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FABA BEAN PROTEIN STABILIZED EMULSION AND GEL

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Tiivistelmä — Referat — Abstract

The literature review detailed the theoretical background for this study, identified the possibility about development of faba bean protein stabilized emulsion for food products and studied the effect of increased oil addition on the structure of emulsions. The practical work was divided into three parts: emulsion analysis, emulsion gel analysis and sensory evaluation. Various research methods were applied to support this study, including droplet size distribution, droplet charge (ζ - potential), texture evaluation, and rheological analysis.

This study showed that oil addition increased emulsion droplet size, as well as the absolute value of ζ – potential. Emulsifying activity index (EAI) and emulsion stability index (ESI) decreased with increasing oil concentration. Faba protein stabilized emulsions were better stabilized by oil addition, compared with those without oil addition. From texture analysis of gel properties, firmness improved with increasing oil concentrations (0% - 30%). In addition, rheological analysis for faba-yogurt samples showed that increasing oil concentrations improved the viscosity of the faba-yogurt samples. The results also illustrated that increasing oil concentrations led to increased deformation, meaning the emulsion gels were more easily destroyed with increasing oil concentrations (0 - 30%). Oil addition resulted in higher whiteness when compared with samples without oil. Sensory evaluation by a professional panel for faba-yogurt samples indicated that samples with 20% oil concentration were the most preferred and had the highest overall quality.

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PREFACE

This study was carried out in the Cereal Technology Group at the Department of Food Technology, University of Helsinki during the years 2012-2013.

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I thank my friends for many ways they tried to support my study and life, especially when I was at a life bottom during the same time while struggling this study work. I thank my dear friend Jiao for making time staying with me even though her PhD work is so hard as well. I also thank my lovely friend Yuchen for every pure smiling.

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Thank you!

Helsinki, May 2013

Yi Xu

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1 INTRODUCTION

Proteins, as one of the three typical food energy sources for humans, play an important role in our daily diet. Basically, proteins could be classified into animal based and plant based according to the sources. Human beings used to eat mainly plant sourced diet in the old days. However, with the rapid development of global economy and society progress, our lifestyle has greatly changed. Animal sourced food started to largely replace plant sourced food in our dietary structure, for instance, meat products and dairy products, etc. On the other hand, the significance of getting back plant based eating habits for human beings has been widely reported and promoted nowadays, for some reasons as healthy issues, ecological issues, as well as economic issues.

Faba bean (*Vicia faba* L.) is the fourth most widely grown and used pulse crop around the world, with various utilizations such as being the low-cost high-quality protein for human diet, as well as being cheap source of the animal feed (Saxena et al. 1993). As the big group in plant family, pulses should be taken into account to a large extent particularly for those impoverished area with severe malnutrition problems. As also reported by Boye, Zare and Pletch (2010) that pulses not only provide diet nutrients for us, but also play a role to reduce risks of many diseases, such as cancer, diabetes, gastrointestinal disorders, and cardiovascular, etc. Tiwari et al. 2011 also stated that legumes or pulses can grow fairly well even in poor cropping condition. And they are reasonably economic and environment-friendly due to their ability to offer nitrogen which help increasing the soil fertility. What is more, they are widely recommended as the staple food due to the favorable amount of nutrients. Specifically, pulses contain various types of carbohydrates, including dietary fibers, resistant starch and oligosaccharides, which are believed as a more balanced choice for carbohydrate supply. Therefore, the starting point of this study aims to see the feasibility of faba bean based food development.

For many food products, the proteins play a key role in keeping the structures, which greatly affect the sensory properties, shelf life for products, as well as the consuming motivation for people. Emulsion system is one of the most widely existed structure in food products, protein components usually act as emulsifiers in these products, for example, milk, yogurt, salad dressing, etc. In the water in oil or oil in water systems, proteins adsorb onto the surface of emulsion droplets, thus stabling the emulsions as emulsifiers (Barfod et al. 1991; Courthaudon et al. 1991; Gelin et al. 1996; De Feijter et al. 1987; Goff 1997).

Oil is another key element in emulsion food products, which is believed to have great effect on emulsion formation and stabilization. Because the oil droplets could be entrapped into the associated protein network and further stabilize the emulsions (Roesch and Corredie 2002). In a gel matrix where oil droplets are embedded in, it is also believed that the volume proportion of oil droplets and the interaction with the gel matrix play a main role in the formation and stabilization of the gel structure (Kim et al. 2001). The oil to protein concentration ratio in emulsion system also affects a lot. Take soy protein (SP) stabilized emulsions (soy milk) for instance, Roesch and Corredie (2002) reported that the properties of emulsions prepared with SP concentrate (concentrations from 1% to 10% (w/v)) varied on account of the oil (10% w/w soy bean oil) added or not.

Literature review aimed to detail the theoretical background for this study, as well as to identify the role of faba protein stabilized emulsion in food development and the effect of oil on emulsion structural properties. The practical work of this study was divided into three parts, including emulsion analysis, emulsion gel analysis and sensory evaluation. In addition to the feasibility of faba bean based food development, this work principally focused on the scientific point of how emulsion and gel structures were affected by various oil concentrations.

2 LITERATURE REVIEW

2.1 Faba bean, other legumes/pulses and relevant products

2.1.1 Faba bean and other legumes/pulses

Faba bean (*Vicia faba* L.) is the fourth most widely grown and used pulse crop around the world, with various utilizations such as being the low-cost high-quality protein for human diet, as well as being cheap source of the animal feed (Saxena et al. 1993). Plant proteins has been reported as an important and significant nutrient source for human diet, which could be digested more easily compared to animal source proteins, could prevent obesity problems, even could turn off the cancer promotion or cancer (Campbell et al. 2006). Therefore, as the big group in plant family, pulses should be taken into account to a large extent particularly for those impoverished area with severe malnutrition problems. As also reported by Boye, Zare and Pletch (2010) that pulses not only provide diet nutrients for us, but also play a role to reduce risks of many diseases, such as cancer, diabetes, gastrointestinal disorders, and cardiovascular, etc.

Based on the introduction of Tiwari et al. 2011, legumes (or pulses), as members of the family Leguminosae, are considered as the major source of food in addition to cereal grains. Legumes of food are those in the family Leguminosae that human beings or animals consume as dry seeds. Faba bean, as the main object in this study, belongs to both "legumes" and "pulses". Although "legumes" and "pulses" are often used interchangeably, they are not totally the same. As defined by Codex Alimentarius Commission, pules are "dry seeds of leguminous plants which are distinguished from leguminous oil seeds by their low fat content". In Food and Agriculture Organization (FAO), "pulses" are particularly used for the dry seed of leguminous plants. Therefore, the green beans and green peas, as well as some seeds containing oil like soybean, are the exclusions from "pulses".

Tiwari et al. (2011) also stated that legumes or pulses can grow fairly well even in poor cropping condition. And they are reasonably economic and environment-friendly due to their ability to offer nitrogen which help increasing the soil fertility. What is more, they are widely recommended as the staple food due to the favorable amount of nutrients. Specifically, pulses contain various types of carbohydrates, including dietary fibers, resistant starch and oligosaccharides, which are believed as a more balanced choice for carbohydrate supply. Due to the low content of methionine, cysteine and tryptophan, but high level of lysine, leucine, aspartic acid, glutamic acid and arginine, legumes and pulses are considered as a perfect complement with cereal, providing a complete protein supply for human diet (Kiosseoglou et al. 2011; Farooq and Boye 2011). In addition, pulses contain necessary vitamins and minerals (i.e. Vitamin B, folates, iron, etc.) as well as other beneficial substance as antioxidant and polyphenols (Tiwari et al. 2011). In recent years, the food market has shown increasingly interests in development of pulses-based products and by-products, as well as the relevant cost-saving processing technologies (Tiwari et al. 2011).

Legumes contain diverse nutrients for ingredients and food production. They have very favorable amount of protein, commonly ranging from 20% to 50% (Cai et al. 2001). This protein content is far more than other plant-sourced raw materials (cereals, vegetables, etc.). Therefore, legumes have been believed as the important supply of proteins for impoverished and malnourished areas in the world (Cai et al. 2001). As was reported, the protein proportions in soybean and faba bean flour both show high level (49.9% and 35.7%). Whereas it differs apparently for lipid content, which gives 23.3% in soy bean, but only 1.2% in faba bean (Cai et al. 2001). The same results were given by Karaca et al. (2011), which shows the protein proportions are 45.41% and 23.94% in soy and faba bean raw materials (flours).

For most pulses, the protein content ranges from 17% to 30%, while the fat content of pulses is usually very low (between 0.83% to 6.6%), depending on the type of the dry

beans (Boye and Zare et al. 2010). As introduced by Kiosseoglou et al. (2011), storage proteins take the biggest part of pulse proteins, mainly including albumins, globulins and glutelins, in which the globulins are the major protein constitution. In addition to storage proteins, various type of other proteins naturally exist in the seeds as well (i.e. Enzymes, enzyme inhibitors etc.). Kiosseoglou et al. (2011) also stated that different constitution of these proteins could give rise to various physicochemical properties for the pulses, shown in their primary molecular structure. Specifically, the water soluble albumin, with low molecular weight, is believed as the result of its hydrophilic surface. While for the salt soluble globulin with high molecular weight, the relatively hydrophobic surface leads to the limited solubility in water. Therefore, the ratio of albumin/globulin of different types of pulses is often used to describe the involved variation and functionality.

Legumes and pulses could be exploited for food production, both on their own or mixed with other materials. When mixed with other food materials, legumes and pulses are always utilized as ingredients and good source of nutrients. Recent years, the isolates of these grains are widely produced due to the high value and low cost, especially for those by-products from the damaged material during harvesting (Sánchez-Vioque et al. 1999). In addition, it is notable that pulse proteins have been proved to be allergenic and provoke cause allergic reactions among a group of food sensitive populations. This probably confines the utilization of these crops to some extent (Boye and Zare et al. 2010).

2.1.2 Bean protein based products (emulsions and gels)

Soy bean, as one of the most widely consumed legumes in the global food market, has achieved huge success in respect of applications of milk imitation. However, very limited amount of reports could be found on the utilization of pulse proteins in the development of milk imitative and alternative products. This might be due to the relatively high amount of fiber and starch content in many pulses, hence providing viscous property which is not suitable for beverage production (Boye and Zare et al. 2010). Even though the unfavorable beany flavor probably limits the utilization with respect to the imitation application as well,

bean protein has been widely studied with respect of food productions which are milk and yogurt like. In the study of Drake and Gerard (2003), soy protein isolates were added into the yogurt products, which were proved to be consumer acceptable. While Ferragut et al. (2009) had a great study of physical characteristics of set-type soy yogurt made from soy milk with ultra-high pressure homogenization treatment, and this product was also proved to be of typical yogurt properties that could be stabilized for at least 28 days. More researches and studies about the bean protein based yogurt like products had been conducted during the past years (Donkor et al. 2007; Pyo 2009; Rivera-Espinoza 2010), showing the great feasibility and acceptance of the non-dairy products in food market.

Curd products based on soybean (Tofu) has been widely studied and investigated. Three main types of coagulants, calcium sulfate, magnesium sulfate and glucono-δ-lactone (GDL) are involved for tofu production during the formation of its curd structure (Kohyama et al. 1992). The process of making tofu usually starts with heating the soymilk to denature the proteins and inactivate the inhibitors (trypsin). Then the coagulant is applied and the soymilk is separated into un-coagulated part (whey) and coagulated part (curds). The curd is further pressed, thus forming tofu.

Cai et al. (2001) prepared bean curds from protein fractions from six legumes (chickpeas, lentils, smooth peas, mung beans and faba beans), induced by heat denaturation of the protein solutions and calcium sulfate as coagulant. It was concluded in this study that the curd texture properties (springiness and cohesiveness) of chickpea and faba beans were quite close to soy curds, whereas the curds of other three bean flour had lower texture properties. A few studies on preparation of curds from legume and pulse had been conducted as well by Kantha (1983) and Gebre-Egziabher (1983). Boye and Zare et al. (2010) indicated that further detailed studies about the coagulation and gelation properties of pulse proteins should be necessary to dig out the possibility to form curd-like products as imitation or even alternatives of tofu and cheese.

As to the study on use of pea and lentil proteins for milk imitation has been studied by Swanson (1990), which demonstrated less desirable quality was found in milk imitations from both protein isolates compared to that from soy protein isolates. Even so, the application of pulse proteins in milk imitation products is greatly feasible as long as enough further studies, on the rheological, color and flavor properties, as well as the relevant processing technologies, are conducted in the future (Boye and Zare et al. 2010).

2.2 Protein-stabilized emulsions

2.2.1 Emulsion systems (mainly of oil and water phases)

A colloidal system comprises dispersed phase and continuous phase and it may be liquid, solid or gaseous, according to the different state combination of the two phases. Emulsion is traditionally classified as one typical form of colloid, in which both the dispersed phase and continuous phase are in a liquid state and immiscible (Rousseau 2000). As described by Dalgleish (2003), oil-in-water (O/W) emulsions and water-in-oil (W/O) emulsions are widely found in food products, of which the former ones are typically fluid (whole milk, cream, etc.), while the later ones are mostly solid-like (butter, margarine, etc.). As the thermodynamically unstable systems, emulsions require the positive free energy to enlarge the interface area between oil and water (Figure 1). The energy is generated from the interaction between oil and water. Since the water molecules could form quite strong hydrogen bonds but not with oil molecules, emulsions tend to decrease the surface area between the two immiscible phases of oil layer on top of water layer (Rousseau 2000).

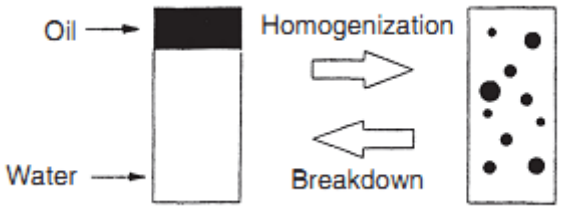


Figure 1. Emulsions are thermodynamically unstable systems that tend to revert back to the individual oil and water phases with time. To produce an emulsion, energy must be supplied (Rousseau 2000).

To keep a stable emulsion system, which could resistant the physical change as much as possible, the kinetic stability plays an important role (Bergenstahl and Claesson 1997). A few methods may be utilized to achieve this stability, including the addition of proteins (e.g. egg proteins), small surfactant molecules (e.g. phospholipids) or thickening agent (e.g. gums) to the emulsion system (Dickinson and Woskett 1989). These emulsifiers are surface-active and they are believed to play two roles in the stability of emulsions: first, they could reduce the interfacial tension between the dispersed and continuous phase, therefore facilitates the breaking down of emulsion droplets during further process (i.e. homogenization); second, they could form, at the same time, cohesive interfacial film in a mechanical way around the droplets, thus keeping from the coagulation (Dickinson 1992). Usually, lower temperature is favorable for stabilizing emulsions due to the higher viscosity (Rousseau 2000). In addition, a few mechanical means, for instance, homogenization which could reduce the droplet size, can be utilized to achieve the stability for emulsions as well (Swaisgood 1996).

As thermodynamically unstable systems, emulsions tend to return back to the initial separated oil and water phases and attempt to minimize the contact surface area (Rousseau 2000). This process depends on multiple factors such as the environmental condition during the emulsion forming and physicochemical properties of the emulsions. Emulsion instability could be attributed into five mechanisms (Figure 2): (1) creaming, (2) flocculation, (3) Oswald ripening, (4) coalescence and (5) phase inversion (McClements 2008). These five mechanisms could affect interactively in which there is always one take the dominant effect on the emulsion properties.

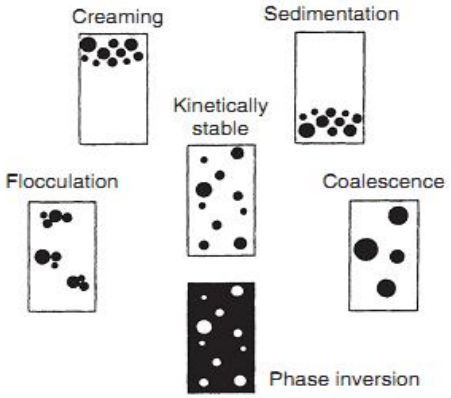


Figure 2. Mechanisms of emulsion instability (McClements 2008).

Creaming and sedimentation

Emulsion creaming and sedimentation could be attributed to the density difference between droplets and the continuous liquid phase. As long as most oils have lower densities than water, the oil droplets tend to accumulate on top of the emulsion (Figure 1). Therefore, creaming tend to occur in an O/W emulsion, whereas sedimentation tend to occur in an W/O emulsion (McClements 2008). The Stokes equation has depicted the creaming rate of single isolated spherical droplet in a viscous liquid:

$$\upsilon = -\frac{2gr^2(p_2 - p_1)}{9\eta_1}, \textbf{ Equation 1}$$

where
υ is the creaming (setting) rate
g is the acceleration due to gravity
r is the droplet radius
p is the droplet density
η is the shear viscosity of the dispersion medium (continuous phase)
the subscripts 1 and 2 refer to the continuous phase and droplets, respectively
The sign of υ determines whether the droplet moves up (+) or down (-)
(McClements 2008)

Stokes equation significantly indicated several means to retain emulsion structure against creaming, which are reducing the density difference between droplets and continuous phase, decreasing the droplet size, as well as increasing the viscosity of the liquid phase.

