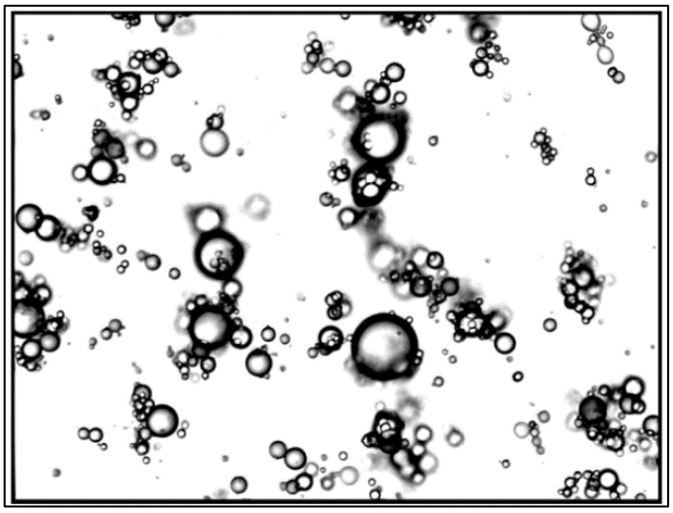

Figure 3.5. A polydisperse emulsion composed of various range oil droplets [2]

Sodium caseinate emulsions were prepared. 5% (w/w) oil, 0.5% (w/w) SC, deionized water at 45ºC were used. Firstly, SC was dissolved in water, oil phase was added slowly into aqueous phase. Then, emulsifier and water were pre-emulsified for 1 minute and emulsification time was completed up to 5 minutes. Seperation was observed during 24 hours again.

As it is shown in figures 3.6, 3.7, 3.8, 3.9; separation is same for 1 h, 6 h, 24 h time intervals. Therefore, 5% oil, 0.5% SC was not enough keep emulsions stable for 24 h. Moreover, after emulsification was achieved, zeta potential and droplet size measurements were made. In zeta potential and droplet size experiments, peaks could not be seen clearly because of the multiscattering effect. The concentrations and emulsifier/oil ratio should have been optimized or increasing SC ratio could be a solution to stabilize the emulsions.

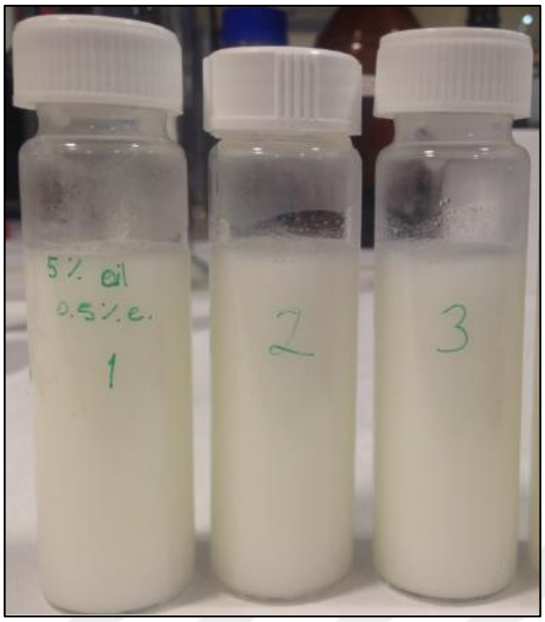

Figure 3.6. Appearance of SC emulsion after 0 h

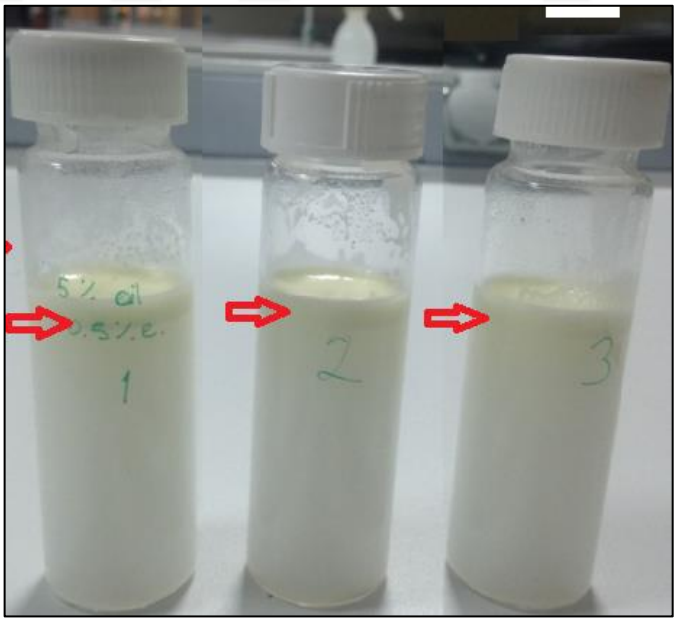

Figure 3.7. Appearance of SC emulsion after 1 h

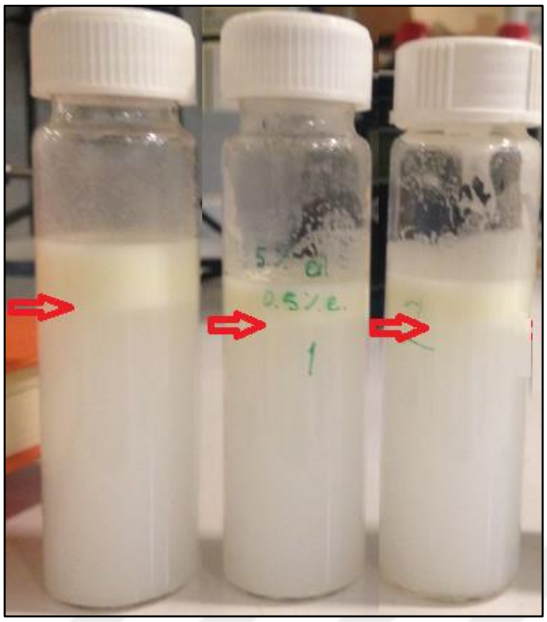

Figure 3.8. Appearance of SC emulsion after 6 h

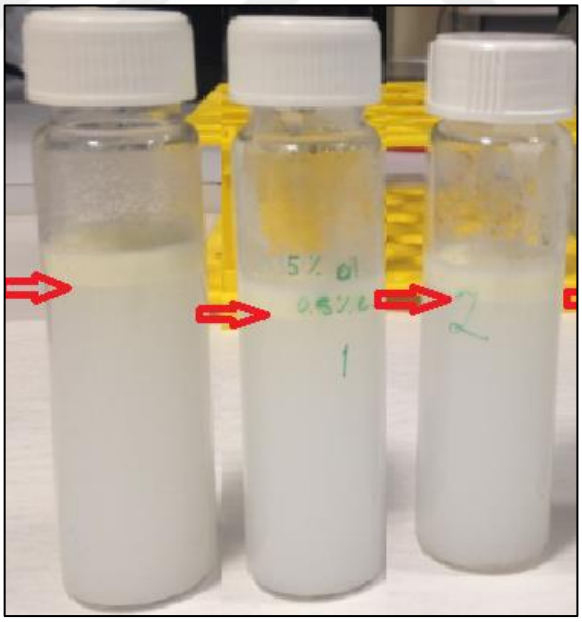

Figure 3.9. Appearance of SC emulsion after 24

As another emulsifier trial, 5% (w/w) oil, 0.5% WPI were emulsified for 15000 rpm. Stability could not achieved for 24 hours again. In addition, zeta potential and droplet size

had multiscattered graphs again. Trials were continued with increased ratios for both emulsifiers and oil ratios.

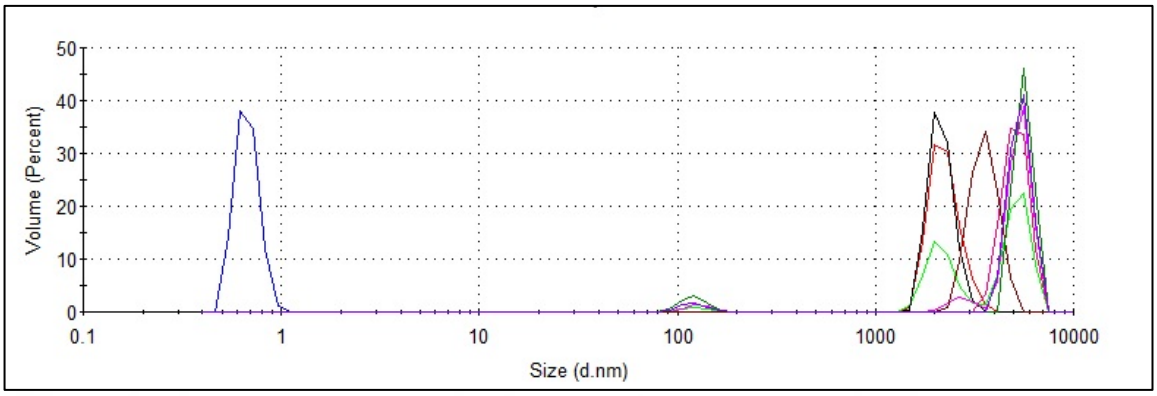

Figure 3.10. Droplet size distribution of a multiscattered sample

Another trial was achieved with 2% (w/w) WPI and 5% (w/w) oil. This time, zetasizer and droplet size were measured in first 1 hour. Emulsions were diluted in 1:9, 1:14 and 1:19 for avoiding multiple scattering effect in droplet size and zetasize measurements because as it can be seen in figure 3.10, precise data can not be extracted from a multiscattered sample measurement. Also, in order to have accurate data from software, zero shear viscosity was measured by using rheometer and entered to zetasizer software. These precautions was resulted in success and multiscattering effect was not seen. As a result, it was understood that, if measurements were achieved in first 1 hour after preparation by diluting samples at least 1:14, multiscattering effect was not seen. According to McClements (2007) [2], droplet size has also high impact on viscosity of emulsions. Therefore, viscosity measurements achieved and obtained precise data triplicate.

For oil type selection, olive oil 1:1 mixture and sunflower oil were investigated individually. Since, olive oil is nutritionally rich and its positive health effects on human health, it was desired to add to formulation but then, both their droplet size and viscosity of emulsions were measured. It was found that these values did not change significantly dependent on oil type. Moreover, sunflower oil is cheap compared to olive oil and also it is feasible, reachable and widely used in many goods like mayonnaise, toppings, salad dressings and so on. In addition, it was thought that removing olive oil from formulation may extend shelf lives of

goods and prevents them from risk of undesirable sensory properties may sourced from short chain fatty acids in olive oil.

While planning the further experiment, 1, 2, 4% (w/w) emulsifier ratios and d 5, 20, 40% (w/w) oil concentrations which have high impact on emulsion stability were planned.

In this study, the oxidative stability change between 1:1 BCD: most stable emulsifier at most stable oil ratio will be compared and effectiveness of BCD addition to system will be evaluated in quality aspect.

In oil-in-water emulsion systems, oil phase is dispersed in continuous phase. If density of dispersed droplets is lower than the surrounding liquid, they have tendency to rise upwards. This movement is named as creaming. Creaming is expressed as creaming index which was calculated by "(Height of inferior turbid layer/ Height of whole emulsion)*100". As it reaches to zero, emulsions can be named as physically stable [2].

There are studies present in literature about addition of xanthan gum to emulsifier systems. For example, Sun et al. conducted a research on 2% wt WPI, 20% (v/v) oil included emulsions at different XG concentrations (0 - 0.5% wt). Droplet size, viscosity, microstructure, creaming and oxidative stability of emulsions were investigated. It was reported that at 0.2 wt% XG , creaming was observed at the end of 70 hours. At 0.5 wt % XG in aqueous phase, no creaming was shown for Although physically stable emulsions were obtained, lipid oxidation of the emulsions was inhibited by addition of 0.15- 0.2 wt % XG. As a result of this study, it was found that even XG addition did not change dropley size distribution significantly, creaming, emulsion viscosity and oxidative stability was affected significantly [36] . Therefore, due to the effect of XG on oxidative stability, its usage would not be correct. Since the aim was to investigate the oxidative stability change, another material which has effect on this parameter could not be used.

After removing xanthan gum from formulation, using texture modifiers might be another solution for keeping emulsions stable for 1 day. Texture modifiers are surface active ingredients which increases the viscosity of continuous phase and slows down the gravitational seperation [20, 29].

Creaming index which determines the physical stability of emulsions was measured for 1%, 2%, 4% whey protein isolate (WPI) included model emulsions were prepared and observed

during 1 day stability period. At the end of 1 hour, 6 hours and 24 hours time intervals, creaming index was measured and most of emulsions were observed in two distinct phases as cream layer and turbid layer because of seperation of oil and water fractions from each other with a distinct line (Fig 3.11.).

Figure 3.11. WPI model emulsions showing cream layer and turbid layer

Preparation stages and reasons an consequences were explained above in Table 1. In order to make model emulsions prevent from aggregating in the means of make them thermodynamically stable, maltodextrin as a texture modifier was determined to add into the system. Maltodextrins are starch hydrolysis products with various dextrose equivalent values. DE also called as “percentage of D-glucose of average degree of polymerization (DP) of anhydro glucose units” [25]. Since, maltodextrins are surface active agents, they modify the viscosity by increasing the viscosity of aqueous phase.

Figure 3.12. 1% WPI, 5% oil contained o/w emulsions which are mixed md solution after homogenization (left) and prepared with md included aqueous phase (right)

Udomrati et.al. (2011) [27] also investigated and reported about to the effects of tapioca maltodextrins on the stability of oil-in-water emulsions. Emulsions were stored at 25 C for 7 days and it was reported that as maltodextrin concentration increased, the turbid (serum) layer thickness decreased. On the other hand, they found that there were no phase separation more than 35% (w/w) for DE 9, 40% (w/w) for DE 12. Also Turchiuli et.al.(2013) [76] reported that the studies conducted with DE 12 was efficient than DE 21 maltodextrin in fine emulsion production. In addition, Udomrati et.al. (2013) [27] also studied on Tween 80 included oil-in-water emulsions and investigated their stability with DE 16, DE 12 and DE 9 MD in aqueous phase. According to literature examples and percentage trials, it was decided to use DE 12 MD in 40% (w/v).

Figure 3.13. Emulsions prepared with 9% MD (first from left) and 15% MD (second from left) included aqueous phase

Glucidex IT 12 (Barentz Food and Chemistry) is generally used as texturizer, powder carrier or fermentable substrate and also it is suitable for ice cream, confectionary, soups, beverages and flavourings as applications. After several percentages were investigated (Fig.3.12 and 3.13), it was thought that, stability could be achieved at 40% (w/v) Glucidex IT 12 maltodextrin of aqueous phase.

WPI is a surface active protein in globular shape which have the ability of adsorbed to oil droplets' surfaces and produces a single layer of molecules. Moreover, its emulsification properties changes according to pH, ionic strenght, net charge, formed layer on oil droplets' viscosity, thickness and elasticity [36].

3.1.1. Physical Analysis of Emulsions

Creaming index was measured as percent of division of turbid layer (HL) to total emulsion height (HE). As CI approaches to zero, 24 h stability of emulsion is increases and emulsion gets more thermodynamically stable. .

In this part of study, WPI O/W emulsion stability is improved with a texture modifier - maltodextrin. Also its effect on creaming, droplet size, zeta potential and viscosity was investigated on model emulsions prepared with 1, 2, 4% (w/w) WPI ratios at 5, 20, 40 (w/w) oil concentrations both for 40% (w/w) MD and pure water aqueous phases.

3.1.1.1. Visual Observation

After emulsion preparation with high speed stirring, they were stored at room conditions for 24 hours. Its height data was obtained at 1, 6 and 24 hours. Here, CI can be expressed as percent separation of emulsion oil phase and aqueous phase from each other. Emulsions were prepared with high speed homogenization, however homogenization equipment and type such as high shear or pressure is very crucial on emulsion stability. Since, it is related with oil droplet dispersion in aqueous phase, it directly effects the creaming and droplet size [92].

In table 3.2., for WPI emulsions, at 5% (w/w) oil concentration and 1% (w/w) WPI ratio, there is manual experimental measuring error is shown as time passes, actually CI should be increased. Therefore, it is not possible to comment about this part. For MD improved emulsions, it seems that stability was achieved for 1 hour for all emulsifier ratios also for 6 h at 4% emulsifier ratio with MD addition. Also there is a dramatic change for 1h, 1% emulsifier ratio when system was improved with MD addition as well as emulsifier ratio increased, creaming was disappeared for 6 h. There is no significant change between 24 h stability at 5% oil concentration.

