ENCAPSULATION OF VITAMIN B_1 USING DOUBLE EMULSION METHOD

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ABSTRACT

ENCAPSULATION OF VITAMIN B_1 USING DOUBLE EMULSION METHOD

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The main objective of the study was to encapsulate Vitamin B_1 in the inner aqueous phase of water-in-oil-in-water (W/O/W) type double emulsion containing hazelnut oil as oil phase and to transfer it to food products for enrichment. It was also aimed to replace the synthetic Polyglycerol Polyricinoleate (PGPR) with lecithin and to study the influence of homogenization methods on double emulsion characteristics.

The expected type of emulsion, water in oil (W/O), could not be obtained by using only lecithin so lecithin-PGPR mixture at different ratios was used as hydrophilic emulsifier. It was found that addition of lecithin (1.5 g/100 g) to PGPR (1.5 g/100 g) enhanced the stability of the double emulsion. Three different homogenization methods were applied as High Speed Homogenization (HSH), Ultrasound and Microfluidization to produce primary emulsion. It was found that the homogenization methods used in

the preparation of primary emulsion influenced the physiochemical characteristics of the double emulsion. The most stable double emulsion with the smallest droplet size was obtained by HSH.

Vitamin B_{12} was used as a marker for water soluble compounds to study the encapsulation properties. It was found that higher than 96.7% of the vitamin could be entrapped by the prepared double emulsion. Considering the results, Vitamin B_{12} was replaced by pH sensitive Vitamin B_1 and added to carrot juice. It was determined that specific structure of double emulsion could reduce vitamin loss during storage. After two days storage, in the double emulsion system vitamin loss was 12% while it was 46% when vitamin was added directly to the juice.

Key words: Emulsifier, Double emulsion, Stability, Encapsulation, Enrichment

ÖZ

İKİLİ EMÜLSİYON YÖNTEMİ KULLANILARAK B_1 VİTAMİNİNİN HAPSEDİLMESİ

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Bu çalışmanın amacı, yağ fazı olarak fındık yağı içeren Su-Yağ-Su (S/Y/S) tipi ikili emülsiyonun iç su fazında B_1 vitamininin hapsedilmesi ve hapsedilebilen vitaminin zenginleştirme amacı ile gıdaya eklenmesidir. Ayrıca, lesitinin sentetik Poligliserol Polirisinolat (PGPR)'ın yerine kullanılması ve homojenizasyon tekniklerinin ikili emülsiyon özelliklerine olan etkilerinin incelenmesi de amaçlanmıştır.

Sadece lesitin kullanılarak beklenilen tipte, yağ içinde su (S/Y), emülsiyonu oluşturulamadığından hidrofilik emülgatör olarak değişik oranlarda lesitin-PGPR karışımı kullanılmıştır. Lesitinin (1.5 g/100 g) PGPR (%1.5 g/100 g) ile birlikte kullanılmasının ikili emülsiyonun kararlılığını arttırdığı bulunmuştur. Birincil emülsiyonun hazırlanması için yüksek hızda homojenizasyon (YHH), ultrasonikasyon ve mikroakışkanlı homojenizasyon olmak üzere üç farklı yöntem uygulanmıştır. Birincil

emülsiyon hazırlamada kullanılan homogenizasyon yönteminin ikili emülsiyon özelliklerini etkilediği bulunmuştur. En stabil ikili emülsiyon YHH yöntemi ile elde edilmiştir.

Enkapsülasyon özellikleri üzerine çalışmak için suda çözünen bileşikleri temsilen B_{12} vitamini kullanılmıştır. Hazırlanan ikili emülsiyon ile vitaminin % 96.7'den daha fazlasının hapsedilebilindiği bulunmuştur. Elde edilen sonuçlar doğrultusunda, B_{12} vitamini yerine pH'a karşı duyarlı olan B_1 vitamini hapsedilmiş ve havuç suyuna eklenmiştir. İkili emülsiyonun özel yapısının depolama sırasında vitamin kaybını azalttığı tespit edilmiştir. İki günlük saklama sonunda, ikili emülsiyon sisteminde vitamin kaybı %12 iken, vitamin direk olarak havuç suyuna eklendiğinde bu kayıp %46 olmuştur.

Anahtar Kelimeler: Emülgatör, İkili Emülsiyon, Kararlılık, Hapsedebilme, Zenginleştirme

To my Family and my LOVE…

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INTRODUCTION

1.1 Double Emulsions

Double emulsion is a very complex system because the emulsion contains the droplets which also contain dispersed droplets within themselves, so it is defined as emulsions of emulsions (Vasiljevic et al., 2009). According to the type of the dispersed phase, they can be classified as oil in water in oil (O/W/O) double emulsions and water in oil in water (W/O/W) double emulsions.

Although double emulsions have many possible applications, there is no valuable product made of double emulsion existing in the food market yet. The basic reason is the difficulty of the production of double emulsions and the instability of the products during the storage. However, many researchers are focusing on the topic of double emulsion due to their potential applications in many industries such as food, pharmaceutical, biomedical and cosmetic. Yan and Pal (2001) studied on the removal of toxic materials by the help of entrapment inside W/O/W double emulsions. Malone et al. (2003) investigated the encapsulation of aroma compound and release of them by using double emulsions. Kaimainen et al. (2015) tried to encapsulate betalain into W/O/W double emulsion system and studied the effect of encapsulation system on the intestinal lipid digestion. Moreover, double emulsions were tried to be used as an alternative way for production of low-calorie and low fat foods (Oppermann et al., 2015).

Composition of emulsion is a very vital issue in obtaining double emulsion because type and amount of surfactants, nature and concentration of oil, presence of electrolytes, method of the emulsion production and the nature of entrapped materials are the factors affecting the stability and characteristics of double emulsions.

Understanding of the formation and structure of the double is a requirement to prepare double emulsions and to use them in complex food system. Therefore, the studies related to the selection of surfactant type and identification of surfactant properties like surface activity are gathering great interests nowadays. There should be a good relation between the nature of the oil and selected surfactants in order to obtain a stable emulsion. Moreover, the process conditions in preparation of double emulsions also affect the stability of double emulsions.

1.2 Preparation of Double Emulsions

Preparation of the double emulsion is a challenging issue due to the nature of emulsions which consist of relatively large droplets having tendency of coalescing more quickly. There are two main approaches for the preparation of double emulsions, which are one-step emulsification method and two-step emulsification method. In the one-step method, the inner emulsion is prepared by mixing of a very excess amount of hydrophobic emulsifier and fewer amounts of hydrophilic emulsifier and then the system is heated. At the suitable temperature, with the convenient hydrophilic-lipophilic balance (HLB) of the emulsifiers, the system forms the double emulsion. However, the use of the one-step emulsification method is not widespread due to the difficulty in the reproducing the emulsion which has the same characteristics.

The common and more controlled way of preparation of double emulsion is two-step emulsification method. Many of the double emulsions used in the food industry are usually obtained from the two-step emulsification method by the application of homogenization methods. In two-step emulsification method, surfactants are used at different stages of the process. A hydrophobic emulsifier is used for the water-in-oil emulsion part which is considered as primary emulsion, while a hydrophilic emulsifier is used for oil-in-water part. At the first stage, primary emulsion is obtained by application of high shear to system containing large amount oil, less amount of water and a hydrophobic emulsifier. On the other hand, at the second stage fewer amounts of the prepared water-in-oil emulsion, high amount of water and a hydrophilic emulsion are mixed gently to get W/O/W.

Manufacturing of commercial double emulsion is difficult because when the system becomes more complex, behavior of double emulsion, especially if the emulsion contains numerous ingredients, is also being more aptness to departure (Dalgleish, 2001).

Homogenization is a critical step in preparation of double emulsions. Many researchers applied different homogenization methods to obtain double emulsion and studied on the influence of these methods on the characteristics of emulsions (Schuch et al., 2014; Kobayashi et al., 2005; Vladisavljevic et al., 2003). Homogenization method has an important role on the emulsion characteristics such as stability, appearance, color, rheological properties and also the cost of the product (McClements, 2004). The most widely used homogenization methods in the production of double emulsions are high speed homogenization, high pressure homogenization and ultrasonic homogenization.

1.2.1 High Speed Homogenization

High speed homogenization (HSH) method is generally used for dissolving, blending, dispersing, mixing and emulsifying purposes in the food industry. In HSH method, the homogenization energy was supplied by the rotary head, which can turn with speed of 10-50 m/s, generating mechanical agitation resulting in the homogenization effects (Zhang et al., 2012).

Although rotational speed and homogenization time are the most important parameters for the emulsification by HSH; there are many factors affecting the homogenization process like viscosity of the solutions, type and concentration of ingredients, process temperature (McClements, 2004). In the HSH, two different types of force were dominant for the emulsification as mechanical impingement against the wall because of the accelerated fluid and shear stress in the gap (Jafari et al., 2008). By combination effect of these forces, the solutions are mixed and desired emulsion is obtained.

In the literature, there are many of studies in which HSH was applied for the production of double emulsion (Sapei et al., 2012; Fechner et al., 2007) because it is a simple and an effective method for obtaining emulsions. Maa & Hsu (1999) reported that HSH was more convenient than the other homogenization methods, namely microfludization or sonication, by considering the easy of cleaning of equipment, easy of sterilization of the products, low cost of production and easy of controlling of the product properties.

1.2.2 Microfluidization

Microfluidization (MF) is also called as high pressure homogenization. In food industry, emulsions can be prepared by the high pressure homogenization process, in which droplets of the emulsions are disrupted by the combination of turbulence and intense shear flow.

MF includes the application of extremely high velocity, high frequency vibrations, high pressure and intense shear in a very short time (Liu et al., 2009). In the literature, there are many studies dealing with the application of MF method such as the production of cheddar cheese with microfluidized whey (Lebeuf et al., 1998), the comparison of yoghurts made from microfluidized milk and conventional homogenized milk (Ciron et al., 2010) and the production of nanoemulsions (Jafari et al., 2007). These studies showed that MF could be used as an alternative treatment for the homogenization of food systems.

It was shown that usage of MF in application for the preparation of double emulsions resulted in course emulsions having monodisperse structure (Okushima et al., 2004; Sugiura et al., 2004). One of the main problems during the production of emulsions by the application of MF was over processing, which produced emulsions having very small droplets and these droplets could come together to form new droplets. Jafari et al. (2007) studied on the production of emulsion by MF and observed that the produced emulsion were instable because of intensive energy input by MF. Jafari et al. (2008) reported that MF for the emulsion production might cause to re-coalescence of newly produced droplets due to slower emulsifier adsorption and higher droplet collisions with shorter resistance time. Therefore, the processing conditions such as pressure and number of cycle should be selected properly by considering this specific problem.

1.2.3 Ultrasonic Homogenization

Ultrasonic (US) homogenization can be defined as the homogenization process by ultrasound waves. The ultrasound waves, above 20 kHz, produce shear and pressure gradients inside the sample by means of turbulence and cavitation effects (Behrend et al., 2000). The produced turbulence and cavitation result in more homogenized system.

Many reserachers studied on the effect of US homogenization method on the production and characteristics of emulsions and also the comparison of the emulsions produced by different homogenization methods including US. Huck- Iriart et al. (2013) tried to prepare the emulsion by using US treatment and found that the droplet size of the emulsions treated with US was significantly smaller than the ones prepared with HSH. Although US treated samples produced more stable emulsions, further application caused destabilization of the samples by creaming, flocculation and phase separation. Therefore, in the application of US to formulation of emulsions, over processing should also be taken into account.

Mun et al. (2011) produced W/O/W double emulsions by US homogenization method including enzymatically modified starch, inside the inner aqueous phase, to see the effect of it on the stability and encapsulation efficiency. It was found that sonication time was an important parameter on the mean diameter of the oil droplets. When the sonication time increased, the mean diameter decreased. The reason of that result could be the positive correlation of energy density with sonication time. The higher sonication energy density caused destabilization and disruption of the droplets (Jafari et al., 2007).

Ultrasound frequency is also an important parameter in US treatments. In the food emulsion systems, the sound ranges are classified as

high-frequency referring to low-energy ultrasounds and low-frequency referring to high-energy ultrasounds.

High-frequency ultrasounds, having frequencies above 1 MHz at intensities below 1 W/cm^2, are mainly used for process control by considering composition of the material, structure and interactions of the compounds inside the system or molecular properties of the food products. These ultrasounds are non-destructive and their application is rapid and inexpensive so they are also used for food quality assurance (Piko, 2012). These properties make US to be useful in the emulsification of fat based food products, evaluation of the composition and improvement of the texture (Awad et al., 2012). Therefore, US could be also used for monitoring the conditions of the products during the processing, like emulsification.

On the other hand, low-frequency ultrasounds have the frequencies in the interval of 20 and 500 kHz, which results in the mechanical, physical, chemical and biochemical changes due to the cavitation caused by the treatment. Therefore, low-frequency ultrasonication is a disruptive method (Awad et al., 2012). The aforementioned cavitation showing up during the application of ultrasound provides a physical shear force which can be used in destruction of microorganisms, extraction and emulsification processes (Abismail et al., 1999). Moreover, Tang et al., (2012) studied preparation of nanoemulsions by application of US method and concluded that low frequency ultrasound could be applied to form well established emulsions.

Application of high frequency ultrasound for the formation of "emulsifier free emulsion" is a new technique and attracts interest of many researchers nowadays. In the treatment, discontinous phase droplets can be dispersed in the continuous phase without using emulsifiers. Kaci et al. (2014) used high frequency ultrasound as a non-denaturing and non-destructive process to

generate emulsions without using any surfactant and concluded that it was a successful method to prepare emulsion by deformation and breakage of the droplets during the application.

Homogenization method is a vital factor for the preparation of the emulsions. Scherze et al. (2006) studied the effect of different emulsification methods on the properties of water in oil emulsions prepared with lecithin or PGPR as surfactants. They prepared emulsions with different devices such as ball valve, orifice valve, high pressure homogenizer, rotor-stator, ultra-turrax and ultrasonic equipment. It was concluded that the properties of the emulsions in which lecithin was used as a surfactant depended more strongly on the method than the emulsion in which PGPR was used as surfactant. In addition, the emulsions containing were not found to be stable when they were treated with rotor- stator equipment.

1.3 Characteristics of Double Emulsions

The physicochemical characteristics of the double emulsions have vital duties in the complex food systems since they directly influence the sensory, nutritional and textural properties of final product.

One of the significant characteristics of double emulsion is stability, which means the resistance to variation of the properties over the time. The physicochemical mechanisms such as flocculation, ostwald ripening, coagulation, coalescence, gravitational effects due to the density differences of phases and phase inversion can be considered as threating factors of stability of double emulsions (McClements, 2004; Dickinson, 2011).

The structural forces like Van der Waals forces cause individual droplets to attact with each other, come together and form bigger droplets. However,

the presence of the suitable surfactant affects these forces and slows down the phenomena by introducing a repulsive force and a mechanical barrier. The repulsive force can be considered as an energy barrier decreasing the rate of serum separation while mechanical barrier forms at the interface and decreases the rate of coalescence of droplets (Kong et al., 2003). In short, surfactants used in the formulation of the double emulsions provide more stable complex by decreasing the interfacial tension.

Addition of a thickening or gelling polymer like xanthan and gelatin to the inner dispersed aqueous phase of the double emulsion is used as an approach for providing long-term stability of emulsion. When preparing the double emulsion, addition of gelatin to the inner aqueous phase to solidify it results in an increase of encapsulation efficiency and emulsion stability (Muschiolik et al., 2006). Multiple emulsions containing bovine serum albumin (BSA) or gelatin inside the inner aqueous phase represented almost the same phase separation, same globule size but better encapsulation efficiency (Nakhare and Vyas, 1996). Moreover, W/O/W double emulsions containing gelatin and sodium chloride (NaCl) in the inner aqueous phase of emulsion were stable against sedimentation for a month while phase separation was observed in the emulsions containing no NaCl or gelatin. In addition to the positive contribution of NaCl or gelatin to the stability of double emulsion, they also increased the encapsulation efficiency of double emulsions (Sapei et al., 2012).

The other parameter that has an important role on the stability of the W/O/W double emulsion is the osmotic pressure difference between the inner aqueous phase and outer aqueous phase of emulsion (Kanouni et al., 2002). In W/O/W double emulsions, the presence of the electrolytes inside the inner aqueous phase can decrease the stability of the emulsion. Therefore, the concentration of the electrolytes must be adjusted to provide

a good relationship between Laplace pressure and osmotic pressure of the systems (Jiao et al., 2002).

Thermodynamic instability of double emulsion restricts their application in the food industry leading to leakage of inner aqueous phase and also the captured material from the inner aqueous phase, separation of phases and flocculation of the droplets inside the emulsions during the application of process and storage (Benichou et al., 2004). The main reasons of destabilization of double emulsions are their composition and microstructure which cause the breakdown of droplets. The mentioned breakdown mechanisms can be listed as coalescence of outer droplets, coalescence of inner droplets but no change in outer part, coalescence of inner droplets and outer droplets together, shrinkage of inner droplets and swelling of outer droplets. All of these breakdowns could occur at the same time and could play positive role on the rate of destabilization of double emulsion (Dickinson & McClements, 1995).

Droplet size of the dispersed phase is another significant characteristic of double emulsion because it affects stability, appearance, texture and sensory properties (McClement, 2004). Factors affecting the droplet size of emulsions are type andamount of surfactants used, the volume ratio of the phases, interfacial properties, viscosity of the system and the emulsification method (Weiss & Muschiolik, 2007; Jafari et al., 2007; Jafari et al., 2008; Behrend et al., 2000; Dickinson, 2011; Bou et al. 2014).

Researchers studied on the production of double emulsions reported that generally unimodal and bimodal droplet size distributions were observed in the double emulsion systems (Mun et al., 2010; Cofrades et al., 2013; Hemar et al., 2010; Sapei et al., 2012). Bou et al. (2014) tried to obtain food grade double emulsions by using the PGPR and sodium caseinate as the surfactants and olive oil and pork lard as the oil phase. They stated that the

fresh double emulsions had unimodal distributions but distributions of some double emulsions changed from unimodal to bimodal during the storage. As a result, the storage conditions are also an important factor in affecting the droplet distribution characteristics of the double emulsions.

Double emulsions prepared with high-intensity mixing or valve homogenization show polydisperse structure and heterogeneous distributions of droplets which is a problem for the characterization of the droplet size distribution (Dickinson, 2011). Characterization of double emulsion in terms of the droplet size is very important step for the emulsion production because emulsion droplet size has an effective role on the properties of the emulsion like stability, rheology and color. Stirring application (Okochi & Nakano, 1997), membrane emulsification method (Van der Graaf et al., 2005), valve homogenizers (O'Regan & Mulvihill, 2009), couette cell operations (Bonnet et al., 2009) and rotor-stator dispersers (Carrillo-Navas et al., 2012) have been applied to produce the double emulsion with ranging droplet sizes. It was observed that the emulsions obtained from the stirring application and rotor-stator dispersers had larger droplets.

Many of the researchers reported that there was a correlation between the droplet size and stability of emulsion. Emulsions containing small droplets are generally more stable than the ones having large droplets. The continuous movement of the droplets causes to come together of them and formulation of the larger ones, resulting in the increase of possibility of destabilization. The density differences between the phases and droplets also influence the upward/downward direction motion. The mentioned upward/downward direction motion of the droplets has an important role on the destabilization of the double emulsions (Tadros, 2013). Therefore, droplet characteristics of the double emulsions have influence on the stability.

1.4 Surfactants

Double emulsion has an additional distinct bulk phase and an oil-water interface compared to the single ordinary emulsion. The presence of this additional interface means that two different surfactants are needed to formulate the double emulsion: one is required for primary oil-water interface and another is for outer oil-water interface. Ability of surfactants to stabilize the systems containing interfaces is related to the chemical structure of them especially, amphiphilic properties. In the structure of the surfactant, two domain groups are present together: hydrophilic and lipophilic groups. Lipophilic group is attracted to oil phase of the emulsion while the hydrophilic group is attracted to the aqueous phase so they can bring these immiscible fluids together in the same medium.

The selection of the surfactant type is a very critical step for preparation of double emulsion. According to their performance and health restriction, lots of surfactants have been used for different composition of double emulsions. Low-mass surfactants, like lecithins, glycolipids, monoglycerides and fatty acids, can reduce the interfacial tension at the interface and move freely so they can behave as coating agents for oil-water interface during the formation of emulsions. High-mass surfactants contain mainly two groups: polysaccharide group and protein group. According to the composition and properties of the mentioned groups the characteristics of the surfactants are identified. On the other hand, usage of the double emulsions in the food applications is limited by the lack of appropriate food-base surfactants for the phases. Many of the surfactants used in the other industries are synthetic polymeric surfactants. Therefore, progress in the production of stable double emulsions for food applications mainly depends on the use of food grade ingredients to replace the aforementioned synthetic surfactants. Natural polysaccharides which have the property of a stabilizer can be used in W/O/W double emulsions. These polysaccharides could be used in the outer aqueous phase.

Surfactants are adsorbed at the layer interphases which result in the formation of a stabilizing layer at the droplet surface so they have a vital role on the selection of the surfactant type for the formation of a stable emulsion. Therefore, the nature of layers and nature of the surfactant should be correlated and the solubility of the surfactant in the layers should be taken into account for the behavior at the stabilizing layer of the system.

As a consequence, double emulsions are very complex systems and preparation and maintaining their stability are more difficult as compared to single ordinary emulsions (Garti, 1997). For the non-food-base products, these difficulties could be overcome by using synthetic surfactants in high amount. However, for the food emulsions usage of the type and amount of the surfactants are restricted. The most commonly used surfactants in the food systems are lecithin, mono and digliseride, polysorbate, esters, gums and phosphates.

1.4.1 Polyglycerol Polyricinoleate

Polyglycerol polyricinoleate (PGPR) is a lipophilic emulsifier, commonly used for obtaining stable water-in-oil emulsions. It is a synthetic emulsifier produced industrially. Its manufacturing process is based on the esterification reaction of polymerized glycerol with condensed castor oil fatty acid (Wilson et al., 1998). The commercial samples include fatty acids and polyricinoleic acid esters of polyglycerol and it has a long hydrophilic polyglycerol chain making it notable in water-binding so high emulsifying property (Dedinaite & Campbell, 2000). The common area of PGPR in the food industry is chocolate production sector due to high water-binding capacity of PGPR that prevent thickening of the chocolate. PGPR is used with lecithin in order to adjust the viscosity of the chocolate products (Banford et al., 1970).

PGPR is generally recognized as GRAS (Substance Generally Recognized as Safe) according to the Food and Drug Administration (FDA, 2006). Like other food additives, the use and amount of the PGPR has to be labelled and the maximum amount of the allowed intake dosage is 2.6 mg / kg of body weight per day (Wilson et al., 1998). Therefore, this synthetic emulsifier should not be used in high amount in food systems because of the regulations. On the other hand, PGPR appears to be the most effective hydrophobic emulsifier used for the preparation of W/O/W double emulsions (Wilson et al., 1998). In order to get stable double emulsions PGPR should be in the range of from 4% to 8% (w/v) (Mun et al., 2010). Therefore, reducing the concentration of PGPR seems very desirable. Su et al. (2006) reduced the PGPR concentration by introducing sodium caseinate in the inner aqueous phase and reported that W/O single emulsion and W/O/W double emulsion could be produced by the aforementioned replacement and they maintained the stability. It was also reported that sodium caseinate and PGPR might act synergistically at the interfacial where they could form an enhanced viscoelastic interfacial layer.

1.4.2 Lecithin

Among the hydrophobic food-grade emulsifiers lecithin is the only natural emulsifier (Dickinson, 1993) used in the food industry as a functional compound for the process of the many products such as bakery products, chocolates and fats due to properties of viscosity regulator and dispersing agent. It is a low molecular weight surfactant composed of various phospholipid species. Low molecular weight of lecithin makes itself to reach to the surface of droplets easily during emulsification process. Lecithin is mainly obtained from the egg yolk or soy beans so according to the origin and degree of purity it can be used in the industry. Commercial lecithin refers to the mixture of phospholipids mainly phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (Zhu & Damodaran,

2013). Although its function in the emulsion is not well understood, it can be used in the food emulsion systems undoubtly.

According to the data obtained from the Codex Alimentarius Commission-CAC (1981) lecithin and monoacylglycerol are not protein based emulsifiers and they are in the class of low-molecular weight surfactants. Zou and Akoh (2013) studied the effect of lecithin and monoacylglycerol as surfactant for the formulation of lipid- based infant formula emulsion representing the milk consumed by the infants. Therefore, the emulsions included dairy proteins, lactose, vitamins, minerals and other micronutrients. They found that both of the surfactants: lecithin and monoacylglycerol played a dominant role on the emulsion stability.

Lecithin was used as emulsifier in water in oil emulsion formulation and it was found that it improved the emulsifying properties of emulsion (Weete et al., 1994). Fang and Dalgleish (1993) realized that lecithin enhanced the stability of oil in water emulsions. Moreover, Pan et al. (2002) used the sunflower lecithin to observe the effect of lecithin on the stability of water in oil and oil in water single emulsions and concluded that lecithin with high phospholipids content in terms of the phosphatidylethanolamine and phosphatidylinositol concentration was the best surfactant for the water in oil suspensions. Therefore, it might be possible to replace the synthetic emulsifiers like PGPR with lecithin for the preparation of primary emulsion of double emulsions.

1.4.3 Sodium Caseinate

Sodium Caseinate (NaCN), produced from milk protein, is a commercially available food emulsifier and used as secondary hydrophilic surfactant in outer aqueous phase of W/O/W double emulsions (Dickinson, 2011).

