SEPTEMBER 2019 M.Sc. in Food Engineering NEBAHAT TAŞ

REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

THE EFFECT OF ULTRASOUND ON THE PYSICOCHEMICAL AND EMULSIFYING PROPERTIES OF SOY PROTEINS

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BY

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M.Sc. Thesis
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Supervisor
Assoc. Prof. Dr. Emine ERÇELEBİ

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Nebahat TAŞ
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Nebahat TAŞ

ABSTRACT

EFFECT OF ULTRASOUND ON THE PYSICOCHEMICAL AND EMULSIFYING PROPERTIES OF SOY PROTEINS

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In this study, the effect of ultrasound application on the physicochemical and emulsifying properties of soy protein emulsions was investigated. Ultrasound was treated to a soy protein isolate (SPI) solution at 5, 10 and 15 amplitude. Results showed that the ultrasound treatment reduced the droplets size of emulsions and caused an increase in the emulsifying activity and stability of emulsions at 15 amp. The effect of pre-heating and pH treatment on the ultrasound treated SPI emulsion was also analyzed. Increased heat treatment from 60 to 70 and 80°C showed better emulsification properties. The best functional properties were observed at pH 9 in comparison to pH 5 and pH 7. On the other hand, $CaSO_4$-induced SPI gel-like emulsions were prepared by the cold-set and heat-set method. Ultrasound pre-treatment of SPI emulsions caused an increase in functional properties of $CaSO_4$-induced SPI gel-like emulsions. Gel-like emulsions were heated at 95°C for 20 min. Heat treatment, due to the structural changes in SPI molecules, led to smaller droplet size and uniform microstructure. And also, water holding capacity (WHC) and fat holding capacity (FHC) of gel-like emulsions increased.

Key Words: Soy Protein, Ultrasound, Gel-like Emulsion

ÖZET

ULTRASONUN SOYA PROTEİNİN FİZİKOKİMYASALVE EMÜLSİYON OLUŞUM ÖZELLİKLERİNE ETKİSİ

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Bu çalışmada, ultrason uygulamasının soya proteini emülsiyonlarının fizikokimyasal ve emülsifiye edici özellikleri üzerine etkisi araştırılmıştır. Ultrason 5, 10 ve 15 amplitude değerlerinde, soya proteini izolatı (SPI) çözeltilerine uygulanmıştır. Analizlerin sonuçları, ultrason uygulamasının emülsiyonun tanecik boyutunu azalttığını, emülsiyon aktivitesi ve stabilitesini ise arttırdığını göstermiştir. Ayrıca 60, 70 ve 80°C'de ön ısıtmalı işlem görmüş SPI çözeltilerinin emülsiyon özellikleri üzerindeki etkisi de incelenmiştir. Artan ısıl işlem ile daha iyi emülsifikasyon göstermiştir. Emülsiyonların pH 9'da pH 5 ve pH 7 ile karşılaştırıldığında daha iyi fonksiyonel özellik gözlenmiştir. Diğer yandan, $CaSO_4$ kaynaklı SPI jel benzeri emülsiyonlar, soğuk ayarlı ve 95°C'de ısıtma ayarlı olmak üzere iki ayrı metotla hazırlanmıştır. Ultrason ile ön muamele SPI emülsiyonlarının, $CaSO_4$ kaynaklı SPI jel benzeri emülsiyonların fonksiyonel özelliklerinde bir artışa neden olmuştur. Jel benzeri emülsiyonlar 95 °C'de 20 dakika ısıtıldı. Emülsiyona uygulanan ısıl işlem SPI moleküllerinde meydana gelen yapısal değişiklikler nedeniyle, daha küçük damlacık boyutuna ve homojen dağılımlı bir mikro yapıya sebep olmuştur. Ayrıca su tutma kapasitesi (WHC) ve yağ tutma kapasitesi de (FHC) artmıştır.

Anahtar Kelimeler: Soya Proteini, Ultrason, Jel-benzeri Emülsiyon

"Dedicated to my family"

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

APPENDICES

LIST OF SYMBOLS

A	Absorbance
L	Liter
M	Molarity
N	Normality
g	gram
h	hour (time)
in	inch
J	joule
kcal	kilocalorie
kDa	atomic mass unit
kg	kilogram
kHz	kilohertz
kJ	kilojoule
ml	milliliter
Mml	micro milliliter
min	minute

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
EAI	Emulsifying activity index
ESI	Emulsifying stability index
FAO	Food and Agriculture Organization
FHC	Fat holding capacity
HCl	Hydrochloric acid
HIU	High-intensity ultrasound
LIU	Low-intensity ultrasound
NaOH	Sodium hydroxide
SDS	Sodium dodecyl sulphate
SPI	Soy protein isolate
WHC	Water holding capacity

CHAPTER 1

INTRODUCTION

1.1. Soy Protein

Soy protein has been increasing popularity by the day due to its nutritional value, high protein content (38-40 %) and usage in a wide range of formulated foods (Nishinari and Fang, 2014). Some important functions of soy proteins in the food industry are emulsification such as sausages, meat burgers, texturizing meats, forming fiber in bologna, giving elastic texture to baked goods (Al-Bakkush, 2008).

Functional foods have become favorite among people due to increasing knowledge of functional components and their influence on human health. People would like to overcome health problems by consuming healthy food products rather than using drugs. The functional food has great importance due to the carry of active compounds such as antioxidants and antimicrobials (Milani and Golkar, 2019).

There is increasing interest in developing gel-like emulsions oil-in-water (O/W) emulsions stabilized by food-grade particles, due to their compatibility with food and some outstanding characteristics, e.g., extraordinary stability against coalescence, sustained release of some encapsulated ingredients, and enhancement of stability against lipid oxidation.

Ultrasound technology has much attraction due to using in emulsification, encapsulation or bioactive material releasing and nano-emulsion technology. In the future computationally used ultrasound processes can lead to generating a new design of food products in case of special needs and specific conditions (Jambrak et al., 2017).

Ultrasound is one of the most innovative methods as food processing technique can potentially alter the physical and chemical properties of food components like taste, aroma, appearance and quality parameters. Ultrasound sonication technology is based on mechanical waves which can be used to change the physical and chemical properties of food. Depending waves ranges can be divided into two branches; one is low frequency (high intensity) (16-100 kHz, power in the range 10-1000 W cm^{-2}) which is used for forming emulsifying system, extraction system chemically and physically changing food material. The other is high frequency (low intensity) ultrasound (100 kHz - 1 MHz, power <1 W cm^{-2}) which is non-destructive and can be used to obtain and classify food content. (Hu et al., 2013; Jambrak et al., 2009).

Although it's well known that soy protein has excellent emulsification properties, soy protein gel-like emulsions were poorly analyzed. In literature, few studies exist on the effect of ultrasound treatment on emulsions. O'Sullivan et al. (2016) have detailed the effect of ultrasound upon cereal proteins, wheat gluten, and rice protein isolate. And a few studies are related to the effect of ultrasound on the gelation ability of soy protein, Li-Chanet al. (2013) researched the effect of high-intensity ultrasound on soybean protein isolate gel while Zang et al. (2016) worked on physicochemical properties transglutaminase-catalyzed soy protein isolate gel.

The aim of this study was to give a deep insight into the mechanism of heat-treated and cold-set gel-like emulsions. Firstly, soy protein emulsions were prepared at different protein concentrations and sonication amplitude in order to analyze the effect of ultrasound on the functional properties of emulsions. The effect of heat treatment and the pH in emulsions were also examined. Then, the physical and chemical properties of these stabilized emulsions were compared. As a second part, gel-like emulsions were prepared by heat-treated and cold-set methods in two protein and fat concentrations. Finally, functional properties of gel-like emulsions were analyzed.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Proteins

Proteins are polymers composed of 19 different amino acids, which are linked by amide bonds which are called peptide bonds. The amino acids forming only differ in the chemical nature of the side chain group. The physicochemical properties such as the charge, solubility and chemical reactivity of amino acids depend on the chemical structure of the side chain group (Phillips and William, 2011). Proteins are highly complex and diverse polymers due to consist of various chemical structures. Various combinations of polypeptides and amino acid sequences are distinct factors in protein (Damodaran, 1997).

The common protein sources for humans are muscle proteins, blood proteins, milk proteins, egg proteins which are the animal origin. Crop-based proteins are wheat, corn, barley, oats, rice, peas, soybeans, lupins, lentils, potato (Damodaran, 1997).

2.1.1 Food Protein Structure

Proteins made up of amino acids. Proteins have both cationic and anionic charges at the presence of ions, the net charge can be zero which is called isoelectric point (Phillips and William, 2011). Each amino acid has a primary amine and carboxylic acid group as shown in Figure 2.1. The general formula of amino acid:

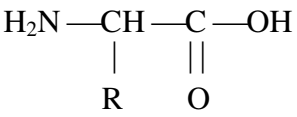


Figure 2.1 Chemical structure an amino acid (Phillips and William, 2011).

The R group is different for each amino acid and determines the character of the amino acid such as polar, non-polar, anionic or cationic properties. In literature, proteins contain 15-10,000 amino acids (Phillips and William, 2011). Primary structure of amino acid and bond formation is shown in Figure 2.2.

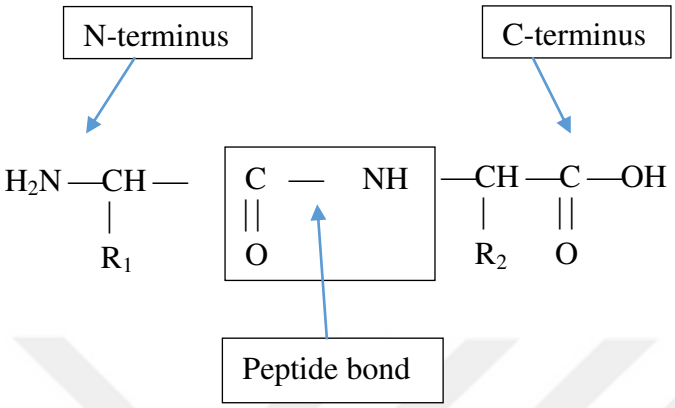


Figure 2.2 Primary structure of amino acid and bond formation (Phillips and William, 2011).

Since amino acids contain nonpolar chains, the aliphatic and aromatic amino acids exhibit low solubility in water. Aliphatic amino acids are Ala, Ile, Leu, Met, Pro, and Val while aromatic amino acids are Phe, Trp, and Tyr.

The charged amino acids, Arg, Lys, His, Glu, and Asp and the uncharged amino acids (Ser, Thr, Asn, Gln, and Cys) are quite soluble in water due to containing polar side chain. Proteins contain just an imino acid that is Proline. An imino acid is which consists =NH group (e.g. L-Proline), but an amino acid consists -NH_2 group (e.g. L-Glycine).

The net charge of a protein at any pH is determined by the relative numbers of the basic group (Arg, Lys, and His) and acidic (Glu and Asp) amino acid group in the protein (Gromiha, 2010).

Table 2.1 Some properties of most common 20 amino acids (Yada, 2000).

Amino acid			Mass	Sidechain type
Alanine	Ala	A	71.09	aliphatic hyrocarbon
Arginine	Arg	R	156.19	basic guanidyl
Aspartic acid	Asp	D	114.11	acidic carboxyl
Asparagine	Asn	N	115.09	acidic amide
Cysteine	Cys	C	103.15	thiol
Glutamic acid	Glu	E	129.12	acidic carboxyl
Glutamine	Gln	Q	128.14	acid amide
Glycine	Gly	G	57.05	hydrogen
Histidine	His	H	137.14	basic imidazole
Isoleucine	Ile	I	113.16	aliphatic hyrocarbon
Leucine	Leu	L	113.17	aliphatic hyrocarbon
Lysine	Lys	K	128.17	basic -amino
Methionine	Met	M	131.19	thio-ether
Phenylalanine	Phe	F	147.18	aromatic phenyl
Proline	Pro	P	97.12	heterocyclic imino
Serine	Ser	S	87.08	polar hyroxyl
Threonine	Thr	T	101.11	polar hydroxyl
Tryptophan	Trp	W	186.12	aromatic indole
Tyrosine	Tyr	Y	163.18	aromatic phenol
Valine	Val	V	99.14	aliphatic hyrocarbon

There are four levels of hierarchical characterization are used to define protein structure or architecture, as can be seen in Figure 2.3.

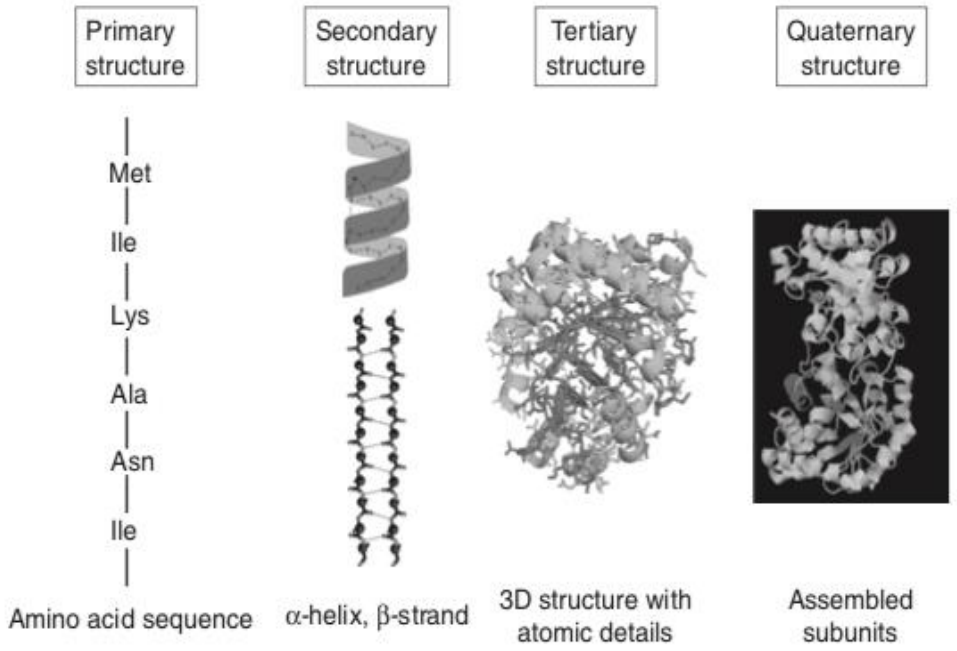


Figure 2.3 Main structure of a protein (Gromiha, 2010)

The primary structure of proteins depends on the characteristic sequence of amino acids of the polypeptide chain and also includes other covalently bonded structures, such as the location of disulfide bridges and the modifications of side chains (Li-Chan, 2004; Walstra and Vliet, 2003). The peptide bonds give shape and flexibility of the amino acid and to the polypeptide chains so that the primary structure indicates the three-dimensional structure of a protein molecule (Li-Chan, 2004).

The secondary structure is defined by the pattern of hydrogen bonds between the carboxyl oxygen (C=O) acceptor and amino hydrogen (N-C) donor groups and atoms in the peptide backbone and also, dihedral angles in rotation around N-C_α and C_α-C bonds (Li-Chan, 2004). Particular amino acids can give shape to secondary structures such as the alpha-helix and beta-sheet (Phillips and William, 2011). The main interactions of protein chains were shown in Figure 2.4.

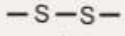
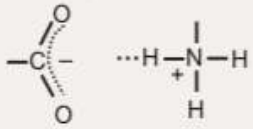
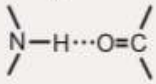
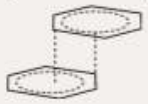
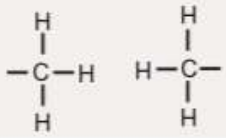
Type	Energy (kJ/mol)	Interaction distance (Å)	Example
Covalent bond	330–380	1–2	
Electrostatic interaction	42–84	2–3	
Hydrogen bond	8–40	2–3	
Hydrophobic interaction	4–12	3–5	
Van der Waals interaction	1–9	2–3[b]	

Figure 2.4 Interactions of protein chains (Li-Chan and Lacroix, 2018)

2.1.2 Functional Properties of Proteins

In addition to nutritional functions, proteins play an important role in the expression of the sensory qualities of foods. Consumer food preferences are predominantly based on the organoleptic properties of foods, such as color, flavor, and texture (Damodaran, 1997). Whilst it is clear that proteins have a major function as food

additives and ingredients. But also, as a special feature is the ability to form gels, to stabilize emulsions and foams and to form films (Phillips and William, 2011).

The functionality of proteins depends on its primary structure, but also on a range of external conditions. Some conditions during the application of proteins can affect the structure of the protein and this can cause markedly effect on the functionality. Some important conditions are briefly discussed.

Proteolysis; peptide bonds can be broken, at a high temperature which greatly changes their properties. Some of the enzymes capable of hydrolyzing various peptide bonds with the pH and temperature effect. And also, heat treatment can cause inactivation of some enzyme and chemically change in peptide bonds. Breaking of peptide bonds has a huge impact on the properties of the protein (Walstra and Vliet, 2003).

Denaturation of globular proteins is referred to the unfolding of the native structure of the protein. The denaturalizing agents are high temperature, pH, pressure and high concentrations of surfactants or polar lipids that bind to hydrophobic side chains. Denaturation causes some consequences such as loss of natural functionality of protein, easier proteolysis of protein, the reactivity of the side group, protein aggregation (Walstra and Vliet, 2003). Some important food protein and their functional properties are described in Table 2.2.

2.1.2.1 Gelation of Protein

Protein solutions are formed to gels through a series of processes that expand intermolecular interactions. Disulfide bonds are important because they generate covalent interaction, iso-amide bonds also have a significant role that can be formed by using the enzyme transglutaminase (Foegeding and Davis, 2011). The gel is formed by processes that break down secondary and higher structures with various degrees of breakdown of the polypeptide backbone (Haug and Draget, 2011). When the degree of intermolecular bonding reaches a point where a continuous network is formed, the macroscopic property of flexibility is improved and the system is considered a gel (Foegeding and Davis, 2011).

Table 2.2 Functional properties of proteins in the food system (Damodaran and Paraf, 1997)

Functions	**Mechanisms**	**Food**	**Protein type**
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water holding, hydrodynamic size, and shape	Soups, food sauces, salad dressings, desserts	Gelatin
Water binding	Hydrogen bonding, ionic hydration	Meat, sausages, cakes, bread	Muscle and egg proteins
Gelation	Water entrapment and immobilization, network formation	Meat products, gels, cakes, bakery products, and dairy products	Muscle, egg, and milk proteins
Cohesion-adhesion	Hydrophobic, ionic, hydrogen bonding	Meat and meat-based products, pasta, bakery products	Muscle, egg, and whey proteins
Elasticity	Hydrophobic bonding, disulfide cross-links	Meats, bakery	Muscle and cereal proteins
Emulsifying properties	Adsorption and film formation at interface region	Meat and meat-based products, bologna, soup, bakery sweet products, dressing	Meat, dairy and animal sources proteins
Foam properties	Interfacial adsorption and film formation	Whipped toppings, ice cream, cakes, desserts	Animal sources such as egg and milk proteins
Fat and flavor binding properties	Hydrophobic bonding, entrapment	Low-calorie bakeries, cookies donut	Dairy products, and cereal proteins

Some gelation factors that increase critical protein concentration and intermolecular interactions; interactions with other components, such as polymers; and protein-protein interactions leading to a micro-phase separated or single-phase system. These will depend on protein size, shape, isoelectric point, types of intermolecular interaction and structural properties (Foegeding and Davis, 2011). The properties of the gels will depend on some parameters, the degree of the unfolding of the protein

chains and the extent and kinetics of chain aggregation. The unfolding of protein can occur by heating, change in pH and ionic strength (Phillips and William, 2011).

The linear combination of denatured molecules leads to the formation of uniform fine-stranded three-dimensional network structures. The association tends to be driven by the interaction between hydrophobic domains along the protein chain. When the electrostatic charge on the protein chains is significantly reduced, micron-sized protein aggregates are formed which then combine to form coarse network structures (Phillips and William, 2011).

2.1.3 Some Important Proteins in Food Industry

Milk has traditionally been a major source of good nutritional protein which is mainly casein and whey-based components. The liquid structure of the milk ensures the effective separation of these components. Caseins are the main protein group in bovine milk. Whey proteins are another by-product of the dairy industry which contains up to 90 % protein. Whey proteins have a wide range of food area which can supply essential nutritional value. Nowadays, whey proteins have been taken much attention due to being used to stabilize foam and emulsions. Seafood is one of animal protein sources that are still provided in significant amounts to the human diet from wild sources. Seafood proteins have important biological value and as a source of essential amino acids. Muscle proteins are very nutritious and include all essential amino acids necessary for the human body. In addition, giving specific functions (gelling, emulsification, water holding, etc.) muscle proteins are very useful in the use of meat and meat products (cattle meat, poultry, and seafood meat) (Phillips and William, 2011).

Some functional mechanism of muscle protein are myofibrillar protein in texturizing of meat products, collagen water holding, juiciness of meat products, gelation emulsion stabilization of ‘surimi’ that is made from the seafood and uses for water-binding and fat emulsification (Xiong, 2004). Soybean is one of the main protein sources which compose of 38-40 % protein content. Soy protein has a wide variety of usage in human life such as nutrition and physiological aspects, physicochemical and functional properties of food systems, sweeteners, allergens, and so on (Li-Chan, 2004). In further chapter, soy protein has been explained with details.

2.2 Soybean: As a Storage Protein Sources

The soybean [*Glycine max* (L.) Merrill family Leguminosae, subfamily Papilionoidea] originated from Eastern Asia cultivated from 2000 years ago as the main food like traditional culture (FAO, 2017). Soybean is the most important cereal when we compare it with others who have high protein content. A grown soybean has about 38 % protein composition, 15 % soluble carbohydrates composition, 15 % insoluble carbohydrate compositions (dietary fiber), 18 % oil, and 14 % moisture content, ash, and vitamins and minerals compositions (WISHH, 2019).

Production of soybean is spread worldwide because of high nutritional content and isoflavones. Also, soybeans can boost the immune system and has a role and safe barrier against diseases such as diabetic, high blood pressure and some type of cancers (Zang et al., 2016).