Table 3.1. Creaming index measurements of WPI and WPI + MD emulsions during 24 hours

C_{Oil}(%)		5			20			40		
C_e (%)	time(h)	1	2	4	1	2	4	1	2	4
WPI	1	96.25 ± 0.22	96.84 ± 0.60	97.701 ± 0.59	2.96 ± 0.49	3.81 ± 1.00	1.00 ± 0.00	3.05 ± 0.59	1.98 ± 0.70	0.97 ± 0.39
	6	94.26 ± 1.00	95.02 ± 0.62	93.69 ± 0.44	28.60 ± 3.37	28.19 ± 2.66	22.147 ± 2.22	15.19 ± 1.26	3.77 ± 0.87	2.68 ± 0.40
	24	91.55 ± 1.42	91.67 ± 0.17	90.50 ± 0.83	31.74 ± 2.16	33.51 ± 1.70	31.53 ± 0.86	36.41 ± 1.23	38.05 ± 5.31	33.63 ± 3.50
WPI+MD	1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6	96.40 ± 0.20	97.73 ± 0.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	24	94.57 ± 0.34	95.85 ± 0.36	96.60 ± 0.38	1.50 ± 0.40	1.26 ± 0.34	0.38 ± 0.06	0.39 ± 0.24	0.52 ± 0.21	0.16 ± 0.19

At 20 % (w/w) oil concentration, for WPI emulsions, there is a slight change as emulsifier ratio increases for 1 hour. As it can be understood from table, while there was no change observed between 1% and 2% WPI for 6 hours, as emulsifier ratio increases, creaming decreases in 6% percent. There is no significant change observed with 1 to 4% emulsifier change for 24 hours stability. When the total percent of creaming is considered, it decreased with increasing the oil amount as approximately 60 %. For MD improved samples, there was no phase separation observed for 1 h and 6 h. While a little bit creaming was observed for 24 hours, this value is decreasing as emulsifier ratio increases. Significant change can be seen between two cases approximately 30% for 24 hours stability.

When the oil concentration was doubled to 40%, stability was achieved for 1 h and 6 h samples. In addition, in WPI emulsions, there is still phase separation was observed more than 35%. This separation value was going down under 1 % with MD addition.

When the oil increment is evaluated, there is a dramatic fall between 5% oil samples with others in CI means for WPI emulsions. As Sun et.al. (2016) [36] explained before, this may sourced from inadequate amount of oil present in system for adsorbing the WPI to form a monolayer between aqueous phase and oil phase below 5 % oil concentration including 5% also. There is no sufficient data obtained from the experiment data points however adequate amount of oil for production of monolayer should be between 5-20 % (w/w) oil concentration

In addition, statistical evaluation of data was achieved in t-test which measures the differences between two cases and gives their significancy level. Results of test is similar with experimental data. There is a significant change between the cases with maltodextrin presence and its absence at system in 95% confidence interval (sig 0.005 < 0.05). Also according to test results, stability was improved 21% in CI means; t test differences between hours with respect to % CI. While the biggest difference is seen for 20% oil at and 2% WPI and 20% oil at 1% WPI, 20% oil at 4% WPI and 40% oil and 1% WPI follow them. There is a little difference seen for 40% oil at 2% WPI and 40 % oil at 4% WPI. Therefore, at 20% oil concentration, MD worked likely more than 40% oil included samples for 6 hours stability. When the 6-24 hours change is evaluated, 40% oil at 2% WPI and 40% oil and 2% WPI shows the biggest creaming improvements.

While 40% oil at 1% and 20% oil and 4%WPI are following them, the smallest improvements is seen for 20% oil at 1% and 2% WPI. As a result, between 6-24 hours, MD worked with 40% oil than 20% oil concentration.

Emulsion instability is the ability of emulsion resistance against physicochemical changes could occur as time passes [2, 3]. It was reported that the MD increases viscosity of aqueous phase so make system do more stable [20, 29]. Also, MD interact with fat and also emulsifiers's aliphatic part and increases the stability [77]. In emulsion systems which includes both polysaccharide and a surfactant, stability and strenght of emulsion is based on the interaction between them [20]. On the other hand, protein molecules create a protective film around emulsion particles by giving a series of reactions. During stirring or whipping, they are diffused and adsorbed by the newly forming water-oil interfaces due of their amphiphilic properties (possess hydrophobic and hydrophilic residues itself) [78]. After their adsorption, they partially unfold and interact between non aqueous medium of structure with their hydrophobic amino acid residues [79].

Improvement strategy of emulsion stability can be achieved by understanding the major driver psycochemical and chemical change mechanisms in system [1, 3, 20, 80] such as gravitational separation (creaming/sedimentation), flocculation, coalescence , partial coalescence, Ostwald ripening and phase inversion [3]. McClements (2005) [80] , expressed the flocculation breakdown of emulsions where two or more droplets come together in early stages by losing their individual presence. Also it was expressed the coalescence as an emulsion breakdown happens in later stages of storage is coming together of flocculated droplets merge into a single droplet.

In food formulations, proteins and polysaccharides both have major roles. Milk proteins like WPI is adsorbed by oil droplets as an excess layer and lowers the interfacial tention. As a result of this situation of WPI have contribution on emulsion stability due to steric and electrotatic repulsion interaction results. On the other hand, polysaccharides like maltodextrin are not adsorbed well by oil-water interface and due to its poor surface active properties. They generally contribute the stabilization effect by modifying the viscosity. If there is strong interaction between the protein and polysaccharide, this case promote stability of emulsion. Inversely, if the relationship between adsorbed polysaccharide and protein at

the droplet interface is low, this situation may lead destabilization by flocculation [20, 81-83].

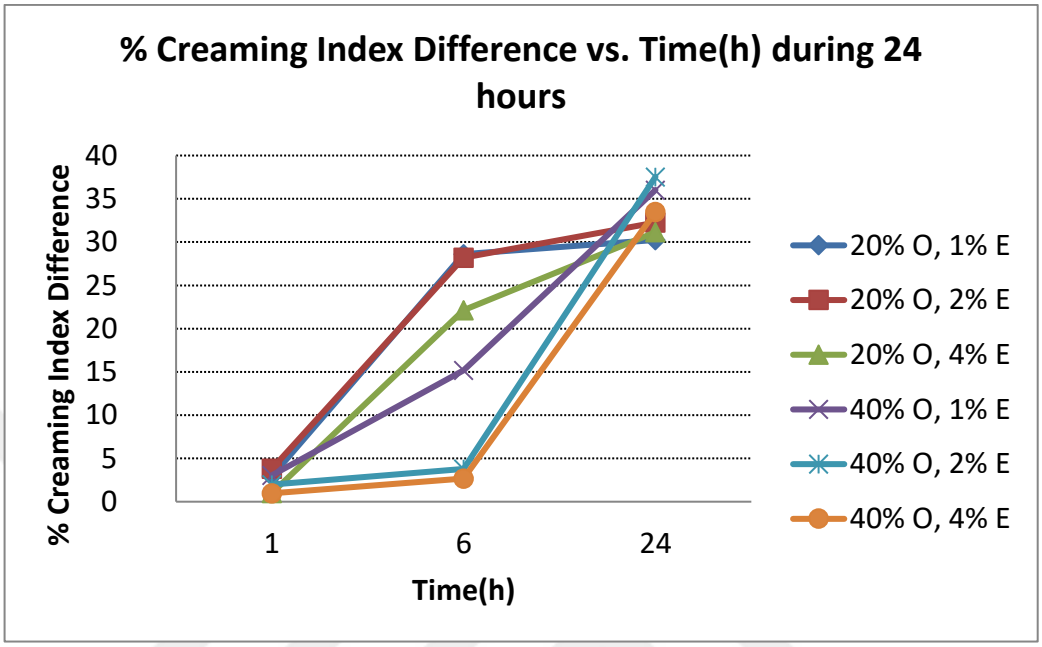

Figure 3.14. Creaming index difference between md presence and absence during 24 hours in percentage (O: oil concentration, E: emulsifier ratio)

According to figure 3.14., the stability improvements with maltodextrin addition can be evaluated as following. For 1-6 hours stability, 20% oil concentration seems that maltodextrin oil interaction is strong than than 40% oil concentration-matodextrin interaction between oil-water interface. However, when the 24 hours stability improvement percentages are evaluated, 40% oil concentration-maltodextrin conjugation seems stronger than 20% oil-maltodextrin interaction. Therefore, in this case, protein-polysaccharide interaction becomes stronger as time passes for 40% oil concentration samples. Also it can be said that the relationship between adsorbed maltodextrin and WPI at the droplet interface is getting lower as time passes and this situation causes destabilization of emulsions by flocculation.

In addition, while 2% and 1% WPI ratio gave the best result for 1-6 hours stability at 20% oil concentration, 2% and 4% WPI ratio was found best improved samples for 40% oil concentration for 6-24 hours stability due to the strong molecular interaction.

3.1.1.2. Particle Size

Droplet size is one of the main characteristics which gives information about emulsification efficiency of an emulsion system. It directly affects its rheology and sensory attributes [1]. Emulsions are divided and named as monodisperse (if droplets are uniformly distributed at the same size) and polydisperse (if various size of droplets are present in emulsion) [2]. Also, if an emulsion is polydisperse, its particle size is expressed as droplet size distribution and calculated by average droplet size (Z_{AVG}). In this experiment, droplet size measurements were achieved by Zetasizer ZS (Malvern Instruments) which uses DSL (Dynamic Light Scattering) principle while measuring droplet size (figure 3.15). This method measures the light scattering with a fixed angle 90 and its diffusion coefficients of particles into water phase. Samples were diluted in order to prevent results from multiscattering effect on vegetable droplets distributed in diluted water phase.

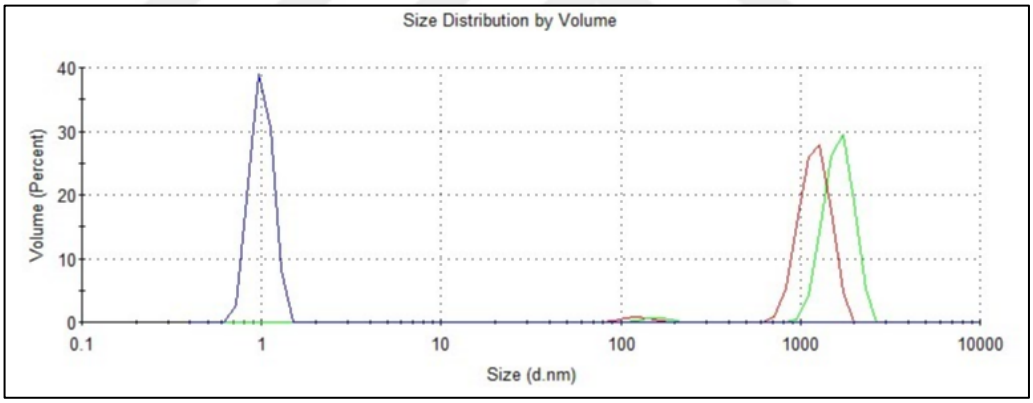

Figure 3.15. Droplet size distribution of a polydisperse emulsion

When without MD samples are considered, as oil concentration is increasing, droplet size is increasing proportionally for all emulsifier concentrations. At 5% WPI concentration, droplet size may be affected by emulsifier change from 2% to 4%. In addition, at 5% oil concentration, according to one factor ANOVA results, droplet size is significantly low (p=0.001 < 0.05) at 95% confidence interval. However, it can not be evaluated as reaching to stability because, it was understood that 5% oil concentration causes the WPI – oil droplet inefficiency between interface at creaming index part. Also according to statistical

evaluation, there is no significant change for emulsifier concentration increment (p=0.46 > 0.05).

When the MD added samples are considered, droplet size changes from 5% (around 2 μm) to 20% and 40% oil concentration (around 4 μm). It is supported with the one factor ANOVA results which droplet size change found significant (p=0.002 < 0.05). Also, droplet size does not change significantly (p=0.49 > 0.05) as emulsifier ratio increases. Therefore, it is not possible to say that as emulsifier ratio increases, droplet size decreases.

As an allover evaluation to data, droplet size was affected by decreasing from DE 12 maltodextrin addition to system especially for 20 % and 40 % oil concentrations. According to statistical evaluation, t-test results, droplet size changed significantly with 40% DE 12 MD addition to system at 95 % confidence interval (p = 0.005 < 0.05). It was affected by mainly oil concentration increment. Emulsifier ratio did not have main effect on system improvement. These results may suggest that when emulsions were produced without MD, coalescence was occurred at very early stages after preparation and average droplet size increased until measurement time which is 1 hour period after preparation. Also, coalescence tendency make emulsions more polydisperse with more different sizes oil droplets. Moreover, MD improved samples were found more stable compared to just WPI produced ones. This indicates that, MD increases the viscosity of aqueous phase and prevents oil droplets from coalescence and this results in comparably small oil droplets with smaller average droplet sizes. This situation is similar with L. Dokic- Baucal et. al. (2004) [25] ; they investigated the influence of different maltodextrins on O/W emulsions and found that the strong dependency of maltodextrin on droplet size. They suggested that low MD concentrations gave bigger droplet size than high concentrations and this leads instability means creaming. In addition, Drapala et.al. (2016) [84] was suggested that MD conjugates gave better stability outputs including droplet size.

Table 3.2 Analysis results of physical droplet characteristics for WPI and WPI + MD emulsions

C_{oil} (%)		5%			20%			40%		
C_e (%)		Visc.(cP)	D.size (μm)	Z. Pot. (mV)	Visc.(cP)	D.size (μm)	Z. Pot. (mV)	Visc.(cP)	D.size (μm)	Z. Pot. (mV)
WPI	1%	3.73 ± 0.19	2.81 ± 0.12	-37.31 ± 1.22	4.46 ± 0.14	6.20 ± 0.38	-52.63 ± 1.23	8.95 ± 0.65	6.92 ± 1.21	-59.33 ± 1.01
	2%	3.753 ± 0.04	2.51 ± 0.60	-35.16 ± 1.45	4.65 ± 0.12	6.027 ± 0.54	-50.22 ± 1.34	10.45 ± 0.62	5.69 ± 0.06	-55.22 ± 1.11
	4%	4.11 ± 0.07	1.74 ± 0.60	-43.42 ± 4.71	6.85 ± 0.04	7.95 ± 0.81	-56.24 ± 1.25	12.08 ± 0.58	7.43 ± 0.51	-48.94 ± 4.53
WPI+MD	1%	17.24 ± 0.68	2.58 ± 0.86	-45.33 ± 2.59	26.70 ± 0.71	4.37 ± 0.65	42.68 ± 0.66	77.09 ± 2.174	3.43 ± 0.56	-37.03 ± 1.42
	2%	17.88 ± 0.96	1.76 ± 0.15	-45.79 ± 4.18	28.77 ± 2.87	4.17 ± 0.22	-40.52 ± 2.43	85.32 ± 1.86	3.62 ± 0.74	-39.97 ± 1.26
	4%	21.20 ± 0.57	1.76 ± 0.15	-50.58 ± 4.45	34.68 ± 1.102	3.42 ± 0.29	-42.38 ± 0.96	111.28 ± 4.583	3.85 ± 0.82	-52.47 ± 1.13

3.1.1.3. Surface Charge

The surface charge of emulsions were measured with ZetaSizer ZS Nano Series (Malvern Instruments, Worcestershire, UK) by using DTS 1070 capillary cuvettes by entering proper parameters for vegetable oil [17]. DTS 1070 cells have two parallel plates that provides particles move in applied electric field supplied by Zetasizer. In that electric field, velocity and direction of particles are determined [21]. In addition, there is an energy barrier present should be altered in order to prevent particles collide which is +/- 30 mV suitable threshold accepted for producing more stable emulsions .

When oil droplets come together, they collide. In order to prevent this collision, the charge associated with the zeta potential should be high. This can be achieved by attractive and repulsive forces combination of following mechanisms [50].

There are two main mechanisms explaining the polymer and particle surface association as steric stabilization and electrostatic stabilization. When the macromolecules adsorb to the particle surface, steric stabilization occurs as a result of closing two particles to each other because of repulsive interaction. In addition, electrical stabilization occurs at two mutual particles' electrical double layer. As electrolyte concentration increases, electrical double layer between two such particles decreases and electrostatic repulsion also decreases. When two described mechanisms occur at the same time, electrostatic repulsion arises. Quantification of electrostatic repulsion is named as zeta potential [85]. Zeta potential of a particle can be measured as dissolving it in a polar medium in order to quantify its charge. In this experiment, another reason was that we diluted an amount of emulsion in water in order to avoid the multiple scattering effect.