However, since this equation describes for completely strict spherical particles dispersed in liquid with infinite viscosity, other factors, such as droplet fluidity, droplet aggregation, droplet crystallization and the adsorbed layer, etc., should be taken into account when studying the stability of food emulsions (McClements 2008).

Flocculation and coalescence

Flocculation and coalescence occur in emulsions due to the collision of the droplets, which may lead the droplets to either move dispersedly or get collected, being up to the attractive and repulsive force interaction between the droplets. Specifically, in flocculation, two or more droplets aggregate but remain individual small droplets, while in coalescence, two or more droplets aggregate and merge together to form a new larger droplet (Figure 2). Interestingly, the structures of the flocculent gels differ from the attractive force. The relatively strong attractive force leads to immediate aggregation as soon as droplets encounter, thereby the flocs structure becomes quite open. As for the relatively weak attractive force, the more firm structure is obtained whereby the droplets could move around each other and interact sufficiently after a collision (McClements 2008). This phenomenon is quite similar to the two types of gels formed from filamentous and particulates (Figure 6). It is noteworthy that the formation of flocs in emulsions influence pronouncedly on the physicochemical properties. To be specific, emulsions including flocculated droplets normally have higher viscosity than those without flocs due to the increased amount of water trapped, which amplifies the effective diameter thus volume fraction of the particles. According the Equation 2 which represents the increase of viscosity of a dilute solution of particles, when the concentration of particles increases:

$$\eta = \eta_0(1 + 2.5\phi),$$ **Equation 2**

where
η is the viscosity of the solution
η_0 is the viscosity of the pure solvent
ϕ is the volume fraction of particles in solution
(McClements 2008)

In addition, emulsions with flocculated droplets also demonstrate the strong behavior of shear-thinning: the flocculent structures are disrupted whereby the shear rate is increased, thus decreasing the effective volume fraction, the viscosity of emulsion eventually decrease (Equation 2). What is more, when the concentration of droplets is low, the flocculation affects the creaming rate as well, by increasing the effective size of the particles in Equation 1. However in most food emulsions, wherein the droplets are usually quite densely concentrated, flocculation confines the creaming in that the flocs are enclosed into the emulsion network of aggregated droplets (McClements 2008).

As to coalescence, it should be noted that this aggregate would ultimately lead to the emulsion revert of the thermodynamic state of stability. Because coalescence has the tendency to minimize the surface area of oil exposed to the continuous phase, which means the oil droplets would be amplified as much as possible, when there is no emulsifier membrane, or the interaction potential of droplets is very weak, or high shear force is applied which rupture the protector films and the interaction potential, therefore the free oil finally move onto the top of the emulsion (McClements 2008).

Partial coalescence

Partial coalescence is a particularly important application in food products (usually dairy products), and it occurs among partly crystalline droplets when they encounter each other. A prerequisite for this aggregation is that crystals should be accessible at the interface (Rousseau 2000). The newly formed droplet via partial coalescence is in irregular shape, because the crystal part of one oil droplet would stick out into the liquid phase, and then penetrate into another partially crystalline droplet that is adjacent to it, through their intervening membranes (Figure 3). The crystalline parts of droplets are originally surrounded by water, but after they penetrate each other, they become surrounded by oil. Through time, the surface area of oil exposed into the liquid region is decreased. When the magnitude of crystalline nucleation becomes high enough until more crystal aggregate in the aqueous phase, the phase inversion could even occur.

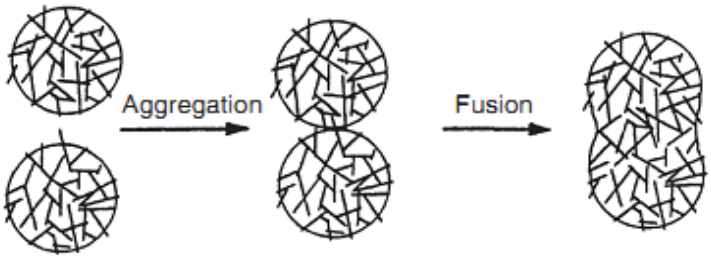


Figure 3. Partial coalescence occurs when two partly crystalline emulsion droplets collide and aggregate because a crystal in one droplet penetrated the other droplet (McClements 2008).

A certain ratio of solid fat and liquid oil is needed for this aggregation, whereas it could not occur under either too low or too high amount of solid fat in the droplets (McClements 2008). In some food productions involved partial coalescence, the O/W emulsions with partially crystalline droplets are obtained by cooling treatment at a certain temperature, then the shear force is applied (i.e. stirring) to cause this type of aggregation (McClements 2008). This is probably the principle of making one of the two major types of yogurt, set and stirred yogurts, of which the former is more curd-like product with gel network while the latter is of apparent viscosity obtained by stirring or pumping and then being cooled to become more viscous for the former gel product.

Ostwald ripening

As to Ostwald ripening, it is the growth of large droplets at the expense of smaller ones due to the solubility gradient among big and small droplets, which is the solubility increases as size of droplet decreases (McClements 2008; Rousseau 2000). However in food emulsions, this aggregation is of less importance in that the solubility difference between liquid and oil phases is very limited (Rousseau 2000). As to phase inversion, the emulsion system changes from an O/W emulsion to a W/O emulsion, or vice versa. This inversion could be achieved by altering the composition or environmental conditions of the emulsion system, such as the type and concentration of emulsifier, temperature, mechanical forces applied, volume fraction of dispersed phase (McClements 2008). It is believed that the mechanism of phase inversion is a complicated process involves all the aggregation types mentioned above, in which different systems of emulsion may exist until the complete inversion

occurs (McClements 2008).

2.2.2 Emulsifying properties of legume protein

Legume/Pulse proteins

It has been reported in many studies that, during the formation of emulsions, different proteins, as well as between surfactants and proteins, will compete for adsorption on the surface of emulsion droplets, thus affecting stability of emulsions (Barfod et al. 1991; Courthaudon et al. 1991; Gelin et al. 1996; De Feijter et al. 1987; Goff 1997). In order to reduce the interfacial tension, or increase the viscosity or form a gel network in the continuous phase of the emulsion, thus improving the kinetic stability, many types of ingredients have been widely used as thickening or gelling agents in food emulsions, including starch, cellulose, and soy protein (Cui 2005; McClements 2005).

In grain legumes and pulses, proteins can be classified into two groups, metabolic proteins and storage proteins. Basically, the storage proteins are classified into five groups based on their solubility in different solvents as following (Oomah et al. 2011):

1. Albumin: soluble in water
2. Globulins: soluble indilute salt solution
3. Prolamin: soluble in 70% ethanol solution
4. Glutelin: soluble in dilute alkali
5. Residue: left-over protein.

In most cases, the storage protein in dry beans usually refers to globular proteins (globulins), because they are the most abundant type of storage protein in those seeds and their globular like shape (Oomah et al. 2011; Kiosseoglou et al. 2011). Generally, globulins could be further classified as 7S and 11S globulins based on their sedimentation coefficients (S). Furthermore, the 7S are usually trimeric proteins while the 11S are normally hexameric proteins. For most legumes, the major storage proteins are 11S proteins with the MW (molecular weight) of 300-400kDa, containing six non-covalently interacted subunit paris (Galazka 1999; Oomah et al. 2011). Each of the pairs comprises one acidic subunit of MW 40kDa and a basic subunit of MW 20kDa, and the two subunits

are connected by a disulfide bond. On the other hand, the trimeric 7S proteins are not able to form SS-bond due to the lack of cysteine, and the MW range from 150kDa to 190kDa (Oomah et al. 2011).

Concerning pulse proteins, as indicated above that many factors affect their emulsifying properties, for instance, the methods applied for emulsion preparation (isoelectric precipitation, ultrafiltration, etc.), the environmental parameters (pH, ionic strength), as well as the type of the pulses. As reported by Fuhrmeister and Meuser (2003), pea protein isolate prepared by ultrafiltration shown much more favorable emulsifying activity than that obtained by acid or heat-acid isoelectric precipitation (with the EAI values of 27.4m^2g^{-1}, 10.1 or 14m^2g^{-1} respectively). As in another research conducted by Zhang et al. (2009), the researchers concluded that the isoelectric precipitation at alkaline pH gave rise to the apparently higher EAI compared to that at a pH close to the protein isoelectric point. Besides, they also stated that the EAI reduced at a lower ionic strength condition and increased back as increasing the ionic strength of the protein solution.

The role of protein molecules in emulsion

It is normal that most food emulsions consist of various elements, for instance, cake batter comprises sugar, baking soda, fine wheat flour all kinds of powder, except for oil and water. Therefore, it is important to understand and control the interactions among these different elements thus promoting the stability of the emulsions (Rousseau 2000). As mentioned above, the interface plays a role in stabilizing the emulsions. It is believed that high viscosity and rigidity interfacial film works better on stabilizing emulsion due to the reducing rate of film drainage (Edwards 1991; MacKay 1973).

As one of the most important biopolymers, proteins are widely used as functional ingredients in food emulsions, mainly to stabilize the emulsions, increase viscosity, as well as to form gels (McClements 2008). The three-dimensional structures formed by globular biopolymers have quite strict compact structures that could be found in many types of food

products. This category of proteins usually have considerable amount of nonpolar groups that tend to fold to globular structures. Although theoretically the nonpolar groups tend to locate away from the water (interiorly) and the polar groups in contact with water (exteriorly), it has proved that some hydrophobic groups (nonpolar) exist on the surface as well, due to other different molecular interactions (McClements 2008). The capability of the protein molecules to transit the conformation to another is fairly important in food productions, with respect of functional properties such as emulsification and gelation. Many environmental conditions could lead to this transition, such as pH, temperature, ionic strength, etc. (McClements 2008).

Proteins are widely used ingredients as effective emulsifiers in foods because protein molecules tend to be surface active, due to the high proportion of nonpolar groups that could accumulate at the oil-water interfaces (McClements 2008). This adsorption mainly in response to the hydrophobic interaction and it minimize the contact area between the oil and water molecules at the interface, thus reducing the interfacial tension (McClements 2008). Therefore it can be seen that the structure of the protein molecules predominantly determine the physicochemical properties of the membrane. As long as the proteins adsorb to the interface, their nonpolar parts (hydrophobic) tend to face the oil phase, therefore, the structural rearrangements of the protein molecules at the interface tend to increase the contacts between nonpolar parts and oil as shown in Figure 4 (McClements 2008). Due to the rigid compact structure, the unfolding of a globular protein at the interface is very slow. The structural unfolding of protein molecules thus requires additional treatment (i.e. heating, enzyme, etc.). The unfolding tends to expose amino acids which were enclosed interiorly in the hydrophobic area of the protein molecules, thus increasing the possibility to interact with other adjoining protein molecules, whereby the hydrophobic attraction and disulfide bond could make a contribution. In this way, thin and firm membranes are formed from globular proteins, with favorable viscoelastic property and more resistance to break (McClements 2008).

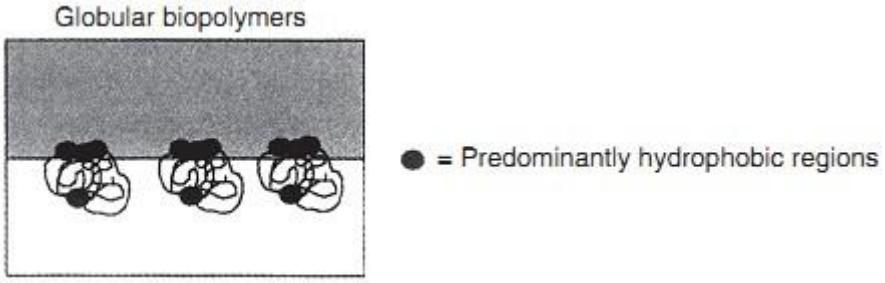


Figure 4. Conformation and unfolding of proteins (globular biopolymers) at oil-water interface depend on their molecular structure (McClements 2008).

Disulfide bond is important in keeping the network of protein gels. The intermolecular disulfide bond (R-S-S-R) is formed from the oxidation of free sulphydryl groups (-SH) of two cysteine residues (Walstra 2002). As was stated by Visschers (2005), the unfolding of proteins could be caused from two mechanisms. On one hand, the formation of disulfide bond rarely occurs in most native protein, but the formations rate could be increased when applying heating, high pressure or shear. Because these treatments could change the tertiary structure of the proteins, the sulfhydryl and cystine become exposed and reactive. Importantly, this chemical reactivity plays a role in many food applications with respect of gel formation. On the other hand, this unfolding could be explained in another mechanism, that most proteins take the preference to partition at air-water or oil-water interfaces, hence -SH and S-S groups exposure to the interfaces and the reactivity of sulphydryl is promoted. Ronald et al. also stated that there might have two possible ways to explain the protein association from heat-induced unfolding. First, cysteine residues and disulphide bonds become accessible after heating treatment, thus could involve in the intermolecular cross-links formation. But it is not definite if activation is needed, or the exposure could automatically cause the reaction. Secondly, in the physical conception, the more proteins aggregate, the less hydrophobic parts exposed, which is considered as the unfavorable exposure.

It is necessary for emulsifying molecules to adsorb onto the surface of newly formed emulsion droplets as fast as possible, so that the protective membranes are formed promptly to prevent the droplets aggregating back. Various types of physical mechanisms

are involved in this stabilization property. Specifically, the short-range steric repulsive forces are provided by these molecules to protect droplets against aggregation, while the membrane could prevent the flocculation if it is thick enough. The electrostatic repulsion could prevent the flocculation as well when the molecules are electrically charged. In this case, the charged biopolymers are usually quite sensitive to the condition of pH and ionic strength in solutions. When the pH is near the isoelectric points of the relevant proteins, or the ionic strengths are high enough, it becomes very easy for the droplets to aggregate because the electrostatic repulsion is not sufficient (McClements 2008).

Characterization of emulsion properties

For legume protein stabilized emulsions, the step of mixing or homogenization of oil is often applied to create oil-water interface, meanwhile, the oil droplets are formed as well. The oil droplets are protected from many structural changes (i.e.coalescing, flocculation, creaming or sedimentation) due to the film or skin formed from the adsorbed protein molecules around these droplets (Boye and Zare et al. 2010). The ability to keep the relatively small size of droplets is accepted to describe the emulsifying ability, which intimately depends on the protein adsorption properties. In addition to the size of droplet, the adsorption properties of protein molecules are characterized normally by solubility, surface hydrophobicity (or the hydrophobicity/hydrophilicity ratio), structural flexibility and constraints, which describe the extent of which these proteins can unfold to form the protective film or skin (Boye and Zare et al. 2010). Also the preparation methods for protein extraction could give rise to various differences on functional aspect of these protein concentration or isolates. To express the emulsifying properties of legume proteins, two indexes, emulsifying activity index (EAI) and the emulsifying stability index (ESI) are often used (Kiosseoglou et al. 2011). The effect factors mentioned above are introduced and discussed in more details as follows.

(i) Droplet size (distribution and change)

It is believed that emulsions with relatively smaller droplet size have satisfactory

emulsifying ability (Kiosseoglou et al. 2011). The measurement of emulsion oil droplet size distribution and change have been widely used in many studies (Liu et al. 1999; Karaca et al. 2011; Chove et al. 2001; Tsoukala et al. 2006; Papalamprou et al. 2005; Kim et al. 2001; Roesch and Corredig 2002; Chapleau et al. 2003; Papalamprou et al. 2010). Homogenization is the process of reducing the size of the droplets in an emulsion (Walstra 1993). In food industry, homogenization is achieved by the mechanical device applying intense agitation to the mixed liquid, which is known as homogenizer (i.e. microfluidizer, ultrasonic homogenizer, etc) (McClements 2005). In emulsion-based food products, the average diameter ranges from 0.1 to 100 μm (McClements 2007).

(ii) Particle size covering on droplet surface

As reported by Walstra (1987), microstructure significantly affects the droplet coverage, which further influences the emulsion stabilization properties. Walstra concluded that the high droplet coverage, the better stabilization properties, based on O/W emulsions. As described by Briggs (1921), the full coverage of droplet surface could be achieved by small particles. Tens of years after Briggs's study, Schulman and Leja (1954) drew a conclusion that to obtain BaSO4 stabilized O/W emulsions, the size of the particles used for stabilization should be smaller to a great extent than the size of emulsion droplets. To be specific, in their study, particles with the size of less than 1 μm were favorable to form a "very good" emulsion. Particles with large size usually exist freely and coagulate in the continuous phase of the emulsion rather than adequately adsorb onto the interface of emulsion droplets. Buchheim and Dejmek (1997) had viewed the phenomenon that, seen in Figure 5 A and B, smaller size of particles (fat crystals in this case) tend to provide better coverage of emulsion droplet than those of larger size. What is more, Figure 5 C demonstrates that a full coverage could be achieved when fat crystallizes directly on the interface of the dispersed and continuous phases.