The cultivation of soybean is highly increasing in the world. In 2017, the total production is 352 million tons. The largest producers are the USA (119 million tons), Brazil (114 million tons), Argentina (54 million tons), China (mainland) (13 million tons), and India (10 million tons). The annual amount of world soybean protein production will be sufficient to one-third of the need for protein content of humans. This large quantity has been one of the dietary protein sources (FAO, 2017). As can be seen in Table 2.3, big countries have a large production area in the world.

Table 2.3 Production of soybean in the world (FAOSTAT, 2017).

Countries	**Production (tones)**	**World Production (%)**
United States of America	119 518490	33.9
Brazil	114 599168	32.5
Argentina	54 971626	15.6
China (mainland)	13 149 485	3.7
India	10 981 000	3.1
Others	39 423 779	11.1
World Total	352 643 548	100

Big population countries have a large production area in the world. Soybeans can meet the basic nutritional needs of largely populated countries due to their nutritional and economic advantages. Therefore, soybean production in the last 35 years has shown a big increase in the world. This shows the importance of soy protein in human life (FAO, 2017). Soybean based food products in food industry have shown in Table 2.4.

Table 2.4 Soybean based goods in the food manufacturing sector (FAO, 2017)

Soybean products	**Usage in industry**
Soybean meal	Feed: Milk products, Swine feed, Poultry feed, Beef-cattle feed, Dairy-cattle feed, bee foods, pet foods, Aquaculture diet
Soybean flour, Protein concentrate, and Soy protein isolates	Food; Bakery ingredients, Noodles, Meat analogs, Breakfast cereal, Baby food, Hypo-allergenic milk, sweets, sausage, Fish sausage and surimi, Yeast culture, imitation dairy products, Flavoring, Infant formula, Salad condiments, coffee whiteners, Liquid whipped toppings, Emulsified food condiments, Non-dairy frozen sweet.
Nutraceuticals	Isoflavones, Saponins and Protease inhibitors

2.2.1 Soybean Protein

Soy protein has been used in a wide range of food formulation. For example, the seafood category can be used in fish sausage and *surimi* which made from fish flesh with combination other soy protein ingredients. Another consumption of soy protein is in the cereal bakery and milk products also, usage in infant feeding products to lactose-intolerant babies, usage in confectionery and deserts production, diet formulate for losing weight and pos-operative nutrition are also some important usage of soy protein for human life (FAO, 2017).

Production of soy protein isolate (SPI) is made from soy flour and soy flakes by acid-base precipitation of protein. Soy protein isolates are produced from soybean meal

flour by removing soluble non-soy protein compounds. During processing, undesirable soybean flavors are removed. Final product compositions are 90 % soy protein (dry basis), 0.5 % fat and 4.5 % ash (FAO, 2017).

A widely used method in soy protein extraction is precipitation reaction from soy flakes or soy meal, shown in Figure 2.5. The first reaction is precipitation reaction of glycinin which has an isoelectric point at pH 6.4, then precipitation of conglycinin from supernatant at pH 4.8 takes place (Kinsella, 1979).

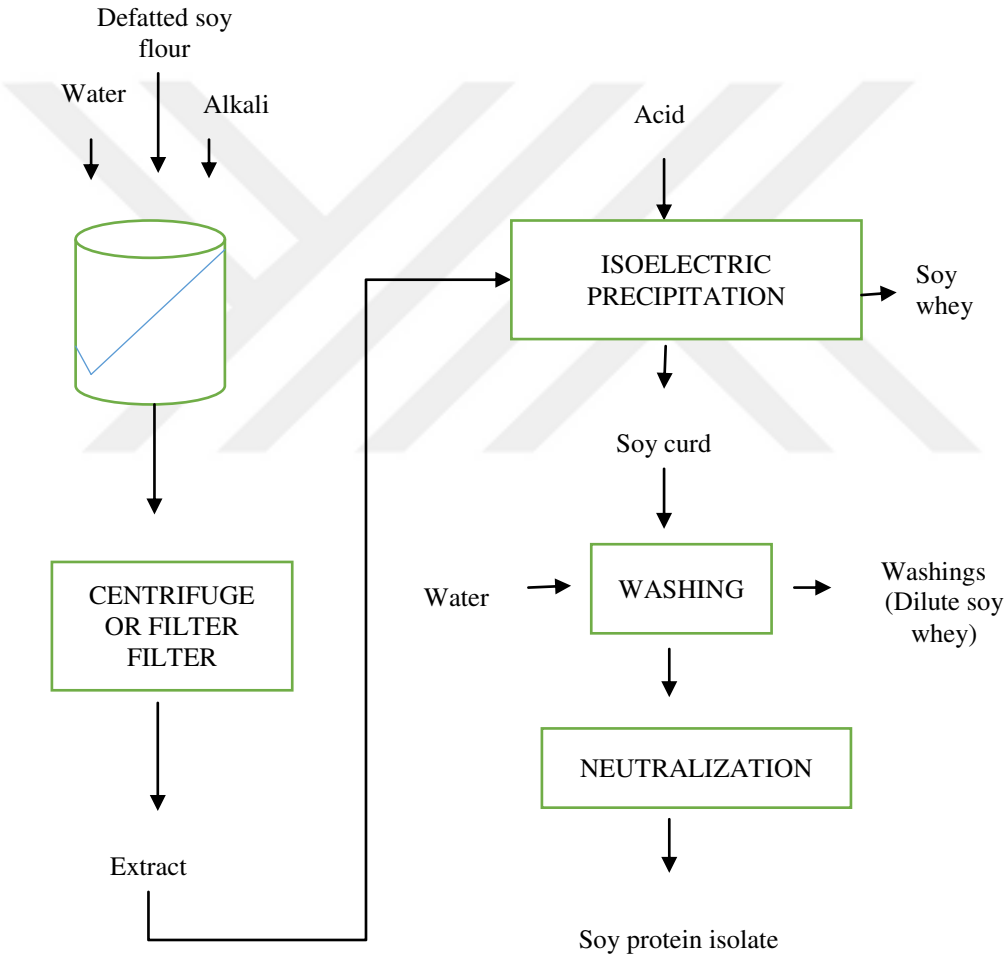


Figure 2.5 Soy protein isolate (SPI) production (Membrane Consultants, 2019).

2.2.1.1 Component of Soybean Protein

Nishinari and Fang (2014) reported that soy protein isolate (SPI) includes several proteins according to sedimentation coefficients; 2S (15%), 7S (34%), 11S (41.9%)

and 15S (9.1%), depending to sedimentation fraction. Glycinin and β-conglycinin are the significant soy proteins which occupy 80 % of soy protein (Renkema, 2001).
The sedimentation coefficient of β-conglycinin is 7S and the sedimentation coefficient of glycinin is 11S (Nishinari and Fang, 2014). β-conglycinin is a glycoprotein. β-conglycinin combine three subunits of ά (71 kDa), α (67 kDa), β (50 kDa) and a minor subunit (γ) which has a similar amino acid composition with others. Another subunit of conglycinin has been found and that leads to association or dissociation in changes of pH and ionic strength of the solution (Fukushima, 2004; Nishinari and Fang, 2014).

Glycinin (11S) has a heterogeneous structure, due to including acidic and basic polypeptide parts that are connected by a disulfide bond (Chen et al., 2014). Glycinin is hexamers and each subunit has acidic and basic polypeptide. Glycinin can be dissociated to their constituent polypeptides, subunits by the effect of extrinsic parameters such as pH, ionic strength, and heating degrees (Fukushima, 2004).

Recently, Nishinari and Fang (2014) stated some studies have done to define the amino acid composition of β-conglycinin and glycinin but the result could not be obtained due to the difficult crystallization. Native glycinin shows low foaming capacity than β-conglycinin due to the strong disulfide bonds in the structure of glycinin.

As a result of the studying of Utsumi et al. (1997), they found that; glycinin gels were harder than β-conglycinin gel due to glycinin has a large amount of SH group. And also, they reported that β-conglycinin is more reliable in forming gel due to higher hydrophobicity and easily unfolding structure.

Ren et al. (2009) reported that when heat treatment applied to protein in soy milk, the basic and acidic subunit of glycinin (11S) and a small number of acidic polypeptides in β-conglycinin (11S) dissociated, rearranged, and aggregated.

Renkema (2001) stated that the 2S subunits contain Bowman-Birk and Kunitz trypsin inhibitors that have to be inactivated by heating to eliminate unwanted and anti-

nutritional effects. Unfortunately, this heating may also cause low solubility and functionality of proteins.

2.2.1.2 Functionality of Soybean Protein

Nishinari and Fang (2014) reported that many researchers have found that the heating of glycinin (11S) at 80°C caused aggregation. The addition of isolated 7S protein to the emulsion was prevented aggregation, due to the increase in electrostatic forces between subunits of β-conglycinin and glycinin. The addition of conglycinin caused a new bond interaction of the basic part of glycinin. In Table 2.5 functional properties of proteins have shown.

Table 2.5 Functional properties of proteins (Fukushima, 2004).

Functionality	**Proteins or subunits**	**Properties**
Gel formation	β-conglycinin	Transparent, soft, but rather elastic gel.
	Glycinin	Muddy, firm, and not brittle gel form.
	A_2B_{1a}subunits	A_2provides gel firmness
	A_3B_4 subunits	A_3provides gel firmness
	$A_5A_4B_3$ subunits	$A_5A_4B_3$providesquick gel formation.
Thermal stability	Soybean storage proteins, β-conglycinin subunits	β-conglycinin> Glycinin
Emulsification	Soybean storage proteins, β-conglycinin subunits	β-conglycinin> Glycinin

2.2.2 Soybean Oil

Soy oil is also important in emulsification, which is necessary for food emulsions. Soybean oil is quite a good ingredient in emulsions due to excellent functional properties in food emulsification. According to the Food and Agriculture

Organization soybeans in oilseed production has a bigger area, as we can see in Figure 2.6 (FAOSTAT, 2017).

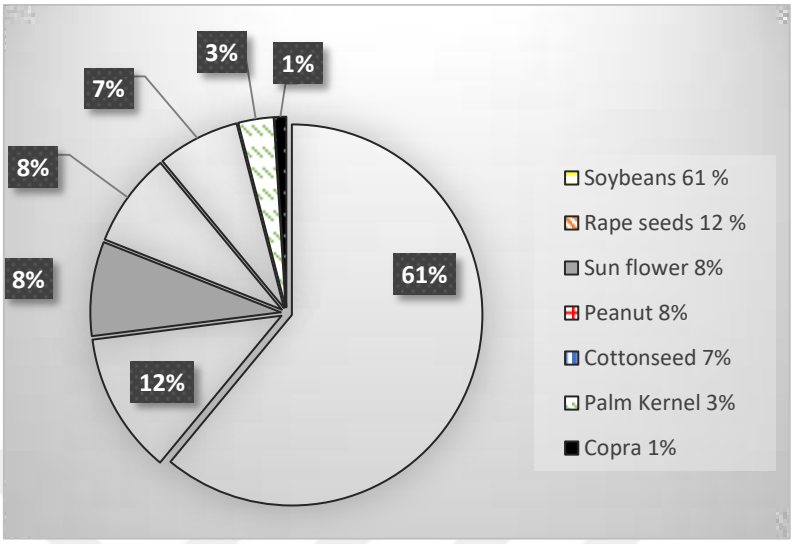


Figure 2.6 World vegetable oil consumption (FAOSTAT, 2017).

2.3 Food Emulsions

The emulsion can be defined as a colloidal system that is formed by dispersing an immiscible (dispersing or internal) phase as droplets in the other (continuous or external) phase (Chung et al., 2018).

All proteins are surface-active agents owing to consisting of the polar and nonpolar sidechains. Due to the amphiphilic structure of the protein, they can adsorb onto oil/water and air/water interfaces. Some chains in protein are quite polar and some others are hydrophobic (Walstra and Vliet, 2003).

2.3.1 Types of Emulsions

I. Oil in Water Emulsion (O/W):

In this type of emulsions, oil is a dispersed phase and water is a continuous phase. Water in oil emulsion known as ‘reverse’ emulsions in the oil industry (Pal, 2011). In food industry oil-in-water emulsions are widely used such as cheese, cream, desserts, flavors, milk, salad dressings, sauces, soups, and yogurts, mayonnaise, that can be an example of oil in water emulsions.

II. Water in Oil Emulsion (W/O):

In this type of emulsions, the dispersed phase is water and the continuous phase is oil. A water-in-oil emulsion is a crucial process in producing crude oil in the petrol industry. Edible oil, margarine, butter, dyes are the main examples.

III. Multiple Emulsions

Multiple emulsions where the dispersed phase is already an emulsion. They made up of a complex mixture of ingredients including proteins, polysaccharides, emulsifiers, water, and oil (Robinson and Wilde, 2003). Water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) are double emulsions such as emulsion inside another continuous phase. It is widely used in petroleum, pharmaceutical, cosmetics, and food industry. Ice cream is an emulsion which is combined oil and air in a water solution with solid particles (Pal, 2011). The type of emulsions is shown in Figure 2.7.

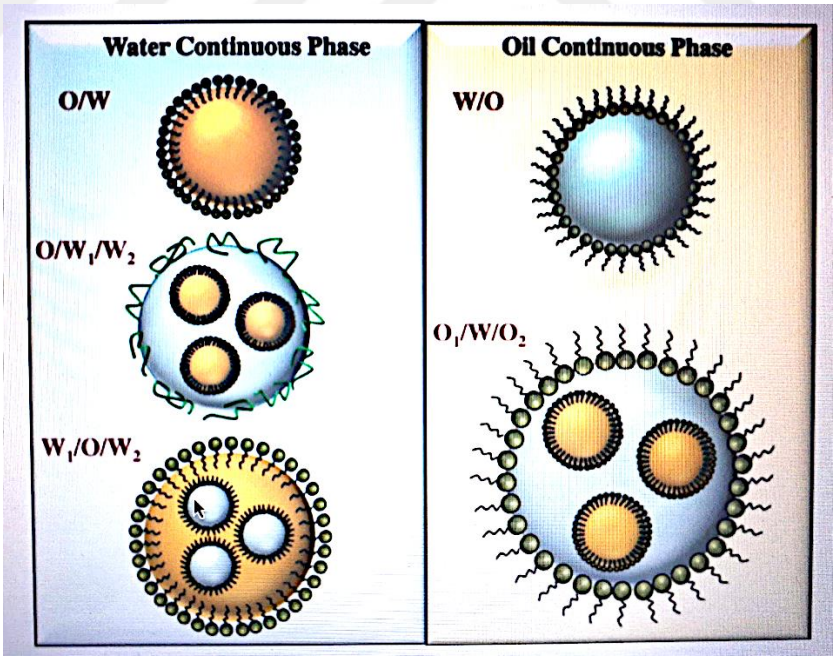


Figure 2.7 Type of emulsions (Chung and McClements, 2014)

2.3.2 Emulsions Phases

2.3.2.1 Aqueous Phase

The aqueous phase is a solvent of water-soluble components (salts, emulsifiers, proteins, carbohydrates, etc.), as either a continuous phase in O-W emulsions or as dispersed phase in W-O emulsions. Water molecules exhibit strong orientation due to attractive hydrogen bonds. They cause an increase in the interfacial tension and distribution of dispersed phase into smaller droplets in emulsion. Therefore, the interfacial tension forces can decrease by the attendance of emulsifiers. The pH, ionic strength and emulsifier concentration affect the formation of the emulsion by changing the size of the droplets and the interactions between phases (Robinson and Wilde, 2003).

2.3.2.2 Oil Phase

In food emulsions, the oil phase is found as triglycerides. Also, the oil has monoglycerides, polar lipids, and free fatty acids. These compounds tend to be surface-active materials and they can provide a role as food emulsifiers. The well-known fatty acid chains are saturated palmitic acid, stearic acid, and unsaturated oleic and linoleic acid. Plant oils possess more unsaturated fat composition than animal origin oil. Plant oils are liquid at room temperature (Robinson and Wilde, 2003).

For making an emulsion; oil, water, a surfactant, and mechanical energy are required. It is easy to make drops, but splitting the drops into small pieces requires higher energy, very strong mixing or homogenization (Walstra and Vliet, 2003). The characters of food emulsions provide by particles and interactions between particles, which are organized according to the characteristic of the interface region. In order to stabilize emulsion and droplet distribution, the oil-water interface can be enclosed with an emulsifier (Robinson and Wilde, 2003).

2.3.3 The Role of Emulsifier in Emulsions

The role of emulsifiers in emulsions is to form an adsorbed layer around the emulsion droplets. And the formed layer prevents flocculation and coalescence in the

emulsion system by reducing the interfacial tension that stabilizes the droplets. Emulsifiers are surface-active substances and they are naturally collected interface. They show amphiphilic properties that have both hydrophilic and hydrophobic parts (Robinson and Wilde, 2003).

Emulsifiers may be low molecular weight emulsifiers or macromolecular polymers. Low molecular weight emulsifiers contain tiny hydrophilic head group and one or more hydrocarbon chains; these include mono and diglycerides, lecithin, polysorbates, and sugar esters. The most common polymeric emulsifiers in food systems are mainly proteins derived from milk, but also other sources are soybean, meat, and fish products (Robinson and Wilde, 2003).

2.3.4 Homogenization of Emulsion

Homogenization is a well-distribution of the dispersed phase, split into small droplets by using mechanical movements. There are three methods of homogenization. The first method, mechanical methods such as rotor-stator systems induce high shear fields to break up droplets. The second method, high-pressure homogenizers are now very common, and simply force the premix through a narrow orifice or valve at high pressures (typically 10 – 100 MPa or 1500 – 15 000 lb in^{-2}). The third method, forcing the emulsion through a valve at high pressure creates turbulence and very high shear force, thus breaking up the droplets. The most energy-efficient process is the microporous approach, where the dispersed phase is forced through a porous substrate into the continuous phase. The droplet size produced is dependent on the interfacial tension and the pore sizes. These microporous methods are still being developed and have not yet been widely utilized in the food industry, due to the low throughput (Robinson and Wilde, 2003).

2.3.5 Mechanism of Emulsions Stability

Emulsion stability can be defined as the system's ability to resist changes in its physicochemical properties over time. From a thermodynamic point of view, an emulsion is an unstable system, since there is a natural tendency for a liquid-liquid system to separate and reduce its interfacial energy. Many emulsion systems which are very stable in terms of bonding are susceptible to flocculation. The flocculation

phenomenon occurs when the dual free energy of interaction between adjacent droplets become significantly negative (Dickinson, 1998). Flocculation of an initially stable emulsion can be induced, by lowering the pH towards the isoelectric point, by increasing the ionic strength, by the addition of divalent cations (especially Ca^{2+}), by lowering of solvent quality, by bridging with an adsorbing polymer, or by depletion with nonadsorbing polymer. Any process, heating, high-pressure treatment or enzyme action that allows the protein molecules on different droplets to become cross-linked, may also cause flocculation. Several mechanisms such as creaming, flocculation, coalescence, and phase separation cause emulsion breakdown. A physically stable emulsion must resist to breakdown mechanisms ((McClements, 2015; Abdel-Raouf, 2012). Breakdown mechanisms are shown in Figure 2.8.

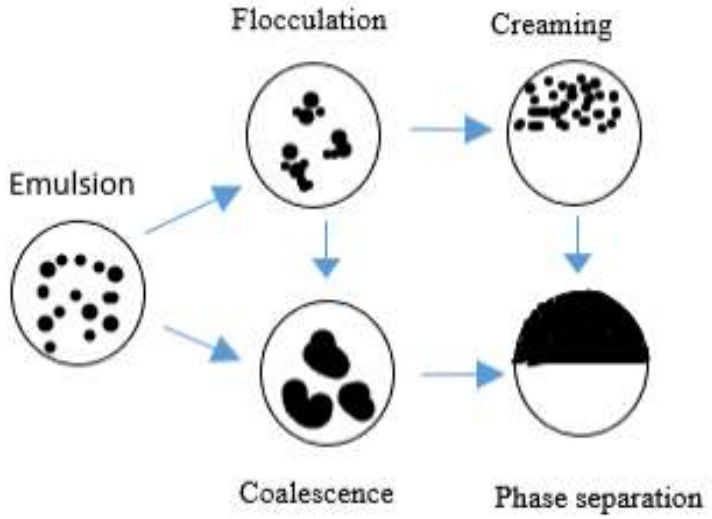


Figure 2.8 Breakdown mechanism of an emulsion (McClements, 2015).

2.3.5.1 Flocculation

Flocculation is the process of two or more droplets stick together (without losing their individual identity) to form an aggregate. If the attractive forces between the particles are stronger than the driving forces, the flocculation occurs. The attraction may also be electrostatic: between mutually charged groups at the surface of the two parts or between the same charges by divalent ions (e.g. Ca^{2+} ion bridges between -COO- groups) (Walstra and Vliet, 2003). Repulsion is related to electrostatic nature. Repulsion decreased when the pH becomes closer to the isoelectric point and the ionic strength increased (Walstra and Vliet, 2003).

Oil in water tends to flocculation due to interactions between droplets in emulsions and density differences between phases (Dickinson, 1998). When Van der Waals attractions are weak in the emulsion system since there is not enough repulsive force and the droplets to form a cluster of connected droplets without droplet destruction by the effect of gravitational force (Tadros, 2013). As a general rule, if the adsorbed protein dissolves well in the continuous phase of the dispersion, stability is generally provided (Walstra and Vliet, 2003).

2.3.5.2 Coalescence

Coalescence is the process in which two or more droplets merge together to form a single larger droplet. Coalescence occurs when surface or film fluctuations drive droplets to lose their identity and form bigger droplets. The strong van der Waals attractions also affect to form coalescence in emulsion (Tadros, 2013; Robinson and Wilde, 2003). Coalescence occurs when two emulsion droplets are close together for a long time and the thin film between the drops may break and cause the drops to flow together. If the drops are bigger, the interfacial tension is smaller and coalescence occurs easier (Walstra and Vliet, 2003).