For WPI emulsions (table 3.3, previous page) , at 5% oil concentration, when emulsifier concentration was increased from 1% to 2%, ZP was decreased and when it was increased from 2% to 4%, there is an increment for zeta potential from around -36 to -43.

When oil concentration rises from 5% to 20%, it was observed that ZP values are higher than 5% oil concentration. While it was decreased from 1% to 2% WPI, there it was increased from 2% to 4% WPI. In addition, ZP was observed at highest value at 40% oil concentration and 1% WPI. After that point, ZP is continuously decreasing through to 4% oil concentration. It can be said that at 40% oil concentration with 1% WPI concentration, adsorption of protein based emulsifier is maximum and this may lead to distinct oil droplets and low coalescence rate. For 40% oil concentration range, zeta potential has the highest

value at 1% emulsifier concentration with -59.33 and has the lowest value with -48.94 with 4% WPI concentration. Even at this point, droplet size is similar to 4% emulsifier concentration at same oil concent., this point was found more stable because of its high zeta potential.

It seems that, zeta potential increased when emulsifier concentration increased from 1% to 2% for all oil concentrations. There is just one exception for 4% emulsifier concentration at 40% oil concentration with continuously decreasing data parallel to decreasing emulsifier concentration. This exception is also parallel with a study mentions about fish oil-in-water emulsions which were came with a protein based emulsifier again. It was suggested that the decrease of zeta potential parallel to emulsion concentration decrease may be lead from the stretching of protein based emulsions over the surface. Also, at low concentrations, there are more charged groups related with polarity in water [21]. Therefore, adsorbed portion of WPI may resulted in well polarity and high ZP for this proportions.

For WPI + MD emulsions, at 5% oil concentration, ZP was increased from 2% to 4% WPI. While at 20% concentration, there is not a big difference between values, the lowest value of ZP was seen at 40% oil and 1% WPI. In addition, at 40% oil and 4% WPI. This may be lead from again adsorbed portion of WPI. When MD was added to system, it was observed that the adsorbed portion of WPI increased. As adsorbed proportion was increased, oil droplets was thought as separately and distinct distributed in emulsion system. This situation resulted in more polar position in dilution phase at experimental set up.

In order to evaluate the results statistically, t-test was applied to data series for different oil concentrations and emulsifier ratios assumed with equal variances. According to results, it was found that at 5 % and 20 % oil concentrations, emulsifier concentration has a significant effect on zeta potential with 0.02 and 0.01 *p*-values respectively. On the other hand, at 40% oil concentration, it does not have significant effect with $p = 0.056 > 0.05$. Moreover, at 1%, 2% and 4% emulsifier concentrations, zeta potential was not affected significantly from oil concentration changes with 0.15, 0.24 and 0.41 *p*-values respectively which are higher than 0.05 at 95 % confidence interval.

According to experimental data, between whole range; the highest and lowest ZPs were observed as -59 and -35 (5 % oil , 2 % WPI) mV respectively. Also, values were inversely proportional. Therefore, when ANOVA single factor test was applied to results in order to understand the MD addition effect on in general, MD addition was not found significant

factor on ZP change. However, when different concentrations were investigated, there are many significantly changed values in detail. Even though there are instabilities about the ZP, there is no value under +/- 30 mV suitable energy threshold which was accepted for producing more stable emulsions.

3.1.1.4. Rheology

Measurements were achieved by concentric cylinder which is suitable for viscous materials and provides highly sensitive data. Rheology of emulsions is important in food processing such as product development, sensory evaluation, consumer acceptability and also at quality control. There is another term flow behavior is very critical for engineering calculations, design and evacuation of food processing equipments, their handling and so on [27, 86]. The viscosity of materials are measured by applying force on them. The material responses as stress named as shear stress which is expressed as measure of deformation as a function of time. Measures of responses to forces are shown with the help of rheograms [50]. Emulsion systems behaves Newtonian at low oil concentrations. However, emulsion systems start to behave non Newtonian as oil droplet concentration increases.

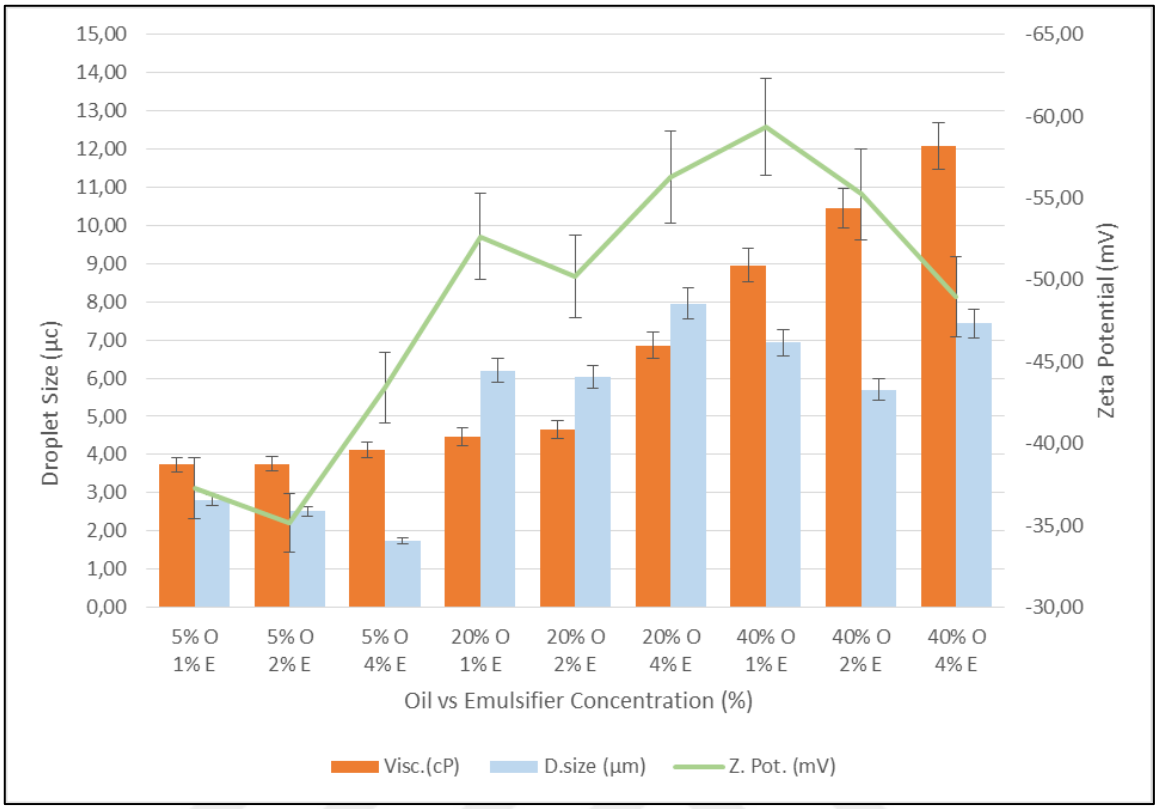

Figure 3.16. Viscosity (cP), d.size (µm) and zeta potential (mV) distribution for WPI samples

In this study, viscosity was obtained with the help of alternative flow curves; x-axis is shear rate and y-axis is shear stress. In figure 3.16., shear stress-shear rate flow curves of emulsions are shown; all of 5%, 20% and 40% oil emulsions show newtonian behaviour. Although there are noisy curves for WPI emulsions, they did not tend to show shear thinning behavior. It was suggested that O/W water emulsion systems show newtonian flow behavior under 57.14% v/v oil concentration [87, 88]. Also, Udomrati et.al (2013) [27] studied with DE 9, 12 and 15 MD values potato maltodextrin at 5% - 35% concentration range and suggested that the tapioca maltodextrin solutions shows newtonian flow behavior. Emulsions gain tendency to shear thinning flow behavior as coalescence increases. Since weakened attractive forces between larger oil droplets results in shear thinning flow behavior emulsions [89]. The noisy flow behaviours may show the coalescences and instable oil droplets dispersed in continuous phase. Addition of MD to system increases the viscosity of continuous phase and provides regularly disperse oil droplets by preventing them from

coalescence for longer time. This case can be shown clearly in figure 3.16.; while red curves show instability tendency, blue MD + WPI curves show high stability with high viscosity.

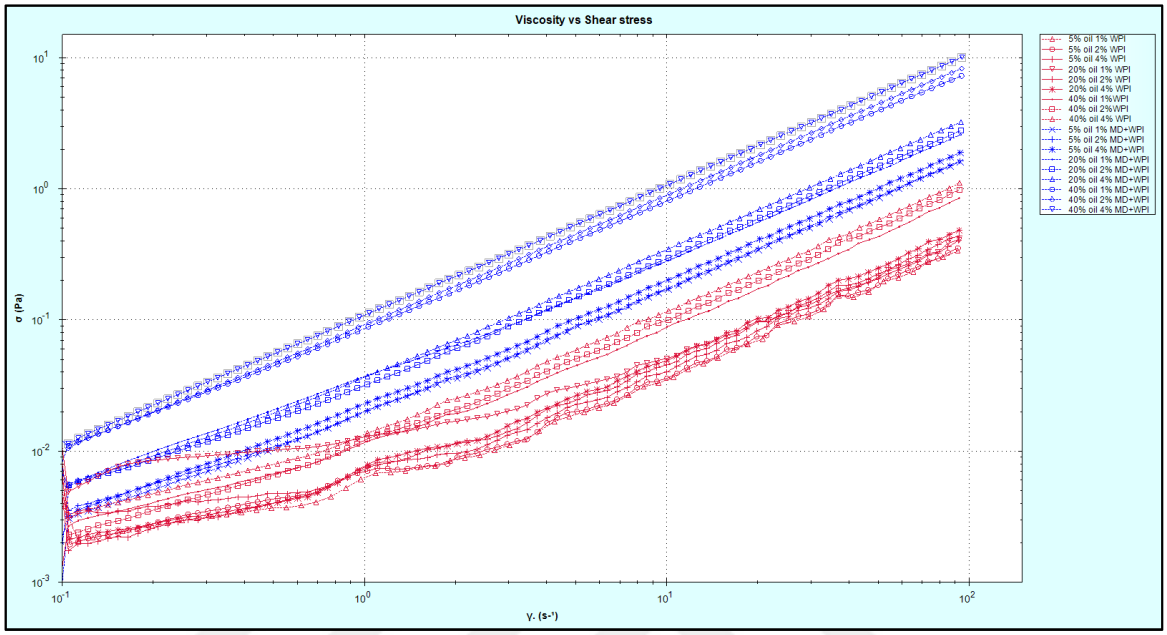

Figure 3.17. WPI (red) and WPI+MD (blue) flow curves of emulsions

Viscosity curves were obtained with alternative flow curve test by using concentric cylinder (cub and bob) probe 0.1–100 s^{-1} shear rate range. Obtained curves can be shown in Figure 3.17. According to experimental data, for WPI emulsions, while at 5% and 20% oil concentrations, there was no change dependent on emulsifier increase from 1% to 2%, there was a small difference with emulsifier concentration increase to 4%. At 40% oil concentration, viscosity increase was found directly proportional with emulsifier increment. The highest viscosities were found at 4% emulsifier concentration ; 4.11 ± 0.07, 6.75 ± 0.04, 12.08 ± 0.58 with respect to 5%, 20% and 40% oil concentrations. In addition, according to double factor ANOVA statistics, there is not significant change with emulsifier concentration change (p=0.09 > 0.05) at 95% confidence interval. However, oil concentration change has significant effect on viscosity change (p=0.0011 < 0.05). Statistical results proved the experimental data.

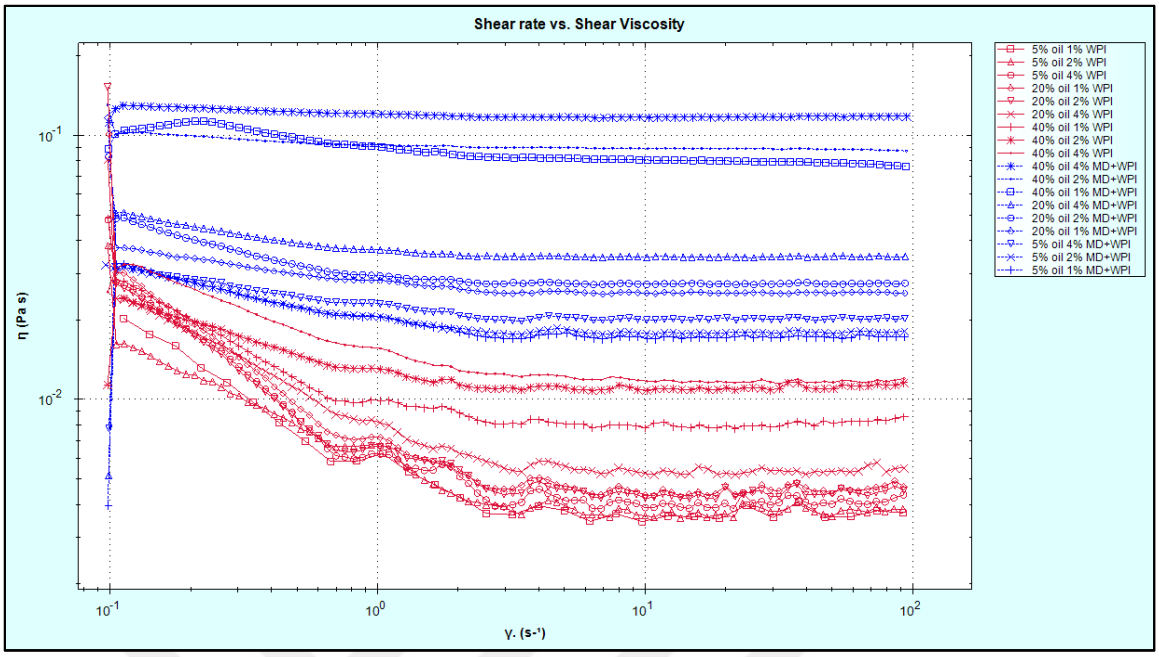

Figure 3.18. Shear rate vs. viscosity curves of WPI and MD+WPI emulsions

For WPI+MD emulsions, at 5 % and 20 % oil concentrations, there is no significant viscostiy change between 1 % and 2 % emulsifier concentrations. When emulsifier concentration increased to 4%, a small difference was observed. At 40% oil concentration, viscosity inreased directly proportional with emulsifier increase. The highest viscosity values reached 21.40 ± 0.57, 34.68 ± 1.10 and 111.28 ± 4.58 respectively for increasing oil concentration. Moreover, according to double factor ANOVA statistics, while viscosity did not change significantly with emulsifier addition to system while MD is present in aqueous phase (p=0.1852>0.05), its change is found significant with oil concentration change (p=0.00096<0.05) at 95% confidence interval. Statistical results proves experimental data.

In addition, according to t test results, viscosity change found significant with MD addition to system with 0.00013, 0.00035 and 0.00072 p-values (p<0.005) for 5%, 20% and 40% oil concentrations respectively.