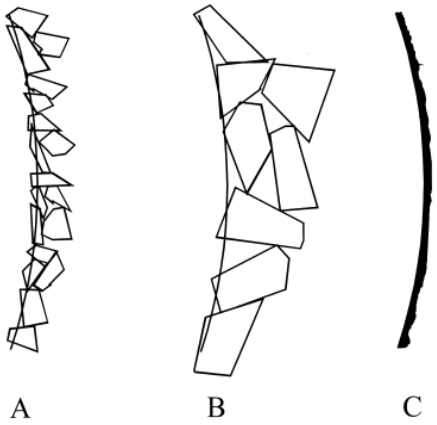


Figure 5. Effect of fat crystal size on droplet coverage; (A) ~1 μm crystals; (B) ~5 μm crystals; (C) sub-micron crystals (Rousseau 2000).

(iii) EAI and ESI (emulsion activity and stability of pulse proteins)

To express the emulsifying properties of legume proteins, two indexes, EAI and ESI are often used. In which the former one is the emulsifying activity index (EAI), and the latter one is the emulsifying stability index (ESI). EAI is defined as the maximum surface area of the oil droplet (or the amount of oil) covered or emulsified per unit of protein, and usually assessed by using method of turbidimetry of emulsion samples that are highly diluted (Kiosseoglou et al. 2011). The initial surface layer of the droplet is formed during the emulsion preparation. Then, the protein surface becomes mechanically strengthened as more protein molecules adsorb onto the surface. This layer could keep stability of the emulsion for a quite long time against association of the droplets, not only due to its innately elastic layer, but might also due to the ability to prevent the aggregation and interaction which result in some unexpected changes for emulsions (i.e. Creaming, or droplet coalescence) (Kiosseoglou et al. 2011). On the other hand, ESI measures the ability to resist the change of emulsion structure over a certain storage period, and is often used as an assessment of stability for legume protein-stabilized emulsions (Boye and Zare et al. 2010). ESI value is measured by testing and analyzing the turbidity decreased with the further dilution of emulsion samples. It should be noted that the ESI is determined in a short period that the only change would be the serum separation, resulting from the droplets moving upwards. Therefore, the ESI could actually be taken as the index of stability against creaming of emulsions (Kiosseoglou et al. 2011).

In many studies, method from Pearce and Kinsella (1978) was usually followed to measure EAI and ESI to demonstrate the emulsifying properties of proteins (Liu et al. 1999; Molina et al. 2001; Karaca et al. 2011; Chove et al. 2001). Although EAI and ESI are fairly useful in depicting the emulsifying capability of various types of proteins, they are actually very empirical properties due to the lack of information about the emulsion droplet size distribution and change. Therefore, the detailed studies about droplet size distribution and change could help researchers go deep to the rheological and textural properties of emulsions (Kiosseoglou et al. 2011).

Interestingly, according to Vose (1980), it was reported that the emulsifying properties of faba bean (horsebean) protein isolates was generally lower than that of soy protein isolates, no matter the proteins were prepared by either isoelectric precipitation or ultrafiltration. However, when saline solution (4% w/v NaCl) was applied, the EAI value became comparable. Different protein fractions, together with the constitution of protein mixtures, give rise to different emulsifying properties. Lefebvre et al. (1987) indicated that vicilin (7S) showed better emulsifying properties among legumin (11S) and pea globulin (vicilin-legumin mixtures). Cserhalmi et al. (1998) gave the similar conclusion in their study that the globulin and 7S fractions had higher protein surface hydrophobicity and better emulsifying properties (EAI and ESI) than 11S fractions and albumin. Besides, technique of hydrolysis also plays a role in emulsifying properties. Sijtsma et al. (1998) reported that a certain degree of hydrolysis could give a favorable improvement of the EAI value of a pea protein isolate. While Braudo et al. (2001) indicated that a treatment of hydrolysis with use of chitosan could improve the ESI value of faba bean.

(iv) Emulsion rheology

The rheological properties of emulsions are quite important general and integral characteristics with respect to the relevant food products, significantly determining their mouthfeel, stability and liquidity of the emulsion foods (McClements 2008). Capillary viscometers are used for strictly ideal liquids with low viscosities, while the dynamic shear

rheometers for non-ideal liquids or emulsions with more complicated viscoelastic properties. The relationship between the stress and strain could be measured and analyzed with the help of these instruments to represent the interactions between emulsion droplets (McClements 2008). Moreover, this measurement also provides the useful physical information about the emulsions such as their textures properties.

2.2.3 Gelation of protein-stabilized emulsions

The ability to cause gel formation in aqueous has made protein molecules as a type of important functional ingredients in many productions of food emulsions (i.e Cheese, tofu and meat, etc.). Gelation imparts favorable textural and sensory characteristics to products, as well as protecting the emulsion droplets against aggregation such as creaming (McClements 2008). Gels could be classified as particulate and filamentous (Figure 6). Particulate gels are usually of opacity property because the particles are able to scatter light significantly. The biopolymer molecules are easily to aggregate to particles or clumps in this case, because the attractive forces around the whole surface of the molecules are very strong. Particulate gels also tend to syneresis due to the large sizes of inter-particle pore, which means the water molecules could not be held tightly enough in the gel network (McClements 2008). Many protein-based emulsion products belong to this case, including dairy proteins (cheese) and legume proteins (tofu) etc. Notably, the syneresis property might be one of the most important limits for legume based yogurt imitation products, due to the unfavorable product state with water out of the structure.

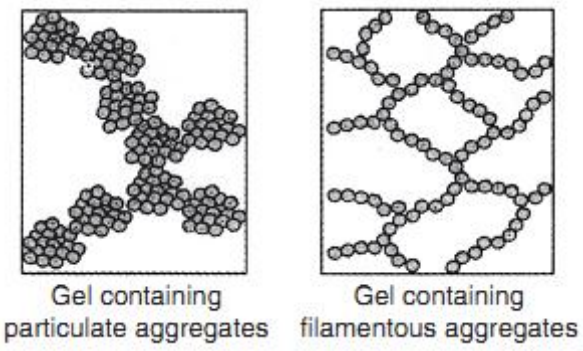


Figure 6. Biopolymer molecules or aggregates can form various types of gel structures, such as particulate or filamentous (McClements 2008).

Gelation ability is one of the most widely used functional properties of pulse protein with respect to food production (i.e. Jellies, meat applications and puddings, etc.), due to the formation of the self-supporting gel network structure which is consumer friendly and generally accepted (Boye and Zare et al. 2010; Kiosseoglou et al. 2011). Furthermore, this ability to form gel network is affected variously by factors like the type of pulse, the method of protein extraction, the constitution, as well as the storage condition. Heating treatment is always applied for gelation when the temperature is higher than the protein denaturation point. Besides, the least gelling concentration (LGC), the minimum concentration of protein required to form a self-supporting gel, is normally used to express the protein gelation ability (Boye and Zare et al. 2010; Kiosseoglou et al. 2011). The relevant determination should be absolutely conducted by a standard method, either by visual, or more exactly by rheological analysis (Kiosseoglou et al. 2011). The lower LGC indicates the greater gelling capacity (Farooq and Boye 2011). Notably, it was reported that LGC of faba bean isolates recovered by isoelectric precipitation was lower (14%) than pea (18%) or soybean (16%) (Fernández-Quintela et al. 1997). Ten years after this study, the similar values were obtained by Kaur and Singh (2007) that the LGC of various chickpea protein isolates ranged from 14% to 18%.

As for the effect of methods applied to prepare pules proteins on gelation, it has been generally reported in many previous studies that the method of ultrafiltration for protein preparation could give rise to much better gelation ability by showing much lower LGC

values, when compared to the isolates obtained by isoelectric precipitation (Boye and Aksay et al. 2010; Kiosseoglou et al. 1999; Papalamprou et al. 2009). Besides, it was further stated by Papalamprou et al. (2009) that there was no significant change of LGC value between the globulin and albumin isolate extracted by ultrafiltration. In addition to the effect on LGC value, the methods of protein preparation affect the final gelling structure as well. Also in the study of Papalamprou et al. (2009), compared to isoelectric precipitation, ultrafiltration gave rise to a higher elasticity of gels made from chickpea protein isolates with certain protein concentration. Moreover, they specified that the much less rough property given by ultrafiltration leaded to less changes of protein structure, as well as better organized interactions among proteins during gelation, whereas the isoelectric precipitation was suggested to result in more aggregation of protein subunit which might reduce the amount of effective protein and cause higher LGC value and lower elastic property.

In addition to methods for recovering protein isolate, the environmental factors (pH, sodium chloride and calcium chloride, etc.) play a role as well for gelling properties of pulse proteins. Kiosseoglou et al. (1999) reported that NaCl addition to lupin protein isolate with the concentration up to 0.5 M gave rise to increased storage modulus and reduced tanδ, which meant the increasing of elasticity. But as the salt concentration increased further, both of the values turned to opposite. Similarly, Zhang et al. (2007) found that the addition of 0.1 M NaCl would cause decreasing of LGC value of chickpea protein isolate. They also had the conclusion that NaCl or $CaCl_2$ content had effect on both elastic property and strength parameters of the gels. Still according to study of Zhang et al. (2010), it was concluded that the gelation ability of kidney bean proteins was markedly influenced by ionic strength, shown as increasing storage moduli and decreasing LGC when applying the ionic strength increasingly from 0 to 300 mM. For the formation of gel network based on pulse proteins, the non-covalent hydrophobic interactions, hydrogen bonds and ionic bonds, should be the dominating factors, while the covalent disulfide might make some contribution as well (Kiosseoglou et al. 1999; Clark et al. 1986, Doi et al.

1989; Yamagishi 1987). Specifically, Clark et al. (1986) indicated that the non-covalent cross-link (hydrogen bonds, ionic bonds, hydrophobic interactions) take the main responsibility to keep the three-dimension gel structure, since the disulphide cross-links were broken down by applying aqueous urea and SDS (sodium dodecyl sulphate) solutions. Doi (1989) asserted that the heat set glycinin (11S of soy globulin) gels should be attributed to both disulphide bonds and hydrophobic interactions, while hydrogen bonds contributed further during the cooling. Yamagishi (1987) also found that the hydrophobic interactions functioned during the beginning of gelation with heating, and disulphide bond appeared to work during the subsequent heating. What is more, it could probably be accepted that the protein charge, influenced by pH or ionic strength, plays a role in keeping balance between intermolecular repulsive and attractive forces of protein, thus establishing an stable gel network with elastic structure (Kiosseoglou et al. 2011).

The gelation of protein-stabilized emulsions has been industrially utilized in food production and been scientifically studied in many researches. For instance, tofu making is one of the typical applications, in which glucono-δ-lacton (GDL) or calcium sulfate are usually induced as coagulants (Hou and Chang 2003). In the soy protein-stabilized emulsion (soy milk), the negatively charged parts of soy protein, which denatured by heating treatment, are decreased by inducing the coagulants (i.e. GDL, calcium sulfate, and magnesium chloride, etc.), thus adding the cations, neutralizing protein molecules and reducing the electrostatic repulsion (Hou and Chang 2003). Ultimately, the protein molecules could draw together and complete the gelation. In addition to coagulants, other factors, the concentration of coagulant, the pH of protein-stabilized emulsions (soymilk), and the temperatures also effect on the formation of gel (Hou and Chang 2003).

In the great study of bean curds conducted by Cai et al. (2001), the protein fractions of six legumes were used as solubles (protein milks) to prepare curds. The protein concentrations ranging from 1% to 4% were made to study the effect on yield, moisture and texture. Meanwhile, a protein content of approximately 3% of bean protein was always used as the

optimum protein concentration for the curd preparation. A coagulant dosage of 1.5% calcium sulfate was used as the best dosage in this study. It is interesting to note that an increasing dosage of coagulant could result in the decreasing moisture content and the higher hardness of curds, which indicated that to a certain extent, a higher amount of coagulant would promote the dehydration of bean curds. What was more, the protein concentration also effected significantly on the hardness of bean curds. Specifically, increased protein concentration generally lowered the hardness of curds.

It was reported that the gelation capability of soy protein should be intimately related to various types of forces, including hydrogen bonds, hydrophobic interactions, electrostatic interactions and disulfide bonds (Cai et al. 2001; Kinsella 1979; Utsumi 1985). The gelation of 7S mainly associates with hydrogen bonding and hydrophobic interactions, whereas the gelation of 11S involves disulfide bonding (Nakamura et al. 1986; Cai et al. 2001). In addition, the gesl of 7S are characterized as harder than that of 11S from soy proteins (Utsumi 1985).

2.3 The role of oil in protein-stabilized emulsions and gels

It has been elucidated in the previous section (2.2.1) that an emulsion system comprises two immiscible liquids, one of which is dispersed as small droplets in the other continuous liquid phase. The emulsions utilized for food production are normally types of O/W or W/O which means the two immiscible liquid phases are usually water and oil, with some exceptions (W/W emulsions) (Norton 2001; Norton 2006). In a gel matrix which oil droplets are embedded, it is believed that the volume proportion of oil droplets and the interaction with the gel matrix play a main role in the formation and stabilization of the gel structure (Kim et al. 2001).

Take soymilk for instance, it comprises about 30% lipids (dry basis) and plays a fairly important role in the texture formation and gelation progress of soymilk-based food products (i.e. Tofu, soy yogurt, etc.). Ono (1996) reported that large amount of lipids are

found in the protein particles in uncooked soymilk, whereas in cooked soymilk, only 3% of total lipids are associated within these particles. Specifically, when the heating temperature is 65°C, a portion of lipids in the protein particles start to liberate into the soluble part, while almost all neutral lipids are released into the soluble fraction when heated above 90°C. As will be discussed below, the addition of coagulant could result in the protein aggregation. Besides, this addition also causes the association of lipids into the protein gel matrix, which has been observed that the oil droplets are embedded in the networks of the gel (Saio 1969; Yamano 1981).

According to the study of soy protein concentrate (SPC) emulsion from Roesch and Corredie (2002), emulsions prepared with SPC (concentrations from 1% to 10% (w/v)) behaved in big difference on account of the oil (10% w/w soy bean oil) added or not. Specifically, for the suspensions which were prepared without oil addition, not only the high amount of large particles (10 μm to 100 μm) were presented, but also the separation of the homogenized suspensions were shown after being stored for only 3 d. However, for the oil added emulsions, the distribution of particles size from 1 μm to 10 μm, even < 1μm, were presented. Besides, it is also interesting to note that for those emulsions prepared with 1 or 2% SPC and 10% oil, the phase separation was observed after only 2 d of storage at 6 °C, whereas the serum separation was found after 5 d storage for emulsions with 4% SPC, and at higher SPC concentration (>4%), the emulsions shown quite good stability to resist the creaming. Therefore, it is believed that oil is one of the major points of emulsion formation and stabilization. Because the oil droplets could be entrapped into the associated protein network and further stabilize the emulsions. This significant effect by oil could also be proved by another group of results in the same study, showing that the w/w percentage of insoluble pellet in emulsion (with oil) was higher than that in suspension (without oil), which indicated that perhaps the small oil droplets were largely embedded into the emulsion network to keep its stability.

In addition to provide the component of the oil phase for O/W or W/O emulsions, the fat or oil imparts significant attributes to the properties, depending on its ability to crystallize or its degree of crystallization (Dalgleish 2003). Oils normally affect the emulsion property to only a small extent because they basically act as the fillers (droplets) in the emulsion. Moreover, for those unsaturated oils, it is susceptible to encounter some unfavorable reactions such as oxidation and other chemical reactions between the denatured oil and the emulsifier molecules. This probably further change the whole functional properties of the emulsion, as well as create some unacceptable flavors to the products (Dalgleish 2003). On the other hand, the fat crystallization occurs when emulsion products are always subjected to cold storage at certain temperature, this thus bring about significant functionality for the emulsions. The partial coalescence based on fat crystals is so far one of the best known and classic instance. The crystal parts of the partially crystallized droplets tend to move to and reside on the interfacial surface, stick to the interface, and cause the rupture of the membrane. The newly formed fat droplets are in irregular shape due to the tendency of the fat crystals to inhibit the wholly aggregation from two spherical droplets into one (van Aken 2003).