Coalescence can prevent an increase in protein concentration. Proteins are a very good stabilizer against the coalescence of small droplets. Proteins cause strong repulsion between the droplets and high interfacial tension to prevent coalescence. Moreover, several globular proteins also form a coherent surface layer that can prevent film tear. Protein cover droplets and acts as an emulsifier. Coalescence occurs when the mechanical force applied to combined droplets for example centrifugation (Walstra and Vliet, 2003).

2.3.5.3 Creaming

Creaming of emulsions is related to the Stokes equation. According to this equation, if the dispersed phase density is less than the continuous phase density, droplets move up and collect on the top of the emulsion. Creaming is considered to be the movement of droplets towards up of emulsion. The rate of creaming is inversely proportional to the viscosity of the emulsion. The physical stability of the emulsion can be increased by decreasing the viscosity differences of phases and reducing

droplet size. Creaming and sedimentation mechanism is similar due to the density difference but in sedimentation, droplets move to the bottom of the emulsion (Chiralt, 2019).

2.3.5.4 Phase Separation

Phase separation is the process of complete separation of phase from each other. The phase of an emulsion depends on the droplet size, the viscosity of the dispersion medium and the phase volume ratio (Dickinson, 1998; McClements, 2015). Phase separation occurs at the last limit of coalescence. (Tadros, 2013). Phase separation and creaming should be considered different; because creaming is reversible, although phase separation is an irreversible mechanism.

2.4 Gel-like Emulsions

Gelation refers to the transformation of a protein in the solid-state by heat or other agents (Damadoran, 1997). Proteins can be used to obtain given rheological properties. They are generally not suitable as thickening agents since quite high concentrations are needed to obtain a high viscosity as compared to polysaccharides. Most proteins can, however, be used to make gels (Walstra and Vliet, 2003). A gel consists of a matrix, i.e. a space-filling structure, and a continuous liquid phase. A gel has solid (elastic) properties, although the major component is a liquid, generally an aqueous solution. A gel matrix can be built of protein. There are three main types of gels made of protein solutions, as well as mixed systems containing other matrix material besides protein (Maltais et al., 2009).

Gel-like emulsions are different than gel, they are in the semi-solid phase. Gel-like emulsions are generally considered to be one of the most suitable delivery systems for many lipophilic bioactive components, including highly unsaturated fatty acids, antioxidants, and carotenoids. The delivery behavior of a lipophilic bioactive in emulsion-based systems is generally determined by the molecular nature of the lipid molecules, the droplet size and interface structure of the dispersed phase, and the structure and composition of the food matrix. Furthermore, the release of encapsulated lipophilic molecules from protein stabilized emulsions may also be

affected by the chemical structure of the bioactive agents applied (Liu and Tang, 2016).

There is increasing interest in developing gel-like emulsions, oil-in-water (O/W) emulsions stabilized by food-grade particles, due to their compatibility with food and some outstanding characteristics, e.g., extraordinary stability against coalescence, sustained release of some encapsulated ingredients, and enhancement of stability against lipid oxidation. The association or aggregation of particles in the continuous phase (which is more favorable at higher particle concentrations) may lead to the formation of a gel-like network in the emulsions, as in the chitin nanocrystal case. The gel-like network formation can thus impart extraordinary stability against creaming to the emulsions (Liu and Tang, 2014). Soy protein isolate acts as a macromolecular surfactant to stabilize oil in water emulsions system in food products such as soups, sausages, coffee whitener, hypo-allergenic milk, fish sausage, salad condiments, liquid whipped toppings, emulsified sour cream or cheese dressings, non-dairy frozen desserts (Utsumi et al., 1997).

Tang and Liu (2013) reported that emulsions were formed by high-pressure homogenization at 80 MPa that emulsions containing >4% SPI showed stability to creaming, and the oil phase was aggregated and entrapped in a structured network of protein. They also reported that all the formed emulsions exhibited a gel-like behavior and stability to creaming upon storage, and the heat treatment (at 82 C for 2 min) before homogenization greatly increased the gel strength. The gel-like structure formation was due to emulsion destabilization by a depletion mechanism.

Arrese et al. (1991) studied the effect of protein composition and degree of protein denaturation on the solubility, water-holding capacity, viscosity, and gelation capacity of commercial soy protein isolates. They stated that the degree of denaturation of soy protein is an effective factor in functional properties of soy protein isolate gel such as solubility, water absorption, viscosity, and capacity for gel formation. And the result of studies demonstrated that the degree of denaturation may affect protein solubility, but also high denaturation causes high solubility and completely denatured proteins showed low gelation capacity. They reported as this characteristic is closely related to the relative number of the 7S and 11S proteins,

since ß-7 S subunit and basic 11S polypeptides, were present in decreased concentrations in the soluble protein fraction. They found that the high degree of denaturation and intermediate solubility values caused an increase in water holding capacity (WHC). The results confirmed that the apparent viscosity of soy protein dispersion is intimately related to WHC.

2.4.1 Heat-Set Emulsions

Heat-set gels of globular proteins. These can be either fine-stranded (although the strands are significantly thicker than a peptide chain) or more like a particle gel. The proteins denature before a gel is formed. Since denaturation and gelation do not depend in the same manner on pH and temperature, gelation can in principle also be achieved by altering the pH after heat denaturation and cooling (this is termed cold gelation) (Tang and Liu, 2013). In typical heat-set gelation, a structural transition occurred prior to the gelation. With progressive increase in temperature, the particles in the system grew with the aggregation of the partial unfolding molecules, leading to an opaque appearance.

In the food industry, there are many heat-sensitive nutraceutical components, such as vitamin B1 and probiotics, which will lose activity under high temperatures. However, from the process of soy protein tofu hydrogels making, it can be observed that heating is usually applied in the second step, which of course, limits soy protein tofu hydrogels' application as nutraceutical component carrier to provide heat-sensitive nutraceutical components (Zang et al., 2016; Hu et al., 2013; Nishinari et al., 2014). A recent study showed that soy protein tofu hydrogels had potential to be used as controlled delivery devices for nutraceutical compounds. However, the process of making traditional soy protein tofu hydrogels involves two steps: firstly, heating soy protein at neutral pH to dissociate soy protein so as to expose the functional groups (such as hydrophobic group) from the interior of the molecule to the surface.

Nishinari (2004) that the formation of b-sheet structures, heating temperature for glycinin and b- conglycinin began to increase above their denaturation temperature, 65 and 80 C, respectively, and was found to be consistent with the change in

mechanical spectra induced by heating. Isolated glycinin aggregates when heated at 80 C. The ultracentrifugal pattern showed that heating of acid-precipitated soy protein at 80 C resulted in the disappearance of glycinin and the concurrent appearance of soluble aggregates and protein components of 2S and 4 S. They found a similar function for egg albumin and bovine serum. They attributed the prevention of aggregation by b-conglycinin to the formation of a soluble complex between the subunits of b-conglycinin and the basic subunits of glycinin via electrostatic interaction (Nishinari et al. 2015)

Utsumi et al. (1984) analyzed that heat-induced interaction between soy protein's 7S and 11S globulins. Heating caused dissociation of both 7S and 11S globulins. The dissociated subunits of 7S and 11S globulins subsequently interacted with each other and forming soluble macro completes. The analyzing of macro completes revealed that the macro completes contained predominantly the basic subunits of 11S globulin and the β subunit of 7S globulin; the little amount of the α and ά subunits of 7S globulin. This analyzing showed that the basic subunits of 11S have a higher affinity for the β subunit. The results also indicated that the interaction between the basic subunits and the β subunit is predominantly electrostatic in nature. Furthermore, disulfide bonds between the basic subunits are also involved in the formation of soluble macro completes.

2.4.2 Cold-Set Emulsions

Since soy protein has excellent gelation properties, there is some study on the gelation of soy protein. Interestingly, soy protein can form gels at a cold temperature under certain conditions, which are called cold-set soy protein gels. The mechanism of the gel-like structure formation is due to colloidal destabilization, especially droplet aggregation through strong attractive interactions between emulsion droplets. If the rate of droplet aggregation is much higher than the creaming rate in the systems, the formation of a space-filling network of droplets (gel formation) may occur. In some cases, the formation of a gel-like structure within the emulsions greatly improves their stability against creaming. Highly concentrated protein-stabilized emulsions usually exhibit some gel-like viscoelastic properties, due to droplet packing effect and inter-droplet electrostatic repulsion (Tang and Liu, 2013).

Cold set gelation has potential applications in the food industry. A commercial whey protein product has been manufactured. This ingredient can be used in various foods such as surimi, pressed ham, dressings, spreads, and bakery products. A few patents have also been awarded for cold-set gelation (Damodaran, 1997). In the food industry, there are many heat-sensitive nutraceuticals components, such as vitamin B1 and probiotics, which lose their activity at high temperatures. In this cold-set, gel-like emulsions can be used as a delivering system and releasing by controlling (Kadam et al. 2015; Zang, et al. 2016).

A recent study showed that Zang et al. (2016) studied on the physicochemical properties of transglutaminase-catalyzed soy protein isolate cold set gel (TSCG) under ultrasound treatment. They reported that significantly increased in the gel strength, water-holding capacity and gel yield of TSCG o and a more uniform and denser microstructure of TSCG by inter-molecular covalent and non-covalent interactions.

Tang and Liu (2013) reported the rheological and microstructural properties of a kind of novel cold, gel-like soy protein isolate (SPI) emulsions obtained by means of microfluidization. The gel-like network was formed by bridging flocculation of oil droplets, mainly through inter-droplet hydrophobic interactions between the proteins adsorbed at the interface. These results suggested that soy proteins exhibit the excellent potential to produce cold, gel-like emulsions, especially through a heat pretreatment followed by microfluidization, which might be of vital importance for the development of soy protein-based formulations, especially as carriers for heat-labile ingredients with health effects.

2.5 Ultrasound Technology in Food System

The use of ultrasound in a food system can be explained by cavitation phenomena (Soria and Villamiel, 2010). When ultrasound is applied to liquid medium at above the human hearing frequency (<16 kHz) causes bubbles in the medium. The bubbles called cavitation and cause some changes in the food system such as mass or heat transferring and formation of free radicals as an undesired chemical reaction (Kentish, 2017). When power ultrasonic waves reach the medium by the probe

(sonotrode, horn) which is a contacting part of ultrasound device with food, high energy enters the medium system, cavitation phenomena occurs and cavitation causes acoustic pressure inside a medium (Tao and Sun, 2015). In addition, when sonication applied in surface, bubbles converts to microjets that spurt out the surface but can lead harmful surface erosion and also a fine mist may also be formed by a process called acoustic atomization. Classification of the ultrasound wave range is shown in Figure 2.10 (Kentish, 2017).

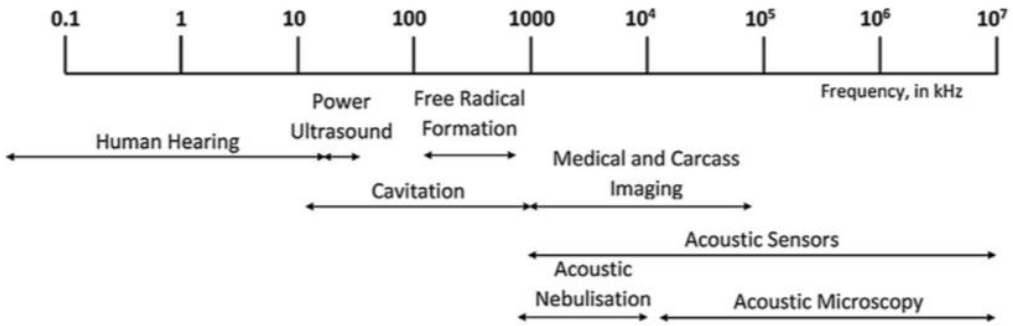


Figure 2.9 Classification of ultrasound waves range (Kentish, 2017).

An ultrasound device (Figure 2.11) has basically generators, transducers, and probe; the function of the generator produces electrical energy to transform into the current at high or low ultrasonic frequency and transmit to the transducer which is an important part while pass through transducer current transform to mechanical waves (Silva and Sulaiman, 2017).

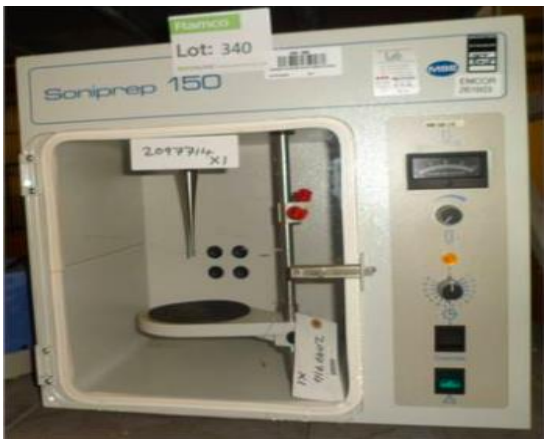


Figure 2.10 An ultrasonic processor, model MSE Soniprep 150 (Ramco Co. Ltd, Lincolnshire, the UK)

When high-power ultrasound (20 kHz or higher) applies to a fluid system due to the cavitation phenomena shown in Figure 2.12, bubbles occur. Due to the high energy, the collision of bubbles causes a movement to the surface of fluids and collapsed bubbles. Collapsed bubbles release free energy to the medium which converts heat energy and induces some chemical and physical reactions (Mason et al., 2005).

Power ultrasound (20 kHz–100 kHz) also has other application areas in food science; nano-emulsification, freezing, thawing, extraction, drying, deactivation of enzymes, sanitation, encapsulation of active substances, and can be used as an antimicrobial agent, etc. Low-energy ultrasound (high frequency, 5 - 10 MHz range is non-destructive, which can be used as an analytical technique to clarifying properties of the materials (Mason et al., 2005).

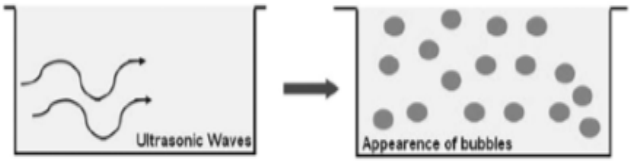


Figure 2.11 Cavitation phenomena in the fluid system (Majid et al., 2015)

During applying high-frequency ultrasound to the medium, because of cavitation, some changes occur in protein conformation. Breakdown of quaternary and tertiary structure increase the bioactivity, hydrolysis of protein, effects on hydrogen bonding interactions in medium, and modified protein structure cause some changes in the functional properties of protein, sensory and physical attributes of protein (Jambrak, 2017). Also, there are ultrasound studies in carbohydrates fields and research has reported on starch, extraction of polysaccharides, and obtain nanoparticles of lignin. Solubility and consistency of starch increased with the intensity of ultrasound intensity (Jambrak, 2017).

2.5.1 Application of Ultrasound on Emulsification

O'Sullivan et al. (2016) investigated the effect of ultrasound on the emulsion formation properties of wheat and soy protein. They found ultrasound treatment provide a considerable reduction in the aggregate size of proteins, while there was no

destruction in the primary structure. Ultrasonic energy was insufficient to break down the peptide bond. The ultrasound treated emulsions were showed decreased size of emulsion droplets and high stability. Thus, the results showed that ultrasound treatment can increase the emulsifying properties of whey protein and soy protein.

Hu et al. (2013) study are showing the emulsification properties of ultrasound treated soy protein $CaSO_4$-gels. They found that high-intensity ultrasound (20 kHz) affects particle distribution and decrease particle size of protein. And increased the surface hydrophobicity and the free sulfhydryl (SH) of soy protein isolate which induced disulfide bond formation during gel formation. Furthermore, water-holding capacity and gel strength were positively correlated with free SH content of heated SPI and surface hydrophobicity of unheated SPI, and negatively correlated with the particle size of heated SPI and free SH content of gel. The result of studies was shown that the high-intensity ultrasound-induced structural changes in SPI molecules, leading to different microstructure and improved water holding capacity and gel strength of gel.

Zang et al. (2016) were also studied the effects of high-intensity ultrasound (HIU). In his study, the effect of high-intensity ultrasound pretreatment of soy protein isolate (SPI) on the functional properties of transglutaminase-catalyzed soy protein isolates cold-set gel (TSCG) was analyzed. They found the gel strength and water-holding capacity increased remarkably after HIU pretreatment and resulted in uniform and dense microstructure. After HIU treatment, the content of free sulfhydryl (SH) groups, hydrophobic amino acid residues were higher and also changed in polypeptide backbone conformation and secondary structure of transglutaminase-catalyzed soy protein isolate cold-set gel (TSCG). These results showed that increased inter-molecular lysine isopeptide bonds, disulfide bonds, and hydrophobic interactions might have contributed to the HIU treated TSCG gel network. As a result, HIU changed the physicochemical and structural properties of SPI. HIU treated gels showed a better form due to the great involvement of covalent and non-covalent interactions between SPI molecules and aggregates.

2.5.2 Encapsulation Systems

Encapsulation is the preserving of an active compound which is enclosed in another substance (wall material). The use of ultrasound to encapsulate nutraceuticals is important to the protection of the target substance when it's susceptible to light, heat, and oxygen (Nedovic et al., 2011). The use of ultrasound technology in the emulsification process has shown significant results in enhancing emulsion systems. Furthermore, as a result of the ultrasonic process associated with the use of emulsifiers has shown high adsorption rates at the oil-water interface. Therefore, using ultrasound can improve the nanometer scale emulsion formation (Silva et al., 2017).

Another ultrasound treatment was studied by Hu et al. (2015), ultrasound applied to form transglutaminase-catalyzed soy protein gel for using riboflavin in functional foods. They confirmed that the high-intensity ultrasound treated soy protein isolate increased the hydrophobic nature of transglutaminase gels and increased the gel yield, riboflavin encapsulation, and gel strength. They found that the encapsulation of riboflavin is possible with cold gel method. This resulted in reduced riboflavin release rate and altered the release mechanism in simulated gastrointestinal fluids both in the absence and presence of digestive enzymes. In conclusion of this study, HIU may facilitate covalent cross-linking, increase hydrophobicity and change the 3D network of transglutaminase-catalyzed soy protein hydrogel. They led to differences in hydrogel stability, as well as riboflavin encapsulation and release profiles.

Ultrasonic technology has a wide range of food industrial applications, such as in the extraction of compounds, inhibition of microorganisms, inactivation of enzymes, sanitation of materials, filtration and drying (Nedovic et al., 2011). Currently, the most widely used method of inactivation of microorganisms is thermal pasteurization and sterilization. The thermal method is effective when used in high-temperature hat can kill the vegetative microorganisms and some spores. However, the magnitude of the high temperature can also kill the beneficial nutrients in food. Side effects of the conventional method can cause destruction in food, undesired taste, and flavor (Dolatowski et al., 2007).

Noteworthy research on ultrasound technology found that by high-intensity ultrasound, microorganisms in food can be inactivated by destroying the cell wall of a biological cell (Dolatowski et al., 2007). Unfortunately for the usage of ultrasound in microbial sterilization is required to be combining with other methods due to knowing high-intensity ultrasound can cause an undesired reaction in food (Mason et al., 1996). The other application of ultrasound with the comparison of the conventional method can be seen in Table 2.6.

Table 2.6 Comparison of ultrasound technology with the conventional method (Chematet al., 2011).

Application	***Conventional method***	***Ultrasound principle***	***Advantage***	***Product***
In cooking	Stove Fryer Water bath	Uniform heat transfer	Less time, increasing heat transfer and organoleptic quality	Meat based products, vegetables
Freezing / Crystallization	Freezer Freezing by immersion	Uniform heat transfer	Provide tiny crystals, increasing diffusion, quick temperature decreasing	Meat Vegetables Fruits Milk products
Drying	Atomization	Uniform heat transfer	Decrease in time, decreasing organoleptic quality	Dehydrated product (fruits, vegetables)
Pickling / marinating	Hot gas stream	Increasing mass transfer	Decreasing time, increasing organoleptic quality and stability	Vegetables Meat Fish cheese
Degassing	Mechanical treatment	Compression - rarefaction phenomenon	decreasing time, increasing hygiene	Chocolate Fermented products
Filtration	Filters	Vibrations	decreasing time, decreasing filtration	Liquids (juices)
Defoaming	Thermal treatment Chemical treatment Electrical treatment	Cavitation phenomenon	Decreasing time, decreasing hygiene	Carbonated drinks, Fermented products
Emulsification	Mechanical treatment	Cavitation phenomenon	decreasing time, emulsion stability	Emulsions
Oxidation	Contact with air	Cavitation phenomenon	decreasing time	Alcohols

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Soy protein isolate, sodium dodecyl sulfate (SDS) and calcium sulfate were purchased from Sigma- Aldric Chemicals Co (St. Louis, MO). Commercially available soy oil was obtained from the local market and used without further purification. Distilled water was used in all analyses. All pH arrangements were made with 0.1 N NaOH or 0.1 N HCl. Sodium hydroxide (NaOH ≥ 97 and hydrochloric acid (HCl, 37.0 %) was obtained from Merck Chemicals Co. (Darmstadt, Germany). All other reagents were obtained from Sigma- Aldric Chemicals Co (St. Louis, MO).

3.2 Methods

3.2.1 Preparation of Soy Protein Isolate (SPI) Solutions

10 ml of soy protein isolate solutions were prepared with distilled water at 1 and 3 % (w/v) protein concentration. Solutions were stirred at by using a magnetic stirrer for 15 min at room temperature (model MS300HD MTops Co. Ltd, Korea).

3.2.2 Ultrasound Treatment

Ultrasound treatment was applied according to the method of Zang et al. (2016). An ultrasound processor model MSE Soniprep 150 (Ramco Co. Ltd, Lincolnshire, the UK) with a 0.636 cm diameter titanium probe was used for sonication. Prepared protein solutions were sonicated by using ultrasonic disintegrator at an amplitude of 5, 10 and 15 for 1 min.

3.2.3 Preparation of Emulsions

Ultrasound treated protein solutions (1 and 3 %) were taken and 10 % (v/v) soybean oil was added. Then samples were homogenized at 10,000 rpm for 2 min by using homogenizer (model IKA T-18, Digital ULTRA TURRAX, Sigma- Aldric Company, St. Louis, MO) with S18N-10G model stainless steel dispersing elements. Ultrasound treated emulsions were analyzed for emulsifying activity index (EAI), emulsifying stability index (ESI), creaming behavior and microscopic droplet size.