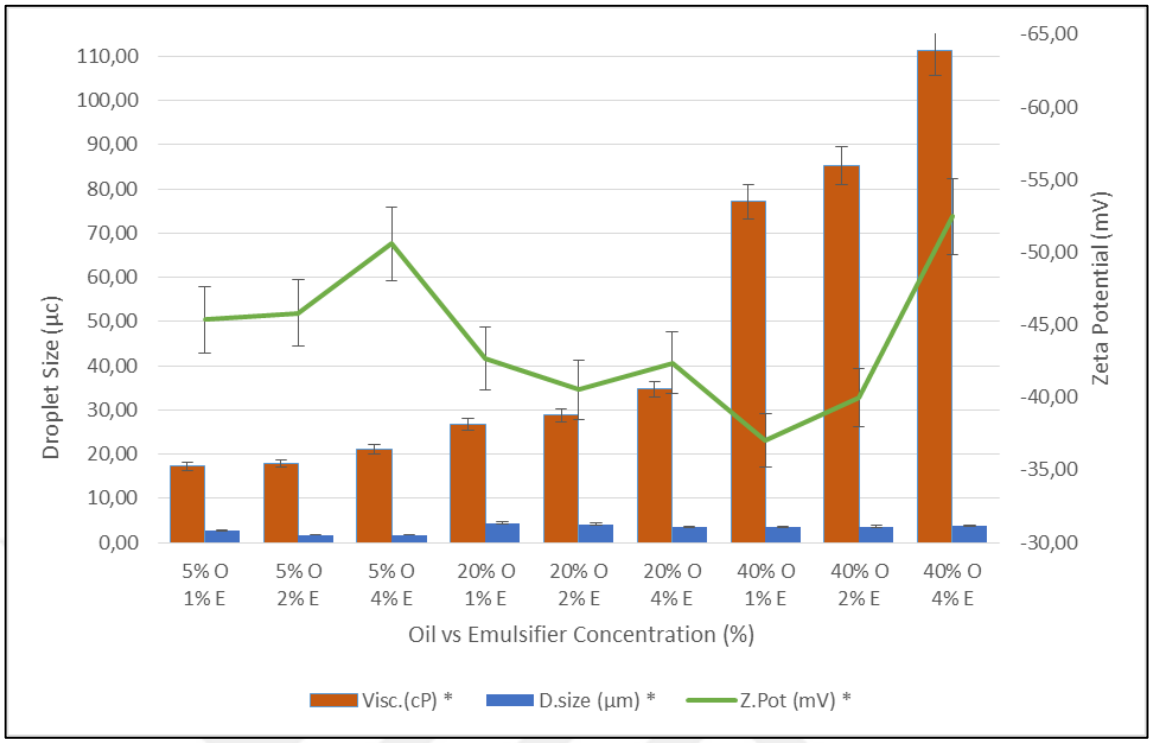

Figure 3.19. Viscosity (cP), d.size (µm) and zeta potential (mV) distribution for WPI+MD samples

It was found that, viscosity change is directly related to oil concentration rather than emulsifier concentration. When the aqueous phase of WPI O/W emulsions was improved with 40% v/v DE 12 MD, viscosity of system increases ten fold as it can be seen in Figure 3.16 and 3.17. Droplet size is not directly related with viscosity. It affects the flow behaviour of emulsions; when oil droplets come togetherthis was resulted in coalescence and flow curves were found noisy such as red WPI emulsion curves in Figure 3.16. According to flow curves, it was obviously proved that MD addition to system, made emulsions more physically stable and prevent them from coalescence by increasing the viscosity of continuous phase. It was also mentioned by Dickinson (2003) [20] and Klinkeson et.al (2004) [29] that maltodextrin increases the viscosity of continuous phase surrounding oil droplets. In addition, this was resulted in uniform distribution of oil droplets surrounded by WPI. This may resulted in lower attractive forces between oil droplets and better creaming stability, improved viscosity. In contrast, zeta potential was reduced at 20% and 40% oil concentrations. This shows us WPI polarity in water was reduced also and this may be caused by maltodextrin WPI interaction .

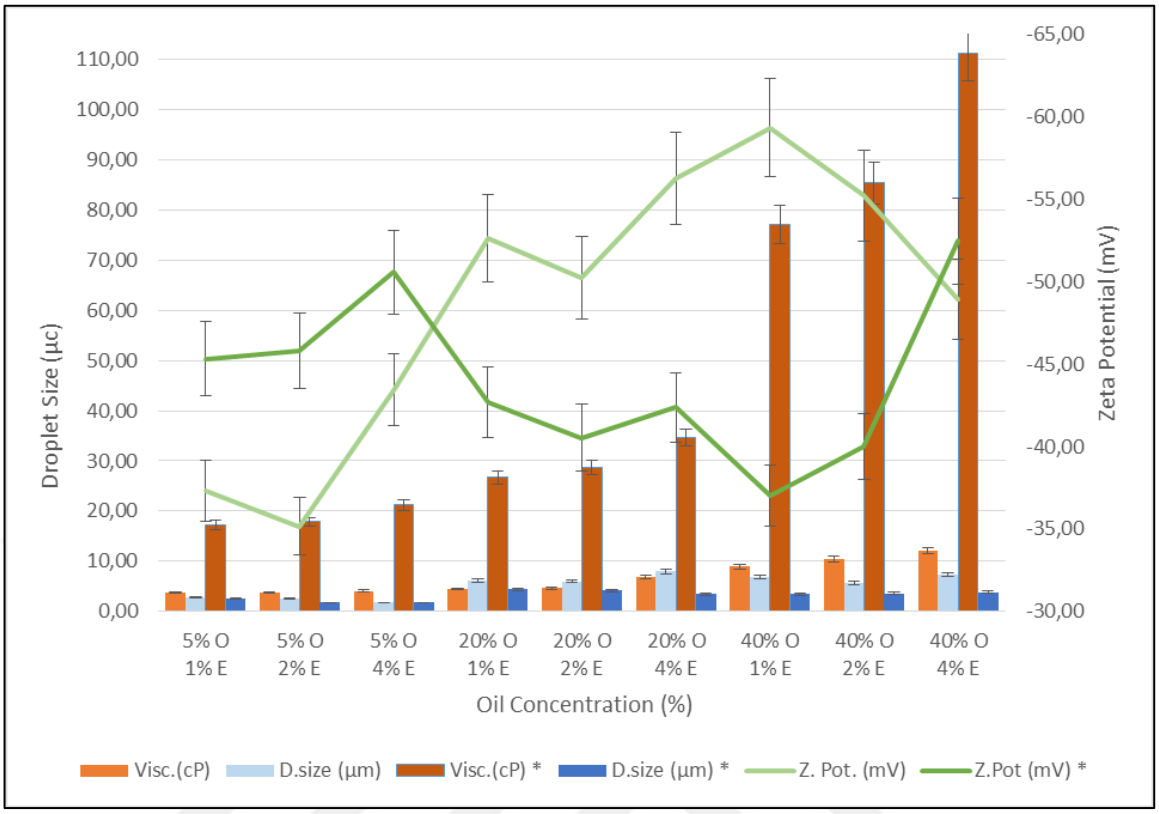

Figure 3.20.Viscosity (cP), d.size (µm) and zeta potential (mV) distribution combination for WPI and WPI + MD included samples together

In summary, it is considered that due to inefficient adsorption of WPI at 5% oil concentration, creaming values were not found as expected. However, MD addition to system obviously improved the samples; while 20% oil concentration kept its stable form for 6 hours, 40% oil samples were found to have better creaming status compared to whole range (more than 30%). In addition, MD as a polysaccharide structured stabilizator contribute creaming stability by increasing viscosity of aqueous phase up to 10 fold. Moreover, droplet size was achieved to be smaller by MD addition and its coalescence tendency was reduced by increasing the viscosity of aqueous phase. Furthermore, ZP of WPI + MD 20 % and 40 % emulsions' were found higher than WPI formulations. It was considered that the protein-emulsifier coated oil droplets have high polarity in water and MD addition may contribute to wider electrical double layer forming between oil droplets. According to statistical evaluations, creaming stability was changed significantly with MD addition ($p = 0.005 < 0.05$). While viscosity and droplet size were affected significantly with

oil concentration level ($p_{viscosity} = 0.0011 < 0.05$; $p_{droplet\text{-}oil} = 0.005 < 0.05$) , MD addition did not cause any significant differences on viscosity, droplet size and zeta potential.

3.2. MOST STABLE EMULSION FORMULATION

In food industry, generally formulated foods are in the form of a dispersed phase in an aqueous phase as a model. Surface active molecules adsorbed by oil water interface are used to keep phases together. In this part, emulsions prepared with most widely used food emulsifiers in food industry which are whey protein isolate (WPI) , sodium caseinate (SC), soy lecithin (Lec) and citric acid esters of mono and di glycerides (CITREM) will be presented in the means of physical stability parameters which are CI, droplet size, ZP and viscosity . Emulsifiers are generally divided by two groups as; low and high molecular weight emulsifiers (LMWEs and HMWEs). LMWEs are small surface active groups which have 10-20 hydrocarbone backbone with hydrophilic headgroup and hydrophobic tail group. HMWEs includes both hydrophobic and hydrophilic parts on its structure. When a hydrophobic part was located in oil droplet, other parts are located in aqueous phase [17]. CITREM is a water dispersible but poorly soluble low molecular surfactant and soluble anionic emulsifier. SC is easily dispersible and heat stable protein which has a number of functional properties includes formation of structure, water binding, emulsification, water binding, foaming and viscosity. As explained before, WPI is a surface active protein in globular shape which have the ability of adsorbed to oil droplets' surfaces and produces a single layer of molecules. Lec is a lipid soluble emulsifier which is generaly polar lipid mixtures (phospholipids, glycolipids, sphingolipids and residual triachylglycerols from different sources) [17, 59]. While CITREM and Lec are LMWEs, sodium caseinate and WPI are classified as HMWEs [17].

Oil droplet surface properties has a main role on emulsion stability. The concentration and type of emulsifiers directly affects the adsorbtion mechanism and adsorbed ratio of emulsifiers. Physical chemistry such as pH, ionic strenght, ions etc. controls the electrostatic charge and thickness of interfacial layer [17, 90].

From production to consumers' fork, foods containing oil are exposed many factors such as light, heat, acidity changes during processes and shelf life and they become prone to oxidation. Oxidation reaction and free radical formation causes undesired sensory properties

and also nutrient losses. This may lower the consumer demand to food. Although there are many technological and packaging solutions prevent food from factors may cause oxidation reactions in foods, there are also product development solutions are present.
In this part of study, four different types of common food emulsifiers will be compared in the means of physical stability in order to understand its physical chemistry which has a main role on oxidative stability mechanism of oil-in-water emulsions. After its physical chemistry was understood in the terms of CI, ZP, droplet size and viscosity, the most physically stable one will be treated against oxidation. Therefore, as it was mentioned above studies, these parameter has significant effects on lipid oxidation.

Many studies uses several buffers while preparing aqueous phase of oil-in-water emulsions, improvement of physical stability with DE 12 MD rather than buffer was previously discussed and decided to use stabilizer in situations which pressured homogenization is not possible to achieve. In addition, as it was mentioned in [62], understanding the oxidation kinetics with a wide range includes fruit beverages (<1% oil), sauces and even mayonnaise is very important. Therefore, model emulsions were prepared with 5, 20 and 40% (w/w) sunflower oil concentrations and 1, 2 and 4% (w/w) emulsifier concentrations for each emulsifier individually. Other factors ZP, viscosity and droplet size were measured in order to how these parameters affect and stability mechanism of emulsifiers behave at these oil and emulsifier concentrations in order to compare them in physically stability means [59, 60].

Data was obtained from emulsions prepared triplicate and also with tree parallel measurements. In addition, measurements were achieved in the following 1 hour after emulsion preparations in order to obtain accurate data protected from coalescence effect.

3.2.1. Physical Characterization of Emulsions

3.2.1.1. Visual Observation

Creaming index of emulsions were observed for 24 hours and 1, 6 and 24 hours creaming stabilities were measured. 24 hours stability of emulsions were evaluated. WPI was evaluated in previous part. It was found that oil concentration is more responsible of better creaming stability of emulsions rather than emulsifier concentration (Table 3.4 next page).

SC emulsions at 5% oil concentrationdid not keep stable form even 1 hour and at the end of 1 hour, CI stays same at around 95 %. At 20 % oil, while CI was increasing directly proportional as time passes, it was increasing as emulsifier concentration decreases. At this oil concentration range, the lowest value was observed for 4 % SC as 2.35 ± 0.40 at the end of 1 hour. On the other hand, the highest value was observed as 61.97 ± 12.31 for 24 hours stability. In addition, at 40% oil concentration, both 1% and 2% emulsifier concentrations, there were no creaming observed. 4% SC emulsions could not be achieved because of physical equipment insufficiency. According to statistical evaluation, time change has significant effect on CI (p=4.39 x 10^{-7} < 0.05).

When Lec emulsions with 5% oil were examined, while oil droplets were stable for 6 hours, layers were seperated from each other completely at the end of 24 hours independently from emulsifier concentration. When oil concentration was increased, better stability was achieved; while maximum creaming was observed at 1% Lec with 1.99 ± 0.20, it was slightly reduced as emulsifier concentration increases. Moreover 40% oil concentration emulsions kept their stable form more than 24 hours. Also in statistical evaluation, CI was affected significantly as time passes (p = 8.5 x 10^{-6} < 0.05) .

Creaming was observed at 1 hour in emulsions produced with CITREM and 5 % oil concentration,. While it was not observed for 20% oil emulsions at the end of 1 and 6 hours; at the end of 24 hours, 0.7 % creaming was observed. Increasing emulsifier concentration helped to achieve better stability at this oil range. At 40 % oil concentration, all the emulsions were observed stable. Also it was understood that, time was not a significant parameter for creaming stability of CITREM emulsions (p = 0.99 > 0.05).

Table 3.3. Creaming index measurements of WPI, SC, Lec and CITEM for 24 hours

Type	C_{oil} %	5			20			40		
	Ce % / time(h)	1	6	24	1	6	24	1	6	24
WPI	1	96.25 ± 0.22	94.26 ± 1.00	91.55 ± 1.42	2.96 ± 0.49	28.60 ± 3.37	31.74 ± 2.16	3.05 ± 0.59	15.19 ± 1.26	36.41 ± 1.23
	2	96.84 ± 0.60	95.02 ± 0.62	91.67 ± 0.17	3.81 ± 1.00	28.19 ± 2.66	33.51 ± 1.70	1.98 ± 0.70	3.77 ± 0.87	38.05 ± 5.31
	4	97.70 ± 0.59	93.69 ± 0.44	90.50 ± 0.83	1.00 ± 0.00	22.147 ± 2.22	31.53 ± 0.86	0.97 ± 0.39	2.68 ± 0.40	33.63 ± 3.50
SC	1	0.00 ± 0.00	80.60 ± 4.71	82.44 ± 2.64	11.53 ± 4.71	31.14 ± 17.50	61.97 ± 12.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2	0.00 ± 0.00	90.39 ± 2.29	85.81 ± 1.53	6.68 ± 2.69	42.31 ± 0.69	52.77 ± 2.48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	4	0.00 ± 0.00	98.30 ± 0.13	88.49 ± 2.56	2.35 ± 0.40	11.85 ± 0.50	41.06 ± 1.38	-	-	-
Lec	1	0.00 ± 0.00	0.00 ± 0.00	97.07 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	1.99 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2	0.00 ± 0.00	0.00 ± 0.00	98.46 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	1.29 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	4	0.00 ± 0.00	0.00 ± 0.00	97.95 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	1.24 ± 0.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CITREM	1	94.06 ± 1.68	94.06 ± 1.68	94.06 ± 1.68	0.00 ± 0.00	0.00 ± 0.00	0.71 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2	98.89 ± 0.49	98.89 ± 0.49	98.89 ± 0.49	0.00 ± 0.00	0.00 ± 0.00	0.76 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	4	98.62 ± 0.87	98.62 ± 0.87	98.62 ± 0.87	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

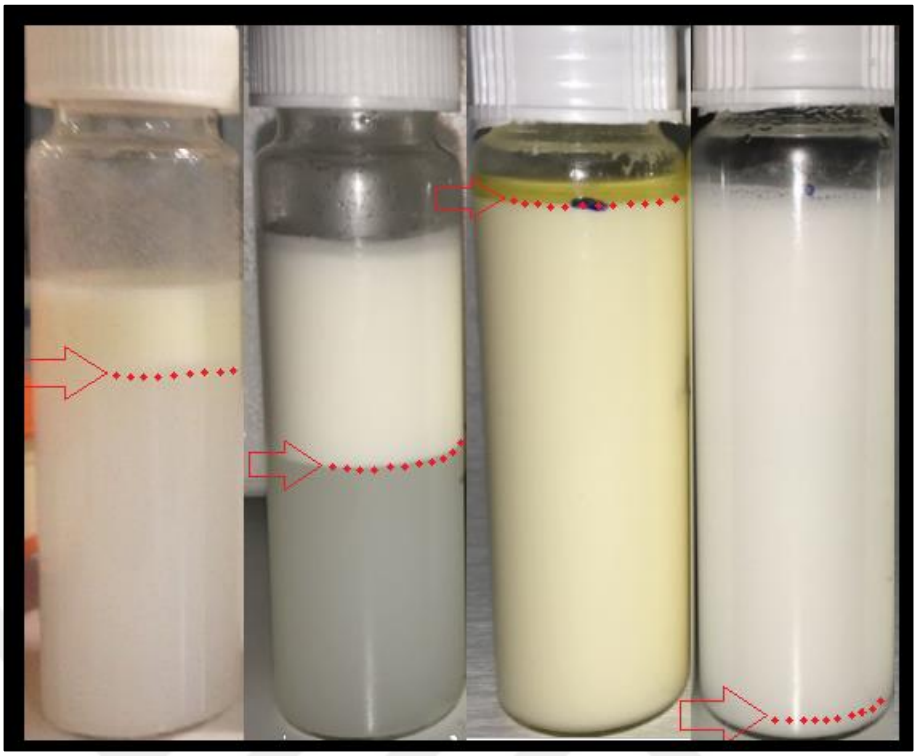

Figure 3.21. Creaming behaviour of WPI, SC, Lec, CITREM emulsions

When four different type of model emulsions' creaming behaviour was observed (figure 3.21); WPI and SC which are protein based emulsifiers form 2 distinct phases as cream layer and a turbid layer, Lec emulsions show oil release behaviour measured as creaming. In addition CITREM phase seperation is seen as slight phase seperation.