3 EXPERIMENTAL RESEARCH

3.1 Aims

This experimental research aimed to (1) compare and clarify the effect of different oil ratios (v/v) on properties of faba protein stabilized emulsions (oil in water liquid) and gels (set style and stirred style); (2) describe and explain the changes of structural properties during making the yogurt imitation products (from emulsions to gels).

3.2 Materials and Methods

3.2.1 Materials

Faba beans of cultivar Divine were of French origin, produced by company Agri Obtentions. The faba beans were grown in 2011 in Viikki, Finland by University Lecturer, Docent, Fred Stoddard from Department of Agricultural Sciences

3.2.2 Preparation of emulsions and emulsion gels

O/W emulsion preparation

Faba beans (400 g) were soaked for 16 h in 4 L Milli-Q water at room temperature (25 °C). After soaking, water was discarded and the testa was removed by hand. The drained seeds were heated at 140 °C for 10 min in drying oven (Termaks, Norway) to remove the beany flavor from the volatile substance which is produced from the oxidation reaction of lipoxygenase and poly-unsaturated fatty acid in the beans. After the heat treatment, the seeds were then blended (Bamix, Switzerland) with 2 L Milli-Q water for 5 min. The resulting slurry was centrifuged using the RC-5C high-speed refrigerated centrifuge (Thermo Fisher Scientific, USA) at 20 °C, 10000 rpm for 15 min and filtrated through double-layered cloth and approximately 1.5 L of faba protein solution was obtained per

400 g of faba beans in 2 L of Milli-Q water.

To prepare faba bean milk (BM), the obtained faba protein solution was first boiled for 10 min to further unfold the protein structure so that protein molecules could better interact with the dispersed and continuous phases and stabilize the emulsion system (McClements 2008). After that, 70 ml of the boiled solution was collected for each emulsion sample preparation with various oil additions (0, 5%, 10%, 15%, 20%, 25%, 30% respectively). Milli-Q water was used to ensure the total amount of each sample was 100 ml. After that, the mixture was pre-homogenized using a high-speed dispersing and emulsifying unit (Ultra-Turrax® T25 basic, IKA® Works Inc., Wilmington, NC) at 20,000 rpm for 2 min, thus obtaining the BM samples.

Emulsion Gel preparation

The bean milk samples (BM) with various oil concentrations were inoculated with ABT-1 including bacterial cultures of *L. acidophilus, Bifidobacterium* sp. *And S. thermophilus* (Chr. Hansen Pty. Ltd., Bayswater, Australia). In all cases, 0.05% (w/v) of starter was added into each BM sample. Fermentation was performed at 37 °C for 16-18 h within incubator (Incubator IPP 500, Memmert®, Germany). In the end of the incubation the pH reached about 4.4. Thus the set style faba-yogurts (GY) were obtained and used for texture analysis (3.2.4) after 24 h storage at 5 °C. To prepare the stirred type faba-yogurts (SY), an injector was used to disrupt the gel network of set type faba-yogurts (10 to 20 times up and down for about 30 sec). Rheological analysis, colorimetric analysis and sensory evaluation were carried out for these stirred type faba-yogurts.

3.2.3 Evaluation of emulsion characteristics

Droplet size distribution

Emulsion droplet size distribution was measured using a particle counting system of a

standard instrument PAMAS SBSS (PAMAS GmbH, Leonberg, Germany), borrowed from the division of Agritechnology, department of Agriculture Sciences. Emulsion samples (faba bean milk, BM) with various oil concentrations (from 0% to 30%) were 100-fold diluted for the test. Two batches of BM dilution samples were prepared and measured, one of which was made from the freshly prepared BM samples, while the other was made from the BM samples that had been stored at 6°C for four days. Data was recorded and exported using PAMAS PMA program.

Surface charge (ζ - potential measurement)

To prepare 100-fold dilutions for this test, 50 µl of each freshly prepared BM samples were mixed, with 4.95 ml Milli-Q water. After that, the ζ - potential (Zeta-potential) measurement was performed using a Zetasizer (Nano ZS, Malvern Instruments Ltd., England) at room temperature (25 °C) and equilibration time was 2 min for each test. The Zetasizer was also borrowed from the division of Agritechnology, department of Agriculture Sciences. The measurement was controlled by DTS Nano software. The cells were filled with diluted samples and the test was operated following the manual. Emulsion stability could be outlined by Table 1, according to their ζ potential values.

Table 1. Outline of relationship between ζ potential values and corresponding stability (Greenwood and Kendall, 1999; Hanaor, et al. 2012)

Absolute Zeta potential / mV	Stability behavior of the colloid
0 ~ 5	Rapid coagulation of flocculation
10 ~ 30	Incipient instability
30 ~ 40	Moderate stability
40 ~ 60	Good stability
> 61	Excellent stability

Emulsifying activity index (EAI) and emulsion stability index (ESI)

Emulsifying activity and stability indexes of BM samples were determined by the method described by Pearce and Kinsella (1978). A 500 μl of 100-fold dilution of the ζ - potential measurement for each sample was immediately taken and further diluted in 12 ml SDS (sodium dodecyl sulfate) solution to give final 2500-fold dilutions. And the SDS concentration is 0.1%. The absorbance of the diluted faba bean milk samples at various oil concentrations was determined at a wavelength of 500 nm in a spectrophotometer (UV-1800, Shimadzu, Japan) using plastic cuvettes (1 cm path length). The two indexes were calculated by using Equation 3 and 4.

$$\text{EAI}\ (m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10000}$$

Equation 3. A_0 is the absorbance of the diluted emulsion immediately after pre-homogenization, N is the diluted factor (2500 in our case), c is the weight of protein per volume (g/ml), φ is the oil volume fraction of the emulsion (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3).

$$\text{ESI}\ (\text{min}) = \frac{A_0}{\Delta A} \times t$$

Equation 4. ΔA is the change in absorbance between 0 and 1 h, and t is the time interval, 1 h in this case.

3.2.4 Textural evaluation of emulsion gel characteristics

Texture test of set type faba-yogurt samples (GY) were performed using a TA-XT2i texture analyzer (Stable Micro System, Surrey, UK) equipped with a ϕ 25 mm cylindrical probe. Test was operated at a constant speed of 2 mm/s, until a depth of 20mm, to obtain the slope of the force-distance curves and the values of gel firmness (breaking force).

3.2.5 Evaluation of stirred emulsion gel (faba-yogurt)

Rheological analysis

(i) Steady shear viscosities

Analysis of steady shear viscosity of the stirred type faba-yogurts (SY) were followed by

the method described by Liu and Tang (2011), using a Rheometer (HAAKE RheoStress600, HAAKE Co., Germany), equipped with a cone (C35 1° Ti, 35 mm diameter, 1° angle) and a parallel plate (PP 35 Ti, 35 mm diameter). The equipment was controlled and driven through RheoWin Pro. Sample was deposited onto the plate of the Rheometer using an injector with the amount of 1 ml for each sampling. Shear rate was ramped from 1 to 100 s^{-1}. Data of shear stress, shear rate, steady shear (apparent) viscosity (η) were exported from the program. Test was performed at 10 °C.

(ii) Viscoelastic properties

Three types of tests were conducted following the method described by Saint-Eve (2006), using the same Rheometer, cone and plate. The first one was a frequency sweep test at an increasing frequency (ω=0.1-100 rad/s) in order to describe the degree of organization of the yogurt samples. Measurements were verified to ensure that they were performed in the linear viscoelastic domain. The second test measured the complex viscosity by the stress sweep. A slop of constrains from 0.1-100 Pa, with a frequency of 1 Hz was used. The third test measured the flow curve of stirred yogurt by increasing the shear rate from 0 to 100 s^{-1} (upward curve), with a 30 s pause, and then followed by decreasing the shear rate from 100 to 0 s^{-1} (downward curve). Test was performed at 10 °C.

Colorimetric analysis

Colorimetric analysis of faba-yogurt samples with different oil concentrations was carried out using a colorimeter (Minolta CR-400, Konica Minolta Sensing, Singapore), which provided ‘L*’, ‘a*’, and ‘b*’ values, three replications of the test were performed with each yogurt samples. Based on the L*a*b*, the Whiteness Index (WI) was calculated using Equation 5 (Thakur, et al. 2010):

$$\mathrm{WI} = 100 - [(100 - \mathrm{L}*)^2 + a*^2 + b*^2]^{0.5}$$

Equation 5. Calculation equation of Whiteness Index (WI), where L* is the lightness component ranging from 0 (black) to 100 (white), and parameters a* (from greenness to redness), and b* (blueness to yellowness) are the two chromatic components.

Sensory evaluation

Faba-yogurt samples (stirred type) were evaluated by a panel of seven professionals. Four separated factors of sensory characteristics (whiteness, firmness, flowability and greasiness) and the overall preference of the samples were also asked to be evaluated by the panel. The sensory evaluation form test used in the test is attached in Appendices section as ‘Appendix 1’.

3.2.6 Statistic Analysis

Data from emulsifying activity index (EAI) and emulsion stability index (ESI) were normalized before producing out figure. Specifically, the original values of each bar chart were valued 1, and the relative values of the rest of original values could be calculated.

For the results of sensory evaluation, the score of each factor for each sample was obtained by a calculational transition. Specifically, lengths of the marks of each horizontal line were measured. According to the ratio of length of mark to length of the horizontal line, score of each factor of each sample could be obtained based on a ten-point scoring system. Therefore, for each sensory characteristic factor, each sample could be evaluated by each evaluator as a certain score out of ten points. Subsequently, for each sensory characteristic factor, faba-yogurt samples could get a total score separately by adding scores from seven evaluators together.

3.3 RESULTS

3.3.1 Droplet size distribution of emulsions with different oil concentrations

The freshly prepared bean milk emulsion BM_0 without oil addition showed similar droplet distribution as other samples with oil addition, with relatively high proportion at droplet size around 10 μm (Figure 7). Whereas the BM_0 sample that has been stored for four days, over half of the droplets gave the size less than 1μm (Figure 8).

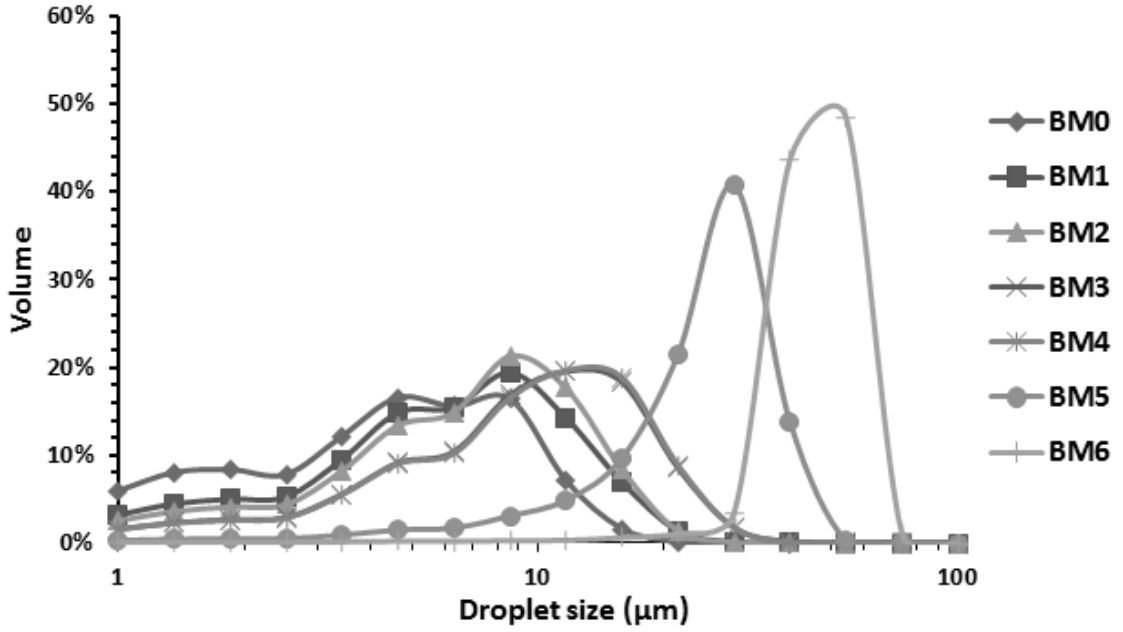


Figure 7. Droplets size distribution of freshly prepared emulsion samples with various oil concentrates: BM_0 (0%), BM_1 (5%), BM_2(10%), BM_3 (15%), BM_4 (20%), BM_5 (25%), BM_6 (30%).

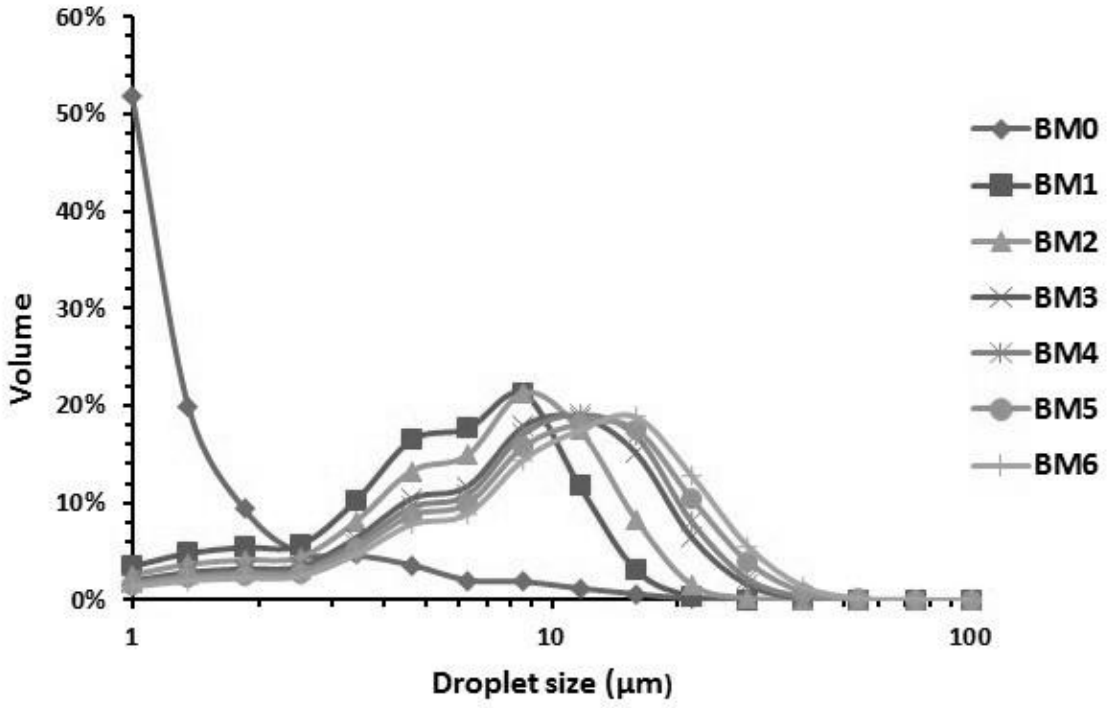


Figure 8. Droplets size distribution of emulsion samples have been stored for 4 days after freshly prepared, with various oil concentrates: BM_0 (0%), BM_1 (5%), BM_2(10%), BM_3 (15%), BM_4 (20%), BM_5 (25%), BM_6 (30%).

3.3.2 Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions with different oil concentrations

For faba bean protein stabilized emulsions (BM) with various oil concentrations, the relative EAI values decreased with the increasing oil concentration (Figure 9). ESI values of these BM samples decreased with the increasing oil concentrations (Figure 10). The emulsion sample with 5% (v/v) oil content was an exception. It could not give a result due to the absorbance stay unchanged during the standing period.

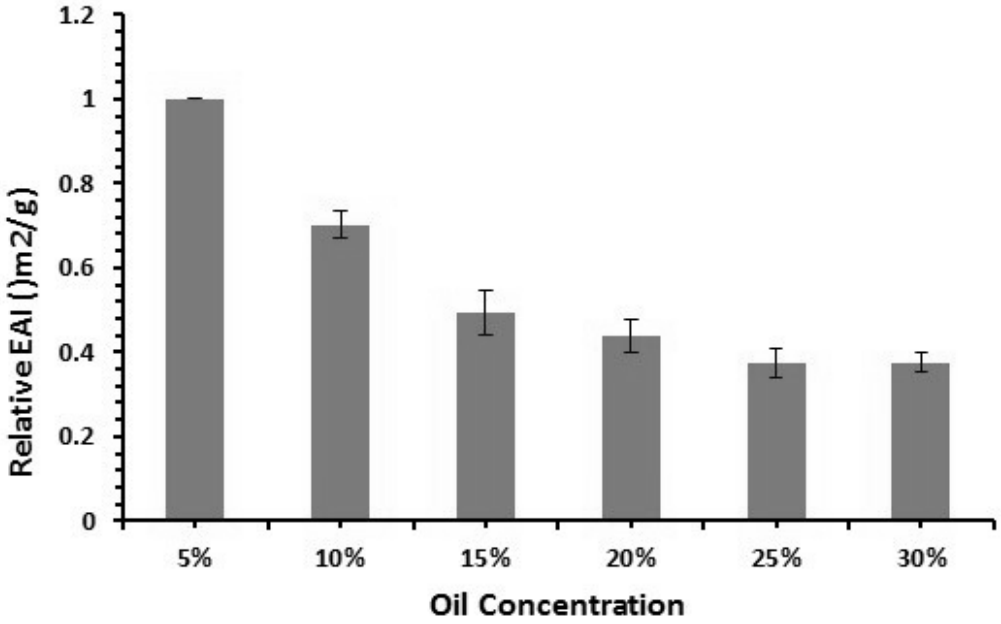


Figure 9. Emulsifying activity index (EAI) as a function of oil concentration of faba bean protein stabilized emulsions. Error bars indicate the standard deviation of the samples.

