ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

INFLUENCE OF EMULSIFIER TYPE ON THE STABILIZATION OF PALM OLEIN DOUBLE EMULSION (W/O/W)-BASED DELIVERY SYSTEMS FOR REDUCED CALORIE FOODS

M.Sc. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KALORİSİ AZALTILMIŞ GIDALARA YÖNELİK OLUŞTURULAN PALM OLEİN ÇOKLU EMÜLSİYON (S/Y/S) TAŞIYICI SİSTEM STABİLİTESİNE FARKLI EMÜLGATÖRLERİN ETKİSİ

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Gamze Hanbeyoğlu Aktürk, a M.Sc./a Ph.D. student of İTU Graduate School of Science Engineering and Technology student ID 506131509, successfully defended the thesis/dissertation entitled "INFLUENCE OF EMULSIFIER TYPE ON THE STABILIZATION OF PALM OLEIN DOUBLE EMULSION (W/O/W)-BASED DELIVERY SYSTEMS FOR REDUCED CALORIE FOODS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 12 September 2017
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To my mother, father, spouse and son,

FOREWORD

I would like to thank all people who made this work possible.
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I dedicate this thesis to my loving son, Tarık Efe, since he has been a very calm boy and has made me taste the motherhood.

September 2017 Gamze HANBEYOĞLU AKTÜRK
(Food Engineer)

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ABBREVIATIONS

ANOVA	**:** Analysis of variance (varyans analizi**)**
DSC	**:** Differantial scanning calorimetry
MWD	**:** Molecular weight distribution
PGPR	**:** Polyglycerol polyricinoleate
SC	**:** Sodium caseinate
SSL	**:** Sodium stearoyl lactylate
W/O	**:** Water-in-oil emulsion
W_1/O/W_2	**:** Water-in-oil-in-water emulsion

SYMBOLS

°C	: Centigrade degree
ml	: Mililiter
M	: Molarity
nm	: Nanometer
mV	: Milivolt
Pa	: Pascal
rpm	: round per minute
min	: Minute
h	: Hour
G’	: Storage modulus
G’’	: Loss modulus
w/w	: Wight/weight ratio
p	: Statistical probability

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INFLUENCE OF EMULSIFIER TYPE ON THE STABILIZATION OF PALM OLEIN DOUBLE EMULSION (W/O/W)-BASED DELİVERY SYSTEM FOR SOME BIOACTİVE COMPONENTS

SUMMARY

Nowadays, there is an increasing interest among the people towards to natural and health promoting lifestyles. Consumers are more conscientious especially in their food choices. They are looking for natural, less calorie, value added and high quality food products. In this respect, ingredients from natural sources, and health promoting valuable bioactive compounds are very popular and there is an increasing market of products that are made from these ingredients. However, usage of these ingredients into the food systems are not easy most of the time due to their low effectiveness, poor bioavailability and instability. Thus, food scientist and also food industry pay attention in this research area.

Emulsion-based delivery systems are promising methods for protecting biologically active materials from the environment and process conditions and by this way increasing their bioavailability. Among the emulsion-based delivery systems, double emulsion systems are very interesting and appealing for different reasons. First of all, different type of active ingredients can be delivered at the same time within these systems. Secondly, by incorporating more healthier fatty acids inside the oil droplets of the O/W systems, the total fatty acid composition of the system can be improved. Additionally, fat reduction can be done by incorporation of water inside the oil phase of the O/W emulsion.

In this study, W/O/W double emulsion system is studied comparing the natural emulsifiers with synthetic ones to form a stable palm-based double emulsion which will be latter applied in bakery products to reduce the calorie by a physical method. While, lecithin and PGPR is examined for simple W/O emulsions, sodium caseinate and SSL is examined for double W/O/W emulsions. The simple emulsions are characterized by droplet size, droplet charge and interfacial tension measurements. On the other hand, the double emulsions are characterized in terms of droplet size, droplet charge, interfacial tension, gravitational stability, rheology, and emulsion efficiency. Finally, storage stability of the double emulsions are analyzed after 7 days.

Lecithin is found to be unsuitable for emulsification of water-in-palm oil simple emulsion. Moreover, increasing PGPR content has decreased the tension of water-oil interface and as a result of that the droplet size of the simple emulsion lowered from 616,95 nm to 451,2 nm. Zeta potential values of all the PGPR concentrations are lower from the value of -20 mV, which was reported as the treshold value for stable emulsions. As the emulsifier concentration of PGPR increased, the zeta potential decreased as expected. These results confirms the droplet size results at 7th day of storage.

The droplet size results of the double emulsions are not statistically affected by the concentration of the SSL and SC except 3% of SC usage. 3% of SC decreased the double emulsion droplet size significantly. Besides, SSL containing double emulsions

show smaller droplet size distributions than SC ones. On the other hand, initial droplet size results have good relationship between the interfacial tension and emulsion efficiency results. Moreover, droplet size results at 7^{th} day of storage have good correlation between zeta potential and gravitational stability results. Microscopic images and rheology results have confirmed the droplet size results at 0^{th} and 7^{th} day. Best emulsion efficiencies are obtained from 4% PGPR-4%SSL and 4% PGPR-3% SC combinations as 99,02% and 95,02 respectively. Interfacial tension between oil and hydrophilic emulsifier (SSL and SC) containing water phase results found to be inversley proportional to the emulsion efficiency results as expected. Furthermore, microscopic images of double emulsions confirm the emulsion efficiency results. Gravitational stability of double emulsions showed that, SSL containing double emulsions (ranges 40-45% up to 100%) are much more stable than SC containg ones (ranges approximetaly 40% to 70%). While, zeta potential of oil droplets in SSL containing double emulsions range approximately -50 mV to -64 mV, zeta potential of oil droplets in SC containing double emulsions range approximately -30 mV to -48 mV. Zeta potential results confirm the gravitational stability results. On the other hand, as viscosity values of SSL containing double emulsions range between 21-65 mPa.s, SC containing ones range between 16-20 mPa.s. This is one of the reasons why the gravitational stability of SSL containing double emulsion is higher than SC containing double emulsions. Besides, according to the oscillatory frequency sweep test results, SSL containing double emulsions has narrower MWD, but SC containing ones has a broad MWD range. Also, microscopic images of the double emulsions confirm these rheological test results, since, SC containing double emulsions have both larger and smaller oil droplets.

As a result, the natural emulsifiers evaluated for the palm-based multiple emulsion systems in this study were not found suitable for stabilizing both in-oil-water and water-in-oil-in-water emulsions. It has been determined that lecithin disperses the phases in the oil-in-water emulsion but can not trap the water phase in the oil phase. Although sodium caseinate can trap up to 95% of the water in the oil phase in the multiple emulsion system, it has been found not to exhibit sufficient stability during storage. In addition, according to the results of different emulsifier combinations, the combination of 4% PGPR- 4% SSL showed the best results. It is considered that further studies with different hydrocolloids and stabilizers can be made so that sodium caseinate can be used as natural emulsifier in palm-based double emulsion systems. In addition, it will be appropriate to examine the thermal stability of this obtained palm-based double emulsion in order to evaluate the applicability of the resulting emulsion in bakery products.

Finally, this study provides useful data on the use of different emulsifier types to stabilize the palm-based double emulsion system at pH 6.0. This study also includes the most suitable emulsifier combination that stabilizes the palm-based double emulsion system at pH 6.0.

FARKLI EMÜLGATÖRLERİN PALM OLEİN ÇOKLU EMÜLSİYONLARI (S/Y/S) STABİLİTESİNE ETKİSİ VE BU EMÜLSİYONLARIN BAZI AKTİF BİLEŞENLER İÇİN TAŞIYICI SİSTEM OLARAK KULLANILMASI

ÖZET

Son yıllarda insanlar arasında doğal ve sağlıklı yaşam tarzlarına oldukça artan bir ilgi bulunmaktadır. Tüketiciler, özellikle gıda konusunda oldukça özenli seçimler yapmaktadırlar. Doğal, düşük kalorili, besin değeri arttırılmış, olumlu sağlık etkisi eklenmiş ve yüksek kaliteli ürünler rağbet görmektedir. Bu bağlamda, doğal kaynaklı hammaddeler ve sağlığa olumlu etki yarattığı bilinen değerli biyoaktif bileşenlerin gıda ürünlerinde kullanımı popülerlik kazanmakta ve bu hammddeler ile üretilen ürünler hızla gelişen bir market haline gelmektedir. Ancak doğal kaynaklı ve biyoaktif bileşenlerin gıda ürünlerinde kullanılması, bu bileşenlerin düşük etkinliği, düşük biyoyararlılıkları ve düşük stabiliteleri sebebiyle her zaman kolay olmamaktadır. Bu bileşenlerin özelliklerinden yararlanabilmek adına bahsedilen bu zorlukları elimine etmek için derinlemesine araştırmalar ve yeni teknolojiler geliştirmek gerekmektedir. Bu sebeplerle bu konu gıda araştırmacıları ve gıda endüstrisi için ilgi çekici bir araştırma alanı olarak karşımıza çıkmaktadır. Biyoaktif bileşenlerin biyoyararlılıklarının arttırılması sadece gıda alanı için değil, ayrıca farmasötik alanda da oldukça popüler bir konudur.

Biyoaktif bileşenlerin biyoyararlılıklarının arttırılması amacıyla çok çeşitli yöntemler çalışılmış ve halen çalışılmaktadır. Bunlar arasında emülsiyon bazlı taşıyıcı sistemler, biyoaktif bileşenlerin taşınması için oldukça elverişli sistemlerdir. Emülsiyon bazlı taşıyıcı sistemler ile bu bileşenler çevre etkileri ve proses koşullarından korunarak hedeflenen bölgelere taşınarak biyoyararlılıkları arttırılabilmektedir. Emülsiyon bazlı taşıyıcı sistemler içerisinde çoklu emülsiyonlar bazı üstünlükleri sebebiyle ilgi çekici olabilmektedir. Öncelikle bu sistemler hem yağ hem de suyu iç faz olarak barındırdığı için hem hidrofilik hem de lipofilik biyoaktif bileşenler rahatlıkla aynı sistemde enkapsüle edilebilmektedir. Bunun yanı sıra bu sistemler ile yağ asit kompozisyonu değiştirilerek sağlıklı yağ asitlerinin miktarı arttırılabilmektedir. Ayrıca bu sistemlerde yağ fazı içerisine yerleştirilen su globülleri ile toplam yağ miktarı azaltılabilmektedir.

Bu çalışmada, daha sonra fırıncılık ürünlerinde kalori düşürmek amacı ile kullanılmak üzere palm-bazlı su-içinde-yağ-içinde-su (S/Y/S) çoklu emülsiyonu sentetik emülgatörler ile doğal kaynaklı emülgatörler kullanılarak çalışılmıştır. Tekli yağ-içinde-su (S/Y) emülsiyonu için lesitin ve PGPR değerlendirilirken, çoklu su-içinde-yağ-içinde-su (S/Y/S) çoklu emülsiyonu için sodyum kazeinat (SC) ve SSL (Sodium stearoyl lactylate) değerlendirilmiştir. Tekli emülsiyonlar partikül boyutu, partikül yükü, ara yüzey gerilimi analizleri ile karakterize edilirken, çoklu emülsiyonlar partikül boyutu, partikül yükü, ara yüzey gerilimi, reoloji, ve emülsiyon verimi analizleri ile karakterize edilmiştir. Hem tekli hem de çoklu emülsiyonların mikroskop görüntüleri de ayrıca incelenmiştir. Son olarak, 20 °C'de 7 gün depolanan tekli ve çoklu emülsiyonların depolama stabilitesi partikül boyutu ve yerçekimi stabilitesi analizleri ile değerlendirilmiştir.

Lesitin, palm yağı ile oluşturulmuş tekli emülsiyonları stabilize etmek için uygun bulunmamıştır. Bunun dışında, PGPR konsantrasyonunun artması ile su-yağ ara yüzey gerilimi azalmış ve buna bağlı olarak partikül boyutu 616,95 nanometreden 451,2 nanometreye düşmüştür. Ayrıca, tüm PGPR konsantrasyonlarına ait partikül yükü değerleri -20 mV değerinden düşük elde edilmiştir ki bu değer stabil emülsiyonlar için eşik değer olarak rapor edilmiştir. PGPR konsantrasyonunun artması ile partikül yükü değerleri beklendiği gibi düşüş göstermiştir. Azalan partikül yükü değerleri ile 7 gün sonundaki partikül boyutu sonuçları birbirini doğrulamaktadır.