In order to choose the most stable oil and emulsifier concentrations and emulsifier type, CIs at 24 hours were compared. According to single factor ANOVA results, for all oil concentrations, emulsifier type was found as a significant factor affecting creaming stability ($p_{5\%\ oil} = 1.1 \times 10^{-4} < 0.05$; $p_{20\%\ oil} = 4{,}3 \times 10^{-6} < 0.05$; $p_{40\%\ oil} = 0{,}3 \times 10^{-2} < 0.05$). In addition, when intergroup averages belonged to emulsifiers at specific oil concentrations were calculated, lowest CIs between oil ranges were found as 86 for SC at 5% oil; 0.49 for CITREM at 20% oil and 0 for SC, Lec and CITREM at 40% oil concentration range. On the other hand, intergroups averages were calculated belonged to oil concentrations, they were found as 95.15 , 13.74 and 0.08. Therefore, oil concentrations can be arranged from most stable to less stable as 40 % > 20 % > 5 % respectively. It was understood that the most stable oil concentration was found as 40% oil with SC, Lec and CITREM emulsions,

which is in contrast to Fomuso et.al. (2002) [63] who compared lecithin, WPI, mono and di glycerols and sucrose fatty acid esters in term of stability and found the lecithin emulsions to be less stable.

3.2.1.2. Droplet Characteristics and Rheology of Different Formulations

Emulsion stability is not a term only related with creaming stability as mentioned before in Famuso et.al. (2002) [63] ; Osborn et.al. (2004) [62] ; Sorensen et al. (2010) [60]; Nielsen et.al (2013) [21]. Therefore, it was decided to measure viscosity, droplet size and zeta potential in order to further explain the changes occured between oil-water interface related to emulsion stability (Table 3.5).

Table 3.4. Analysis results of physical droplet characteristics for WPI, SC, Lec and CITREM emulsions

Type	C_{Oil} / C_{Emul} %	5			20			40		
		Visc.(cP)	D.size (µm)	Z. Pot. (mV)	Visc.(cP)	D.size (µm)	Z. Pot. (mV)	Visc.(cP)	D.size (µm)	Z. Pot. (mV)
WPI	1%	17.24 ± 0.68	2.58 ± 0.86	-45.33 ± 2.59	26.70 ± 0.71	4.37 ± 0.65	-42.68 ± 0.66	77.09 ± 2.174	3.43 ± 0.56	-37.03 ± 1.42
	2%	17.88 ± 0.96	1.76 ± 0.15	-45.79 ± 4.18	28.77 ± 2.87	4.17 ± 0.22	-40.52 ± 2.43	85.32 ± 1.86	3.62 ± 0.74	-39.97 ± 1.26
	4%	21.20 ± 0.57	1.76 ± 0.15	-50.58 ± 4.45	34.68 ± 1.102	3.42 ± 0.29	-42.38 ± 0.96	111.28 ± 4.583	3.85 ± 0.82	-52.47 ± 1.13
SC	1%	18.10 ± 0,68	1.82 ± 0.40	-53.40 ± 2.44	28.13 ±0.44	4.56 ± 0.75	-47.53 ± 2.19	94.90 ± 3.86	2.44 0.15	-41.12 ± 3.30
	2%	22.24 ± 1.20	1.20 ± 0.16	-55.21 ± 2.97	34.62 ± 5.51	4.71 ± 1.15	-50.92 ± 4.57	130.68 ± 5.56	4.40 ± 0.00	-42.70 ± 0.00
	4%	34.99 ± 1,75	0.71 ± 0.09	-55.38 ± 2.83	65.80 ± 0.00	4.58 ± 1.34	-64.57 ± 7.17	-	-	-
Lec	1%	17.06 ± 0.73	0.47 ± 0.05	-52.49 ± 6.11	26.07 ± 0.87	1.82 ± 0.11	-42.00 ± 2.70	64.50 ± 0.36	5.99 ± 0.82	-47.44 ± 6.27
	2%	18.34 ± 0.13	0.37 ± 0.04	-53.62 ± 2,34	30.06 ± 1.37	2.00 ± 0.51	-42.52 ± 4.59	86.76 ± 1.01	3.30 ± 1.45	-41.89 ± 5.69
	4%	21.45 ± 0.67	0.31 ± 0.04	-45.43 ± 5.28	37.91 ± 1.16	2.51 ± 0.56	-46.88 ± 0.99	113.78 ± 3.66	4.84 ± 0.82	-35,99 ± 3.88
CITREM	1%	17.08 ± 0.64	3.34 ± 1.02	-45.62 ± 1.24	31.16 ± 1.91	0.97 ± 0.07	-48.81 ± 1.27	66.94 ± 0.72	4.48 ± 0.50	-48.68 ± 0.35
	2%	20.35 ± 1.97	0.26 ± 0.08	-48.56 ± 1.52	31.72 ± 1.02	0.64 ± 0.03	-40.77 ± 1.89	89.39 ± 3.40	2.49 ± 0.08	-37.73 ± 1.59
	4%	29.30 ± 3.35	0.34 ± 0.08	-51.39 ± 1.32	42.60 ± 0.87	0.55 ± 0.06	-43.42 ± 1.38	138.49 ± 3.83	1.96 ± 0.08	-41.22 ± 2.49

3.2.1.2.1. Sodium Caseinate Emulsions

In SC emulsions, viscosity showed an increasing proportional to emulsifier concentration for all oil concentrations (figure 3.22).

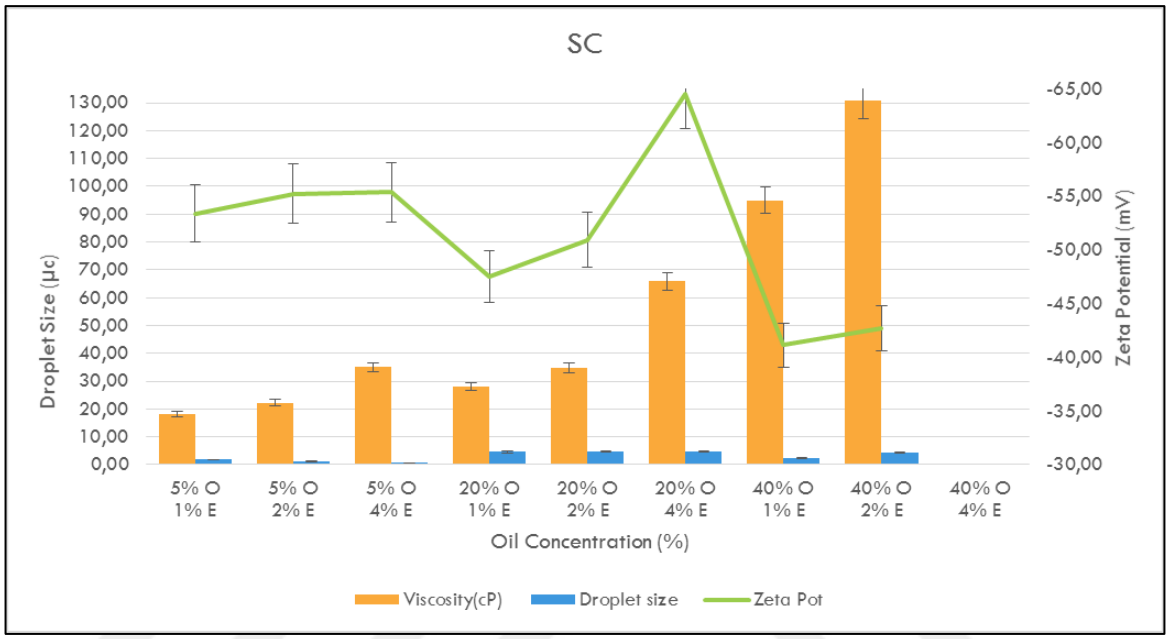

Figure 3.22. Viscosity, droplet size and zeta potential of SC emulsions

Between these oil ranges, viscosity has increased significantly with 0.02 *p*-value. However, emulsifier concentration did not affect viscosity significantly with 0.26 *p*-value > 0.05. While droplet size decreased for 5% oil concentration, it did not change for 20 % oil concentration and it increased for 40% oil concentration. According to statistical evaluation, droplet size was affected significantly while oil concentration increases 20 % from 5 %. In addition, while zeta potential did not changed for 5% and 40% oil concentrations, it increased for 20 % oil as emulsifier concentration increases. Statistical results showed that Zp has significantly affected from oil change for 1 % and 2 % emulsifier concentrations (p= 0,0006 < 0.05). With 20 % oil concentration, while zeta potential increased as emulsifier concentration increases and also directly proportional to viscosity change. This shows that, oil droplets was not saturated enough at 1 % SC concentration. As it increases at medium, since its polarity is higher than whole emulsions, its zeta potential was measured highest with 64.57 ± 7.17 without droplet size change. Hu et.al. (2016) [5] who studied with WPI,

SC and SPI, suggested that the increasing protein concentration has no effect on zeta potential. Zeta potential indicates the saturated droplet surfaces. In contrast, for 20 % oil concentration, while zeta potential increased as emulsifier concentration increases and also directly proportional to viscosity change. It indicates that there were still unsaturated areas on oil droplets at this oil concentration. Therefore, the high zeta potential indicates that better polarity of SC in water. Also it as metioned in Nielsen et.al. (2013) [21] as at low concentrations, there are more charged groups related with polarity in water. Therefore, adsorbed portion of SC may resulted in well polarity and high ZP.

3.2.1.2.2. Lecithin Emulsions

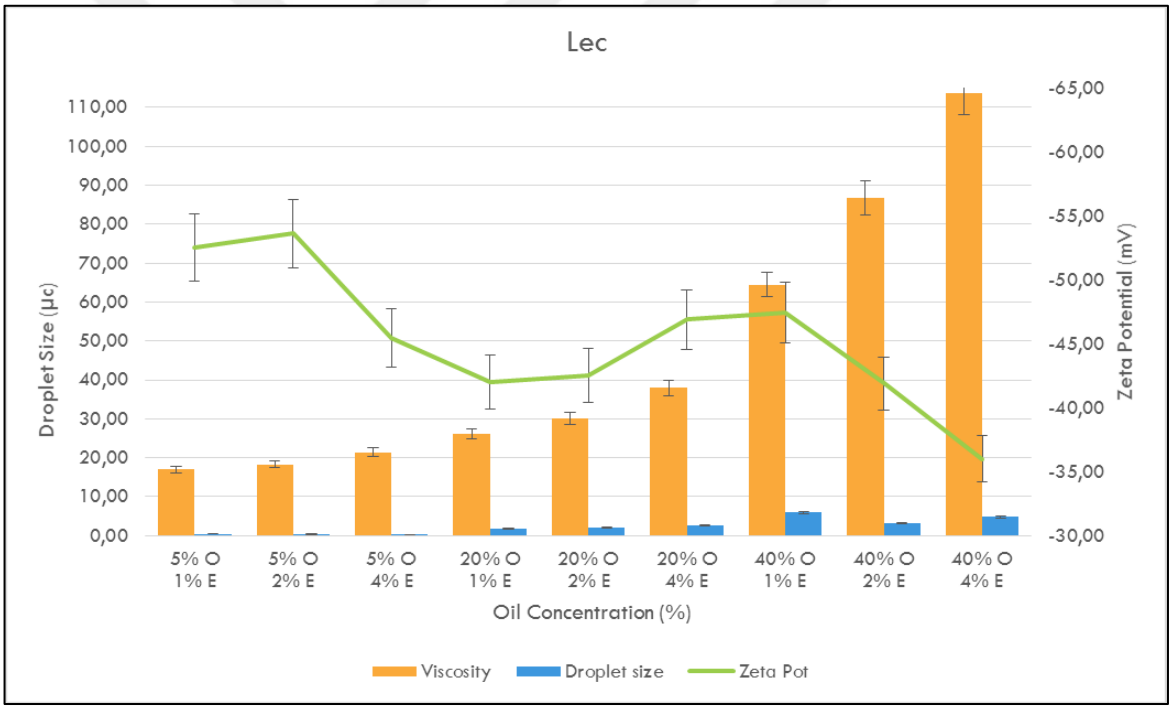

Figure 3.23. Viscosity, droplet size and zeta potential of Lec emulsions

In Lec emulsions, viscosity showed an increasing trend proportional with emulsifier concentration for all oil concentrations (figure 3.23). Statistical evaluations showed that viscosity changed significantly as oil proportion increases with p-value 0.002 < 0.05 and it was not affected significantly from emulsifier change (p=0.46 > 0.05). In addition, while at 5% oil concentration range; the lowest droplet size concentrations even under 1 µm were

observed, as oil proportion increases, these values also increase. Droplet size was not affected significantly by emulsifier concentration increase (p=0.08 < 0.05). Moreover, zeta potential is in a varying behaviour however it can be said that it decreased at 40% oil concentration as emulsifier ratio increased.

3.2.1.2.3. CITREM Emulsions

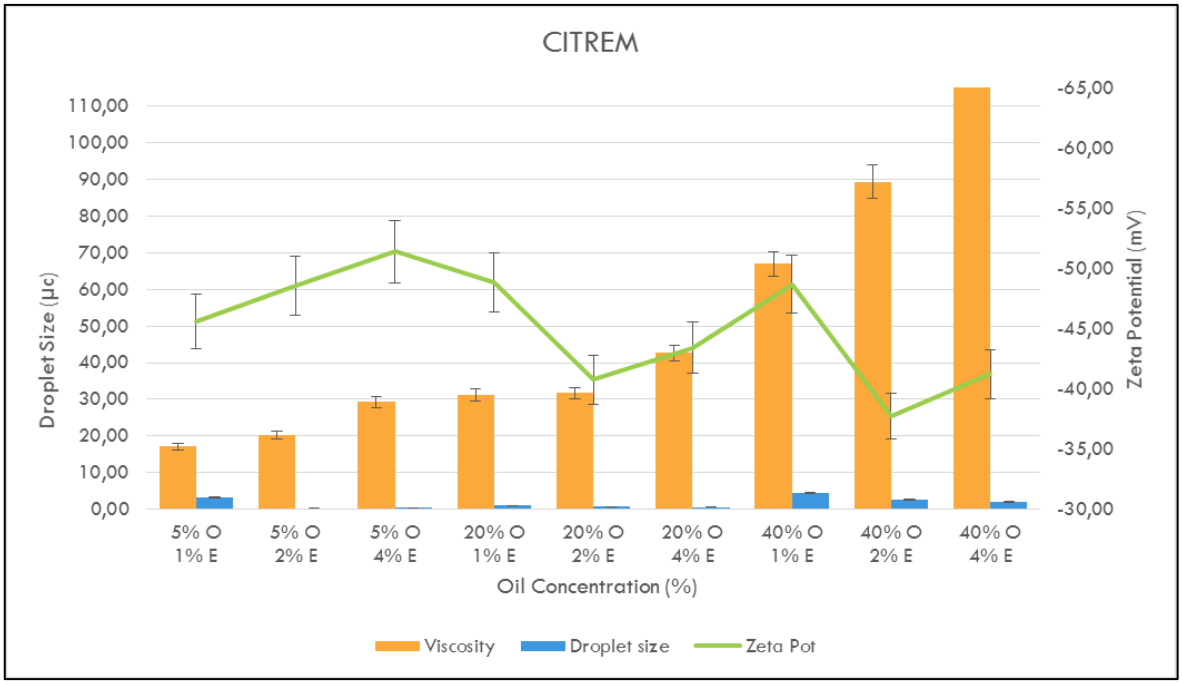

Figure 3.24. Viscosity, droplet size and zeta potential of CITREM emulsions

In CITREM emulsions, viscosity is increasing as oil and emulsifier concentrations increased proportionaly with them (figure 3.24). According to statistical evaluations, viscosity changed significantly related to oil concentrations (p=0.012 <0.05) but its change with emulsifier concentration is not significant (p=0.18 < 0.05). For 5% oil concentration range, droplet size increases about 2 µm when emulsifier concentration increses from 2% and 4 %. For all oil ranges, as emulsifier concentration increases, droplet sizes got smaller however, its change was not significant with p-value 0.07. Also, oil change did not affect droplet size significantly (p=0.057 > 0.05). Zeta potential showed varying trend again, it is hard to relate it with a parameter.