3.2.4 Effect of Heat on Emulsions

The preheated emulsion preparation method was adopted from Gu et al. (2009) with slight modifications. Protein solutions were prepared at 1 and 3 % (w/v) SPI concentrations. The solutions were heated to 60, 70, and 80°C in a water bath for 2 min and were rapidly cooled to room temperature in an ice bath (model SWB15D Camlab, UK). The solutions were sonicated at 15 amplitude for 1 min. Then, 10 % (v/v) soybean oil was added to sonicated solutions and homogenized at 10,000 rpm for 2 min.

3.2.5 Effect of pH on Emulsions

SPI solutions were prepared at 1 and 3 % (w/v) concentrations. The pH of emulsions was adjusted to 5, 7 and 9 by adding 0.1 N NaOH or 0.1 N HCl solutions (Li et al., 2012). The pH measurements were made by a microprocessor pH meter (211 model; Hanna Instrument Co, Woonsocket, USA). Before using, the pH meter was calibrated with standard buffer solutions pH 4 and 9. The pH adjusted solutions were sonicated at 15 amp for 1min. Then, 10 % (v/v) soybean oil was added to the sonicated solution and homogenized at 10,000 rpm for 2 min.

3.2.6 Emulsifying Activity and Stability Index of Emulsions

The emulsifying activity index (EAI) of the emulsions was determined by the method of Pearce and Kinsella (1978). 1 and 3 % (w/v) SPI containing emulsions were prepared. The solutions were taken to sonication for 5, 10 and 15 amplitude for 1 min. Then 10 % of soybean oil was added and homogenized for 2 min. 50 µl of freshly made emulsion were diluted into 0.2 M SDS solution. Then immediately 1 ml

of diluted emulsions were taken to measure absorbance. The absorbance of the diluted emulsions was measured by spectrophotometer (Pharmacia Biotech, Novaspec II, Cambridge, UK) at 500 nm in 1 cm path length cuvettes. The absorbances were read immediately (0 min) and after 10 min. Turbidity and EAI were calculated in terms of m^2/g by using the following equation:

$$T = (2.303\ A)/L$$

where A is the observed absorbance and L is the path length of the cuvette in cm.

$$EAI = 2T/\phi\ C$$

where ϕ is the volumetric fraction of oil and C is the weight of protein per unit volume of the aqueous phase. ESI values were calculated by the following equation:

$$ESI\ (\%)\ \frac{A_0}{(A_0 - A_{10})} x\ 100$$

where A_0 and A_{10} are the absorbance of the diluted emulsion at 0 and 10 min, respectively (Wanget al., 2008).

3.2.7 Determination of Creaming Stability of Emulsions

Creaming stability of emulsions was measured by the method of Wanget al. (2008). 10 ml of 1 and 3 % (w/v) SPI containing emulsions were prepared. Then prepared emulsions were observed visually at room temperature for 10 h. The cream separation was expressed as the percentage of the volume of the serum layer over the total volume of the emulsion in the cylinder.

Creaming (%) = (Volume of the serum layer/volume of the emulsions) x (100)

3.2.8 Viscosity of Emulsion

10 ml emulsions were prepared. 0.5 ml of freshly made emulsions were taken with a pipette into viscometer and measured at a shear rate of 40 s^{-1} by using a DV2T Viscometer (Brookfield Rheometer).

3.2.9 Optical Microscopy

The microscopic images of emulsions were taken by using the method of Erçelebi et al. (2011). For this analysis, a polarized light microscope Olympus BX 51 model (Tokyo, Japan) equipped with a video camera (Pixera PVC 100C, USA) was used. One drop of the freshly made emulsion was put on a microscopic slide and covered with a coverslip and then replaced onto the microscope. Then images were taken with a 10X magnification objective. The droplet size of emulsions was measured by using Bab program that attached to the polarized light microscope. The diameter of 100 droplets was measured and the average diameter was recorded as droplet size.

3.2.10 Preparation of Gel-Like Emulsions

The gel-like emulsion was prepared according to the method of Hu et al.(2013) with some modifications. The soy protein isolate powder was dissolved in the distilled water. The protein solutions were prepared at 1, 3, 5, 6, 7, 8 and 10 % (w/v) SPI concentrations. The solutions were sonicated at 15 amp for 1 min. Soybean oils were added to solutions at a ratio of 10 and 30 % (v/v). Then prepared samples were homogenized at 10,000 rpm for 2 min. Then 0.2 ml 0.2 M $CaSO_4$ was added to the prepared emulsion and mixed well. This process was called cold-set gel-like emulsions and the emulsions were left to form gel-like texture in the refrigerator for 24 h.

For the heat-set gel-like emulsion, after the addition of the 0.2 ml 0.2 M $CaSO_4$, the emulsions were heated at 95°C in a water bath for 20 min. After heat treatment, the emulsions were rapidly cooled to room temperature in an ice bath. The emulsions allowed left for 24 h in a refrigerator to form gel-like texture.

3.2.11 Water Holding Capacity of Gel-like Emulsion

The water-holding capacity (WHC) of gel-like emulsions was measured according to the method of Yang et al. (2013) with modifications. 10 g of gel-like emulsions were taken to the 50 ml centrifuge tubes. The tubes were centrifuged at 8000×g for 30 min at 4°C. The separated water was filtered by using dry filter paper. The tubes with the gel samples before and after the centrifugation was accurately weighed and the

difference between them was taken to represent the weight of liquid released. WHC was calculated as follows:

$$\text{WHC (\%)} = \frac{W_t - W_f}{W_t} \text{x } 100$$

where Wt was the total weight of water added to emulsions (g), Wf was filtered water (g).

3.2.12 Fat Holding Capacity of Gel-Like Emulsion

The fat holding capacity (FHC) of the gel-like emulsion was determined by the method of Gu et al. (2009) with some modifications. 20 ml of SPI gel-like emulsions were prepared. The gel-like emulsions were centrifuged at 10,000 g for 30 min. 10 ml 5 % (w/v) KOH was added to the precipitated gel and homogenized with an Ultra-Turrax homogenizer for 1 min. After 2 days the fat layer was observed on the top. Fat holding capacity calculated as:

$$\text{FHC (\%)} = \frac{F_g}{F_t} \text{x } 100$$

Where and Ft was the oil (ml) added to the emulsion and Fg was oil (ml) in the SPI emulsion gel.

3.3 Statistical Analysis

Samples were triplicated. Results were compared by one-way analysis of variance (ANOVA) to test for significant differences. Means of the groups were compared by using Tukey's multiple range test using an SPSS statistical packet (Version 25). The differences between the groups were to be significant when $p<0.05$. Graphs of the results were drawn by Microsoft Office program.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of Ultrasound Treatment

4.1.1 Emulsifying Activity and Stability Index of Emulsions

The emulsifying activity index (EAI) is a measure of the amount of oil that can be emulsified per unit of protein (Aryee et al., 2018). In our study, the EAI of ultrasound treated emulsions was measured. Emulsions were prepared at 1 and 3 % soy protein isolate (SPI) concentrations. Ultrasound was applied at an amplitude of 5, 10 and 15 for all emulsions. The data were shown in Figure 4.1.

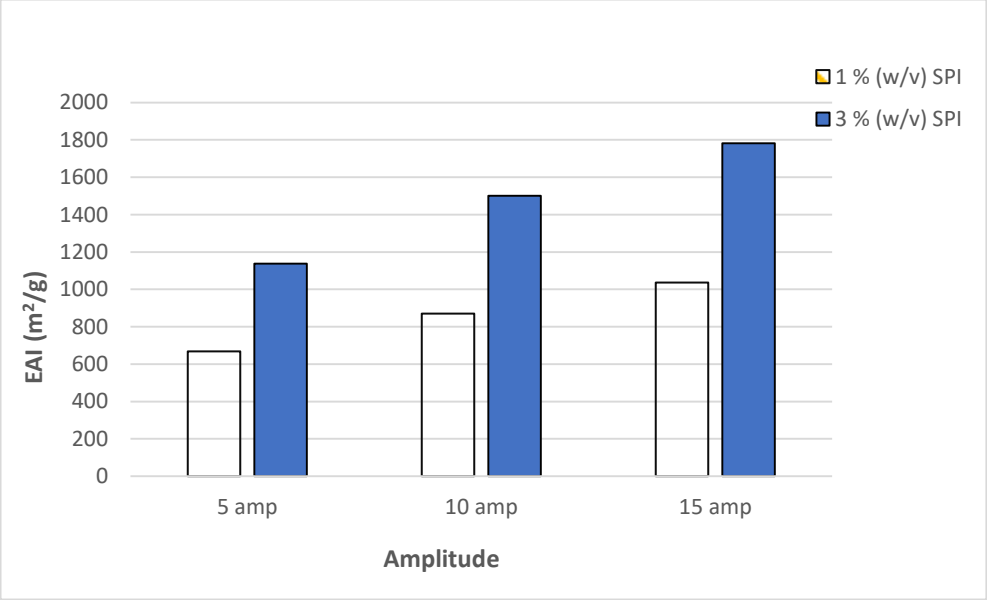


Figure 4.1 EAI of SPI stabilized emulsions at different ultrasound amplitude.

The EAI of emulsions containing 1 % (w/v) SPI sonicated at 5 amp, 10 amp and 15 amp that were 667.87, 870.54 and 1036.35 m^2/g while for emulsions containing 3 % (w/v) SPI were 1137.68, 1501.56 and 1782.52 m^2/g, respectively.

The increase in the SPI concentration from 1 to 3 % (w/v) were increased the EAI values, as were shown in Figure 4.1. An increase in protein concentration has led to more interaction between protein chains (Dickinson, 1998). It caused an increase in EAI values. EAI at an amplitude of 15 was higher than that at 5 and 10, for both SPI concentrations (1 and 3 % (w/v)). The EAI values at 15 amp, were 1036.35 and 1782.52 m^2/g at 1 and 3 % (w/v) SPI concentrations, respectively. They were the highest values among the data. In general, EAI of all emulsions increased significantly ($p<0.05$) with ultrasound treatment.

McClements (1995) stated that an increase in ultrasound intensity causes changes in the structure of emulsion. Sufficient acoustic energy was released to a broke covalent bond. This enhanced activity of ultrasound treated stabilized SPI emulsion, is attributed to an increase in the hydrophobicity and a decrease in the interfacial tension (O'Sullivan et al., 2016).

The emulsifying stability index (ESI) of an emulsion measures the resistance of the change in the structure of the emulsion during a specific time period (Aryee et al., 2018). In our experiments, the absorbance of emulsions was measured immediately at 0 and 10 min. The ESI of ultrasound treated emulsions at 5, 10 and 15 amp in two SPI concentrations (1 and 3 % (w/v)) were shown in Figure 4.2.

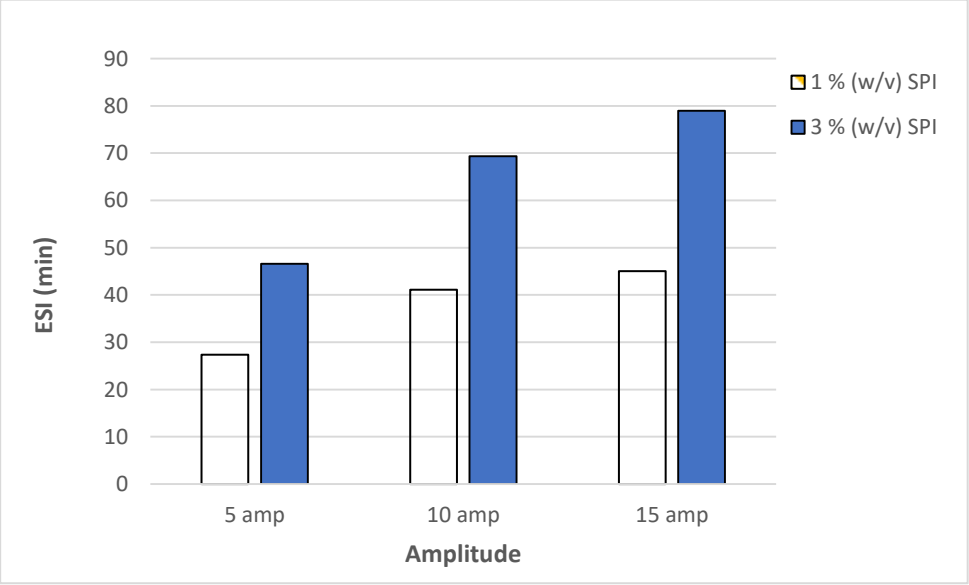


Figure 4.2 ESI of SPI stabilized emulsions at different ultrasound amplitude.

In overall data, ESI values at 3 % (w/v) SPI concentration were dominantly higher than 1 % (w/v) SPI concentration, as expected. Dickinson (1998) has been reported that emulsion stability is directly related to the amount of protein present in the emulsion due to the increase in protein concentration which causes an increase in protein adsorption at the oil-water interface. Therefore, the increase in the SPI concentration at the same amplitude (15 amp), from 1 to 3 % (w/v) was increased the stability of the emulsion from 45 to 78.98 min, respectively.

Increasing the ultrasound intensity showed high stability for both protein concentration of 1 and 3 % (w/v), as shown in Figure.4.2. The lowest ESI values were measured at lowest amplitude (5 amp), as 27.35 and 40.6 min for 1 and 3 % SPI concentration, respectively. An increase in ultrasound amplitude increased significantly ($p<0.05$) ESI values. Ultrasonic treatment was consistent with a significant reduction in droplet size (e.g. increase in surface area-volume ratio) and thus allowed increased protein adsorption at the oil-water interface, therefore increases the stability of emulsions (O'Sullivan et al., 2016).

McClements(1995) reported high-intensity ultrasound can affect the structure of substances and induce chemical reactions in emulsions. For both concentrations (1 and 3 %), ESI showed the highest values at 15 amp. Majid et al.(2015) reported ultrasonication has an efficient method in producing protein conjugates and to improve the hydrolysis of proteins and increase stability of emulsions.

4.1.2 Creaming Stability of Emulsions

Creaming data of ultrasound treated emulsions were shown in Figure 4.3. Creaming is a sign of emulsion instability and subsequent breakdown system. In 1 and 3 % (w/v) SPI containing emulsions that sonicated at 5 amp for the first 30 min time interval, creaming was detected as 26.1 and 24.5 %, and total creaming was recorded as 28.6 and 27 %, respectively.

In 1 % SPI containing emulsion, the highest creaming value was detected as 28.6 % at 5 amp. And this creaming value remained constant during the 24 h observation.

Due to the low intensity of ultrasound (5 amp), the creaming of 3 % SPI containing emulsion was also high compared to other amplitudes (10 and 15 amp).

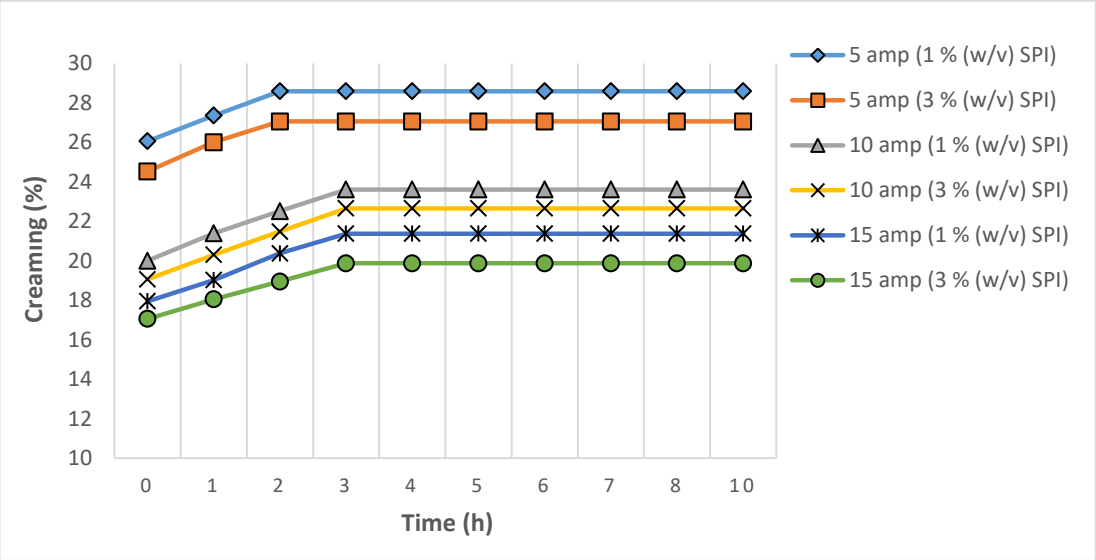


Figure 4.3 Creaming percentage of SPI stabilized emulsions at different ultrasound amplitude.

Dickinson (1998) stated that the increase in protein concentration creates more stable emulsions. At the end of 24 h, the lowest creaming was observed at high protein concentration (3 %) and high-intensity ultrasound (15 amp) which was 19.9 %. High-intensity ultrasound can alter the physical and chemical structure of the protein and this can retard serum separation and increase emulsion stability (Hu et al., 2013).

4.1.3 Microscopic Observation of Ultrasound Treated Emulsions

The microscopic images of ultrasound treated emulsions at 5, 10 and 15 amp were taken by a polarized-light microscope with 10X magnification and were shown in Figure 4.4 and droplet size was shown in Table 4.1. According to our results, larger droplet size was observed at low protein concentration (1 % (w/v)) and low ultrasound treatment (5 amp.)

As shown in Table 4.1, in 3 % SPI containing emulsions that sonicated at 15 amp, droplet size decreases and maintains emulsion stability. The ultrasonic treatment

has a significant effect on reducing the aggregate size of proteins, without destruction of disulfide bonds in protein (Jambrak, 2017). Ultrasound treatment causes reducing the interfacial tension, enhancing emulsion droplet breakup during emulsification and fabricating smaller emulsion droplets (O'Sullivan et al., 2016).

Table 4.1 Droplet size of SPI stabilized emulsions at different ultrasound amplitude.

Ultrasound	1 % (w/v) SPI concentration	3 % (w/v) SPI concentration
5 amp	12.05 μm	9.28 μm
10 amp	8.48 μm	7.88 μm
15 amp	7.72 μm	6.57 μm

As reported by O'Sullivan et al. (2016), the decrease in droplet size after ultrasonic treatment is consistent with a reduction in protein size and cause higher protein adsorption at the oil-water interface and smaller emulsion droplet size.

When we compared the effect of protein concentration, bigger droplets and sparse distribution were observed at low protein concentration (1 % SPI). And the smallest droplets were observed at 3 % SPI containing emulsions. Increasing protein concentration caused more protein-protein interaction inside emulsion. As a result, increasing protein concentration showed a uniform distribution of droplets so increased creaming stability as well. O'Sullivan et al. (2016) stated that the acoustic energy of ultrasound was sufficient to achieve proteolysis of the peptide bond or breaking of disulfide bonds. While the increasing intensity of ultrasound, the droplet size of emulsions was become smaller respectively, from 5 amp to 10 amp and to 15 amp for both protein concentration 1 to 3 % (w/v)

A comparison of increasing ultrasound amplitude from 10 to 15 amplitude led to a decrease in droplet size. For both protein concentration (1 and 3% SPI) at 15 amp, smaller droplets were observed compared to 10 amplitude. Among the images the smaller droplets (6.57 μm) were observed at 15 amp and at 3 % SPI concentration, as seen in Table 4.1.

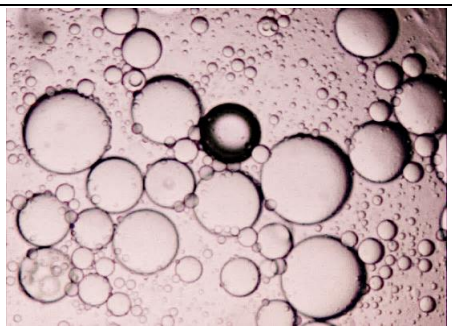
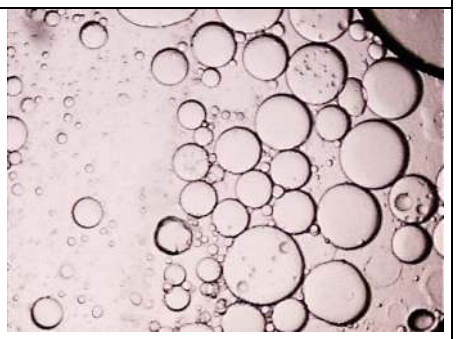
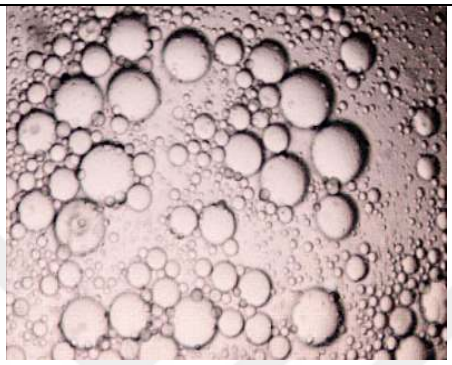
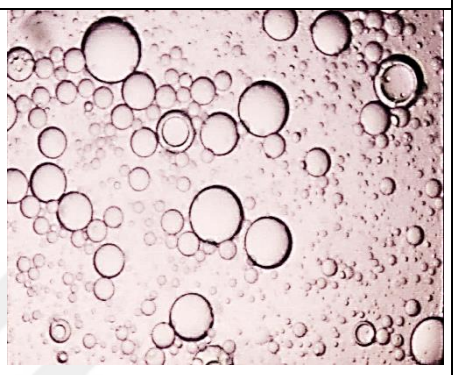
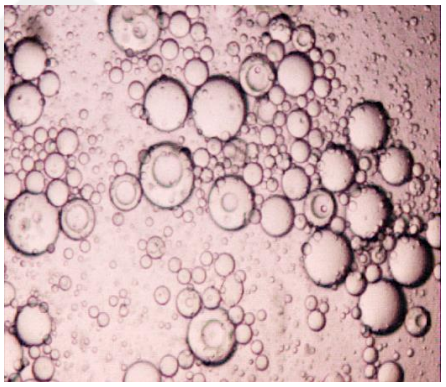
	1 % (w/v) SPI Concentration	3 % (w/v) SPI concentration
5 Amp		
10 Amp		
15 Amp		

Figure 4.4 Microstructure of SPI stabilized emulsions at different ultrasound amplitude.