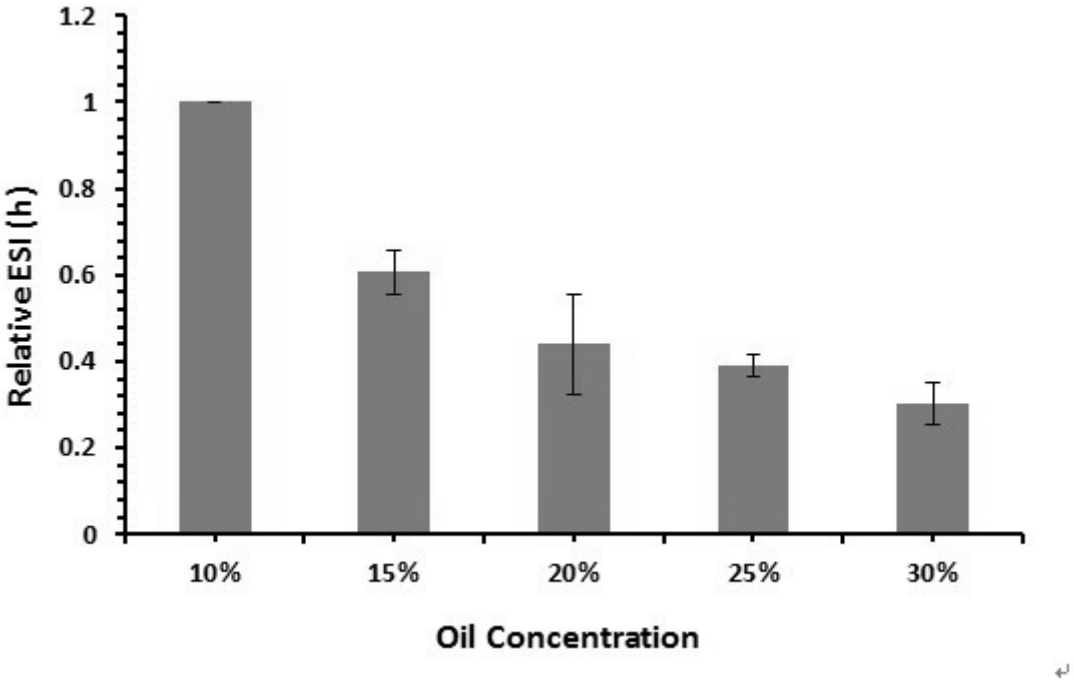


Figure 10. Emulsifying stability index (ESI) as a function of oil concentration of faba bean protein stabilized emulsions. Error bars indicate the standard deviation of the samples.

3.3.3 Surface charge (ζ - potential measurement) of emulsions with different oil concentrations

Droplet surface charge, the zeta potential, of faba protein stabilized emulsions (BM) showed that there was no apparent regular pattern among the emulsion samples with various oil additions from 5% to 30% (Figure 11). However, it is interesting to note that the absolute ζ-potential value in sample without oil addition (0%) is lower than in all the other samples, regardless of the oil concentrations. Furthermore, the absolute ζ-potential values of the samples with oil addition (5% - 30%) all exceed 40 mV, indicating good stability for the emulsions. The emulsion sample without oil addition (0%) also showed moderate stability with the absolute ζ-potential value of 35mV.

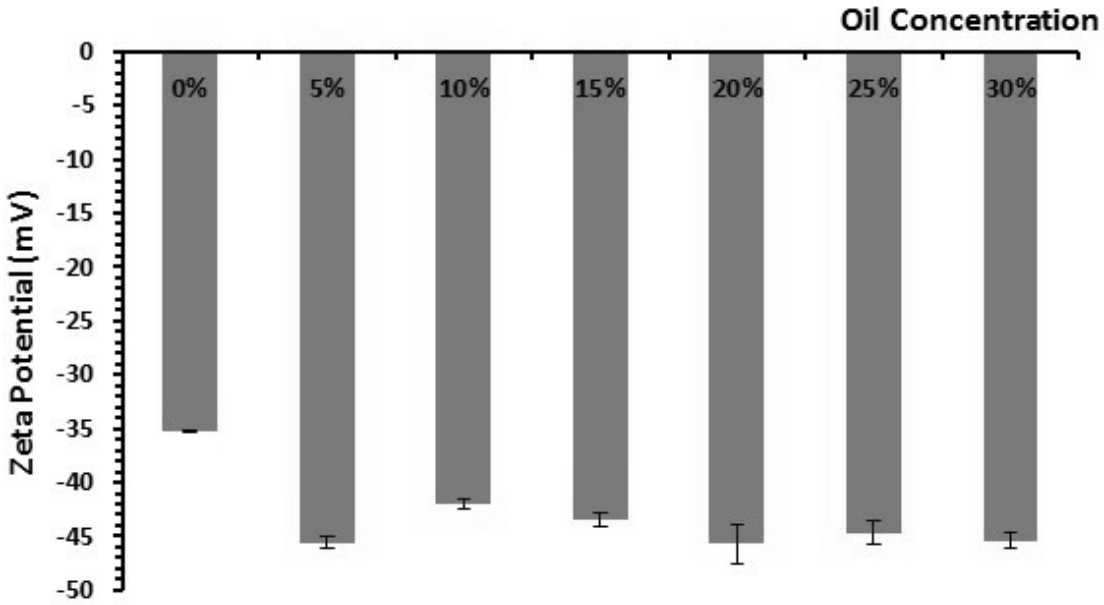


Figure 11. Influence of various oil concentration on the ζ-potential of emulsion samples stabilized by faba bean protein (FP).

3.3.4 Texture evaluation of emulsion gels with different oil concentrations

Texture profile analysis of set style faba-yogurts, or gel yogurts (GY), after 24h cold storage (at 5°C) are demonstrated in Figure 12. There is a regular increase in resistance to deformation for samples with increasing oil concentration. To be specific, samples obtained from faba milk emulsified by low oil concentrations, GY_0 with no oil added and

GY_1 with 5% oil added, exhibited no peaks when the force was added onto the samples, while those samples made from faba milk with higher oil concentrations (GY_2 with 10% to GY_6 with 30%) showed deformation peaks soon after the force was added. The peak values of GY_2, GY_3, GY_4, GY_5, GY_6 and the corresponding points of GY_0 and GY_1 were collected as firmness for each gel sample (Figure 13).

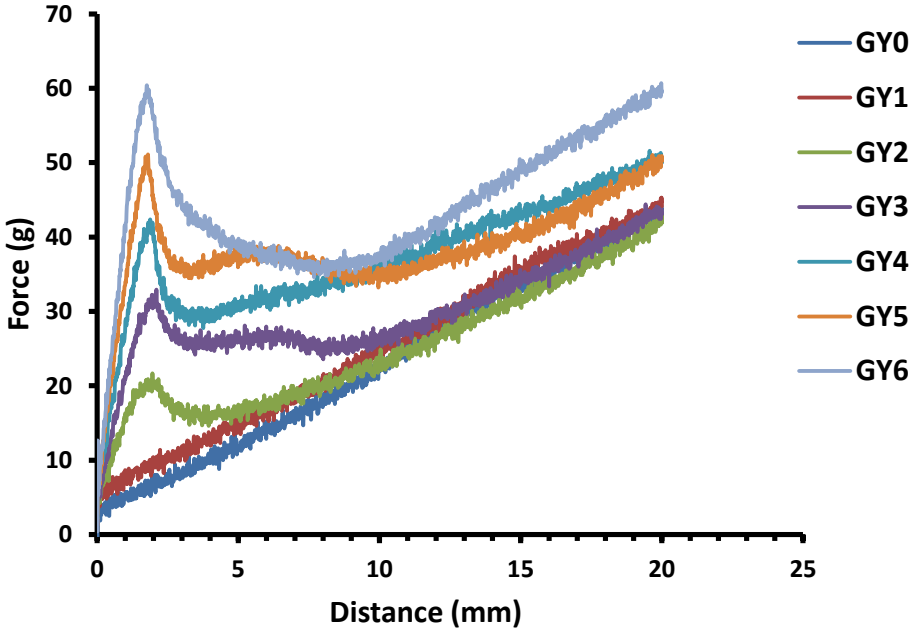


Figure 12. Force-distance curve of set style faba-yogurt, with different oil concentration: GY_0(0%), GY_1(5%), GY_2(10%), GY_3(15%), GY_4(20%), GY_5(25%), GY_6(30%). Experimental details can be seen in section 3.2.4 of Material and Methods part.

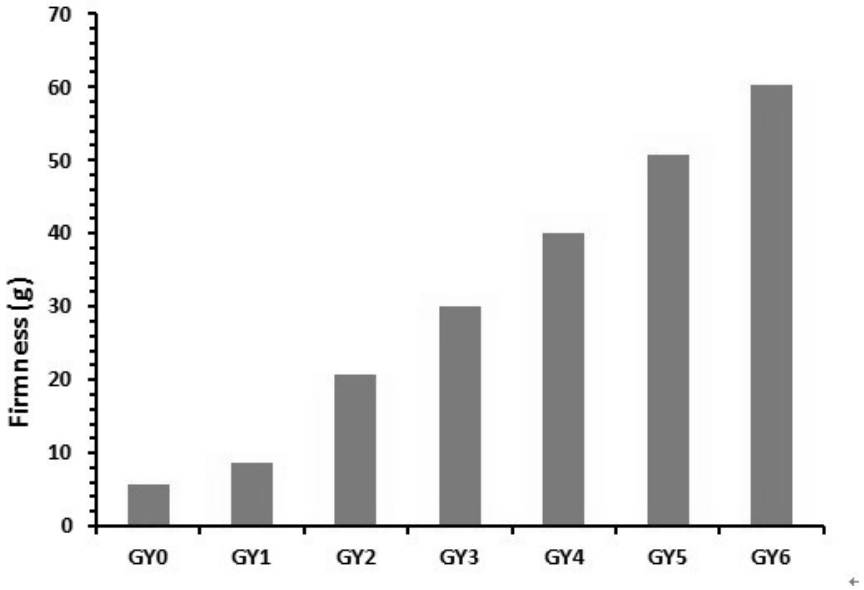


Figure 13. Set style faba-yogurt firmness with different oil concentration: GY_0(0%), GY_1(5%), GY_2(10%), GY_3(15%), GY_4(20%), GY_5(25%), GY_6(30%).

3.3.5 Rheological properties of emulsion gels with different oil concentrations

(i) Visual observations

Visual observations of stirred faba-yogurts (SY) at about 5% (w/w) protein content and various oil fractions (φ = 0-0.3) after cold storage at 5 °C for one month revealed gel structure variations (Figure 14). Emulsion samples with 25% (SY_5) and 30% (SY_6) oil concentrations displayed self-standing gel textures, while the sample without added oil (SY_0) displayed a liquid like state which could be observed in spreading of the sample (Figure 14).

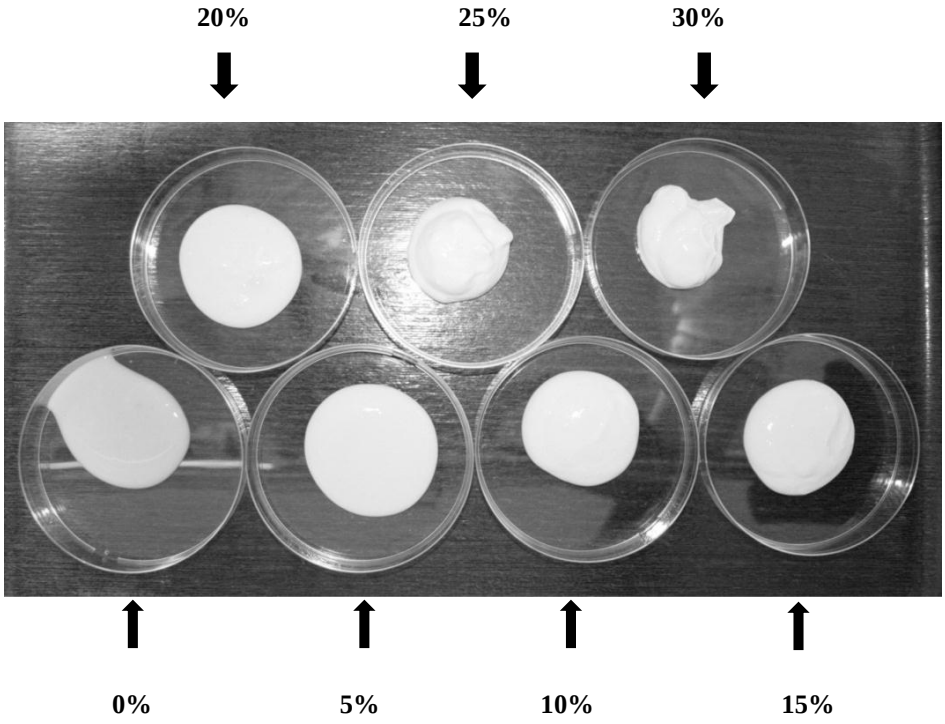


Figure 14. Visual images of stirred faba-yogurt samples at various oil concentrations: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

(ii) Steady shear viscosities

The apparent viscosities (η_a) of faba-yogurts at various oil concentrations (0%-30%) exhibited shear-thinning behaviors of non-Newtonian fluids under the shear rate ranged from 1-100 s^{-1} (Figure 15). The apparent viscosity (η_a) of faba-yogurts increased, to a varying extent, with the increasing oil concentrations from 0% to 30% (Figure 15).

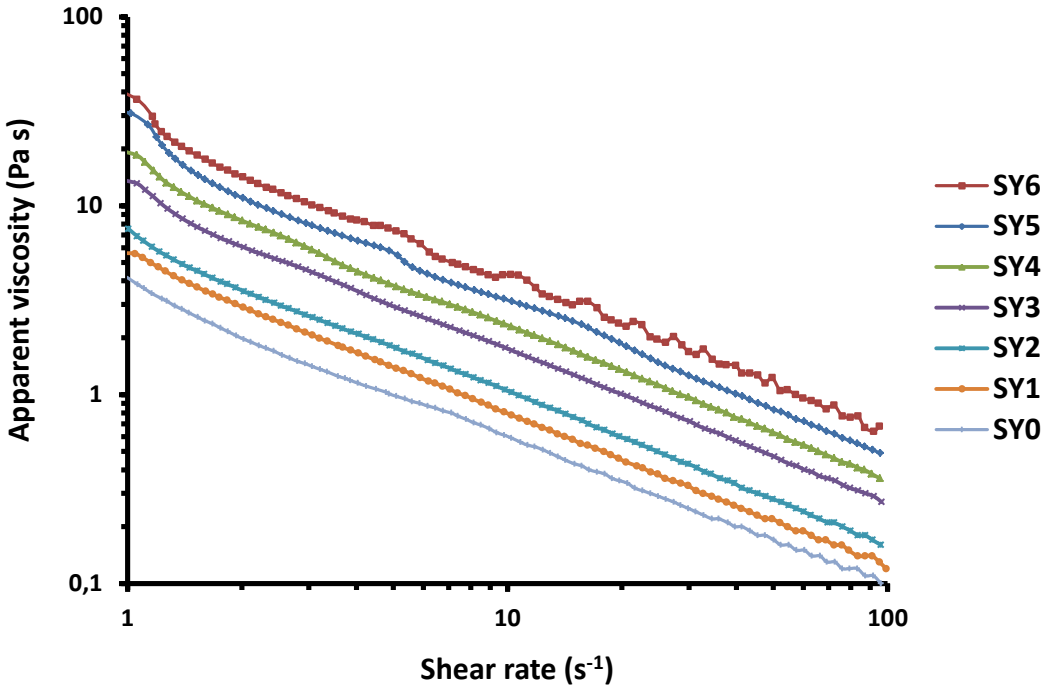


Figure 15. Changes in apparent viscosity (η_a) as a function of shear rate at 10°C for the stirred faba-yogurts (SY) prepared with various oil concentrations: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

(iii) Viscoelastic properties

The viscoelastic properties of stirred faba-yogurts were measured by frequency sweep tests. The storage modulus G' (Figure 16) and the loss modulus G'' (Figure 17) of the seven stirred style faba-yogurts (SY) increased with the increasing oil concentration. The value of G' was higher than that of G'' over the whole frequency range (0.1-10 Hz) (Figures 16 and 17). Furthermore, both G' and G'' values increased gradually and slightly with the increasing frequency. In addition, all the G' slopes increased parallel with G'' slopes.