In milk, caseins are present as large aggregated structures and they are sensitive to pH. As a result, caseins can be removed from the system by adjusting the pH, which leads to precipitation of the caseins. Then, they can be resuspended with the treatment of sodium hydroxide (NaOH) resulting in formulation of NaCN. NaCN is industrially manufactured on the large scale and used in many of the food sectors (Southward, 1989). Industrial NaCN

is a multicomponent mixture including four major phosphoproteins as αs1-casein, αs2-casein, β-casein, and κ-casein and the composition of mixture is variable according to the target aim of the usage. In the aqueous system, the mentioned individual phosphoproteins, except κ-casein, can adsorb to the interface. This adsorbtion results in the establishment of a film which protects newly existed droplets against the deformation such as creaming, flocculation or coalescence (Sanchez et al, 2005).

In food industry NaCN is mainly used as emulsifier, fat binding agent, water binding agent, gelatine contributor and thickener. Many researchers used NaCN as hydrophilic emulsifier in order to prepare double emulsion system because oil-water interface adsorbs it and produces a gel like solution (Delample et al., 2014). Moreover, researchers studying on double emulsion to encapsulate the valuable compounds have used NaCN in the outer aqueous phase (Cofrades et al., 2014, Bou et al., 2014).

Depending on the primary emulsion surfactant type and amount, phase ratio of the primary emulsion to outer aqueous phase, type of oil phase and ingredients of the inner aqueous phase composition; different concentrations of the NaCN were used for the formulation of W/OW type double emulsions (Su et al., 2008; O'Reagan & Mulvihill, 2009). On the other hand, NaCN can also be mixed with the other emulsifiers or compounds having specific properties for the production of multiple emulsions. Perrechil et al. (2013) mixed NaCN and carrageenan for the production of

double emulsion systems. Farshchi et al. (2013) also used NaCN with locus bean gum to obtaine emulsions.

NaCN concentration is a very critical step for the production of the double emulsions because it affects the whole characteristics of the system. The concentration should be high enough to surround new formulated primary emulsion droplets distributed into the outer aqueous phase (O'Dwyer et al., 2013). Dense suspensions of NaCN (>100 g/1 L) have visco-elastic properties and their viscosity increase rapidly with the concentration increment due to the jamming of small caseinate particles (Pitkowski et al., 2008). The jamming can provide contributions for the stability of the double emulsions but it can be a problem for the application of homogenization methods.

1.4.4 Gum Arabic

Gum arabic has a very flexible molecular structure so it is used in the food base systems due to its versatile characteristics (Islam et al., 1997). The main functions of the gum arabic in the food industry can be summarized as emulsification, encapsulation, stabilization, water binding, adhesion and film forming (Dickinson et al., 1991; McNamee et al., 1998; Verbeken et al., 2003).

The composition of the gum arabic is complex including polysaccharide, protein and arabinogalacto protein species. The mentioned protein species which are adsorbed effectively in the oil-water interphase have surfactant behavior in the emulsion systems (Dickinson et al., 1994).

Gum arabic solutions have a rather low viscosity compared to other polysaccharides. The favorable concentration of gum arabic to be used as an

emulsifiying agent is 10-20% for the emulsifying agent (Kibbe, 2006). Standard gum arabic forms thick and viscoelastic layers in the oil-water interphase (Dickinson et al., 1989) but in the literature there is no research on the production of W/O/W type double emulsion using only gum arabic as surfactant. On the other hand, Su et al. (2008) tried to use the gum arabic as a surfactant for production of double emulsion but they used a modified gum arabic product, SUPER GUM, instead of the conventional gum arabic. After the modification of gum arabic, it could be used as outer aqueous phase surfactant for the double emulsion production.

1.5 Encapsulation Properties of Double Emulsions

Double emulsions are more adequate for entrapment of the valuable compounds than the single emulsions in terms of the entrapment yields and stability against degradation (Vasiljevic et al., 2009). Double emulsion system has a specific structure as it contains two different compartments of liquid dispersions. This specific structure provides potential applications. The inner droplets might function as reservoir for the desired compounds entrapped by the double emulsion. Their transfer from the inner droplets to the outer phase might be controlled by the proper transport mechanism conditions of double emulsion. This attribute makes the W/O/W double emulsions convenient for encapsulation of hydrophilic compounds like water soluble vitamins, minerals and some bioactive compounds. As a result, W/O/W emulsion is a potential method to entrap valuable or sensible substances in the primary emulsion because oil phase layer separates the two aqueous phases so inner aqueous phase offers potential for encapsulation of the hydrophilic ingredients.

Many researchers studied on entrapment of water soluble active materials inside W/O/W double emulsion. Bonnet et al. (2009) worked on the encapsulation of magnesium within the double emulsion. McClements et al.

(2009) used oil in water emulsions as a delivery system for lipophilic compounds like beta-carotene. However, water soluble molecules have tendency to migrate from the inner phase to outer phase due to the osmotic pressure gradient difference between the phases, so researchers mainly face with the difficulty to control the diffusion of water soluble molecules (Grossiord et al., 1998).

Encapsulation characteristics of the double emulsion mainly refer to the encapsulation stability and encapsulation efficiency. Encapsulation efficiency means the capability of the percentage amount of entrapped material inside the discontinuous phase of the primary emulsion whereas encapsulation stability means the ability of entrapment of the desired material during the storage of double emulsion. The simplest and the most commonly used method for measurement of the encapsulation efficiency and stability is application of centrifugation method in which the primary emulsion and outer aqueous phase are separated and the desired material content is determined by analyzing them.

There are many approaches to determine the encapsulation properties of double emulsions. Magdassi and Garti (1984) measured concentration of chloride transferring from inner aqueous phase to outer aqueous phase by potentiometric titration method which was applied to outer aqueous phase to measure chloride concentration of it. Sela et al. (1995) studied on the measurement of NaCl concentration in outer aqueous phase of W/O/W double emulsion which shows the releasing of NaCl from inner phase to outer phase by conductivity measurement method. Owusu et al. (1996) tried to measure the encapsulation properties of glucose, which was entrapped inside W/O/W double emulsion, by dialysis membrane method depending on the oxidation-reduction mechanism. Fechner et al. (2007) applied the same membrane method to determine the encapsulation properties of Vitamin B_{12} in W/O/W double emulsion. Benichou et al. (2007) used the

differential pulse polarography to measure the encapsulation efficiency of Vitamin B_1 in double emulsion. Hai and Magdassi (2004) also used the luminescence spectroscopy method to study the entrapment of fluorescent compounds inside the inner aqueous phase.

In order to increase the efficiency and stability of compounds entrapped by double emulsion, different type of the surfactants like monomeric surfactants, polymeric surfactants and polymeric amphiphilic emulsifiers are used (Garti & Aserin, 1996; Grossiord et al., 1998; Dickinson et al., 1994). On the other hand, in order to increase the effectiveness of the entrapment of compounds inside the emulsions, natural macromolecules like proteins, gelatin, hydrocolloids, gums have been used to cover and form a film over the oil and/or water droplets. This film could behave as a barrier for the entrapment compound, decrease diffusion and also increase the stability of the emulsion. Pitchaon et al. (2013) used tamarind kernel powder, gum arabic and maltodextrin as a novel combination for encapsulating agents of phenolic compound entrapment by double emulsion and found that the combination could be a potential method as an encapsulation barrier material.

The double emulsion system focusing on the entrapment of a desirable compound and transfering it to more complex systems should be carefully produced to provide additional functions to the final product. At the first step, the ingredients of the double emulsion should be economic and easy to handle and the production method should be reliable and cheap. On the other hand, the effect of the production method should be studied in detail. For example, the effects of temperature fluctuations, mechanical forces and ingredients interactions should be known. Moreover, the sensory properties of the final product should also be considered. Double emulsion should not adversely affect the sensory properties, rheological properties, optical properties and the shelf life of the final product.

W/O/W double emulsions can be considered as a potential vehicle for the water soluble bioactive compounds (Dickinson, 2011). In the current research, water soluble vitamins (B_{12} and B_1) will be tried to be encapsulated in the inner aqueous phase of the W/O/W type double emulsion.

1.6 Vitamins

Vitamins are essential nutrients for the human body in order to perform chemical and physiological functions. The main reservoirs of them are natural food sources so they can be easily taken from the foods in order to satisfy daily needs. However, vitamin deficiencies can be observed in the population due the insufficient diet so the mentioned essential nutrients should be provided to the body in small amounts on a regular basis (Combs, 2008). Enrichment and fortification of foods in terms of vitamins can help to overcome vitamin deficiency problem.

Vitamins are generally unstable in food systems and the processing conditions result in degradation of the vitamins. The loss is mainly related to the nature of the food and preparation parameters like temperature, light, moisture, pH, and the processing time. According to the nutritional science, there are 13 vitamins and these vitamins are classified into two main categories as water soluble and fat soluble vitamins. Water soluble vitamins are B group vitamins (Thiamine, Riboflavin, Folates, Pantothenic Acid, Niacin, Pyridoxine, Cobalamin and Biotin) and Vitamin C. On the other hand, the category of fat soluble vitamins includes Vitamin A, Vitamin D, Vitamin E and Vitamin K. They have important role on the maintain health and life and also they have individual functions such as reproduction, regulation of metabolic processes, cellular functions and prevention for diseases (Leskova et al., 2006).

Vitamin B_{12} is a vital water soluble vitamin for human health since its deficiency might cause important health problems like pernicious anemia and various neurological disorders (Singh & Sachan, 2011). In order to decrease the deficiency in the population, the commercial food products can be enriched with it instead of taking the supplements. It was found that Vitamin B_{12} from the milk was taken to body more efficiently than the synthetic form (Matte et al., 2012).

O'Regan and Mulvihill (2009) studied on the encapsulation of Vitamin B_{12} with the help of the W/O/W double emulsions and showed that it was a very stable compound and suitable for studying the entrapment properties of the double emulsions. Giroux et al. (2013) studied on the cheese fortification using the W/O/W double emulsion as carrier for the water soluble nutrient. It was found that encapsulation of Vitamin B_{12} in emulsions had very high efficiency and double emulsion prevented the loss of the vitamin during the digestion.

At the current research, Vitamin B_{12} was chosen since it could be used a marker for the water soluble nutrient encapsulation with W/O/W double emulsion. Vitamin B_{12} can be used as a control compound to determine the suitable conditions to formulate double emulsions because it is a very stable vitamin under most food processing operations (Leskova et al., 2006). Love and Prusa (1992) studied the effect of different thermal treatment on Vitamin B_{12} reduction in beef. They noticed that encapsulation prevented the loss of vitamin. Like all other water soluble valuable compounds, the loss of the vitamin was mainly related to the leaching of it into the processing liquids (Leskova et al., 2006).

Vitamin B_1, also known as Thiamin, is one of the most important vitamin B complex vitamins by an essential role as a coenzyme in carbohydrate metabolism and nerve system (Talwar et al., 2000). It is easily drived out of

the human body with biological fluids, so it is not accumulated within the body and deficiency is usually a problem. Therefore, Vitamin B_1 should be supplied to the human body on a regular basis to prevent the deficiency.

Vitamin B_1 is stable at pH 2.0 to 4.0 but it gradually loses the stability as the pH is changed from acidic to neutrality and alkaline (Dwivedi & Arnold, 1973). Ball (1994) reported that alkaline pH during cooking and processing of the food materials including Vitamin B_1 leads to extensive losses of the vitamin. Leskova et al. (2006) also indicated that Vitamin B_1 is unstable in neutral pH values even weak alkaline conditions result in the degradation.

In the literature, there is a conflict about the effect of heat on the degradation of Vitamin B_1. Many researchers mentioned that Vitamin B_1 is a very heat sensitive compound and it is lost mainly through heating so processing methods including heating treatment results in destruction of it (Batifoulier et al., 2005; Martinez-Villaluenga et al., 2009; Mihhalevski et al., 2013; Oseredczuk et al., 2003; Maskova et al., 1996). Moreover, Whitney & Rolfes (2011) mentioned that thermal processing caused to a considerable loss of Vitamin B_1 due to thermal breakdown. On the other hand, Lassen et al., (2002) studied on cooking of pork meat at $72^{\circ}C$ and reported that the mentioned temperature did not cause any thermal degradation of the Vitamin B_1. This is in line with the study of Sierra and Vidal-Valderde (2001) in which milk was exposed to temperature ranging from 90-120 ^0C in order to analyze the effect of the heat on the degradation Vitamin B_1 and reported that heat treatments did not result in the significant loses of the vitamin content of the milk.

Two commercially available products of Vitamin B_1 are thiamine hydrochloride and thiamine mononitrate. Hydrochloride form is a colorless, crystalline powder with a yeasty odor and a salty nut-like taste. It is also more soluble in water (1g/ml) so used in the technical researches and for the

food fortification (Eittenmiller et al., 2008).

The best natural sources of Vitamin B_1 include whole grain cereals, yeast and yeast extract, beans, nuts, egg yolk, poultry, fish and liver (Navarra, 2004). The official recommendation of Vitamin B_1 intake is 1.2 mg/day for men and 1.1mg/day for women but slightly higher levels are required during pregnancy, illness or stress (Whitney & Rolfes, 2011). The mentioned intake amount is easily met by the adequate and balanced nutrition diet. However, as thiamine is not stored in the body and drived out of the human body with biological fluids, failing to requirement intake leads to thiamine deficiency.

Thiamin deficiency can cause the diseases known as Beriberi and Wernicke-Korsakoff Syndrome in the people (Lynch & Young, 2000). In the year 2003, a life threating Vitamin B_1 deficiency was detected in infants due to the under nutrition (Fattal-Valevski et al., 2005). Thus, enrichment of the food materials with the mentioned vitamin to improve the vitamin status in the population could be an interesting study. Moreover, there is no research in the literature in which Vitamin B_1 is encapsulated via W/O/W type double emulsion including natural emulsifiers and transferred to a food matrix.

1.7 Objective of the Study

Nowadays, people want to consume the functional products, which supply bioactive compounds like antioxidant, vitamins and other nutritive compounds due to the increase in diseases related to malnutrition. Vitamins are essential nutrients and should be consumed regularly in small amounts in the diet. The vitamin deficiencies can cause important health problems so enrichment of the foods with vitamins can help to decrease the vitamin deficiencies in the population.

Double emulsions are more adequate for entrapment of the valuable compounds than the single emulsions because they have a specific structure containing two different compartments. Droplets of the primary emulsion can behave as a reservoir for entrapment of the desired compounds by the double emulsion.

The primary objective of this research was the encapsulation of water soluble compounds inside the inner aqueous phase of W/O/W type double emulsion and transferring it to a food product for the enrichment. In this manner, it was aimed to decrease the amount of PGPR in the production of food based double emulsions by replacing it with lecithin due to the limitation of PGPR in the food industry.

Coating of O/W emulsion by sodium caseinate and gum arabic was also tried to be able to prepare stable emulsion using only lecithin.

In the production of double emulsions, hazelnut oil was used as oil phase of W/O/W type double emulsions. Turkey is the leading producer and exporter of hazelnuts (FAOstat, 2010). The fatty acid profile of the hazelnut oil is similar to the olive oil profile (Yalcin et al., 2012). As a result, the incorporation of hazelnut oil in double emulsion study is an interesting research, which can affect the consumer in terms of the healthy aspects.

Another aim of the study was to study the effects of different homogenization methods on the characteristics of double emulsion. Different homogenization methods were applied to produce emulsions and the one resulting in the longest stability with the smallest droplet size was determined to obtain double emulsions in which water soluble vitamins were entrapped.

It was also aimed to study the encapsulation properties of the prepared double emulsion. At this stage, firstly Vitamin B_{12} was used as a marker due

to the stability of the vitamin against enviromental conditions such as heat and oxidizing agents. After that, the marker vitamin was replaced with a sensitive compound, Vitamin B_1. In literature, there is no research in which Vitamin B_1 was encapsulated via W/O/W type double emulsion including PGPR and lecithin combination as primary emulsion surfactant.

Enrichment of different food materials with encapsulated Vitamin B_1 was another objective of this study. Bread and carrot juice were chosen as food products for the enrichment. The reason of choosing bread as a target food product was not only its being an extensively consumed product in our country, but also to analyze the protective effect of specific double emulsion structure for vitamin loss during baking. Carrot juice was another target food product to observe the effects of W/O/W type double emulsion on pH sensitive Vitamin B_1.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

In the current research, edible refined hazelnut oil was used as oil phase of the double emulsions. The mentioned hazelnut oil was purchased from the local market in Ankara (Çotanak, Ankara, Turkey). PGPR was used as lipophilic surfactant and it was supplied by a chocolate factory (ETİ Gıda San. ve Tic. A.Ş., Eskişehir, Turkey). Lecithin was obtained from Lipoid GmbH (Lipoid® S 75, Ludwigshafen, Germany). Vitamin B_{12}, Vitamin B_1, Gelatin (Bloom 180), sodium caseinate (NaCN) and sodium azide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All of the experiments were conducted with distilled water and the other compounds used in the experiments were of analytical grade.

2.2 W/O/W Type Double Emulsion

W/O/W type double emulsions were prepared according to the two-step emulsification method of Fechner et al. (2007) with some modifications.

2.2.1 Preparation of W/O Type Primary Emulsions

Different amounts of surfactants (PGPR, lecithin or PGPR-lecithin mixture), which were represented in Table 2.1, were dissolved in hazelnut oil by means of High Speed Homogenizer (IKA T25 Digital Ultra-Turrax,

Selangor, Malaysia) at 10,000 rpm for 1 min. Then, oil phase containing lipophilic surfactant was kept at 50°C in water bath (GFL 1086, Burgwedel, Germany) for 15 min. NaCl (0.6 g/100 g) and sodium azide (0.02 g/100 g) were dissolved in distilled water to produce inner aqueous phase. Surfactant containing oil phase and inner aqueous phase were mixed at different ratios by high speed homogenization at 18,000 rpm for 7 min to produce the primary emulsion for dilution test. Primary emulsions were prepared at different oil-water ratios (Table 2.1).

Table 2.1: Experimental design for dilution test

Surfactant Type	Surfactant Concentration (g /100 g emulsion)	Phase Ratio (g aqueous/g oil)
Lecithin	8	40/60
Lecithin	5	40/60
Lecithin	2	40/60
Lecithin	1	40/60
Lecithin	5	20/80
Lecithin	2	20/80
Lecithin	1	20/80
Lecithin	3	80/20
Lecithin	6	60/40
PGPR	2	20/80
PGPR	2	40/60
PGPR	3	40/60
Lecithin & PGPR	1.5 & 0.5	40/60
Lecithin & PGPR	1.5 & 1.0	40/60
Lecithin & PGPR	1.5 & 1.5	40/60
Lecithin & PGPR	2.0 & 1.5	40/60
Lecithin & PGPR	0.5 & 1.5	40/60
Lecithin & PGPR	1.5 & 2.5	40/60

Dilution test was applied to determine the type of the obtained primary emulsion whether it is oil-in-water (O/W) or water-in-oil (W/O) type. The method of dilution test is a quick method which depends on the miscibility.

A few drops of the prepared emulsion were splattered into two beakers, one containing the oil phase and the other containing the aqueous phase solution. Then the miscibility of the drops was observed. If they were dispersed in the oil phase, this was a W/O emulsion because easy dispersion could perform only in continuous phase (Vermeir et al., 2014).

After determination of the best surfactant type and oil-water ratios of primary emulsion, three different homogenization methods as High Speed Homogenization (HSH), Ultrasonic Homogenization (US) and Microfluidization (MF) were applied to produce the primary emulsion.

Oil phase containing lipophilic surfactants was kept at 50°C in water bath for 15 min. NaCl (0.6 g/100 g) which was used as osmotic pressure balancer between the phases and sodium azide (0.02 g/100 g) which was a microbial inhibitor were dissolved in distilled water to produce inner aqueous phase. Oil phase and inner aqueous phase were homogenized with the mentioned three different homogenization methods for the production of primary emulsion.

In the case of US (Sonic Ruptor 400, OMNI International the Homogenizer Company, Kennesaw, GA, USA) treatment, the mixture was firstly homogenized by HSH (15,000 rpm for 5 min), then treated by US at 160 W power, 20 kHz frequency and with 50% pulse for 15 min. In the case of MF (M-110Y, Microfluidics, USA) treatment, the mixture was firstly homogenized by HSH (15,000 rpm for 5 min), then 103 MPa pressure was applied for one cycle.

2.2.2 Preparation of W/O/W Type Double Emulsion

Primary emulsion formation was the first step of preparation of double emulsion. The second step was the mixing of prepared primary emulsion with outer aqueous phase as a continuous phase with the help of a hydrophilic surfactant in order to form W/O/W double emulsion. In order to adjust the osmotic pressure balance between the aqueous phases, 0.6 g/100 g NaCl was added to both the inner and outer aqueous phases of the system (Hemar et. al., 2010). The outer aqueous phase was prepared by the addition of NaCN (11%, w/w) dropwise to the solution while mixing with Magnetic Stirrer (MR 3,001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany), and then stirred for at least 2 h. The stirred solution was left overnight at room conditions to ensure the complete dissolution of outer aqueous phase.

In order to obtain the double emulsion, hydrophilic surfactant containing outer aqueous phase and primary emulsion were mixed with two different mixing methods as Magnetic Stirrer (MS) and Home Type Food Processor (Arçelik Robolio, Turkey). The primary emulsion was added to outer aqueous phase gradually in 5 min and then mixed for 15 min in the case of MS, performing at 1000 rpm. In order to obtain the double emulsion by Home Type Food Processor (HTFP), outer aqueous phase was mixed for 1 min by adjusting the power of food processor at level 2, and primary emulsion was added to outer aqueous phase gradually in 2 min and then further mixed for 3 min at the same speed.

2.2.3 Determination of Double Emulsion Characteristics

After the production of double emulsion, the obtained double emulsion was analyzed by optical microscopy, droplet size distribution, stability and encapsulation properties.

2.2.3.1 Optical Microscopy Analysis

To check if the double emulsion was formed, the prepared system was observed under the microscope (Zeiss, Primo Vert, Jena, Germany) and analyzed by TopView software program. The obtained double emulsion was diluted with outer aqueous phase prior to the microscopic analyses to increase the visuality. The images of the emulsions were taken at magnification factor of ×40.

2.2.3.2 Droplet Size Distribution

Distribution of primary emulsion droplets inside the outer aqueous phase was determined by a particle size analyzer (Mastersizer 3000, Malvern Instruments, Worcestershire, UK). Refractive index of hazelnut oil which was 1.4675, absolute index of 0.01, obscuration range of 8-25% and mixing value of 2600 rpm were set. For each sample, triplicate measurements were done. Droplet characteristics of the system were expressed as D[4,3], representing the volume weighted mean droplet size (Eq. 1), span, representing the width of the distributed emulsion (Eq. 2), and specific surface area (SSA) (Elversson et al., 2003).

$$D[4,3] = \sum(ni.di^4)/\sum(ni.di^3) \quad [1]$$

where, n is the number and d is the diameter of droplets, μm.

$$Span=[d(v,90)– d(v,10)]/d(v,50) \quad [2]$$

where, d(v,90), d(v,50) and d(v,10) are diameters at 90%, 50% and 10% of the cumulative volume.

2.2.3.3 Stability of Double Emulsion

Stability of the prepared double emulsions was evaluated by two different approaches as instant stability (sedimentation rate) and storage stability (phase separation rate).

2.2.3.3.1 Instant Stability

In order to analyze the sedimentation rate, the prepared double emulsions were centrifuged (Hettich Mikro 200/200R, Sigma Laborzentrifugen GmbH, Germany) at 5,000 rpm for 15 min. The height of the phase separation was used to calculate the sedimentation rate (Eq. 3) which can be considered as instant stability of the double emulsions.

$$S_c=(h_c /h_0)\times 100 \quad [3]$$

where, h_c is the height of upper phase, cm
h_0 is the height of solution before centrifugation, cm
S_c is sedimentation rate (instant stability), %.

2.2.3.3.2 Storage Stability

In order to analyze the phase separation rate, prepared double emulsions were stored at 30°C and the separated height was measured at specific time intervals as 24 h and 48 h. The measured height was used to calculate the phase separation rate (Eq. 4) which can be considered as storage stability of the double emulsions.

$$S=(h_c /h_0)\times 100 \quad [4]$$

where, h_c is the height of the upper phase, cm
h_0 is the initial height before the storage, cm
S is phase separation rate (storage stability), %.

2.2.3.4 Encapsulation Stability and Efficiency

Encapsulation stability of the double emulsion depends on the amount of vitamin released from the inner aqueous phase to the outer aqueous phase during storage, so storage conditions were important parameters for encapsulation stability analyses. On the other hand, encapsulation efficiency mainly depends on the amount of entrapped vitamin in the production of double emulsion (O'Regan & Mulvihill, 2009).

In this study, two different approaches were used to determine the encapsulation characteristics of the double emulsion which were based on the measurement of the vitamin concentration of outer aqueous phase and inner aqueous phase.

2.2.3.4.1 Determination of Vitamin B_{12} Concentration in Outer Aqueous Phase

In this approach, encapsulation efficiency and stability of the double emulsion was determined based on the vitamin concentration of the outer aqueous phase. It was calculated after 0, 24 and 48 h of storage at 45°C as accelerated shelf life test conditions.

Primary emulsion was prepared by mixing of 1.5% (w/w) lecithin and 1.5% (w/w) PGPR containing oil phase with 0.3% (w/w) Vitamin B_{12} containing aqueous phase by using HSH for 7 min at 18,000 rpm. High concentration of vitamin was used to facilitate the determination of amount of vitamin. Prepared primary emulsion (40%, w/w) was added gradually into the outer aqueous phase (60%, w/w) by mixing them into the home type food processor to obtain double emulsion.

The prepared double emulsion was centrifuged at 5000 rpm for 15 min at 20°C in order to separate the primary emulsion and the outer aqueous phase. The outer aqueous phase, collected at the bottom of the centrifugation tube during the centrifugation, was taken and diluted with distilled water with a dilution rate of 1:2.