4.1.4 Viscosity of Ultrasound Treated Emulsions

The viscosity (cp) of the ultrasound treated sample was shown in Figure. 4.5. The viscosity of 1 % (w/v) SPI containing emulsions that sonicated at 5 amp, 10 amp and 15 amp was measured as 2.72, 3.38 and 4.38 cp, respectively. Increasing in ultrasonic intensity caused a significant ($p<0.05$) increase in viscosity. The same trend was observed in 3 % (w/v) SPI containing emulsions as well and viscosity was 3.34, 5.04 and 9.74 cp at 5, 10 and 15 amp, respectively.

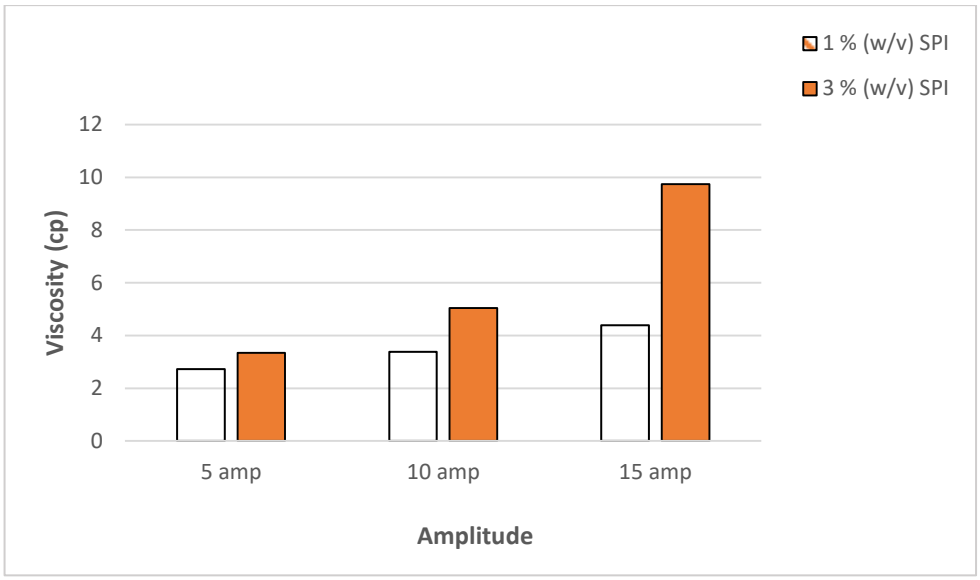


Figure 4.5 Viscosity of SPI stabilized emulsions at different ultrasound amplitude.

O'Sullivan et al. (2016) reported that the significant increase in the hydrophobicity would lead to an increased rate of protein adsorption to the oil-water interface, reducing the interfacial tension, thus improved facilitation of emulsion droplet break-up.

4.2 Effect of Heat Treatment on Emulsions

4.2.1 Emulsifying Activity and Stability Index of Heat-treated Emulsions

Emulsifying activity index of heat-treated emulsions at 60, 70, 80°C at two SPI concentrations were shown in Figure 4.6. In our study, EAI of the 3 % (w/v) SPI containing stabilized emulsions showed obviously higher EAI values than 1 % (w/v) containing emulsions, as can be seen in Figure 4.6. While the EAI values were 884.35, 1063.99 and 1202,17 m^2/g at 1 % SPI containing emulsions and EAI of 3 % SPI containing emulsions were 1317.32, 1561.43, 1625.92 m^2/g at 60, 70 and 80°C, respectively. As can be seen, an increase in SPI concentration from 1 to 3 %, EAI values increased dramatically. Increase in protein concentration, increase protein-protein and protein-oil interactions as well. In generally, EAI increased significantly ($p<0.05$) by increasing protein concentration (from 1 to 3 %) and heating temperature.

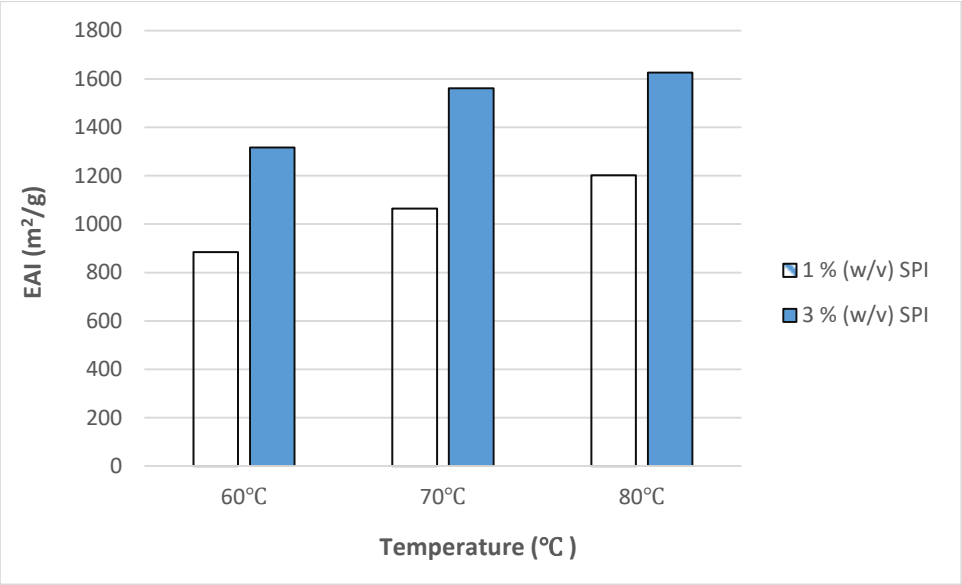


Figure 4.6 EAI of SPI stabilized emulsions at different heating temperatures.

Increasing heating temperature also significantly ($p<0.05$) increased ESI of emulsions as were shown in Figure 4.7. At 60°C, ESI was 45 min for 1 % SPI concentration and 76 min for 3 % SPI concentration. ESI values at 70°C were 55 and 89 min for 1 and 3 % (w/v) SPI concentrations, respectively.

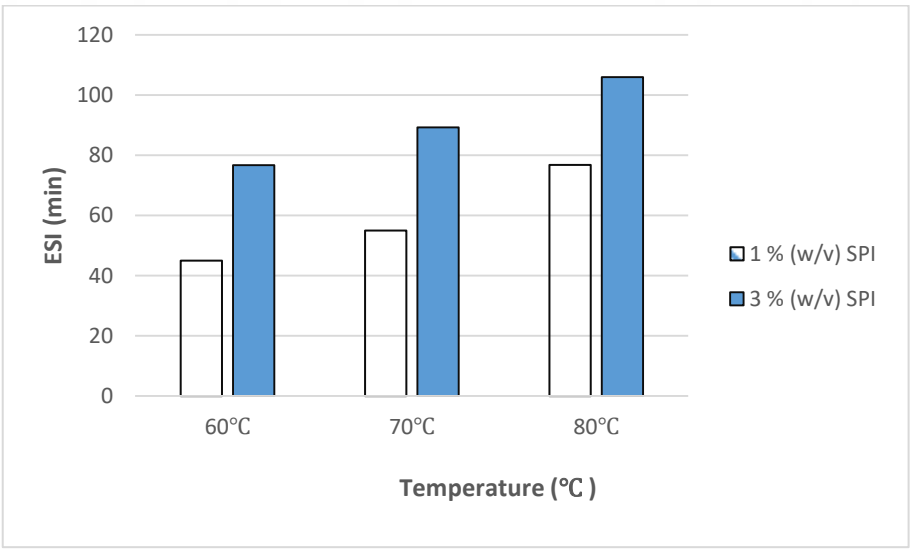


Figure 4.7 ESI of SPI stabilized emulsions at different heating temperatures.

The highest ESI was observed at 80°C for 1 and 3 % (w/v) concentrations. This temperature is very close to the denaturation temperature of the conglycinin subunits. The denaturation temperatures of β, α, and α′ subunits in conglycinin were 90.8, 82.7 and 78.6°C, respectively (Nishinari et al., 2018). And also, as seen in Figure 4.7, ESI

of 3 % (w/v) SPI containing emulsions was higher than 1 % (w/v) SPI containing emulsion. Increasing the protein concentration from 1 to 3 % (w/v) increased the stability of emulsions significantly ($p<0.05$). And also, increasing temperature of heat treatment led to more denaturation in protein that caused more stable emulsions.

4.2.2 Creaming Behavior of Heat-treated Emulsions

As shown in Figure 4.8, the lowest creaming value was observed in the presence of 3 % SPI concentration at 80°C heated emulsions. It started with 8.1 % creaming at the first 30 min then increased to 9.4 %. The changes between the two values were only 1 %, during the 24 h time period. But, creaming for 1 % SPI emulsion was higher than 3 % SPI concentration. Creaming started at 8.6 % and increased to 10.4 %. Those results indicated that an increase in SPI concentration was very essential in forming a stable emulsion.

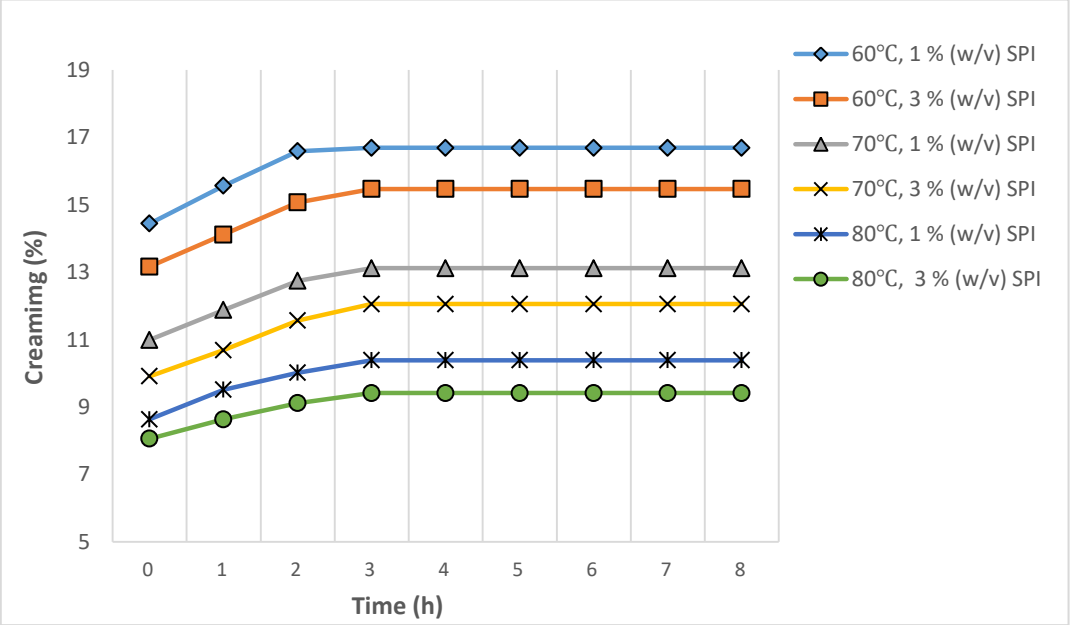


Figure 4.8 Creaming percentage of SPI stabilized emulsions at different heating temperatures.

Creaming stability of 1 % SPI containing emulsion was 16.7 % at 60°C. In increasing temperature to 80°C, creaming decreased to 10.4 %. According to our result, low temperature caused high creaming and low stability in all emulsions. And high temperature caused the lowest creaming which means high stable emulsions.

As reported by Nishinari et al. (2018) some protein subunits in SPI have high denaturation temperature. So, heat treatment of protein solution at high temperatures produces more stable emulsion system. Increasing heating temperature influences emulsion stability and so decreasing creaming.

4.2.3 Microstructure of Heat-treated Emulsions

Images of the microstructure of emulsions that heat-treated at 60, 70 and 80°C temperature were shown in Figure 4.9 and droplet size was shown in Table 4.2.

Increasing SPI protein concentration from 1 to 3 % has an obvious influence on droplet size. In a comparison of soy protein concentrations, 3 % SPI containing emulsion had smaller droplets than 1 % SPI containing emulsion. As increasing protein content improves more friction and bonding in emulsion systems. Heat treatment on protein solution also changed droplet size as shown in Table 4.2. For both SPI concentrations (1 and 3 %), increasing temperature from 60 to 80°C, caused a reduction in droplet size.

Table 4.2 Droplet size of SPI stabilized emulsions at different heating temperatures.

Temperature	1 % (w/v) SPI concentration	3 % (w/v) SPI concentration
60°C	9.84 μm	8.93 μm
70°C	6.7 μm	5.88 μm
80°C	4.54 μm	4.17 μm

Droplet sizes of 1% SPI containing emulsion were 9.84, 6.7 and 4.54 μm at 60, 70 and 80°C temperature, respectively. And droplet size of 3 % SPI containing emulsions was 8.93, 5.88 and 4.17 μm at 60, 70 and 80°C temperature, respectively. Heat treatment was an important factor that changes the droplet size of the emulsions. Nishinari et al. (2014) reported that a good correlation of emulsion stability with surface hydrophobicity of proteins using soy 7S globulin. The surface hydrophobicity of 7S globulin was found to increase with heat denaturation. An increase in hydrophobic groups in emulsion cause more interaction with oil and water, prevent coalescence of droplets and sustain emulsion stability.

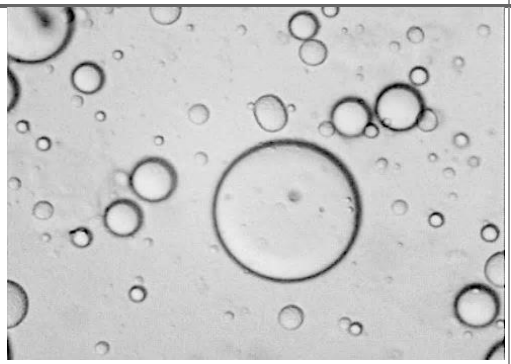
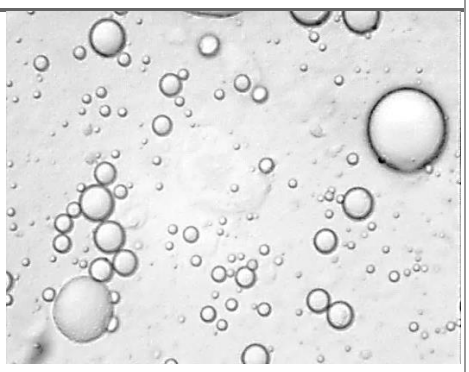
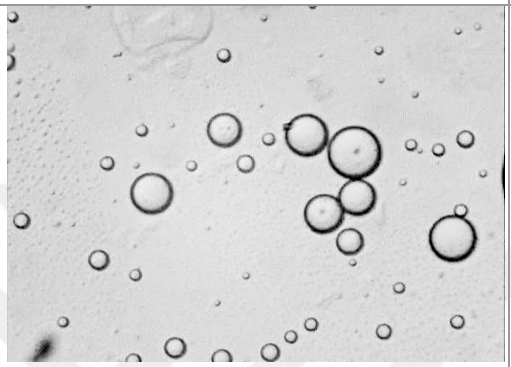
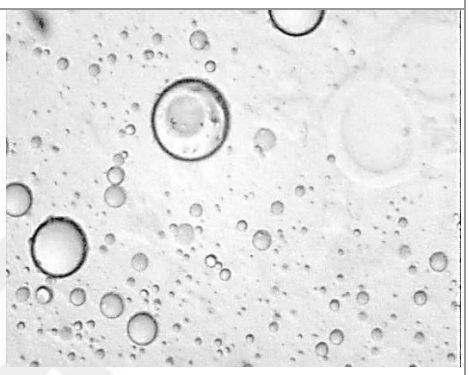
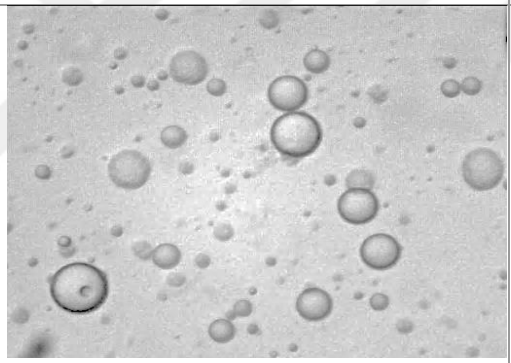
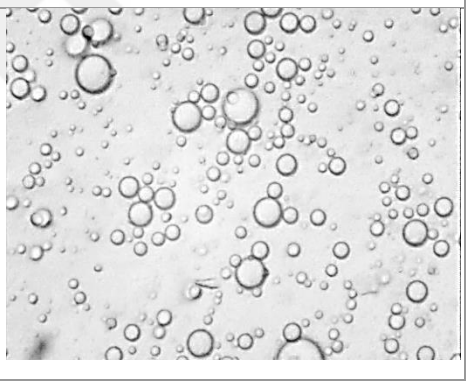
	1 % SPI Concentration	3 % SPI concentration
60°C		
70°C		
80°C		

Figure 4.9 Microstructure of SPI stabilized emulsions at different heating temperatures.

4.2.4 Viscosity of Heat-treated Emulsions

The viscosity of heat-treated emulsions was shown in Figure. 4.10. In the presence of 1 % (w/v) SPI, viscosities of emulsions at 60, 70 and 80°C were measured as 1.6, 1.5 and 1.4 cp, respectively.

In general, increasing in the temperature cause significantly ($p<0.05$) decrease in viscosity. In the presence of 3 % (w/v) SPI, the viscosities of emulsions were 3.0, 2.7 and 2.5 cp for 60, 70 and 80 ° C, respectively. Increasing protein concentration from

1 % to 3 % (w/v) caused more dense emulsions. This causes an increase in viscosity as a result. The lowest viscosity was observed at 80°C for both SPI concentrations (1 and 3 %). Utsumi et al. (1984) stated that the high heat treatment (80 °C) showed more interaction and binding due to rearrangement of the protein structure that affected the solubility of the soy protein isolate and led to better dissolution SPI that consist with increase in viscosity of emulsion.

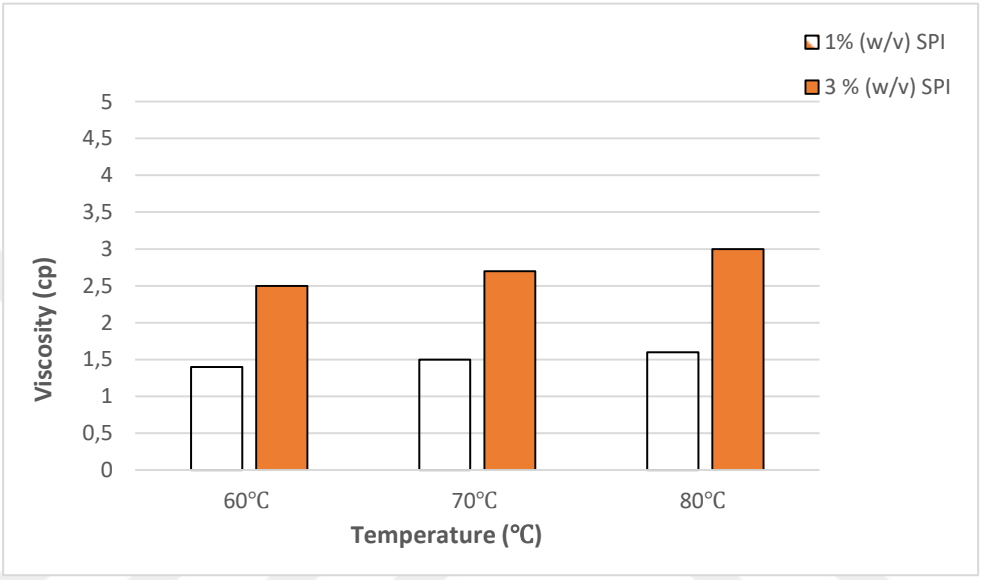


Figure 4.10 Viscosity of SPI stabilized emulsions at different heating temperatures.

4.3 Effect of pH on Emulsions

4.3.1 Emulsifying Activity and Stability Index

The EAI of pH arranged emulsions at different SPI concentrations (1 and 3 % (w/v)) were illustrated in Figure 4.11. The lowest value of EAI was determined at pH 5 and measured as 607.99 and 1229.80 m^2/g for both concentrations (1 and 3 % (w/v)), respectively.

Since pH 5 is closer to the isoelectric point of some subunit (pH 4.8), β-conglycinin might have been precipitated at this pH, as reported by Chen et al. (2014). When EAI of emulsions was compared, changing from pH 5 to 7 caused significant ($p<0.05$) increase for both SPI concentrations (1 and 3 %). EAI values increased from 607.99 to 1114.65 and from 1229.8 to 1741.1 m^2/g, for 1 and 3 % SPI containing emulsions, respectively. The highest EAI values measured at pH 9. EAI values in 1 % SPI

containing emulsions were 1192.95 m^2/g and in 3 % SPI containing emulsions was 1975.97 m^2/g.

At all pH values (pH 5, 7 and 9), 3 % SPI containing emulsions had significantly ($p<0.05$) higher EAI values. Nishinari et al. (2018) reported that protein stabilized emulsions are particularly sensitive to the pH and tend to flocculate at pH values near the isoelectric point of the adsorbed proteins.