Çoklu emülsiyonların partikül boyutları %3 SC kullanımı dışındaki SSL ve SC konsantrasyonundan istatistiki anlamda farklılık göstermemiştir. Buna rağmen,%3 oranındaki SC kullanımı, çoklu emülsiyon partikül boyutunu önemli ölçüde azaltmıştır. Bunun dışında, SSL ile oluşturulmuş çoklu emülsiyonlar, SC ile oluşturulmuşlardan daha küçük partikül boyutu dağılımı göstermiştir. Ek olarak, çoklu emülsiyonların başlangıç partikül boyutu sonuçları, ara yüzey gerilimi ve emülsiyon verimi sonuçları ile iyi bir ilişki göstermiştir. Ayrıca, 7 gün sonundaki partikül boyutu sonuçları ise partikül yükü ve yerçekimi stabilitesi ile doğrusal bir ilişki göstermiştir. Çoklu emülsiyonların başlangıç ve 7. gün mikroskopik ve reoloji sonuçları partikül boyutu sonuçlarını destekler niteliktedir. Öte taraftan, en iyi emülsiyon verimi sonuçları sırasıyla % PGPR-4%SSL ve 4% PGPR-3% SC kombinasyonlarından %99,02 ve %95,02 olarak elde edilmiştir. Yağ fazı ve hidrofilik emülgatör (SSL ve SC) içeren su fazı arasındaki ara yüzey gerilimi sonuçları beklendiği şekilde emülsiyon verimi sonuçları ile ters orantılı olarak bulunmuştur. Bunun yanı sıra, çoklu emülsiyonların mikroskopik görüntüleri emülsiyon verimi sonuçları ile uyumlu olarak elde edilmiştir. Çoklu emülsiyonların yerçekimi stabilitesi sonuçları SSL içeren emülsiyonların SC içeren emülsiyonlardan çok daha stabil olduğunu göstermiştir. Buna ek olarak, SSL içeren çoklu emülsiyonların partikül yükü sonuçları -50 mV ile -64 mV arasında değişirken, SC içeren çoklu emülsiyonların partikül yükü sonuçları -30 mV ile -48 mV değerleri arasında değişmektedir ki bu değerler hem -20 mV değerinden oldukça düşük hem de yerçekimi stabilitesi sonuçları ile uyumluluk göstermektedir. SSL içeren emülsiyonların partikül yükleri daha düşüktür, bu da yerçekimi stabilitesini arttırmaktadır. Bunlara ek olarak, SSL içeren emülsiyonların viskozite değerleri 20-65 mPa.s aralığında iken, SC içeren emülsiyonların viskozite değerleri 16-20 mPa.s değerleri arasında değişmektedir. Görüldüğü üzere SSL içeren emülsiyonların viskozite değerleri SC içerenlerden oldukça yüksektir ve bu da SSL içerenlerin yerçekimi stabilitesinin daha fazla olmasının bir nedeni olarak düşünülmüştür. Ayrıca frekans salınımlı test sonuçları göstermiştir ki SSL içeren çoklu emülsiyonlar daha dar partikül boyutu dağılımı sahipken, SC içeren çoklu emülsiyonlar daha geniş partikül boyutu dağılımına sahiptir. Bu sonuçlar, çoklu emülsiyon mikroskop görüntüleri ile uyumludur.

Sonuç olarak, bu çalışma kapsamında palm-bazlı çoklu emülsiyon sistemleri için değerlendirilen doğal emülgatörler hem yağ-içinde-su hem de su-içinde-yağ-içinde su emülsiyonlarını stabilize etmek için uygun bulunmamıştır. Lesitinin, yağ-içinde-su emülsiyonunda fazları birbiri içinde dağıttığı ancak su fazını yağ fazı içerisine hapsedemediği belirlenmiştir. Sodyum kazeinatın ise çoklu emülsiyon sisteminde %95'e kadar suyu yağ fazı içerisine hapsedebilmesine rağmen depolama süresince yeterli stabiliteyi göstermediği tespit edilmiştir. Bunun yanı sıra, farklı emülgatör kombinasyonları sonuçlarına göre pH 6,0'da %4 PGPR-%4 SSL kombinasyonunun en iyi sonuç verdiği görülmüştür. Sodyum kazeinatın palm-bazlı çoklu emülsiyon sistemlerinde doğal kaynaklı emülgatör olarak kullanılabilmesi için farklı hidrokolloidler ve stabilizatörler ile ileri çalışmalar yapılabileceği düşünülmektedir. Bunun dışında, elde edilen bu palm-bazlı çoklu emülsiyonun fırıncılık ürünlerinde uygulamasının yapılabilirliğinin değerlendirilmesi amacıyla termal stabilitesinin de incelenmesi uygun olacaktır.

Son olarak, bu çalışma, pH 6,0'da palm-bazlı çoklu emülsiyon sistemini stabilize etmek için farklı emülgatör çeşitlerinin kullanımı ile ilgili faydalı veriler sağlamaktadır. Ayrıca yine bu çalışma, palm-bazlı çoklu emülsiyon sistemini pH 6,0'da stabilize eden en uygun emülgatör kombinasyonunu da içermektedir.

1. INTRODUCTION

In recent years, increasing interest has emerged about health and health promoting food consumption. Consumers are looking for healthier, natural, added value and high quality food products. Foods with synthetic additives and overconsumption of calorie-rich foods (with high fat and/or carbohydrate content) are the negative factors that influence consumer's preferences. There is a growing trend toward natural and reduced-calorie foods.

In this respect, usage of natural sources for food additives instead of synthetic ones and incorporating active materials (such as vitamins, minerals, carotenoids, phenolic compounds, microorganisms, amino acids, oils, flavors etc.) into food products are the popular research areas both for scientist and food industry. Functional foods that contains active ingredients has an expanding market, and also can promote public health and welfare and/or lower the risk of disease (Jiménez-Colmenero, 2013). Hence, encapsulation of active materials is an important investigation field not only for food industry, but also for pharmaceutical field.

However, incorporating active ingredients in foods is generally challenging due to their low solubility, inappropriate physical state, poor physicochemical stability, poor biochemical stability, poor flavor profile, and poor handling characteristics. Due to their unique properties and functional requirements, specific delivery systems must be developed for any individual ingredient and application (McClements, 2016).

There are several emulsion-based delivery systems for encapsulation of active ingredients. Conventional emulsions, double emulsions, multilayer emulsions, solid-lipid particles, filled hydrogel particles, colloidomes, microcluesters and nanoemulsions are some examples for emulsion-based delivery systems. Water-in-oil-in-water (W/O/W) double emulsions, also known as double emulsions, are one of the promising method to overcome these limitations of the active material usage in foods. These emulsions are compartmentalized systems that consist both water-in-oil (W/O) and oil-in-water (O/W) systems at the same time and this feature enables the system to deliver both hydrophilic and lipophilic active ingredient together in one emulsion.

Furthermore, W/O/W double emulsions can be used to reduce the fat content of the various food products (such as dairy, bakery, meat, cream etc.) as it contains small water droplets inside the dispersed oil phase.

The present work aims to develop a palm-based W/O/W double emulsion system (which will be applied to a bakery product latter) to reduce the fat content of the total emulsion by physical methods, comparing natural emulsifiers lecithin and sodium caseinate, with synthetic PGPR and SSL emulsifiers respectively.

2. THEORATICAL PART

2.1 Emulsion Based Delivery Systems

Delivering functional components have been done for many years with conventional emulsion systems such as water-in-oil (W/O) or oil-in-water (O/W) emulsions. However these type single emulsion systems have some limitations to deliver active substances. These limitations can be listed as limited resistance to environmental conditions (such as pH, ionic strength, thermal processing, chilling, freezing, drying and ingredient compatibility) and limited capacity to produce innovative and functional food products (ingredient protection systems, controlled release, site-specific release, triggered release) (McClements, n.d.). A number of different kind of novel techniques have been proposed to overcome these limitations and numerous studies have done on this subject. Some of these techniques are shown schematically in Figure 2.1.

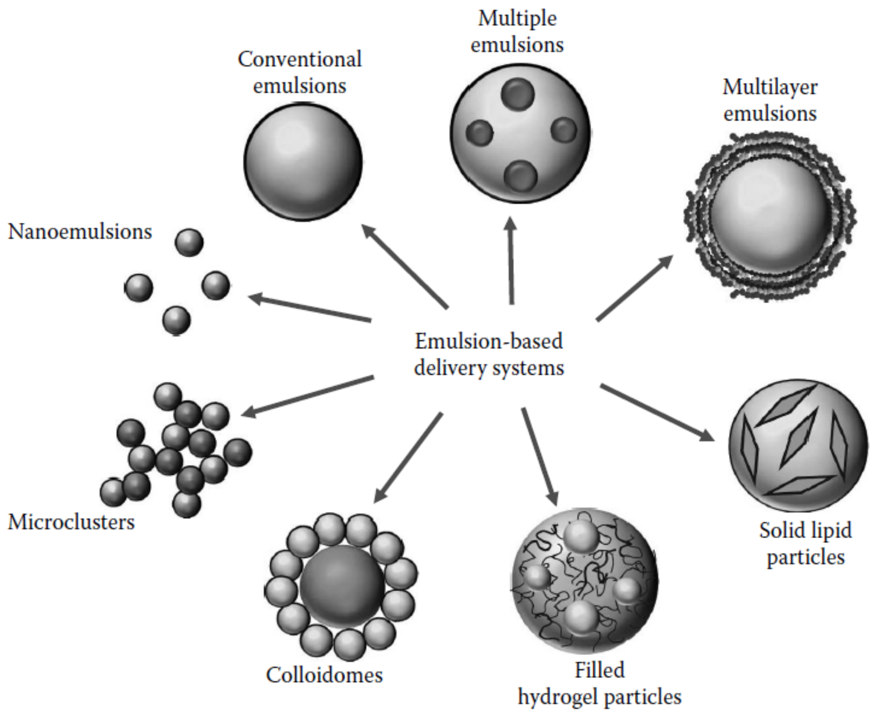


Figure 2.1 : Examples of novel and conventional emulsion based-delivery systems (McClements, 2014).

Formation of a delivery system can be problematic by reason of the specific characteristics of each active materials. A brief summary of most common difficulties that is encountered while incorporating active ingredients into food products is given below.

Low solubility: Solubility of active material within water or oil determines the encapsulation side of the material. Moreover, either low oil and low water soluble materials should be deliver in crystalline form. (McClements, 2016).

Inappropriate physical state: The physical state of the active material can be solid, liquid or even gaseous at ambient temperature. These features can be detrimental for the specific applications and can be change products organoleptic properties or influence the stability. Thus, should be preprocessed before incorporation (McClements, 2016).

Poor physicochemical stability: Active materials can be physically or chemically instable during processing and storage conditions. So, while incorporation the material, the specific attributes of the material should be considered application point of view (McClements, 2016).

Poor biochemical stability: The structure of active materials undergoes some modifications during gastrointestinal digestion. For controlled release of these substances, the composition and functional groups should be taken into account (McClements, 2016).

Poor flavor profile: Bitter or astringent taste can be come up with some active materials. Thus, it is important to mask these undesirable flavours. (McClements, 2016).

Poor handling characteristics: Transporting, storage and handling properties of the active materials can be difficult. Hence, the appropriate conditions should be determined (McClements, 2016).

Although the needs for encapsulation of the specific active ingredients and the needs for application areas are change from one to another, delivery systems have some common features. These are briefly summarized below.

Food grade: Both emulsion ingredients and processing operations should be food grade (McClements, 2016).

Economic production: The delivery system should be cost effective. The achievements from encapsulation of the active material should be more than the costs of encapsulation (McClements, 2016).

Food matrix compatibility: The delivery system and active ingredient should not be influence the organoleptic properties and shelf life of the food material. (McClements, 2016).

Protection against chemical degradation: Chemical and biochemical degradation reactions should be prevented by the delivery system (McClements, 2016).

Loading capacity, encapsulation efficiency and retention: It is expected that, the delivery system encapsulate as much as active material it can carry per unit mass. Also the retention of the encapsulated material until the targeted released location should be maintained by the delivery system (McClements, 2016).

Delivery mechanism: The delivery system should release the active component at the targeted location at a controlled rate or a specific environmental condition such as storage or gastrointestinal tract (McClements, 2016).

Bioavailability/bioactivity: The bioavailability/bioactivity of the active material should be increased by the delivery system (McClements, 2016).

Controlled release: To obtain exact side effect the active material should be released in a desired manner (McClements, 2016).

2.2 Double (multiple) Emulsion Systems

Conventional emulsions consist from a continuous (outer) and a dispersed (inner) liquid phase, which are normally immiscible. These continuous or dispersed phases can be either oil (O) or water (W). On the other hand, double emulsions are systems that have small droplets inside the dispersed phase and thus they are multi-compartmentalized systems. This feature enables these systems to create a wide range of different possibilities for different applications. The most widely studied forms of double emulsions are water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) systems, where water-in-oil-in-water systems found themselves more application areas within food industry. Water-in-oil-in-water usually shown as $W_1/O/W_2$ in which W_1 is the inner dispersed phase, O is the outer disperse phase which separates two water phases, and W_2 is the continuous phase. A schematic illustration of W/O/W is given in Figure 2.2.

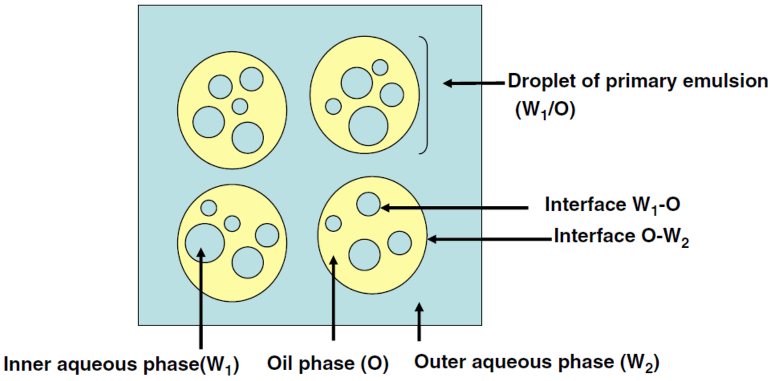


Figure 2.2 : Schematic illustration of W/O/W emulsions (Jiménez-Colmenero, 2013).

Furthermore, W/O/W emulsions have been used as carriers for anti-cancer agents, hormones, steroids, etc. in pharmaceutical industy; easy application of creams with encapsulated compounds in cosmetic industry and many other applications in different industries (Jiménez-Colmenero, 2013).

W_1/O/W_2 emulsions have some superiorities over conventional emulsions which are carrying both hydrophilic (within W_1 phase) and lipophilic (within O phase) compounds at the same time, controlled release of these active substances during eating and digestion, and development of fat-reduced food as the part of the oil phase is replaced by water.