The critical step of this approach was the removal of the NaCN from the system because NaCN resulted in the turbidity, which was a big problem for the spectrophotometric measurement. The proteins were removed by the addition of trichloroacetic acid solution (20%, w/w) with a dilution rate of 1:4. After dilution with acidic solution, the sample was centrifuged at 5000 rpm for 15 min at 20°C to remove the precipitates.

The vitamin concentration was determined by measuring absorbance using a UV-visible spectrophotometer T 70 (PG Instruments LTD, UK) at 361 nm (O'Regan & Muhvihill, 2009). Calibration curve (R^2 =0.999) was prepared with different Vitamin B_{12} concentrations (0, 1, 3, 5 ve 10 mg/100 g) dispersed in the outer aqueous phase (Fig. A.1). The measured vitamin concentration was used to determine the encapsulation efficiency and stability (Eq.5) of the double emulsions.

$$\text{Encapsulation } (E_{time}) = [(M_i - M_o)/M_i] \times 100 \quad [5]$$

where, E_{time} represents the encapsulation efficiency or stability, %

M_o is the vitamin concentration found by spectrophotometry measurement in the outer aqueous phase, mg/ml

M_i is the initial vitamin concentration added to the inner aqueous phase, mg/ml.

2.2.3.4.2 Determination of Vitamin B_{12} Concentration in Inner Aqueous Phase

In this approach, encapsulation efficiency of the double emulsion was determined based on the measurement of entrapped vitamin concentration in the inner aqueous phase. The critical step of this approach was to ensure the complete separation of the phases of double emulsion into primary emulsion and outer aqueous phase because the sample was taken from the separated primary emulsion part.

Primary emulsion was prepared by mixing 1.5% (w/w) lecithin and 1.5% (w/w) PGPR containing oil phase with 0.3% (w/w) Vitamin B_{12} containing aqueous phase by using HSH for 7 min at 18,000 rpm. Prepared primary emulsion (40%, w/w) was added gradually into the outer aqueous phase (60%, w/w) by mixing them using home type food processor to obtain the double emulsion.

After centrifugation of double emulsion at 5000 rpm for different times (5-30 min) at 20°C, the primary emulsion was collected as supernatant. The sample (1.0 ml) was taken from the supernatant and diluted with a ratio of 1:9. The diluted primary emulsion was disrupted by the application of HSH at 15,000 rpm for 5 min. The disrupted system was centrifuged at 5000 rpm for 15 min at 20°C in order to remove the oil phase. The purified inner aqueous phase from the oil phase was diluted again with dilution ratio of 1:4. Then, it was centrifuged at 5000 rpm for 5 min at 20°C to get rid of the remaining oil phase.

The samples were filtered using 0.45 μm filter paper. Vitamin concentration was determined by measuring absorbance of the filtrate using UV-visible spectrophotometer T70 at 361 nm. Distilled water was used as the blank. Calibration curve (R^2 =0.999) was prepared with different Vitamin B_{12}

concentrations (0, 1, 2, 4 ve 6 mg/100 g) dispersed in the distilled water (Fig. A.2). The measured vitamin concentration was used to determine the encapsulation efficiency (Eq.6) of the double emulsions.

$$\text{Encapsulation } (E_{time}) = (M_f / M_o) \times 100 \quad [6]$$

where, E_{time} represents the encapsulation efficiency, %
Mf is the remaining vitamin found by spectrophotometry, mg/ml
Mo is the vitamin initially added to the primary emulsion, mg/ml

2.3 Coating of O/W Type Single Emulsion

In order to coat O/W emulsion, firstly simple O/W type emulsion was prepared. Then, it was dissipated into coating solution.

2.3.1 Preparation of O/W Type Simple Emulsion

Variable amount of lecithin (3%, 5% and 8%, w/w) was dissolved in hazelnut oil and homogenized by HSH to dissipate the surfactant into oil phase. Surfactant containing oil phase was kept at 50°C in water bath for 15 min. 0.6 g/100 g NaCl and 0.02 g/100 g sodium azide were dissolved into distilled water to produce aqueous phase. Surfactant containing oil phase (60 g/100 g) and aqueous phase (40 g/100 g) were homogenized by two different methods which were HSH and US for the production of O/W type simple emulsion. After the application of homogenization methods, the type of the emulsion was checked by dilution test.

In the HSH, the solutions were homogenized at 15,000 rpm for 7 min. In the US, the solutions were firstly homogenized by HSH (15,000 rpm-3 min), then by US at 160 W power, 20 kHz frequency and with 50% pulse for

different exposing times (10, 15 and 20 min) to produce O/W type simple emulsion.

2.3.2 Coating of O/W Emulsion by Sodium Caseinate

O/W type simple emulsion production was the first step of the coating system. The second step was the dissipating of prepared simple emulsion into NaCN solution.

In order to adjust the osmotic pressure balance between the aqueous phase of simple emulsion and coating solution, 0.6 g/100 g NaCl was also added to the coating solution. The coating solution was prepared by adding of NaCN to the solution when it was being mixed with Magnetic Stirrer (MS) and then stirred for at least 2 h. The stirred solution was stored overnight at room conditions to ensure the complete dissolution. In order to analyze the effect of NaCN concentration, different caseinate concentrations (7%, 9% and 13%, w/w) were studied.

O/W type simple emulsion was dissipated into coating solution by MS. The mixing ratio was 40% (w/w) emulsion to 60% (w/w) coating solution. O/W type emulsion was added to coating solution gradually in 5 min when the coating solution was being mixed by MS. After the complete addition of emulsion to the coating solution, the system was mixed for 5 min at 1000 rpm to obtain the coated emulsion.

2.3.3 Coating of O/W Emulsion by Gum Arabic

In order to study the possibility of the coating of O/W type emulsion with gum arabic solution, different gum arabic concentrations were studied as 10%, 15%, 20% and 25%. Gum arabic coating solutions were prepared by

dissolving of gum arabic in the distilled water containing 0.6 g/100 g NaCl and 0.02 g/100 g sodium azide. The obtained O/W emulsion was dissipated into gum arabic coating solution by MS.

2.3.4 Determination of Coated Emulsion Characteristics

Distribution of O/W type emulsion droplets inside coating solution were analyzed with the same procedure as mentioned in the Section 2.2.3.2 with little modification. The mixing value of the particle size analyzer was set 2600 rpm for double emulsion measurements while it was set 1600 rpm for the coating systems.

The method for determining of the instant stability of the coating system was the same with the procedure given for the double emulsion as mentioned in the Section 2.2.3.3.1. In order to analyze the storage stability of the coating emulsion systems, the same procedure for double emulsion was applied as mentioned in the Section 2.2.3.3.2 with little modification. Coated O/W type emulsions were stored at 45°C for 2 days as accelerated shelf life method.

2.3.5 Determination of Vitamin B_{12} Concentration in Coated O/W Type Emulsion

In order to study the encapsulation efficiency and stability of the coated O/W type simple emulsion with the caseinate solution, the O/W type simple emulsion was prepared by dissolution of 5% (w/w) lecithin into the oil phase by the application of HSH treatment. Then, it was coated by 11% (w/w) NaCN solution. The amount of the vitamin passed from the aqueous phase of O/W emulsion to the coating solution was also measured with the same procedure as mentioned in the Section 2.2.3.4.1. The results were compared with the double emulsion system.

2.4 Replacement of Encapsulated Vitamin B_{12} with Vitamin B_1

After analyzing the encapsulation efficiency and stability of double emulsion containing water soluble marker (Vitamin B_{12}), the marker was replaced with more sensitive water soluble compound Vitamin B_1. Double emulsions were prepared as mentioned in the Section 2.2.3.4.2 but 3% (w/w) Vitamin B_1 was dissolved into the aqueous phase solution instead of the Vitamin B_{12}. Encapsulation properties of the double emulsion with Vitamin B_1 were analyzed according to the inner aqueous phase vitamin concentration.

2.4.1 Determination of the Effect of Temperature on Vitamin B_1

In order to study the influence of temperature on the degradation of Vitamin B_1 present in the inner aqueous phase of double emulsion, the prepared double emulsion were kept in water bath at different temperatures (30, 45, 60, 75 and 90°C) for 20 min. After exposing to different temperatures, double emulsions were stored at room conditions for 30 min to cool down. In order to calculate the vitamin amount of primary emulsion, firstly the oil phase was separated by centrifugation at 5000 rpm for 20 min at 20°C. The purified inner aqueous phase from the oil phase was diluted again at a ratio of 1:4 and centrifuged at 5000 rpm for 5 min at 20°C to get rid of the remaining oil phase.

Vitamin B_1 concentration was determined by- Thermo Scientific Finnigan Surveyor HPLC equipped with a Autosampler Plus, LC Pump Plus and UV-VIS Plus Detector (San Diego, CA). The mobile phase was prepared by mixing buffer solution and methanol at a ratio of 96:4 (w/w). Buffer solution was the aqueous solution of hexane sulfonic acid sodium salt, potassium dihydrogen phosphate and triethylamine with a pH value of 3.0. pH value of the buffer solution was adjusted by ortophosphoric acid.

The used column was VARIAN Chromsper5 C18 HPLC Column (150 mm×4.6 mm I.D., 5 μm; MerckKGaA, Darmstadt, Germany) and column temperature was 35°C. Mobile phase flow rate was adjusted 1 $mL.min^{-1}$ and 20 μL sample was injected for the measurement. Samples were run in duplicate.

Calibration curve (R^2 =0.992) was prepared by using aqueous Vitamin B_1 solutions at different concentrations (0, 1.25, 2.50, 5.00 ve 7.50 mg/100 g) (Fig. A.3). The measured vitamin concentration was used to determine the encapsulation efficiency.

2.4.2 Addition of Encapsulated Vitamin B_1 to Bread

The encapsulated Vitamin B_1 within the W/O/W type double emulsion was added to the dough and the amount of vitamin in the baked bread was calculated to analyze the influence of baking conditions on the vitamin loss. As a control, Vitamin B_1 was added directly to the dough. For comparison, primary emulsion containing vitamin was also transferred to the bread dough.

Dough was prepared according to the hamburger bread formulation which contains 100% flour, 8% sugar, 6% milkpowder, 2% salt, 3% yeast, 8% oil and 55% water on flour weight basis (Ozkoc, 2008). The oil composition of the bread was kept constant at 8 %. In order to add the vitamin directly to the dough, vitamin was mixed with the flour.

Firstly dry ingredients were mixed. Then yeast, dissolved into water, and oil or emulsions was added and all the ingredients were mixed by mixer (KitchenAid, 5 K45SS, USA) for 4 min. The prepared dough was placed into incubator (Nüve EN 400, Turkey) at 30°C for 125 min for fermentation. After the fermentation, it was baked in conventional oven (Arçelik, Turkey) at 200°C for 13 min (Ozkoç, 2008).

After baking, the breads were stored at room temperature at least 1 h to decrease the temperature of the breads. The sample was taken from bread crumb and dissolved in distilled water. It was homogenized by HSH at 15,000 rpm for 5 min. In order to get rid of the residues of the breads, it was centrifuged at 5000 rpm for 10 min at 20°C. The supernatant was diluted with distilled water and vitamin concentration was measured by HPLC.

2.4.3 Determination of the Effect of Slightly Alkali or Neutral pH on Vitamin B_1

In order to analyze the effect of medium pH on the Vitamin B_1 degradation, 3% (w/w) vitamin was added to the inner aqueous phase solution and the solution pH was adjusted to different values (4.2, 5.5, 6.0, 7.0, 8.0 and 9.0).

In order to adjust the pH value of the solution having the same ingredients as inner aqueous phase, 0.1 M NaOH and/or 0.1 M HCl was added to the solution. After pH was adjusted, double emulsion (10 %, w/w) was added to medium drop by drop in 3 min and then mixed by MS for 1 min at 7,500 rpm. The double emulsion added solution was stored at room conditions for 2 h. After the storage, the solution was homogenized by HSH at 15,000 rpm for 3 min and centrifuged at 5000 rpm for 15 min at 20°C. The supernatant was diluted with distilled water and vitamin concentration was measured by HPLC.

2.4.4 Enrichment of Carrot Juice with Encapsulated Vitamin B_1

Vitamin B_1 is a pH sensitive vitamin. In order to study the influence of double emulsion on the protection of this vitamin against pH of the medium, Vitamin B_1 containing double emulsion was mixed with carrot juice.

Double emulsion containing 3% (w/w) Vitamin B_1 was added to the carrot juice while it was being mixed by MS at 7,500 rpm. Double emulsion was added (10 %, w/w) to juice drop by drop in 3 min and then mixed for 1 min at the same conditions. The emulsion added juice was stored at refrigerator temperature (4°C) for 2 days and vitamin amount of the juice was measured at specific time intervals (0, 12, 24, 36 and 48 h). After the storage, the carrot juice was homogenized by HSH at 15,000 rpm for 3 min and centrifuged at 5000 rpm for 15 min at 20°C. The supernatant was diluted with distilled water and vitamin concentration was measured by HPLC. Direct addition of vitamin to the carrot juice was also studied as a control to analyze the influence of the double emulsion system on the degradation of vitamin.

2.5 Statistical Analysis

Analysis of variance (ANOVA) was conducted for the determination of differences between independent variables by using SAS Software Version 9.1 (SAS Institute Inc., NC, USA). If significant difference was found, Duncan's Multiple Comparison Test was used for comparison ($p \leq 0.05$). All the results represent the means of two replications. Statistical evaluations were reported in the appendix part (Appendix B).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 W/O/W Type Double Emulsion

3.1.1 Effects of Surfactant Type on Type of Primary Emulsion

It is very common to use PGPR for the primary emulsion surfactant of W/O/W type double emulsion, but there is a limitation for the intake of PGPR. The maximum amount of the allowed intake dosage of PGPR is 2.6 mg/kg of body weight per day (Wilson et al., 1998). Therefore, this synthetic emulsifier should not be used in high amount in food systems. On the other hand, for lecithin usage in the food industry there is no limitation as the maximum level is defined as good manufacturing practices (CODEX, 2013). No research has been performed so far in which PGPR was combined with lecithin for the production of double emulsion system. Therefore, one of the substantial aim of the research was the replacement of PGPR with a suitable food base surfactant, lecithin.

Water in oil (W/O) type emulsion could not be produced when PGPR was replaced completely by lecithin as primary emulsion surfactant. Dilution test results showed that lecithin could produce very stable but only oil in water (O/W) type emulsion as can be seen from Table 3.1. However, in the current research W/O type emulsion was necessary to encapsulate the water soluble vitamin in the inner aqueous phase of double emulsion. For this purpose, different phase ratios and lecithin concentrations were studied to analyze the effect of them. It was found that only phase ratio of 40/60 (%, w/w) could

produce O/W type emulsion. Lecithin could not function when the phase ratio was 20/80 (%, w/w) and as a result cloudy suspensions were obtained. On the other hand, no matter what the phase ratio or the surfactant concentration was in the system, usage of PGPR resulted in W/O type emulsion. Therefore, the mixture of the PGPR and lecithin was decided to be used.

As represented in the Table 3.1, it was realized that both of the surfactants were very powerful but in different function. PGPR produced W/O; but lecithin produced O/W type primary emulsion. Depending on the ratio of the lecithin to PGPR, functionality of the surfactants was modified and the type of the emulsion was determined. When lecithin to PGPR ratio was 1:1, 1:3 or 3:5, W/O type emulsion could be obtained. As can be seen from Table 3.1, the ratio 4:3, which refers to surfactant mixture of 2 g/100 g lecithin and 1.5 g/100g PGPR produced O/W type emulsion.

The stability mechanisms of O/W and W/O type emulsions are different from each other. O/W type emulsions are stabilized by the shared effect of steric and electrostatic repulsion while for W/O type emulsions, the dominant force is only the steric force due to the low electric conductivity of the continuous phase (Claesson et al., 2004). Therefore, the obtained type of the emulsion might be related to different action of surfactants with the electrodes (Mishchuk et al., 2004) since electrode concentration influences the hydrophilic/lipophilic balance and the hydration conditions of the surfactants (Kawashima et al., 1992). In addition, phospholipids provide an electrostatic repulsion barrier to the emulsion droplet (Rydhag, 1979) so the presence of NaCl could have different influence on the lecithin plus PGPR containing systems.

Table 3.1: Dilution test for determining the type of primary emulsion

Surfactant Type	*Surfactant Concentration*	*Phase Ratio*	*Type of Emulsion*	*Stability*
Lecithin	8	40/60	O/W	+
Lecithin	5	40/60	O/W	+
Lecithin	2	40/60	O/W	+
Lecithin	1	40/60	O/W	+
Lecithin	5	20/80	*	-
Lecithin	2	20/80	*	-
Lecithin	1	20/80	*	-
Lecithin	3	80/20	O/W	+-
Lecithin	6	60/40	O/W	+-
PGPR	2	20/80	W/O	+
PGPR	2	40/60	W/O	+
PGPR	3	40/60	W/O	+
Lecithin & PGPR	1.5 & 0.5	40/60	O/W	+
Lecithin & PGPR	1.5 & 1.0	40/60	O/W	+
Lecithin & PGPR	1.5 & 1.5	40/60	W/O	+
Lecithin & PGPR	2.0 & 1.5	40/60	O/W	+
Lecithin & PGPR	0.5 & 1.5	40/60	W/O	+
Lecithin & PGPR	1.5 & 2.5	40/60	W/O	+

"*"	no clear dissolution of prepared sample into both of the phases
"+"	production of stable emulsion
"-"	sedimentation after 30 min and production of cloudy suspension
"+-"	separation of phases in 3 hours storage

Thakur et al. (2007) used lecithin as surfactant and showed that both type of the simple emulsion: O/W or W/O could be produced by changing the salt concentration of the medium. Therefore, salt concentration has an important factor influencing the relationship between the functionality of the surfactant and the produced type of the emulsion. On the other hand, the presence of the electrode was found to be necessary for the stability of the multiple emulsions (Kanouri et al., 2002). Spaei et al., (2012) and Scherze et al., (2006) found that the presence of salt played a key role in the stability and the encapsulation property of double emulsion.

3.1.2 Effects of Second Step Homogenization Methods and Surfactant Concentrations on Double Emulsion Characteristics

The researchers studying on double emulsion to encapsulate the valuable compounds generally used NaCN in outer aqueous phase (Cofrades et al., 2014, Bou et al., 2014). Many researchers dealing with the production of W/O/W emulsion concentration used very low concentrations of NaCN such as 0.5% (Su et al., 2008) and 1% (O'Reagan & Mulvihill, 2009). However, the preliminary studies showed that NaCN concentration was very critical for the production of stable double emulsions. Desired emulsion characteristics could not be achieved with the concentrations below 11% (g NaCN/100 g outer aqueous phase solution) so the outer aqueous phase concentrations of 11% and 15% (w/w) were studied.

Table 3.2 shows the effect of different homogenization methods in the second step of double emulsion preparation. Double emulsion could not be obtained by the application of HSH because this homogenization mechanism was not suitable for the double emulsion production. In addition, MS was not able to mix the emulsion which was very viscous due to the high concentration of NaCN.

Table 3.2: Effect of second step homogenization methods and surfactant concentration on double emulsion formation

Treatment	Surfactant Concentration (%)	Remarks
Magnetic Stirrer	11	W/O/W type double emulsion could be obtained
	15	Homogeneous emulsions could not be obtained because the surfactant concentration was very high and so maximum power of the stirrer failed to mix the double emulsion system properly
Home Type Food Processor	11	W/O/W type double emulsion could be obtained
	15	W/O/W type double emulsion could be obtained
High Speed Homogenizator	11	Homogeneous emulsions could not be produced with low process conditions (between 5,000-8,000 rpm). High process conditions (between 9,000-13,000 rpm) disrupted the primary emulsion and produced O/W type emulsion
	15	Homogeneous emulsions could not be produced with low process conditions (between 5,000-8,000 rpm). High process conditions (between 9,000-13,000 rpm) disrupted the primary emulsion and produced O/W type emulsion

Double emulsions were generally obtained by the two-step emulsification method by the application of conventional equipment like high pressure homogenizer, but membrane emulsification (Vladisavljevic et al., 2003) and microchannel emulsification (Kobayashi et al., 2005) were also applied. In the current research, HTFP and MS were used as the second step of double emulsion preparation. They were compared in terms of stability and droplet size of the prepared double emulsions. Perrechil & Cunha (2013) applied MS for the second step of multilayer emulsion preparation. However, in literature no research including the application of HTFP was available.

Instant stability results showed that there was no significant difference between different homogenization methods ($p>0.05$, Table B.1). However, double emulsions prepared by HTFP were about 5% more stable than the ones prepared by MS after24 h (Fig. 3.1).

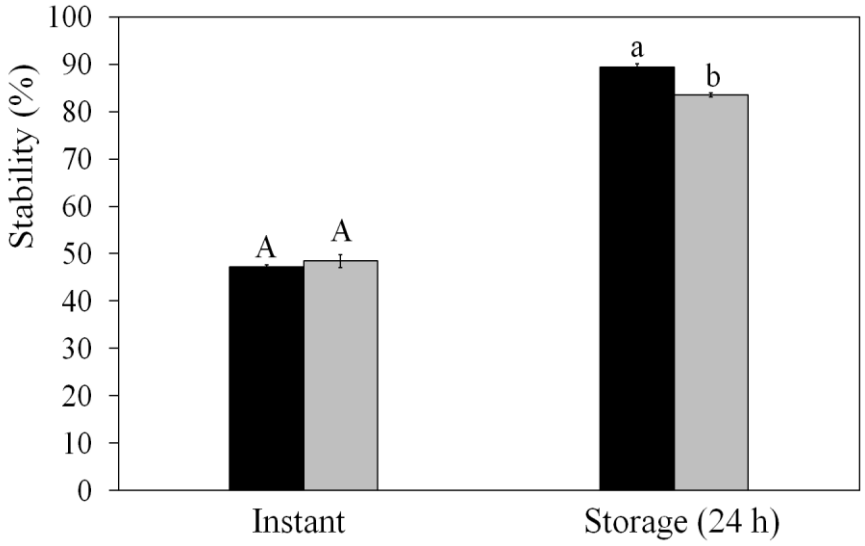

Figure 3.1: Comparisons of stability values of double emulsions produced by MS: (■) and HTFP: (■).Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p\leq0.05$).

Droplet size results showed there was a significant difference between the droplet properties of the emulsions produced with different homogenization method (Table B.3). HTFP produced smaller droplets than the MS. SSA of the primary emulsion droplets produced by HTFP was 5 times higher than the ones produced by MS (Table 3.3).

The droplet size distribution of the double emulsions prepared with different homogenization methods showed that HTFP application produced a bimodal distribution with ~ 7 – 45 μm range and a small distribution in the range of ~ 0.2 – 0.8 μm. On the other hand, in the emulsion produced by MS the dominant mode was unimodal distribution with ~ 5 – 70 μm range (Fig. 3.2). As a result, the mean diameter decreased with the application of HTFP. This also confirmed that HTFP application produced smaller droplets of primary emulsion into the outer aqueous phase.

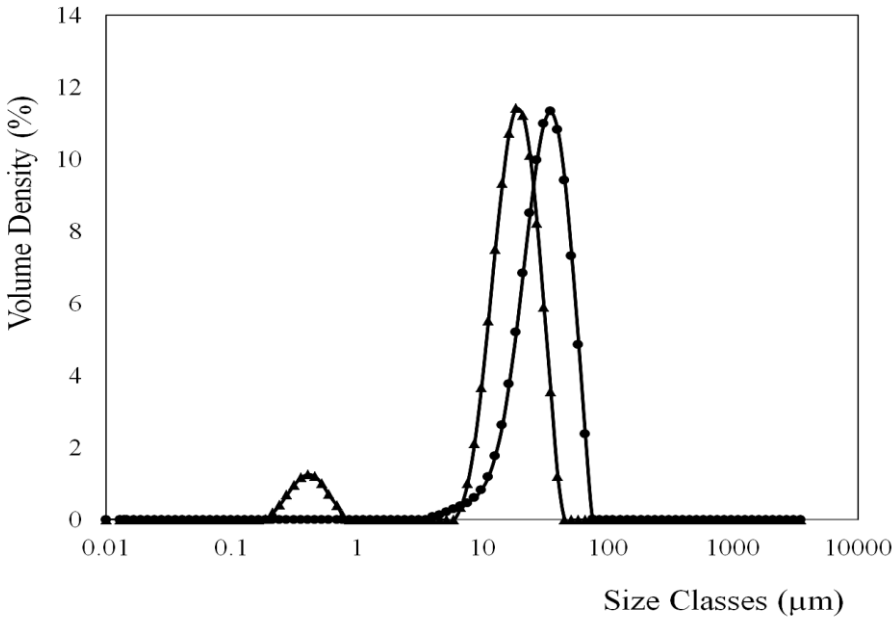

Figure 3.2: Droplet size distribution of double emulsions produced by HTFP (▲) and MS (●).

Therefore, HTFP treatment was selected as the second step of the production of double emulsion in the current research.

Table 3.3: Comparison of homogenization methods in terms of droplet properties

	t=0			t=24 h		
	***D[4,3]* (*μ*)**	***Span***	***SSA* (m^2/kg)**	***D[4,3]* (*μ*)**	***Span***	***SSA* (m^2/kg)**
MS	$35.1\pm0.20^{a,X}$	$1.140\pm0.01^{a,Y}$	$213\pm0.5^{b,Y}$	37.8 ± 1.91^{X}	1.413 ± 1.95^{X}	371 ± 30^{X}
HTFP	$19.5\pm0.15^{b,X}$	$1.195\pm0.00^{a,Y}$	$1481\pm15.0^{a,X}$	18.7 ± 0.05^{Y}	1.294 ± 0.02^{X}	1455 ± 5^{X}

Means within the same column shown by different small letters (a,b) are significantly different ($p\leq0.05$) and means within the same raw shown by different capital letters (X,Y) are significantly different ($p\leq0.05$). Concentration of the NaCN in the outer aqueous phase was 11% (w/w).Values given in this table are means ± standard error of duplicated experiments.

The next step was the determination of NaCN concentration of the outer aqueous phase. NaCN concentrations of 11% and 15% (w/w) were studied and compared in terms of emulsion properties (Table 3.4). As can be seen from the Table 3.4, the double emulsions prepared with 15% (w/w) NaCN concentration could not be separated by the application of the centrifugation force in measurement of the instant stability. It might be related to the higher viscosity of the solution of 15% (w/w) NaCN. Dense suspensions of the NaCN (>100 g/ L) had visco-elastic properties. Their viscosity increased rapidly with the increase in concentration due to the jamming of small caseinate particles (Pitkowski et al., 2008). The increase in NaCN concentration to 15% (w/w) resulted in the increase of the instant stability of the double emulsion since no separation could be achieved by centrifugation. This could cause a problem for the future of the research because the double emulsions must be separable by centrifugation to analyze the encapsulation characteristics.

Table 3.4: Effect of NaCN concentration on double emulsion stability

Concentration (%)	**Instant Stability (%)**	**Storage Stability (%) t=24 h**	**Storage Stability (%) t=48 h**
11	47.1±0.51	89.3±0.51	80.5±0.64
15	100	100	100

Values given in this table are means ± standard error of duplicated experiments.

The increase of the surfactant concentration resulted in more stable double emulsion. As can be seen from the Table 3.4, double emulsions prepared with 11% (w/w) NaCN concentration started to separate into phases within 24 hours storage whereas double emulsion prepared with 15% (w/w) NaCN solution kept its stability.

Table 3.5: Effect of NaCN concentration on droplet properties of double emulsions

	t=0			t=24 h			t=48 h		
Conc. (%)	***D[4,3]* (μ)**	***Span***	***SSA* (m^2/kg)**	***D[4,3]* (μ)**	***Span***	***SSA* (m^2/kg)**	***D[4,3]* (μ)**	***Span***	***SSA* (m^2/kg)**
11	$19.5\pm0.2^{a,X}$	$1.195\pm0.0^{a,Y}$	$1481\pm 15^{a,X}$	18.7 ± 0.1^{Y}	1.294 ± 0.0^{X}	1455 ± 5^{X}	19.1 ± 0.1^{YX}	1.290 ± 0.0^{X}	1388 ± 13^{X}
15	$6.7\pm0.0^{b,X}$	$1.301\pm0.1^{a,X}$	$1242\pm163^{a,X}$	6.2 ± 0.5^{X}	1.522 ± 0.2^{X}	1457 ± 11^{X}	7.0 ± 0.9^{X}	1.342 ± 0.2^{X}	1236 ± 92^{X}

Means within the same column shown by different small letters (a,b) are significantly different ($p\leq0.05$) and means within the same raw shown by different capital letters (X,Y) are significantly different ($p\leq0.05$). Values given in this table are means ± standard error of duplicated experiments.

In terms of the D[4,3] value of the droplets, significant difference was measured between different concentrations of NaCN (Table 3.5). D[4,3] values of double emulsion prepared by lower concentration of NaCN was higher because the produced droplets coalesced together during the production and larger ones were formed due to the insufficient levels of caseinate present in the medium to surround them (O'Dwyer et al., 2013).

Mean droplet size distribution of the primary emulsion into the outer aqueous phase of the solution clearly represented the effect of the outer aqueous phase surfactant concentration on the properties of the double emulsions too. The more concentrated solution provided a distribution of smaller droplets with larger surface area and resulted in the shifting of main peak from ~20 μm to ~5 μm with a monomodal distribution (Fig. 3.3).

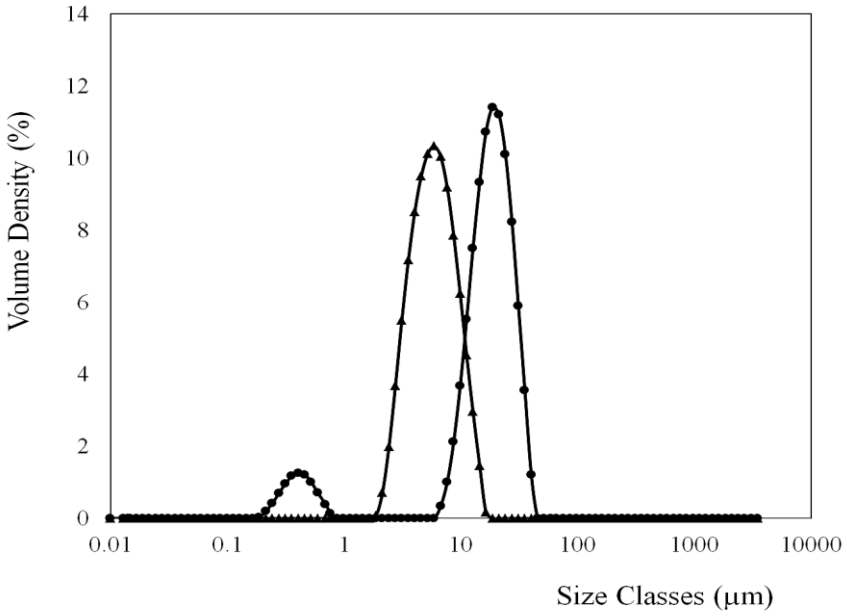

Figure 3.3: Droplet size distribution of emulsions with 11% (w/w) NaCN concentration: (●) and 15% (w/w) NaCN concentration: (▲).

Although 15% (w/w) NaCN containing solution provided more stable double emulsion, 11% (w/w) NaCN concentration was selected for further research. This was due to the impossibility of phase separation of emulsion by centrifugation in the presence of high NaCN concentration which was necessary for further analysis.

3.1.3 Effects of Lecithin-PGPR Combination Ratio on Double Emulsion Characteristics

Since the lecithin itself could not produce the desired type of emulsion, in order to decrease the amount of PGPR, lecithin and PGPR were used together as a mixture surfactant (Table 3.1). The dilution test results also gave information about the limits of the combination ratio. PGPR and lecithin were mixed at different mass ratios as 1:1, 2:3 and 1:3 (lecithin amount: PGPR amount) by keeping the PGPR amount in the primary emulsion system as constant (1.5%, weight basis (w/w)) and varying the lecithin concentration from 0.5 to 1.5 (%, w/w). The effects of the ratio of lecithin:PGPR on the double emulsion characteristics were analyzed and compared with the double emulsions containing only PGPR.

In order to visualize the production of the emulsions, they were observed under the optical microscopy. The microscopy images of the primary and double emulsions are illustrated at the Figure 3.4. The images represent the difference between the primary and double emulsions clearly. As can be seen from Figure 3.4, primary emulsion had two different phases while double emulsion had three different phases located one within the other. This figure also demonstrated that with the convenient combination ratio of lecithin-PGPR for the primary emulsion surfactant and suitable NaCN concentration for the second step surfactant, the desired type of double emulsion (W/O/W) could be obtained.

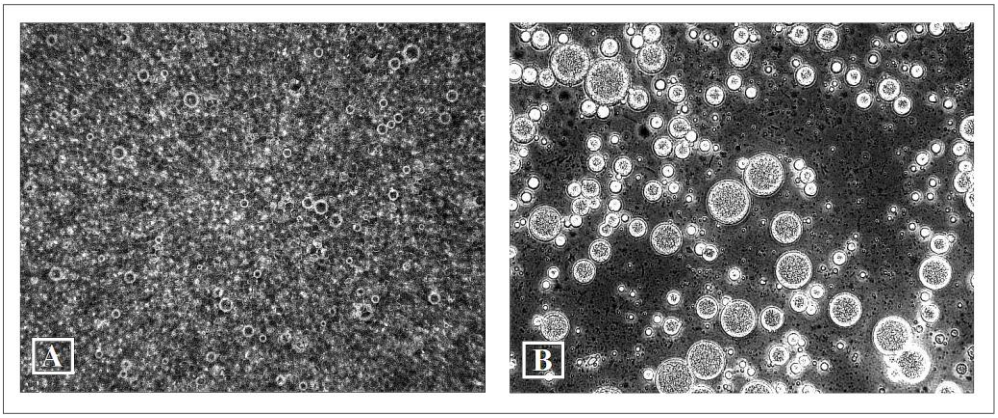

Figure 3.4: Microscopic images of emulsions; A:Primary Emulsion and B:Double Emulsion.

Table 3.6 and Table 3.7 represented the effects of different lecithin:PGPR ratios on the instant and storage stability of the prepared double emulsions; respectively. As the amount of the lecithin in the surfactant mixture increased, more stable double emulsion was obtained.

Table 3.6: Effect of Lecithin – PGPR combination ratio on instant stability of double emulsion

Surfactant	***Surfactant Concentration (g/100 g emulsion)***	***Mixture Ratio***	***Instant Stability (%)***
Lecithin & PGPR	0.5 & 1.5	1:3	47.1 ± 0.51^{dc}
Lecithin & PGPR	1.0 & 1.5	2:3	52.9 ± 1.51^{b}
Lecithin & PGPR	1.5 & 1.5	1:1	56.1 ± 1.69^{a}
PGPR	1.5		44.4 ± 0.11^{de}
PGPR	2.0		42.6 ± 0.79^{e}
PGPR	3.0		48.7 ± 1.41^{c}

Means within the same column having different letters are significantly different ($p \leq 0.05$). Values given in this table are means ± standard error of duplicated experiments.

The comparison of instant stabilities of double emulsions prepared with only PGPR and 0.5 g/100 g lecithin plus 1.5 g/100 g PGPR (1:3 mixture ratio) showed that lecithin could be used in order to decrease the amount of PGPR in the double emulsion system (Table 3.6). Table 3.6 also showed that the surfactant concentration and type of the primary emulsion were important parameters influencing the instant stability of the double emulsion ($p \leq 0.05$).

As can be understood from Table 3.7, no significant difference was determined between storage stabilities of only PGPR (1.5 %, 2.0 % and 3.0 %, w/w) containing and less amount of lecithin (0.5 % lecithin plus 1.5 % PGPR and 1.0 % lecithin plus 1.5 % PGPR) containing double emulsions ($p > 0.05$). However, 1:1 mixture ratio emulsion (1.5 % lecithin plus 1.5 % PGPR (w/w)) produced more stable emulsion. Therefore, the increase in the lecithin amount led to more stable double emulsion system by keeping the PGPR amount as constant (1.5%, w/w).

Table 3.7: Effect of Lecithin – PGPR combination ratio on storage stability of double emulsion

Surfactant	***Concentration (g/100 g emulsion)***	***Mixture Ratio***	***Storage Stability (%) t= 48 h***
Lecithin & PGPR	0.5 & 1.5	1:3	80.5 ± 0.95^{b}
Lecithin & PGPR	1.0 & 1.5	2:3	83.8 ± 0.45^{b}
Lecithin & PGPR	1.5 & 1.5	1:1	93.6 ± 0.53^{a}
PGPR	1.5		80.0 ± 1.26^{b}
PGPR	2.0		80.7 ± 2.00^{b}
PGPR	3.0		82.3 ± 2.05^{b}

Different letters in the column denote difference in the effect of surfactant on the storage stability ($p \leq 0.05$). Values given in this table are means ± standard error of duplicated experiments.

Knoth et al. (2005) reported that lecithin- stabilized W/O emulsions were sensitive to the presence of the other surface-active substance (gelatine, whey protein isolate and xhantan) so the addition of the lecithin might have resulted in stable emulsions by means of decreasing the interfacial tension of phases. Ushikubo & Cunha (2014) also studied on the stability mechanisms of liquid W/O emulsion produced by addition of different surfactants including lecithin and PGPR. They observed that emulsions prepared with PGPR were homogeneous in appearance after the storage of the prepared emulsion for 14 day while the one prepared with lecithin showed a gel-like structure. The same gel structure might have occurred in the current research providing the positive contribution for the stability of the inner aqueous phase of double emulsion.

Although lecithin itself could not produce W/O type emulsion, when it was mixed with a powerful lipophilic surfactant, it enhanced the functionality of the lipophilic surfactant instead of fettering it. This might be explained by the amphiphilic properties of lecithin in such a way that two emulsifiers could coexist in the formulation of double emulsion and could provide positive contribution to the stability properties (Schmidts et al., 2009). The effect of the primary emulsion surfactant type and ratio of lecithin:PGPR on the droplet properties of the obtained double emulsions was also studied. Table 3.8 shows the droplet properties of double emulsion just after the preparation and during storage. It was measured that the type and the amount of surfactant had a significant influence on the D[4,3] values of double emulsions ($p \leq 0.05$, Table B.11). Span values of the double emulsions prepared with only PGPR was about 1.2. When lecithin concentration increased, span values increased to about 1.5. In terms of the SSA, no significant difference was measured between the 1.5% (w/w) lecithin plus 1.5% (w/w) PGPR containing emulsion and 3.0% (w/w) PGPR containing emulsions ($p > 0.05$). However, in the presence of 1.5% (w/w) PGPR increasing lecithin concentration increased SSA (Table 3.8).

Table 3.8: Effect of Lecithin–PGPR combination ratio on double emulsion droplet properties

	t=0			t=24 h			t=48 h		
Surfactant (%, w/w)	**D[4,3] (μ)**	***Span***	***SSA*** **(m^2/kg)**	**D[4,3] (μ)**	***Span***	***SSA*** **(m^2/kg)**	**D[4,3] (μ)**	***Span***	***SSA*** **(m^2/kg)**
PGPR(1.5)&Lecithin(0.5)	$19.5\pm0.2^{c,X}$	$1.195\pm0.0^{b,Y}$	$1481\pm15^{c,X}$	18.7 ± 0.1^{Y}	1.294 ± 0.0^{X}	1455 ± 05^{X}	19.1 ± 0.1^{YX}	1.290 ± 0.0^{X}	1388 ± 13^{Y}
PGPR(1.5)&Lecithin(1.0)	$17.2\pm0.1^{e,X}$	$1.438\pm0.0^{a,X}$	$1545\pm6^{b,X}$	16.6 ± 0.2^{Y}	1.373 ± 0.0^{YX}	1496 ± 27^{X}	17.1 ± 0.1^{X}	1.307 ± 0.0^{Y}	1367 ± 10^{Y}
PGPR(1.5)&Lecithin(1.5)	$16.5\pm0.1^{f,X}$	$1.506\pm0.0^{a,X}$	$1663\pm11^{a,X}$	16.4 ± 0.0^{X}	1.379 ± 0.0^{Y}	1485 ± 13^{Y}	15.7 ± 0.3^{Y}	1.319 ± 0.0^{Y}	1471 ± 17^{Y}
PGPR(1.5)	$23.8\pm0.1^{a,X}$	$1.073\pm0.0^{b,Y}$	$1463\pm2^{c,X}$	22.4 ± 0.4^{Y}	1.246 ± 0.0^{X}	1400 ± 02^{Y}	23.8 ± 0.1^{X}	1.220 ± 0.0^{X}	1324 ± 18^{Z}
PGPR(2.0)	$21.9\pm0.0^{b,X}$	$1.113\pm0.0^{b,Y}$	$1518\pm4^{b,X}$	20.9 ± 0.2^{Y}	1.267 ± 0.0^{X}	1455 ± 06^{Y}	21.6 ± 0.0^{X}	1.247 ± 0.0^{X}	1400 ± 01^{Z}
PGPR (3.0)	$17.8\pm0.3^{d,X}$	$1.166\pm0.0^{b,X}$	$1642\pm22^{a,X}$	16.5 ± 0.4^{X}	1.210 ± 0.0^{X}	1518 ± 30^{Y}	17.3 ± 0.9^{X}	1.294 ± 0.0^{X}	1564 ± 42^{YX}

Means within the same column shown by different small letters (a,f) are significantly different ($p\leq0.05$) and means within the same raw shown by different capital letters (X,Z) are significantly different ($p\leq0.05$). Values given in this table are means ± standard error of duplicated experiments.

One of the expected behavior of the droplets was that droplets would come together during the storage and produce larger ones. However, this could not be observed in the study and droplet size remained constant or decreased. The mentioned constancy of the droplets during storage might be related to the relationships between the emulsifier, droplets and the produced interface by them. McClements (2004) reported that when the droplets were surrounded by a suitable emulsifier membrane, the droplets resisted for the deformation. The decrease in the droplet size during the storage might be due to the phase separation. Droplets might become larger and form a creamy layer. Since the droplet size analysis was performed using the lower layer of emulsion, smaller droplet size was observed.

Khalid et al. (2013) studied the production of W/O/W type double emulsion and observed no change in the average diameters of the oil droplets between 1-35 days. Sapei et al. (2012) found that the average oil phase sizes in W/O/W emulsions, containing salt and gelatin in the inner aqueous phase, did not change for 1 month. Aditya et al. (2015) also did not measure noticable increases in D[4,3] value of the oil droplets inside the double emulsion systems stored for 15 days. The current research results were in accordance with the mentioned studies as the size of the primary emulsion droplets was either constant or decreased slightly during storage.

Bou et al., (2014) studied the effect of storage on the W/O/W emulsion droplet size and reported that globule size did not change during 10 days due to the addition of salt to both of the aqueous phases. In the current research, the same amount of NaCl was added to inner and outer aqueous phases of double emulsion to minimize water transport. This might explain why the droplets of primary emulsion in the double emulsion system did not change with time in the study.

By considering the droplet size and the stability results of the obtained double emulsions, primary emulsion containing 1.5% (w/w) lecithin plus 1.5% (w/w) PGPR was chosen for the further experiment. The next step was to study the effects of homogenization methods in the preparation of primary emulsion on the characteristics of double emulsion.

3.1.4 Comparison of Different Homogenization Techniques Used in Primary Emulsion Preparation on Double Emulsion Characteristics

The most stable double emulsion was obtained when 1:1 mixture ratio of lecithin-PGPR was used (Table 3.7). Three different homogenization methods were applied to produce primary emulsions. To our knowledge no research exists comparing the effect different homogenization methods of primary emulsion preparation on the characteristics of lecithin-PGPR stabilized double emulsions.

Before the comparisons, it was an important point to control whether double emulsions were obtained or not. Therefore, in order to be sure about the production of the double emulsion, the prepared emulsions were observed under the microscopy. Figure 3.5 showed that the inner aqueous phase was surrounded by hazelnut oil droplets that were also dispersed into outer aqueous phase so desirable type primary emulsion and double emulsions were produced by the applications of all homogenization methods. However, it was an important point to mention that among the emulsification method, only HSH treatment could produce primary emulsion directly from the two separate phases, but for US and MF a pre-treatment of HSH was required to produce emulsion.

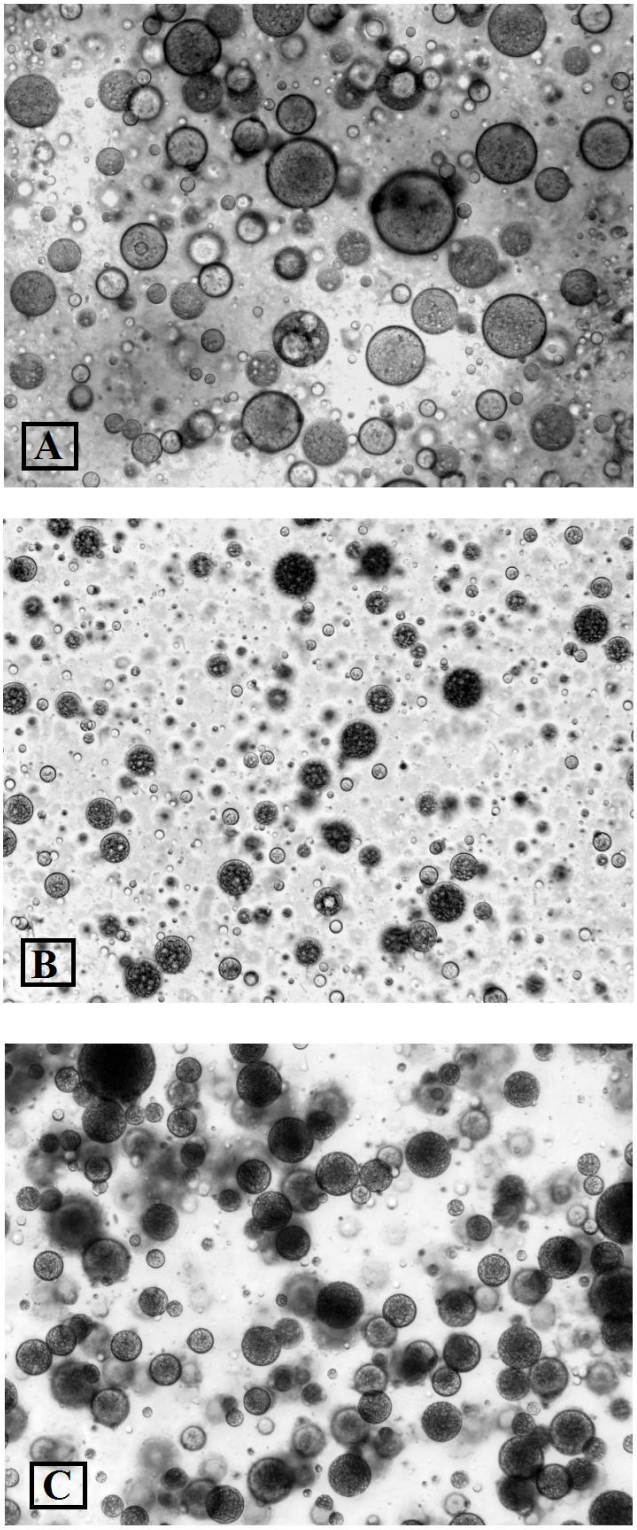

Figure 3.5: Microscopic images of double emulsions prepared by different homogenization methods: MF (A), US (B) and HSH (C).

Figure 3.6 shows the influence of different homogenization methods on the stability of double emulsion. Although no significant difference was observed in the instant stability of double emulsions produced by MF and US ($p>0.05$), they were different in terms of the storage stability ($p\leq0.05$). The most stable emulsion was obtained with the application of HSH. It was followed by emulsions produced by MF and US. Stability value of emulsion produced by HSH was 94% for 48 hours, while that value of emulsions produced by other methods was less than 85%. In the literature, in many of the researches HSH treatment was applied for the production of double emulsion (Sapei et al., 2012; Fechner et al., 2007) while there were limited number of studies about US and MF (Huck-Iriart et al., 2013). This might have a good indicator that HSH could be a simple, convenient and effective method for the double emulsion production.

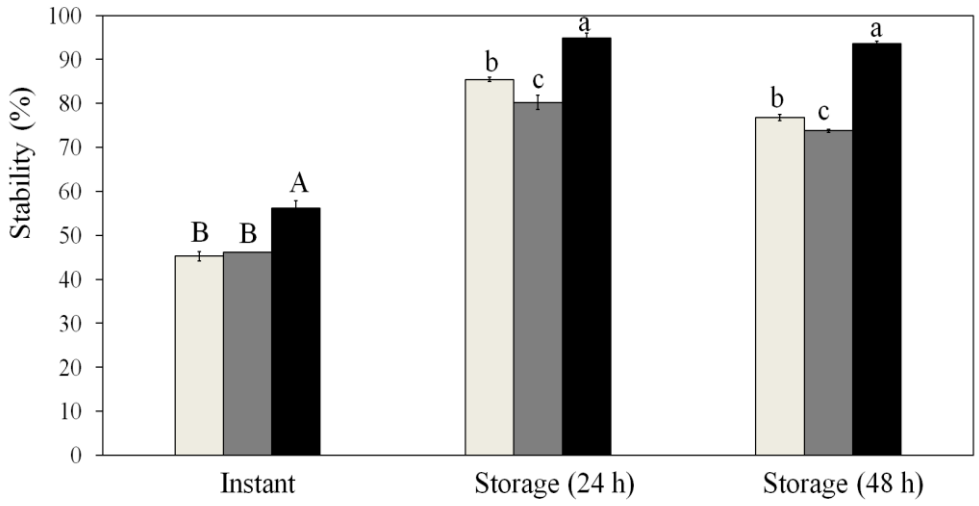

Figure 3.6: Effect of homogenization method of primary emulsion on stability; MF (□), US (■) and HSH (■). Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p\leq0.05$).

The decrease in the stability of the emulsions prepared with MF might be related to the over-processing of the system. One of the main problems of high energy homogenization treatment like microfludization is the "over-processing" which means re-coalescence of the new produced very small droplets due to the low surfactant adsorption rate, limited resistance time with higher frequency droplet collisions and less amount of active surfactant to cover the newly produced droplets with very large surface area (Jafari et al., 2008). The current research conditions might have resulted in the over-processing of the system and could not have produced stable emulsions.

There is a general agreement among the researchers that the emulsions having the smallest droplet sizes have the highest stability. Thus, it would be expected that emulsion produced by HSH would have the smallest droplet distribution. It was observed that there was a correlation between the stability of double emulsion and the droplet size of the primary emulsion distributed into the whole system. Double emulsion prepared with HSH application had the smallest primary emulsion droplets and so the largest specific surface area ($p \leq 0.05$, Table B.16) as represented in Table 3.10. HSH treatment produced a noticeable increase of span value varying from ~1.1 to ~1.5. In HSH, percent reduction in D[4,3] value was 25.7% and 17.1% as compared to US and MF, respectively.Therefore, homogenization method used in primary emulsion production had significant effect ($p \leq 0.05$) on the droplet properties of the systems. On the other hand, for all of the methods, the properties of the primary emulsion droplets in double emulsion was not significantly affected ($p > 0.05$) by the storage time (Table 3.9).

Figure 3.7 and Figure 3.8 represent the effect of surfactant type and homogenization method used in preparation of primary emulsion on the droplet size distribution of the primary emulsion inside double emulsion.

Table 3.9: Effect of homogenization method used in primary emulsion preparation on double emulsion droplet properties

	t=0			t=24 h			t=48 h		
Homogenization method	**D[4,3] (μ)**	**Span**	**SSA (m^2/kg)**	**D[4,3] (μ)**	**Span**	**SSA (m^2/kg)**	**D[4,3] (μ)**	**Span**	**SSA (m^2/kg)**
MF	$19.9\pm0.1^{b,X}$	$1.191\pm0.0^{b,X}$	$875\pm5^{c,Y}$	20.6 ± 0.4^{X}	1.217 ± 0.1^{X}	900 ± 16^{Y}	19.7 ± 0.1^{X}	1.486 ± 0.1^{X}	1070 ± 25^{X}
US	$22.2\pm0.1^{a,X}$	$1.140\pm0.0^{c,X}$	$1354\pm23^{b,X}$	21.9 ± 0.1^{YX}	1.260 ± 0.2^{X}	1322 ± 5^{X}	21.2 ± 0.3^{Y}	1.438 ± 0.0^{X}	1326 ± 25^{X}
HSH	$16.5\pm0.1^{c,X}$	$1.506\pm0.0^{a,X}$	$1663\pm11^{a,X}$	16.4 ± 0.0^{X}	1.379 ± 0.0^{Y}	1485 ± 13^{Y}	15.7 ± 0.3^{Y}	1.319 ± 0.0^{Y}	1471 ± 17^{Y}

Means within the same column shown by different small letters (a,c) are significantly different ($p\leq0.05$) and means within the same raw shown by different capital letters(X,Y) are significantly different ($p\leq0.05$). Values given in this table are means ± standard error of duplicated experiments.

As can be seen from the Figure 3.7, when lecithin to PGPR ratio of 1:1 was used, there was a change in volume size of the primary emulsion inside double emulsion but no change occurred in the volume density. All the homogenization methods produced different range of distributed droplets. When the system became more stable, the curve was shifted to the left side representing the decreasing of volume size of the primary emulsions inside double emulsion.

The efficiency of disruption of the discontinuous phase into the continuous phase of the emulsification equipment depends on the type of the forces acting on the droplets (Scherze et al., 2006) so different homogenization equipment should have provided different disruption mechanisms which resulted in different size and distribution of the droplets.

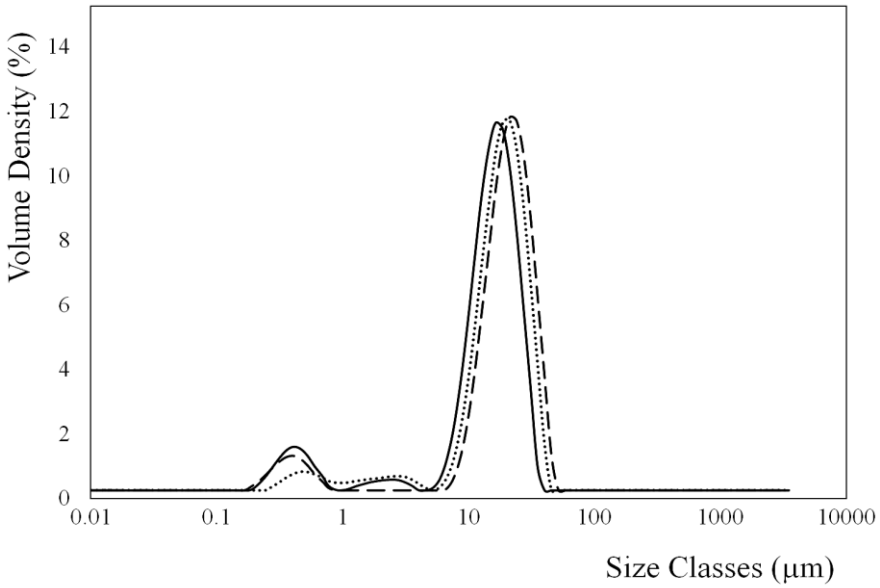

Figure 3.7: Effects of homogenization methods used in primary emulsion preparation on droplet size distribution when lecithin plus PGPR was used as surfactant: HSH (——), US (- - -) and MF (·····).

In the HSH treatment, two different types of forces were dominant for the droplet formation as mechanical impingement against the wall because of

the accelerated fluid and shear stress in the gap between rotor and stator. In the MF treatment, the fluid was forced by high pressure to flow through microchannels and the direct impingement created a rigorous shear so the inertial forces in turbulent flow along with cavitation were the dominant forces for the distribution and disruption of the droplets (Jafari et al., 2008). On the other hand, in US emulsification, high energy was supplied by a sonicator probe which generated mechanical vibrations leading to cavitation and the formation of this cavitation was the key point of the effect of US on the formulation of emulsions (Behrend et al., 2000; Eberth & Merry 1983).

Besides the disruption mechanism, the adsorption rate of emulsifier responsible for the freshly produced droplets was also an important criterion for the characteristics of emulsion. Jafari et al. (2008) reported that in emulsion formation, low adsorption rate of emulsifier was required for HSH treatments while high or middle adsorption rate was needed for MF and US. Therefore, the adsorption rate of the PGPR and lecithin mixture might be more convenient for emulsification by HSH.

Moreover, Dickinson (2011) reported that homogenization conditions of the double emulsion production system resulted in the differences in droplet size and distribution. In the current research, different homogenization conditions depending on the application treatment like pressure, shear, and viscosity were applied to produce double emulsion and the reported differences in droplet size and distribution might be explained by the differences in the homogenization conditions.

One of the main aims of the research was to decrease the amount of PGPR by replacing it with lecithin. Figure 3.8 represents the effect of the production method on double emulsion produced by only PGPR (2%, w/w). As can be seen from figure, there was no change in volume size of the primary emulsion inside double emulsion but the treatment of HSH

enhanced the desired characteristics of double emulsion by increasing the peak of the volume density from ~11 to ~13%.

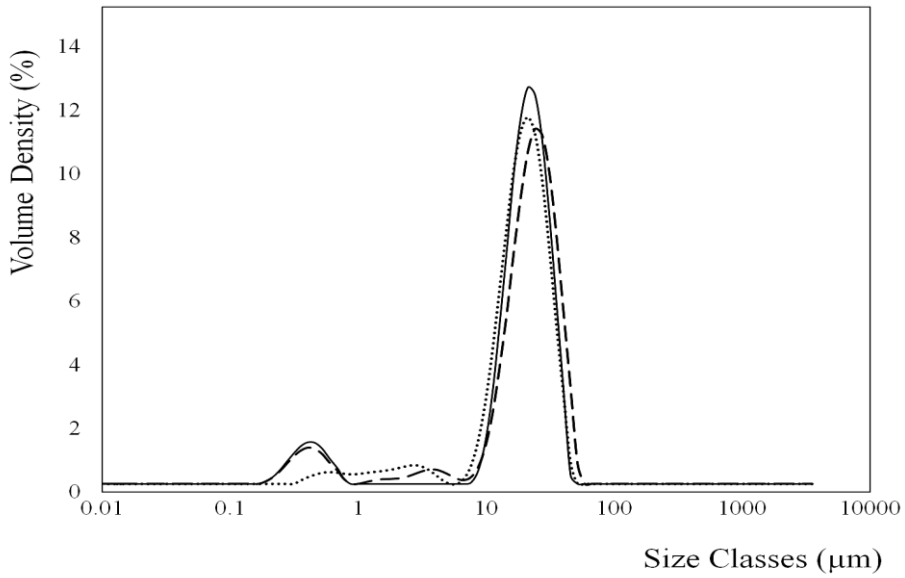

Figure 3.8: Effects of homogenization methods used in primary emulsion preparation on droplet size distribution when only PGPR was used as surfactant: HSH (——), US (- - -) and MF (·····).

As a conclusion, the most stable emulsion was obtained by the application of HSH so encapsulation properties of the double emulsion were studied with the application of mentioned method. Maa & Hsu (1999) also claimed that classical homogenizer was more convenient than the microfludization or sonication by considering the cleaning of equipment, sterilization of the products, cost of production and equipment and controlling of the properties of the product as droplet size distribution.

3.1.5 Encapsulation Properties of Double Emulsion

3.1.5.1 Encapsulation Properties Based on Outer Aqueous Phase

Vitamin B_{12} concentration in the outer aqueous phase of double emulsion was measured to analyze the encapsulation properties. Double emulsion prepared with a mixture of lecithin-PGPR as lipophilic surfactant and NaCN as hydrophilic surfactant was a very effective system to encapsulate Vitamin B_{12} since encapsulation efficiency was determined as 99.9% showing that almost all of the vitamin could be entrapped in the inner aqueous phase by double emulsion system. In the study of Giroux et al., (2013) which was about the encapsulation of Vitamin B_{12} by double emulsion for the cheese fortification, similar result for the encapsulation efficiency was obtained. They compared different production methods and found encapsulated vitamin efficiency between 96 – 99%. Moreover, O'Regan and Mulhivill (2009) also studied whether Vitamin B_{12} would be used as a marker for water soluble compounds and tried to encapsulate measurable amount of Vitamin B_{12} in the inner phase of double emulsion. They found that the mentioned vitamin was equally distributed throughout the aqueous phase of the whole system and 99.3% of the vitamin was recovered. Therefore, the results of the current research were in accordance with these studies.

Besides the encapsulation efficiency, the effect of the storage time on the amount of migrated vitamin from the inner aqueous phase to the outer phase was also studied. Table 3.10 represents the encapsulation stability of prepared double emulsion based on accelerated shelf life test. As can be seen from the table, although there was a slight decrease in the encapsulation stability, 96.5% of the vitamin could still be entrapped inside the inner aqueous phase of the system after being stored for 48 hours.

Table 3.10: Effect of storage time on encapsulation characteristics of double emulsion based on accelerated shelf life conditions

Time (h)	Encapsulation stability/efficiency (%)
0	99.9±0.09[a]
24	99.4±0.07[a]
48	96.5±0.35[b]

Values given in this table are means ± standard error of duplicated experiments. Means within the same column shown by different letters (a,b) are significantly different ($p \leq 0.05$).

The stability of the primary emulsion was a very critical factor in influencing the encapsulation characteristics of the overall system. This is because a stable primary emulsion produces stable double emulsion, which has a positive contribution to the encapsulation efficiency and stability.

In order to measure the vitamin concentration of the outer aqueous phase, centrifugation force was applied to separate the primary emulsion and outer aqueous phase. Figure 3.9 shows the influence of centrifugation force on the double emulsion stored at 45°C for 48 hours. The pink solution shows the primary emulsion containing vitamin and the whitish cloudy solution at the bottom represents the outer aqueous solution containing NaCN which is the second step surfactant of double emulsion. As can be seen from the figure, although the vitamin concentration was very high, it could not diffuse from primary emulsion to the outer phase during the production and storage.

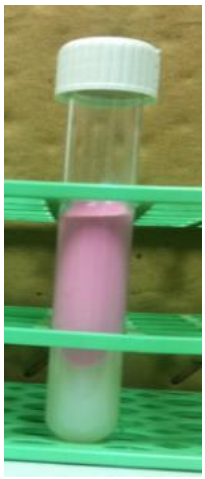

Figure 3.9: Influence of centrifugation force to separate the phases of double emulsion stored at 45°C for 48 h.

There was a good relationship between the stability of the primary emulsion and encapsulation properties of double emulsion prepared by it. Besides the stability of the primary emulsion, other properties like the type of the oil used in the primary emulsion might also have influenced the encapsulation characteristics of the prepared double emulsion. Bonnet et al. (2009) studied on the encapsulation of magnesium in the W/O/W type double emulsion including different type of oils (olive oil, rapeseed oil, olein and miglyol) and observed that primary emulsion oil type affected the released amount of encapsulated material significantly. In the literature no research has been available including hazelnut oil as the oil phase of the double emulsion but the current research showed that hazelnut oil could be suitable for the encapsulation of the water soluble compounds in the W/O/W type double emulsion. The other factor affecting the encapsulation property of double emulsion might be the concentration of NaCN in the outer phase. In the study, the prepared outer aqueous phase was very viscid and sticky by dissolution of the 11% (w/w) NaCN. Therefore, rheological properties of the outer phase should also have influenced the encapsulation characteristics.

3.1.5.2 Encapsulation Properties Based on Inner Aqueous Phase

The encapsulation properties of double emulsion could be analyzed in terms of the vitamin concentration of the outer aqueous phase or vitamin concentration of the inner aqueous phase. Vitamin concentration measurement of the outer part showed that almost all of the vitamin in the inner aqueous phase could be entrapped by double emulsion system (Section 3.1.6.1). However, this approach included the step of removal of caseins by the addition of huge amount of acid to the system for the precipitation of proteins. The changing of the environment to very strong acidic one could have resulted in the loss of the pH sensitive water soluble compounds so the mentioned approach may not be suitable for them. Therefore, after the separation of the phases of double emulsion, the vitamin concentration of the inner aqueous phase was calculated.

The approach of the calculation of the vitamin concentration of the inner phase depended on the disruption of the primary emulsion by HSH treatment and removal of the oil part by centrifugation. At that point, the critical step was to ensure the complete separation of the phases of double emulsion into primary emulsion and outer aqueous phase. Therefore, the obtained double emulsion was centrifuged at different times (5-30 min) at 20°C in order to be ensure the complete separation of the phases.

Figure 3.10 represents the effect centrifugation time applied to separate phases of the double emulsion into primary emulsion and outer aqueous phase and measurement of the vitamin content of the primary emulsion after the disruption of it. As can be seen from the figure, centrifugation of double emulsion for 20 min provided the complete separation. It was expected to obtain vitamin content as 6.0 mg/100 g. The measured vitamin content in primary emulsion was 5.54 mg/100 g meaning that only 7.8% vitamin was lost during the production operations or measurement.

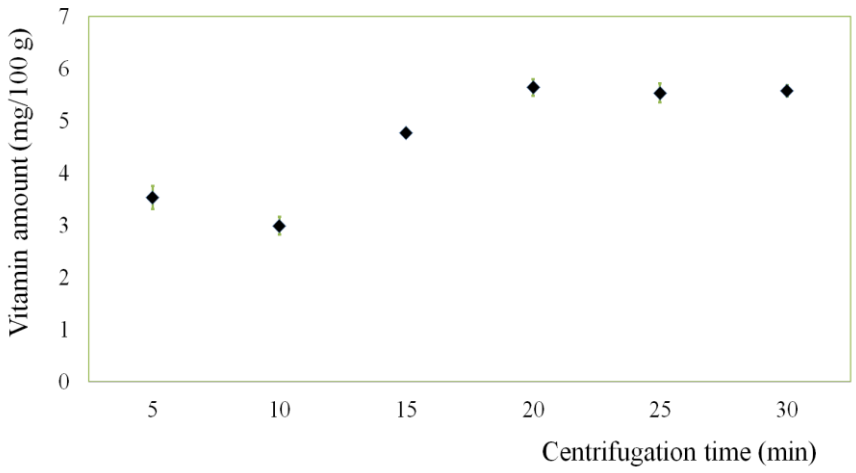

Figure 3.10: Effect of centrifugation time on the vitamin concentration.

This showed that almost all of the vitamin could be encapsulated by double emulsion. This result was similar to what was discussed in Section 3.1.6.1. The findings were very close to the literature studies. Schuch et al. (2014) measured the encapsulation properties of W/O/W emulsion with different methods as photometry (with Vitamin B_{12}), electro conductivity (with NaCl), rheometry and DSC and reported that more than 89% of the samples could be entrapped by the double emulsion system. Khalid et al. (2013) studied the retention kinetics of ascorbic acid in W/O/W emulsions and measured the vitamin concentration of the inner phase by the methanolic extraction treatment. It was found that the retention of ascorbic acid was higher than 90%.

3.2 Coating of O/W Type Single Emulsion with Caseinate

3.2.1 Effects of Surfactant Concentration and Phase Ratio on O/W Emulsion

One of the substantial aims of the research was the replacement of PGPR with a suitable, food base surfactant, lecithin. However, as mentioned in Section 3.1.1 the dilution test showed that only O/W type simple emulsion could be produced when lecithin was used alone. Thus, the produced emulsion containing lecithin was tried to be coated by NaCN solution. In this approach, the first step was to produce stable O/W type simple emulsion before coating. Different amounts of lecithin were used to formulate 20% (w/w) aqueous phase containing emulsions with different methods of processing (Table 3.11).

Table 3.11: Production of simple O/W emulsion with different processes and lecithin concentration

Processing method	5% (w/w) lecithin	2% (w/w) lecithin
HSH	-	+
US	-	-
HTFP	-	-

"+" represents the success of emulsion production while "-"illustrates the failure of emulsion production

The critical step for the preparation of emulsions which contain 80% (w/w) oil phase was the concentration of lecithin inside the oil phase. The lecithin could not function when the amount was high (5%, w/w) and a cloudy suspension was obtained after the operation. The most homogeneous solution was obtained with the application of HTFP but it was not an

emulsion as can be seen in the images (Fig. 3.11, A&B). Only a cloudy suspension could be obtained. When solutions, containing either 5% or 2% lecithin were prepared by US, non-homogeneous systems were obtained (Fig. 3.11, C).

A. System containing 5% (w/w) lecithin prepared by HTFP just after preparation

B. System containing 5% (w/w) lecithin prepared by HTFP after 2 h storage

C. System containing 2% (w/w) lecithin prepared by US just after preparation

Figure 3.11: Production of emulsion by different homogenization methods and lecithin concentration.

After analyzing the effects of lecithin concentration and production methods on the emulsion consisting of 80% (w/w) oil phase, the next step was determination of the ratio of aqueous phase to oil. Emulsions could be obtained when 20/80 and 40/60 phase ratios were used. Two different phase ratios were studied with emulsion contanining 2% (w/w) lecithin and observations were given in Table 3.12. However, 40/60 phase ratio was chosen for the study in order to compare the effects of coating system and double emulsion system on encapsulation efficiency.

Table 3.12: Effect of phase ratio on characteristics of emulsions containing 2% (w/w) lecithin and produced by HSH treatment

Phase Ratio (w/w, inner aqueous /oil)	**Characteristics**
20/80	➢ More viscous like mayonnaise ➢ Difficult to pour ➢ Bright white color
40/60	➢ Viscous like PGPR containing emulsions ➢ Easy to pour ➢ Bright white color

The stability of the emulsion, which would be coated, was the most important factor affecting the total system properties. It was observed that emulsion containing 2% (w/w) lecithin with phase ratio of 40/60 was unstable and showed significant destabilization after 2 days storage but emulsion containing 5% (w/w) lecithin produced a homogeneous system and could stay stable longer. However, 5% (w/w) lecithin concentration was not suitable for the production of the emulsion containing 80% oil phase.

As a result, 40/60 (w/w, aqueous phase/oil phase) phase ratio and 5% (w/w) lecithin concentration were selected for the production of O/W type emulsion to be coated with NaCN solution.

3.2.2 Effects of NaCN Concentration on Coated O/W Emulsion Characteristics

It was possible to obtain a stable system by coating of O/W emulsion with 5% (w/w) NaCN solution. In order to increase the stability of the coated emulsion by NaCN solution, different caseinate concentrations were studied. Table 3.13 represents the influence of NaCN concentration on the droplet size of oil phase of the coated emulsion. While no significant difference was measured between 7% and 9% (w/w) NaCN concentration ($p>0.05$, Table B.20), 13% (w/w) NaCN solution resulted in the about 50% reduction of D[4,3] values and so produced droplets having larger SSA.

Table 3.13: Effect of NaCN concentration on oil phase droplet size of coated emulsion

NaCN Concentration (%)	D[4,3] (μ)	Span	SSA (m^2/kg)
7	10.95±0.495[a]	1.436±0.0170[a]	1073±47.4[b]
9	10.55±0.071[a]	1.465±0.0035[a]	1144± 5.7[b]
13	5.66±0.643[b]	1.356±0.1626[a]	1738±75.7[a]

Values given in this table are means ± standard error of duplicated experiments. Different letters (a,b) in the same column denote difference in the effect of NaCN concentration on the droplet properties ($p\leq0.05$).

Besides the droplet size, stability was another important criterion for the coated emulsions. Figure 3.12 clearly showed that the highest stability could be obtained when the concentration was the highest. On the other hand, coated emulsion by high concentration of NaCN (13%, w/w) could not be separated by centrifugation for determination of instant stability. However, in order to analyze the effect of the application parameters on the emulsion system, the coated emulsion system should be separable by the application

of centrifugal force. There was no significan difference between emulsions containing 5% and 7% (w/w) NaCN in terms of stability values ($p>0.05$, Table B.23). However, stability increased significantly as NaCN concentration increased from 7% to 9% (w/w) ($p\leq0.05$) indicating that protein concentration of the coating solution influenced the system properties. Storage stability of the emulsion coated by 11% NaCN solution was more stable than the emulsion coated by 9% NaCN solution.

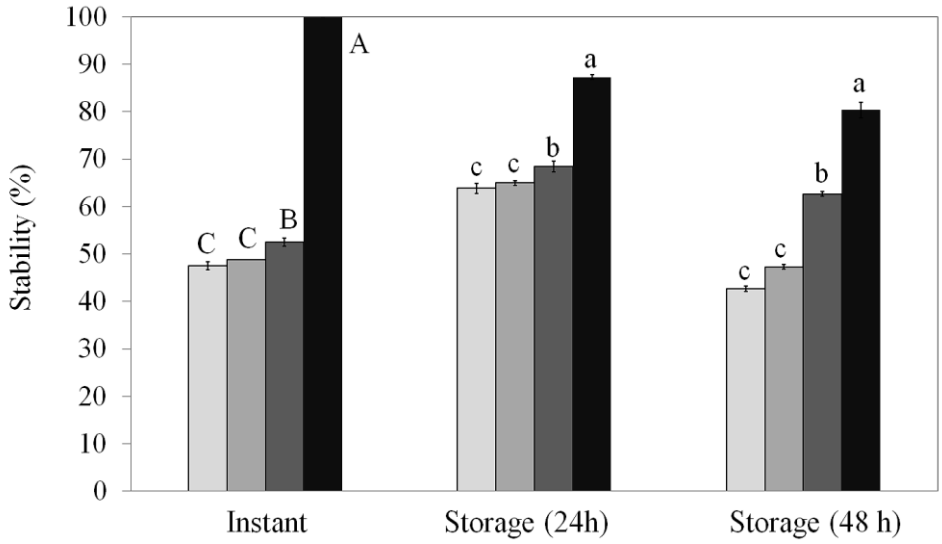

Figure 3.12: Effect of NaCN concentration (w/w) on stability of coated emulsion; 5 % (▫), 7 % (▪), 9 % (▪) and 13 % (■). Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p\leq0.05$).

As a result, 11% NaCN (w/w) concentration was determined as the coating concentration of O/W type emulsion.

3.2.3 Effects of Lecithin Concentration on Coated O/W Emulsion Characteristics

The droplet properties of the emulsions in the systems was very important because it played a key role in characteristics of emulsions like stability, color, appearance, texture and rheology (Jafari et al., 2008). Table 3.14 represents the influence of the lecithin concentration of the simple emulsion on the oil droplet size inside the coated emulsion. As emulsifier concentration increased, droplets having larger SSA were obtained ($p \leq 0.05$, Table B.30).

Table 3.14: Effect of lecithin amount on oil droplet size of coated emulsion

Lecithin Conc. (%, w/w)	D[4,3] (μ)	Span	SSA (m^2/kg)
3	13.20 ± 0.000^a	1.486 ± 0.0064^b	1025 ± 2.1^c
5	9.06 ± 0.085^b	1.465 ± 0.0085^b	1320 ± 29.0^b
8	7.36 ± 1.874^b	1.557 ± 0.0085^a	1755 ± 150.6^a

Values given in this table are means ± standard error of duplicated experiments. Different letters (a,c) in the same column denote difference in the effect of lecithin concentration on the droplet properties ($p \leq 0.05$).

Besides increasing the specific area of the oil droplets, the stability results showed that increment of the emulsifier concentration of the emulsion had a positive influence on the stability of the coated system. Lecithin concentration had a statistically important influence on the instant and storage stability ($p < 0.05$). The increase in the lecithin concentration in the simple emulsion resulted in the increase in stability (Fig. 3.13).

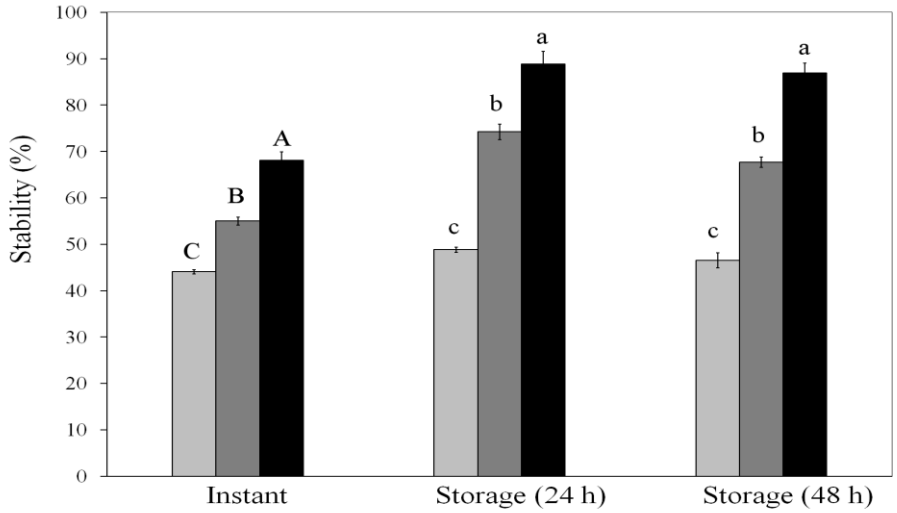

Figure 3.13: Effect of lecithin amount (w/w) on stability of coated emulsion; 3% (▫), 5% (▪) and 8% (■). Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$).

There was a positive correlation with the stability and lecithin concentration of emulsions. No phase separation was observed in any of lecithin containing O/W simple emulsions for 1 week storage. However, 3% (w/w) lecithin containing emulsion started to decompose before the others. Although it was not a clear separation, decomposition of it could be noticed after 2 weeks storage. When the emulsions were stored for 1 month, decomposition of 5% (w/w) lecithin containing emulsion occurred. Results of the stability of the coated emulsions showed that in the presence of stable O/W type simple emulsion stable coated emulsion could be obtained.

3.2.4 Effect Ultrasonication on the Coated Emulsion Characteristics

Table 3.15 shows the influence of the US exposing time on the oil droplet properties of the coated emulsion.

Table 3.15: Effect of US exposing time on oil droplet properties of coated emulsion

Time (min)	D[4,3] (μ)	Span	SSA (m^2/kg)
10	10.70±0.00^a	1.735±0.015^c	1384± 32^c
15	10.50±0.14^a	2.284±0.013^b	1832± 23^b
20	8.62±0.10^b	2.842±0.033^a	2517±106^a

Values given in this table are means ± standard error of duplicated experiments. Different letters (a,c) in the same column denote difference in the effect of US exposing time on the droplet properties ($p \leq 0.05$).

As can be seen from table, in terms of the D[4,3] value of the droplets there was no significant difference between US time of 10 and 15 min ($p > 0.05$). However, 20 min operation provided smaller droplets ($p \leq 0.05$). Moreover, the US exposing time had an important influence on the span and SSA of the systems. Increase in US time increased span and SSA ($p \leq 0.05$). There was a good correlation between the droplet properties and the stability of coated emulsion as can be seen from Figure 3.14. When the sonication time increased, stability increased. The reason of that result could be the positive correlation of energy density with sonication time. The higher sonication energy density caused destabilization and disruption of the droplets (Jafari et al., 2007).

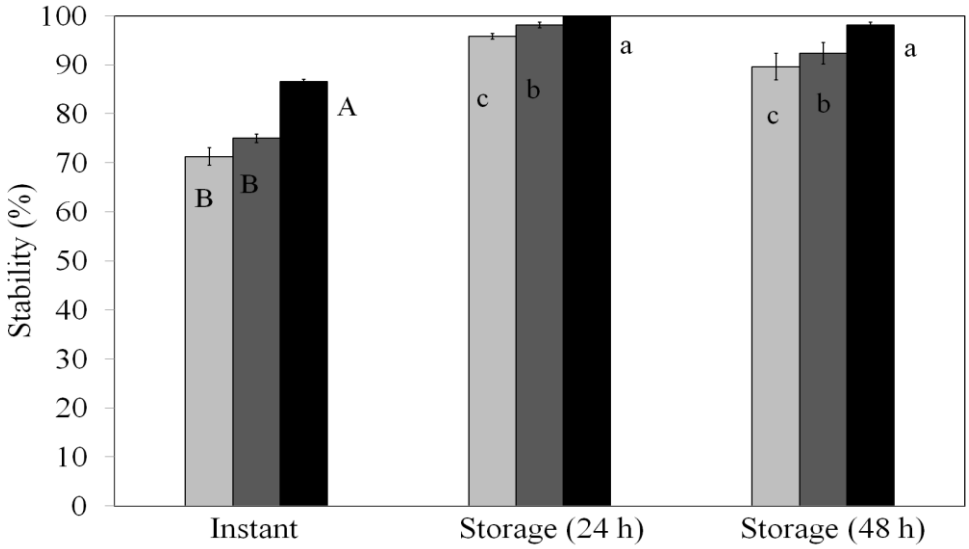

Figure 3.14: Effect of US exposing time on stability of coated emulsion; 10 min (■), 15 min (■) and 20 min (■). Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$).

3.2.5 Effects of Homogenization Methods on Coated O/W Emulsion Characteristics

The coated emulsion was prepared by two different homogenization methods (HSH and US). Then, these production methods were compared in terms of droplet size (Table 3.16) and stability (Fig. 3.15).

The oil droplet size of the distributed emulsion prepared with different production processes showed that homogenization method influenced the properties of the coating system significantly. As can be seen from the Table 3.16, there was a significant difference between the distributed oil droplets characteristics ($p \leq 0.05$, Table B.33, Table B.34 and Table B.35). US homogenized emulsion had larger SSA and span. Scherze et al. (2006) studied the effect of different production methods (high pressure, rotor-stator, ball valve, orifice valve and ultrasonic) on the W/O emulsion

properties and reported that due to the different disruption mechanism of the equipment the produced emulsion droplets had different specific surface areas.

Table 3.16: Effect of homogenization method on oil droplet size

Production method	D[4,3] (μ)	Span	SSA (m^2/kg)
HSH	7.35±0.304[b]	1.401±0.0622[b]	1482± 17.0[b]
US	8.62±1.099[a]	2.842±0.0332[a]	2517±106.1[a]

Values given in this table are means ± standard error of duplicated experiments. Different letters (a,b) in the same column denote difference in the effect of emulsion production method on the droplet properties ($p \leq 0.05$).

The other important criterion to investigate the difference between the homogenization methods was the stability. Figure 3.15 represented the effect of homogenization methods on the stability. The results were in good agreement with the oil droplet properties as represented in Table 3.16. The homogenization method of O/W type simple emulsion had a statistically important influence on the stability ($p \leq 0.05$, Table B.36). US homogenization produced more stable coated system. It might have related to the destabilization mechanisms of the applied processes. According to the Huck-Iriart et al. (2013), the destabilization mechanism of HSH treated emulsion was creaming. On the other hand, US application changed the destabilization mechanism from creaming to flocculation.

Although US provided longer stability, HSH was applied to capture the vitamin inside the O/W emulsion in order to compare the outcomes with the W/O/W type double emulsion encapsulation.

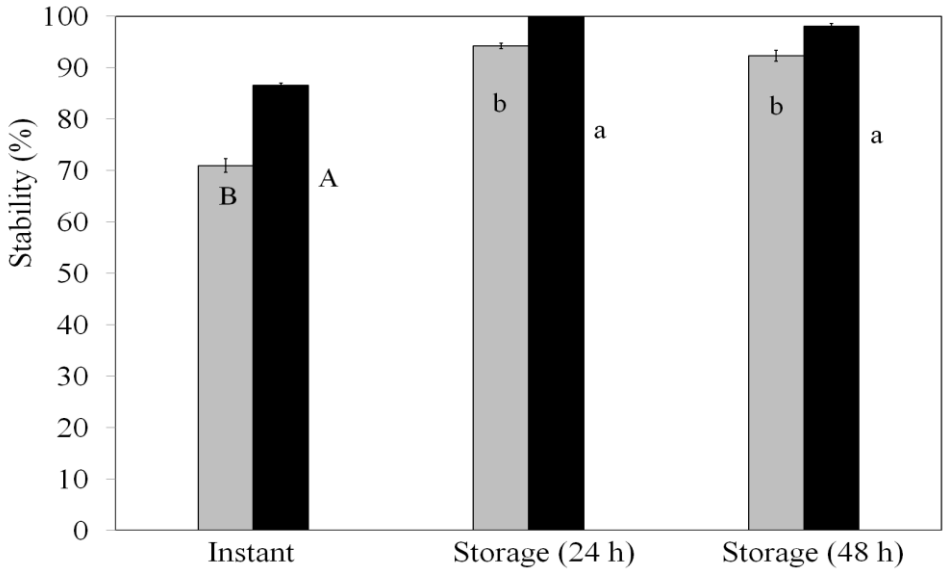

Figure 3.15: Effect of emulsion production method on stability of coated system; HSH (■) and US (■). Capital and small letters corresponds to individual statistical analysis. Bars with different letters represent significant difference ($p \leq 0.05$).

3.2.6 Encapsulation Efficiency and Stability of Coated O/W Emulsion

The encapsulation efficiency of the coated system refers to the Vitamin B_{12} amount of the O/W simple emulsion coated by the caseinate solution. On the other hand, the influence of the storage time on the migration of the vitamin from simple emulsion to the coating agent could be represented by the encapsulation stability.

The capsulation properties of the coated system were measured to compare with the double emulsion system in which almost all of the vitamin could be entrapped.

Table 3.17 shows the capsulation efficiency and stability of the coated O/W emulsion with caseinate solution. Although the O/W type single emulsion was very stable (92.3%), coated emulsion could stay stable for 2 days storage. The vitamin could easily transfer from the O/W emulsion aqueous

phase to the covering material both during the coating process and storage. This showed that the mentioned coated system was not suitable for the capsulation of water soluble compound. However, coating of O/W emulsion with the caseinate solution system might be used for the protection of the fat soluble and valuable compounds like retinols, Vitamin D and carotenes. The fat soluble compound could be covered firstly simple emulsion and then coated by the caseinate solution.

Table 3.17: Capsulation characteristics of coated emulsion

Storage Time (h)	Capsulation stability/efficiency (%)
0	72.2 ± 0.06^{a}
24	55.7 ± 4.29^{b}
48	27.1 ± 1.45^{c}

Values given in this table are means ± standard error of duplicated experiments. Means within the same column shown by letters (a,c) are significantly different ($p \leq 0.05$).

3.3 Effects of Usage of Gum Arabic on Coated O/W Emulsion Characteristics

After coating of the emulsion with NaCN, replacement of NaCN with gum arabic was also studied. Table 3.18 showed the effect of gum arabic concentration on droplet properties.

Table 3.18: Effect of gum arabic concentration on droplet properties of coated O/W type emulsion

Gum Arabic Conc. (%)	D[4,3] (μ)	Span	SSA (m^2/kg)
10	29.40±0.764[b]	1.728±0.7128[b]	376±56.9[b]
15	28.50±0.523[b]	1.893±0.4158[b]	472±10.3[b]
20	26.35±0.191[b]	1.939±0.2199[b]	661±40.6[a]
25	232.50±1.626[a]	19.070±0.5112[a]	451±53.0[b]

Values given in this table are means ± standard error of duplicated experiments. Different letters (a,b) in the same column denote difference in the effect of gum arabic concentration on the droplet properties ($p \leq 0.05$).

Up to concentration of 25%, the increase in the concentration did not affect droplet size of coated system. When the amount of the gum arabic was passed this aforementioned point, the oil droplets started to become bigger. The same trend was also observed for the other droplet properties like span. However, SSA of the droplets did not give significant differences. This could be related to the low viscosity of emulsions in the presence of gum arabic (Kibbe, 2006).

The stability experiments also showed that gum arabic was not a suitable surfactant for coating of the emulsion containing 5% (w/w) lecithin with 40/60 phase ratio. Figure 3.16 represents the act of the centrifugation force on the separation of the coated emulsion systems prepared with gum arabic (numbered as 1) and sodium caseinate (numbered as 2) as coating material. Although the concentration of the gum arabic was about three times higher than the NaCN concentration, the coated O/W emulsion with the gum arabic was very prone to dissociation. As can be seen in the figure, after centrifugation very transparent coating solution was obtained for gum arabic while cloudy suspension was separated for the NaCN solution. Gum arabic

solutions are known to have a lower apparent viscosity compared to other polysaccharides used for encapsulated agents (Kibbe, 2006). This explained why gum arabic solution containing system was more prone to separation.

Figure 3.16: Separation of covered systems prepared with different stabilizers, by centrifugation force: 20% (w/w) gum arabic (1) and 7% (w/w) NaCN (2).

The other indication was the storage stability. Emulsions coated by gum arabic started to dissociate immediately and showed destabilization after 6 hours. Su et al. (2008) tried to use the gum arabic as a surfactant for the capsulation of the W/O type emulsion but they used a modified gum arabic product (SUPER GUM) instead of the conventional gum arabic. They changed the protein composition, which was mainly related to the emulsifying property of the gum arabic, by accelerated aggregation process and produced gum arabic with larger molecular-weight protein aggregates.

Although, the coated emulsions were initially homogeneous and it was not possible to separate the phases, they could not keep the homogeneity during

the storage. Finally heterogeneous unstable solutions were obtained. When the sodium caseinate was used to coat the emulsion, due to their flexible structure, it could be adsorbed on the interface of the emulsion (Euston & Hirst, 2000). The same function might not be performed by the gum arabic.

3.4 Comparison of the W/O/W Type Double Emulsion and Coated O/W Emulsion Systems in Terms of Water Soluble Vitamin Capsulation

In the current research, two different encapsulation systems were analyzed and the encapsulation efficiency of water soluble vitamin by double emulsion was compared with coated of simple O/W type emulsion. As clearly represented in Table 3.19, the water soluble vitamin could be entrapped almost entirely by double emulsion system. However, coating of O/W type simple emulsion with NaCN system was not suitable in terms of the same purpose.

Table 3.19: Encapsulation efficiency and stability of prepared systems

	Efficiency (%)		
	t=0	**t=24 h**	**t=48 h**
W/O/W Type Double Emulsion	$99.9\pm0.09^{a,X}$	99.4 ± 0.07^{X}	96.5 ± 0.35^{Y}
Coated O/W Emulsion	$72.8\pm0.66^{b,X}$	55.7 ± 4.29^{Y}	27.1 ± 1.45^{Z}

Different letters (a,b) in the same column denote difference in the effect of encapsulation method ($p\leq0.05$) on efficiency and different letters (X,Z) represent difference in the effect of storage time ($p\leq0.05$) on stability. Values given in this table are means ± standard error of duplicated experiments.

The continuous phase of the O/W type simple emulsion was the aqueous phase so the water soluble vitamin was present in the outer part of the emulsion. When the simple emulsion was coated with the NaCN solution,

the mentioned aqueous phase could contact directly with coating solution in which NaCN was dissolved into the aqueous phase. This might have provided opportunity for passing of the water soluble vitamin from emulsion phase to the coating solution. On the other hand, for the double emulsion system, the inner aqueous phase was distributed into the oil phase and this oil phase was dissipated into the NaCN solution so the oil phase between the aqueous phases might have acted as a barrier for transfer of the water soluble vitamin from the inner phase to the outer one (Pitchaon et al., 2013).

Vasijevic et al. (2009) reported that double emulsions were more adequate for entrapment of the valuable compounds than the single emulsions due to the multi-compartment interphase boundaries. As can be seen from the Table 3.20, in the presence of coated O/W emulsion encapsulation could not be achieved efficiently. The water soluble compound passed easily from the emulsion aqueous phase to the coating solution. It might be related to the higher solubility of the Vitamin B_{12} in the water. O'Regan and Mulhivill (2009) studied whether Vitamin B_{12} would be used as a marker for water soluble compounds in the encapsulation systems and found that the mentioned vitamin was equally distributed throughout the aqueous phase of the whole systems. This study also supported the higher and easily dissolution of the aforementioned vitamin in the water base systems. Therefore, the vitamin in the simple O/W type emulsion could directly contact with the water base coating solution and could diffuse to the coating solution while oil might have been an obstacle for the transfer due to its insolubility.

3.5 Replacement of Encapsulated Vitamin B_{12} with Vitamin B_1

After analyzing the encapsulation efficiency of the double emulsion system with a water soluble marker, Vitamin B_{12}, the next step was the replacement of it with Vitamin B_1 which was a more sensitive compound. Therefore, Vitamin B_1 was encapsulated by the same manner and the effects of temperature and pH on the degradation of Vitamin B_1 were studied.

3.5.1 Addition of Encapsulated Vitamin B_1 by Double Emulsion Method to Bread

Vitamin B_1 was encapsulated by double emulsion system and the prepared system was transferred to the bread in order to study the thermal effect. It was thought that the specific structure of the double emulsion could protect the heat sensitive compounds during the baking process. However, it was observed that Vitamin B_1 did not decrease during the baking process ($p>0.05$, Table B.40). No vitamin reduction was measured in bread when vitamin was added directly and double emulsion containing vitamin was added (Table 3.20).

Table 3.20: Effect of baking process on the vitamin amount of different systems

	Amount of Remaining Vitamin B_1 (mg vitamin/100 g dough)*
Direct vitamin addition	86.2 ± 4.71^a
Primary emulsion addition	85.2 ± 0.74^a
Double emulsion addition	87.3 ± 1.02^a

Values given in this table are means ± standard error of duplicated experiments. Means within the same column shown by different letters are significantly different ($p \leq 0.05$).

*The amount of added vitamin was 90 mg vitamin/100 g dough.

3.5.2 Effect of Temperature on Vitamin B_1 Encapsulated by Double Emulsion

In the current research, it was expected that the specific structure of double emulsion, which had two different interphases such as water-oil (primary emulsion) and oil-water (double emulsion) interphases, could provide a protective layer for the thermal effects. Therefore, degradation of the heat labile compounds could be decreased by transferring them inside the inner phase of double emulsion.

There is conflicting information in the literature about the effect of thermal prosesses on the loss of Vitamin B_1. Some researchers reported that thiamin was highly sensitive to the heat treatments (Whitney & Rolfes, 2011; Oseredczuk et al., 2003; Maskova et al., 1996). The loss of Vitamin B_1 as a result of cooking was 20% - 56% (Batifoulier et al., 2005; Martinez-Villaluenga et al., 2009; Mihhalevski et al., 2013). On the other hand, there were studies stating that the thermal processes did not decrease the vitamin content. Lassen et al., (2002) studied the Vitamin B_1 reduction in pork meat cooked at 72°C and reported that the mentioned temperature did not cause any thermal degradation of the vitamin.

In order to study the effect of double emulsion system on the degradation of vitamin, the suitable temperature range should have been selected properly by focusing on the emulsion characteristics. In the current research, it was realized that storage of the prepared double emulsions at 90°C for 1 hour resulted in the break down of the caseinate and production of a heterogeneous solution. Preliminary experiments showed that the temperatures could be 30°C–90°C and exposing time could be 20 min. Then, double emulsions containing 3% (mg vitamin /100g emulsion) Vitamin B_1 were exposed to mentioned temperatures. However, no degradation of the vitamin was observed.

In order to investigate the heat stability of the mentioned vitamin, 3% (w/w) Vitamin B_1 was dissolved into distilled water and exposed to 90°C. As can be seen in the Figure 3.17, no vitamin loss was observed during 3 hours. After 4 hours the vitamin content decreased due to the thermal effect.

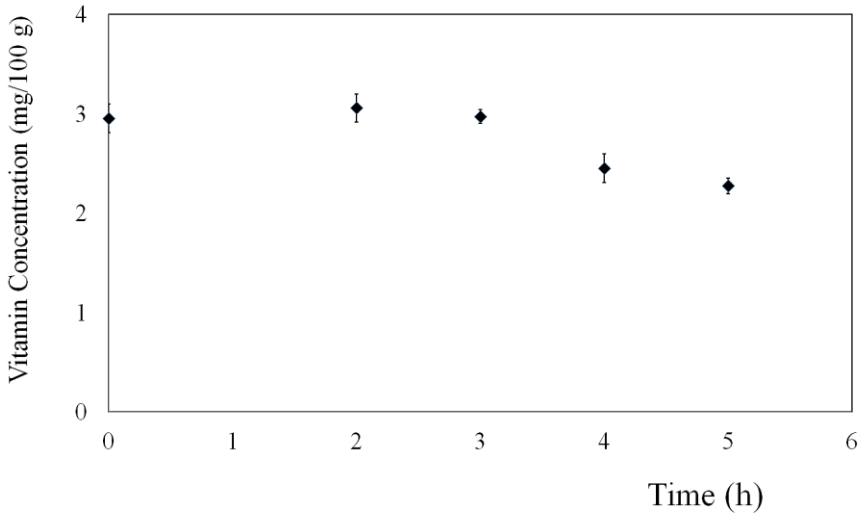

Figure 3.17: Degradation of Vitamin B_1 exposed to 90°C

This was accordance with the study of Sierra and Vidal-Valderde (2001) in which milk was exposed to temperature ranging from 90-120°C in order to analyze the effect of the heat on the degradation Vitamin B_1. It was reported that heat treatment did not result in any significant losses of the vitamin content of the milk.

It is known that Vitamin B_1 is lost during the production process of bakery products where it is present in small quantities. The reduction of the vitamin content of the bakery products might be related with the addition of baking powder as ingredients which make the medium more alkali (Leskova et al., 2006). Furthermore, preliminary studies showed that basic medium caused

loss of the vitamin. Therefore, the effect of pH on the vitamin concentration was also studied.

3.5.3 Loss of Vitamin B_1 in Slightly Alkali or Neutral Medium

After dissolving Vitamin B_1 in the inner aqueous phase, the pH of the system was measured as 4.20 which was acidic. Preliminary studies showed that Vitamin B_1 concentration decreased with the increment of pH value of the medium. In order to be sure, Vitamin B_1 was exposed to different pH values and as can be seen from the Figure 3.18, pH of the medium influenced the vitamin concentration. The alkali conditions resulted in considerable loss of the Vitamin B_1. Vitamin B_1 started to degradate when the medium was neutral or slightly alkali whereas the acidic medium did not affect the vitamin concentration. This result was in accordance with the studies of the Ball (1994) and Leskova et al. (2006). Ball (1994) reported that alkaline pH during cooking and processing of the food materials including Vitamin B_1 led to extensive losses of the vitamin. Leskova et al. (2006) indicated that Vitamin B_1 was unstable in neutral pH values even weak alkaline conditions resulted in the degradation of it.

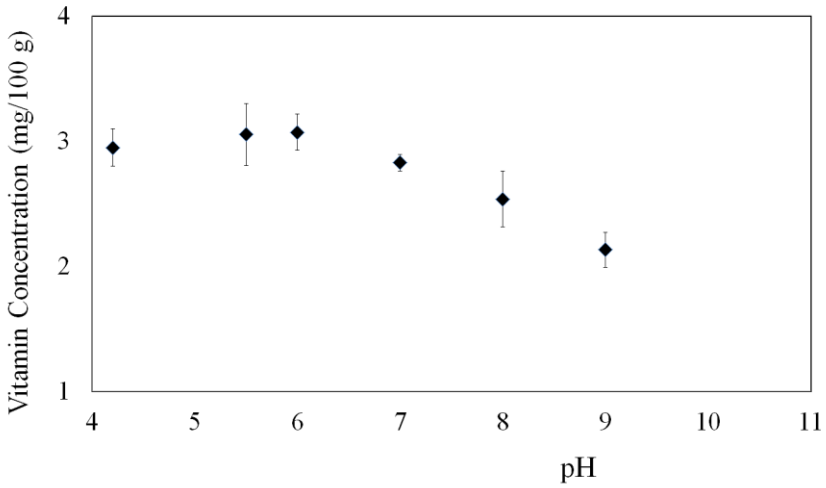

Figure 3.18: Effect of medium pH on degradation of Vitamin B_1

On the other hand, it was observed that the color of the solution became yellowish at higher pH values. It should be related to the complex degradation of vitamin at more alkali conditions. The matrix conditions above pH 9 might cause turning of the solution containing Vitamin B_1 rapidly in yellow color due to formation of the various complex degradation products (Farrer, 1948).

3.5.4 Enrichment of Carrot Juice with Encapsulated Vitamin B_1 Using Double Emulsion as Carrier

As mentioned before, the prepared double emulsion system contained two different aqueous phases and an oil phase. The oil phase locating between the aqueous phases might have provided a protective layer for direct contact of the pH sensitive compounds present inside the inner phase of the emulsion to food matrix. Therefore, degradation of the pH sensitive compounds could be prevented by transferring them into the food matrix by using the double emulsion as carrier.

Based on this approach, the prepared double emulsion containing Vitamin B_1 was added to the freshly squeezed carrot juice which had pH value as 6.6±0.14 and the loss of the vitamin during 2 days was recorded. Direct addition of vitamin to the juice was also studied as control to analyze the influence of the double emulsion system on the degradation of vitamin.

As can be seen clearly from Figure 3.19, when uncapsulated vitamin was directly added to carrot juice vitamin concentration decreased fast during the storage. At the end of 2 days storage, about 46% of the vitamin was lost in the sample. On the other hand, double emulsion protected the pH sensitive Vitamin B_1 and only 12% of the vitamin was lost during the storage. The results showed that double emulsion structure provided a

barrier for the direct contact of the inner phase of the system and the food matrix and so the degradation of pH sensitive compound, Vitamin B_1, could be prevented.

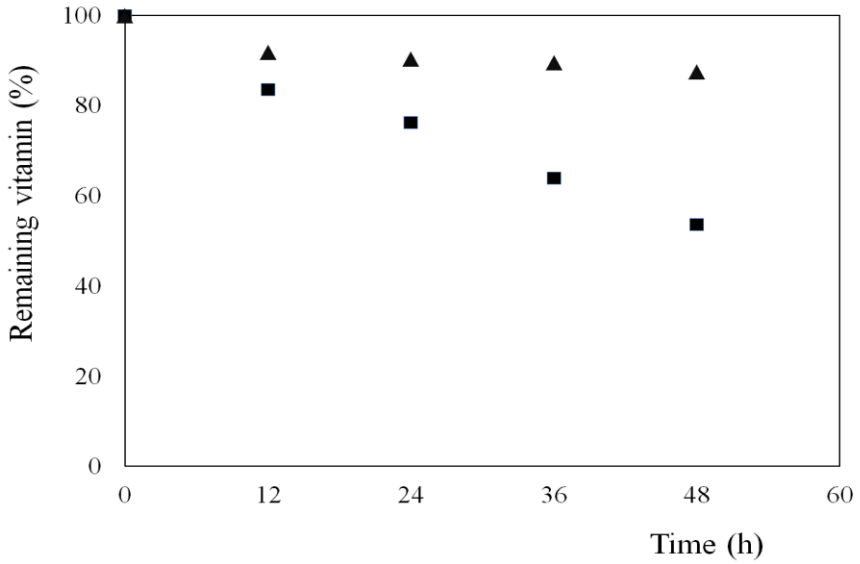

Figure 3.19: Addition of vitamin to the carrot juice; direct vitamin addition: (■) and encapsulated vitamin by double emulsion: (▲).

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

The present study showed that W/O/W type double emulsion can be produced by combining lecithin and PGPR. Type of the primary emulsion and physicochemical properties of double emulsion were influenced by the mass ratio of lecithin to PGPR. PGPR produced W/O type but lecithin produced O/W type primary emulsion. The most stable emulsion system was obtained when 1.5 g/100 g lecithin was combined with PGPR at concentration of 1.5 g/100 g. It was found that HSH treatment produced more stable double emulsion which contained smaller droplets as compared to US and MF treatments. This study showed that the concentration of synthetic emulsifier PGPR can be reduced by using a natural emulsifier lecithin in W/O/W double emulsions to be used in food systems.

W/O/W type double emulsion system was found to be an effective system to encapsulate water soluble compounds. On the other hand, coating of O/W type simple emulsion with sodium caseinate was determined to be not suitable for entrapment of Vitamin B_{12}. Moreover, the specific structure of double emulsion could provide a barrier for the direct contact of the inner aqueous phase of the W/O/W type emulsion and the food matrix and so the pH sensitive compound, Vitamin B_1, was prevented from the degradation.

The reason for the selection of the carrot juice was the pH of the juice which was about 7 because the aforementioned vitamin was stable under acidic conditions. However, in the food industry many of the food systems are acidic and many of the nutritive vitamins are lost due to the sensitivity to

acidic environments. Therefore, in the future the analyzed double emulsion system could be used to encapsulate nutritive acid-sensitive compounds and transfer it to the specific water base food matrix to prevent the loss of the compounds due to the acidic conditions of the matrix.

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APPENDICIES

APPENDIX A

CALIBRATION CURVES

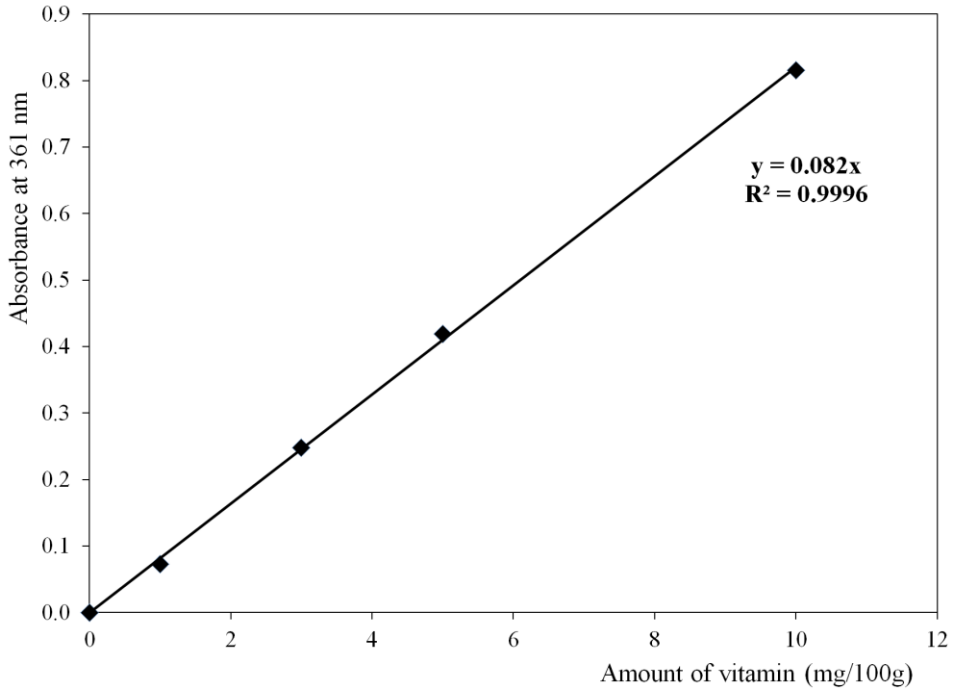


Figure A.1: Calibration curve of Vitamin B_{12} dispersed in the outer aqueous phase

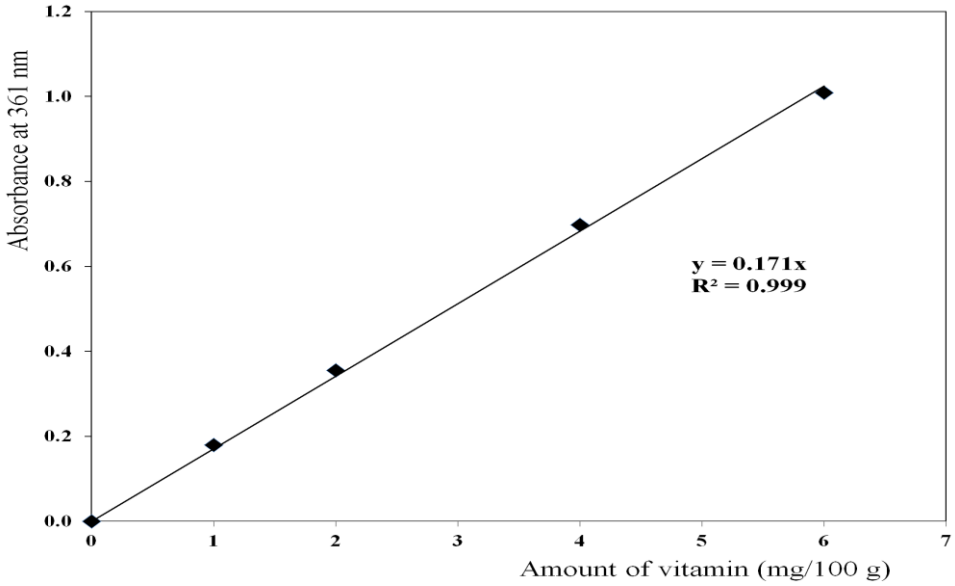


Figure A.2: Calibration curve of Vitamin B_{12} dispersed in the distilled water

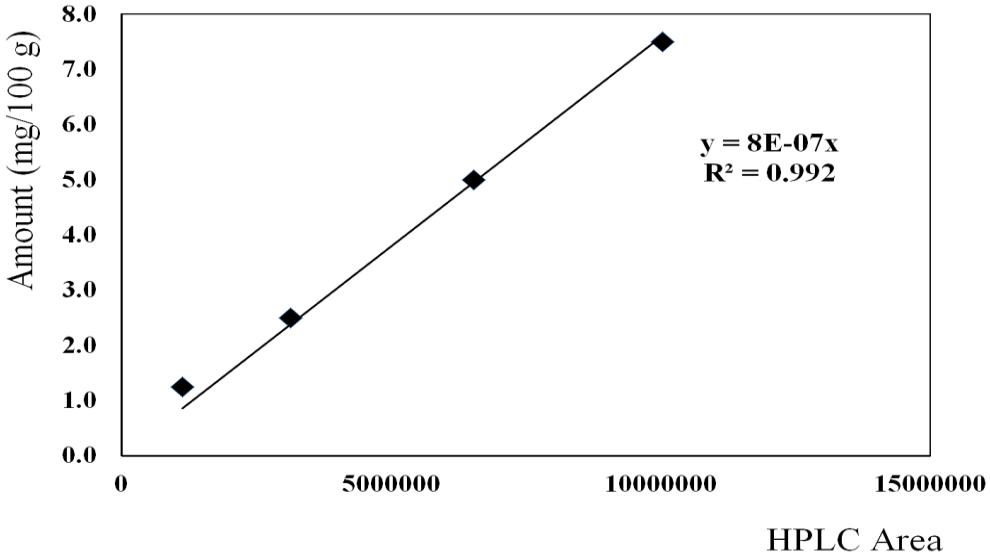


Figure A.3: Calibration curve of Vitamin B_1

APPENDIX B

STATISTICAL ANALYSIS

Table B.1: One way ANOVA and Duncan's Multiple Range Test for instant stability of double emulsions prepared by MS and HTFP

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2

Number of Observations Read	4
Number of Observations Used	4

The SAS System
The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.69000000	1.69000000	1.65	0.3278
Error	2	2.05000000	1.02500000		
Corrected Total	3	3.74000000			

R-Square	Coeff Var	Root MSE	Y Mean
0.451872	2.118039	1.012423	47.80000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1.69000000	1.69000000	1.65	0.3278

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1.69000000	1.69000000	1.65	0.3278

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	1.025

Number of Means	2
Critical Range	4.356

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	48.450	2	2
A	47.150	2	1

Table B.2: One way ANOVA and Duncan's Multiple Range Test for storage stability of double emulsions prepared by MS and HTFP

The GLM Procedure
Class Level Information

Class	Levels	Values
X1	2	1 2

Number of Observations Read 4
Number of Observations Used 4

The SAS System
The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	31.36000000	31.36000000	125.44	0.0079
Error	2	0.50000000	0.25000000		
Corrected Total	3	31.86000000			

R-Square	Coeff Var	Root MSE	Y Mean
0.984306	0.579374	0.500000	86.30000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	31.36000000	31.36000000	125.44	0.0079

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	31.36000000	31.36000000	125.44	0.0079

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.25

Number of Means 2
Critical Range 2.151

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	89.1000	2	1
B	83.5000	2	2

Table B.3: Two way ANOVA and Duncan's Multiple Range Test for D[4,3] value of double emulsions prepared by MS and HTFP; time of 0 and 24 h

The GLM Procedure
Class Level Information

Class	Levels	Values
X1	2	1 2
X2	2	1 2

Number of Observations Read 8
Number of Observations Used 8

The SAS System
The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	605.4925000	302.7462500	110.60	<.0001
Error	5	13.6862500	2.7372500		
Corrected Total	7	619.1787500			

R-Square	Coeff Var	Root MSE	Y Mean
0.977896	5.964718	1.654464	27.73750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1.7112500	1.7112500	0.63	0.4650
X2	1	603.7812500	603.7812500	220.58	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1.7112500	1.7112500	0.63	0.4650
X2	1	603.7812500	603.7812500	220.58	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 5
Error Mean Square 2.73725

Number of Means 2
Critical Range 3.007

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	35.050	4	2
B	19.425	4	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	2.73725
Number of Means	2
Critical Range	3.007

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	27.425	4	1
A	28.050	4	2

Table B.4: Two way ANOVA and Duncan's Multiple Range Test for span of double emulsions prepared by MS and HTFP; time of 0 and 24 h

The GLM Procedure
Class Level Information

Class	Levels	Values
X1	2	1 2
X2	2	1 2

Number of Observations Read	8
Number of Observations Used	8

The SAS System
The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00980100	0.00980100	30.77	0.0514
Error	5	0.00033700	0.00016850		
Corrected Total	7	0.01013800			

R-Square	Coeff Var	Root MSE	Y Mean
0.899762	1.112318	0.012981	1.167000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.00302500	0.00302500	17.95	0.0504
X2	1	0.07452900	0.07452900	105.19	0.0494

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.00302500	0.00302500	17.95	0.0504
X2	1	0.07452900	0.07452900	105.19	0.0494

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	0.000168

```
                          Number of Means          2
                          Critical Range       .05585

                Means with the same letter are not significantly different.

                 Duncan Grouping          Mean      N    X1

                              A       1.19450      2    2
                              A       1.13950      2    1

                          Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                             experimentwise error rate.

                          Alpha                        0.05
                          Error Degrees of Freedom        5
                          Error Mean Square         0.000168

                              Number of Means          2
                              Critical Range       .05585

                Means with the same letter are not significantly different.

                 Duncan Grouping          Mean      N    X1

                              A       1.31250      2    2
                              B       1.14950      2    1
```

Table B.5: Two way ANOVA and Duncan's Multiple Range Test for SSA of double emulsions prepared by MS and HTFP; time of 0 and 24 h

```
                                The GLM Procedure
                              Class Level Information

                            Class         Levels    Values
                            X1                 2    1 2
                            X2                 2    1 2

                           Number of Observations Read           8
                           Number of Observations Used           8

                                The SAS System
                              The GLM Procedure
Dependent Variable: Y
                                    Sum of
       Source             DF        Squares       Mean Square    F Value    Pr > F
       Model               2     1606556.250     1606556.250    7132.33    0.0001
       Error               5     450.500         225.250
       Corrected Total     7     1607006.750

                         R-Square     Coeff Var      Root MSE        Y Mean
                         0.999720      1.771417      15.00833      847.2500
```

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1522496.000	24964.00000	26.83	0.0001
X2	1	702.250	702.250000	2.86	0.2327

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1522496.000	24964.00000	26.83	0.0001
X2	1	702.250	702.250000	2.86	0.2327

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	225.25
Number of Means	2
Critical Range	64.58

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1481.00	2	2
B	213.50	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	225.25
Number of Means	2
Critical Range	64.58

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	847.50	2	2
A	913.00	2	1

Table B.6: Two way ANOVA and Duncan's Multiple Range Test for D[4,3] value of double emulsions prepared by 11% and 15% NaCN concentration; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

```
                          Number of Observations Read          12
                          Number of Observations Used          12

                                    The SAS System
                                  The GLM Procedure
Dependent Variable: Y
                                        Sum of
       Source                DF         Squares     Mean Square    F Value    Pr > F
       Model                  3     462.2450000     154.0816667     530.17    <.000
       Error                  8       2.3250000       0.2906250
       Corrected Total       11     464.5700000

                     R-Square     Coeff Var      Root MSE        Y Mean
                     0.994995      4.195303      0.539096      12.85000

       Source                DF       Type I SS     Mean Square    F Value    Pr > F
       X1                     1     461.2800000     461.2800000    1587.20    <.0001
       X2                     2       0.9650000       0.4825000       1.66    0.2494

       Source           DF     Type III SS     Mean Square    F Value    Pr > F
       X1                1     461.2800000     461.2800000    1587.20    <.0001
       X2                2       0.9650000       0.4825000       1.66    0.2494

                            Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                          experimentwise error rate.

                             Alpha                        0.05
                             Error Degrees of Freedom        8
                             Error Mean Square        0.290625

                                 Number of Means          2
                                 Critical Range       .7177

                  Means with the same letter are not significantly different.

                   Duncan Grouping          Mean      N    X1
                                 A       19.500      6    2
                                 B        6.650      6    1

                        Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                          experimentwise error rate.

                             Alpha                        0.05
                             Error Degrees of Freedom        8
                             Error Mean Square        0.290625

                                 Number of Means          2          3
                                 Critical Range       .8790      .9160

                  Means with the same letter are not significantly different.

                   Duncan Grouping        Mean      N    X2
                                 A       6.075      4    1
                                 A       6.025      4    3
                                 A       5.450      4    2
```

Table B.7: Two way ANOVA and Duncan's Multiple Range Test for span of double emulsions prepared by 11% and 15% NaCN concentration; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read	12
Number of Observations Used	12

The SAS System
The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.05554900	0.02777450	0.36	0.7262
Error	3	0.23369500	0.07789833		
Corrected Total	5	0.28924400			

R-Square	Coeff Var	Root MSE	Y Mean
0.948825	1.193468	0.015028	1.259167

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.01256133	0.00628067	27.81	0.0716
X2	2	0.01123600	0.01123600	3.39	0.2071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.01256133	0.00628067	27.81	0.0716
X2	2	0.01123600	0.01123600	3.39	0.2071

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.003318

Number of Means	2
Critical Range	.2479

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.30050	2	2
A	1.19450	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rat

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.003318

Number of Means	2	3
Critical Range	.04783	.04798

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	1.408	4	1
A	1.306	4	3
B	1.248	4	2

Table B.8: Two way ANOVA and Duncan's Multiple Range Test for SSA of double emulsions prepared by 11% and 15% NaCN concentration; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	57360.2500	57360.2500	2.15	0.2799
Error	2	53262.5000	26631.2500		
Corrected Total	4	110622.7500			

R-Square	Coeff Var	Root MSE	Y Mean
0.818521	11.98831	163.1908	1361.250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	6413.69048	3206.84524	2.68	0.1824
X2	2	9692.333	989846.167	1.40	0.3711

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	6413.69048	3206.84524	2.68	0.1824
X2	2	9692.333	989846.167	1.40	0.3711

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	26631.25

Number of Means	2
Critical Range	702.2

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1481.0	2	2
A	1241.5	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	26631.25

Number of Means	2	3
Critical Range	2671	2680

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1456.0	2	2
A	1311.5	2	1
A	1302.5	2	3

Table B.9: One way ANOVA and Duncan's Multiple Range Test for instant stability of double emulsions prepared by different PGPR and PGPR-Lecithin concentrations

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	6	1 2 3 4 5 6

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	259.7600000	51.9520000	38.15	0.0002
Error	6	8.1700000	1.3616667		
Corrected Total	11	267.9300000			

R-Square	Coeff Var	Root MSE	Y Mean
0.969507	2.398571	1.166905	48.65000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	5	259.7600000	51.9520000	38.15	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	5	259.7600000	51.9520000	38.15	0.0002

Duncan's Multiple Range Test for instant stability

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	1.361667

Number of Means	2	3	4	5	6
Critical Range	2.855	2.959	3.011	3.037	3.048

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	56.100	2	3
B	52.900	2	2
C	48.700	2	6
D C	47.150	2	1
D E	44.400	2	4
E	42.650	2	

Table B.10: One way ANOVA and Duncan's Multiple Range Test for storage stability of double emulsions prepared by different PGPR and PGPR-Lecithin concentrations

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	6	1 2 3 4 5 6

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	41.01416667	8.20283333	10.90	0.0057
Error	6	4.51500000	0.75250000		
Corrected Total	11	45.52916667			

R-Square	Coeff Var	Root MSE	Y Mean
0.900833	0.953873	0.867468	90.94167

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	5	41.01416667	8.20283333	10.90	0.0057

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	5	41.01416667	8.20283333	10.90	0.0057

Duncan's Multiple Range Test for Storage stability

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.7525

Number of Means	2	3	4	5	6
Critical Range	2.123	2.200	2.238	2.257	2.266

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	93.5500	2	3
B	83.7500	2	2
B	82.8000	2	6
B	80.6500	2	5
B	80.5000	2	4
B	80.2000	2	1

Table B.11: Two way ANOVA and Duncan's Multiple Range Test for D[4,3] value of double emulsions prepared by different surfactant types and concentrations; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	6	1 2 3 4 5 6
X2	3	1 2 3

Number of Observations Read	36
Number of Observations Used	36

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	246.6750000	35.2392857	218.54	<.0001
Error	28	4.5150000	0.1612500		
Corrected Total	35	251.1900000			

R-Square	Coeff Var	Root MSE	Y Mean
0.982026	2.111619	0.401559	19.01667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	5	242.1233333	48.4246667	300.31	<.0001
X2	2	4.5516667	2.2758333	14.11	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	5	242.1233333	48.4246667	300.31	<.0001
X2	2	4.5516667	2.2758333	14.11	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	28
Error Mean Square	0.16125

Number of Means	2	3	4	5	6
Critical Range	.4749	.4990	.5146	.5257	.5340

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	23.7500	2	4
B	21.9000	2	5
C	19.4500	2	1
D	17.8000	2	6
E	17.1500	2	2
F	16.5000	2	3

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	28
Error Mean Square	0.16125

Number of Means	2	3
Critical Range	.3358	.3528

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	19.4250	12	1
A	19.0667	12	3
B	18.5583	12	2

Table B.12: Two way ANOVA and Duncan's Multiple Range Test for span of double emulsions prepared by different surfactant types and concentrations; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	6	1 2 3 4 5 6
X2	3	1 2 3

Number of Observations Read	36
Number of Observations Used	36

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.03651033	0.01825517	18.29	0.0209
Error	12	0.00299450	0.00099817		
Corrected Total	19	0.03950483			

R-Square	Coeff Var	Root MSE	Y Mean
0.924199	2.254819	0.031594	1.401167

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	5	0.32181967	0.06436393	8.52	0.0107
X2	2	0.01703033	0.00851517	6.97	0.0746

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	5	0.32181967	0.06436393	8.52	0.0107
X2	2	0.01703033	0.00851517	6.97	0.0746

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.007559

Number of Means	2	3	4	5	6
Critical Range	.2127	.2205	.2243	.2262	.2271

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.50600	2	3
A	1.43750	2	2
B	1.19450	2	1
B	1.16600	2	6
B	1.11300	2	5
B	1.07300	2	4

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.007559

Number of Means	2	3
Critical Range	.1113	.1116

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.37750	2	1
B A	1.31250	2	2

Table B.13: Two way ANOVA and Duncan's Multiple Range Test for SSA of double emulsions prepared by different surfactant types and concentrations; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	6	1 2 3 4 5 6
X2	3	1 2 3

Number of Observations Read	36
Number of Observations Used	36

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	69190.41667	13838.08333	63.65	<.0001
Error	6	1304.50000	217.41667		
Corrected Total	13	70494.91667			

R-Square	Coeff Var	Root MSE	Y Mean
0.981495	0.950119	14.74506	1551.917

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	5	33817.33333	16908.66667	30.83	<.0001
X2	2	19369.00000	9684.50000	43.99	0.0060

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	5	33817.33333	16908.66667	30.83	<.0001
X2	2	19369.00000	9684.50000	43.99	0.0060

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	217.4167

Number of Means	2	3	4	5	6
Critical Range	36.08	37.39	38.05	38.37	38.52

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1663.00	2	3
A	1642.00	2	6
B	1544.50	2	2
B	1518.00	2	5
C	1481.00	2	1
C	1463.00	2	4

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	217.4167

Number of Means	2	3
Critical Range	47.22	47.38

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1568.00	2	1
B	1479.50	2	2
C	1394.00	2	3

Table B.14: One way ANOVA and Duncan's Multiple Range Test for instant stabilities of double emulsions prepared by MF, HSH and US

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	145.0233333	72.5116667	54.25	0.0044
Error	3	4.0100000	1.3366667		
Corrected Total	5	149.0333333			

R-Square	Coeff Var	Root MSE	Y Mean
0.973093	2.351477	1.156143	49.16667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	145.0233333	72.5116667	54.25	0.0044

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	145.0233333	72.5116667	54.25	0.0044

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	1.336667

Number of Means	2	3
Critical Range	3.679	3.692

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	56.100	2	3
B	46.150	2	2
B	45.250	2	1

Table B.15: Two way ANOVA and Duncan's Multiple Range Test for storage stabilities of double emulsions prepared by MF, HSH and US; time of 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3
X2	2	1 2

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	735.0683333	245.0227778	57.13	<.0001
Error	8	34.3116667	4.2889583		
Corrected Total	11	769.3800000			

R-Square	Coeff Var	Root MSE	Y Mean
0.955403	2.462521	2.070980	84.10000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	646.5050000	323.2525000	75.37	<.0001
X2	1	88.5633333	88.5633333	20.65	0.0019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	646.5050000	323.2525000	75.37	<.0001
X2	1	88.5633333	88.5633333	20.65	0.0019

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
                    Alpha                          0.05
                    Error Degrees of Freedom          8
                    Error Mean Square          4.288958

                  Number of Means             2          3
                  Critical Range          3.377      3.519

          Means with the same letter are not significantly different.

 Duncan Grouping          Mean      N    X1

                A        94.200      4    3
                B        81.125      4    1
                C        76.975      4    2

                    Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the
                  experimentwise error rate.

                    Alpha                          0.05
                    Error Degrees of Freedom          8
                    Error Mean Square          4.288958

                       Number of Means             2
                       Critical Range          2.757

          Means with the same letter are not significantly different.

 Duncan Grouping          Mean      N    X2

                A        86.817      6    1
                B        81.383      6    2
```

Table B.16: Two way ANOVA and Duncan's Multiple Range Test for D[4,3] value of double emulsions prepared by MF, HSH and US; time of 0, 24 and 48 h

```
                                The SAS System
                              The GLM Procedure

                          Class Level Information

                       Class         Levels    Values
                       X1                 3    1 2 3
                       X2                 3    1 2 3

                     Number of Observations Read          18
                     Number of Observations Used          18

Dependent Variable: Y
                                      Sum of
      Source             DF         Squares     Mean Square    F Value    Pr > F
      Model               4     100.2422222      25.0605556     237.13    <.0001
      Error              13       1.3738889       0.1056838
      Corrected Total    17     101.6161111
```

R-Square	Coeff Var	Root MSE	Y Mean
0.986480	1.681985	0.325090	19.32778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	98.00111111	49.00055556	463.65	<.0001
X2	2	2.24111111	1.12055556	10.60	0.0019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	98.00111111	49.00055556	463.65	<.0001
X2	2	2.24111111	1.12055556	10.60	0.0019

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	0.105684

Number of Means	2	3
Critical Range	.4055	.4247

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	22.2000	2	2
B	19.8500	2	1
C	16.5000	2	3

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	0.105684

Number of Means	2	3
Critical Range	.4055	.4247

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	19.6333	6	2
A	19.5167	6	1
B	18.8333	6	3

Table B.17: Two way ANOVA and Duncan's Multiple Range Test for span of double emulsions prepared by MF, HSH and US; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3
X2	3	1 2 3

Number of Observations Read	18
Number of Observations Used	18

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.15718800	0.07859400	451.69	0.0002
Error	8	0.00052200	0.00017400		
Corrected Total	12	0.15771000			

R-Square	Coeff Var	Root MSE	Y Mean
0.996690	1.031345	0.013191	1.279000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	0.03651033	0.01825517	18.29	0.0209
X2	2	0.08957033	0.04478517	1.46	0.3606

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	0.03651033	0.01825517	18.29	0.0209
X2	2	0.08957033	0.04478517	1.46	0.3606

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.000174

Number of Means	2	3
Critical Range	.04198	.04212

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.50600	2	3
B	1.19100	2	1
C	1.14000	2	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
                    Alpha                          0.05
                    Error Degrees of Freedom          8
                    Error Mean Square          0.000174

                  Number of Means             2          3
                  Critical Range         .5572      .5591

          Means with the same letter are not significantly different.

 Duncan Grouping          Mean      N    X1

               A        1.4378      2    3
               A        1.3605      2    2
               B        1.1291      2    1
```

Table B.18: Two way ANOVA and Duncan's Multiple Range Test for SSA of double emulsions prepared by MF, HSH and US; time of 0, 24 and 48 h

```
                                The SAS System
                              The GLM Procedure

                           Class Level Information

                          Class         Levels    Values
                          X1                 3    1 2 3
                          X2                 3    1 2 3

                        Number of Observations Read          18
                        Number of Observations Used          18

Dependent Variable: Y
                                      Sum of
       Source           DF         Squares     Mean Square    F Value    Pr > F
       Model             4       630577.3333     315288.6667     700.64    <.0001
       Error             3         1350.0000        450.0000
       Corrected Total   7       631927.3333

                       R-Square     Coeff Var      Root MSE        Y Mean
                       0.997864      1.635139      21.21320      1297.333

       Source              DF       Type I SS     Mean Square    F Value    Pr > F
       X1                   2     44790.33333     22395.16667      38.12    0.0074
       X2                   2       984.333333      492.166667       0.57   0.6182

       Source              DF     Type III SS     Mean Square    F Value    Pr > F
       X1                   2     44790.33333     22395.16667      38.12    0.0074
       X2                   2     984.333333      492.166667        0.57    0.6182

                        Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                             experimentwise error rate.

                           Alpha                          0.05
                           Error Degrees of Freedom          3
                           Error Mean Square               450
```

Number of Means	2	3
Critical Range	67.51	67.73

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1663.00	2	3
B	1354.00	2	2
C	875.00	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	450

Number of Means	2	3
Critical Range	103.7	104.0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1954.50	2	2
B	1436.00	1	1
B	1352.00	3	3

Table B.19: One way ANOVA and Duncan's Multiple Range Test for encapsulation characteristics of double emulsions; time of 0, 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	13.48000000	6.74000000	202.20	0.0006
Error	3	0.10000000	0.03333333		
Corrected Total	5	13.58000000			

R-Square	Coeff Var	Root MSE	Y Mean
0.992636	0.185167	0.182574	98.60000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	13.48000000	6.74000000	202.20	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	13.48000000	6.74000000	202.20	0.0006

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.033333

Number of Means	2	3
Critical Range	.5810	.5830

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	99.9000	2	1
A	99.4000	2	2
B	96.5000	2	3

Table B.20: One way ANOVA and Duncan's Multiple Range Test for D[4,3] value of coated emulsion by 7%, 9% and 13% NaCN concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	34.84000000	17.42000000	79.79	0.0025
Error	3	0.65500000	0.21833333		
Corrected Total	5	35.49500000			

R-Square	Coeff Var	Root MSE	Y Mean
0.981547	5.163111	0.467262	9.050000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	34.84000000	17.42000000	79.79	0.0025

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	34.84000000	17.42000000	79.79	0.0025

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.218333

Number of Means	2	3
Critical Range	1.487	1.492

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	10.9500	2	1
A	10.5500	2	2
B	5.6500	2	3

Table B.21: One way ANOVA and Duncan's Multiple Range Test for span of coated emulsion by 7%, 9% and 13% NaCN concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01265633	0.00632817	0.71	0.5593
Error	3	0.02675050	0.00891683		
Corrected Total	5	0.03940683			

R-Square	Coeff Var	Root MSE	Y Mean
0.321171	6.655397	0.094429	1.418833

Source	DF	Type I SS	Mean Square	F Value	Pr >
X1	2	0.01265633	0.00632817	0.71	0.5593

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	0.01265633	0.00632817	0.71	0.5593

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.008917

Number of Means	2	3
Critical Range	.3005	.3015

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.46450	2	2
A	1.43600	2	1
A	1.35600	2	3

Table B.22: One way ANOVA and Duncan's Multiple Range Test for SSA of coated emulsion by 7%, 9% and 13% NaCN concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	533053.0000	266526.5000	99.93	0.0018
Error	3	8001.0000	2667.0000		
Corrected Total	5	541054.0000			

R-Square	Coeff Var	Root MSE	Y Mean
0.985212	3.918286	51.64301	1318.000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	533053.0000	266526.5000	99.93	0.0018

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	533053.0000	266526.5000	99.93	0.0018

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	2667

Number of Means	2	3
Critical Range	164.4	164.9

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1737.50	2	3
B	1144.00	2	2
B	1072.50	2	1

Table B.23: One way ANOVA and Duncan's Multiple Range Test for instant stability of coated emulsion by 7%, 9% and 13% NaCN concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	4	1 2 3 4

Number of Observations Read 8
Number of Observations Used 8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3837.160000	1279.053333	3552.93	<.0001
Error	4	1.440000	0.360000		
Corrected Total	7	3838.600000			

R-Square	Coeff Var	Root MSE	Y Mean
0.999625	0.964630	0.600000	62.20000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	3	3837.160000	1279.053333	3552.93	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	3	3837.160000	1279.053333	3552.93	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	0.36

Number of Means	2	3	4
Critical Range	1.666	1.702	1.711

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	100.0000	2	4
B	52.5000	2	3
C	48.8000	2	2
C	47.5000	2	1

Table B.24: Two way ANOVA and Duncan's Multiple Range Test for storage stability of coated emulsion by 7%, 9% and 13% NaCN concentration; time of 24 and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	4	1 2 3 4
X2	2	1 2

Number of Observations Read	16
Number of Observations Used	16

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	718.4500000	239.4833333	331.46	<.0001
Error	11	2.8900000	0.7225000		
Corrected Total	15	721.3400000			

R-Square	Coeff Var	Root MSE	Y Mean
0.995994	1.194659	0.850000	71.15000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	3	447.3225000	447.3225000	619.13	0.0016
X2	1	615.0400000	615.0400000	93.21	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	3	447.3225000	447.3225000	619.13	0.0016
X2	1	615.0400000	615.0400000	93.21	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	11
Error Mean Square	0.7225

Number of Means	2	3	4
Critical Range	2.360	2.412	2.424

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	87.3000	2	4
B	68.4500	2	3
C	65.0000	2	2
C	63.8500	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
                        Alpha                          0.05
                        Error Degrees of Freedom         11
                        Error Mean Square            0.7225

                            Number of Means              2
                            Critical Range           2.827

                Means with the same letter are not significantly different.

                 Duncan Grouping          Mean      N    X2

                              A         79.450      8    1
                              B         64.050      8    2
```

Table B.25: One way ANOVA and Duncan's Multiple Range Test for D[4,3] value of coated emulsion by 10%, 15%, 20% and 25% gum arabic concentration

```
                                    The SAS System
                                  The GLM Procedure

                                Class Level Information

                              Class         Levels    Values
                              X1                 4    1 2 3 4

                            Number of Observations Read           8
                            Number of Observations Used           8

Dependent Variable: Y
                                        Sum of
       Source              DF         Squares     Mean Square    F Value    Pr > F
       Model                3     62689.08375     20896.36125     236.22    <.0001
       Error                4       353.84500        88.46125
       Corrected Total      7     63042.92875

                         R-Square     Coeff Var      Root MSE        Y Mean
                         0.994387      11.87736      9.405384      79.18750

       Source              DF       Type I SS     Mean Square    F Value    Pr > F
       X1                   3     62689.08375     20896.36125     236.22    <.0001

       Source              DF     Type III SS     Mean Square    F Value    Pr > F
       X1                   3     62689.08375     20896.36125     236.22    <.0001

                              Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                             experimentwise error rate.

                              Alpha                          0.05
                              Error Degrees of Freedom          4
                              Error Mean Square          88.46125

                          Number of Means          2          3          4
                          Critical Range       26.11      26.68      26.82
```

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	232.500	2	4
B	29.400	2	1
B	28.500	2	2
B	26.350	2	3

Table B.26: One way ANOVA and Duncan's Multiple Range Test for span of coated emulsion by 10%, 15%, 20% and 25% gum arabic concentration

The SAS System
The GLM Procedure
Class Level Information

Class	Levels	Values
X1	4	1 2 3 4

Number of Observations Read	8
Number of Observations Used	8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	444.6264464	148.2088155	598.88	<.0001
Error	4	0.9899065	0.2474766		
Corrected Total	7	445.6163529			

R-Square	Coeff Var	Root MSE	Y Mean
0.997779	8.079586	0.497470	6.157125

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	3	444.6264464	148.2088155	598.88	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	3	444.6264464	148.2088155	598.88	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	0.247477

Number of Means	2	3	4
Critical Range	1.381	1.411	1.419

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	19.0690	2	4
B	1.9385	2	3
B	1.8930	2	2
B	1.7280	2	1

Table B.27: One way ANOVA and Duncan's Multiple Range Test for SSA of coated emulsion by 10%, 15%, 20% and 25% gum arabic concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	4	1 2 3 4

Number of Observations Read	8
Number of Observations Used	8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	87745.00000	29248.33333	15.13	0.0120
Error	4	7735.00000	1933.75000		
Corrected Total	7	95480.00000			

R-Square	Coeff Var	Root MSE	Y Mean
0.918988	8.974372	43.97442	490.000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	3	87745.00000	29248.33333	15.13	0.0120

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	3	87745.00000	29248.33333	15.13	0.0120

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	1933.75

Number of Means	2	3	4
Critical Range	122.1	124.8	125.4

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	660.50	2	3
B	472.00	2	2
B	451.50	2	4
B	376.00	2	1

Table B.28: One way ANOVA and Duncan's Multiple Range Test for D[4,3] value of coated emulsion by 3%, 5%, 8% lecithin concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read 6
Number of Observations Used 6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	35.72333333	17.86166667	15.83	0.0255
Error	3	3.38500000	1.12833333		
Corrected Total	5	39.10833333			

R-Square	Coeff Var	Root MSE	Y Mean
0.913446	10.74769	1.062230	9.883333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	35.72333333	17.86166667	15.83	0.0255

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	35.72333333	17.86166667	15.83	0.0255

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	1.128333

Number of Means	2	3
Critical Range	3.380	3.392

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	13.200	2	1
B	9.050	2	2
B	7.400	2	3

Table B.29: One way ANOVA and Duncan's Multiple Range Test for span of coated emulsion by 3%, 5%, 8% lecithin concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00603100	0.00301550	49.03	0.0051
Error	3	0.00018450	0.00006150		
Corrected Total	5	0.00621550			

R-Square	Coeff Var	Root MSE	Y Mean
0.970316	0.518492	0.007842	1.512500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	0.00603100	0.00301550	49.03	0.0051

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	0.00603100	0.00301550	49.03	0.0051

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.000062

Number of Means	2	3
Critical Range	.02496	.02504

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.557000	2	3
B	1.495000	2	2
B	1.485500	2	1

Table B.30: One way ANOVA and Duncan's Multiple Range Test for SSA of coated emulsion by 3%, 5%, 8% lecithin concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	539433.3333	269716.6667	34.39	0.0085
Error	3	23529.5000	7843.1667		
Corrected Total	5	562962.8333			

R-Square	Coeff Var	Root MSE	Y Mean
0.958204	6.482493	88.56165	1366.167

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	539433.3333	269716.6667	34.39	0.0085

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	539433.3333	269716.6667	34.39	0.0085

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	7843.167

Number of Means	2	3
Critical Range	281.8	282.8

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1754.50	2	3
B	1319.50	2	2
C	1024.50	2	1

Table B.31: One way ANOVA and Duncan's Multiple Range Test for instant stability of coated emulsion by 3%, 5%, 8% lecithin concentration

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	580.0900000	290.0450000	216.18	0.0006
Error	3	4.0250000	1.3416667		
Corrected Total	5	584.1150000			

R-Square	Coeff Var	Root MSE	Y Mean
0.993109	2.077674	1.158303	55.75000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	580.0900000	290.0450000	216.18	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	580.0900000	290.0450000	216.18	0.0006

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	1.341667

Number of Means	2	3
Critical Range	3.686	3.699

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	68.150	2	3
B	55.000	2	2
C	44.100	2	1

Table B.32: Two way ANOVA and Duncan's Multiple Range Test for storage stability of coated emulsion by 3%, 5%, 8% lecithin concentration; time of 24 h and 48 h

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3
X2	2	1 2

Number of Observations Read	18
Number of Observations Used	18

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1638.880000	819.440000	234.24	<.0001
Error	13	10.495000	3.498333		
Corrected Total	17	1649.375000			

R-Square	Coeff Var	Root MSE	Y Mean
0.993637	2.647393	1.870383	70.65000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	108.00111111	49.00055556	463.65	0.0005
X2	2	5.29000000	5.29000000	3.66	0.0051

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	108.00111111	49.00055556	463.65	0.0005
X2	2	5.29000000	5.29000000	3.66	0.0051

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	0.105684

Number of Means	2	3
Critical Range	.4055	.4247

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	21.7667	6	2
B	20.0333	6	1
C	16.1833	6	3

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	3.498333
Number of Means	2
Critical Range	5.172

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	78.850	2	1
B	46.550	2	2

Table B.33: One way ANOVA and Duncan's Multiple Range Test for D[4,3] value of coated emulsion prepared by HSH and US

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2

Number of Observations Read	4
Number of Observations Used	4

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.69000000	1.69000000	26.00	0.0364
Error	2	0.13000000	0.06500000		
Corrected Total	3	1.82000000			

R-Square	Coeff Var	Root MSE	Y Mean
0.928571	3.186887	0.254951	8.000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1.69000000	1.69000000	26.00	0.0364

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1.69000000	1.69000000	26.00	0.0364

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.065

```
                    Number of Means          2
                    Critical Range       1.097

          Means with the same letter are not significantly different.

 Duncan Grouping          Mean      N    X1

                A        8.6500      2    2
                B        7.3500      2    1
```

Table B.34: One way ANOVA and Duncan's Multiple Range Test for span of coated emulsion prepared by HSH and US

```
                                The SAS System
                              The GLM Procedure
                           Class Level Information

                          Class         Levels    Values
                          X1                 2    1 2

                       Number of Observations Read           4
                       Number of Observations Used           4

Dependent Variable: Y
                                  Sum of
       Source           DF         Squares     Mean Square    F Value    Pr > F
       Model             1      2.07504025      2.07504025     833.94    0.0012
       Error             2      0.00497650      0.00248825
       Corrected Total   3      2.08001675

                        R-Square     Coeff Var      Root MSE        Y Mean
                        0.997607      2.351555      0.049882      2.121250

       Source             DF       Type I SS     Mean Square    F Value    Pr > F
       X1                  1      2.07504025      2.07504025     833.94    0.0012

       Source             DF     Type III SS     Mean Square    F Value    Pr > F
       X1                  1      2.07504025      2.07504025     833.94    0.0012

                           Duncan's Multiple Range Test for Y

       NOTE: This test controls the Type I comparisonwise error rate, not the
                          experimentwise error rate.

                           Alpha                        0.05
                           Error Degrees of Freedom        2
                           Error Mean Square        0.002488

                           Number of Means          2
                           Critical Range       .2146

                  Means with the same letter are not significantly different.

                 Duncan Grouping          Mean      N    X1
                               A       2.84150      2    2
                               B       1.40100      2    1
```

Table B.35: One way ANOVA and Duncan's Multiple Range Test for SSA of coated emulsion prepared by HSH and US

The SAS System
The GLM Procedure
Class Level Information

Class	Levels	Values
X1	2	1 2

Number of Observations Read 4
Number of Observations Used 4

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1071225.000	1071225.000	185.69	0.0053
Error	2	11538.000	5769.000		
Corrected Total	3	1082763.000			

R-Square	Coeff Var	Root MSE	Y Mean
0.989344	3.798646	75.95393	1999.500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1071225.000	1071225.000	185.69	0.0053

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1071225.000	1071225.000	185.69	0.0053

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	5769
Number of Means	2
Critical Range	326.8

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	2517.00	2	2
B	1482.00	2	1

Table B.36: One way ANOVA and Duncan's Multiple Range Test for instant stability of coated emulsion prepared by HSH and US

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2

Number of Observations Read	4
Number of Observations Used	4

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	244.9225000	244.9225000	246.77	0.0040
Error	2	1.9850000	0.9925000		
Corrected Total	3	246.9075000			

R-Square	Coeff Var	Root MSE	Y Mean
0.991961	1.264669	0.996243	78.77500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	244.9225000	244.9225000	246.77	0.0040

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	244.9225000	244.9225000	246.77	0.0040

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.9925

Number of Means	2
Critical Range	4.286

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	86.6000	2	2
B	70.9500	2	1

Table B.37: Two way ANOVA and Duncan's Multiple Range Test for storage stability of coated emulsion prepared by HSH and US; time of 24 and 48 h

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	2	1 2

Number of Observations Read 8
Number of Observations Used 8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	33.64000000	33.64000000	210.25	0.0047
Error	5	0.32000000	0.16000000		
Corrected Total	7	33.96000000			

R-Square	Coeff Var	Root MSE	Y Mean
0.990577	0.411946	0.400000	97.10000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	1.7112500	1.7112500	0.63	0.0650
X2	1	3.6100000	3.6100000	4.51	0.0676

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1.7112500	1.7112500	0.63	0.0650
X2	1	3.61000000	3.61000000	4.51	0.0676

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 5
Error Mean Square 2.73725

Number of Means 2
Critical Range 3.007

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	100.0000	2	2
B	94.2000	2	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	2.73725
Number of Means	2
Critical Range	3.007

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	96.425	4	1
B	87.050	4	2

Table B.38: One way ANOVA and Duncan's Multiple Range Test for encapsulation characteristics of coated emulsion; time of 0, 24 and 48 h

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2082.813333	1041.406667	147.26	0.0010
Error	3	21.215000	7.071667		
Corrected Total	5	2104.028333			

R-Square	Coeff Var	Root MSE	Y Mean
0.989917	5.141980	2.659261	51.71667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	2082.813333	1041.406667	147.26	0.0010

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	2082.813333	1041.406667	147.26	0.0010

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05	
Error Degrees of Freedom	3	
Error Mean Square	7.071667	
Number of Means	2	3
Critical Range	8.463	8.491

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	72.250	2	1
B	55.750	2	2
C	27.150	2	3

Table B.39: Two way ANOVA and Duncan's Multiple Range Test for encapsulation characteristics of systems as double emulsion and coated emulsion; time of 0, 24 and 48 h

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12
Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	764.5225000	764.5225000	3597.75	0.0003
Error	8	0.4250000	0.2125000		
Corrected Total	11	764.9475000			

R-Square	Coeff Var	Root MSE	Y Mean
0.999444	0.535553	0.460977	86.07500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	480.422500	4809.422500	4209.56	0.0002
X2	2	195.322500	1905.322500	204.82	0.0048

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	489.422500	4809.422500	4209.56	0.0002
X2	2	195.322500	1905.322500	204.82	0.0048

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 8
Error Mean Square 0.2125

Number of Means 2

Critical Range 1.983

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	99.9000	2	1
B	72.2500	2	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.290625

Number of Means	2	3
Critical Range	8.463	8.491

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	87.250	2	1
B	75.750	2	2
C	61.150	2	3

Table B.40: One way ANOVA and Duncan's Multiple Range Test for baking process on the vitamin amount of different systems as direct addition of vitamin, primary emulsion addition and double emulsion addition

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.20333333	2.10166667	0.26	0.7852
Error	3	24.03000000	8.01000000		
Corrected Total	5	28.23333333			

R-Square	Coeff Var	Root MSE	Y Mean
0.148878	3.280751	2.830194	86.26667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	4.20333333	2.10166667	0.26	0.7852

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	4.20333333	2.10166667	0.26	0.7852

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	8.01

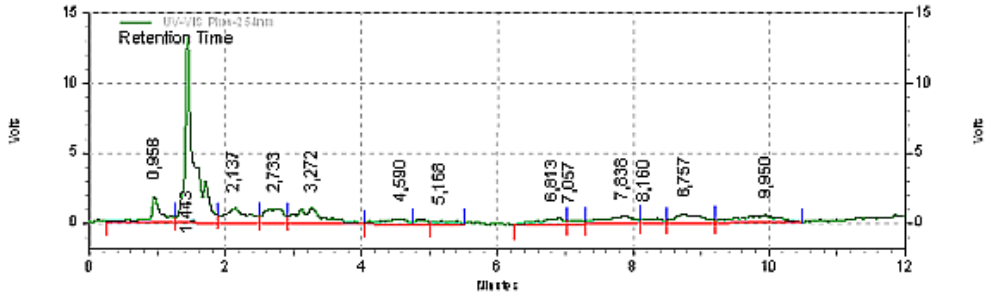
Number of Means	2	3
Critical Range	9.007	9.037

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	87.300	2	3
A	86.250	2	1
A	85.250	2	2

APPENDIX C

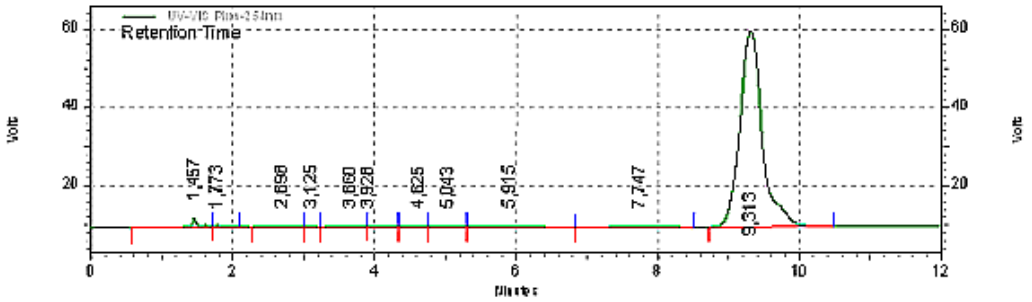
HPLC ANALYSIS



UV-VIS
Plus-254nm
Results (System
(14.10.2015
15:35:46)
(Original))

Retention Time	Area	Area %	Height	Height %
0,958	23258	6,90	1857	8,40
1,443	125668	37,28	13321	60,23
2,137	25017	7,42	1064	4,81
2,733	20520	6,09	985	4,45
3,272	32153	9,54	1081	4,89
4,590	10915	3,24	443	2,00
5,168	6779	2,01	297	1,34
6,813	13979	4,15	488	2,21
7,057	5167	1,53	367	1,66
7,838	20840	6,18	570	2,58
8,160	8678	2,57	424	1,92
8,757	19628	5,82	659	2,98
9,950	24479	7,26	561	2,54
Totals	337081	100,00	22117	100,00

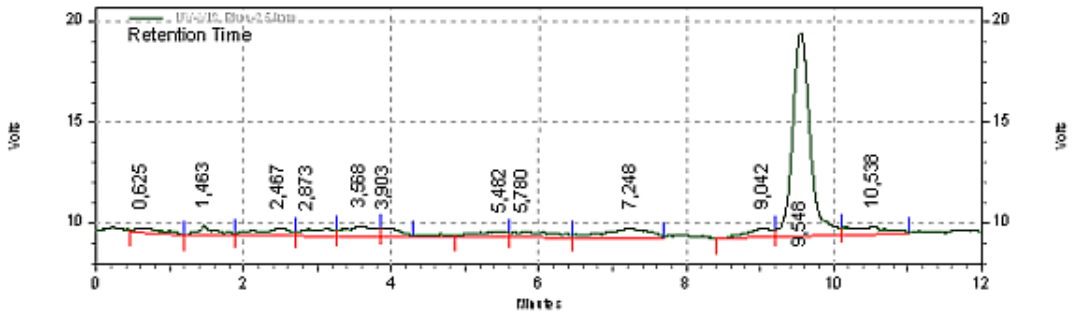
Figure C.1: HPLC choromatogram of fresh carrot juice



UV-VIS
Plus-254nm
Results (System
(09.10.2015
13:55:30)
(Original))

Retention Time	Area	Area %	Height	Height %
1,457	25875	2,06	2574	4,50
1,773	9615	0,77	769	1,34
2,698	17858	1,42	550	0,96
3,125	5328	0,42	617	1,08
3,660	13474	1,07	484	0,85
3,928	9839	0,78	416	0,73
4,625	8798	0,70	421	0,74
5,043	10933	0,87	391	0,68
5,915	28863	2,30	564	0,98
7,747	17725	1,41	431	0,75
9,313	1107040	88,19	50045	87,40
Totals	1255348	100,00	57262	100,00

Figure C.2: HPLC choromatogram of carrot juice enriched by Vitamin B_1 via double emulsion and stored 48 h



UV-VIS
Plus-254nm
Results (System
(09.10.2015
14:08:52)
(Original))

Retention Time	Area	Area %	Height	Height %
0,625	7765	2,56	255	1,75
1,463	9395	3,10	512	3,51
2,467	12786	4,22	401	2,75
2,873	10610	3,50	367	2,52
3,568	14663	4,84	502	3,45
3,903	8152	2,69	422	2,90
5,482	9660	3,19	299	2,05
5,780	11806	3,90	294	2,02
7,248	22771	7,52	537	3,69
9,042	11860	3,92	491	3,37
9,548	167725	55,37	10083	69,21
10,538	15743	5,20	406	2,79
Totals	302936	100,00	14569	100,00

Figure C.3: HPLC choromatogram of carrot juice enriched by Vitamin B_1 via direct addition of vitamin and stored 48 h

CIRRICULUM VITAE

PERSONAL INFORMATION

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WORK EXPERIENCE

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2009-2012	METU Department of Food Engineering	Research Assistant