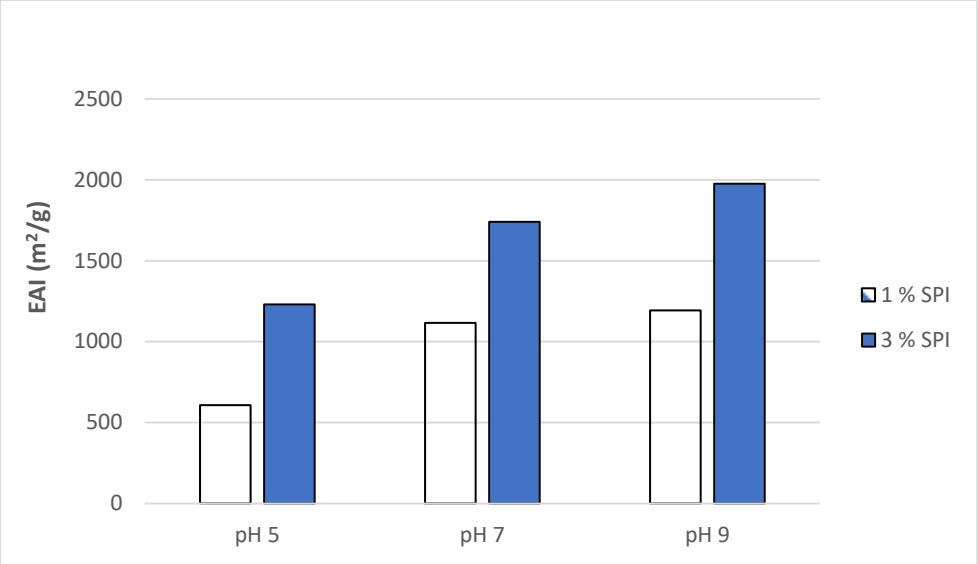


Figure 4.11 Effect of pH on EAI of SPI stabilized emulsions at different protein concentrations.

The effect of pH on emulsions stabilized with different SPI concentrations (1 and 3 % (w/v)) illustrated in Figure.4.12. ESI values were 53.96 and 146.93 min for 1 and 3 % SPI containing emulsions, respectively. The highest ESI value was measured at pH 9. It can be concluded that some subunit of glycinin has affected by basic condition and show more interactions that increase in bonding and increase stability.

At pH 7, ESI was higher than pH 5 and lower than pH 9 for both two concentrations. It was close to soy protein solution without any processing as its nature pH which was pH 7.24 according to our measurements. Figure 4.12 demonstrated most destabilization at pH 5 due to the denaturation of proteins near the isoelectric point of soy protein isolate. As a result, pH changing from pH 5 to 9 demonstrated significant ($p < 0.005$) changes at EAI and ESI of emulsions.

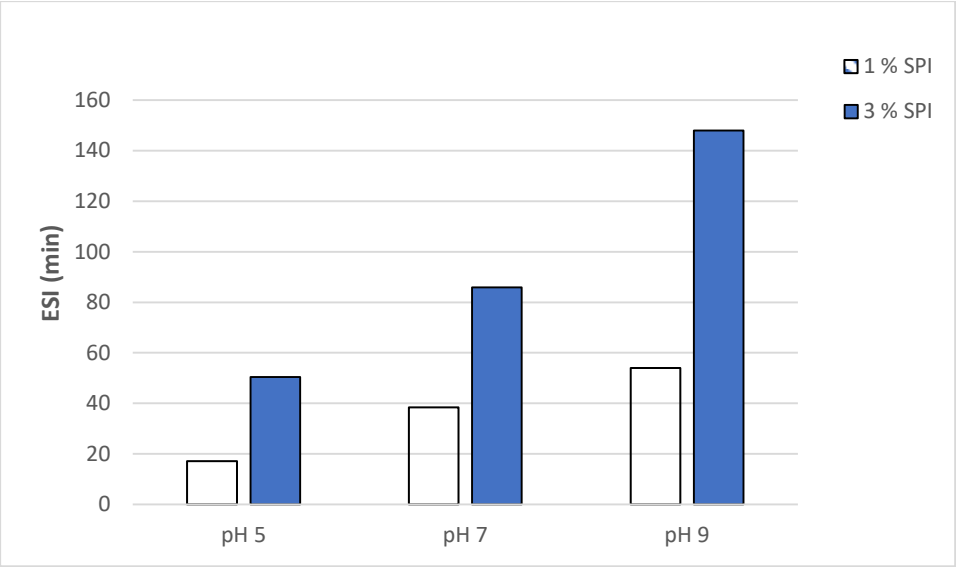


Figure 4.12 Effect of pH on ESI of SPI stabilized emulsions at different protein concentrations**.**

4.3.2 Creaming Stability of Emulsions

Figure 4.13 illustrates the creaming % of emulsions stabilized in two different SPI concentrations at different pH values. Creaming percentage of emulsions of pH 5 was highest for both protein concentration (1 and 3 % (w/v)).

Creaming of emulsions at pH 9 showed 6 and 10 % of creaming values for 1 and 3 % SPI concentration, respectively. When we compare the creaming of emulsions at pH 5, 7 and 9, the lowest creaming was observed at the emulsion of pH 9. Emulsions containing 1 and 3 % (w/v) SPI showed lowest creaming and high stability. Nishinari et al., (2018) reported that the composition of the basic part of glycinin has a bigger mass than the acidic part. Therefore, SPI emulsions for both concentration 1 and 3 % (w/v) were shown higher stability in basic conditions (pH 9).

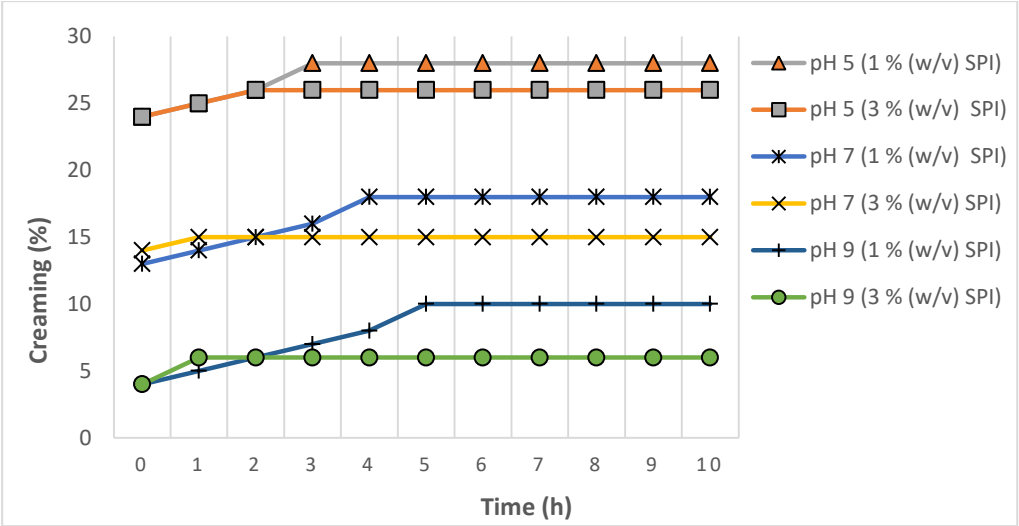


Figure 4.13 Effect of pH on creaming of SPI stabilized emulsions at different concentration protein concentrations.

4.3.3 Microscopic Structure of Emulsions

The droplets of emulsion images were taken under a 10X magnification lens. The data of particle size are illustrated to Table 4.3 and images were shown in Figure.4.14. In both SPI concentrations (1 and 3 % (w/v), the finer droplets and uniform distribution of particles were observed at pH 9 in comparison to pH 7 and 5. The SPI emulsions showed the best qualification in the basic environment due to the dominant basic part of SPI.

Table 4.3 Effect of pH on droplet size of SPI stabilized emulsions.

	1 % (w/v) SPI concentration	3 % (w/v) SPI concentration
pH 5	10.91 µm	9.78 µm
pH 7	9.99 µm	6.82 µm
pH 9	8.93 µm	6.17 µm

The isoelectric point of conglycinin is 4.8, therefore proteins were precipitated at pH 5 and this led to deformation of linkage between protein-oil droplets. Proteins dissociated at acidic conditions due to the breaking linkage between the protein-oil bond and lead to aggregation which is called de-emulsification (Gu, et al., 2009).

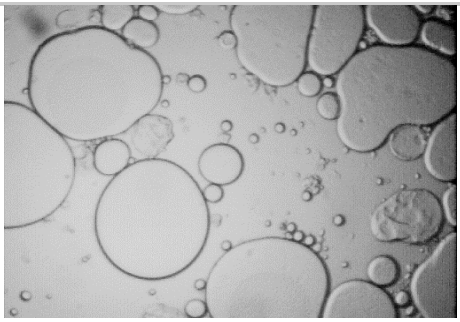
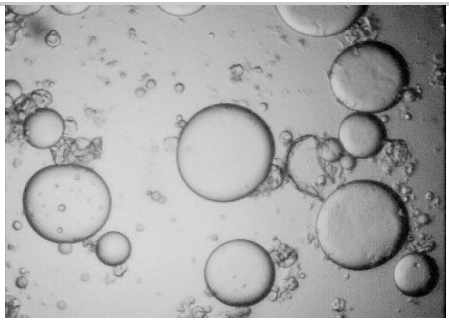
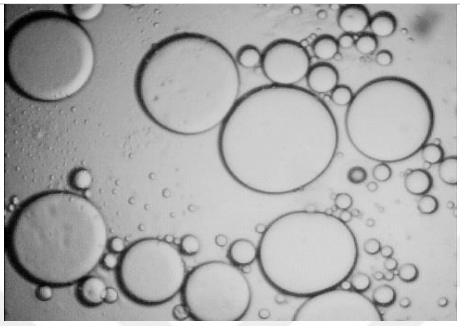
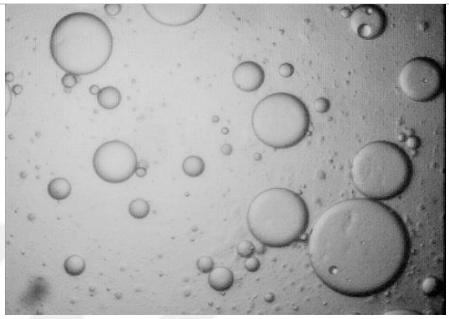
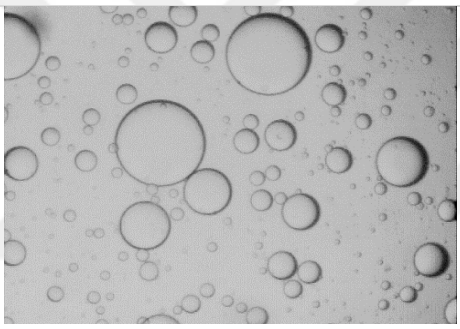
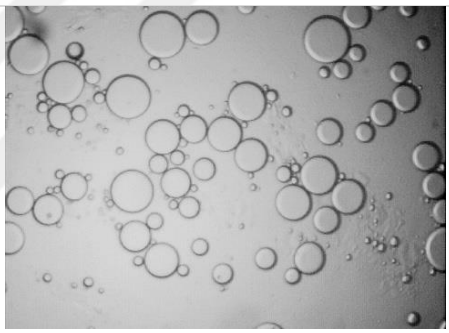
	1 % (w/v) SPI concentration	3 % SPI (w/v) concentration
pH 5		
pH 7		
pH 9		

Figure 4.14 Effect of pH on microscopic images of SPI stabilized emulsions at different protein concentrations.

4.3.4 Viscosity of Emulsions

The effect of pH on the viscosity of emulsions was shown in Figure 4.15. In our experiments, nature pH of emulsions was measured as 7.24. In the presence of 1 % (w/v) SPI, the viscosity of emulsions at pH 5, 7, 9 were found as 0.36, 0.51 and 0.57 cp, respectively. While in the presence of 3 % (w/v) SPI, it was measured as 0.76, 1.11 and 1.23 cp, respectively.

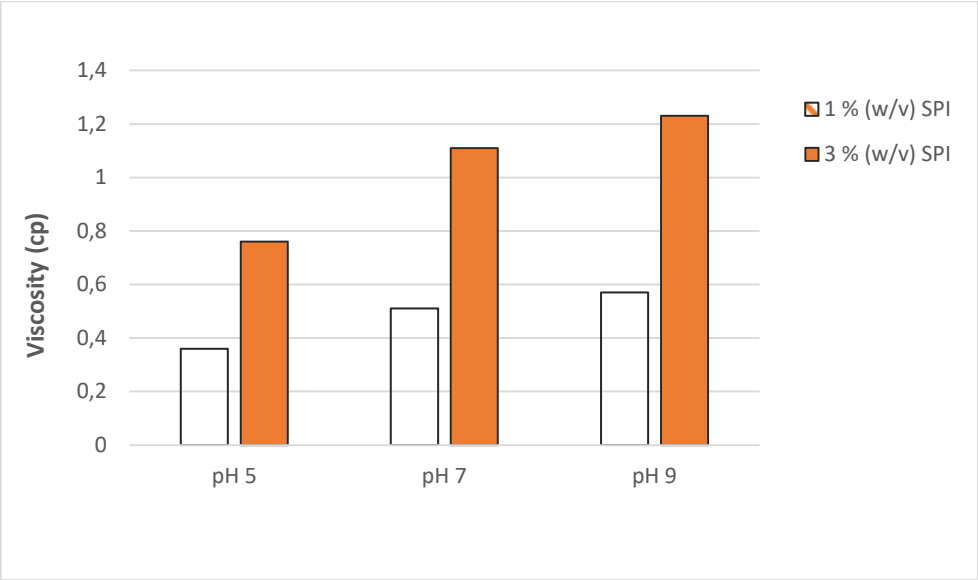


Figure 4.15 Effect of pH on the viscosity of SPI stabilized emulsions at different protein concentrations.

The lowest viscosities for both two SPI concentrations were measured at pH 5. The isoelectric point (pI) of conglycinin is pH 4.8, at pH was conglycinin might be precipitated and caused to the destabilization of emulsion that consists of low viscosity. The viscosity of emulsions at pH 7 showed intermediate values in comparison to pH 5 and 7. The highest viscosity obtained at emulsion of pH 9 since high stability was also obtained at emulsion of pH 9.

Gu et al. (2009) stated that if the pH of an emulsion drops to its iso-electric point, the magnitude of electrostatic repulsive forces between protein molecules is reduced and hydrophobic attractions lead proteins to aggregate. This was caused by the destabilization of emulsions which decreased the flow rate of emulsions and increase viscosity.

4.4 Properties of Gel-like Emulsions

4.4.1 Effects of Protein Concentration on the Gel-like Emulsions

The gel formation studies were tried with various soy protein isolate concentrations, 1, 3, 5, 6, 7, 8 and 10 % (w/v) at two different oil concentrations, 10 and 30 % (v/v). After 24 h, desired gel formation was observed at 6 and 7 % SPI concentration. It was concluded that protein concentrations have a significant effect on the gelation

formation of protein stabilized emulsions. The critical soy protein isolate concentration for heat-induced gel formation was determined as 6 and 7 %, which was supported by findings of Campbell et al. (2009). As a result, 6 and 7 % SPI stabilized gel-like emulsions were prepared. Then prepared gel-like emulsions were analyzed for water holding capacity, fat holding capacity, microstructure and droplet size.

Since gel-like emulsions are semi-solid structure, EAI and ESI of gel-like emulsions are hard to be measured. In structure of gel-like emulsion during gel formation fat entrap into the three dimensions structure, so creaming cannot be observed. Gel-like emulsions formed by higher amount of protein than emulsions. Proteins act as a stabilizer in an emulsion. An increase in protein concentration reduces the possibility of coalescence, bringing higher stability to gel-emulsions.

Nishinari et al. (2018) reported that the critical gel concentration for SPI was higher than 5 % (w/v) at pH 7. In our studies, the desired gel structure formation was observed at 6 and 7 % (w/v). The gel-like emulsions were prepared by two methods; cold-set and heat-set method.

Zang et al. (2016) were studied the effect of high-intensity ultrasound on transglutaminase-catalyzed soy protein isolate cold-set gel. And they found that after ultrasound pretreatment, gel formation and functional properties of gel such as water-holding capacity, protein solubility increased remarkably.

Zang et al. (2016) stated that the denatured soy protein tends to have negative charge at neutral pH. Protons induced by coagulants (such as $CaSO_4$ and glucono-d-lactone) that can neutralize the net charge of the soy protein and hydrophobic interactions of the neutralized protein becomes more predominant and then induce aggregation. Therefore, in our experiment, for making a cold-set emulsion, ultrasound treatment was applied to protein and $CaSO_4$ was used as a coagulant. For heat-set gel-like emulsions, heat treatment was applied (95°C, for 20 min) after adding $CaSO_4$.

The heating of a protein solution which induces unfolding of the protein globular structure, thus exposing reactive groups and, after cooling, the second step consists

essentially of adding calcium salts. Ca^{2+} ions neutralize electrostatic repulsion and form salt bridges between protein aggregates, allowing them to form a space-filling network (Maltais et al., 2009)

4.4.2 Water Holding Capacity (WHC) of Gel-like Emulsions

Figure 4.16 and Figure 4.17 illustrate the WHC data of gel-like emulsions at two SPI concentrations (6 and 7 % (w/v)), and oil concentrations of 10 and 30 % (v/v).
Increasing protein concentration from 6 to 7 % SPI showed the highest ($p<0.05$) WHC for both method and two oil concentration. Increasing protein concentration in emulsion caused more bonding water molecules. The WHC of the gel-like emulsions at 7 % SPI and 30 % oil concentration, significantly ($p<0.05$) increased from 55 to 75 % by heating effect, as shown in Figure 4.16. Increasing WHC of the gels is closely associated with the heating of SPI gel-like emulsions. Farooq and Boye (2011) reported that in their studies, when heat applied to pulse protein isolate, showed higher WHC due to proteins contribute to swelling under heat treatment

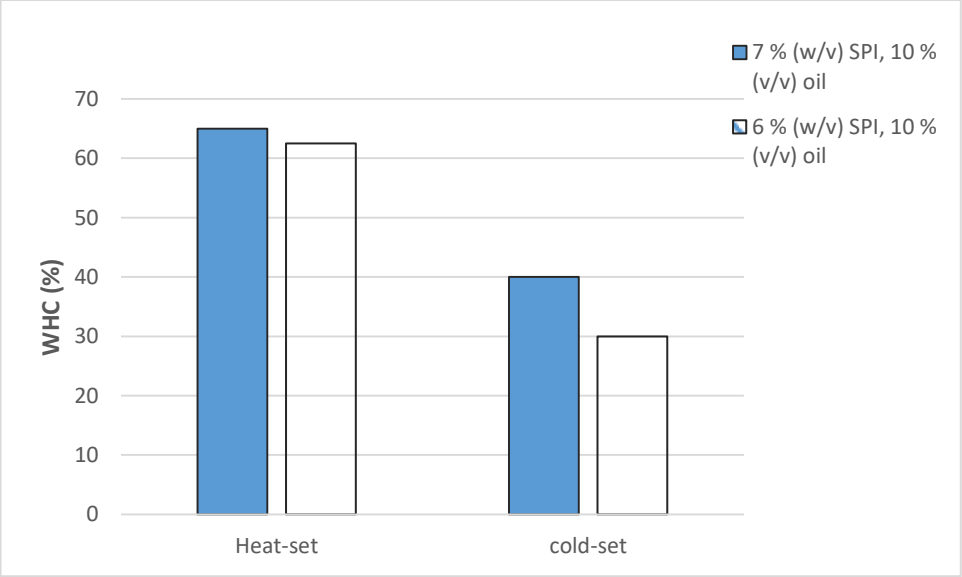


Figure 4.16 Water holding capacity of heat-set and cold-set gel-like emulsions at 10 % oil concentrations.

Campbell et al. (2009) also founded that surface dispersibility, hydrophobicity and WHC increased after heat treatment. They also reported that when soy protein heated shows complex behavior since they consist of oligomers. The lowest WHC of

emulsions among all samples was observed at low protein (6 %) and low oil (10) concentration.

When we increased oil concentration, WHC is also significantly increased ($p<0.05$). WHC of emulsions at 10 % oil concentration was 65. After oil concentration increased to 30 % of oil concentration, WHC increased to 75 %. In the comparison of heat-set and cold-set method, heat-set showed significantly ($p<0.05$) higher WHC, as seen in Figure 4.16 and Figure 4.17.

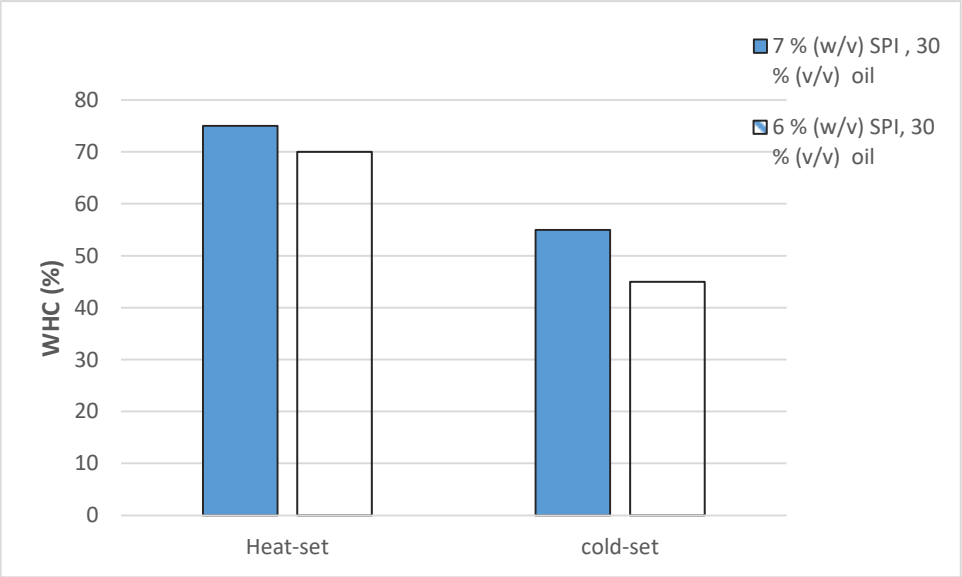


Figure 4.17 Water holding capacity of heat-set and cold-set gel-like emulsions at 30 % oil concentration.

4.4.3 Fat Holding Capacity (FHC) of Gel-like Emulsions

Figure 4.18 and Figure 4.19 illustrate the fat holding capacity (FHC) of gel-like emulsions that containing 6 and 7 % (w/v) SPI at two oil concentrations (10 and 30 % (v/v)).