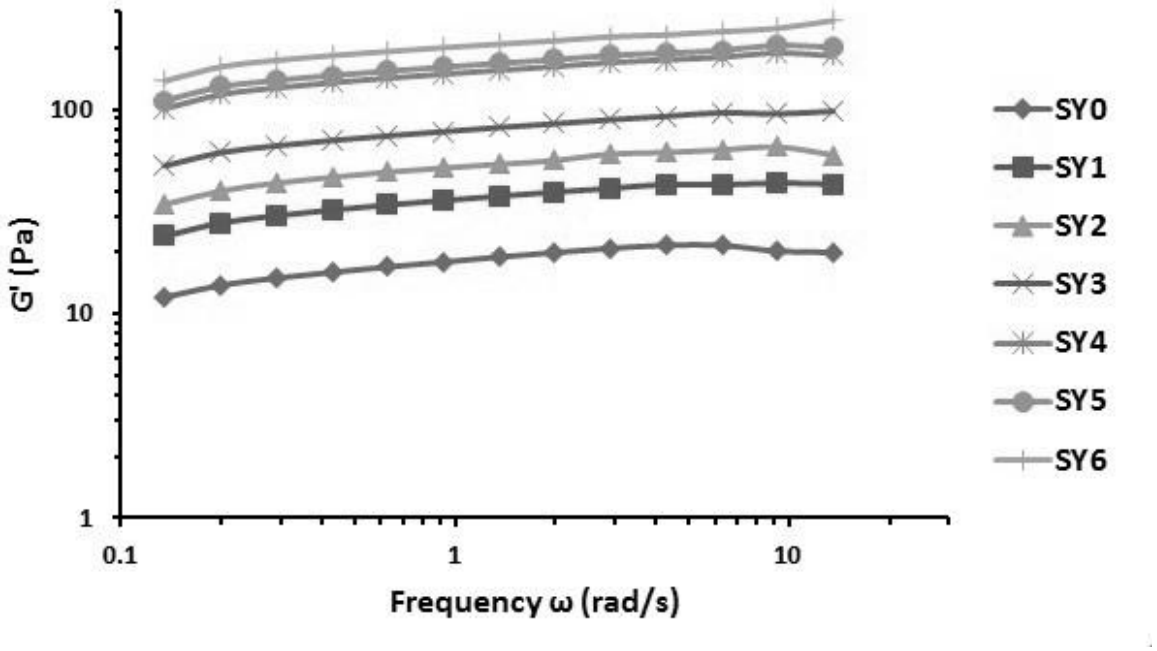


Figure 16. Variations of storage modulus G' which represents the elastic property of stirred faba-yogurt (SY) at various oil fractions: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

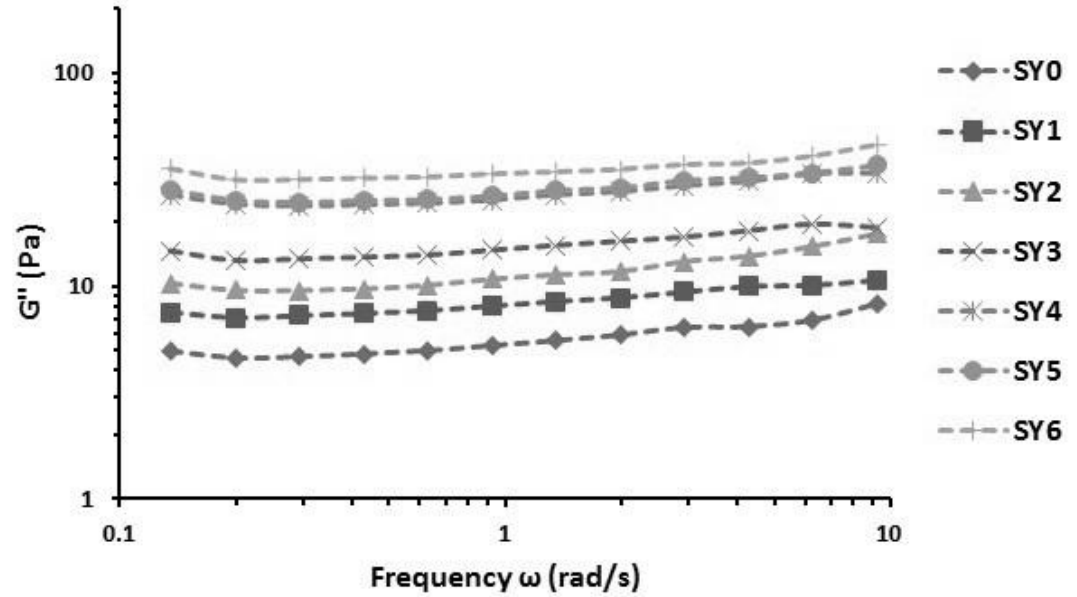


Figure 17. Variations of loss modulus G'' which represents the viscous property of stirred faba-yogurt (SY) at various oil fractions: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

Figure 18 displays the changes of complex viscosity as a function of frequency for stirred faba-yogurts with various oil fractions. The complex viscosity increased with the increasing oil concentration, regardless of frequency. Moreover, the stress sweep test

illustrated the influence of various oil fractions on the complex viscosity at various shear stresses.

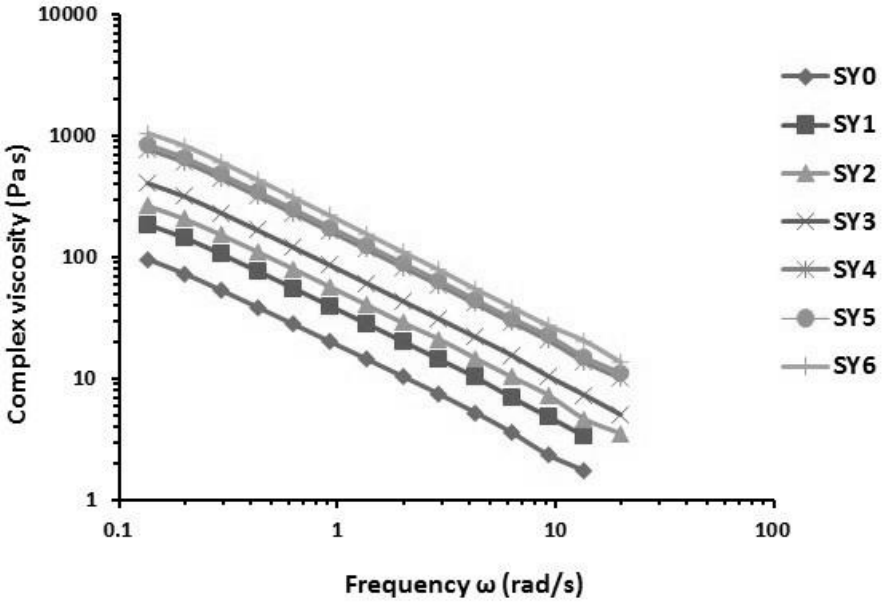


Figure 18. Changes of complex viscosity as a function of frequency at 10°C for stirred faba-yogurts prepared with different oil concentrations: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

The complex viscosities under 0.1 and 50 Pa were presented in Table 2. These stress values were selected according to Saint-Eve (2006). Where the results at a low shear stress of 0.1 Pa corresponded to the thickness of the yogurt observed with a spoon, while the complex viscosity at the shear stress of 50 Pa corresponded to yogurt products under mastication conditions. The complex viscosity of faba-yogurts increased with increasing oil concentration in both shear stresses (Table 2).

Table 2. Complex viscosity (Pa s) at a low shear stress of 0.1 Pa ($\eta_{0.1}$*), correspond to spoon condition and at 50 Pa (η_{50}*), correspond to mastication condition shear stress of stirred faba-yogurts (SY) at 10°C.

Yogurts	$\eta_{0.1}$*/Pa s	η_{50}*/Pa s
SY_0	6.86	0.36
SY_1	7.44	0.39
SY_2	11.10	0.49
SY_3	16.53	0.68
SY_4	27.28	1.10
SY_5	42.39	2.06
SY_6	55.90	2.91

The flow curves of stirred faba-yogurts with various oil concentrations displayed that samples with high oil concentration (15-30%) had high shear stress values during the increasing and decreasing shear rate sweep (Figure 19). It is also interesting to note that the area of the hysteresis loop, which represents the structural breakdown condition of yogurts during shearing, became higher with the increasing oil fractions.

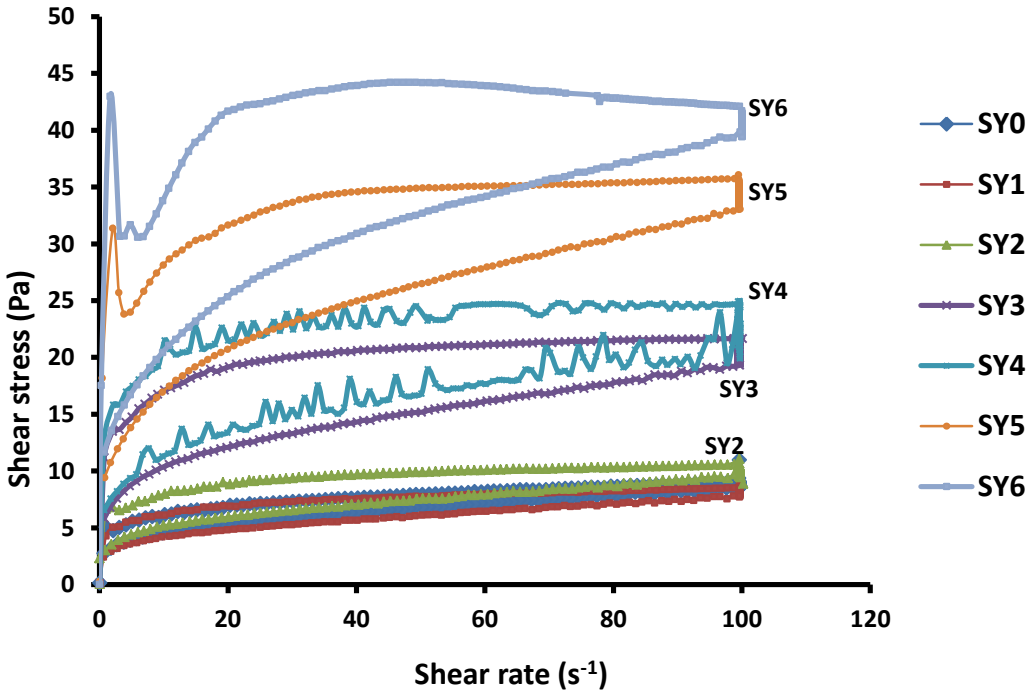


Figure 19. Shear stress as a function of increasing and decreasing shear rate for faba-yogurts at 10°C with various oil fractions: SY_0(0%), SY_1(5%), SY_2(10%), SY_3(15%), SY_4(20%), SY_5(25%), SY_6(30%).

3.3.6 Colorimetric analysis of faba-yogurt samples

Color analysis of stirred type faba-yogurts was performed to evaluate whiteness changes as a function of oil concentrations. There was no significant change of whiteness index (WI) among yogurt samples with oil concentrations from 5% to 30% (Figure 20). However, the sample without oil showed dramatic lower WI compared to others.

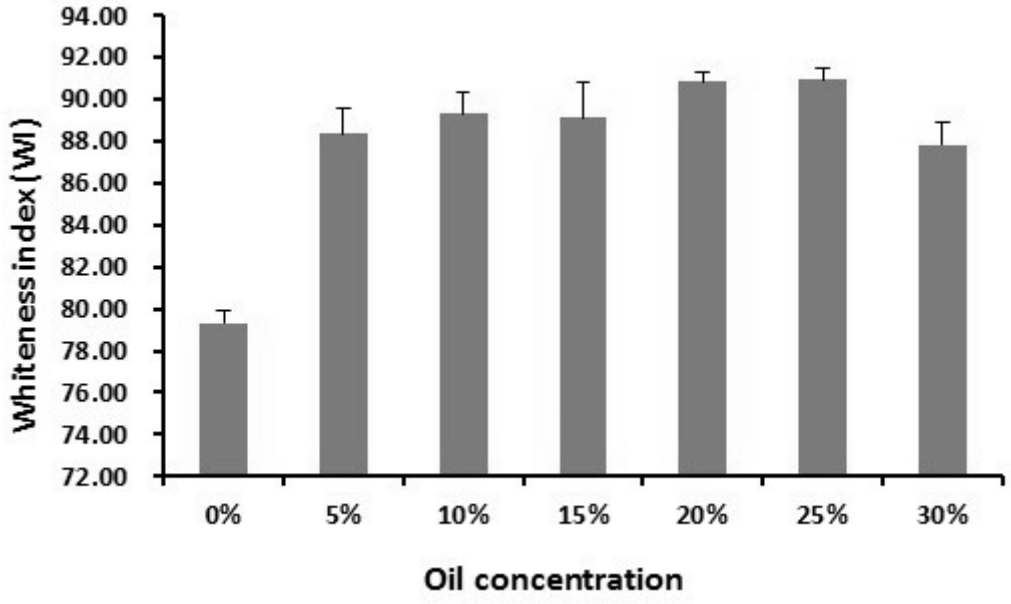


Figure 20. Whiteness index (WI) of stirred type faba-yogurts (SY) at various oil concentrations. Error bars indicate the standard deviation of the samples with triplicate measurement.

3.3.7 Sensory evaluation of faba-yogurt samples

Four separated factors of sensory characteristics (yellowness, firmness, flowability and greasiness) and the overall preference of the samples were evaluated by a panel of seven professionals. The results of sensory evaluation of faba-yogurt samples with various oil concentrations (0% - 30%). Faba-yogurt sample without oil addition gave the highest score of yellowness, followed by samples with oil concentrations of 5% and 10%. Samples with high oil concentrations (20%, 25% and 30%) showed relatively low scores in yellowness. As for the evaluations of other three factors (firmness, flowability and greasiness), it is interesting to note that samples with high oil concentrations basically gave higher scores than those with lower oil concentrations. The scores of greasiness showed less difference than, other factors among the seven samples (Figure 21). As to the overall preferences, samples with 0% and 5% oil concentrations were the least preferred, while the yogurt sample with 20% oil concentration was preferred the most, followed by those with 30% and 25% oil concentrations (Figure 22).

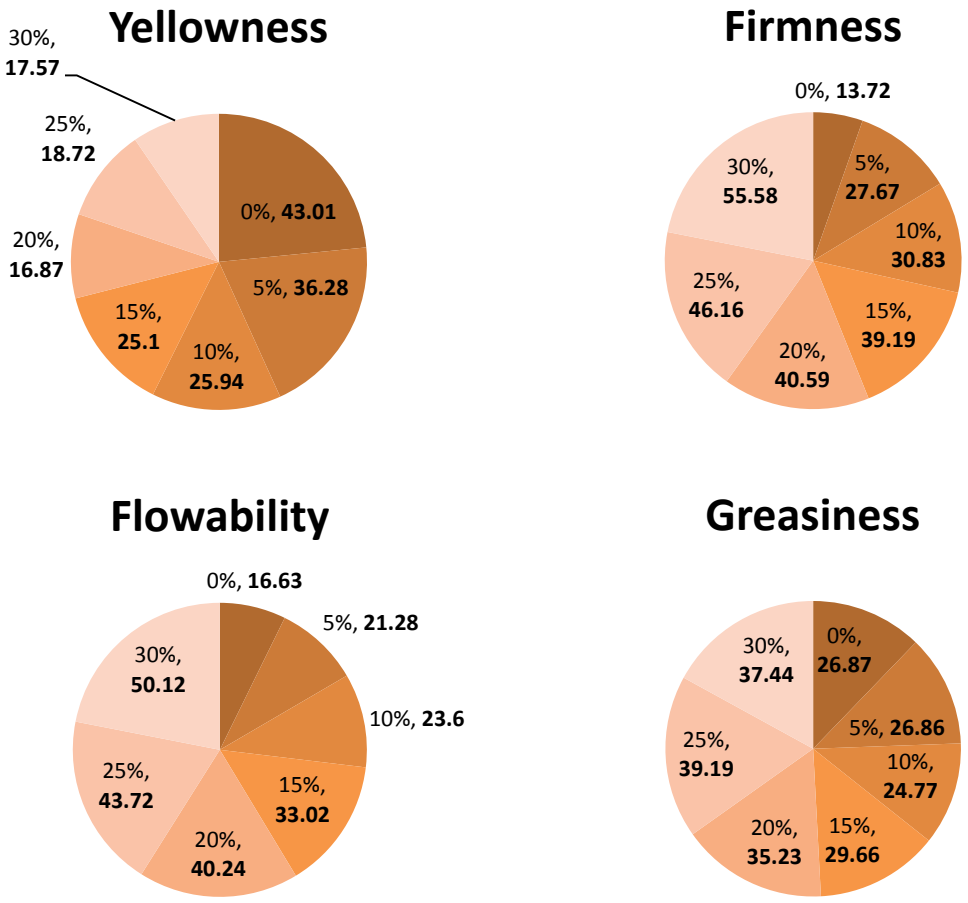


Figure 21. Sensory evaluation results of faba-yogurt samples with various oil concentrations (0% - 30%). Samples were scored from low to high as following descriptions: yellowness: natural white – light yellow; firmness: thin like milk – firm like cream cheese; flowability: smooth like milk – ropy like mayonnaise; greasiness: water – oil.