3.2.1.3.Comparison of Physical Characteristics

Viscosity values of emulsifiers were compared in figure 3.25. It increased generally for all formulations. While the highest value was observed for CITREM, it was predicted to be seen in SC 40% oil and 4% emulsifier concentrations, if this formulation would have been achieved. Even its 2% emulsifier concentration has close value with currently most viscous formulation which is 40% oil and 4% emulsifier concentration as 138.49 ± 3.83 cP. Viscosity shows an increasing trend proportional to increasing oil and both protein and oil based emulsifier concentrations. In contrast to these observable differences between them, emulsifier type is not a significant parameter affecting emulsion viscosity for whole ranges (p=0.96 < 0.05). It was suggested that oil concentration influenced the viscosity directly. When oil ranges were evaluated one by one, emulsifier increase was found as a significant factor on viscosity with 0.01, 0.03 and 0.02 p-values respectively for oil concentrations.

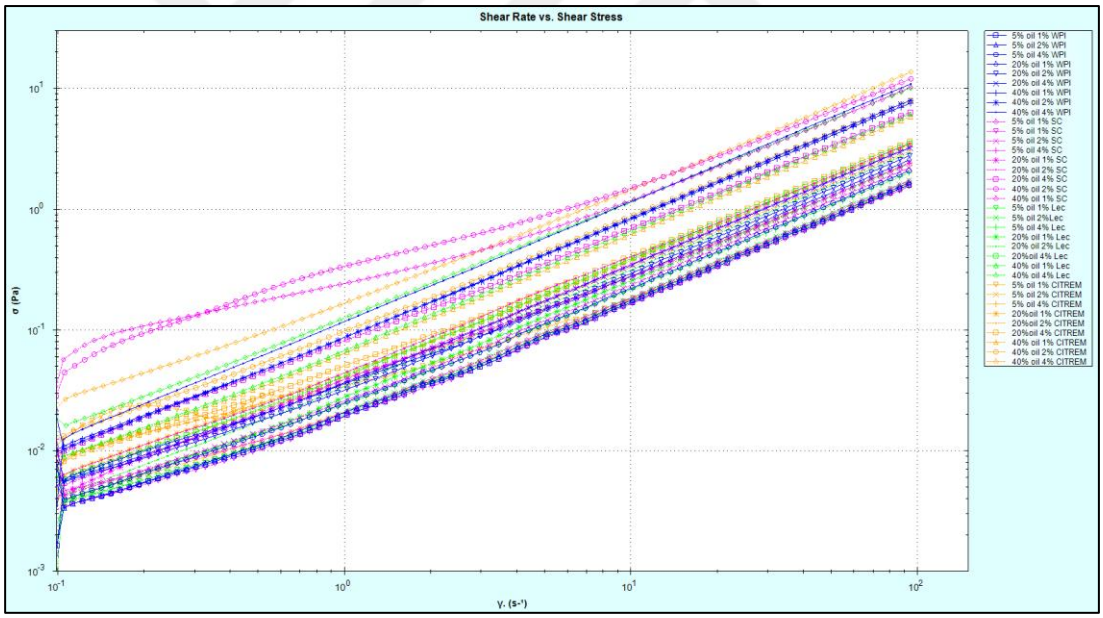

Figure 3.25. Flow behaviour curves of WPI (blue), SC (pink), Lec (green), CITREM (orange) O/W emulsions

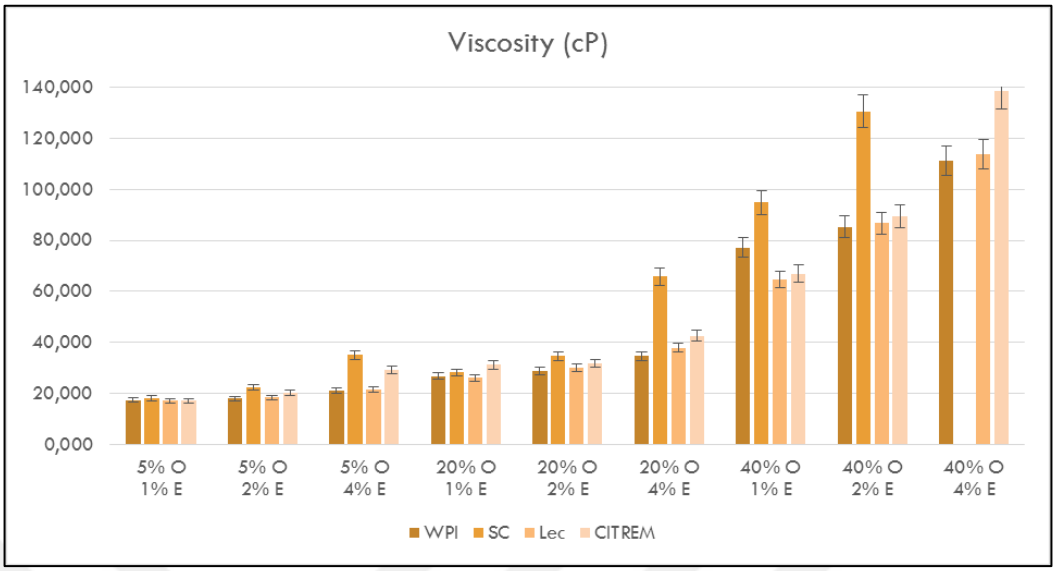

Figure 3.26. Comparison of viscosity of 4 different emulsifiers' emulsions

When the flow curves were evaluated, all formulations behaved Newtonian (Figure 3.26). According to curves, while 1 % and 2 % SC and 4 % CITREM with 40 % oil-in-water emulsions showed shear thinning behaviour when pressure first applied on them 10^{-1}-10^{0} s^{-1} range, their behaviour was converted themselves as pressure increases.

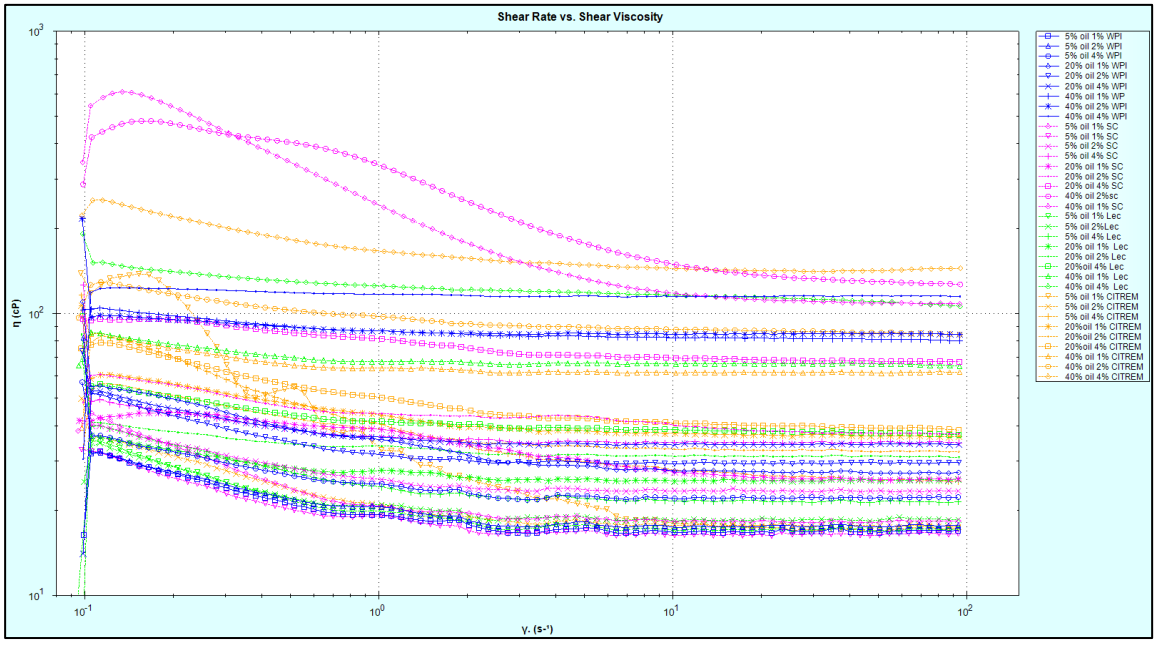

Figure 3.27. Viscosity curves of WPI (blue), SC (pink), Lec (green), CITREM (orange) O/W emulsions

It was explained before that the viscosity change depends on oil portion of emulsion. When viscosity curves are evaluated (Figure 3.27), it was obtained noisy viscosity curves for lowest oil concentrations independent from emulsifier concentration. Although they are noisy, they have a long smooth region at the end to obtain viscosity. Also, it is clearly shown that as oil concentration increases, curves were getting smoother.

According to figure 3.28. ; among 5 % oil included samples, Lec and CITREM was shown to have the smallest oil droplets. Especially Lec emulsions' droplet size was found under 0.5 µm. In this range, 1% Lec was an exception with around 3 µm droplet size. This may have resulted from inefficient bonding between oil and water interface. Moreover, in this range, generally as emulsifier concentration increases, oil dropletsgot smaller. In addition, for 20% oil concentration range, CITREM again resulted in the smallest oil droplets. WPI and SC showed almost same droplet sizes independent from emulsifier concentration. Droplet size decrease was just achieved with emulsifier increase for CITREM in this range. Also, at 40% oil concentration range, protein based emulsifier formulations' droplet size was found around 3µm. The smallest oil droplets were obtained for 4% CITREM with 1.96 ± 0.08 µm.

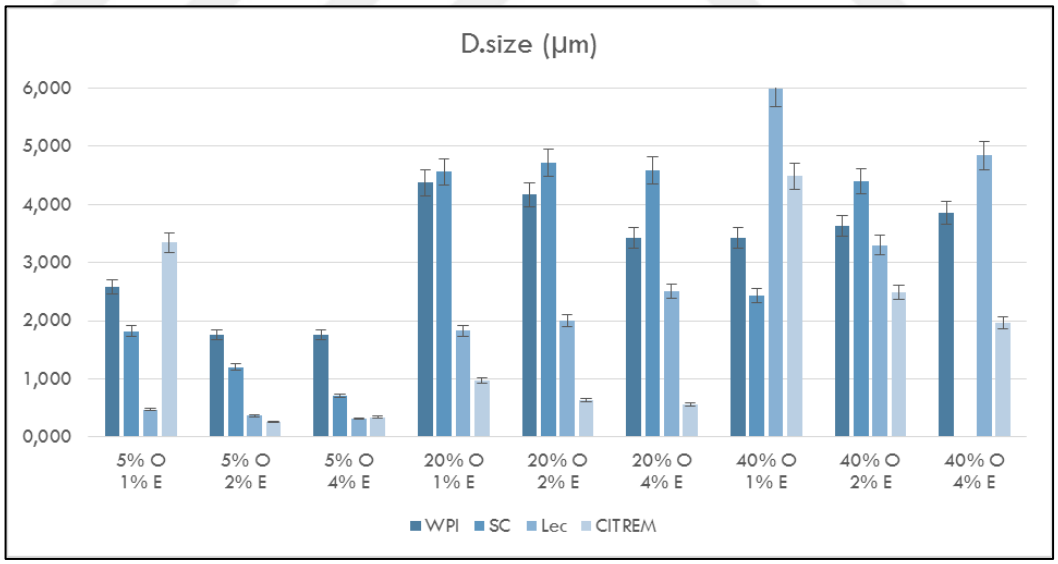

Figure 3.28. Comparison of droplet size of 4 different emulsifiers' emulsions

Among the whole range, while the smallest droplet size was obtained from 2% oil based emulsifier CITREM with 20 % oil concentration formulation, the largest droplets were obtained from 1% oil based emulsifier Lec with 40 % oil formulation. This indicates that the oil droplet size is not related with oil or protein based emulsifiers. Also, WPI as a protein based emulsifier formed around 3μm oil droplets independent from oil or emulsifier concentrations. However it can be said that CITREM can make effective bonds with its hydrophilic tails between oil-aqueous phase interface as a LMWE dissolved in oil portion of emulsion prior to emulsification.

According to statistical evaluation, emulsifier type did not affecte droplet size significantly (p=0.17 > 0.05). Intergroup averages was calculated; while CITREM was found the smallest oil droplets forming emulsifier with 1.67 μm, the larger oil droplets forming emulsifier was calculated as SC with 3.21 μm. In addition, emulsifiers dependent on oil droplets averages can be ordered as WPI > SC > Lec > CITREM. However, change in oil concentration affected droplet size significantly (p = 0.00014 < 0.05). As it can be seen in figure 3.28, while the low oil concentration increases, oil droplet sizes changed relative with it.

Among 5 % oil range, the highest ZP values were found for SC and Lec and it does not dependent on emulsifier concentration change directly (figure 3.28). When 20% oil was used, SC emulsions were observed as to have a ZP increasing proportional to emulsifier concentration while other emulsions' ZP are varying. Between this range, the highest value was observed for SC with 4% emulsifier concentration. At 40% oil range, the ZP of WPI emulsions were increasing as emulsifier concentration increased. Moreover, Lec emulsions showed a decreasing trend with increasing emulsifier concentration. Here, the highest ZP was shown for 4% WPI emulsions. When whole range was considered, the highest value was observed for 4% SC and 20% oil with -64.57 ± 7.17; the lowest values were observed for 40% oil with 1% WPI and 40% oil 4% Lec emulsions with -37.03 ± 1.42 and -37.73 ± 1.59 respectively. According to statistical evaluation, it was found that ZP was not affected significantly with emulsifier type change (p=0.06 > 0.05). When the intergroups averages were calculated, the highest was found with -51.35 mV and Lec followed it with -45.36 mV. This indicated that zeta potential is not directly related with oil or protein based emulsifier usage. Furthermore, emulsifier type and concentration change was not found as significant. Also, it was proven that the oil concentration change affected ZP significantly (p = 0.002 <

0.05). However, all values were observed out of -30 and +30 range. Therefore, all the ZP can be classified as stable and can be used in food emulsions effectively protective to coalescence effect. In contrast to Haahr et.al (2008) [59], CITREM's zeta potential values were rarely found higher than SC emulsions.

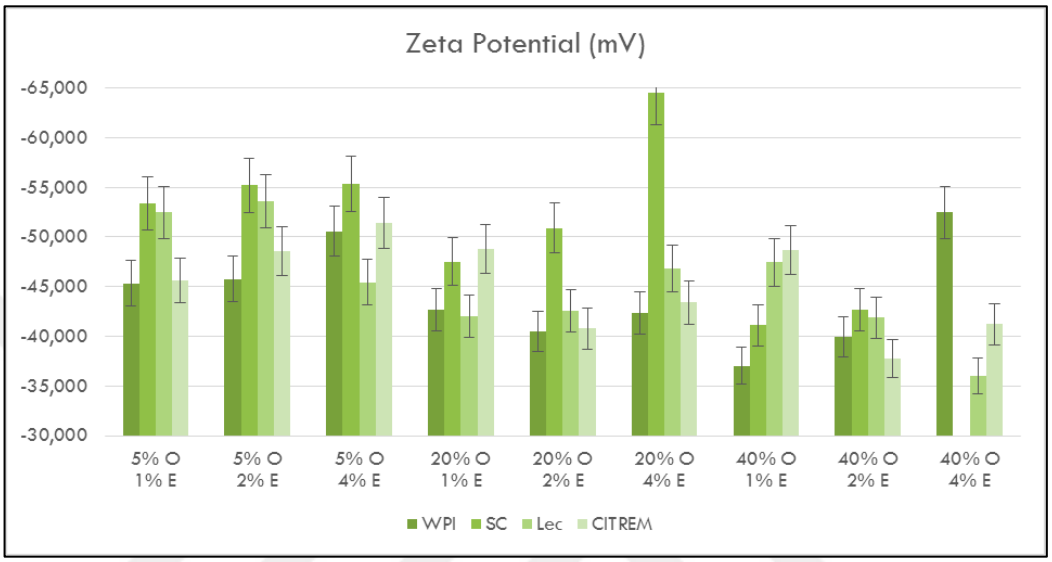

Figure 3.29. Comparison of zeta potential of 4 different emulsifiers' emulsions

In general, as oil concentration increased in formulation, vicosity and droplet size also increased. It indicates that oil droplet size is dependent on homogenization and effective bonding. It can be seen that when emulsifier is oil based, its adsorbtion between oil-water interface is relatively high and it leads to more bonding relative to oil droplet size (CITREM). Moreover, there were noisy viscosity curves observed for low emulsifier concentrations and oil concentrations. This may have sourced from inefficient bonding because of low oil concentration or much emulsifier relative to oil concentration. Also it was seen that varying droplet size did not affect the flow behaviour of formulations. Furthermore, while the highest ZP were observed for SC, lowest values were observed for WPI and Lec. This may be because polarity in water changes with effective bonding at specific oil and emulsifier concentrations. Emulsifier concentrations may be set according to oil concentration of a product. For ex. if a 20 % oil product are trying to be formulated, 4 % will give better stability.

When overall ranges were evaluated, 40% oil range was found to be the most stable. However, it was hard to say physically most stable formulation in order to improve its

oxidative stability. Therefore, 20 % oil range was evaluated in the means of both creaming stability and droplet size. 4 % CITREM emulsions was found to have lowest droplet size. It was mentioned in Fomuso et. al (2002) [63] as droplet size decreases, droplets' surface area increases. More surface area of small droplets increases the tendecy of lipid oxidation rather than large droplets. It was also known that oxidative stability was strongly affected by emulsifier type [59]. In Haahr's study, oxidative stability was found in decreasing order CITREM > Lec > SC. Moreover, there are a few papers evaluated protein stabilized emulsions' better oxidative stability than oil based emulsions and increasing protein portion leads less oxidized systems [63, 64].

According to aim of this study, most physically stable emulsion was aimed to be examined with addition of BCD as a protective against lipid oxidation biological material. Suprisingly, lowest creaming index was observed for CITREM and the lowest droplet size was measured for 4 % CITREM at 20 % oil concentration. Also it was reported as the less oxidative stable one [59]. Therefore, CITREM was determined to be examined in changes of lipid oxidation by using it as a blend such as some other studies present in literature [60, 61].

3.3. PHYSICAL AND CHEMICAL STABILITY OF BLEND OF BETA CYCODEXTRIN-CITREM

In the previous part, the most stable and oxidation tended formulation was selected as 20% oil and 4% CITREM in order to understand its physical and oxidative stability. It was used as blend with BCD-CITREM and its physical and oxidative stability was evaluated. This study can be seen as a novelty to current studies. Here, as a LMWE ; CITREM's emulsification ability was investigated with combination of inclusion complex forming BCD with a commonly used sunflower oil in food industry. Its physical stability and chemical stability was evaluated as well.

In food industry, fridge conditions (4ºC) and supermarket (21ºC) conditions are very crucial for food product quality. Also, while exporting emulsion based food products, they are exposed to temperatures up to 55ºC. However, there are very rare studies which evaluate creaming stability of CITREM and BCD emulsions at 4, 21 and 55º C. In present study, this conditions were evaluated for the first time in means of creaming stability which indicates physical quality of food products.

Emulsions were prepared by using individually CITREM and 1:1 CITREM + BCD mixture to achieve 4% emulsifier concentration and 20% oil concentration in final product. Besides creaming stability, viscosity, zeta potential and droplet size measurements were achieved in order to have detailed information about physical structure and to determine the oxidation induced parameters. In order to understand its oxidation behaviour, the emulsions were exposed to 55º C for 15 days at. Moreover, while, CI measurement was not achieved during heat stability test, creaming stability was also investigated on 21º C and 4º C samples at the same time.

Moreover, light is a very crucial parameter that causes lipid oxidation. Wide range of food products have transparent packages such as beverages, some mayonnaises, milk product, salad dressings and many other products. In order to understand how light affects these emulsions, Suntest XLS (Atlas) Climateric Conditions Test Equipment was used for 1 stronger sunlight cycle which is equal to exposure of direct sunlight for 10 days as another novelty. At the end of the cycle, creaming stability of emulsions were measured. Also, their oxidative stability was investigated.

3.3.1. Physical Characterization

3.3.1.1. Visual Observation

When figure 3.30 and table 3.5. was considered, generally CIs are increasing depend on temperature change until 6 days and they stayed almost the same for rest of storage days. It was clearly seen that BCD affected creaming stability in a positive way. Especially at 4º C, emulsions could keep their stable form until 12 days (figure 3.31). At 21º C, creaming was improved around 65 %. There is a sudden stability change observed for 55ºC samples without BCD. This may have caused from homogenization error while preparation. Most stable conditions were observed for fridge conditions with BCD addition and room conditions with BCD addition followed it. While room conditions without BCD has the highest creaming stability, 55º C without BCD could not be evaluated. In addition, creaming stability was not affected by light exposure. At the end of 1 cycle (24 hours), while CITREM emulsions seperated similar to 1 day stability results of without BCD samples with room temperature and fridge conditions without BCD addition.

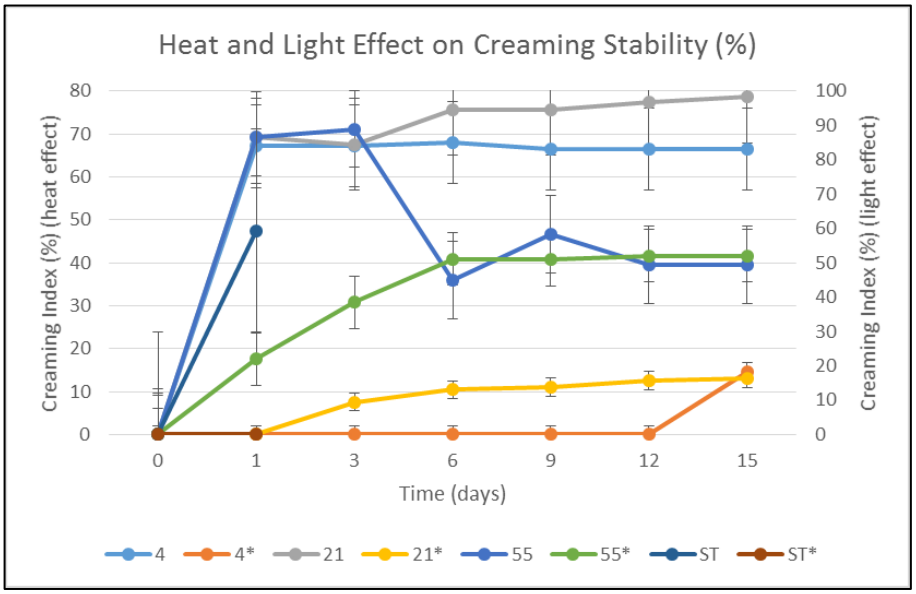

Figure 3.30. Heat and light effect on creaming stability at 4, 21 and 55º C (* indicates the BCD added samples)

Changes with BCD addition was evaluated by t-test. Statistical evaluation results are parallel with experimental results (Table 3.6). While BCD addition affected creaming stability of fridge and room conditioned samples significantly (p_{4^oC} =5.38 x 10^{-5}; p_{21^oC} =1.3 x 10^{-4}), at 55ºC and light conditions samples were not affected significantly (p_{light} = 0.21; p_{55^oC} = 0.13). Also, results are similar with Cheong.et.al. (2016) [72] ; with presence of BCD resulted in more stable emulsions. It indicated that synergictic effect occured between BCD-CITREM or BCD-MD.

Figure 3.31. Samples at fridge conditions after 15 days

Furthermore, temperature change for similar type of emulsions were analyzed with ANOVA. According to results, stability of samples without BCD was not affected from temperature change significantly (p = 0.34 > 0.05). However, temperature change affected BCD+CITREM samples significantly (p= 1.64 x 10^{-4}).

Table 3.5. Creaming index measurements of CITREM and CITREM + BCD emulsions stored at 4, 21, 55ºC and direct sunlight

Temp.(ºC)	4		21		55		ST (=10 days direct sunlight)	
Time(days)	CITREM	CITREM+BCD	CITREM	CITREM+BCD	CITREM	CITREM+BCD	CITREM	CITREM+BCD
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	67.15 ± 3.25	0.00 ± 0.00	69.16 ± 2.55	0.00 ± 0.00	69.20 ± 4.01	17.55 ± 3.47	59.41 ± 8.29	0.00 ± 0.00
3	67.28 ± 3.49	0.00 ± 0.01	67.59 ± 2.86	7.58 ± 0.92	71.18 ± 5.18	30.75 ± 13.67		
6	68.07 ± 3.91	0.00 ± 0.02	75.72 ± 8.59	10.40 ± 0.61	36.01 ± 5.97	40.91 ± 7.81		
9	65.56 ± 3.41	0.00 ± 0.03	75.72 ± 8.59	11.00 ± 0.61	46.54 ± 6.61	40.68 ± 5.62		
12	66.56 ± 3.41	0.00 ± 0.04	77.39 ± 7.77	12.50 ± 0.11	39.54 ± 7.35	41.68 ± 6.13		
15	66.56 ± 3.41	14.60 ± 0.87	78.60 ± 7.75	13.02 ± 0.23	39.54 ± 7.35	41.68 ± 6.13		

3.3.1.2. Viscosity, Droplet Size and Zeta Potential

As it can be seen in Table 3.6 and Figure 3.32., when BCD was added to emulsion system, emulsion viscosity increases around 5 cP; droplet size decreases more than 2 fold and also zeta potential was slightly decreased. Also in statistical evaluation with t-test, significant change on droplet size was proven with $p = 0.006 < 0.05$ and zeta potential change was not found significant ($p = 0.15 > 0.05$)

Table 3.6. Vicosity, droplet size and zeta potential of CITREM and CITREM+BCD emulsions

Emul. Type	Viscosity (cP)	Droplet size(d.nm)	Zeta Pot(mV)
CITREM	26.08 ± 0.88	4.34 ± 0.51	-51.63 ± 1.80
CITREM+BCD	33.76 ± 0.36	2.09 ± 0.76	-46 ± 8.21

Cheong.et.al (2016) [72] reported that high viscosity is related with hight zeta potential and relatively resulted in more stable emulsions. In contrast, in our study, while viscosity was increasing, zeta potential was decreasing. However, BCD addition to system was lowered the droplet size similarly with present study. This indicates that when oil droplets were coated with aqueous phase, possiby there were a MD-CITREM synergistic effects. When BCD was added to system, BCD was interared with aqueous phase and MD portion in emulsion. This caused lower zeta potential than without BCD emulsions.

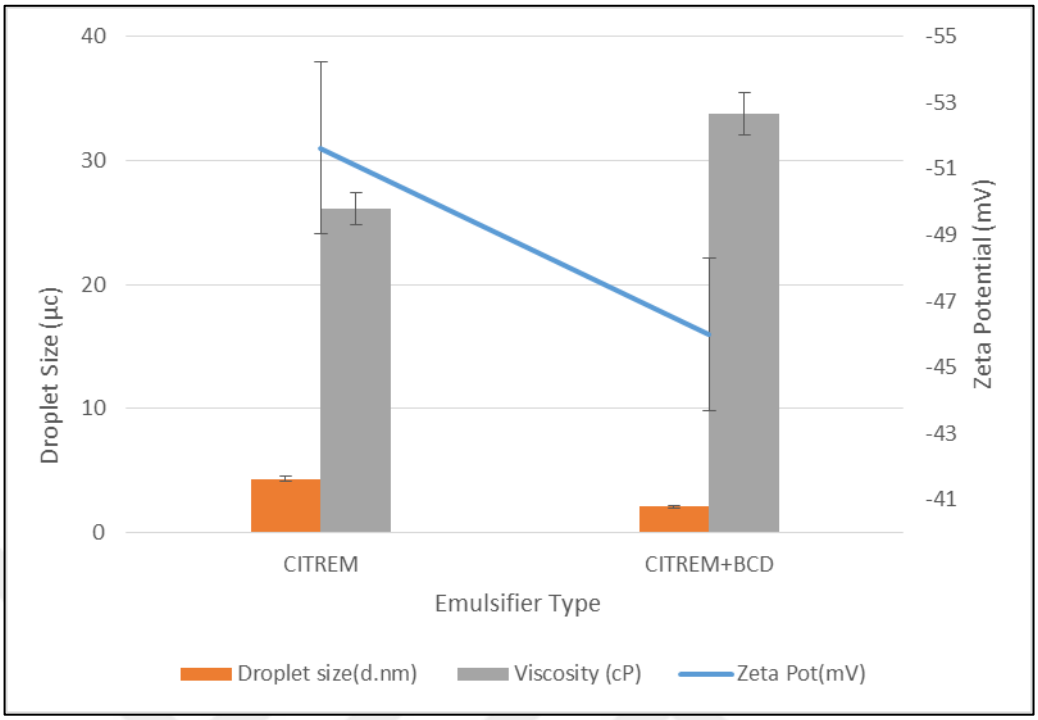

Figure 3.32. Droplet size, viscosity and zeta potential distributions of CITREM and CITREM+BCD emulsions

Flow behaviour of BCD blends have not been reported before. When the flow behaviours was observed (figure 3.33.), it was obviously seen that, BCD addition to system improves the flow behaviour of formulation. While the CITREM emulsion's curve was noisy and showed phase seperations and tendencies to seperation, as force was continued to apply on it, it reaches Newtonian behaviour. BCD added formulation showed a very stable and smooth curve. This also contributed to the creaming stability of BCD emulsions.

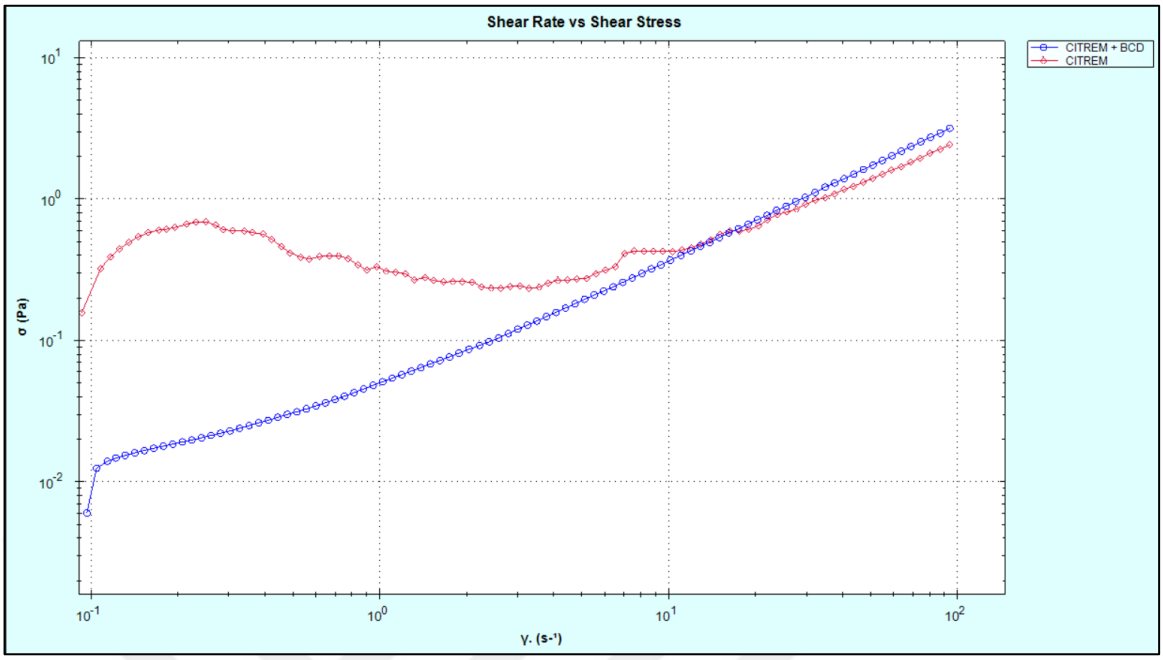

Figure 3.33. Flow behaviour of CITREM (red) and CITREM+BCD (blue) emulsions

According to figure 3.34, it can be said that the formulations'final viscosity are close to each other, and CITREM emulsions showed high tendency of seperation with its noisy curve.