FHC of SPI emulsions increased significantly ($p<0.05$) with increasing protein concentration from 6 to 7 % (w/v) at 10 % (v/v) oil concentration. WHC of 6 % SPI containing emulsions increased from 30 to 40 %. Increasing protein led to more bonding of fat into the emulsion.

In both methods, cold-set and heat-set FHC of emulsions increased after increasing oil concentration from 10 to 30 % (v/v). The quantity of oil trapped in the precipitated protein gel network might have been influenced by an increased in the oil concentrations. The results indicate that the fat adsorption capacity increased as the oil concentration increased for all the emulsion gels. At the same oil concentration, the high protein concentration emulsion gels bound more fat than low protein concentration.

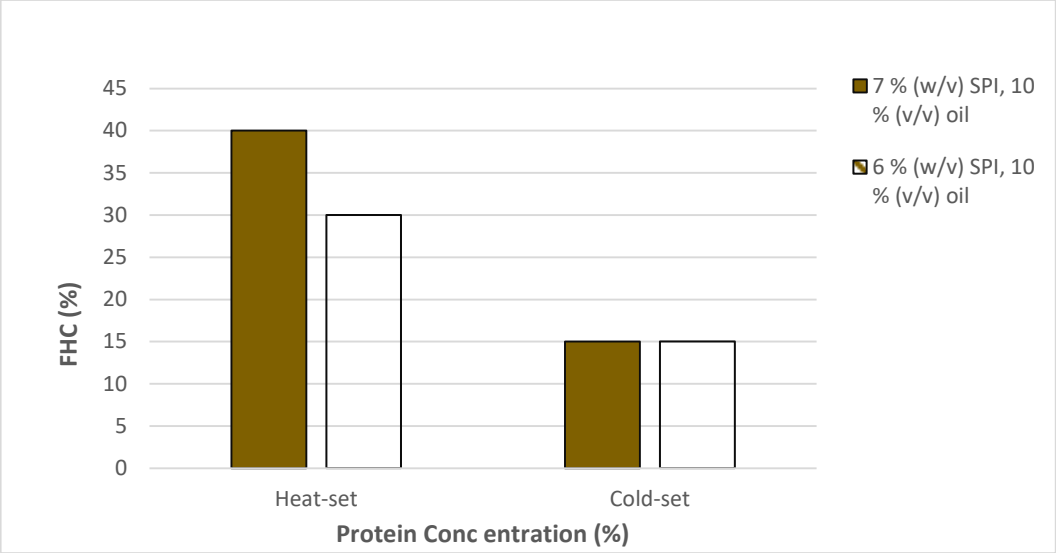


Figure 4.18 Fat holding capacity of SPI gel-like emulsions at 10 % oil concentration.

FHC in cold-set gel-like emulsions was 15 %, at heated-set gel-like emulsions was 30 %. This can be interpreted that the FHC of the heat-set emulsion was in double ratio of cold-set emulsion. The lowest FHC (15 %) was observed at cold-set method that emulsion containing 6 % (w/v) SPI at 10 % (v/v) oil concentration. in concentration.

The highest FHC (75 %) was observed in the heat-set gel-like emulsion that containing 7 % SPI and 30 % oil concentration. This showed that the heat treatment at 95°C caused a remarkable effect on the FHC of gel-like emulsions. After heating the emulsion gels at 95°C for 20 min, more fat was trapped in the gel matrix in comparison to the cold-set emulsification method as was shown in Figure 4.18. Fat adsorption capacity is the binding of fat by non-polar amino acid present in the side chains of proteins. The higher fat adsorption capacity of the emulsion gels obtained

after heat treatment could be due to protein rearrangement and altered protein-to-protein and protein-to-fat interactions (Gu et al., 2009).

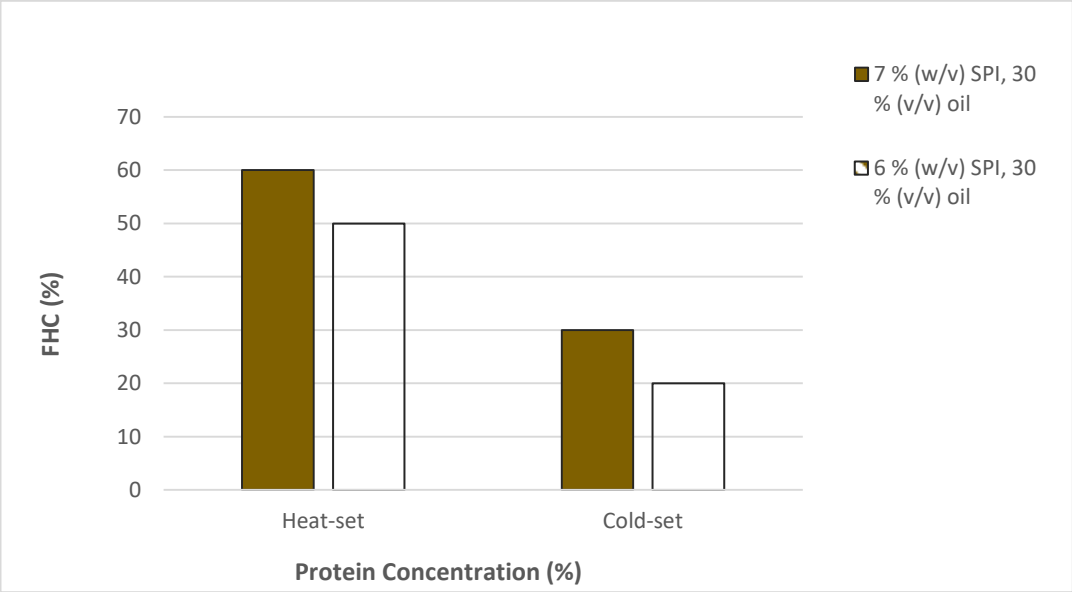


Figure 4.19 Fat holding capacity of SPI gel-like emulsions at 30 % oil concentration.

According to Utsumi et al. (1984) studies, glycinin hexamer dissociates to their constituents under heat treatment, which led to an increase in the gel network. Heating of soy isolate at 80°C showed the dissociation of both 7S and 11S globulin fractions. The dissociated subunits of 7S globulin subsequently interact electrostatically with the basic subunits of 11S globulin and led to form soluble complexes.

4.4.4 Microstructure of Gel-like Emulsions

Gel-like emulsions were prepared in two SPI concentrations 6 and 7 % (w/v), and two oil concentrations, 10 and 30 % (v/v). The gel-like emulsions were prepared by two methods heat-set and cold-set. The heat-set gel-like emulsions were formed by heat-treatment at 95°C for 20 min. The cold-set gel-like emulsions were formed without heat treatment. All gel-like emulsions were allowed to gel-forming in the fridge immediately after forming.

Increasing protein concentration for cold-set gel-like emulsions from 6 to 7 at 10 % oil concentration caused a reduction from 7.97 to 6.83 μm in droplet size. Increasing oil concentration from 10 to 30 % in 7 % SPI containing emulsion caused a reduction

from 6.83 to 5.98 μm in droplet size, as seen in Table 4.4. Heat-set emulsion showed a similar trend as the protein concentration in emulsion increased from 6 to 7 % decreased droplet size, as seen in Table 4.4.

Table 4.4 Droplet size of gel-like emulsions at the different protein and oil concentration.

Method	Oil conc. (v/v)	6 % (w/v) SPI conc.	7 % (w/v) SPI conc.
cold-set	10%	7.97 μm	6.83 μm
cold-set	30%	7.11 μm	5.98 μm
heat-set	10%	6.15 μm	5.64 μm
heat-set	30%	6.92 μm	4.67 μm

In general, when we compared droplet size of heat-set gel-like emulsions showed smaller droplets than the cold-set gel-like emulsions, as can be seen Table 4.4, Figure 4.20 and Figure 4.21. According to Nishinariet al. (2018) heat treatment of emulsion cause denaturation which was a prerequisite for gelling of soy proteins.

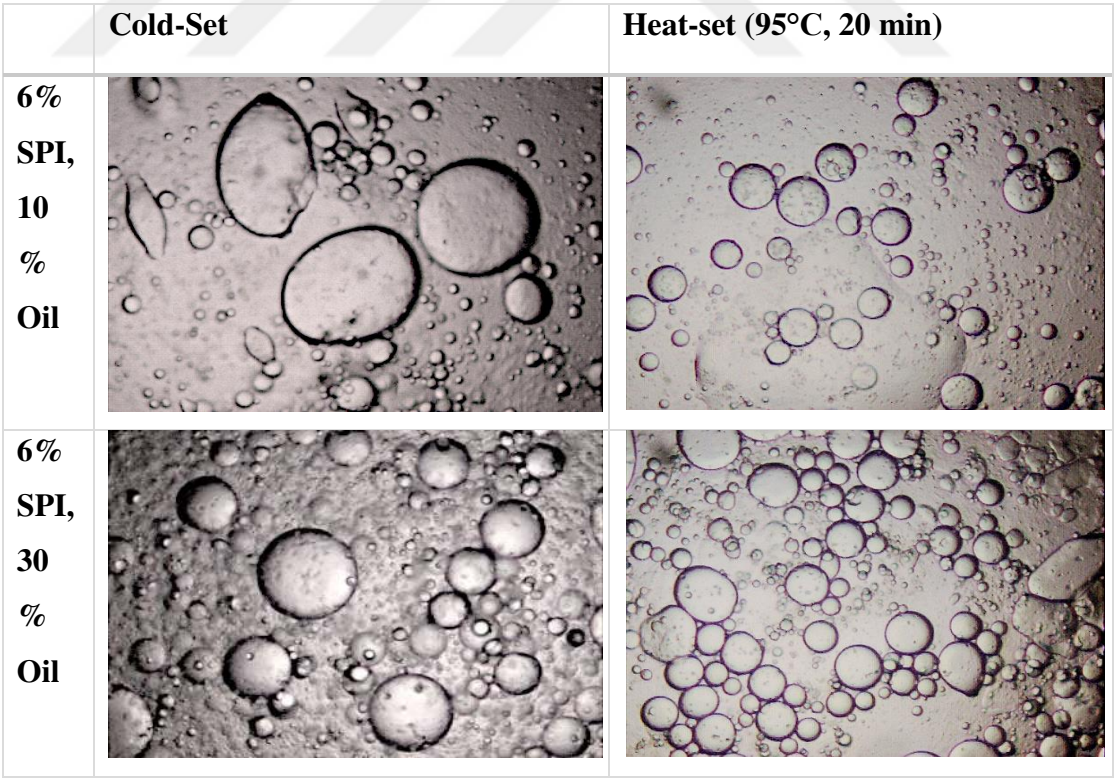


Figure 4.20 Microstructure (10X magnification) of cold-set and heat-set gel-like emulsions at 6 % protein concentration.

In cold set and heat-treated gel-like emulsion, the increasing of oil concentration from 10 to 30 % (v/v) decreased the droplet size in emulsions. This can be interpreted as that the heating gel led to more oil trapped into the gel network, so increased density and cause more interaction (Gu et al., 2009). The smallest droplets observed at heated-set emulsions images containing 7 % SPI and 30 % oil concentration as were shown in Figure 4.21.

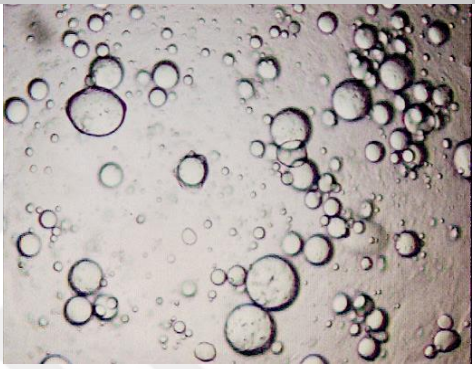
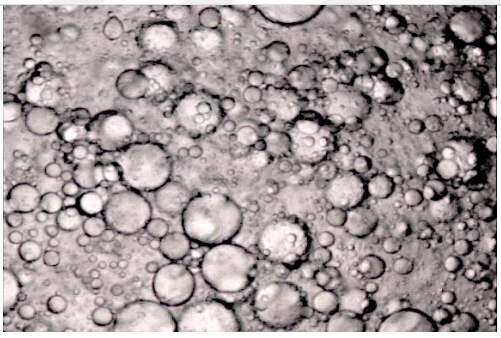
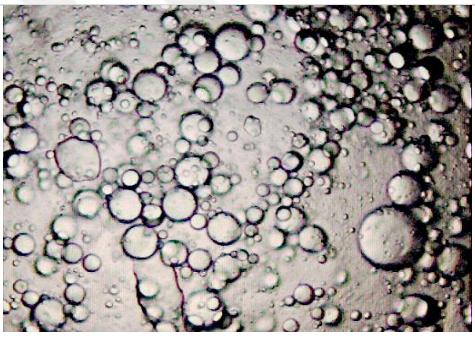
	Cold-Set	**Heat-set (95°C, 20 min)**
7% SPI, 10 % Oil		
7% SPI, 30 % Oil		

Figure 4.21 Microstructure (10X magnification) of cold-set and heat-set gel-like emulsions at 7 % protein concentration.

CHAPTER 5

CONCLUSION

Ultrasound treatment showed a significant ($p<0.005$) increase in the EAI and ESI of emulsions at 15 amplitudes. Also, ultrasound treatment at 15 amp increased in viscosity of emulsions. Creaming of emulsions decreased with increasing ultrasound amplitude from 5 to 15 amplitude. 3 % SPI containing emulsions that sonicated at 15 amplitude showed more stable emulsions in overall data. The microstructure of emulsions that sonicated at 15 amp showed a denser and more uniform structure. Ultrasound led to dissociation and partial unfolding of SPI containing emulsions. Therefore, ultrasound treatment can be used for improving the emulsification properties of emulsions.

In our study, the effect of pH changes was investigated. The results showed that emulsions at pH 9 exhibited more stable emulsions compared to pH 5 and pH 7. And also, high protein concentration increased the emulsification properties of emulsions. The effect of temperature on the emulsion showed that increasing temperature from 60°C to 80°C caused a significant ($p<0.05$) increase in the EAI and ESI values. Increasing temperature caused an increase in viscosity and a decrease in the creaming of emulsions. Heat treatment at 80°C showed the most stable emulsions. Therefore, increasing temperature from 60°C to 80°C showed better emulsification properties in emulsions.

SPI gel-like emulsions were formed in two methods; cold-set and heat-set (95°C) methods in two protein concentrations (6 and 7 % (w/v)). Heat treatment at 95°C caused a significantly ($p<0.05$) increase in the WHC and FHC of SPI stabilized gel-like emulsions. High temperature could cause a rearrangement and more interactions between protein-protein and protein-oil bonding. Heat treatment caused a reduction

in particle size that leading to the formation of a uniform and dense gel network. In the cold-set method, more uniform and denser microstructure observed at 7 % SPI containing stabilized gel-like emulsions.

Increasing protein concentration caused an increase in EAI and ESI of emulsions. Also, WHC and FHC of gel-like emulsions were increased with increasing protein concentration. Generally, the stability of emulsions and gel-like emulsion increased with increasing protein concentration. Heat treatment on emulsions improved physicochemical properties such as EAI, ESI, and viscosity, also in gel-like emulsions increased functional properties such as WHC and FHC.

REFERENCES

Abdel-Raouf, M. E.-S. (2012). Factors Affecting the Stability of Crude Oil Emulsions. *Crude oil Emails. Stab. Charact.*IntechOpen Publishing: Rijek.

Akbari, S., and Nour, A. H. (2018). Emulsion Types, Stability Mechanisms, and Rheology: A Review. *International Journal of Innovative Research and Scientific Studies* **(1)**, 14-21.

Al-Bakkush, A.-A. A. (2008). *Improvement of Functional Properties of Soy Protein.* Edinburgh: Heriot-Watt University School of life Sciences.

Arrese, E. L., Sorgentini, D. A., Wagner, J. R., Anon, M. C. (1991). Electrophoretic, Solubility, and Functional Properties of Commercial Soy Protein Isolates. *Journal of Agricultural and Food Chemistry*, **39 (6)** 1029-1032.

Aryee, A., Agyei, D., Udenigwe, C. C. (2018). *Proteins in Food Processing (Second Edition)*. Woodhead Publishing: Cambridge.

Campbell, l. J., Gu, X., Dewar, S. J., Euston, S. R. (2009). Effect of Heat Treatment and Glucono-δ-lactone-induced Acidification on the Characteristic of Soy Protein Isolate. *Food Hydrocolloids* **(23)**, 344-351.

Chemat, F., Huma, Z. E., Khan, M. K. (2011). Application of Ultrasound in Food Technology: Processing, Preservation, and Extraction. *Ultrasonics Sonochemistry*, **18 (4)**, 813-835.

Chen, W., Li, X., Al-Hajj, N. M., Chandra Dey, K., Raqib, S. M., M. R. (2014). Review: Emulsification Properties of Soybean Protein. *Nusantara Bioscience*, **(6)**, 196-202.

Chiralt, A. (2019). *Food Emulsions.* Retrieved from UNESCO-EOLSS: https://www.eolss.net/Sample-Chapters/C10/E5-10-03-05.pdf, 17.07.2019.

Chung, C., McClements, D. J. (2014). Structure-function Relationship in Food Emulsions: Improving Food Quality and Sensory Perception. *Food Structure* **(1)**, 106-126.

Damodaran, S. (1997). Food Proteins: An Overview. In S. Damodaran, A. Paraf, *Food Proteins, and Their Applications*, Marcel Dekker Publishing: New York.

Damodaran, S., Paraf, A. (1997). *Food Proteins and Their Application.* Marcel Dekker Publishing: New York.

Dickinson, E. (1998). *Advances in food emulsions and foams.*Elsevier Science Publishers: London.

Dickinson, E. (1998). Structure, Stability, and Rheology of Flocculated Emulsions. *Current Opinion in Colloid & Interface Science*, **3 (6)**, 633-638.

Dolatowski, Z. J., Stadnik, J., and Stasiak, D. (2007, 09 01). Application of Ultrasound in Food Technology. *Acta Scientiarum Polonorum: Technologia Alimentaria, (***6),** 89-99.

Erçelebi, E. A., Kara, Ş., İbanoğlu, E. (2011). Stability of Bitter Orange Juice-Olive Oil Salad Dressings with Polysaccharides. *Journal of Food Science and Engineering,* **(1)**, 297-302.

FAO. Food and Agriculture Organization of the United Nations. 2017. Chapter 1 The Soybean. Retrieved from: http://www.fao.org/3/t0532e/t0532e02.htm, 20.07.2019.

FAO. Food and Agriculture Organization of the United Nations. 2017. Chapter 6 Isolated Soybean Protein.http://www.fao.org/3/t0532e/t0532e07.htm, 20.07.2019.

FAO. Food and Agriculture Organization of the United Nations.2017. World Vegetable Oil Consumption.FAOSTAT: http://www.fao.org/faostat/en/#data/QC, 20.07.2019.

FAO. (2017). Production of Soybean in the World.*FAOSTAT*.: http://www.fao.org/faostat/en/#data/QC. 20.07.2019.

Farooq, Z., Boye, J. I. (2011). Novel food and industrial application of pulse flours and fractions. In B. K. Tiwari, B. McKenna, A. Gowen, *Pulse Food; Processing, Quality, and Nutraceutical*. Academic Press: London.

Foegeding, E. A., Davis, J. P. (2011). Food rotein functionality: A comprehensive approach. *Food Hydrocolloids* **(25)**, 1853-1864.

Fukushima, D. (2004). Soy Proteins. In R. Y. Yada, *Protein in food processing*. Woodhead Publishing Limited: Cambridge.

Gromiha, M. M. (2010). Chapter 1: Proteins. In M. M. Gromiha, *Protein Bioinformatics*. Academic Press: New Delhi.

Gu, X., Campbell, L. J., Euston, S. R. (2009). Effect of Different Oils on the Properties of Soy Protein Isolate Emulsions and Gel. *Food Research International* **(42)**, 925-932.

Guo, J., Yang, X.-Q., He, X.-T., Wu, N.-N., Wang, J.-M., Gu, W., Zhang, Y.-Y. (2012). Limited Aggregation Behavior of β-Conglycinin and Its Terminating Effect on Glycinin Aggregation during Heating at pH 7.0. *Journal of Agricultural and Food Chemistry*.(**60)**, 3782-3791.

Haug, I. J., Draget, K. I. (2011). Gelation. In G. O. Phillips, P. A. Williams, *Handbook of Food Protein*. Woodhead Publishing Limited: Cambridge.

Hu, H., Li-Chan, E. C., Wan, L., Tian, M., Pan, S. (2013). The Effect of High-intensity Ultrasonic Pretreatment on the Properties of Soybean Protein Isolate Gel Induced by Calcium Sulfate. *Food Hydrocolloids*. (**32)**, 303-311.

Hu, H., Zhu, X., Hu, T., Cheung, I. W., Pan, S., Li-Chan, E. C. (2015). Effect of Ultrasound Pretreatment on the Formation of Transglutaminase-catalyzed Soy Protein Hydrogel as a Riboflavin Vehicle for Functional Foods. *Journal of Functional Foods.* **(19)**, 182-193.

IJAIR. (2015). Manufacturing Soy-Protein Concentrates and Isolates by Membrane Technology. *International Journal of Agriculture Innovations and Research*.

Jambrak, A.R., Lelas, V., Mason, T.J., Kresic, G. Badanjak, M.(2009). Physical Properties of Ultrasound Treated Soy Proteins. *Journal of Food Engineering.* **(93)**, 386-393.

Jambrak, A. R. (2017). Physical Properties of Sonicated Products: A New Era for Novel Ingredients. In D. Bermudez-Aguirre, *Ultrasound Advances in Food Processing and Preservation*. Academic Press, London.

Kadam, S. U., Tiwari, B. K., Alvarez, C., O'Donnell, C. P. (2015). Ultrasound applications for the extraction, identification, and delivery of food proteins and bioactive peptides. *Trends in Food Science & Technology*. **46 (1)**, 60-67.

Kentish, S. E. (2017). Engineering Principle of Ultrasound Technology. In D. Bermudez-Aguirre, *Ultrasound: Advances in Food Processing and Preservation.* Academic Press: London.

Kinsella, J. E. (1979). Functional Properties of Soy Proteins. *Journal of the American Oil Chemists' Society.* (**56)**, 242-258.

Li, F., Kong, X., Zhang, C., Hua, Y. (2012). Gelation behavior and rheological properties of acid-induced soy protein-stabilized emulsion gels, *Food Hydrocolloids.* **(29)**, 347-355.

Li-Chan, E. C. (2004). Properties of protein in food systems: an introduction. In R. Y. Yada, *Proteins in food processing.* Woodhead Publishing Limited & CRC Press LLC: Cambridge.

Li-Chan, E. C. Y. Lacroix, I. M. E., (2018). Properties of proteins in food systems: An introduction. *Proteins in Food Processing.* Woodhead Publishing: Cambridge.

Liu, F. Tang, C. (2014). Emulsifying Properties of Soy Protein Nanoparticles: Influence of Protein Concentration and Emulsification Process. *Journal of Agriculture and Food Chemistry.* **(62)**, 2644-2654.

Liu, F. and Tang, C. (2016). Soy Glycinin as Food-grade Pickering stabilizers: Part. III. Fabrication of gel-like emulsions and their potential as sustained-release delivery systems for b-carotene. *Food Hydrocolloids*, **(56)**, 434-444.

Majid, I., Nayik, G. A., Nanda, V. (2015). Ultrasonication and Food Technology: A Review. *Cogent Food & Agriculture*, **1(1)**, 1-11.

Maltais, A., Remondetto, G.E., Subirade, M., (2009). Soy Protein Cold-Set Hydrogels As Controlled Delivery Devices For Nutraceutical Compounds. *Food Hydrocolloids*. (**23)**, 1647-1653.

Mason, T. J., Riera, E., Vercet, A., Lopez-Buesa, P. (2005). Application of Ultrasound. In D.-W. Sun, *Emerging Technologies for Food Processing*. Elsevier Academic Press: London.

Mason, T., Paniwyk, L., Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry,***(3)**, 253-260.

McClements, D. J. (1995). Advances in The Application of Ultrasound in Food Analysis and Processing. *Trends in Food Science &Technology*. **6(9)**, 293-299.

McClements, D. J. (2015). *Food Emulsions: Principles, Practices and Techniques.*CRC Press:London.

Membrane Consultants. 2019. Solutions For Separation of Soybean. http://www.membraneconsultants.com/soy-proteins.html, 17.07.2019.

Milani, J. M., Golkar, A. 2019. *Introductory Chapter: Some New Aspects of Colloidal System in Food.*IntechOpen: https://www.intechopen.com/books/some-new-aspects-of-colloidal-systems-in-foods. 25.07.2019.

Nedovic, V., Klausevic, A., Manojlovic, V., Levic, V., Bugarski, B. (2011). An Overview of Encapsulation Technologies for Food Applications. *Procedia Food Science.* **(1)**, 1806-1815.

Nishinari, Fang. (2014). Soy Proteins: A Review on Composition, Aggregation, and Emulsification. *Food Hydrocolloids*. **(39)**, 301-318.

Nishinari, K., Fang, Y., Nagano, T., Guo, S., Wang, R. (2018). Soy as a Food Ingredient. In R. Y. Yada, *Proteins in Food Processing (Second Edition)*. Woodhead Publishing: Cambridge.

O'Sullivan, J., Park, M., Beevers, J. (2016). The Effect Of Ultrasound Upon The Physicochemical and Emulsifying Properties of Wheat and Soy Protein Isolates. *Journal of Cereal Science*. **(69)**, 77-84.

Pearce, K. N., Kinsella, J. E. (1978). Emulsifying Properties of Proteins: Evaluation Of A Turbidimetric Technique. *J. Agric. Food Chem.* **26 (3)**, 716-723.

Pal, R. (2011). Rheology of Simple And Multiple Emulsions. *Current Opinion in Colloid & Interface Science*,**16 (1)**, 41-60.

Phillips, G. O., William, P. A. (2011). Introduction to Food Proteins. In G. O. Phillips, & P. A. William, *Handbook of Food Proteins*. Woodhead Publishing: Oxford.

Ren, C., Tang, L., Zang, L., Guo, S. (2009). Structural Characterization of Heat-Induced Protein Particles in Soy Milk. *Journal of Agricultural and Food Chemistry,* **(57)**, 1921-1926.

Renkema, J. M. (2001). Formation, Structure and Rheological Properties of Soy Protein Gels. Wageningen: Wageningen Universiteit. Retrieved from http://edepot.wur.nl/121265, 25.07.2019.

Robinson, M. M., Wilde, P. J. (2003). Colloids and Emulsions. In B. Caballero, *Encyclopedia of Food Science and Nutrition (Second Edition)*. Academic Press: Cambridge.

Silva, E. K., Zabot, G. L., Toledo Hijo, A. A., Meireles, M. A. (2017). Encapsulation of Bioactive Compounds Using Ultrasonic Technology. In D. Bermudez-Aguirre, *Ultrasound Advances in Food Processing and Preservation*. Academic Press: London.

Silva, F. V., Sulaiman, A. (2017). Advances in Thermosonication for Inactivation of Endogenous Enzymes in Foods. In D. Bermudez-Aguirre, *Ultrasound Advances in Food Processing and Preservation*. Academic Press: London.

Soria, A. C., Villamiel, M. (2010). Effect of Ultrasound on the Technological Properties and Bioactivity of Food: A Review. *Trends in Food Science & Technology* **(21)**, 323-331.

Tadros, T. F. (2013). *Emulsion Formation and Stability and Rheology, First Edition.* Wiley Publishing: Dresden.

Tao, Y., Sun, D.-W. (2015). Enhancement of Food Processes by Ultrasound: A Review. *Food Science and Nutrition*, **55 (4)**, 570-594.

Tornberg, E. (1978). Functional Characterization of protein stabilized emulsions: Emulsifying Behavior of Proteins in a Valve Homogenizer. *Science of Food and Agriculture,* **29 (10)**, 867-879.

Utsumi, S., Damodaran, S., Kinsella, J. E. (1984). Heat-Induced Interactions Between Soybean Proteins: Preferential Association of 11S Basic Subunits and Beta Subunits of 7S. *Journal of Food Agricultural and Food Chemistry*, **32 (6)**, 1406-1412.

Utsumi, S., Matsumura, Y., Mori, T. (1997). Structure-Function Relationships of Soy Proteins. In S. Damodaran, A. Paraf, *Food Proteins and Their Applications*. Marcel Dekker Publishing: New York.

Walstra, P., Vliet, T. v. (2003). Chapter 2 Functional Properties. *Progress in Biotechnology,*(**23**), 9-30.

Wang, X., Tang, C. H., Li, B. S., Yang, X. Q., Li, L., Ma, C. Y. (2008). Effect of High-Pressure Treatment on Some Physicochemical and Functional Properties of Soy Protein Isolate. *Food Hydrocolloids,*(**22**), 560-567.

WISHH. (2019). *World Initiative for Soy in Human Health*. Retrieved from Composition of Soybean: http://www.wishh.org, 27.07.2019.

Xiong, Y. L. (2004). Muscle proteins. In R. Y. Yada, *Protein in food processing*. Woodhead Publishing & CRC Press LLC: Cambridge.

Yada R. Y., (2000). *Proteins in Food Processing*. Cambridge: Woodhead Publishing and CRC Press LLC

Yang, M., Liu, F., Tang, C.-H. (2013). Properties And Microstructure of Transglutaminase-Set Soy Protein-Stabilized Emulsion Gels. *Food Research International*, **52 (1)**, 409-418.

Zang, P., Hu, T., Feng, S., Xu, Q., Zheng, T., Zhou, M., Hu, H. (2016). Effect of High-Intensity Ultrasound on Transglutaminase-Catalyzed Soy Protein Isolates Cold Set Gel. *Ultrasonics Sonochemistry,* **(29)**, 380-387.

APPENDICES

Table A.1 Anova of results of emulsifying activity and stability index of ultrasound treated emulsions containing 1 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI1	Between Groups	204223.378	2	102111.689	2205.31	0.000
	Within Groups	277.816	6	4.303		
	Total	204501.194	8			
ESI1	Between Groups	524.940	2	262.470	81.792	0.000
	Within Groups	19.254	6	3.209		
	Total	544.194	8			
Viscosity1	Between Groups	4.287	2	2.143	3507.50	0.000
	Within Groups	0.004	6	0.001		
	Total	4.291	8			

Table A.2 Multiple range test of emulsifying activity and stability index of ultrasound treated emulsions containing 1 % SPI.

Tukey HSD							
Dependent Variable	(I)Amp 1	(J)Amp 1	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bound	Upper Bound
EAI1	5	10	-203.95467*	5.555	0.000	-221.001	-186.9075
		15	-368.27333*	5.555	0.000	-385.320	-351.2262
	10	5	203.95467*	5.555	0.000	186.907	221.0018
		15	-164.31867*	5.555	0.000	-181.365	-147.2715
	15	5	368.27333*	5.555	0.000	351.226	385.3205
		10	164.31867*	5.555	0.000	147.271	181.3658
ESI1	5	10	-13.92616*	1.462	0.000	-18.414	-9.4384
		15	-17.78050*	1.462	0.000	-22.268	-13.2927
	10	5	13.92616*	1.462	0.000	9.4384	18.4140
		15	-3.85435	1.462	0.086	-8.3421	0.6335
	15	5	17.78050*	1.462	0.000	13.292	22.2683
		10	3.85435	1.462	0.086	-0.6335	8.3421
Viscosity1	5	10	1.00333*	0.020	0.000	0.9414	1.0653
		15	1.68000*	0.020	0.000	1.6181	1.7419
	10	5	-1.00333*	0.020	0.000	-1.0653	-0.9414
		15	0.67667*	0,020	0.000	0.6147	0.7386
	15	5	-1.68000*	0.020	0.000	-1.7419	-1.6181
		10	0.67667*	0.020	0.000	0.7386	-0.6147
*. The mean difference is significant at the 0.05 level.							

Table A.3 Anova of results of emulsifying activity and stability index of ultrasound treated emulsions containing 3 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI3	Between Groups	627570.500	2	313785.250	8344.28	0.000
	Within Groups	225.629	6	37.605		
	Total	627796.129	8			
ESI3	Between Groups	1648.534	2	824.267	160.078	0.000
	Within Groups	30.895	6	5.149		
	Total	1679.429	8			
Viscosity1	Between Groups	4.287	2	2.143	3507.50	0.000
	Within Groups	0.004	6	0.001		
	Total	4.291	8			

Table A.4 Multiple range test of emulsifying activity and stability index of ultrasound treated emulsions containing 3 % SPI.

Tukey HSD							
Dependent Variable	(I) Amp 3	(J)Amp 3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EAI3	5	10	-364.93133*	5.006	0.000	-380.294	-349.5685
		15	-644.96333*	5.006	0.000	-660.326	-629.6005
	10	5	364.93133*	5.006	0.000	349.5685	380.2941
		15	-280.03200*	5.006	0.000	-295.394	-264.6692
	15	5	644.96333*	5.006	0.000	629.6005	660.3261
		10	280.03200*	5.006	0.000	264.6692	295.3948
ESI3	5	10	-23.25598*	1.852	0.000	-28.9408	-17.5712
		15	-32.08861*	1.852	0.000	-37.7734	-26.4038
	10	5	23.25598*	1.852	0.000	17.5712	28.9408
		15	-8.83263*	1.852	0.007	-14.5174	-3.1478
	15	5	32.08861*	1.852	0.000	26.4038	37.7734
		10	8.83263*	1.852	0.007	3.1478	14.5174
Viscosity1	5	10	1.00333*	0.020	0.000	0.9414	1.0653
		15	1.68000*	0.020	0.000	1.6181	1.7419
	10	5	-1.00333*	0.020	0.000	-1.0653	-0.9414
		15	0.67667*	0.020	0.000	0.6147	0.7386
	15	5	-1.68000*	0.020	0.000	-1.7419	-1.6181
		10	-0.67667*	0.020	0.000	-0.7386	-0.6147
*. The mean difference is significant at the 0.05 level.							

Table A.5 Anova of results of emulsifying activity and stability index of heat-treated emulsions containing 1 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI1	Between Groups	152382.550	2	76191.275	15243.853	0.000
	Within Groups	29.989	6	4.998		
	Total	152412.539	8			
ESI1	Between Groups	1538.139	2	769.069	534.142	0.000
	Within Groups	8.639	6	1.440		
	Total	1546.778	8			
Viscosity1	Between Groups	0.066	2	0.033	7.000	0.027
	Within Groups	0.028	6	0.005		
	Total	0.095	8			

Table A.6 Multiple tests of emulsifying activity and stability index of heat-treated emulsions containing 1 % SPI.

Tukey HSD							
Dependent Variable	(I) Temp 1(°C)	(J)Temp 1 (°C)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EAI1	60	70	-178.36800*	1.825	0.000	-183.96	-172.767
		80	-317.94133*	1.825	0.000	-323.542	-312.340
	70	60	178.36800*	1.825	0.000	172.767	183.968
		80	-139.57333*	1.825	0.000	-145.174	-133.972
	80	60	317.94133*	1.825	0.000	312.340	323.542
		70	139.57333*	1.825	0.000	133.972	145.174
ESI1	60	70	-9.82333*	0.979	0.000	-12.8294	-6.8172
		80	-31.30667*	0.979	0.000	-34,3128	-28.3006
	70	60	9.82333*	0.979	0.000	6.8172	12.8294
		80	-21.48333*	0.979	0.000	-24.4894	-18.4772
	80	60	31.30667*	0.979	0.000	28.3006	34.3128
		70	21.48333*	0.979	0.000	18.4772	24.4894
Viscosity1	60	70	0.04333	0.056	0.733	-0.1292	0.2159
		80	0.20000*	0.056	0.028	0.0274	0.3726
	70	60	-0.04333	0.056	0.733	-0.2159	0.1292
		80	0.15667	0.056	0.071	-0.0159	0.3292
	80	60	-0.20000*	0.0562	0.028	-0.3726	-0.0274
		70	-0.15667	0.0562	0.071	-0.3292	0.0159
*. The mean difference is significant at the 0.05 level.							

Table A.7 Anova of results of emulsifying activity and stability index of heat-treated emulsions containing 3 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI3	Between Groups	15889.322	2	79445.661	299.283	0.000
	Within Groups	159.301	6	26.550		
	Total	159050.623	8			
ESI3	Between Groups	1284.261	2	642.131	242.115	0.000
	Within Groups	15.913	6	2.652		
	Total	1300.174	8			
Viscosity3	Between Groups	0.422	2	0.211	69,827	0.000
	Within Groups	0.018	6	0.003		
	Total	0.440	8			

Table A.8 Multiple tests of emulsifying activity and stability index of heat-treated emulsions containing 3 % SPI.

Tukey HSD							
Dependent Variable	(I) Temp 3(°C)	(J) Temp3 (°C)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EAI3	60	70	-245.24600*	4.20715	0.000	-258.1547	-232.3373
		80	-307,92400*	4.20715	0.000	-320.8327	-295.0153
	70	60	245,24600*	4.20715	0.000	232.3373	258.1547
		80	-62,67800*	4.20715	0.000	-75.5867	-49.7693
	80	60	307,92400*	4.20715	0.000	295.0153	320.8327
		70	62,67800*	4.20715	0.000	49.7693	75.5867
ESI3	60	70	-12,50400*	1.32970	0.000	-16.5839	-8.4241
		80	-29.16200*	1.32970	0.000	-33.2419	-25.0821
	70	60	12.50400*	1.32970	0.000	8.4241	16.5839
		80	-16.65800*	1.32970	0.000	-20.7379	-12.5781
	80	60	29.16200*	1.32970	0.000	25.0821	33.2419
		70	16.65800*	1.32970	0.000	12.5781	20.7379
Viscosity 3	60	70	0.33667*	0.04489	0.001	0.1989	0.4744
		80	0.52333*	0.04489	0.000	0.3856	0.6611
	70	60	-0.33667*	0.04489	0.001	-0.4744	-0.1989
		80	0.18667*	0.04489	0.014	0.0489	0.3244
	80	60	-0.52333*	0.04489	0.000	-0.6611	-0.3856
		70	-0.18667*	0.04489	0.014	-0.3244	-0.0489
*. The mean difference is significant at the 0.05 level.							

Table A.9 Anova of results of emulsifying activity and stability index of pH-treated emulsions containing 1 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI1	Between Groups	605005.752	2	302502.876	10630.012	0.000
	Within Groups	170.745	6	28.457		
	Total	605176.497	8			
ESI1	Between Groups	1744.353	2	872.177	283.388	0.000
	Within Groups	18.466	6	3.078		
	Total	1762.819	8			
Viscosity1	Between Groups	0.070	2	0.035	93.294	0.000
	Within Groups	0.002	6	0.000		
	Total	0.073	8			

Table A.10 Multiple tests of emulsifying activity and stability index of pH-treated emulsions containing 1 % SPI.

Tukey HSD							
Dependent Variable	(I) pH1	(J) pH 1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EAI1	5	7	-507.17000*	4.35564	0.000	-520.5343	-493.8057
		9	-584.62400*	4.35564	0.000	-597.9883	-571.2597
	7	5	507.17000*	4.35564	0.000	493.8057	520.5343
		9	-77.45400*	4.35564	0.000	-90.8183	-64.0897
	9	5	58.,62400*	4.35564	0.000	571.2597	597.9883
		7	77.45400*	4.35564	0.000	64.0897	90.8183
ESI1	5	7	-20.35661*	1.43240	0.000	-24.7516	-15.9616
		9	-33.87183*	1.43240	0.000	-38.2668	-29.4768
	7	5	20.35661*	1.43240	0.000	15.9616	24.7516
		9	-13.51521*	1.43240	0.000	-17.9102	-9.1202
	9	5	33.87183*	1.43240	0.000	29.4768	38.2668
		7	13.51521*	1.43240	0.000	9.1202	17.9102
Viscosity1	5	7	0.07333*	0.01587	0.009	0.0246	0.1220
		9	0.21333*	0.01587	0.000	0.1646	0.2620
	7	5	-0.07333*	0.01587	0.009	-0.1220	-0.0246
		9	0.14000*	0.01587	0.000	0.0913	0.1887
	9	5	-0.21333*	0.01587	0.000	-0.2620	-0.1646
		7	-0.14000*	0.01587	0.000	-0.1887	-0.0913
*. The mean difference is significant at the 0.05 level.							

Table A.11 Anova of results of emulsifying activity and stability index of pH-treated emulsions containing 3 % SPI.

		Sum of Squares	df	Mean Square	F	Sig.
EAI3	Between Groups	793681.068	2	396840.534	331.884	0.000
	Within Groups	7174.320	6	1195.720		
	Total	800855.388	8			
ESI3	Between Groups	14274.442	2	7137.221	2936.591	0.000
	Within Groups	14.583	6	2.430		
	Total	14289.025	8			
Viscosity 3	Between Groups	0.406	2	0.203	56.882	0.000
	Within Groups	0.021	6	0.004		
	Total	0.427	8			

Table A.12 Multiple tests of emulsifying activity and stability index of pH-treated emulsions containing 3 % SPI.

Tukey HSD							
Dependent Variable	(I) pH3	(J) pH3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EAI3	5	7	-479.72533*	28.233	0.000	-566.3543	-393.0963
		9	-713.40067*	28.233	0.000	-800.0297	-626.7717
	7	5	479.72533*	28.233	0.000	393.0963	566.3543
		9	-233.67533*	28.233	0.000	-320.3043	-147.0463
	9	5	713.40067*	28.233	0.000	626.7717	800.0297
		7	233.67533*	28.233	0.000	147.0463	320.3043
ESI3	5	7	-34.32058*	1.2729	0.000	-38.2262	-30.4149
		9	-96.24123*	1.2729	0.000	-100.1469	-92.3356
	7	5	34.32058*	1.2729	0.000	30.4149	38.2262
		9	-61.92065*	1.2729	0.000	-65.8263	-58.0150
	9	5	96.24123*	1.2729	0.000	92.3356	100.1469
		7	61.92065*	1.2729	0.000	58.0150	65.8263
Viscosity3	5	7	0.05000	0.0487	0.589	-0.0996	0.1996
		9	0.47333*	0.0487	0.000	0.3237	0.6229
	7	5	-0.05000	0.0487	0.589	-0.1996	0.0996
		9	0.42333*	0.0487	0.000	0.2737	0.5729
	9	5	-0.47333*	0.0487	0.000	-0.6229	-0.3237
		7	-0.42333*	0.0487	0.000	-0.5729	-0.2737
*. The mean difference is significant at the 0.05 level.							

Table A.13 Anova of results of WHC and FHC of gel-like emulsions.

		Sum of Squares	df	Mean Square	F	Sig.
WHC-Coldset10	Between Groups	149.500	1	149.500	48.415	0.002
	Within Groups	12.352	4	3.088		
	Total	161.852	5			
WHC-Heatset10	Between Groups	9.375	1	9.375	2.813	0.169
	Within Groups	13.333	4	3.333		
	Total	22.708	5			
FHC-Coldset10	Between Groups	2.042	1	2.042	2.579	0.184
	Within Groups	3.167	4	0.792		
	Total	5.208	5			
FHC-heatset10	Between Groups	160.167	1	160.167	384.40	0.000
	Within Groups	1.667	4	0.417		
	Total	161.833	5			

Table A.14 Multiple tests of results of WHC and FHC of gel-like emulsions.

		Sum of Squares	df	Mean Square	F	Sig.
WHC-Coldset30	Between Groups	165.375	1	165.375	113.400	0.000
	Within Groups	5.833	4	1.458		
	Total	171.208	5			
WHC-Heatset30	Between Groups	45.375	1	45.375	28.658	0.006
	Within Groups	6.333	4	1.583		
	Total	51.708	5			
FHC-Coldset30	Between Groups	145.042	1	145.042	25.409	0.007
	Within Groups	22.833	4	5.708		
	Total	167.875	5			
FHC-heatset30	Between Groups	140.167	1	140.167	129.385	0.000
	Within Groups	4.333	4	1.083		
	Total	144.500	5			