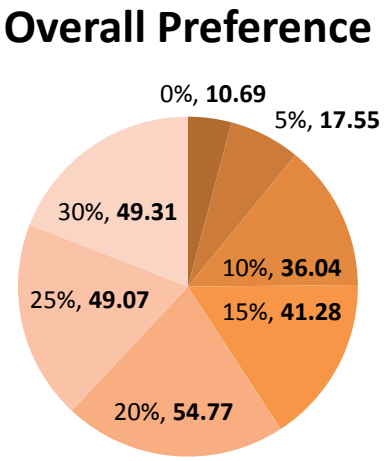


Figure 22. Overall preference of faba-yogurt with various oil concentrations (0% - 30%).

3.4 Discussion

3.4.1 Effect of oil concentrations on faba protein emulsifying properties

The droplets in the emulsions are kept in and move with continuous migration due to some factors including their thermal energy, gravitational forces, as well as some applied mechanical forces. Therefore, frequent collisions would happen among the droplets when they move around each other. Accordingly, the forces acting among the droplets effect greatly on the emulsion stability, which could be reflected by whether the droplets get flocculated, coalesced or not. To be specific, droplets tend to get aggregated when the attractive force dominates, while they remain un-aggregated when the net force is greatly repulsive (McClements 2008).

Differences can be seen that the freshly prepared faba bean milk, BM_0 without oil addition, showed similar droplet distribution as other samples with oil addition, with relatively high proportion at droplet size around 10 μm. Whereas for the BM_0 sample that had been stored for four days, over half of the droplets gave the size less than 1μm. This difference could be attributed to the low oil content in raw material that there were not enough oil-water interfaces for protein molecules to be adsorbed, therefore the size distribution of protein molecules was seen of size less than 1 μm rather than oil droplets which were around 10 μm.

To lower the density difference between droplets and the continuous liquid phase helps the emulsion system to gain stability (McClements 2008). Therefore, with the help of oil addition and homogenization which enlarged the oil-water interface, the oil droplets resisted aggregation and creaming. Due to the accumulated protein molecules, the density of droplets decreased, thus this decreased the density difference between the dispersed and continuous phases and the stability of the emulsion system was improved.

The average droplet diameter increased with increasing oil concentration in the emulsion

samples, regardless of the storage period of the samples. This corresponds to the study of Gu et al. (2009) which showed that the average oil droplet size increased with the increasing oil (or fat) concentration regardless of the type of oil (or fat) used in the emulsion. In addition, Gu et al. (2009) also mentioned that coalescence/flocculation could be indicated in the larger droplet diameters and the oil surface tensions, which could influence the stability, droplet size or other emulsion properties.

Hydrophobic interactions play an important role in food emulsions which are often stabilized by protein emulsifiers. The surfaces of droplets in the emulsion system may not be totally adsorbed by protein emulsifiers, the attractive hydrophobic interactions between the droplets could affect greatly on the stability of the emulsion system (McClements 2008). Therefore, back to our study, the increased oil concentrations indicated the decreased ratio of oil to protein, which further indicated the more oil-water interface exposure without being covered by protein molecules. Thus the increased average droplet diameters could be attributed to the increased attractive hydrophobic interactions among the droplets as the stabilizing capability of protein emulsifiers decreases. Similar conclusion has been presented in previous studies, Wong and Kitts (2003) attributed the increased droplet size to the increased hydrophobic attraction between oil droplets resulted from the increasing oil to protein ratio. In the study of Sourdet et al. (2003) on milk protein emulsions, the formation of larger droplet size was resulted from the decreasing protein concentrations. Similarly in Parker's study (1987), the smaller droplets were formed due to the reduced interfacial tension on the interface facilitated by the increased protein concentration.

The emulsifying activity index (EAI) is often utilized to describe the emulsifying ability of legume proteins, which is defined as the maximum surface area created per unit protein (Kiosseoglou 2011). Results of this measurement revealed that the relative EAI values decreased with the increasing oil concentration. Interestingly, it was reported in previous study in 2009 (Gu et al.) that for samples emulsified with sunflower and soy oil, the results

were corresponding to our study (samples emulsified with rapeseed oil), whereas for those samples emulsified with palm stearin, the reversed results obtained. This distinction of EAI between liquid oil and solid fat indicates their different emulsifying properties and further research is required for studying on oil (fat) function in bean protein stabilized emulsion system.

In this study, the protein concentrations were constant in all emulsion samples, therefore the decreasing values of EAI indicated the decreasing emulsifying efficiency of the protein involved, regardless of the oil concentrations. This interesting and noticeable finding could be attributed to the increasing hydrophobic interactions among emulsion droplets, when oil concentration increased and more droplet surface exposed into the continuous phase.

The emulsifying stability index (ESI) is often utilized for evaluating the stability of legume protein-stabilized emulsions and is measured by monitoring the decrease in turbidity of a diluted emulsion within a certain standing period (Kiosseoglou 2011). Waniska et al. (1981) defined it as the ability of droplets in emulsion system remaining dispersed without creaming, flocculating, coalescing, or oiling off. ESI of emulsion samples decreased with the increasing oil concentrations. The emulsion sample with 5% (v/v) oil content was an exception, it could not give a result due to the absorbance stay unchanged during the standing period. This result agrees with the explanation of Gu et al. (2009) that ESI decreased with the increasing oil (fat) concentrations regardless of type of oil (fat) used in the samples. In addition, the results also indicate a positive correlation between ESI and protein to oil ratio, indicating the stability of emulsions decreased with the decreasing protein concentrations, which corresponds to Parker's (1987) results and discussion.

Droplet charge (absolute value of ζ – potential) of emulsion samples without oil was considerably lower than those with oil additions, regardless of the concentrations. This corresponded to the droplet size distribution that the oil assisted keeping and stabling the emulsion droplet structure, providing more oil-water interface for protein molecules to

cover on. However, for those samples with oil additions, the droplet charge represented irregularly, without any gradient tendency as the results of droplet size gave. Nonetheless, all the emulsion samples gave approving stabilities, of which the sample without oil addition showed moderate stability with the absolute ζ – potential value of 35mV, whereas the emulsion samples with oil addition showed good stability with the absolute ζ – potential values exceeding 40mV (Greenwood and Kendall, 1999; Hanaor, et al. 2012).

3.4.2 Effect of oil concentrations on textural and rheological properties of faba protein gels

The texture profile tests showed that there was a regular increase in resistance to deformation for samples with increasing oil concentrations. Faba-yogurts made from faba milk with higher oil concentrations (GY_2 with 10% to GY_6 with 30%) showed apparent deformation peaks, indicating that they were apparently deformable with high gel firmness. It can be observed that there was a tendency to increase gel firmness in set type faba-yogurts as oil concentrations increase. Besides, emulsification with progressively higher oil concentrations not only give progressively stronger gels, but also keep higher moduli and show higher viscosity after the set style faba-yogurts were processed to stirred type faba-yogurts.

As to the visual observation of stirred type faba-yogurts, the samples with self-standing gel textures and samples with liquid like state implied that thicker yogurts were obtained for those with higher oil concentrations, which correspond to the rheological analysis of these samples. In addition, Liu and Tang (2011) have already reported the similar observation that a more self-standing and gel-like texture was displayed for those emulsions with higher oil fractions.

The changes in apparent viscosities (η_a) as a function of shear rate of faba-yogurts at various oil concentrations. All the yogurt samples exhibited shear-thinning behaviors of non-Newtonian fluids under the shear rate ranged from 1 to 100 s^{-1} regardless of oil

fractions. Interestingly, in the previous works of Liu and Tang (2011), Manoi and Rizvi (2009) and Boutin et al. (2007), the similar shear-thinning flow behaviors of emulsions at various oil fractions have been observed. Specifically, shear-thinning behaviors were developed as the oil content increased, which were largely attributed to emulsion flocculation that gave result to greater deletion of flocculation as shear rate increased. In addition, such shear-thinning behaviors have been found in emulsions with various preparation treatments as well (Demetriades 1997; Demetriades 1998).

Figure 18 also demonstrates that the η of faba-yogurts increased, to a varying extent, with the increasing oil concentrations from 0% to 30%, which was regarded to be a typical behavior of the protein-stabilized emulsions (Liu and Tang 2011; Manoi and Rizvi 2009; Boutin et al. 2007). This is expected and unvarying with the fact that the oil droplets become close enough as its fraction increased, thus being able to interact with each other and getting aggregated. It is interesting to note that even for sample SY_0 that no oil added, the typical shear-thinning behavior was still observed clearly as other samples with oil addition. Although in many previous works, the shear-thinning behaviors were predominantly attributed to the flocculation in high oil content emulsions, and for those protein-stabilized emulsions with relatively low oil content, or even without oil, the non-Newtonian behaviors were always observed (Boutin et al. 2007). But in our study, sample SY_0 was prepared from sample GY_0, which was fermented from faba protein solution without any oil added, which means the emulsion network in SY_0 (also GY_0) was mainly kept by protein-protein interactions.

The viscoelastic properties of stirred faba-yogurts were measured by frequency sweep tests. The results implied the typical gel structures that our stirred faba-yogurts possess. Besides, all the G’ slopes increased parallel with G’’, indicating that these seven stirred faba-yogurts did not vary greatly in terms of the overall bond nature (Saint-Eve 2006).

In the frequency sweep test, there was an apparent tendency that the complex viscosity

increased as increasing oil fraction, regardless of frequency. Moreover, the stress sweep test illustrated the influence of various oil fractions on the complex viscosity at various shear stresses, and the results under 0.1 and 50 Pa showed that complex viscosity tend to increase with increasing oil concentrations regardless of the magnitude of shear stress. According to Saint-Eve (2006), the results at a low shear stress of 0.1 Pa correspond to the thickness of the yogurt observed with a spoon, while the complex viscosity at the shear stress of 50 Pa correspond to yogurt products under mastication conditions.

It is also interesting to note in the test of shear stress as a function of shear rate that the area of the hysteresis loop, which represents the structural breakdown condition of yogurts during shearing, became higher as the increasing oil fractions as well. As described in previous study by Saint-Eve (2006), these results suggest that oil addition could lead to greater structure deformation and make the product to be damaged more easily. Moreover, it can be also interpreted that in this study, faba-yogurt samples with lower oil concentration was more stable as increasing shear rate. This is also corresponding to the evaluation of emulsion characteristics (EAI and ESI indexes) in this study, indicating that when oil is drawn into and successfully stabilize the emulsion system, the higher the concentration of oil, the lower the emulsifying activity and stability. This is promising in an industrial production prospect for manufactures, which manifests that small amount of added oil for stabilization is actually required during production as long as it is sufficient to stabilized the emulsion system for a favorable period.

3.4.3 Effect of oil concentration on colorimetric and sensory properties of faba-yogurt

Colorimetric analysis of stirred type faba-yogurts was performed to evaluate whiteness changes as a function of oil concentrations. There was no significant change of whiteness index (WI) among yogurt samples with oil concentrations from 5% to 30%, whereas for the only sample without oil added, a dramatic decrease of WI was observed. McClements

(2002) reported that both droplet concentration and droplet radius effect on the color of concentrated oil-in-water emulsions. Interestingly, McClements (2002) reported in this study that emulsion lightness (L^*) increased with increasing oil droplet content, which was attributed to the fact that more oil droplet could multiply scatter more light backwards. Furthermore, similar result was found among the research of McClements (2002) and us that the lightness increased dramatically when the oil droplet fraction increased from 0 to 0.05. However, McClements (2002) reported that the lightness of emulsions was much less steeply increased at higher oil droplet contents, whereas there was not such regular result obtained in our research. This is to be expected resulted from another factor, droplet radius, which was discussed in previous section (3.4.1) and proved to be various level of distribution, influences together with droplet content on the color properties.

From a food development perspective, consequences above significantly suggest a dramatic change of product appearance when the oil droplet fraction decreased to a certain level, which might lead to sacrifice of perceived quality (McClements 2002). This could be seen in a typical case that the skim milk appears quite watery while the full fat milk appears creamy, which is attributed to the much higher oil droplet content in full fat milk (~4%) than skim milk (~0.1%). Therefore, it is believed that oil content plays an important role in food emulsion system for product appearance improvement.

Results of firmness from sensory evaluation mostly corresponded to the texture evaluation, indicating the higher oil concentration, the more firm structure of the emulsion gel. Meanwhile, results of flowability from sensory evaluation corresponded to the rheological analysis, indicating the higher oil concentration, the easier for structure deformation and damage. Yellowness results from sensory evaluation was also corresponding to the colorimetric analysis, manifesting that increasing oil concentration leaded to less whiteness. Additionally, it is interesting to note that there was no significant difference in terms of greasiness among faba-yogurts with various oil concentrations.

Besides, it is also interesting to note that, faba-yogurt sample with 20% oil concentration obtained most preferences from the sensory evaluation, closely followed by those with 30% and 25% oil concentrations. This is far more than the oil concentration of normal cow milk yogurt (~3.5%). Therefore, to a commercial perspective, it is significant to continue further study about how different cow milk protein and bean protein effect on the fermented products, thus balancing the consumer preference and commercial purpose.

4 CONCLUSIONS

Oil addition into faba protein stabilized emulsions led to oil droplets in the emulsion system and this greatly helped keeping the structure stable than those without oil addition. As to emulsifying properties, both emulsifying activity index (EAI) and emulsifying stability index (ESI) decreased with increasing oil concentrations (0% - 30%). Oil addition improved oil droplet surface charge ability (ζ-potential) in the emulsion system. As to faba protein stabilized gels, firmness of both set style and stirred style gels were improved with increasing oil concentrations. The rheological analysis also gave results that the apparent viscosity (η), storage modulus (G') and loss modulus G'' increased with increasing oil concentrations, as well as the gel deformation ability. In addition, whiteness index of gels without oil addition was much less than those with oil additions. As to the faba-yogurt products, those with 20% oil concentrations were most preferred in sensory analysis.

This study focused on the structural effect of various oil concentrations on faba protein stabilized emulsions. Interesting relevance between the oil addition and emulsion properties were found, which gave a significant perspective for both technological research and development of legume based foods. Sensory properties are taken into account as an important factor for food development. The promising results from the sensory evaluation of this study encourage to faba bean application in food formulations. Moreover, as the plant based food is increasingly popular, accepted and demanded, this study developed a new application area of faba bean for legume protein stabilized emulsions.

REFRENCES

Barfod NM, Krog N, Largsen G, Buchheim W. 1991. Effects of emulsifiers on protein-fat interaction in ice cream Mix during Ageing I: Quantitative Analyses. Fett Wissenschaft Technologie/Fat Science Technology 93(1):24-9.

Bergenstahl BA, Claesson PM. 1997. Surface forces in emulsions. In: S. E. Friberg, K. Larsson, Editors. Food emulsions. 3rd ed. New York: Marcel Dekker.

Boutin C. 2007. Characterization and acid-induced gelation of butter oil emulsions produced from heated whey protein dispersions. Int Dairy J 17(6):696-703.

Boye JI, Aksay S, Roufik S, Ribereau S, Mondor M, Farnworth E, Rajamohamed SH. 2010. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Res Int 43(2):537-46.

Boye J, Zare F, Pletch A. 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Res Int. 43(2):414-31.

Braudo EE. 2001. Plant protein interactions with polysaccharides and their influence on legume protein functionality - A review. Nahrung-food 45(6):382-4.

Briggs TR. 1921. Emulsions with Finely Divided Solids. Ind Eng Chem 13(11):1008-10.

Buchheim W, Dejmek P. 1997. Milk and dairy-type emulsions. In: S. E. Friberg, K. Larsson, Editors. Food emulsions. 3rd ed. New York: Marcel Dekker.

Cai R, Klamczynska B, Baik B. 2001. Preparation of bean curds from protein fractions of six legumes. J Agric Food Chem 49(6):3068-73.