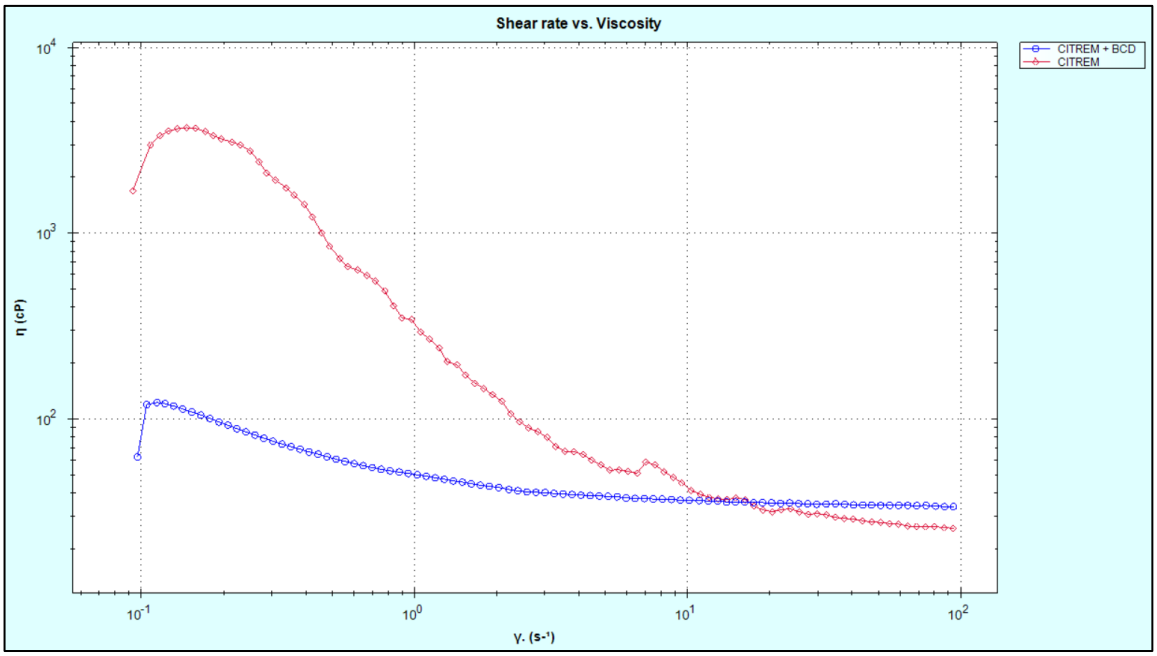

Figure 3.34. Viscosity Curves of CITREM and CITREM+BCD Emulsions

In addition, the smooth, uniform and foaming structure can be observed below (Figure 3.35).

Figure 3.35. CITREM+BCD emulsion (left) and CITREM emulsion after preparation

3.3.1.3. Oxidative Stability

As it was explained before, oxidative stability against heat and light was measured. Heat was applied to approximately 20 ml samples in glass containers (figure 3.36) at 55 ºC for 15 days. 3 replicates of individual emulsions were taken from incubator at days 1, 3, 6, 9, 12 and 15. Until analysis, they were stored in -80 ºC.

Figure 3.36. 3 Days oxidized emulsions in glass containers

Primary oxidation of samples were measured spectrophotometrically. Ferric ion complex methodmeasures the ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions. In order to analyze samples, oil was extracted with a little modification of Blight and Dyer (1959). An amount of emulsion was homogenized with 2:1 chloroform-methanol and centrifuged at 15 000 rpm and layer separation was achieved [91]. Seperated bottom layer was extracted and analyzed according to Shanta and Decker's method [57]. The reason of modification in extraction was impurity of oil (figure 3.37.). In prior studies, oil samples were obtained however since it is not clear, it could not be analyzed. Moreover, secondary oxidation experiments in order to measure p-Anisidine value were achieved according AOCS (Cd-10-90) spectrophotometric method. This method measures the content of aldehydes generated during the decomposition of hydroperoxides. Prior to p-An experiments, an amount of emulsion was mixed with 2:1 hexane-methanol in order to achieve phase separation. Mixture was centrifuged at 15 000 rpm for 10 minutes. It was aimed that all the oil in emulsion solubilized in hexane layer. At the end of centrifugation,

upper hexane layer was extracted and analyzed by adding on iso-octane according to experimental procedure.

Figure 3.37. Impure extracted oil samples from emulsions

After peroxide value (pOV) and p-Anisidine value (pAn), total oxidation was calculated (TOTOX). TOTOX is a measure of total oxidation calculated by pOV and pAn values. During lipid oxidation, firstly pOV products as hydroperoxides are forming in oxidized structure. Then, as hydroperoxides decompose, pOV rises and pAn increases. Therefore, TOTOX value gives the both hydroperoxides and its breakdown products and provides an approximate value of progressive oxidation values.

Table 3.6. Peroxide, p-Anisidine and TOTOX values of 55º C and sunlight stored samples

Type	Oxidation value	ST	Days					
		1 cycle	1	3	6	9	12	15
CITREM	pOV	8.20 ± 1.88	3.80 ± 0.71	5.92 ± 1.11	10.18 ± 1.18	8.73 ± 1.51	15.11 ± 0.52	15.82 ± 0.77
	pAV	2.59 ± 0.14	14.46 ± 0.23	17.85 ± 0.78	15.49 ± 0.57	15.50 ± 1.37	16.05 ± 4.40	11.25 ± 0.39
	TOTOX	18.99	22.08	29.07	29.11	46.49	46.29	42.9
CITREM + BCD	pOV	7.12 ± 1.14	5.12 ± 0.57	11.50 ± 0.78	13.21 ± 1.23	15.70 ±1.30	15.67 ± 0.73	14.94 ± 0,15
	pAV	6.56 ± 0,.82	42.41 ± 8.79	48.94 ± 4.58	52.19 ± 0.01	56.05 ± 0.01	59.25 ± 0.01	66.92 ± 9.72
	TOTOX	20.81	52.67	71.96	78.63	87.46	90.61	96.82

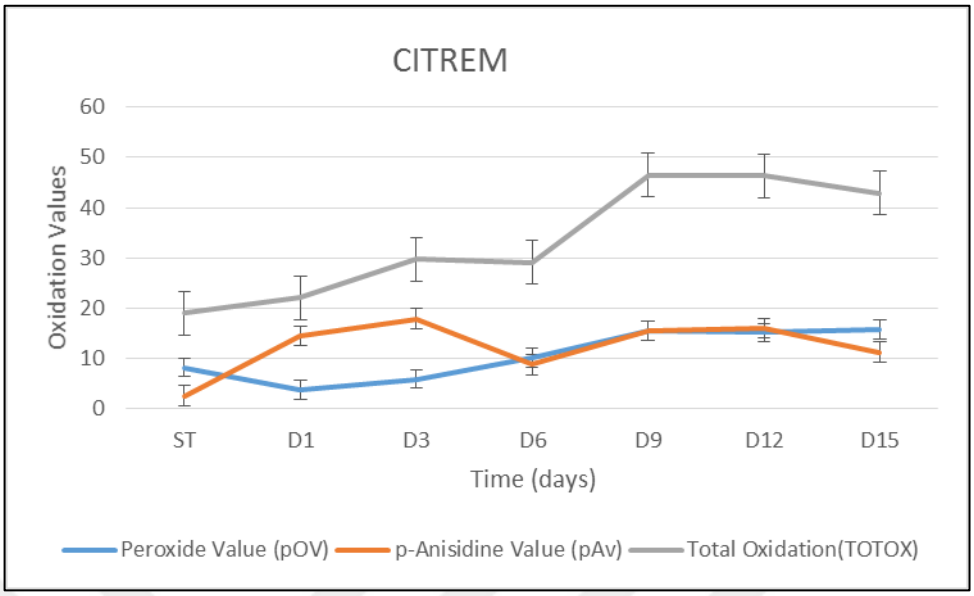

Figure 3.38. pOV, pAn and TOTOX values of CITREM emulsions during ST and 55ºC storage conditions

In CITREM emulsions, exposure of direct sunligt affected emulsions less than 1 day heat exposure. As it can be seen in Figure 3.38, suntest samples' pOV value was found higher than p-AV, it indicated that light did not affect emulsions as much as 1 day of heating. After 6 days, decrease in hydroperoxide formation and its equivalence with aldehyde value was observed.

When 1:1 CITREM and BCD was evaluated, pOV and pAV of light exposed samples are close to each other. In addition, pAV value is high from 1st day to 15th day (figure 3.41).

Statistical evaluation to measure the significancy level of change, t-test was applied on data. According to t-test results, BCD addition and CITREM decrease in system did not changed pOV significantly (p = 0.31 > 0.05). In addition, this caused significant change in pAV (p = 0.0003 < 0.05).

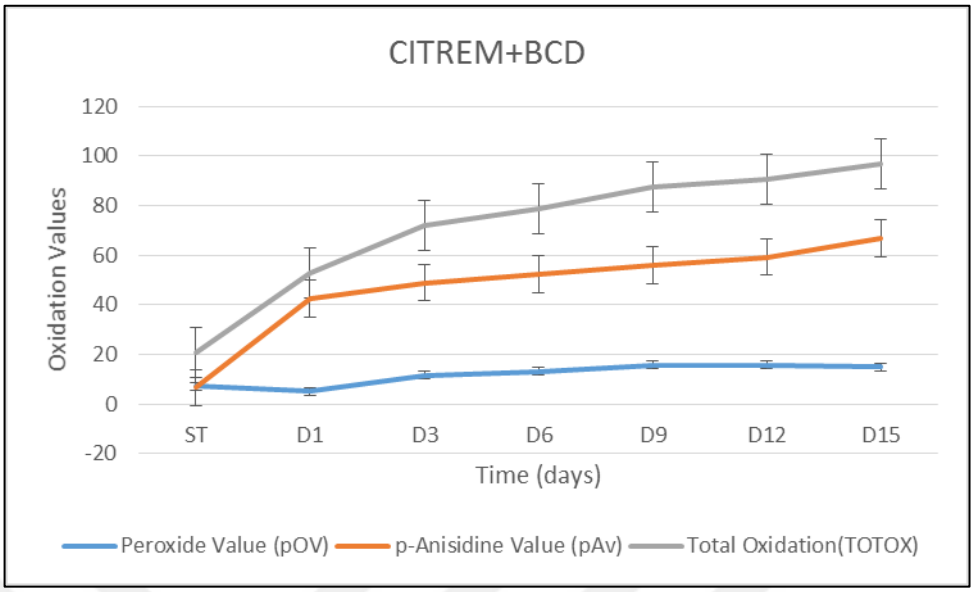

Figure 3.39. pOV, pAn and TOTOX values of CITREM+BCD emulsions during ST and 55ºC storage conditions

When total oxidation was compared by means of these changes (Figure 3.39) , it increases and this increase was found as significant ($p = 0.002 < 0.05$) in 95% confidence interval. It almost increased 2 fold.

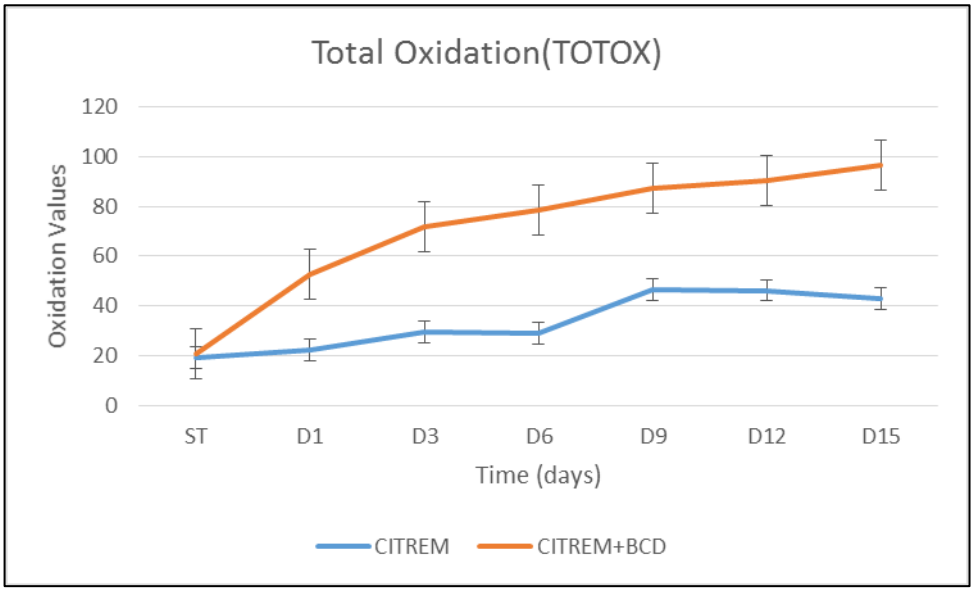

Figure 3.40. Comparison of TOTOX values of CITREM and CITREM+BCD emulsions during ST and 55ºC storage conditions

It was reported that lipid oxidation is affected significantly by the emulsifier type [59]. Althogh there are many studies mentioning the antioxidative properties of protein based emulsifiers [5, 54, 61] and also more improvement on them with CDs [70] ; there are less studies evaluating LMWE such as lecithin [23] and CITREM. Suprisingly, in contrast to be expected, BCD addition to the system did not improve the oxidative stability. Even, it was reported in [59] as second highest oxidation value following Tween, its individually decrease have not studied yet.

As a final discussion to oxidation angle, there may be a few reasons of increasing oxidative stability. Firstly, decreasing LMWE concentration in emulsion system may lead to released oil droplets resulted in unsaturated oil droplets and they could not form inclusion complexes in a correct way with BCD because of inefficient mechanical forces and temperature (BCD inclusion complexes are formed by mechanical forces and a specific temperature and resting time are needed to form inclusion complexes). Secondly, it may be because of competition phenomenon between LMWE and water soluble emulsifiers [61]. In addition it may have caused from inefficient stirring time, temperature and shear due to the emulsification equipment. Furthermore, another reason may be that, since MD polysaccharide has saturated the aqueous phase, BCD could not be solubilized in aqueous phase prior to forming inclusion complexes in water. Therefore, excess emulsifiers may have caused inefficient emulsification and may have caused the released oil droplets similar to emulsifier competition case. As uncoated oil droplets are increasing in emulsion, it gets more prone to oxidation. Other comment can be the reduced oil droplet size increases surface area of oil droplets and emulsion were more prone to oxidation. In order to understand which reason is more related with suprising consequent, these parameters should be studied further.

In summary, the content of the present study was producing physically stable emulsions with common emulsifiers used in food industry, evaluating parameters affecting the physical stability and examining the oxidation status of most physical stable emulsion and its 1:1 mixture with BCD which is able to form inclusion complexes and hinder biologically active ingredients in its hydrophobic cavity by behaving as a host. In progress of study, 40 % DE 12 MD was used as a natural and commonly used polysaccharide stabilizer. It was achieved to form stable emulsions with its addition to WPI emulsions. Also, emulsions were produced by using 2 protein and 2 oil based emulsifiers and it was tried to understand how emulsifier type affected the physical stabilization mechanism by comparing their creaming stability,

viscosity, zeta potential and droplet size. Most stable emulsions were produced with 40% oil and 1, 2, 4% emulsifier concentrations. Towards the aim of examining oxidative stability, higher creaming stability, lowest droplet size with highest zeta potential emulsion (20% oil and 4% CITREM) was selected to use as 1:1 blend with BCD. While oxidative stability was evaluated, creaming stability at 4 C, 21C and 55 ºC which are crucial temperatures in food production, transportation and storage were examined. At the end of study, it was found that while BCD addition to system improves physical stability by increasing viscosity, decreasing droplet size; it did not affect the oxidative stability in a positive way. In order to understand the mechanism of MD + CITREM + BCD, some parameters should be studied further as explained above.

There is no literature reporting BCD-CITREM conjugation and sun light exposure as one of main action mechanism of lipid oxidation of O/W emulsions. Present study, Suntest which is an equipment generally used material science application on food was achieved for the first time This study may be beneficial to understand the competitive mechanism that occur in LMWE emulsions and BCD. Therefore, strategies to increase BCD absorption may decrease lipid oxidation and further studies may generate solutions for both physical and chemical stability improvement.

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