2.2.1 Double emulsion systems for improving fat content of food

Double emulsions are useful systems for fat containing food products to add innovative features. For instance by double emulsification, some part of the oil phase can be replaced by water, but the volume fraction of the oil phase do not influenced. Hence, reduced-fat food products with similar physicochemical and organoleptic properties as full-fat counterparts can be achieved (Garti, 1997;McClements et al., 2009). Some studies that have been done on double emulsions as fat-replacers are listed in Table 2.1. It is seen that 15-26% fat reduced cheese-like products have been produced by W/O/W emulsion systems. On the other hand, it is observed that any studies haven't done on bakery products. The effect of W/O/W emulsion systems as fat-replacers on bakery product is still unknown.

Table 2.1 : Studies on double emulsions as fat-replacers (Jiménez-Colmenero, 2013).

Application	Content of W_1 phase	Oil phase (O)	Lipophilic emulsifier (%)	Hydrophilic emulsifier (%)	Reference
Reduced-calorie food emulsion	NaCl (0.1 M)	Olive oil	Glycerol mono-dioleate (3%), and sucrose palmitate-stereate (5%)	Sucrose palmitate–stereate (3%)	De Cindio & Cacace, 1995
Reduced-fat white fresh cheese-like products	Gellan gum (0.1%), and Panodan SDK® (1.6%)	Canola oil	PGPR (6.4%)	Different biopolymers of gum arabic, carboxymethyl cellulose and low-methoxyl pectine	Lobato-Calleros et al., 2006
Reduced-fat white fresh cheese-like products	Gellan gum (0.1%), and Panodan SDK® (1.6%)	Canola oil	PGPR (6.4%)	Different biopolymers of gum arabic, carboxymethyl cellulose and low-methoxyl pectine.	Lobato-Calleros et al., 2008
Formulation of low-fat stirred yogurt	8% Panodan SDK® + 0.5% gellan gum	Canola oil	PGPR (8%)	Skim milk with amidated low-methoxy pectin (1%) or carboxymethyl cellulose (0.5%)	Lobato-Calleros et al., 2009
Whipped dairy cream with encapsulated Ca	Calcium salts	Sunflower oil	PGPR (0.5, 1.0 and 2.0%)	Soybean milk containing 0.02% xanthan gum	Márquez & Wagner, 2010
Improve fat content of meat systems	NaCl 0.6%	Olive oil	PGPR (6%)	Sodium caseinate (0.5%) or whey protein concentrate (6%)	Cofrades et al., 2013

W/O/W emulsions are not only a promising method for reduced-fat products, but also improvement in fatty acid compositions of the product can be done by these systems. It is known that many scientific authorities such as World Health Oraganization (WHO), have recommended a ratio for total and saturated fat intake per day. In this respect, food manufacturers try to optimize the fat content and fat compositions of the food products. Replacement of animal based lipids by plant (olive, sunflower, linseed, chia, etc.) and marine (fish and algae) origin; changing saturated fatty acids with monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids; obtaining better w-6/w-3 ratios; formation of cholesterol free products can be listed for the use of improvement of fatty acid compositions of the food products by W/O/W emulsions (Jiménez-Colmenero, 2013).

2.2.2 Double emulsion systems for encapsulation of active ingredient

Numerous studies have been done on delivering active materials by double emulsions (Table 2.2). W/O/W double emulsion systems have several advantages about carrying active ingredients. First of all, these emulsions are suitable for controlled release of functional substances at targeted area in gastrointestinal tract such as mouth, stomach or small intestine. Also, by encapsulating the material inside the inner water phase, the oil phase acts as a protective coating for the active material, and prevent the chemical reactions between other water-soluble molecules within the outer water phase. Furthermore, the undesirable flavor components, such as bitterness, astringency and metallic flavors, which are come up with the active ingredients, can be entrapped in the inner water phase (Jiménez-Colmenero, 2013).

Table 2.2 : Studies on W/O/W double emulsions as delivery systems (Jiménez-Colmenero, 2013).

Main purpose	Content of inner aqueous solution (W1)	Lipid oil (O)	Lipophilic emulsifier (% in oil phase)	Hydrophilic emulsifier (% in external aqueous solution,W2)	Reference
Formation of multiple emulsions	PTSA	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (1–10%)	Decaglycerol monolaurate	Shima et al., 2004
Encapsulation of Lactobacillus acidophilus	MRS broth containing the washed bacteria	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (10%)	Decaglycerol monolaurate (3%)	Shima et al., 2006
Encapsulation of Lactobacillus acidophilus	MRS broth containing the washed bacteria	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (10%)	Decaglycerol monolaurate (3%)	Shima et al., 2009
Encapsulation of vitamin B12	Vitamin B12 (1%), and NaCl (0.22%)	Tripalmitin	Tetraglycerin condensed ricinoloeic acid ester (10%)	Decaglycerin condensed stearic acid ester (1%)	Kukizaki & Goto, 2007
Encapsulation of aroma compounds	Aromatic compound (0.025%) and glycerin	Rapeseed oil	SpanTM 80 (3%)	WPC, SC, skim milk power, soy protein, modified starch, maltodextrin, and arabic gum theW1/O/W2 were spray dried	Brückner, Bade, & Kunz, 2007
Encapsulation of carotenoids	Gellan gum, Panodan SDK and saponified oleoresin	Commercial blend of sunflower–canola–cartamo oils with oleoresin	PGPR (6.4%)	Gum arabic (17%), mesquite gum (66%), and maltodextrin (17%) the W/O/W were spray dried	Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, & Vernon-Carter, 2004
Formation of multiple emulsions	NaCl (0.1 M), gelatin (5%), and Poly R-478 (0.5%)	Sunflower oil	PGPR (4%)	WPI	Scherze, Knöfel, & Muschiolik, 2005
Encapsulation of L-tryptophan	NaCl (0.1 M), gelatin (5%), and L-tryptophan (0.05 M)	Semicrystalline oils (different oils with high melting points)	PGPR (2–8%)	WPI (1%). theW1/O/W2 were embedded in alginate gels	Weiss et al., 2005
Encapsulation of lactoferrin	Lactoferrin with sodium lactate, and sodium bicarbonate	22% butter fat, 78% corn oil	PGPR (0.1%)	WPI (30%) + 0.02% xanthan gum the W1/O/W2 were freeze-dried	Al-Nabulsi, Han, Liu, Rodriguez-Vieira,&Holley, 2006

Formation of multiple emulsions	Sodium caseinate (0.5%), and Poly R-478	Soybean oil	PGPR (0.5–8%)	SC (0.03–2%), phosphate buffer (0.1 M),	Su et al., 2006
Formation of multiple emulsions	0 and 15%WPI, without and with heating	Corn oil	PGPR (8%)	Tween 20 (0.5%)	Surh, Vladisavljevic, Mun, & McClements, 2007
Encapsulation of vitamin B12	0.1 M NaCl + 5% gelatin + 1.0% vitamin B12	MCT	PGPR (2, 8%)	0.7% SC or 2.1% SC–dextran conjugates	Fechner et al., 2007
Formation of multiple emulsions	SC (0.5%) or gum arabic, and Poly R-478	Soybean oil	PGPR (0.5–2%)	SC (0.5%) or gum arabic (0–15%)	Su, Flanagan, & Singh, 2008
Encapsulation of vitamin B12	5% gelatin (hydrated) + NaCl 0.6% + vitamin B12 0.03%	MCT	PGPR (2%)	SC (1% protein)	O'Reagan & Mulvihill, 2009
Encapsulation of magnesium	0.1 M Mg Cl2	Olive oil, rapeseed oil, and MCT	PGPR (5%)	0.3 M lactose + 12% SC	Bonnet et al., 2009
Encapsulation of iron	WPI (15%), with 0.1% Fe	Corn oil	PGPR (8%)	Tween 20 (0.5%)	Choi et al., 2009
Encapsulation of vitamin C	Glucose (11.9%), NaCl (4.4%) and WPI. Sodium ascorbate (1–30%)	MCT R(+) limonene	PGPR (10%)	WPI/pectine (4/0.5%)	Lutz et al., 2009
Encapsulation of Ca	Calcium salts	Sunflower oil	PGPR (0.5, 1,0 2.0%)	Soybean milk containing 0.02% xanthan gum	Márquez & Wagner, 2010
Encapsulation of resveratrol	0.25% resveratrol in sol of 0.1 M NaCl, ethanol, and 2.5% WPI	Canola oil	PGPR (8%)	0.5% SC, and 0.1 M NaCl	Hemar et al., 2010
Formation of multiple emulsions	0.28 M NaCl	Sunflower oil	PGPR (4%)	Tween 20 + glucose	Pawlik, Cox, & Norton, 2010
Encapsulation of vitamin B12	Hydrating gelatin (5%), NaCl (0.06%), and vitamin B12 (0.03%)	MCT	PGPR (2%)	SC or SC–maltodextrin conjugate	O'Reagan & Mulvihill, 2010
Formation of multiple emulsions	PTSA	Soybean oil	PGPR (4–8%)	WPI (2–6%)	Mun et al., 2010
Encapsulation of Mg	MgCl2, hemimagnesium salt, phosvitin, gluconate, and lactose	Olive oil	PGPR (5%)	SC (12%), and lactose (0.2 M)	Bonnet, Cansell, Placin, David-Brian, et al., 2010

Encapsulation of Mg	MgCl2 (0.1 M), lactose (0.02 M–0.2 M), and SC (1.9%)	Olive oil, and MCT	PGPR (5%)	SC (1.9–12.0%), and lactose (lactose 0.20 or 0.3 M)	Bonnet, Cansell, Placin, Anton, et al., 2010
Encapsulation of magnesium	0.1 M Mg Cl2	Olive oil, and MCT	PGPR (5%)	Lactose (0.3 M), and SC (3.1–12%)	Bonnet, Cansell, Placin, Monteil, et al., 2010
Formation of multiple emulsions	D-glucose (40%) and NaCl (8%)	Sunflower oil	PGPR (1–6%)	Gum arabic (20%), xanthan gum (0.025–1.0%), and D-glucose	Leal-Calderón, Homer, Goh, & Lundin, 2012
Formation of multiple emulsions	Gelatin (0–10%) and NaCl (0–8%)	Canola oil	PGPR (6%)	Polysorbate 80	Sapei, Naqvi, & Rousseau, 2012
Encapsulation of vitamin E and vitamin B2	Vitamin B2 (0.5 mg/mL)	Soy oil containing vitamin E (20%)	PGPR (8%)	WPI (10%), polysaccharides (0.2–1%), methoxyl peptin, -carrageenan	Li et al., 2012
Encapsulation of anthocyanin extract	Anthocyanin extract, peçtin and CaCl2	Rapeseed oil	PGPR (2.5%)	Bile extract, whey protein isolate or protein– polysaccharide-conjugate with peptin amide	Frank et al., 2012
Encapsulation of glucose and B1	Glucose (2%) + glycerol B1 (1%)	MCT	PGPR (8%) + monoglyceride oleate (2%)	WPI (5%)-polysaccharide complexes (xanthan gum 0.5–6%, guar gum and locust bean gum)	Benichou et al., 2007
Encapsulation of ferrous bisglycinate	Ferrous bisglycinate (30%)	Mineral oil (food grade)	5% of PGPR/Panodan SDK® (6:4)	WPC:polysaccharides (gum arabic or mesquite gum or low methoxyl peptin) 2:1	Jiménez-Alvarado et al., 2009
Encapsulation of Lactobacillus rhamnosus	Sweet whey containing 8.02 log cfu/mL	Canola oil	8% (1 part of Panodan SDK® + 4 parts of PGPR)	Sweet whey or concentrate of sweet whey Dispersions	Pimentel-González et al., 2009
Encapsulation of ascorbic acid	Ascorbic acid (30%), gellan gum (0.5%), and Panodan SDK® (8%)	Chia oil	6% (1 part of Panodan SDK® + 4 parts of PGPR)	Mesquite gum, maltodextrin, and WPC	Carrillo-Navas et al., 2012

Several active ingredients can be incorporated into the food products by the W/O/W double emulsions, such as vitamins, minerals, carotenoids, phenolic compounds, microorganisms, antimicrobials, flavors, oligosaccharides and fibers, amino acids, bioactive lipids, bioactive peptides, etc. (Jiménez-Colmenero, 2013; McClements, 2016).

Phenolic compounds also known as polyphenols, are organic chemicals which have large number of phenol groups within its structure. They are usually found in nature but synthetic or semisynthetic forms are also commercialized. These compounds can be used as strong antioxidants. Also they can inhibit cancer cell proliferation and cholesterol uptake; regulate transcription, expression and mode of action of different enzymes including telomerase, cyclooxygenase, and lipoxygenase; participate in several signal transduction pathways and modulate cell cycle and platelet functions and preventing endothelial dysfunctions (Lu et al., 2016).

On the other hand, polyphenol usage within food structure is limited due to their low bioavailability and instability during handling, processing, digestion and absorption. They are very prone to extreme environmental conditions such as light, heat and oxygen and also their bioavailabilities are low as a result of insufficient gastric residence time, low permeability, and water-solubility (Wildman and Kelly 2007; Munin & Edwards-Levy, 2011).

2.3 Emulsion Ingredients

Emulsions are consists of a several different ingredient such as oils, emulsifiers, thickening agents, gelling agents, buffering systems, preservatives, antioxidants, chelating agents, sweeteners, salts, colorants, flavors, etc. Ingredient composition and their interactions with each other is directly influence the physiochemical, organoleptic and nutritional properties of emulsion-based food products. In this respect, emulsion ingredients should be selected carefully by considering the factors that characteristics of each ingredient, high quality, available, easy to use, economically feasible, and also may be label friendly (McClements, 2006).

Although a wide range of edible fats and oils can be used in double emulsions, the most appropriate one should be selected based on the nutritional, physicochemical, sensory and economic aspects for the each application area. Palm oil is extracted from ripe palm fruit which contains 45% to 55% oil, mainly composed from saturated fatty acids. Fatty acid composition and some physiochemical properties of palm oil is given in Table 2.3 and 2.4 respectively. 90% of palm oil production is used for food source (e.g., margarine, deep fat frying, shortening, ice creams, cocoa butter substitutes in chocolate); the remaining 10% is used for soap and oleochemical manufacturing (fatty acids, methyl esters, fatty nitrogenous derivatives, surfactants and detergents) (Edem, 2002). In this study, palm oil is selected due to its widespread application area, mostly in bakery industry.

Table 2.3 : Fatty acid composition of palm oil (Edem, 2002).

Fatty Acids	Composition (%)
Lauric 12:0	0–0.2
Myristic 14:0	0.8–1.3
Palmitic 16:0	43.1–46.3
Palmitoleic 16:1	Trace–0.3
Stearic 18:0	4.0–5.5
Oleic 18:1	36.7–40.8
Linoleic 18:2	9.4–11.9
Linolenic 18:3	0.1–0.4
Arachidic 20:0	0.1–0.4
Saturates	50.2
Monounsaturates	39.2
Polyunsaturates	10.5

Table 2.4 : Physiochemical properties of palm oil (Edem, 2002).

Physiochemical properties	Value
Melting point (◦C)	34.2
Relative density (50 ◦C/water at 25 ◦C)	0.89–0.92
Refractive index (η)	Oca.46
Moisture and impurities (%)	0.1
Iodine value	47–55.83
Saponification value (mg KOH/g)	196–208.2
Unsaponifiable matter (%)	0.01–0.5

In order to form a stable emulsion, emulsifier selection is crucial. Choosing appropriate inner and external phase emulsifier and their combination within the double emulsion should be considered very carefully. Literature survey shows that in double emulsions usually PGPR used as a lipophilic emulsifier (see Table 2.2). For the outer emulsifier a variety of emulsifiers are used. In addition to that, using 'label friendly' ingredients is seems to more appealing for consurmers. In this respect, for emulsification, natural emulsifiers are used against synthetic emulsifiers in this study. For the inner droplets of W_1/O, lecithin versus polyglycerol polyricinoleate (PGPR) used in the oil phase as an lipophilic emulsifier; for the outer final emulsion of $W_1/O/W_2$ sodium caseinate versus sodium stearoyl lactylate (SSL) used in the outer water phase as an hydrophilic emulsifier.

One of the other factor that affects the stability of the emulsion is the viscosity of the inner dispersed phase (W_1). In order to increase the inner viscosity of W_1 water phase, xanthan gum is used in this study. Thus, it is assumed that the mobility and diffusivity of the inner phase to the outer W_2 water phase will be decreased. Xanthan gum is an extracellular polysaccharides that is produced by bacteria of the genus Xanthanomonas. It is a linear and anionic polysaccharide. It can be easily dispersed in hot and cold water and increase the viscosity of the solution. Xanthan solutions are acid, salt and heat stable even at low concentrations. Furthermore, these solutions exhibit pseudoplactic propertiy and as a result of this, fluidity, good mouthfeel and adhesion. Xanthan gum can be used a wide range of food applications such as batter and bakery products, meat and seafood products, edible coatings for fruits, dairy products, salad dressings, dry mixes and frozen foods, canned foods, sauces, syrups, soups, beverages, chocolate, encapsulated nutrients, desserts, and in the structure of biodegradable food packaging as thickener, stabilizer, and suspending agent (Habibi and Khosravi-Darani, 2017; McClements, 2016).

2.4 Emulsion Formation

W/O/W emulsions can be formed by one or two-step emulsification (Garti, 1997). The most widely applied method is two step method. In this method, inner dispersed phase (W_1) and oil phase are emulsified with a lipophilic emulsifier to form a simple W/O emulsion. After that, prepared W/O emulsion and W_2 continuous phase are also emulsified with a hydrophilic emulsifier to finally create a W/O/W emulsion (Figure 2.5).

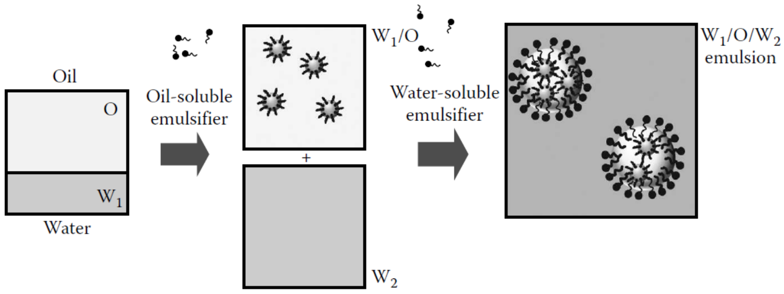


Figure 2.3 : Schematic representation of W/O/W formation (McClements, 2016).

For the reason of encapsulation of the inner water phase, the mean diameter of the oil droplets of W/O/W emulsion is higher than conventional O/W emulsions. Thus, the mean droplet diameter of the internal aqueous phase may be around 100–1000 nm, whereas the mean droplet diameter of the oil droplets may be from 1 to 100 μm (McClements, 2016).

The first homogenization step which one is formation of W_1/O emulsion can be done with high energy equipments such as high shear mixers, high-pressure homogenizers, colloid mills, ultrasonic homogenizers, and membrane homogenizers. On the other hand, the second homogenization step should be less destructive in order to avoid breaking of the W_1/O droplets. Thus, in the second step a blender or a membrane homogenizer can be used in the second emulsification step (McClements, 2016).

The size distribution of the droplets in double emulsion can be controlled by the emulsifier type and concentration and homogenization conditions. Also the concentrations of the W_1 phase and W_1/O phase can be controlled by the W_1 to O phase ratio and W_1/O to W_2 phase ratio.

Finally, various active ingredients can be encapsulated within the double emulsion structure. For example; lipophilic materials can be incorporated into the oil phase, whereas hydrophilic ones can be incorporated into the inner water phase.

2.5 Emulsion Stability and Controlling Emulsion Stability

Stability is defined as the ability of an emulsion to preserve its physiochemical properties over time. Emulsion instability can be driven by physical or chemical forces. Physical instability mechanisms can be creaming, flocculation, coalescence, partial coalescence, phase inversion, and Ostwald ripening, whereas oxidation and hydrolysis can be listed as chemical instability mechanisms. An illustration of physical instability mechanism is shown in Figure 2.6.

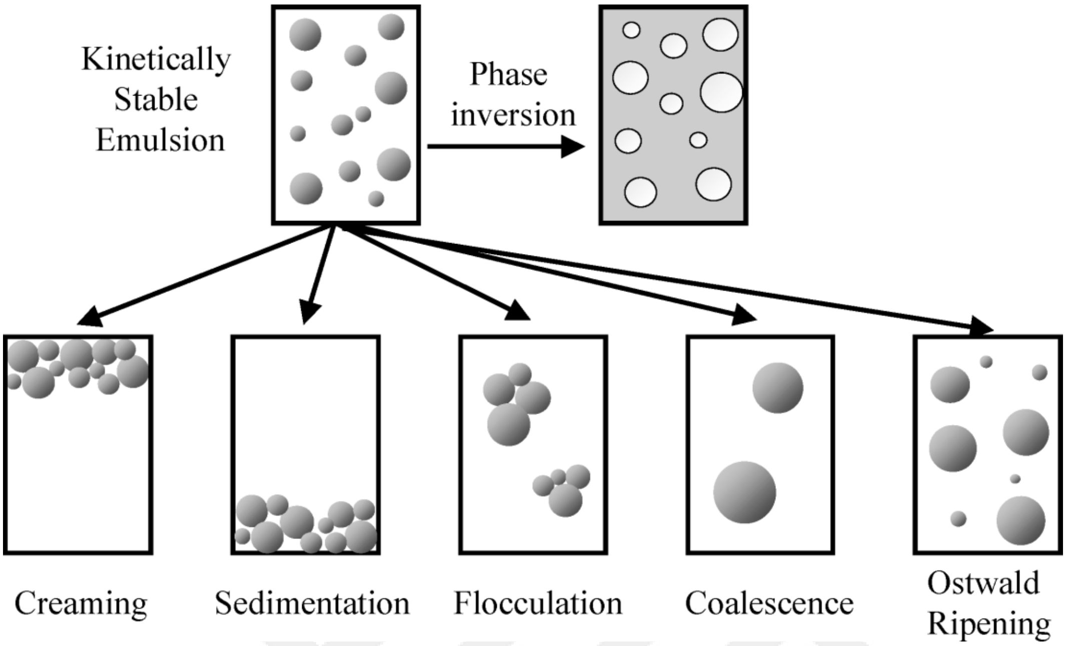


Figure 2.4 : Illustration of most common instability mechanisms that occur in food emulsions (McClements, 2007).

The type of the product determines the stability time of the emulsion. For example; cake batter, ice cream mix, and margarine premix are intermediate products during manufacturing. Therefore, these examples should be remain stable for a few seconds, minutes or may be hours. On the other hand, some food emulsions should be remain stable much more longer such that days, months or even years. Some examples can be given to the long stable emulsions are dressings, sauces, dips, creams.

Stability of emulsions is classified into two different category which are thermodynamic stability and kinetic stability. Thermodynamic stability refers to the phase separation or even initiation of the phase separation. It deals with the occurrence of the phase separation. On the other hand, kinetic stability is interested in the rate of the process. It gives information about the how fast the emulsion properties change over time, the type of changes, or the mechanisms that is underlying behind these changes.

Gravitational stability is the most common instability form. It occurs because the fact that emulsion droplets usually have different densities. So that, the gravitational force acts on the droplets are different than each other. This leads to separating the phases, which the low density droplets goes up to the emulsion surface leading to creaming.

Flocculation is described as association of the two or more droplets still preserving their individuality. Flocculation fastens the gravitational separation. And also flocculation increases the density of the emulsion. Thus the flocculation should be controlled to have the desired texture in different applications.

Coalescence is the process which two or more liquid droplets joined each other and form a bigger droplet. The droplets rapidly moves toward their thermodynamically stable state and thus creaming or sedimentation of the phases.

Oswalt ripening is another instability processes that bigger droplets of the dispersed phase grows by the merging the smaller ones into the bigger droplets. Long chain fatty acid containing oils (such as canola, corn, fish, olive, palm, peanut, or sunflower oil) have low water solubility and thus their interaction through water phase is usually negligible.

These instability mechanisms should be fully understood and considered when evaluating and controlling the emulsion stability.

Controlling stability of double emulsions can be done by different methods; gelling or thickening internal or external water phase, crystallizing or semi crystallizing the oil phase, covering the oil droplets with a protective coating and osmotic swelling (McClements, 2016).

In addition to these, usually 0.1 M NaCl added to the internal aqueous phases in all emulsions, to provide inner droplets stability. Emulsion stability increases due to the fact that electrolytes weaken the attractive forces between water droplets, reducing the difference between dielectric constants of aqueous and oil phases, and so reducing collision (Matos et al., 2015).

To conclude, this study aims to produce a stable palm-based double emulsion to produce a reduced calorie emulsion which will be latter applied in a bakery product. The effect of inner and outer emulsifier type and concentration on the stability of the palm based double emulsion will be studied.

3. MATERIALS AND METHODS

3.1 Materials

Palm olein, polyglycerol polyricinoleate (PGPR), soy lecithin, sodium stearoyl lactylate (SSL), sodium caseinate (SC) and xanthan gum are provided from ELVAN Co. (Istanbul, Turkey) and used as received.

Sodium chloride (NaCl) and sodium azide (N_3Na) are analytical grade.

All other reagents and solvents used were of a suitable grade for spectrophotometric analyses.

Distilled water was used to prepare all solutions and emulsions.

3.2 Methods

3.2.1 Preparation of solutions

W_1 stock solution is prepared by dissolving 1M NaCl (0,584 gr NaCl in 100 ml water), 0,01% sodium azide (a non-food grade antimicrobial) and 0,1% xanthan gum in the distilled water. To dissolve xanthan gum completely, solution is mixed with high speed blender (Ultra-Turrax T18 basic IKA, Staufen, Germany) at 15500 rpm for 30 s.

W_2 stock solution is prepared by dissolving 1M NaCl, 0,01% sodium azide, 0,1% xanthan gum and the corresponding ratio of the hydrophilic emulsifier in the distilled water. The SSL containing water phase is warmed at 50 °C for 45 min to ensure complete dissolution of the SSL. After that emulsion is mixed with Ultra Turrax 1 min at 15500 rpm. The SC containing water phase either warmed at 40 °C for 2 h followed by mixing with Ultra Turrax 1 min at 15500 rpm to ensure complete hydration of SC. pH of the water solutions is adjusted on 6,0 by using HCl and NaOH. pH 6,0 which is the optimal pH level for bakery products to lower microbial growth.

The oil phase was prepared by adding corresponding ratio of hydrophobic emulsifier in the palm oil followed by 30 min stirring at around 60 °C (Matos et al., 2015).

According to literature survey the experimental design for emulsion formation is determined as seen in Table 3.1.

Table 3.1 : Experimental design for emulsion formation.

Emulsion no	Emulsifier combinations for SSL	Emulsion no	Emulsifier combinations for SC
1	3% PGPR – 2% SSL	1	3% PGPR – 1% SC
2	3% PGPR – 3% SSL	2	3% PGPR – 2% SC
3	3% PGPR – 4% SSL	3	3% PGPR – 3% SC
4	4% PGPR – 2% SSL	4	4% PGPR – 1% SC
5	4% PGPR – 3% SSL	5	4% PGPR – 2% SC
6	4% PGPR – 4% SSL	6	4% PGPR – 3% SC
7	5% PGPR – 2% SSL	7	5% PGPR – 1% SC
8	5% PGPR – 3% SSL	8	5% PGPR – 2% SC
9	5% PGPR – 4% SSL	9	5% PGPR – 3% SC

3.2.2 Preparation of W_1/O emulsion

Primary W_1/O single emulsions were prepared using 30% (v/v) of inner aqueous phase (W_1) and 70% (v/v) of continuous oily phase (O). Both continuous and dispersed phases were emulsified in glass vessels while increasing the mixing speed of high speed blender (Ultra-Turrax T18 basic, IKA, Staufen, Germany) from 3500 to 11000 within 2 min. Mixing was continued for 6 min at 15500 rpm (Oppermann et al., 2015).

3.2.3 Preparation of $W_1/O/W_2$ double emulsion

Double emulsions were prepared by dispersing 30 wt% (v/v) of the primary (W_1/O) emulsion into 70 wt% (v/v) of outer W_2 aqueous phase while increasing the mixing speed of the high speed blender (Ultra-Turrax T18 basic, IKA, Staufen, Germany) from 3500 rpm to 7000 rpm within 1 min. Mixing was continued for 4 min at 7000 rpm (Oppermann et al., 2015).

3.3 Emulsion Characterization

3.3.1 Light microscopy

The morphology of W_1/O and $W_1/O/W_2$ emulsions are observed by a light microscope Olympus CX21 (Olympus, Japan). Samples are placed between microscopic slides. Observation is done by 100x magnification at room temperature (Matos et al. 2014). More than 5 images has taken. Among these, one of the most representative one is shown.

3.3.2 Dynamic light scattering and zeta potential measurements

A commercial instrument, which combined dynamic light scattering and microelectrophoresis measurements (Nano-ZS, Malvern Instruments, Worcestershire, UK) is used to determine the droplet size distribution and the ζ-potential of the samples. Samples are diluted to a droplet concentration of approximately 0.05 wt% using pure water for double emulsions and paraffin oil for simple W/O emulsions prior to analysis. Measurements are performed at room temperature. (Chang and McClements, 2016).

3.3.3 Interfacial tension measurements

The equilibrium interfacial tensions between water and oil phases were measured with a tensiometer (DCAT11EC, DataPhysics, Germany) at 20 ℃ using the Du Noüy's platinum ring method.

3.3.4 Emulsion yield (DSC)

Emulsion yield is determined based on the method that is reported by Schuch et al. (2013). The method is slightly modified. 10 mg of the sample hermetically sealed and introduced into the calorimeter (DSC Q10, New Castle, USA). The sample conditioned at +10 ℃ for1 min, and then cooled to -60 ℃ at a cooling rate of 5 ℃ per minute. The area under the corresponding peak in the thermogram was intergrated using Universal Analysis 2000 data analysis software (TA Instruments Ltd., New Castle, DE), to calculate the energy required for the freezing of the water in the inner dispersed phase. The yield is defined as the ratio of the amount of water in the inner dispersed phase (m_{w_1}(t)) to the amount of inner dispersed phase that is initially emulsified ($m_{w_1}(t_0)$) and calculated as follows;

$$Yield = \frac{m_{w_1}(t)}{m_{w_1}(t_0)} = \frac{\frac{H_{c,w_1(wow)}}{\varphi_{w_1\,in\,wow}}}{\frac{H_{c,w_1(wo)}}{\varphi_{w_1\,in\,wo}}} x100 \qquad (3.1)$$

m_{w_1}(t) : The mass of inner dispersed water at the moment of interest t

$m_{w_1}(t_0)$: The initial mass of inner dispersed water

$\Delta H_{c,w_1(wow)}$: The change in enthalpy during the crystallization of the inner aqueous dispersed phase of the ($W_1/O/W_2$) double emulsion in J g $^{-1}$

$H_{c,w_1(wo)}$: The change in enthalpy during the crystallization of the inner aqueous dispersed phase of the primary water-in-oil emulsion, in J g $^{-1}$

$\varphi_{w_1\,in\,wow}$: The mass fractions of inner dispersed phase in the ($W_1/O/W_2$) double emulsion

$\varphi_{w_1\,in\,wo}$: The mass fractions of inner dispersed phase in the primary (W_1/o) emulsion, (Oppermann et al., 2015).

3.3.5 Gravitational stability

The gravitational stability of the samples are measured in quadruplicate from 15 mL sample aliquots placed in glass test tubes. The gravitational stability (creaming) is calculated in terms of phase separation and expressed as % of total sample height (Flaiz et al., 2016).

3.3.6 Rheology

Rheological properties of emulsions were determined with a rheometer (RheoStress 1, Thermo Scientific, Karlsruhe, Germany) using a cone and plate probe (cone diameter 35 mm, angle 2°, and gap 1 mm). All measurements were performed at 20°C. All measurements were replicated twice and done in 24 hours after preperation of the emulsion

Shear rates increased from 0 to 500 s^{-1} within 300 s. The apparent viscosity was recorded as function of the shear rate. Oscillatory frequency sweep tests were carried out from 0.1 to 10 Hz at a constant shear stress of 1 Pa. The storage modulus (G') and loss modulus (G'') were recorded versus frequency (rad/s) (Matos et al., 2015).

3.3.7 Storage stability

To test storage stability, 50 ml of double emulsions samples were placed falcon test tubes and stored for 7 days at 20 °C. Samples are analyzed in terms of gravitational stability and droplet size distribution (Bai et al. 2016).

3.4 Statistical Data Analysis

All measurements were performed on two samples with at least two measurements per sample and are reported as means and standard deviations. For multiple comparisons, data were subjected to statistical analysis using Minitab software (Version 16, Minitab

Pennsylvania, USA) for the analysis of variance (ANOVA). Means were separated by Tukey's HSD test ($p < 0.05$).

4. RESULTS AND DISCUSSION

4.1 Emulsion Characterisation

4.1.1 Simple W/O emulsion characterization

The effect of PGPR concentration and storage time on droplet diameter in the simple emulsions are analyzed. Besides that, the effect of PGPR concentration on the interfacial tension and zeta potential of the simple emulsions are also studied.

The average droplet size of emulsions are influenced by several factors such as composition of the phases, the viscosity of the phases, disperse to continuous phase volume ratio, homogenizetion method and conditions and finally type and concentration of the emulsifiers. Emulsifiers are surface active materials which reduces the interfacial forces of the phases and thus at same homogenization conditions results smaller droplet size. Likewise, the more ratio of the emulsifier increases the more interfacial tension decreases. According to the Laplace theory (McClements, 2016), droplet diameter decreases as interfacial tension decreases for constant homogenization conditions.

Simple W/O emulsion's physical properties are given in Table 4.1. While increasing the emulsifier ratio, the average droplet size has decreased from 616,95 nm to 451,2 nm as expected. However, these values were found not to be statistically different than each other. Moreover, increased emulsifier ratio decreased the interfacial tension between oil and W_1 phases. Interfacial tension values declined from 20,436 mN/m to 15,036 mN/m. In other words, interfacial tension and droplet size results verifys each other.

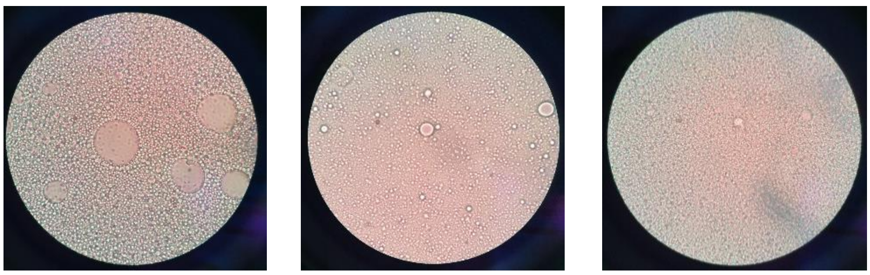
Smaller droplet sizes and lower zeta potential values improve the emulsion stability. Higher surface charge keeps the smaller droplets away from each other through electrostatic forces and prevents coalesence of the emulsions. Zimmermann and Müller (2001) reported that for producing stable emulsions, approximately -20 mV zeta potential is sufficient. As seen in Table 4.1, all zeta potential values (between -50,7 to -817 mV) are higher than -20 mV, meaning that all simple emulsions are stable over time. Moreover, higher emulsifier concentrations lowered the zeta potential value, thus increased stability. These values confirm the average droplet size results at 7 days of storage except 5% PGPR concentration. For usage of 3% or 4% of PGPR, small droplet size and higher zeta potential values resulted stable emulsions over time and the average droplet size values at 7th day of storage is not affected by aging. On the other hand, in the case of 5% PGPR concentration, after 7 days of storage the average droplet size of the simple W/O emulsion increased from 451,2 nm to 610,9 nm but it is found that this increase is not statistically significant.

Table 4.1 : Mean droplet diameters at 0 and 7th days, interfacial tensions and zeta potential values of simple W/O emulsions.

PGPR (%)	Z-average (nm) 0th day	Z-average (nm) 7th day	Interfacial Tension (mN/m)	Zeta Potential (mV)
3%	616,9 ± 119^{a,x}	608,9 ± 49^{a,x}	20,44 ± 4,62^{a}	-50,7 ± 806^{a}
4%	585,0 ± 25^{a,x}	538,5 ± 17^{a,x}	16,71 ± 4,76^{a,b}	-697 ± 595^{a}
5%	451,2 ± 44^{a,x}	610,9 ± 93^{a,x}	15,04 ± 6,37^{b}	-817 ±265^{a}

Values are means ± standard deviation. Different letters in the same column (a, b, c, d, e) and in the same row (x, y) designate significant differences (P < 0.05).

In addition to these, microscopic images of simple emulsions are shown in Figure 4.1. It is obviously seen that as the emulsifier ratio increases, the droplet size of the emulsion decreases as expected.



3% PGPR **4% PGPR** **5% PGPR**

Figure 4.1 : Microscopic images of simple W/O emulsions with different PGPR concentrations.

4.1.2 Double W/O/W emulsion characterization

The effect of the type and concentration of the hydrophilic emulsifiers (SSL and SC) and storage time on droplet diameter in the double emulsions is evaluated. Additionally the effect of type and concentration of the emulsifier on the interfacial tension between oil phase and W_2 phase, zeta potential of the oil droplets, double emulsion efficiency, rheology and gravitational stability are investigated.

According to the droplet size results in Table 4.2, the concentration of the SSL and SC does not affect the droplet size of the double emulsions statistically except 4% PGPR – 3% SC and 5% PGPR – 3% SC concentrations. Usage of 3% SC has decreased the droplet size of the double emulsions significantly. Besides that, it is obviously seen that the droplet size of the SSL containing double emulsions are much more smaller than SC containing ones. SSL containing double emulsions droplet sizes range from 398,4 nm to 713,9 nm. On the other hand, SC containing double emulsions droplet sizes range from 330,8 nm to 2471 nm. This results also correlate with the microscopic images and rheological results. While, SC results a wide range of distribution, SSL exhibits a narrower distribution.

Moreover, droplet size results of the double emulsions at 0^{th} day has a good correlation with interfacial tension and results. As the interfecial tension between oil phase and W_2 phase decreases, the droplet size of the double emulsions has decreased as expected. On the other hand, a decline in the droplet size results higher emulsion efficiencies as predicted.

Additionally, the droplet size results of the double emulsions at 7^{th} day of storage exhibit a great correlation with both zeta potential and gravitational stability results. While an increase has been seen in the droplet size, the gravitational stability of the emulsions become lower. Likewise, as lowered zeta potential values indicate more stable emulsions, those emulsions with smaller zeta potentials have lower droplet sizes increase during storage.

Furthermore, it is expected that after 7 days of storage droplet size of double emulsions should become larger due to aggregation of the droplets or at least stay similiar with initial droplet size. However, as seen in Table 4.2, some droplet size results of 7^{th} day of storage is lower than initial droplet size of the relevant double emulsions. This might be occured as a result of phase separation (Yuce-Altuntas et al., 2017).

Table 4.2 : Mean droplet diameters at 0^{th} and 7^{th} days, interfacial tensions, zeta potentials, emulsion efficiencies, gravitational stability and viscosity values of double W/O/W emulsions with hydrophilic (in W_2 phase) emulsifiers SSL (A) and SC (B).

(A)

PGPR (%)	SSL (%)	Z-average (nm) 0^{th} day	Z-average (nm) 7^{th} day	Interfacial Tension (mN/m)	Zeta Potential (mV)	Emulsion Efficiency (%)	Gravitational Stability (%)	Viscosity (mPa·s)^{a}
	2%	620,0 ± 127^{ax}	42683 ± 4570^{a,y}	14,27 ± 4,50^{a}	-54,2 ± 4,38^{a,b}	64,47 ± 1,61^{a,b}	47,26 ± 7,8^{a}	21,46 ± 3,70^{a}
3%	3%	713,9 ± 32^{a,x}	1773 ± 510^{b,y}	20,29 ± 3,58^{a}	-48,4 ± 1,25^{a}	53,40 ± 1,13^{c}	70,73 ± 5,9^{b}	25,81 ± 2,05^{a}
	4%	449,9 ± 31^{a,x}	632,3 ± 39^{c,x}	15,74 ± 4,3^{a}	-60,0 ± 2,55^{a,b}	95,14 ± 2,31^{d}	94,07 ± 1,1^{c,d}	41,38 ± 0,08^{b}
	2%	409,4± 37^{a,x}	22860 ± 10880^{a,y}	12,80 ± 4,17^{a}	-51,6 ± 3,18^{a,b}	70,27 ± 1,73^{a}	41,12 ± 2,6^{a}	26,74 ± 1,16^{a}
4%	3%	656,0 ± 85^{a,x}	633,5 ± 54^{c,x}	20,31 ± 4,41^{a}	-56,0 ± 5,89^{a,b}	57,98 ± 1,48^{c,e}	82,78 ± 3,0^{e}	37,45 ± 1,16^{b}
	4%	398,4 ± 54^{a,x}	488,9 ± 23^{c,y}	16,88 ± 4,93^{a}	-58,0 ± 4,45^{a,b}	99,02 ± 0,86^{d}	100,00 ± 0,0^{c}	61,25 ± 0,84^{c}
	2%	621,5 ± 44^{a,x}	57870 ± 843^{a,y}	14,15 ± 4,47^{a}	-64,1 ± 9,87^{b}	57,48 ± 1,16^{c,e}	43,53 ± 4,7^{a}	29,11 ± 1,09^{a}
5%	3%	680,2 ± 17^{a,x}	508,3 ± 103^{b,c,x}	23,89 ± 4,03^{a}	-48,4 ± 4,45^{a}	53,51 ± 1,16^{c}	89,16 ± 3,0^{d,e}	28,79 ± 0,63^{a}
	4%	553,8 ± 16^{a,x}	414,7 ± 32^{c,x}	17,30 ± 5,13^{a}	-50,6 ± 7,11^{a,b}	60,57 ± 1,54^{b,e}	93,06 ± 0,7^{c,d}	65,17 ± 3,43^{c}

(B)

PGPR (%)	SC (%)	Z-average (nm) 0^{th} day	Z-average (nm) 7^{th} day	Interfacial Tension (mN/m)	Zeta Potential (mV)	Emulsion Efficiency (%)	Gravitational Stability (%)	Viscosity (mPa·s)^{a}
	1%	1047,6 ± 136^{a,x}	565,4 ± 48^{a,y}	24,22 ± 4,43^{a,b,c}	-31,0 ±8,49^{a}	80,24 ± 1,29^{a}	63,34 ± 3,5^{a,b}	16,03 ± 0,82^{a,b}
3%	2%	904,5 ± 346^{a,x}	642,8 ± 98^{a,x}	15,25 ± 3,76^{c}	-52,3 ± 0,55^{b}	81,04 ± 1,16^{a}	64,08 ± 2,8^{a,b}	17,24 ± 0,70^{a,b}
	3%	732,9 ± 492^{a,x}	81383 ± 71585^{b,y}	13,38 ± 3,42^{c}	-44,6 ± 0,67^{a,b}	88,84 ± 1,16^{b,c}	39,12 ± 0,6^{d}	17,07 ± 0,28^{a,b}
	1%	1382,7 ± 362^{a,b,x}	679,8 ± 173^{a,x}	20,62 ± 4,09^{a,b,c}	-34,4 ± 14,70^{a,b}	83,53 ± 1,54^{a,d}	61,78 ± 1,5^{b}	16,95 ± 0,44^{a,b}
4%	2%	826,6 ± 223^{a,x}	893,2 ± 466^{a,x}	16,39 ± 4,17^{b,c}	-34,0 ± 7,43^{a,b}	93,66 ± 1,44^{b,e}	51,42 ± 7,0^{c}	17,99 ± 0,74^{b}
	3%	330,8 ± 22^{c,x}	46223 ± 6900^{b,y}	15,94 ± 3,61^{c}	-48,3 ± 5,26^{a,b}	95,02 ± 1,54^{e}	36,23 ± 0,5^{d}	17,89 ± 0,90^{b}
	1%	2471,0 ± 920^{b,x}	768,0 ± 112^{a,y}	33,08 ± 0,05^{a}	-35,3 3,45^{a,b}	94,37 ± 1,67^{e}	66,84 ± 1,0^{a,b}	15,17 ± 0,34^{a,b}
5%	2%	1394,8 ± 757^{a,b,x}	969,9 ± 287^{a,x}	31,33 ± 0,05^{a}	-45,0 ± 4,56^{a,b}	86,00 ± 1,91^{c,d}	70,03 ± 2,3^{a}	16,65 ± 0,83^{a,b}
	3%	496,7 ± 167^{c,x}	1954,0 ± 1239^{a,b,y}	27,61 ± 3,59^{a,b}	-30,9 ± 6,04^{a}	77,62 ± 1,16^{a}	36,41 ± 3,4^{d}	20,74 ± 0,80^{c}

Values are means ± standard deviation. Different letters in the same column (a, b, c, d, e) and in the same row (x, y) designate significant differences ($P < 0.05$).

^{a} Average viscosity at shear rates between 100 s−1 and 500 s−1.

Interfacial tension and emulsion efficiency results show great correlation both for SSL and SC in our study. They are inversely proportional to each other. When the interfacial tension of the phases increases, the emulsion efficiency decreases as well. As seen in Table 4.2, in the case of SSL containing double emulsions, maximum emulsion efficiency results are obtained from 4% SSL concentration for 3%, 4% and 5% PGPR concentration as 95,14%, 99,02% and 60,57% respectively. For SC containing double emulsions, along with increased SC concentration an increase is seen in emulsion efficiency results for 3% and 4% of PGPR concentration from 80,24% to 95,02%, whereas a decrease is seen for 5% of PGPR concentration from 94,37% to 77,62%.

Microscopic images of double emulsions confirm the emulsion efficiency results. As seen in Figure 4.3, in the case of SSL, 4%PGPR-4% SSL containing double emulsion has encapsulated more water inside the oil phase than other emulsifier concentrations. On the other hand, SC containing double emulsions' efficiency results are higher than SSL containing ones. This can be also seen from microscopic images. SC can able to entrap more water inside the oil droplets. However, again it can be observed from the microscopic images that, SC creates larger oil droplets than SSL. Also these larger oil droplets stay very close to each other. This leads aggregation of the oil droplets over storage period and as a consequence of this, phase seperation occurs over time in SC containing double emulsions.

The phase seperation of the double emulsions are evaluated by measuring the phase seperation after 7 days of storage at 20 ℃ and results are given in Table 4.2. Also, visual inspection of double emulsions before and after storage is shown in Figure 4.2. For SSL, increasing emulsifier ratio increases the gravitational stability from approximately 40-45% up to 100% . On the other hand, for SC increasing emulsifier ratio generally decreases (approximately 20%) the gravitational stability. The maximum gravitational stability results for SC containing double emulsion is obtained as 70,03% for 5% PGPR-2% SC emulsifier ratios. It can be clearly said that SSL containing double emulsions are much more stable than SC containing double emulsions.

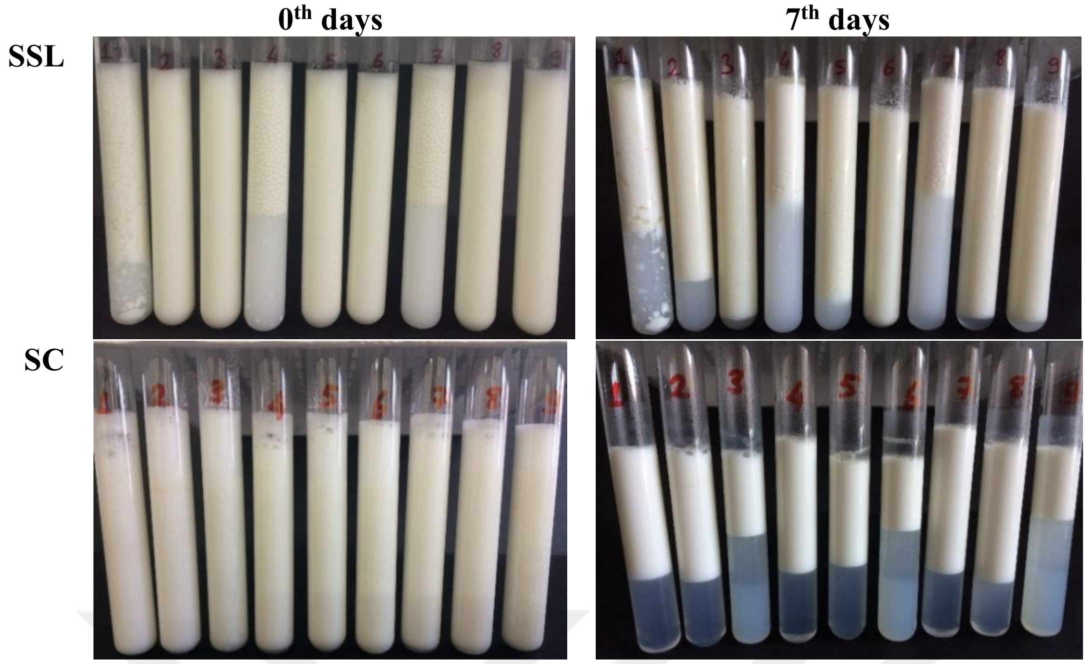


Figure 4.2 : Visual inspection of WOW emulsions for gravitational stability.

The zeta potential of the oil droplets of double emulsions are not significantly affected by the concentration of the SSL and SC. While, zeta potential of oil droplets in SSL containing double emulsions range approximately -50 mV to -64 mV, zeta potential of oil droplets in SC containing double emulsions range approximately -30 mV to -48 mV. These results confirm the gravitational stability results as SSL containing double emulsions has lower zeta potential values than SC containing ones.

In our study, in the case of SSL, while emulsifier concentration increases, the viscosity of the emulsion increases about 21 mPa.s to 65 mPa.s. On the other hand, increase in emulsifier concentration of the SC does not influence significantly the viscosity values of the double emulsion except 4% PGPR-2% SC, 4% PGPR-3% SC and 5% PGPR-3% SC emulsifier concentrations. Viscosity ratios of SC containing double emulsions range between 16,03 mPa.s to 20,74 mPa.s. This values are much more lower than SSL containing double emulsions viscosity ratios. Higher emulsion viscosities prevents movement of oil droplets and keeps them away from each other in order to not form aggregates. In other words, higher emulsion viscosities yield higher gravitational stability. Particularly, viscosity results verify the gravitational stability results.

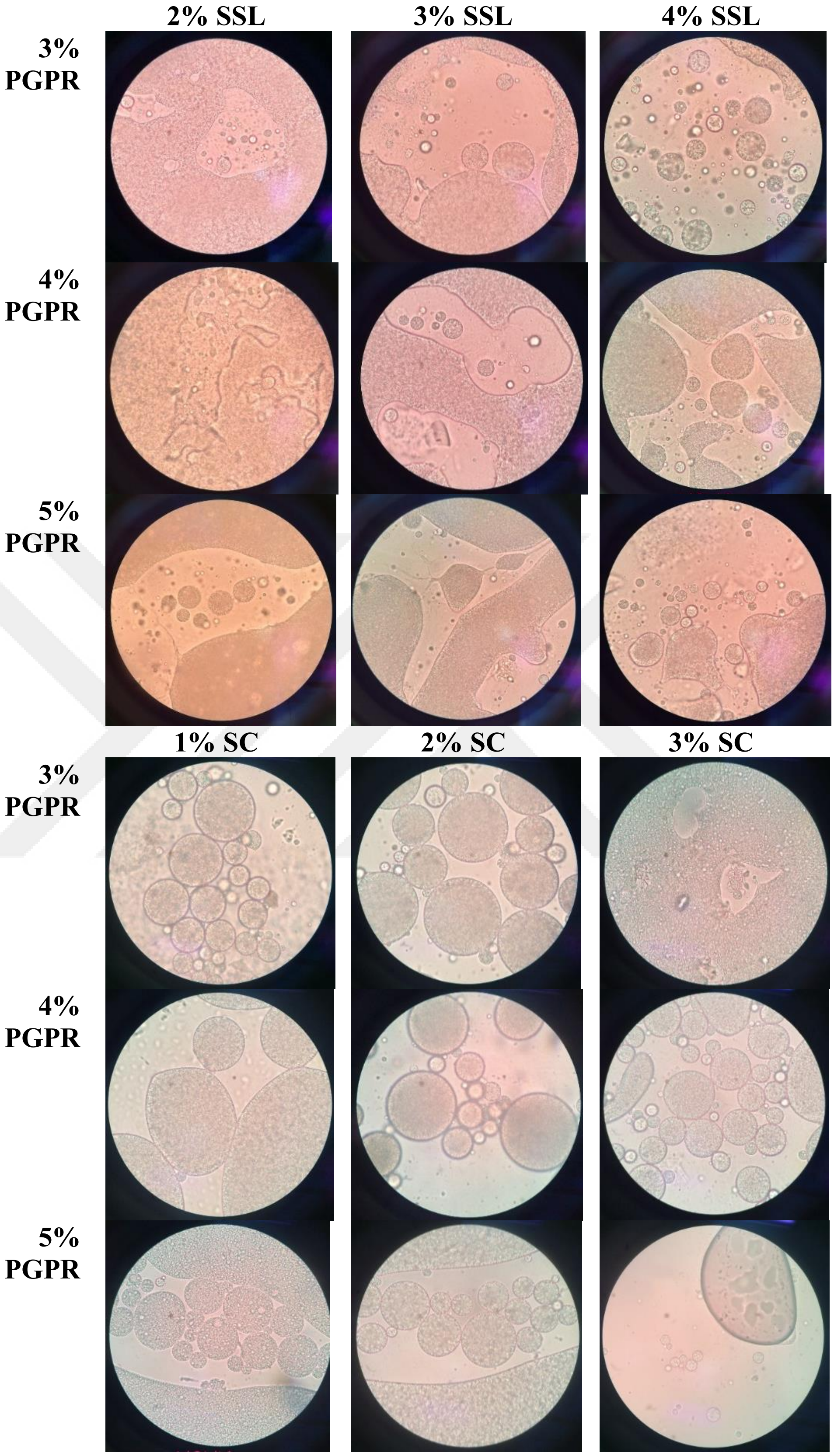


Figure 4.3 : Microscopic images of double emulsions.

Rheological measurements not only give important parameters about emulsions flow behaviour, but also they tell us some good informations about molecular weight (MW) and molecular weight distributions (MWD) of emulsions. Storage modulus (G') and loss modulus (G'') are two of the valuble results of oscillatory frequency sweep tests. When a higher modulus at the coross-over point achieved from oscillatory frequency sweep tests, it means that the emulsion has a narrower MWD. On the other hand, if the loss modulus is obtained higher than the storage modulus, it can be said that the emulsion exhibits more fluid-like behaviour.

Our oscillatory frequency sweep tests results indicate that, all loss modulus results are higher than storage modulus. To give an example, 3%PGPR – 2%SSL and 5%PGPR – 1%SC emulsifier combinations' oscillatory sweep test results are shown in Figure 4.4 and Figure 4.5 respectively. Without regard to the type of the emulsifier (SSL or SC), all emulsions exhibit liquid like behaviour. Furthermore, for SSL containing double emulsions, the cross-over point is obtained from higher modulus values. On the other hand, in the case of SC containing double emulsions, the cross-over point is obtained at lower modulus values. It means that, while SSL containing double emulsions has narrower MWD, but SC containing ones has a broad MWD range. Microscopic images of the double emulsions also confirm these rheological test results. As seen in Figure 4.3, SC containing double emulsions have both larger and smaller oil droplets.

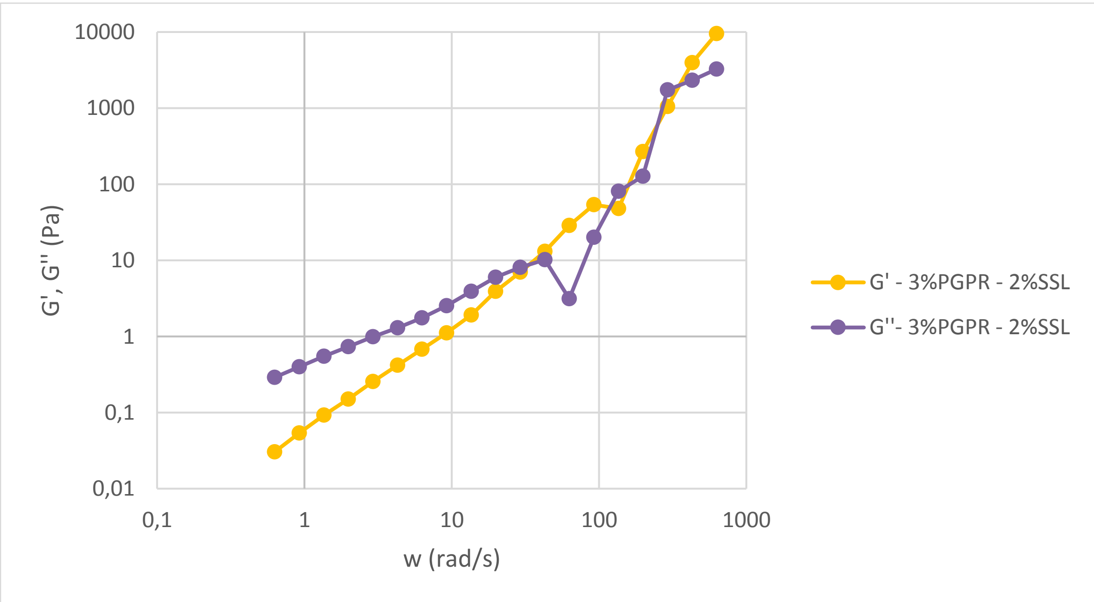


Figure 4.4 : Frequency sweep test results of 3%PGPR – 2%SSL.

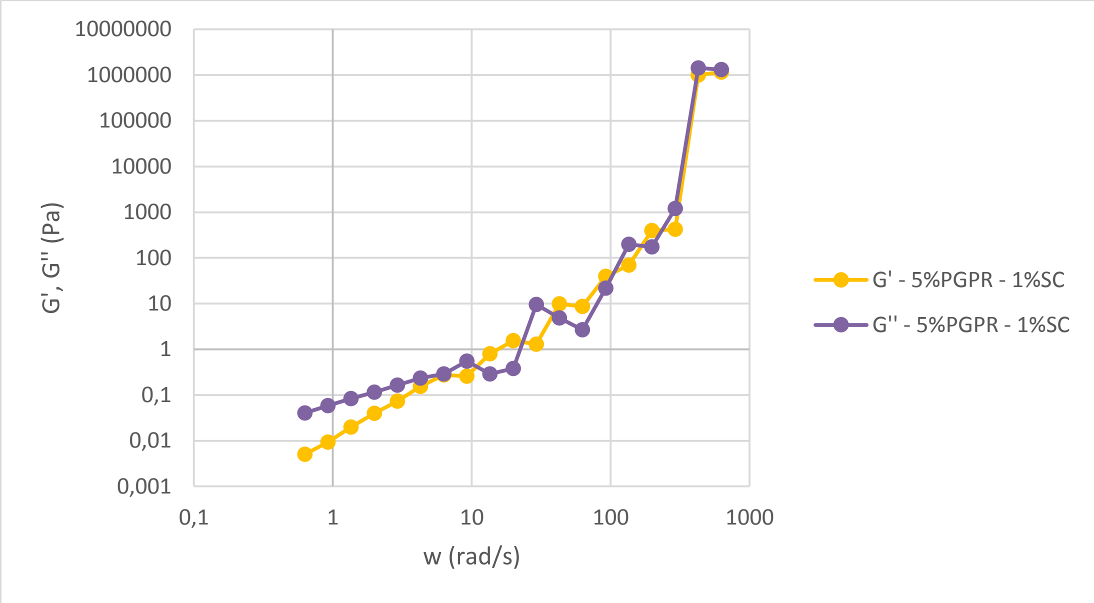


Figure 4.5 : Frequency sweep test results of 5%PGPR – 1%SC.

To sum up all emulsion characterization results, it can be easily said that 4% PGPR – 4% SSL emulsifier combination is the most stable one for the palm-based double emulsion system at pH 6.0.

5. CONCLUSIONS AND RECOMMENDATIONS

This study aims to form a stable palm-based double emulsion system by comparing the natural emulsifiers (lecithin for W/O emulsions and sodium caseinate for W/O/W emulsions) with synthetic ones (PGPR for W/O emulsions and SSL for W/O/W emulsions) to reduce the calorie of total emulsion. Emulsions with different ratios of emulsifiers are characterized in terms of droplet size, zeta potential, interfacial tension, emulsion efficiency, gravitational stability and rheological parameters.

In conclusion, in this study both for simple W/O and double W/O/W emulsions natural emulsifiers failed to stabilize the emulsions. It is found that, any ratio of lecithin is not able to form an emulsion. Lecithin helps to disperse two phases but could not able to entrap the water phase into the oil phase. On the other hand, while sodium caseinate is able to entrap up to 95% of water inside the oil phase, it is not able to stabilize the double emulsion over time. However, combination of 4% PGPR-4% SSL is found to stabilize the palm-based double emulsion at pH 6,0. Further studies can be done on the usage of sodium caseinate in palm-based double emulsions as hydrophilic emulsifier. In this respect, different hydrocolloids and stabilizers can be studied. Besides of that, further studies should be done on the thermal stability of the double emulsion in order to understand if the double emulsion is suitable for baking process.

This study provides a valuable data on the usage of different kinds of emulsifiers to stabilize the palm-based double emulsions. It contributes an emulsifier combination which is able to stabilize a system at pH 6.0.

REFERENCES

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion based delivery systems for curcumin: encapsulation and release properties. *Food Chemistry,* 132, 799-807.

Aditya, N. P., Aditya, S., Yang, H., Kim, H. W., Park, S. O., & Ko, S. (2015). Co-delivery of hydrophobic curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion. *Food Chemistry,* 173, 7–13.

Almajano, M. P., Delgado, M. E. & Gordon, M. H. (2007). Albumin causes a synergistic increase in the antioxidant activity of green tea catechins in oil-in-water emulsions. *Food Chemistry,* 102, 1375–1382.

Al-Nabulsi, A. A., Han, J. H., Liu, Z., Rodriguez-Vieira, E. T., & Holley, R. A. (2006). Temperature-sensitive microcapsules containing lactoferrin and their action against Carnobacterium viridans on bologna. *Journal of Food Science*, 71, M208–M214.

Anand, P., Nair, H. B., Sung, B., Kunnumakkara, A. B., Yadav, V. R., Tekmal, R. R., et al. (2010). Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo. *Biochemical Pharmacology,* 79, 330-338.

Apak, R., Guclu, K., Ozyurek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry,* 52, 7970–7981.

Bai, L., Huan, S., Gu, J., & McClements, D. J. (2016). Fabrication of oil-in-water nanoemulsions by dual-channel microfluidization using natural emulsifiers: Saponins, phospholipids, proteins, and polysaccharides. *Food Hydrocolloids,* 61, 703-711.

Bala, I., Bhardwaj, V., Hariharan, S., Kharade, S. V., Roy, N., & Ravi Kumar, M. (2006). Sustained release nanoparticulate formulation containing antioxidant-ellagic acid as potential prophylaxis system for oral administration. *Journal of Drug Targeting,* 14, 27-34.

Barras, A., Mezzetti, A., Richard, A., Lazzaroni, S., Roux, S., Melnyk, P., et al. (2009). Formulation and characterization of polyphenol-loaded lipid nanocapsules. *International Journal of Pharmaceutics,* 379, 270-277.

Benichou, A., Aserin, A., & Garti, N. (2007). W/O/W double emulsions stabilized with WPI-polysaccharide complexes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 294, 20–32.

Berendsen, R., Güell, C., & Ferrando, M. (2015). Spray dried double emulsions containing procyanidin-rich extracts produced by premix membrane emulsification: Effect of interfacial composition. *Food Chemistry,* 178, 251–258.

Bonnet, M., Cansell, M., Berkaoui, A., Ropers, H. M., Anton, M., & Leal-Calderón, F. (2009). Release rate profiles of magnesium from multiple W/O/W emulsions. *Food Hydrocolloids*, 23, 92–101.

Bonnet, M., Cansell, M., Placin, F., Anton, M., & Leal-Calderón, F. (2010). Impact of sodium caseinate concentration and location of magnesium release from multiple W/O/W emulsions. *Langmuir*, 26, 9250–9260.

Bonnet, M., Cansell, M., Placin, F., David-Brian, E., Anton, M., & Leal-Calderón, F. (2010). Influence of ionic complexation on release rate profiles from multiple waterin- oil-in-water (W/O/W) emulsions. *Journal of Agricultural and Food Chemistry*, 58, 7762–7769.

Bonnet, M., Cansell, M., Placin, F., Monteil, J., Anton, M., & Leal-Calderón, F. (2010). Influence of the oil globule fraction on the release rate profiles from multiple W/O/W emulsions. *Colloids and Surfaces. B, Biointerfaces*, 78, 44–

Brückner, M., Bade, M., & Kunz, B. (2007). Investigation into the stabilization of a volatile aroma compound using a combined emulsification and spray drying process. *European Food Research and Technology,* 226, 137–146.

Carrillo-Navas, H., Cruz-Olivares, J., Varela-Guerrero, V., Alamilla-Beltrán, L., Vernon-Carte, E. J., & Pérez-Alonso, C. (2012). Rheological properties of a double emulsion nutraceutical system incorporating chia essential oil and ascorbic acid stabilized by carbohydrate polymer-protein blends. *Carbohydrate Polymers*, 87, 1231–1235.

Chang, Y., & McClements, D. J. (2016). Influence of emulsifier type on the in vitro digestion of fish oil-inwater emulsions in the presence of an anionic marine polysaccharide (fucoidan): Caseinate, whey protein, lecithin, or Tween 80. *Food Hydrocolloids,* 61, 92-101.

Chen, F. Liang, L., Zhang, Z., Deng, Z., Decker, E. A., & McClements, D. J. (2017). Inhibition of lipid oxidation in nanoemulsions and filled microgels fortified with omega-3 fatty acids using casein as a natural antioxidant. *Food Hydrocolloids,* 63, 240-248.

Chen-yu, G., Chun-fen, Y., Qi-lu, L., Qi, T., Yan-wei, X., Wei-na, L., et al. (2012). Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. *International Journal of Pharmaceutics,* 430, 292-298.

Choi, S. J., Decker, E. A., & McClements, J. (2009). Impact of iron encapsulation within the interior aqueous phase of water-in-oil-in-water emulsions on lipid oxidation. *Food Chemistry,* 116, 271–276.

Cofrades, S., Antoniou, I., Solas, M. T., Herrero, A. M., & Jiménez-Colmenero, F. (2013). Preparation and impact of multiple (water-in-oil-in-water) emulsions in meat systems. *Food Chemistry,* 141, 338–346.

De Cindio, B., & Cacace, D. (1995). Formulation and rheological characterization of reduced-calorie food emulsions. *International Journal of Food Science and Technology,* 30, 505–514.

Donsì, F., Sessa, M., Mediouni, H., Mgaidi, A., & Ferrari, G. (2011). Encapsulation of bioactive compounds in nanoemulsion- based delivery systems. *Procedia Food Science,* 1, 1666-1671.

Edem, D. O. (2002). Palm oil: biochemical, physiological, nutritional, hematological and toxicological aspects: a review. *Plant Foods for Human Nutrition*, 57 (3-4), 319-341.

Fechner, A., Knoth, A., Scherze, I., & Muschiolik, G. (2007). Stability and release properties of double-emulsions stabilised by caseinate–dextran conjugates. *Food Hydrocolloids*, 21, 943–952.

Flaiz, L., Freire, M., Cofrades, S., Mateos, R., Weiss, J., Jiménez-Colmenero, F., & Bou, R. (2016). Comparison of simple, double and gelled double emulsions as hydroxytyrosol and n-3 fatty acid delivery systems. *Food Chemistry,* 213, 49–57.

Frank, K., Walz, E., Gräf, V., Greiner, R., Köhler, K., & Schuchmann, H. P. (2012). Stability of anthocyanin-rich W/O/W emulsions designed for intestinal release in gastrointestinal environment. *Journal of Food Science,* 77, N50–N57.

Garti, N. (1997). Progress in stabilization and transport phenomena of double emulsions in food applications. *Lebensm.-Wiss. u.-Technol.,* 30, 222–235.

Gültekin-Özgüven, M., Karadağ, A., Duman, Ş., Özkal, B., & Özçelik, B. (2016). Fortification of dark chocolate with spray dried black mulberry (*Morus nigra*) waste extract encapsulated in chitosan-coated liposomes and bioaccessability studies. *Food Chemistry,* 201, 205–212.

Habibi, H., & Khosravi-Darani, K. (2017). Effective variables on production and structure of xanthan gum and its food applications: A review. *Biocatalysis and Agricultural Biotechnology,* 10, 130–140.

Hemar, Y., Cheng, L. J., Oliver, C. M., Sanguansri, L., & Agustin, M. (2010). Encapsulation of resveratrol using water-in-oil-in-water double emulsions. *Food Biophysics,* 5, 120–127.

Italia, J., Datta, P., Ankola, D., & Kumar, M. (2008). Nanoparticles enhance per oral bioavailability of poorly available molecules: epigallocatechin gallate nanoparticles ameliorates cyclosporine induced nephrotoxicity in rats at three times lower dose than oral solution. *Journal of Biomedical Nanotechnology*, 4, 304-312.

Jiménez-Colmenero, F. (2013). Potential applications of multiple emulsions in the development of healthy and functional foods. *Food Research International,* 52, 64–74.

Jiménez-Alvarado, R., Beristain, C. I., Medina-Torres, L., Román-Guerrero, A., & Vernon-Carter, E. J. (2009). Ferrous bisglycinate content and release in W1/O/W2 multiple emulsions stabilized by protein–polysaccharide complexes. *Food Hydrocolloids,* 23, 2424–2433.

Kukizaki, M., & Goto, M. (2007). Preparation and evaluation of uniformly sized solid lipid microcapsules using membrane emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 293, 87–94.

Kumari, A., Yadav, S. K., Pakade, Y. B., Kumar, V., Singh, B., Chaudhary, A., et al. (2011). Nanoencapsulation and characterization of *Albizia chinensis* isolated antioxidant quercitrin on PLA nanoparticles. *Colloids and Surfaces B: Biointerfaces*, 82, 224-232.

Kumari, A., Yadav, S. K., Pakade, Y. B., Singh, B., & Yadav, S. C. (2010). Development of biodegradable nanoparticles for delivery of quercetin. *Colloids and Surfaces B: Biointerfaces,* 80, 184-192.

Leal-Calderón, F., Homer, S., Goh, A., & Lundin, L. (2012). W/O/W emulsions with high internal droplet volume fraction. *Food Hydrocolloids*, 27, 30–41.

Li, B., Jiang, Y., Liu, F., Chai, Z., Li, Y., Li, Y., et al. (2012). Synergistic effects of whey protein–polysaccharide complexes on the controlled release of lipid-soluble and water-soluble vitamins inW1/O/W2 double emulsion systems. *International Journal of Food Science and Technology*, 47, 248–254.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews,* 64, 4-17.

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science & Technology,* 47, 1-9.

Lutz, R., Aserin, A., Wicker, L., & Garti, N. (2009). Release of electrolytes from W/O/W double emulsion stabilized by a soluble complex of modified pectin and whey protein isolate. *Colloids and Surfaces. B, Biointerfaces*, 74, 178–185.

Lobato-Calleros, C., Recillas-Mota, M. T., Espinosa-Solares, T., Álvarez-Ramírez, J., & Vernon-Carter, E. J. (2009). Microstructural and rheological properties of low-fat stirred yoghurts made with skim milk and multiple emulsions. *Journal of Texture Studies*, 40, 657–675.

Lobato-Calleros, C., Rodríguez, E., Sandoval-Castilla, O., Vernon-Carter, E., & Álvarez-Ramírez, J. (2006). Reduced-fat white fresh cheese-like products obtained fromW-1/O/W-2multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Research International,* 39, 678–685.

Lobato-Calleros, C., Sosa-Pérez, A., Rodríguez-Tafoya, J., Sandoval-Castilla, O., Pérez-Alonso, C., & Vernon-Carter, E. (2008). Structural and textural characteristics of reduced-fat cheese-like products made from W1/O/W2 emulsions and skim milk. *LWT—Food Science and Technology,* 41, 1747–1856.

Márquez, A. L., & Wagner, J. (2010). Rheology of double (W/O/W) emulsions prepared with soybean milk and fortified with calcium. *Journal of Texture Studies,* 41, 651–671.

Matos, M., Gutierrez, G., Coca, J., & Pazos, C. (2014). Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol. *Colloids and Surfaces A: Physicochem. Eng. Aspects,* 442, 69– 79.

Matos, M., Gutierrez, G., Iglesias, O., Coca, J., & Pazos, C. (2015). Enhancing encapsulation efficiency of food-grade double emulsions containing resveratrol or vitamin B12 by membrane emulsification. *Journal of Food Engineering,* 166, 212–220.

McClements, D. J. (n.d.). Emulsion-based Delivery Systems: Novel or Improved Performance Through Emulsion Technology. Department of Food Science University of Massachusetts , Amherst.

McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition*, 47, 611–649.

McClements, D. J. (2014). Nanoparticle- and microparticle-based delivery systems: Encapsulation, protection and release of active compounds. CRC Press. Boca Raton, FL.

McClements, D. J. (2016). Food emulsions: Principles, practices, and techniques. CRC Press. Boca Raton FL.

McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceutical and functional foods. *Critical Reviews in Food Science and Nutrition,* 49, 577–606.

Mehrina, M. A., Jafari, S. M., Makhmal-Zadeh, B. S. & Maghsoudlou, Y. (2017). Rheological and release properties of double nano-emulsions containing crocin prepared with Angum gum, Arabic gum and whey protein. *Food Hydrocolloids,* 66, 259-267.

Miller, N. J., & Rice-Evans, C. (1997). Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. *Free Radical Research,* 26, 195–199.

Mun, S., Choi, Y., Rho, S. -J., Kang, C. -G., Park, C. -H., & Kim, Y. -R. (2010). Preparation and characterization of water/oil/water emulsions stabilized by polyglycerol polyricinoleate and whey protein isolate. *Journal of Food Science*, 75, E116–E125.

Munin, A., & Edwards-Levy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics,* 3, 793-829.

Nagle, D. G., Ferreira, D., & Zhou, Y. D. (2006). Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. *Phytochemistry*, 67, 1849-1855.

Onoue, S., Ochi, M., & Yamada, S. (2011). Development of (-)-epigallocatechin-3-gallate (EGCG)-loaded enteric microparticles with intestinal mucoadhesive property. *International Journal of Pharmaceutics,* 410, 111-113.

O'Reagan, J., & Mulvihill, D. M. (2009). Water soluble inner aqueous phase markers as indicators of encapsulation properties of water-in-oil-in-water emulsions stabilized with sodium caseinate. *Food Hydrocolloids,* 23, 2339–2345.

O'Reagan, J., & Mulvihill, D. M. (2010). Sodium caseinate–maltodextrin conjugate stabilized double emulsions: Encapsulation and stability. *Food Research International,* 43, 224–231.

Oppermann, A. K. L., Renssen, M., Schuch, A., Stieger, M., & Scholten, E. (2015). Effect of gelation of inner dispersed phase on stability of (w1/o/w2) multiple emulsions. *Food Hydrocolloids,* 48, 17-26.

Pawlik, A., Cox, P. W., & Norton, I. T. (2010). Food grade duplex emulsions designed and stabilized with different osmotic pressure. *Journal of Colloid and Interface Science,* 352, 59–67.

Pimentel-González, D. J., Campos-Montiel, R. G., Lobato-Calleros, C., & Pedroza-Islas, R. (2009). Encapsulation of Lactobacillus rhamnosus in double emulsions formulated with sweet whey as emulsifier and survival in simulated gastrointestinal conditions. *Food Research International,* 42, 292–297.

Rodríguez-Huezo, M. E., Pedroza-Islas, R., Prado-Barragán, L. A., Beristain, C. I., & Vernon-Carter, E. J. (2004). Microencapsulation by spray drying of multiple emulsions containing carotenoids. *Journal of Food Science*, 69, E351–E359.

Schuch, A., Köhler, K., & Schuchmann, H. P. (2013). Differential scanning calorimetry (DSC) in multiple W/O/W emulsions. *Journal of Thermal Analysis and Calorimetry,* 111, 1881–1890.

Sapei, L., Naqvi, M. A., & Rousseau, D. (2012). Stability and release properties of double emulsions for food applications. *Food Hydrocolloids*, 27, 316–323.

Scalia, S., & Mezzena, M. (2009). Incorporation of quercetin in lipid microparticles: effect on photo- and chemical-stability. *Journal of Pharmaceutical and Biomedical Analalysis,* 49, 90-94.

Scherze, I., Knöfel, R., & Muschiolik, G. (2005). Automated image analysis as a control tool for multiple emulsions. *Food Hydrocolloids*, 19, 617–624.

Shao, J., Li, X., Lu, X., Jiang, C., Hu, Y., Li, Q., et al. (2009). Enhanced growth inhibition effect of resveratrol incorporated into biodegradable nanoparticles against glioma cells is mediated by the induction of intracellular reactive oxygen species levels. *Colloids and Surfaces B: Biointerfaces*, 72, 40-47.

Shima, M., Kobayashi, Y., Fujii, T., Tanaka, M., Kimura, Y., Adachi, S., et al. (2004). Preparation of fine W/O/W emulsion through membrane filtration of coarse W/O/W emulsion and disappearance of the inclusion of outer phase solution. *Food Hydrocolloids,* 18, 61–70.

Shima, M., Matsuo, T., Yamashita, M., & Adachi, S. (2009). Protection of Lactobacillus acidophilus from bile salts in a model intestinal juice by incorporation into the inner-water phase of a W/O/W emulsion. *Food Hydrocolloids,* 23, 281–285.

Shima, M., Morita, Y., Yamashita, M., & Adachi, S. (2006). Protection of Lactobacillus acidophilus from the low pH of a model gastric juice by incorporation in a W/O/W emulsion. *Food Hydrocolloids,* 20, 1164–1169.

Siddiqui, I. A., Adhami, V. M., Bharali, D. J., Hafeez, B. B., Asim, M., Khwaja, S. I., et al. (2009). Introducing nanochemoprevention as a novel approach for cancer control: proof of principle with green tea polyphenol epigallocatechin-3- gallate. *Cancer Research,* 69, 1712-1716.

Sonaje, K., Italia, J., Sharma, G., Bhardwaj, V., Tikoo, K., & Kumar, M. R. (2007). Development of biodegradable nanoparticles for oral delivery of ellagic acid and evaluation of their antioxidant efficacy against cyclosporine A-induced nephrotoxicity in rats. *Pharmaceutical Research,* 24, 899-908.

Souguir, H., Salaün, F., Douillet, P., Vroman, I., & Chatterjee, S. (2013). Nanoencapsulation of curcumin in polyurethane and polyurea shells by an emulsion diffusion method. *Chemical Engineering Journal,* 221, 133-145.

Su, J., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effect of polyglycerol ester polyricinoleic acid and sodium caseinate on the stabilization of water–oil–water emulsions. *Food Hydrocolloids*, 20, 261–268.

Su, J., Flanagan, J., & Singh, H. (2008). Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUM™). *Food Hydrocolloids,* 22 (112), 120.

Surh, J., Vladisavljevic, G. T., Mun, S., & McClements, D. J. (2007). Influence of pH, emulsifier concentration, and homogenization condition on the production of stable oil-in-water emulsion droplets coated with fish gelatin. *Journal of Agricultural and Food Chemistry,* 55, 175–184.

Tsai, Y.-M., Jan, W.-C., Chien, C.-F., Lee, W.-C., Lin, L.-C., & Tsai, T.-H. (2011). Optimised nano-formulation on the bioavailability of hydrophobic polyphenol, curcumin, in freely-moving rats. *Food Chemistry*, 127, 918-925.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry,* 46, 4113-4117.

Walle, T. (2004). Absorption and metabolism of flavonoids. *Free Radical Biology and Medicine,* 36, 829-837.

Weiss, J., Scherze, I., & Muschiolik, G. (2005). Polysaccharide gel with multiple emulsions. *Food Hydrocolloids*, 19, 605–615.

Wenzel, S. & Somoza, V. (2005). Metabolism and bioavailability of trans-resveratrol. *Molecular Nutrition and Food Research*, 49, 472-481.

Wildman, R. E. C. & Kelly, M. (2007). Nutraceuticals and functional foods. In Handbook of Nutraceuticals and Functional Foods. (pp. 1-21). (2nd ed.). CRC Press. Boca Raton FL.

Wu, T.-H., Yen, F.-L., Lin, L.-T., Tsai, T.-R., Lin, C.-C., & Cham, T.-M. (2008). Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. *International Journal of Pharmaceutics*, 346, 160-168.

Yuce-Altuntas, O., Sumnu, G., & Sahin, S. (2017). Preparation and characterization of W/O/W type double emulsion containing PGPR–lecithin mixture as lipophilic surfactant. *Journal of Dispersion Science and Technology*, 38 (4), 486–493.

Zimmermann, E., & Müller, R. H. (2001). Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN™) dispersions in artificial gastrointestinal media. *European Journal of Pharmaceutics and Biopharmaceutics,* 52 (2), 203–210

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