Campbell TC, Campbell TM. 2006. The China Study. Dallas, Texas: Benbella books.

Chapleau N, de Lamballerie-Anton M. 2003. Improvement of emulsifying properties of lupin proteins by high pressure induced aggregation. Food Hydrocoll 17(3):273.

Chove BE, Grandison AS, Lewis MJ. 2001. Emulsifying properties of soy protein isolate fractions obtained by isoelectric precipitation. J Sci Food Agric 81(8):759-63.

Clark AH, Lee-Tuffnell CD. 1986. Gelation of globular proteins. p203-272. In: J. R. Mitchell, D. A. Ledward, Editors. Functional Properties of Food Macromolecules. London: Elsevier Applied Science Publishers.

Courthaudon J-, Dickinson E, Dalgleish DG. 1991. Competitive adsorption of β-casein and nonionic surfactants in oil-in-water emulsions. J Colloid Interface Sci 145(2):390-5.

Cserhalmi Z. 1998. Emulsifying properties, surface hydrophobicity and thermal denaturation of pea protein fractions. Acta Aliment 27(4):357-63.

Cui SW. 2005. Food Carbohydrates Chemistry, Physical Properties, and Applications. London : CRC Press.

Dalgleish D. 2003. Food Emulsions. chapter 1. In: Stig E. Friberg, Kåre Larsson, Johan Sjöblom, Editors. Food Emulsions. CRC Press.

De Feijter JA, Benjamins J, Tamboer M. 1987. Adsorption displacement of proteins by surfactants in oil-in-water emulsions. Colloids and Surfaces 27(1-3):243.

Demetriades K. 1998. Influence of pH and heating on physicochemical properties of whey protein-stabilized emulsions containing a nonionic surfactant. J Agr Food Chem 46(10):3936-42.

Demetriades K. 1997. Physical properties of whey protein stabilized emulsions as related to pH and NaCl. J Food Sci 62(2):342-7.

Dickinson E, Woskett CC. 1989. Competitive adsorption between proteins and small-molecule surfactants in food emulsions. In: R. D. Bee, P. Richmond, J. Mingins, Editors. Food Colloids. London: Royal Society of Chemistry.

Dickinson E. 1992. Structure and composition of adsorbed protein layers and the relationship to emulsion stability. J Chem Soc Faraday T 88(20):2973-83.

Doi E. 1989. Structure of glycinin and ovalbumin gels. Food Hydrocoll 3(4):327-37.

Donkor ON. 2007. Rheological properties and sensory characteristics of set-type soy yogurt. J Agric Food Chem 55(24):9868-76.

Drake MA, Gerard PD. 2003. Consumer attitudes and acceptability of soy-fortified yogurts. J Food Sci 68(3):1118-22.

Edwards DA. 1991. A micromechanical model of linear surface rheological behavior. Chem Eng Sci 46(5-6):1247-57.

Farooq Z, Boye JI. 2011. Novel food and industrial applications of pulse flours and fractions. chapter 11, p5-11. In: Brijesh K. Tiwari, Aoife Gowen, Brian McKenna, Editors. Pulse Foods: Processing, Quality and Nutraceutical Applications. Academic Press.

Fernández-Quintela A, Macarulla MT, Del Barrio AS, Martínez JA. 1997. Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. Plant Food Hum Nutr 51(4):331-42.

Ferragut V, Cruz NS, Trujillo A, Guamis B, Capellas M. 2009. Physical characteristics during storage of soy yogurt made from ultra-high pressure homogenized soymilk. J Food Eng 92(1):63-9.

Fuhrmeister H, Meuser F. 2003. Impact of processing on functional properties of protein products from wrinkled peas. J Food Eng 56(2):119.

Galazka VB. 1999. Emulsifying behaviour of 11S globulin Vicia faba in mixtures with sulphated polysaccharides: comparison of thermal and high-pressure treatments. Food Hydrocoll 13(5):425-35.

Gebre-Egziabher A. 1983. Preparation of high protein curd from field peas. J Food Sci 48(2):375.

Gelin J-, Poyen L, Rizzotti R, Le Meste M, Courthaudon J-, Lorient D. 1996. Interactions between food components in ice cream. Part 1: unfrozen emulsions. Food Hydrocoll 10(4):385-93.

Goff HD. 1997. Instability and partial coalescence in whippable dairy emulsions. J Dairy Sci 80(10):2620-30.

Greenwood R, Kendall K. 1999. Selection of suitable dispersants for aqueous suspensions of zirconia and titania powders using acoustophoresis. J Eur Ceram Soc 19(4):479-88.

Gu X, Campbell LJ, Euston SR. 2009. Effects of different oils on the properties of soy protein isolate emulsions and gels. Food Res Int 42(8):925-32.

Hanaor D, Michelazzi M, Leonelli C, Sorrell CC. 2012. The effects of carboxylic acids on the aqueous dispersion and electrophoretic deposition of ZrO2. J Eur Ceram Soc 32(1):235-44.

Hou H, Chang K. 2003. Science and Technology of Tofu Making. p16-17. In: Anonymous Handbook of Vegetable Preservation and Processing. CRC Press.

Kantha SS. 1983. Laboratory scale production of winged bean curd Psophocarpus tetragonolobus. J Food Sci 48(2):441-4.

Karaca AC, Low N, Nickerson M. 2011. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Res Int 44(9):2742-50.

Kaur M, Singh N. 2007. Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. Food Chem 102(1):366-74.

Kim K-, Renkema JMS, Van Vliet T. 2001. Rheological properties of soybean protein isolate gels containing emulsion droplets. Food Hydrocoll 15(3):295-302.

Kinsella JE. 1979. Functional-properties of soy proteins. J Am Oil Chem Soc 56(3):242-58.

Kiosseoglou V, Paraskevopoulou A. 2011. Functional and physicochemical properties of pulse proteins. p1-19. In: Brijesh K. Tiwari, Aoife Gowen, Brian McKenna, Editors. Pulse Foods: Processing, Quality and Nutraceutical Applications. Academic Press.

Kiosseoglou A, Doxastakis G, Alevisopoulos S, Kasapis S. 1999. Physical characterization of thermally induced networks of lupin protein isolates prepared by isoelectric precipitation and dialysis. Int J Food Sci Tech 34(3):253-63.

Kohyama K, Yoshida M, Nishinari K. 1994. Erratum: Rheological study on gelation of soybean 11S protein by glucono-delta-lactone (Journal of Agricultural and Food Chemistry (1992) 40, (740-744)). J Agric Food Chem 42(9):2076.

Lefebvre J, Dagorn-Scaviner C, Gueguen J. 1987. Emulsifying properties of pea globulins as related to their adsorption behaviors. J Food Sci 52(2):335.

Liu F, Tang C-. 2011. Cold, gel-like whey protein emulsions by microfluidisation emulsification: Rheological properties and microstructures. Food Chem 127(4):1641-7.

Liu M, Lee D-, Damodaran S. 1999. Emulsifying properties of acidic subunits of soy 11S globulin. J Agric Food Chem 47(12):4970-5.

Mackay G. 1973. The formation of water-in-oil emulsion subsequent to an oil spill. J Inst Petrol 59(568):164-172.

Manoi K, Rizvi SSH. 2009. Emulsification mechanisms and characterizations of cold, gel-like emulsions produced from texturized whey protein concentrate. Food Hydrocoll 23(7):1837.

McClements DJ. 2002. Theoretical prediction of emulsion color. Adv Colloid Interface Sci 97(1-3):61.

McClements DJ. 2005. Emulsion ingredients. Food Emulsions: Principles, Practices, and Techniques. chapter 4. 2nd. ed. CRC Press.

McClements DJ. 2007. Critical Review of Techniques and Methodologies for Characterization of Emulsion Stability. Crit Rev Food Sci Nutr 47(7):611-49.

McClements DJ. 2008. Lipid-Based Emulsions and Emulsifiers. chapter 3, p3-30. In: Casimir C. Akoh and Dacid B. Min, Editors. Food Lipids: Chemistry, Nutrition and Biotechnology. 3rd. ed. CRC Press.

Molina E, Papadopoulou A, Ledward DA. 2001. Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. Food Hydrocoll 15(3):263-9.

Norton IT. 2006. Fluid gels, mixed fluid gels and satiety. Food Hydrocoll 20(2):229-39.

Norton IT. 2001. Microstructure design in mixed biopolymer composites. Food Hydrocoll 15(4-6):543-53.

Ono T. 1996. Interaction of protein particles with lipids in soybean milk. Biosci Biotechnol Biochem 60(7):1165-9.

Oomah BD, Patras A, Rawson A, Singh N, Compos-Vega R. 2011. Chemistry of pulses. chapter 2, p1-7. In: Brijesh K. Tiwari, Aoife Gowen, Brian McKenna, Editors. Pulse Foods: Processing, Quality and Nutraceutical Applications. Academic Press.

Papalamprou EM, Doxastakis GI, Kiosseoglou V. 2010. Chickpea protein isolates obtained by wet extraction as emulsifying agents. J Sci Food Agric 90(2):304-13.

Papalamprou EM, Doxastakis GI, Biliaderis CG, Kiosseoglou V. 2009. Influence of preparation methods on physicochemical and gelation properties of chickpea protein isolates. Food Hydrocoll 23(2):337-43.

Papalamprou EM, Makri EA, Kiosseoglou VD, Doxastakis GI. 2005. Effect of medium molecular weight xanthan gum in rheology and stability of oil-in-water emulsion stabilized with legume proteins. J Sci Food Agric 85(12):1967-73.

Parker NS. 1987. Properties and functions of stabilizing agents in food emulsions. Crit Rev Food Sci Nutr 25(4):285-315.

Pearce KN, Kinsella JE. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J Agric Food Chem 26(3):716-23.

Pyo YH. 2009. Physicochemical and sensory characteristics of a medicinal soy yogurt containing health-benefit ingredients. J Agric Food Chem 57(1):170-5.

Rivera-Espinoza Y. 2010. Non-dairy probiotic products. Food Microbiol 27(1):1-11.

Roesch RR, Corredig M. 2002. Characterization of oil-in-water emulsions prepared with commercial soy protein concentrate. J Food Sci 67(8):2837-42.

Rousseau D. 2000. Fat crystals and emulsion stability - a review. Food Res Int 33(1):3-14.

Saint-Eve A. 2006. Complex viscosity induced by protein composition variation influences the aroma release of flavored stirred yogurt. J Agric Food Chem 54(11):3997.

Saio K. 1969. Protein-calcium-phytic acid relationships in soybean. III. effect of phytic acid on coagulative reaction in tofu-making. Agr Biol Chem (1):36-42.

Sánchez-Vioque R, Clemente A, Vioque J, Bautista J, Millán F. 1999. Protein isolates from chickpea (Cicer arietinum L.): Chemical composition, functional properties and protein characterization. Food Chem 64(2):237-43.

Saxena MC. 1993. Faba bean production and research in China : proceedings of an international symposium : 24-26 May 1989, Hangzhou, China / editors, Mohan C. Saxena, Susanne Weigand, Lang Li-juan ; sponsors, Chinese Academy of Agricultural Sciences, Zhejiang Academy of Agricultural Sciences, International Center for Agricultural Research in the Dry Areas. Aleppo, Syria: International Center for Agricultural Research in the Dry Areas, 1993.xi, 179 pp. xi.

Schulman JH, Leja J. 1954. Control of contact angles at the oil-water-solid interfaces: Emulsions stabilized by solid particles (BaSO4). Transactions of the Faraday Society 50(6):598-605.

Sijtsma L, Tezera D, Hustinx J, Vereijken JM. 1998. Improvement of pea protein quality by enzymatic modification. Nahrung/Food 42(3,4):215-6.

Sourdet S. 2003. Effects of milk protein type and pre-heating on physical stability of whipped and frozen emulsions. Colloids Surf B 31(1-4):55.

Swaisgood HE. 1996. Characteristics of milk. In: Fennema OR, Editor. Food Chemistry. 3rd ed. New York: Marcel Dekker.

Swanson BG. 1990. Pea and lentil protein extraction and functionality. J. Am. Oil Chem Soc. 67(5):276-80.

Thakur G, Mitra A, Basak A, Rousseau D, Pal K. 2010. Characterization of oil-in-water gelatin emulsion gels: Effect of homogenization time. International Conference on Systems in Medicine and Biology, ICSMB Dec.2010. p305-8

Tiwari BK, Gowen A, McKenna B. 2011. Introduction. p1-6. In: Brijesh K. Tiwari, Aoife Gowen, Brian McKenna, Editors. Pulse Foods: Processing, Quality and Nutraceutical Applications. Academic Press.

Tsoukala A. 2006. Adsorption at the air-water interface and emulsification properties of grain legume protein derivatives from pea and broad bean. Colloids Surf B 53(2):203-8.

Utsumi S. 1985. Forces involved in soy protein gelation: Effects of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S, and soy isolate. J Food Sci 50(5):1278-82.

van Aken GA. 2003. Coalescence mechanisms in protein-stabilized emulsions. p21-22. In: Friberg Stig, Larsson Kare, Sjoblom Johan. Food Emulsions. CRC Press..

Visschers RW. 2005. Disulphide bond formation in food protein aggregation and gelation. Biotechnol Adv 23(1):75-80.

Vose JR. 1980. Production and functionality of starches and protein isolates from legume seeds (field peas and horsebeans). Cereal Chem 57(6):406-10.

Walstra P. 2002. Proteins. chapter 7, p11-38. Physical Chemistry of Foods. CRC Press.

Walstra P. 1987. Overview of emusion and foam stability. p242-257. In: E. Dickinson, Editor. Food Emulsions and Foams. London: Royal Society of Chemistry.

Walstra P. 1993. Principles of emulsion formation. Chem Eng Sci 48(2):333-49.

Waniska RD, Shetty JK, Kinsella JE. 1981. Protein-stabilized emulsions: Effects of modification on the emulsifying activity of bovine serum albumin in a model system. J Agric Food Chem 29(4):826.

Wong PYY, Kitts DD. 2003. A comparison of the buttermilk solids functional properties to nonfat dried milk, soy protein osolate, dried egg white, and egg yolk powders. J Dairy Sci 86(3):746.

Yamagishi T. 1987. Covalent polymerization of acidic subunits on hear-induced gelation of soybean glycinin. Cereal Chem 64(4):207-12.

Yamano Y. 1981. Incorporation of lipid into soybean protein gel and the role of 11S-protein and 7S-protein. Japan Soc Food Sci Technol 28(3):136-41.

Zhang T, Jiang B, Wang Z. 2007. Gelation properties of chickpea protein isolates. Food Hydrocoll 21(2):280-6.

Zhang T, Jiang B, Mu W, Wang Z. 2009. Emulsifying properties of chickpea protein isolates: Influence of pH and NaCl. Food Hydrocoll 23(1):146-52.

Zhang Y, Tang C, Wen Q, Yang X, Li L, Deng W. 2010. Thermal aggregation and gelation of kidney bean (*Phaseolus vulgaris* L.) protein isolate at pH 2.0: Influence of ionic strength. Food Hydrocoll 24(4):266-74.

APPENDICES

Appendix 1. Sensory Evaluation Form

Product: Faba-Yogurt **Date:** 31.08.2012

Instructions:

You are asked to mark in the horizontal line with a single cross (×) that corresponds to your criticism in between 1 to 5, where the descriptions of 1 and 5 have been shown in the brackets.

There are 7 samples to be evaluated and the whole test will be separated into two parts, 3 and 4 samples for each respectively, with a 15min break.

Enjoy!

Factors

1. Yellowness (natural white – light yellow)

Score / Sample	1 5	Comments
A	I---------- I	
B	I---------- I	
C	I---------- I	
D	I---------- I	
E	I---------- I	
F	I---------- I	
G	I---------- I	

2. Firmness (thin like milk – firm like cream cheese)

Score / Sample	1 5	Comments
A	I---------- I	
B	I---------- I	
C	I---------- I	
D	I---------- I	
E	I---------- I	
F	I---------- I	
G	I---------- I	

3. Flowability (smooth like milk – ropy like mayonnaise, aiming to evaluate how quick samples melt and swallowed)

Score / Sample	1 5	Comments
A	I---------- I	
B	I---------- I	
C	I---------- I	
D	I---------- I	
E	I---------- I	
F	I---------- I	
G	I---------- I	

4. Greasiness (water - oil)

Score / Sample	1 5	Comments
A	I---------- I	
B	I---------- I	
C	I---------- I	
D	I---------- I	
E	I---------- I	
F	I---------- I	
G	I---------- I	

5. Overall preference

Score / Sample	1 5	Comments
A	I---------- I	
B	I---------- I	
C	I---------- I	
D	I---------- I	
E	I---------- I	
F	I---------- I	
G	I---------- I	

Thank you very much for your time! We do appreciate for your participation! :)

Free Talk Time please feel free to share your opinions about the ‘products’ with others and also leave your feedbacks.

Feedbacks: