CHARACTERIZATION OF OIL-IN-WATER EMULSIONS PREPARED WITH SOY PROTEIN CONCENTRATE BY HIGH PRESSURE HOMOGENIZATION.

by

RODRIGO ROESCH

(Under the direction of Milena Corredig)

ABSTRACT

Soy protein concentrate (SPC) is a low cost ingredient containing about 70% protein and 20% fiber. In spite of their widespread utilization, there is a lack of understanding of the functional properties of commercial soy isolates. A better knowledge of the behavior of SPC in-oil-in-water emulsions will optimize its utilization in many soy-based formulations. For this reason, a model oil-in-water emulsion prepared by high-pressure homogenization was studied. Emulsions were prepared with SPC and soybean oil and homogenized at 80 MPa. Samples were characterized for stability, microstructure, particle size, rheological properties and protein adsorption behavior.

The increment of volume fraction of soybean oil from 0.1 to 0.2 resulted in emulsions system with very little similarities. All emulsions containing 20% oil showed a creamy texture, gel-like behavior, good water holding capacity and stability to creaming. Emulsions prepared with >4% SPC and 10% oil were stable and showed flow behavior. Heating of 20% oil-in-water emulsions before homogenization resulted in emulsions with higher elasticity than those prepared with no heat.

INDEX WORDS: Soy protein concentrate, high-pressure homogenization

oil-in-water emulsions, soy protein, microstructure.

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RODRIGO ROESCH

Licentiate, San Carlos University of Guatemala, Guatemala, 1998

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2002

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RODRIGO ROESCH

Approved:

Major Professor: Milena Corredig

Committee: Louise Wicker
Rakesh Singh

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
May 2002

Dedicated To:

God, My mother Lily Palomo, my father Rodolfo Roesch and my brothers Ivan and Alexis

ACKNOWLEDGMENTS

To triumph in life, it is always necessary a light, a guide and well directed orientation. Therefore, I sincerely want to express my appreciation and gratitude to my advisor and friend Dr. Milena Corredig for her encouragement to think in a linear way, and all the interest and dedication given to me in the trajectory of my studies. With her support and knowledge offered to me, today I see my goals achieved. Thank you Milena for your teachings, your patience, and hard and good moments.

I wish to thank the support of my committee members, Dr. Louis Wicker and Dr. Rakesh Singh. I thank Dr. Romeo Toledo for his advices. I would like to thank Dr. William Monagle (Central Soya Research and Development, Fort Wayne, Indiana, USA) for his advices and valuable contribution. I thank Central Soya, for donating the soy protein concentrate.

I acknowledge the help of Dr. John Shields and Dr. Mark Farmer from The Center for Advanced Ultrastructural Studies at the University of Georgia.

My special thanks to my cousins Kerry and Wayne Parrot for their support, trust and helping me to enroll in a graduate program. This has opened new opportunities for my carrier.

Special thanks goes to my mother Lily for her love and support.

I would like to thank my friend Yusuf Yilmaz for his help in the statistical analysis and format checking. His friendship is appreciated.

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CHAPTER 1

INTRODUCTION

1.1. Introduction

The recent health claim on soy proteins by FDA (FDA, 1999) has caused an increased consumer demand for formulated foods containing soy (U.S. retail sales of soyfoods were $ 0.85 billion in 1992 and are projected to rise to $ 3.7 billion in 2002) (FDA, 2002).

Soy protein concentrate (SPC) is a low-cost ingredient containing low molecular surface-active components, about 70% protein and 20% polysaccharides, the latter mostly insoluble (with a solubility of about 1.5%). SPC derives from a conventional alcohol-wash process of hexane-extracted soy flakes and is employed as an ingredient in foods because of its functionality (it is claimed to impart stability and increase water binding) and nutritional value (high in protein and fiber). In addition, the low cost makes SPC a widespread ingredient in many formulated foods, especially in meat products.

SPCs are commercially advertised as ingredients that maintain their functionality in "stressed" conditions such as high fat and high moisture formulations, freeze/thaw cycles and pre-cooked foods to be reconstituted (Anonymous, 1998). The presence of soy fiber makes SPC different from soy isolates; SPC seems to perform better under shear conditions than SPI (SPC is the ingredient of choice for many extruded products). In spite of its widespread utilization, limited information is available on the utilization of SPC in formulated foods, the effect of processing on the functionality of SPC, and the

role played by the different components in food products. A better understanding of the functional properties of SPC will help to optimize its utilization in new soy-based formulations and further increase the demand for such ingredient.

The objectives of this research are as follows:

i) To study the effects of dynamic high-pressure homogenization on oil-in-water emulsions prepared with soy protein concentrate (SPC).

ii) To understand the role played by the different components of SPC in oil-in-water emulsions by microstructural characterization.

To achieve these objectives the effect of SPC and oil concentration on the stability, microstructure and rheological properties of oil-in-water emulsions prepared by high-pressure homogenization were evaluated. In addition, the changes occurring upon heating before homogenization were also studied.

The hypotheses of this research are:

i) SPC is able to produce stable emulsions.

ii) Oil-in-water emulsions containing SPC are unique in their microstructure and viscoelastic properties.

Results from this research will contribute to evaluate the potential utilization of SPC as an ingredient in many novel soy-based foods. In addition, while much research has been focused in understanding properties of purified soy proteins and to a less extent soy protein isolates, little or no work is available on the functionality and interactions of commercial SPC. Therefore, these results will provide information on SPC functionality that will help understanding differences in functional behavior between SPC and other soy ingredients reported in the literature.

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CHAPTER 2

LITERATURE REVIEW

2.1 Soy History

Humans have been consuming soybeans for more than 5,000 years. The first report of crops grown by men dates around 2838 B.C. when the Chinese Emperor Cheng-Nung began promoting soy agriculture in his kingdom. The ancient Chinese honored soybeans as one of the five sacred grains (referred as "Wu Ku") essential to the existence of Chinese civilization, and considered the soybean both as a food and as a medicine (Anonymous, 2001a).

By the first century AD, soybeans spread to central and southern China and Korea. In the VII century, soybeans made their way to Japan, and then to Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal and northern India. This plant remained a secret of the Orientals until the XVII century, when European visitors to the East became aware of this crop. Soy first reached the Western shores as imported soy sauce, and by early 1700 soybeans were grown in Europe (Anonymous, 2002a; Anonymous, 2002b).

Soybeans were used in North America to make soy sauce and soy noodles for export to England. Benjamin Franklin contributed to the introduction of soybeans to America, by sending seeds from London to North America in 1770 to a botanist friend (Anonymous, 2002a; Anonymous, 2001b).

From 1804 to 1890 numerous introductions of soybean varieties were made from China and Japan to the U.S. After 1890, soybean research intensified and the first publication devoted solely to soybean was published by the USDA in 1899. Before 1898 only eight soybean varieties were known in the U.S.; by the year 1910, 280 varieties and types were available. More than 11,000 introductions of soybean germplasm have come to the United States since then (Anonymous, 2002a; Anonymous, 2002b).

Gradually, soybeans spread to farming areas, and were grown primarily as animal feed. By 1935 soybean meal was conventionally used as of livestock and poultry feed. Even though extensive research began to reveal the soybean's superior nutritional properties, it did not become a recognized food crop until the beginning of XX century (Anonymous, 2001a; Anonymous, 2001b).

By World War I, soybeans became a valuable source of oil and of inexpensive, high-quality protein. In fact, less than 20% of the seed is oil and 35-40% is protein meal. By 1938 the U.S. was a net exporter of soybean meal to Canada and Europe. The U.S. approximately produces 50% of the total production of soybean in the world, while Brazil is the number two-world producer ranging from 17-19%; Argentina and China rank third or fourth producing 10-12% of the world production (Anonymous, 2001a). Soybean is presently the major world oilseed, for example, in the U.S the soybean oil production for the year 2001 was 24,751 million lb (USDA, 2002).

2.2 Soy Protein Health Claims

In October 1999, the FDA authorized the use of health claims on labeling of food containing soy protein. The agency reviewed results from various research laboratories demonstrating the effect of soy protein value in lowering the levels of total blood

cholesterol and low-density lipoproteins (LDL) (Food and Drug Administration, 1999). Soy protein can reduce plasma concentrations of total and LDL cholesterol without adversely affecting the levels of the high density lipoproteins (HDL).

Other studies have indicated the benefits of soy consumption in preventing other chronic diseases (Food and Drug Administration, 1999). Soy contains isoflavones which have been shown to prevent osteoporosis and menopausal symptoms and may inhibit enzymes necessary for the growth and spread of many types of cancer such as breast, prostate, colon cancer (Anonymous, 2002b).

The FDA authorized claim is as follows "Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease". To qualify for the claim foods must contain: "6.26 g of soy protein, less than 3 g of fat, less than 1 g of low saturated fat, less than 20 mg of cholesterol, less than 480 mg of sodium"(Food and Drug Administration, 1999). Foods that may be eligible for the health claim include soy beverages, tofu, tempeh, soy-based meat alternatives, and some baked goods.

Soy protein products can be good substitutes for animal products because, unlike some other beans, soy offers a complete protein profile. Soybean contains all the amino acids essential to human nutrition; therefore, they can be used without major adjustments elsewhere in the diet (Food and Drug Administration, 1999).

2.3 Soybean Composition

Mature soybean seeds are made of three basic parts: the seed coat, the embryo, and food storage structures. The outer layer of the seed coat constitutes about 8% of the total bean weight. Soybean is one of the highest protein-containing legumes. In the

soybean seed both storage protein and lipid bodies are contained in the cotyledon, while the complex carbohydrates are present in the cell wall. The composition of a raw, dehulled bean depends on the seed variety and is approximately 38% protein, 18% oil, 15% soluble carbohydrates, 15% insoluble carbohydrates, 14% moisture and less than 0.1% ash.

2.4 Processing

After selecting the beans based on color and size; they are cleaned, conditioned, cracked, dehulled and rolled into flakes. The flakes are subjected to a solvent bath to extract the oil. The solvent is then removed and the flakes are dried, creating defatted soy flakes, which are the starting material of all protein products (Figure 2.1).

2.5 Soy Protein Ingredients

2.5.1 Soy flour

Flour is the simplest soy protein ingredient, with a protein content of approximately 50% soy. Flour is produced by simply grinding and screening defatted soy flakes, it still contains many of the original characteristics of the soybean. Flours are high in oligosaccharides, soluble carbohydrates that seem to be responsible for the "beany" flavor, characteristic of soybean, and some of the antinutritional properties of soybean (Liu, 1997). The minimal processing of soy flour makes it highly variable in quality. Soy flours and grits are widely produced and are used most often in baked goods, snacks foods and pet foods (Liu, 1997; Anonymous, 1998).

2.5.2 Soy protein concentrates

The concentrates are prepared by removing the soluble carbohydrate fraction as well as some flavor components from the defatted meal. These products are

approximately 70% protein, containing almost all of the protein available in the bean and retaining much of its dietary fiber. In the 1960s, traditional soy protein concentrates were developed to overcome the problem of soy flours (for example presence of oligosaccharides), without increasing the ingredient cost. Since then, concentrates have been extensively used in processed foods and have become even more widespread in the 1970s when extrusion made textured soy concentrates a reality. Early, less successful, attempts at producing textured soy products were carried out with soy flour (Liu, 1997).

In the 1980s a new generation of soy proteins concentrates "functional protein concentrates" was developed by Central Soya (Anonymous, 1998). They are inexpensive soy ingredients, low in flavor and sodium, high in protein and dietary fiber. The fiber still present in these SPCs is composed of pectin, hemicelluloses (mainly xylose, glucose, galactose and arabinose), hot water soluble polysaccarides (composed of arabinans, arabinogalactans), galactomannans and acidic polysaccharide complexes (composed of galacturonic acid, galactose, arabinose, xylose, rhamnose, fucose) (Furuta et al., 1998; Stombaugh et al., 2000).

Three processes have been used for carbohydrates removal from the protein flour (Liu, 1997): acid leaching, aqueous ethanol (60-80%) extraction, and moist heat-water leaching. All treatments insolubilize the protein fraction while a portion of the carbohydrates remains soluble and is separated by centrifugation. Solids containing mainly proteins and insoluble carbohydrates are then dispersed in water, neutralized (to pH 7.0) and spray-dried to produce soy concentrates (Liu, 1997)

Aqueous alcohol extraction is the method commonly employed for the commercial production of soy protein concentrates (Liu, 1997; Anonymous, 1998). In

this process, alcohol soluble carbohydrates and minor flavor/odor compounds are extracted from the defatted flour using a countercurrent stream of aqueous alcohol. The wet flakes containing proteins and insoluble polysaccharides are continuously removed, desolventized, and dried to yield the protein concentrate. The alcohol is recovered and eventually reused. The protein concentrate produced by this process has low nitrogen solubility (Liu, 1997).

During acid leaching defatted soy flakes are leached with water at pH 4.5 to remove the soluble carbohydrates. Typically an extraction is carried out for 30-45 min at 40^{o}C. The insoluble residue containing proteins is separated by centrifugation, neutralized to pH 7.0, and spray-dried. The soy protein concentrates have a higher nitrogen solubility than those processed alcohol by extraction (Lawhon et al., 1981; Liu, 1997).

In the moist-heat water-leaching process defatted soy flakes are heat-treated to denature proteins and precipitate them. Soluble carbohydrates and salts are then removed from the insoluble protein and polysaccharide material with water (Norris, 1964) or aqueous organic solvents in the temperature range of 66^{o}C-93^{o}C and at a pH ranging from 5.3-7.5 (McAnelly, 1964).

2.5.3 Soy protein isolates

Soy isolates contain about 90% protein and they are free of fiber. Depending on the process, their solubility varies, and they may at times be high in sodium. The production of soy isolates is quite costly when compared to that of protein concentrates. Soy isolates are prepared by various processes such as separation by molecular weight, membrane processing, aqueous extraction, salt extraction, separation of intact protein

bodies (Lawhon et al., 1981; Liu, 1997). Figure 2.2 illustrates a conventional commercial process of production of soy isolates.

2.6 Soy Protein Characterization

Based on their biological function, seed proteins can be distinguished in two types: metabolic proteins and storage proteins. Metabolic proteins are involved in normal cellular activities (for example enzymes). Storage proteins are synthesized for the seed development: following seed germination they provide a source of nitrogen and carbon. The majority of the protein in soybean is found in the protein bodies, and it has a storage function (Liu, 1997).

Seed proteins are often divided into albumins and globulins depending on their solubility. Albumins are soluble in water, whereas globulins are soluble at high ionic strength (Liu, 1997). Most soy proteins are globulin. Globulins are further divided in two distinct types: legumin and vicilin. Compared with vicilins, legumins have larger molecular size, are less soluble in salt solutions, and have higher thermal stability. They also constitute a major part of the total seed globulins.

In soy proteins the two types of globulins are glycinin and conglycinin (Hartwing et al., 1990). These common names are apparently derived from the genus name of soybean plant, *Glycine*. Other proteins are named according to their biological function, for example hemagglutinin, trypsin inhibitor, and lipoxygenase. Soybean proteins characterization is based on the sedimentation coefficient after ultracentrifugation (Howart et al., 1983). During ultracentrifugation, under appropriate buffer conditions, soy proteins separate into four fractions: 2S, 7S, 11S, and 15S (S stands for Svedeburg unit) (Liu, 1997).

The 11S fraction is the soybean glycinin and accounts for at least 30% of the extractable protein. The 15S fraction is thought to be a polymer of glycinin and accounts for about 10% of the extractable protein. Both fractions precipitate at pH 4.5-4.8, and they are called acid-precipitated proteins (Yamauchi et al., 1991). In contrast, the 2S and 7S fractions are heterogeneous proteins. The 7S fraction consists of conglycinin, α-amylase, lipoxygenase, and hemaglutinin (Nielsen, 1985). The 2S fraction accounts for about 20% of the extractable protein and includes the Kunitz and Bowman-Birk trypsin inhibitor and cytocrome C. The complex composition of soy proteins is illustrated in Figure 2.3 where electrophoretic migration of soy protein contained in a soy protein isolate is shown.

2.6.1 Protein fractionation

Several methods have been developed to isolate soy fractions (Thanh and Shibasaki, 1976; Nagano et al., 1992) and subunits (Damodaran and Kinsella, 1982). These methods have been widely used to study the different properties of individual soy fractions. For example, using the Thanh and Shibasaki method, Liu and Coworkers (1999) isolated 11S globulin from a low-heat-treated defatted soy meal and studied its emulsifying properties. A modification of Nagano (1992) has been recently developed by Wu et al. (2000), and it is shown in Figure 2.4.

2.6.2 β-Conglycinin (7S Globulin)

β-conglycinin accounts for about 30% of the total seed protein. 7S globulin is often refereed to as β-conglycinin, however, it also consists of α-conglycinin and the basic 7S globulin. It is a quaternary trimeric glycoprotein containing from 4 to 5%

carbohydrates (Pernollet and Mosses, 1983) with a molecular weight of about 180 kDa consisting of three prevalent types of subunits: α' (57-72 kDa), α (57-68 kDa), and β (42-52 kDa). β-conglycinin forms dimers (9S globulin) at pH 7.6 and ionic strength less than 0.1 (Koshiyama, 1968). The subunits have isoelectric points of 5.3, 5.2, and 5.8-6.2 respectively (Thanh and Shibasaki, 1976). In addition, another subunit named β' is present but only in some soybean varieties (its primary structure is still unknown, but the amino acid composition suggested that this subunit is rich in sulfur-containing amino acids) (Coates et al., 1985; Morita et al., 1996). The subunits are non-covalently associated via hydrophobic interactions and hydrogen bonding (Yamauchi et al., 1991). They can be resolved by ion-exchange chromatography after denaturation of the native complex with urea (Thanh and Shibasaki, 1976) or by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. All three subunits are rich in aspartate/asparagine, glutamate/glutamine, leucine and arginine (Liu, 1997).

2.6.3 15S Globulin

The 15S fraction has a molecular weight of 506 kDa (Marcone et al., 1994) and it is often found during 11S preparation. 15S seems to have similar characteristics as the 11S fraction: it dissociates into subunits at low ionic strengths and in the presence of detergents and urea (Wolf and Briggs, 1958). Catsimpoolas (1977) suggested that the 15S fraction might be a polymer or copolymer of β-conglycinin. It was recently demonstrated that 15S fraction is an aggregate of 11S (Marcone et al., 1994). Nevertheless, it is still not clear whether the soybean 15S exists as a native protein in the seed or it is an artifact formed during protein isolation (Wolf and Nelsen, 1996). It has been suggested that 15S

exists in the meal: it is found in extracts obtained by simply stirring defatted meal in water or salt (Wolf and Briggs, 1956).

2.6.4 Glycinin (11S Fraction)

Glycinin is the largest single fraction of total seed protein (25-35%) (Murphy and Resurrection, 1984). At pH 7.6 and high ionic strength (0.5 M) glycinin is a hexamer (11S globulin) with a molecular weight of about 360 kDa, while at pH 3.8 and low ionic strength (0.03 M) glycinin has trimeric form (7S form) with a molecular weight of 180 kDa (Lakemond et al., 2000). Each monomer is composed of acidic (34-44 kDa and isoelectric point of 5.0) and basic (20 kDa and isoelectric point of 8.0) subunits, which are linked together by disulfide bonds. Its quaternary structure is stabilized by electrostatic and hydrophobic interactions and by S-S between A-B. Six different acid polypeptides (A_{1a}, A_{1b}, A_2-A_4 with a molecular weight of 37-45 kDa, and A_5 with molecular weight of 10 kDa) and five basic polypeptides (B_{1a}, B_{1b}, B_2-B_4; with a molecular weight about 22.5 kDa) have been isolated (Liu, 1997).

2.6.5 Functional properties of soy protein

Soy proteins have been widely used as a source of highly functional proteins in many formulated food products (Molina et al., 2001). Among the most important functional properties attributed to soy protein are solubility, water holding capacity, gelation, emulsification and foaming.

Solubility is governed by intrinsic and extrinsic factors influencing the equilibrium between protein-protein and protein-solvent interactions (Damodaran, 1994); intrinsic factors include hydrophobicity, size, charge, steric properties, and extrinsic

factors are pH, type and ionic strength of various salts, and interactions with other molecules in solution.

Soy proteins are not soluble in their isoelectric region (pH 4.2 to 4.6); their solubility increases above and below this pH range (Liu, 1997). Among salts, sulfite salts have shown the strongest effect in reducing soy protein solubility; for other salts in general, as the ionic strength increases up to 0.1 M, the solubility of soy proteins decreases, while at concentrations > 0.1 M the solubility increases (Boatright and Heattiarachchy, 1995).

Soy proteins foaming properties are closely related to their solubility (Liu, 1997). Foaming can be enhanced by heating at 75-80^{o}C or by limited hydrolysis. For example, it has been reported that with limited hydrolysis by papain, soy proteins had foaming properties similar to those of egg white (Were et al., 1996). Kim et al. (1985) suggested that the foaming properties of glycinin are limited by its closed packed globular conformation. Native glycinin adsorbs at the air water interface with difficulty because of its low surface hydrophobicity, low molecular flexibility, and high molecular size (Wagner and Gueguen, 1999). The surface behavior and functionality of glycinin can be enhanced by chemical modifications in its structure such as reduction, succinylation, acetylation or deamidation (Krause et al. 1996; Wagner and Gueguen, 1999).

Because of its emulsifying properties, soy has been utilized as a functional ingredient in many meat products (Pearson et al., 1965; Inklaar and Fortuin, 1969). Soy proteins have been used in the manufacture of emulsions such as coffee whiteners, non-dairy beverages, mayonnaise (Eida et al., 1978). It is known that the surface and emulsifying properties of proteins are correlated to their structure (Damodaran, 1994).

Environmental and processing conditions such as pH, ionic strength, and temperature affect the structure and functional properties of soy protein (Hutton and Campell, 1977). It has been shown that below the isoelectric point and between pH 6.5 and 7.4 soy proteins produce a viscous emulsion by forming a skin-like cohesive film around the oil droplets (Flint and Johnson, 1981). The same authors have also shown that at pH 7.0, the emulsifying capacity of soy protein increases by increasing the ionic strength of the dispersed phase from 0.03 to 0.05.

Usually, the emulsifying properties of soy protein tend to decrease if the protein solution is heated at temperature above 50^{o}C (Aoki et al., 1980). Studies on individual fractions of soy protein revealed that while the 11S fraction is stabilized against thermal aggregation at high ionic strengths, the 7S fraction is more stable at low ionic strengths (Hashizume et al., 1975). Renkema and Vliet (2002) demonstrated that at pH 7.0 with a 0.5 M NaCl, the onset denaturation temperatures for 7S and 11S proteins were 63^{o}C and 80^{o}C, respectively. These results are also in agreement with previous literature (Damodaran, 1988). In addition, Damodaran (1988) reported that 11S and 7S fractions show different thermal stability depending on their subunit composition.

Not only their subunit composition, but also the ratio and amount of 7S/11S may play a role in the emulsifying properties of soy isolates. To date, the differences in functional properties of 7S and 11S are still to be fully understood. For example, according to Aoki et al. (1980) in a pH range between 2.0 and 10.0, 7S proteins showed higher emulsifying capacity than 11S fractions. Molina et al. (2001) reported that during high pressure treatment, 11S is the fraction responsible of decreasing the surface hydrophobicity and solubility of soy proteins isolates, while 7S protein plays an

important role in improving the emulsifying properties of the isolates. On the other hand, Liu et al (1999) demonstrated that 11S acidic subunits show excellent emulsifying properties, once separated from the basic 11S subunits.

One of the widest applications of soy proteins in food systems is a consequence of their gelling properties. The gelation process involves various steps: denaturation, aggregation, network formation, and gel stiffening (Renkema, 2002). Denaturation is considered the most important step in the heat-induced gelation of globular proteins (Renkema, 2002). Nagano et al. (1994) observed the formation of β-sheet structures during protein-protein interactions of glycinin and β-conglycinin. These results are in agreement with Wang et al. (1991) who suggested that during heat-induced gelation the globular proteins form cross-links with the intermolecular β-sheet structures.

Protein concentration, rate and duration of heating and cooling conditions are the factors that affect soy protein gelation (Liu, 1997). During prolonged heating, gel stiffening is caused by molecular rearrangement. Gel stiffening, occurring upon cooling is a thermoreversible process with no participation of disulfide bonds (Renkema, 2002).

While soy flour and concentrates form soft, fragile gels, soy isolates form firm, hard, resilient gels (Yatsumatsu et al., 1972). 7S and 11S fractions differ in their gelling (purified β-conglycinin gels at lower temperatures than glycinin) texturizing and film-forming properties (Ishino and Kudo, 1977). Disulfide bridges are involved in the gelation process of soy protein isolate and purified glycinin, while they do not play a role in gelation of purified β-conglycinin (Puppo et al., 1995). In heat-induced gels, Fukishima (1991) indicated that 11S subunits play an important role in gel formation; A_5, A_4, and B_3 subunits are closely related to gel formation rate and transparency, while the

A_3 and B_4 subunits are related to the gel hardness (Yagasaki et al., 1997). Renkema et al. (2001) found that the gelation temperature for β-conglycinin is lower that glycinin and more dependent on concentration, glycinin forms firmer and better gels than β-conglycinin. In addition, the same authors suggested that there is a correlation between pH and gelation temperature. For example, at pH 7.6, protein gelled at higher temperature that pH at 3.8.

Water-holding capacity, a measure of the entrapped water, varies depending on the type of soy protein ingredient. Among the factors which affect water binding of proteins are: amino acid composition, protein structure, surface charge and polarity, ionic strength, pH and temperature (Liu, 1997). Fleming et al. (1974) reported water-holding capacities of 2.6, 2.75 and 6.25 g/g of solids for soy flour, concentrates and isolates, respectively. Soy flour performs well in low-moisture systems, holding about twice its weight of water (Liu, 1997). Soy protein isolates are hydrophilic and perform well in systems with higher moisture (Liu, 1997). Heating in the presence of 0.5M urea decreases the water holding capacity of soy isolate to 0.5 g/g of protein (Liu, 1997).

2.7 Food Emulsions

An emulsion consists of two immiscible phases such as oil and water, with one of the phases dispersed in the other (McClements, 1999). A system, which consists of oil droplets dispersed in an aqueous phase, is an oil-in-water (O/W) emulsion, for example, mayonnaise, milk, soups and sauces. On the other hand, a system, which consists of water droplets dispersed in an oil phase, is a water-in-oil (W/O) emulsion, some examples of a w/o emulsion are: margarine, butter, and spreads. It is also possible to

prepare multiple emulsions such as oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) (Dickinson and McClements, 1995; Evison et al., 1995).

The diameter of oil droplets in an oil-in-water emulsion usually is between 0.1 and 100μm (Dickinson, 1992; Walstra, 1996). The concentration of droplets in an emulsion is described in terms of the dispersed phase volume fraction. The process of converting two separate immiscible liquids into an emulsion, or reducing the size of the droplets in an emulsion is called emulsification. Usually the process of homogenization involves the breakup and mixture of the oil and aqueous phases so that droplets of one of the phases become dispersed in the other (Walstra, 1996). The reduction in size of the droplets in an emulsion depends on the type of homogenizer, type and concentration of emulsifier (McClements 1999).

Oil droplets tend to merge with their neighbors upon collision and eventually coalescence will lead to phase separation (McClements, 1999). The driving force for this process is the thermodynamically unfavorable contact between oil and water. Emulsifiers are amphiphilic (surface-active) molecules, which adsorb on the surface of freshly formed droplets during homogenization, lower the surface tension and form a protective membrane. This prevents the droplets from coming in close contact and coalescing (McClements, 1999). Among the most common emulsifiers used in food industry are proteins, small-molecule surfactants, and phospholipids. In addition to emulsifiers, thickening agents are often used to enhance the stability of the emulsions. They increase the viscosity of the continuous phase retarding the movement of the droplets. The most common thickening agents used in the industry are polysaccharides (Dickinson and McClements, 1995).

2.7.1 Emulsion stability

This term describes the "ability of an emulsion to resist changes in its properties with time" (McClements, 1999). The composition and microstructure of the emulsions play a role in determining the rate of change and the mechanisms by which this process occurs (Dickinson and McClements, 1995). There are a variety of physical and chemical mechanisms responsible for alterations in the properties of an emulsion (Fennema, 1996). In addition to intrinsic conditions, the environmental conditions that the emulsion experiences during its lifetime also affect the stability and the mechanisms of change.

Creaming describes the upward movement of droplets, because of the difference in density between the dispersed and continuous phases. If the oil droplets have a higher density than the dispersed phase they move downward (sedimentation).

During flocculation the droplets come together to form an aggregate in which their individuality is maintained (McClements, 1999). The droplets in emulsions are in motion because of the effects of thermal energy, gravity, or applied mechanical forces, and as they move, they frequently collide with their neighbors (Lips et al., 1993; Dukhin and Sjoblom, 1996). Luyten et al. (1993) reported that flocculation accelerates the rate of gravitational separation in dilute emulsions. In addition, flocculation produces an increase in emulsion viscosity and may lead to gel formation (Demetriades et al., 1997) or shear thinning behavior (Bujannunez and Dickinson, 1994; Bower et al., 1997).

During coalescence, two or more droplets merge together to form a larger droplet. Coalescence is affected by the nature of the forces that interact between droplets, and occurs when the droplets are close to each other and the interfacial membrane is disrupted

(Walstra, 1996). Coalecence results in an increment in particle size, and eventually creaming or sedimentation.

2.8 Methods Employed For Physico-Chemical Characterization of Food Emulsion

2.8.1 Integrated light scattering

This method is based on the Mie theory of light scattering (when light is passing through a medium containing colloidal particles, the beam of light can be absorbed, reflected, diffracted, transmitted or scattered) (Hunter, 1993). If the colloidal particles are small, scattering will be the dominant process. The pattern at which a particle or colloid scatters the light depends on particle diameter, shape and volume. This pattern is determined by measuring the intensity of the energy at certain angles. Integrated light scattering devices have a series of detectors that receive the scattering signal from suspended particles at various angles. Light scattering has been widely employed for particle size analysis (Dickinson and Stainsby, 1982), determination of biopolymer interactions (Burchard, 1994), and study of colloidal aggregates (Horne, 1993; Corredig et al., 2001).

2.8.2 Rheology

Rheology studies the relationship between applied forces and the deformation and flow of matter (Macosko, 1994). Rheological properties of foods such as viscosity, creaminess, hardness, thickness, smoothness (Mc Clements, 1999) as well as viscoelasticity (Rao and Steffe, 1992) are important for sensory, shelf life, and design of processing operations. There are different mechanical rheological instruments designed to measure the shear properties of liquids, viscoelastic materials, and solids (Macosko, 1994).

Small deformation rheometers are often used to characterize emulsions. For these analyses usually the sample is located in a thermostatted measurement cell, where it is subjected to a controlled shear stress or strain. Two different types of rheometers exist: constant stress instruments in which constant torque is applied to the sample, and constant strain instruments in which the torque generated in the sample is measured by applying a constant strain.

Dynamic rheological measurements provide information about the structural organization and interaction with the different components in emulsions (Hunter, 1993). By using rheology, the behavior of flocculated emulsions (Dickinson, 1992), and the interactions of protein-polysaccharides in oil-in-water emulsions (Dickinson and Pawlowsky, 1996) have studied. In addition, dynamic oscillatory measurements have been utilized to observe the interaction of soy protein stabilized emulsions droplets in soy protein isolate gels (Kim et al., 2001) and the influence of processing variables on the characteristics of lupin protein-stabilized emulsions (Franco et al., 1998).

2.8.3 Confocal microscopy

In a laser scanning confocal microscope (LSCM) a beam of light is focused on a small portion of the specimen, and a confocal point detector is used to recollect the signal from the sample. In-focus plane images are obtained and the out-focus regions appear as black background (Brakenhoff et al., 1988).

It is possible to distinguish the spatial distribution of the components present in a sample by using fluorescent labeling dyes, such as fluorescein for protein, Bodipy or Nile blue for lipids, Texas red for polysaccharides (Vodovotz, 1996). Confocal microscopy has several advantages: it can work in two modes (fluorescence and reflectance), it has

the ability to scan samples at different depths; it is possible to obtain 3-D images without damage to the sample, high resolution images can be obtained, and it does not require major sample preparation (Vodovotz, 1996).

Confocal microscopy has been a very useful tool in the study of physical aggregation and coalescence, phase separation, effects of processing conditions and ingredient variation on microstructure of food systems (Vodovotz, 1996). For example, Blonk and van Aalst (1993) used confocal microscopy to study the effect of additives and gluten network formation on loaf volume. Wong-Lion and Frank (1995) used CLSM to monitor the migration of Salmonella in eggs. Hassan et al. (1995) studied directly, under the microscope, the microstructure of yogurt following in real time the development of the milk gels. In emulsions, the 3-D spatial distribution of emulsion droplets was observed during separation, by collecting z-images and presenting the reconstructed image as an x-y plane (Brakenhoff et al., 1988).

2.8.4 Scanning electron microscopy

Scanning electron microscopy (SEM) a portion of the sample is bombarded with an electron beam generating backscattered, secondary, Augene electrons and X-rays. Secondary electrons are recollected and measured by a detector. It is also possible to measure backscattered electrons, but the resolution is limited (Slayter and Slayter, 2000). The advantages of this microscope are the large depth of field (all in focus), high resolution, less elaborated sample preparation than other electron microscopy techniques, and provides surface topography images of the sample (Heertje and Paques, 1995).

SEM Microscopy has been widely used to determine microstructure and size emulsion droplets (McClements, 1999). In addition SEM is often employed to observe

the dimension and structure of flocs, morphology of droplets and air bubbles, size and shape of fat crystals, and structural properties and network formation in emulsions (Kalab, 1981; Ogale et al., 2000).

2.8.5 Determination of Protein Adsorption

To determine the type and amount of protein adsorbed at the interface, many researches have used gel electrophoresis. For example, Hunt and Dalgleish (1994) showed differences in preferential adsorption of milk proteins in-oil-in-water emulsions by SDS-PAGE. The principle of electrophoresis is based on the application of an electrical field on charged molecules in a gel, so that they migrate in the direction of an oppositely charged electrode. The speed of migration of the proteins will depend on their mass and size, and will be influenced by field strength, concentration, pH of buffer and type of gel.

To determine protein adsorption at the interface, the different phases of the emulsion are separated by centrifugation. After washing, the collected oil droplets are mixed with sample buffer and the migration by gel electrophoresis is compared to that of proteins present in the whole emulsion. Similarly, migration of protein present in the dispersed phase can be compared to that of the adsorbed phase, to determine if differences exist in the adsorption behavior among the proteins present in the emulsion.

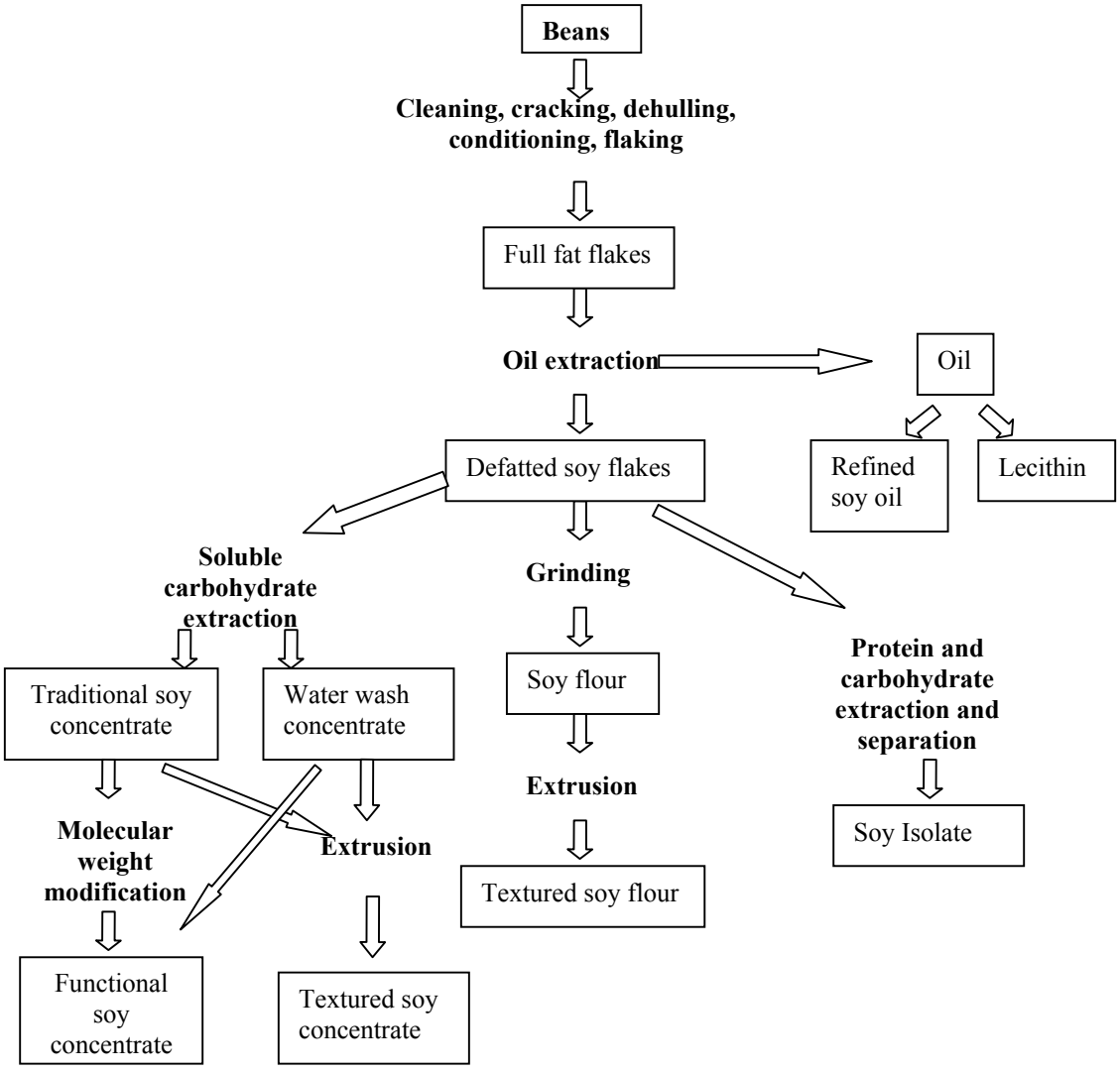

Figure 2.1: Scheme of different protein products obtained from soybeans (adapted from Anonymous, 1998).

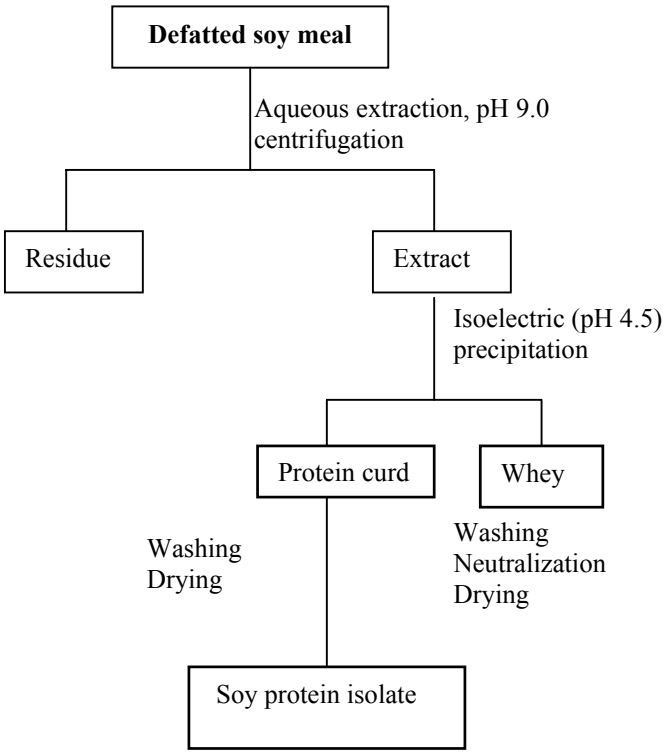

Figure 2.2: Schematic for the production of soy protein isolates (adapted from Liu, 1997).

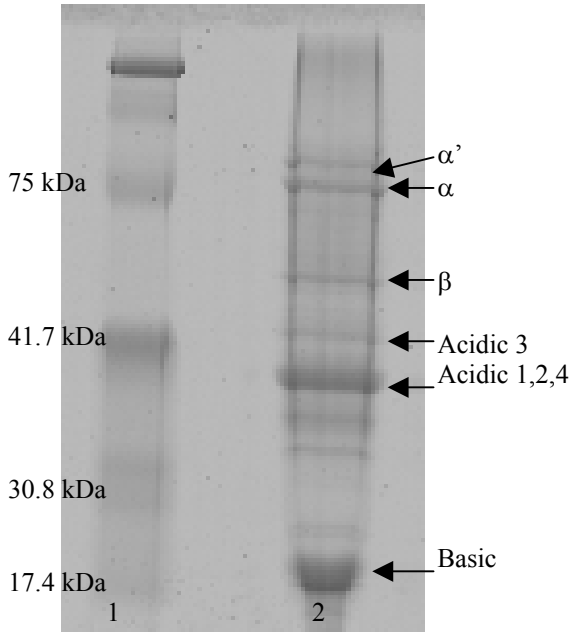

Figure 2.3: Electrophoresis separation of the main subunits of soy protein. Lane 1: molecular weight standard; lane 2: soy protein

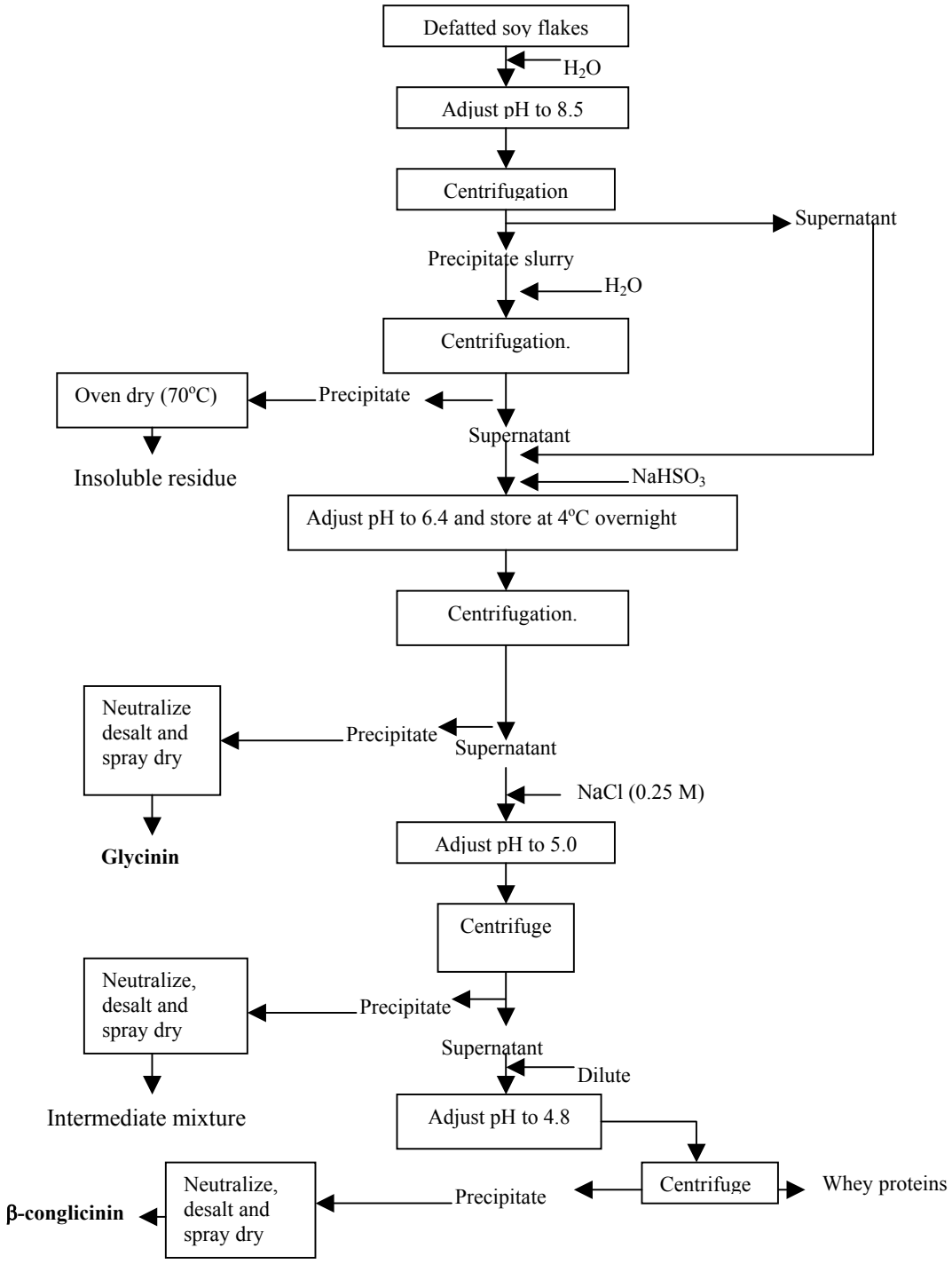

Figure 2.4: Scheme of the process of isolation of glycinin and β-conglycinin (adapted from Wu et al., 2000)

2.9 References

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CHAPTER 3

CHARACTERIZATION OF OIL-IN-WATER EMULSIONS PREPARED WITH COMMERCIAL SOY PROTEIN CONCENTRATE[1]

[1]Roesch, R., and Corredig, M. Submitted to *Journal of Food Science*, 3/02

3.1 Abstract

Oil-in-water emulsions containing a commercial fraction of soy protein concentrate (SPC) were characterized for stability and microstructural differences. Emulsions were prepared with SPC (concentrations between 1% and 10% (w/v) and soybean oil (10% w/w)) and homogenized at 80 MPa. When SPC was added at a concentration high enough to be present in the dispersed phase, the average particle size, as determined by integrated light scattering, reached a plateau value. In addition, emulsions prepared with >4% SPC showed increased viscosity with increasing SPC concentration. The protein formed a continuous network and emulsions were stable to creaming. Microstructural observations showed that phase separation occurred in emulsions prepared with high SPC concentrations.

3.2 Introduction

Soybean proteins play an important role in many food products, not only because of their high nutritional value but also for their contribution to food texture and emulsifying properties. Proper understanding of the behavior of soy ingredients in foods requires an in-depth knowledge of their molecular arrangements and interactions, which determine the consistency and stability of the food products.

Soy protein isolates and concentrates are employed in a wide variety of food applications, and a recent health claim by the Food and Drug Administration (Food and Drug Administration, 1999) has resulted in an increased consumer demand for products containing soy proteins. Sales of soy-based products are growing at a rapid pace, and among the various products dairy alternatives, flavored beverages, yogurts, and nonfrozen desserts are gaining particular attention.

Among the various soy protein groups, soy protein concentrates (SPCs) contain 70% protein on a moisture-free basis. In addition to fat- soluble material, during solvent extraction soluble carbohydrates are also removed from SPCs. SPCs are relatively inexpensive ingredients containing about 20% of polysaccharide material. SPCs are often marketed as ingredients that maintain functionality even in stressed systems such as high-fat and high-moisture formulations, during freeze/thaw cycles, reconstitution of pre-cooked products, and in pre-cooked or cooked products requiring extended holding times (Anonymous, 1998).

In food products, particulate material, fat globules, gas bubbles, polysaccharides, and protein aggregates coexist in polymer networks and all components affect the overall

structural properties (Dickinson and Stainsby, 1982). The surface activities and emulsifying properties of soy proteins have been extensively studied (Wagner and Gueguen 1999; Liu et al., 1999). Even though soy proteins are characterized by lower emulsifying properties when compared to other surface-active proteins such as caseins, soy proteins exhibit high emulsifying properties compared to other plant proteins (Hettiarachchy and Kalapathy, 1998). This difference is mainly related to the compact tertiary structure and the quaternary structure of the protein components present in soy protein concentrates and isolates (Liu et al., 1999).

Only limited information is available from fundamental studies on the surface properties of soy proteins (Dickinson and Matsumura, 1991; Yu and Damodaran, 1991; Krause et al., 1997; Wagner and Gueguen, 1999), and no studies have been reported on the behavior of commercial fractions of soy protein in oil-in-water emulsions, in spite of the increasing interest in the field. The increased use of soybean components for nutritional and functional foods will require a more in-depth understanding of the functionality of the protein used as an ingredient. This improved knowledge will facilitate the utilization of soy protein concentrate in the production of stable foods with the desired functionalities. The work presented herein describes the behavior of a commercial SPC when used as an ingredient in oil-in-water emulsions. The effects of the presence of various SPC concentrations on creaming stability, viscosity, and microstructure of the emulsions will be discussed.

3.3 Materials and Methods

3.3.1 Materials

SPC used in this study was provided by Central Soya (Fort Wayne, IN, USA); it is available in the market under the trade name Promine™ DS 3070. The SPC contained 19.4% dietary fiber (1.2% soluble and 18.2% insoluble fiber) and 70% protein (data provided by the Manufacturer). Soy oil was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and all chemicals, analytical grade, were obtained from Fisher Scientific Co. (Atlanta, GA, USA).

3.3.2 Emulsion preparation

Suspensions were prepared by dispersing SPC (1%-10% w/v) in 50 mM sodium phosphate buffer, pH 7.5, containing 0.02% sodium azide (as antimicrobial). After overnight incubation at 6°C to allow complete hydration of the protein, soy oil was added to the suspensions to a concentration of 10% (w/w). Mixtures were prehomogenized with a hand homogenizer (Polytron Pt 12000; Kinematica, Zurich, Switzerland) at 10000 rpm for 1 min, and emulsions were then prepared by high-pressure homogenization (Emulsiflex C5, Avestin, Quebec, Canada), with 4 passes through the homogenizer at 80 MPa of pressure. Three separate emulsions were prepared for each treatment.

3.3.2 Droplet size distribution

Emulsions were characterized with respect to particle size within a few hours and after 10 days of storage at 6°C using the Malvern Mastersizer S (Malvern Inst., Southborough, MA, USA), using the standard presentation code for samples dissolved in water (1.5295, 0.1000 and 1.3300 for the relative particle refractive index, relative

refractive index and dispersant refractive index, respectively). The average particle size diameter was defined as $D_{3,2}=(\Sigma n_i d_i^3/\Sigma n_i d_i^2)$ and $D_{4,3}=(\Sigma n_i d_i^4/\Sigma n_i d_i^3)$, where d is the diameter and n is the number of particles.

3.3.3 Viscoelastic properties

The viscoelastic properties of the suspensions and the emulsion samples were measured using a controlled stress rheometer (Model SR-5000, Rheometrics Scientific, Piscataway, NJ, USA) with constant temperature of 25^{o}C controlled by a Peltier heating device. Measurements were carried out using a parallel plate of 40 mm diameter and a gap of 0.6 mm. Viscosity measurements were performed in a steady rate sweep mode after 24 h of storage by increasing the shear rate from 0-100 s^{-1} in 300 s and then decreasing to 0 in 300 s. Determination of flow behavior (n) and consistency index (K) were carried out by fitting a power-law relationship of shear stress versus shear rate in the first part of the flow shear analysis during upward sweep. In addition, the frequency dependence of storage (G') and loss moduli in function of an oscillatory frequency was measure by performing a frequency sweep test.

3.3.4 Surface protein coverage

The cream was separated from the serum phase and pellet by centrifugation of emulsions at 19,000xg for 30 min at 25^{o}C in a refrigerated superspeed centrifuge (Sorvall RC-5B, Fisher Sci.). The cream phase and pellet were then re-dispersed in sodium phosphate buffer, to the initial concentration (10% w/w), and centrifuged again under the same conditions, and then dried on a Whatman no.1 filter paper as previously described (Hunt and Dalgleish, 1994). After the first centrifugation, the subnatant was carefully removed and filtered through a 0.22-μm filter (Millex GP; Millipore Corp., Bedford, MA,

USA). 10 mg of cream, and pellet (re-suspended in 90 µl of 0.05M Tris buffer) and 150 µl serum were dispersed in electrophoresis buffer (12.5% Tris-HCl, pH 6.8, 20%(w/v) SDS, 1.25% bromophenol blue, 5% β-mercaptoethanol, 25% glycerol) to a volume of 400 µl and denatured in a boiling water bath. Samples containing cream and pellet were then centrifuged (Eppendorf centrifuge; Brinkmann Instruments, Westbury, NY, USA) at 15,000xg for 3 min. Finally, all samples were loaded onto a 12.5% Tris-HCl acrylamide gel (Biorad, Hercules, CA, USA). Gels were run at 200 V, stained with Coomassie blue and destained according to manufacturer's instructions (Biorad).

3.3.5 Stability studies

Aliquots of emulsions were placed in duplicate in 10-mL graduated tubes (10 mm internal diameter) and stored quiescently at 6°C for 20 days. The serum separated after creaming was recorded as percentage of the total height as a function of time of storage. In addition to creaming, emulsions were centrifuged at 19,000xg for 30 min at 25°C as described above, and the wet weight of the pellet was measured. The percent of the total weight of the emulsions constituted by the pellet was taken as an index of presence of insoluble material. Pellets were further freeze-dried for electron microscopy analysis.

3.3.6 Microscopy

Microstructural observations were carried out with a scanning electron microscope with a cold stage attachment (Leo 982 emission scanning electron microscope, Alto 2500 cryostage and cryoprep; Leo Electron Microscopy Inc., Thornwood, NY, USA). One drop of sample was placed on a specimen stub and frozen with liquid nitrogen. The sample was then introduced into the cryo-unit and fractured

with a knife and coated with gold as previously described by Saio (1981). Observations were carried out within 36 h of emulsion making. Freeze-dried pellet samples were also analyzed by scanning electron microscopy, after coating the specimen with gold. Phase contrast microscopy observations were performed using a light microscope (Carl Zeiss Photomicroscope III, New York, NY, USA) in phase contrast mode with a Ph2 Neufluar 25X objective. This technique uses light diffraction, and does not require any modification of the initial sample. Samples were measured at room temperature.

Results presented are averages of triplicate samples. Significant differences were determined by the general linear model procedure (SAS version 6.1; SAS Institute, Cary, NC, USA), and treatments were considered significant at $p<0.05$.

3.4 Results and Discussion

Figure 3.1 depicts the effect of homogenization at 80 MPa on the particle size distribution of 4% and 8% SPC suspensions. Homogenization decreased the particle size of the SPC suspensions from an average of 100μm to about 50μm diameter; however, suspensions still presented a wide distribution of large particles even after homogenization. No significant differences were shown in the particle size distribution of the suspensions varying in SPC concentration. These homogenized suspensions showed separation only after 3 days of storage at 4^{o}C.

The average particle size distribution of 10% oil-in-water emulsions showed similar trends from those obtained from the homogenized suspensions (Figure 3.2). A wide distribution of particle size was shown also in the emulsions, and upon homogenization a population of particles < 1 μm was present in the samples. At the higher concentrations of SPC (6% and 10%) a shoulder in the distribution became

apparent in the size range 1-10μm. In all the emulsions analyzed, no changes were shown in the particle size distribution after 10 days of storage at 6°C. A high value of mean apparent diameter was shown for 10% oil-in-water emulsions containing 1 and 2% SPC, but no changes were observed at concentrations > 4% (Figure 3.3). The average particle size was unaffected by further increase in SPC concentration.

As already shown with the size distribution, differences in $D_{3,2}$ and $D_{4,3}$ suggested a very heterogeneous and wide particle size distribution in the emulsion samples. Stability studies at 6°C indicated phase separation within the first 48 h of storage for emulsions containing 1 or 2% SPC and 10% oil, while emulsions containing 4% SPC developed a visible cream layer after about 5 days, as shown in Figure 3.4. Serum separation occurred because of the difference in density between the continuous and the dispersed phases (Dickinson and Golding, 1997). Creaming also occurred in emulsions containing 4% SPC; but with creaming delay. At concentrations higher than 4% emulsions were characterized by good stability with respect to creaming. A network of aggregated protein entrapping oil droplets could be the cause of the stability of emulsions prepared with SPCs.

SPC differentiates itself from other soy protein ingredients by the presence of insoluble fiber and partially denatured and insoluble protein, and these components may also play a role in the structure formed around the oil droplets of these emulsions. The amount of insoluble material collected as pellet after centrifugation tests confirmed the hypothesis that only in the presence of un-adsorbed material, delay of creaming of the emulsions occurred (Figure 3.5). The amount of insoluble pellet present in 8% SPC suspensions was smaller than that present in emulsions containing 8% SPC, perhaps

because of the presence of small oil droplets (with a diameter <5 μm) embedded in the pellet of emulsions. This was directly noted during scanning electron microscopy observations (Figure 3.6).

When the protein present in the various phases (cream, subnatant, pellet) of the emulsions was analyzed by SDS-PAGE electrophoresis (Figure 3.7A and B) a similar pattern of bands was shown in cream, serum, and pellet of emulsions containing high amounts of SPC. No preferential adsorption seemed to occur under these conditions. On the other hand, when emulsions contained only 2% SPC, as shown in Figure 3.7B, very little protein was present in the soluble phase; most protein was adsorbed onto the cream phase or was present in the pellet. In addition, in the serum phase a polypeptide migrating at about 35 kDa was shown. A band at about 32 kDa was also shown in the insoluble pellet in a higher amount than in the emulsion sample. Such differences in the electrophoretic migration and protein distribution of the insoluble, cream, and serum phases were not shown in emulsions prepared with a higher concentration of protein.

These results, indicating the non selectivity of the SPC proteins, when present in sufficient amount, are somewhat in disagreement with previous findings on the differences in surface properties of soy proteins (Liu et al., 1999). Differences exist in the functional properties of 11S and 7S fractions, and recently it has been demonstrated that their emulsification behavior after treatment with static high pressure depends on their ratio (Molina el al., 2001). When using SPC as an ingredient in oil-in-water emulsions no differences were shown in the protein distribution in the cream or insoluble fraction. The similar behavior of the various proteins present in SPC shown in this research was most likely caused by the processing history of SPC. The solvent extraction, followed by an

ethanol wash, might have decreased the molecular flexibility that affects the functional properties of such proteins, not showing differences in the extent of the interactions of the different protein fractions with the cream phase of the emulsion.

The effect of processing history on SPC protein functionality has been described before in studies of gelation of commercial isolates and concentrates from soy (Chronakis et al., 1995): differential scanning calorimetry studies have shown that Promine™-D from Central Soya is partially denatured and has a different gelation behavior than its native counterparts (Chronakis et al., 1995).

Figure 3.8 illustrates the effect on viscosity of increasing amounts of SPC present in 10% oil-in-water emulsions and after 10 days of storage at 4°C. The emulsions exhibited a shear-thinning behavior, hysteresis and viscosity values increased with increasing SPC concentration. The shear-thinning behavior may be caused in part by the presence of aggregated droplets as already shown by other authors (McClements, 1999). In addition, phase separation may have increased protein-protein interactions at high concentrations of SPC. This was confirmed by frequency sweep test where higher SPC concentration showed less frequency dependence than concentrations <6% SPC. Emulsion containing 6% SPC showed at low oscillatory frequency a viscous behavior (higher G"); however at high oscillatory frequency it showed a cross over of the viscous and elastic modulus (Figure 3.9) suggesting the formation of some kind of structure (Rao and Steffe, 1992). In addition, emulsions containing 10% SPC showed higher elastic than viscous modulus regardless of the frequency suggesting the formation of a week gel may be due to the increment in density of the continues phase.

When the power law coefficients derived from the flow measurements were analyzed and the mean values compared (Table 3.1), the values of consistency index (K) and flow behavior (n) were significantly affected by the addition of SPC both at day 0 and after 10 days of storage. Emulsions containing 10% SPC had a value of K significantly higher than those of all the other emulsions while emulsions containing 6% SPC showed a n value significantly higher than emulsions containing 8 and 10% SPC. This means that the shear rate dependency of 6% SPC emulsion is higher that 8% and 10% SPC emulsions. When emulsions were compared for storage effect within the same concentrations, no significant differences were shown in the values of K and n after 10 days of storage.

Microstructural observations of emulsions prepared with different amounts of SPC are shown in Figures 3.10 and 3.11. Phase contrast microscopy allowed the observation of differences in the distribution of the oil in the continuous phase. When SPC >4% was added to the emulsions (Figure 3.10B) the oil phase showed a clear phase separation from a protein-containing continuous phase. These observations were in agreement with the flow behavior measurements carried out on emulsions containing various amounts of SPC. In addition, phase contrast microscopy allowed observation of the insoluble fiber. These particles were most likely what was detected as large aggregates during light scattering measurements (Figures 3.1 and 3.2), and they seemed to be only slightly reduced in size by high-pressure homogenization.

By increasing the amount of SPC added to the emulsion, an increase in viscosity was produced, and it was hypothesized that a network was formed which would stabilize the oil droplets against creaming. Scanning electron microscope observations (Figure

3.11) confirmed this hypothesis. A protein network was clearly shown in the emulsion specimens analyzed by cryogenic scanning electron microscopy. The pore size of the network seemed to decrease with the increase in SPC concentration in the emulsion. This was evidenced by the increase in viscosity occurring upon increase in the amount of SPC present in the continuous phase. The partially denatured state of the proteins in SPC, caused by the ethanol extraction, and the presence of the insoluble polysaccharide are a unique combination in SPC, and this combination caused network formation upon homogenization. This structured continuous phase entrapped the oil droplets, causing delay on creaming at SPC concentrations > 4%.

In emulsions made with low concentrations of SPC, no protein was present in the continuous phase, and the presence of oil droplets caused rapid phase separation, because of the density difference between the oil droplet flocs and the surrounding liquid. On the other hand, when the emulsions contained a higher amount of SPC the aggregated droplets formed flocs that were prevented from creaming. This phase separation was evident in the phase-contrast microscopy observations (Figure 3.10).

3.5 Conclusions

In emulsions prepared with SPC and 10% soy oil, phase separation occurred. However, emulsions containing >4% SPC showed stability to creaming, and the oil phase was aggregated and entrapped in a structured network of protein. Rheological measurements and creaming stability studies of the emulsions showed no changes over time within 20 days of storage. Stability of such systems was caused by the interactions occurring between the partially denatured proteins present in the dispersed phase. In addition, microstructural observations showed the presence of large particles of insoluble

fiber, possibly contributing to the phase separation occurring in the emulsions. However, the role played by the insoluble fiber in SPC is far from being understood.

Table 3.1: Means comparison of consistency index (K) and flow behavior (n) of 10% soy oil emulsions prepared with SPC. Values are the mean of three replicate samples. Within a column values for which the first superscript is identical are not significantly different ($p<0.05$), within a row values for which the second superscript is identical are not significantly different.

	Day 0		**Day 10**	
SPC	K ($Pa.s^n$)	n	K ($Pa.s^n$)	n
6%	$0.22^{a\ j}$	$0.55^{d\ x}$	$0.20^{a\ j}$	$0.66^{d\ x}$
8%	$0.70^{a\ j}$	$0.48^{c\ x}$	$0.73^{a\ j}$	$0.49^{c\ x}$
10%	$2.05^{b\ j}$	$0.37^{c\ x}$	$2.51^{b\ j}$	$0.37^{c\ x}$

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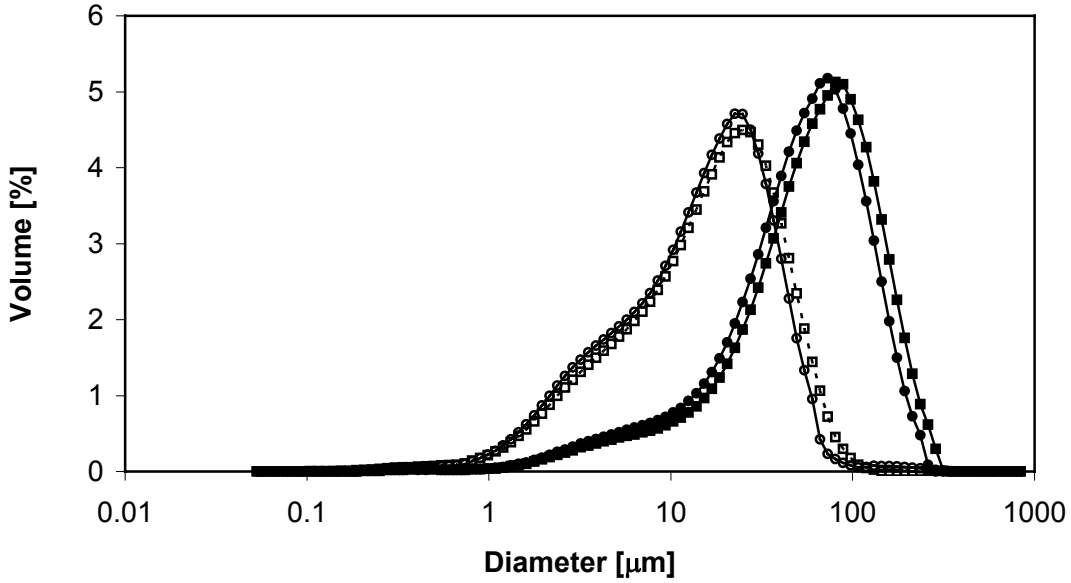

Figure 3.1: Particle size distribution, as measured by integrated light scattering, of suspensions made with 4% (■) and 8% (●) SPC, before (■,●) and after (□,○) homogenization at 80 MPa.

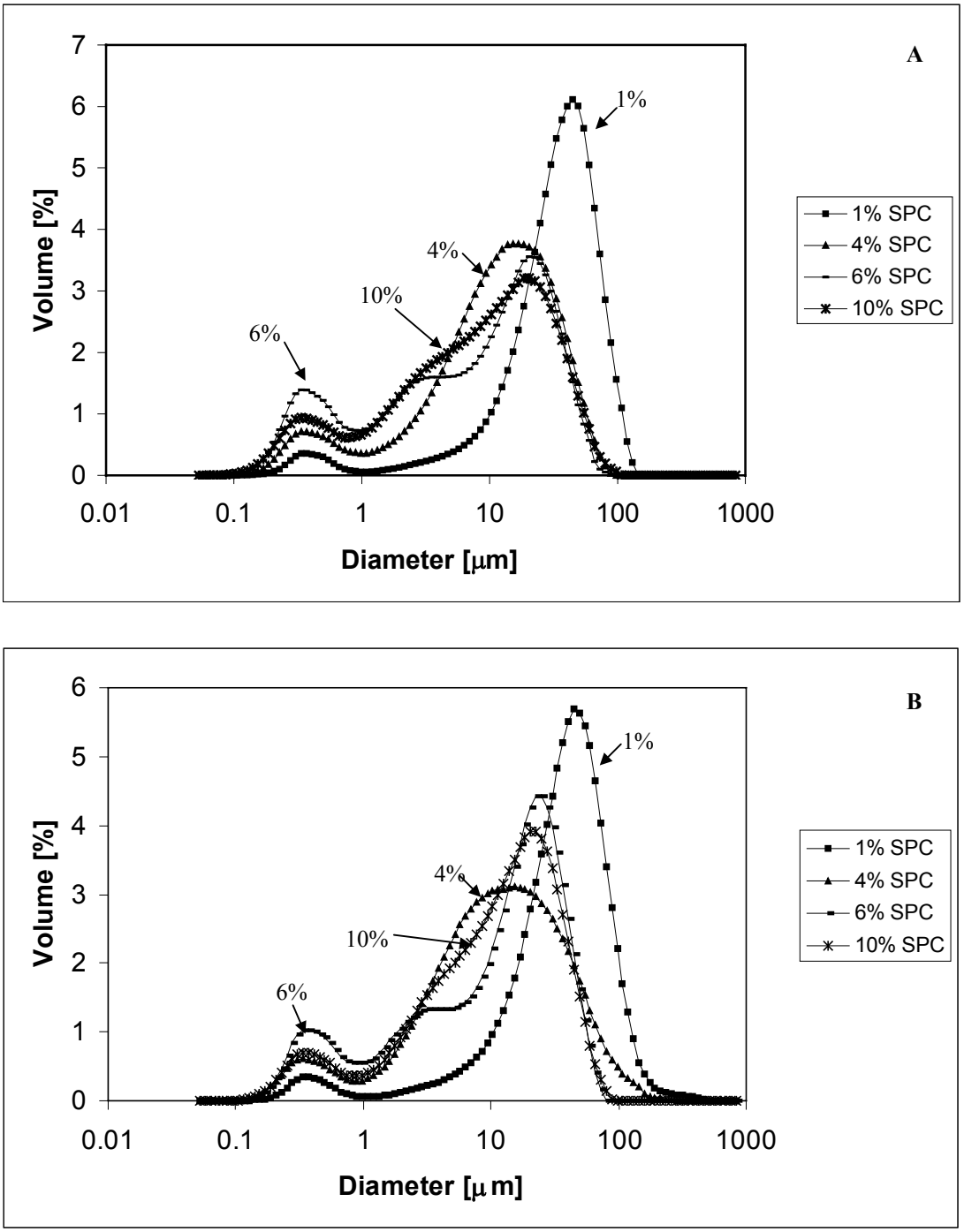

Figure 3.2: Particle size distribution, as measured by integrated light scattering, of emulsions prepared with different concentrations of SPC and 10% oil. Distributions are the averages of 3 replicate measurements conducted at time 0 (A) and after 10 days of storage at 6 °C (B).

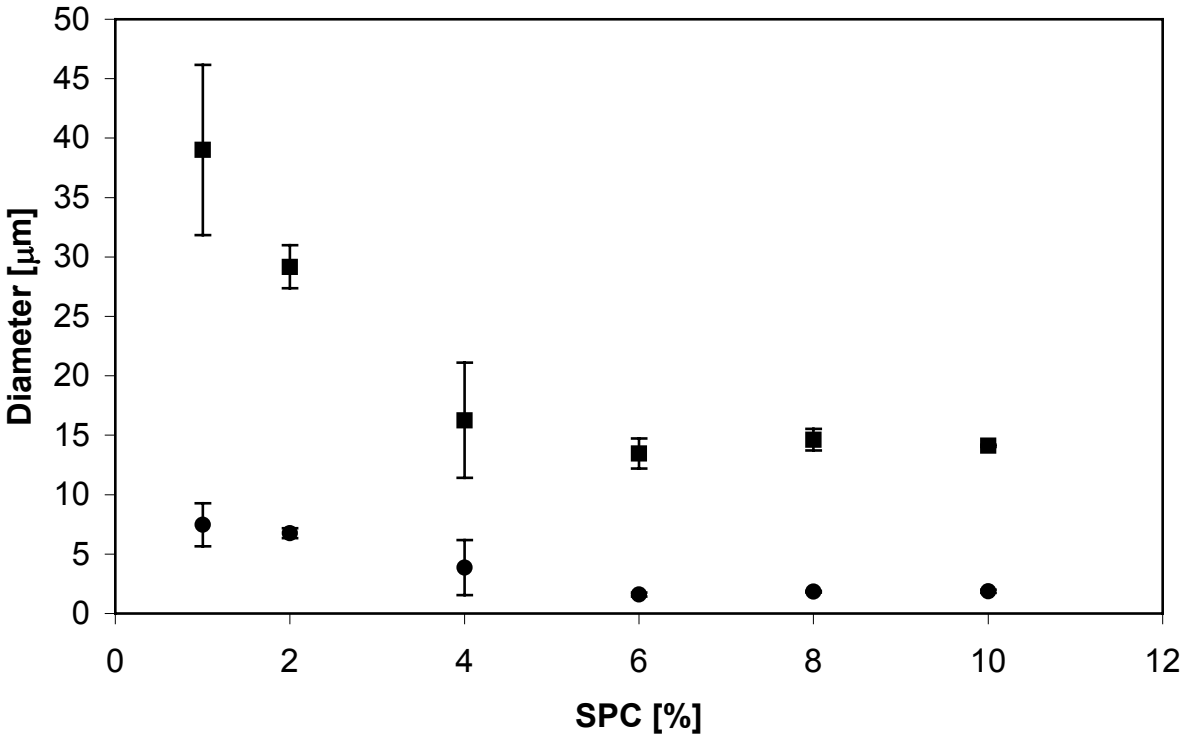

Figure 3.3: Average particle diameter of emulsions as a function of amount of SPC added. Values of $D_{4,3}$ (■) and $D_{3,2}$ (●) are the averages of 3 replicate samples.

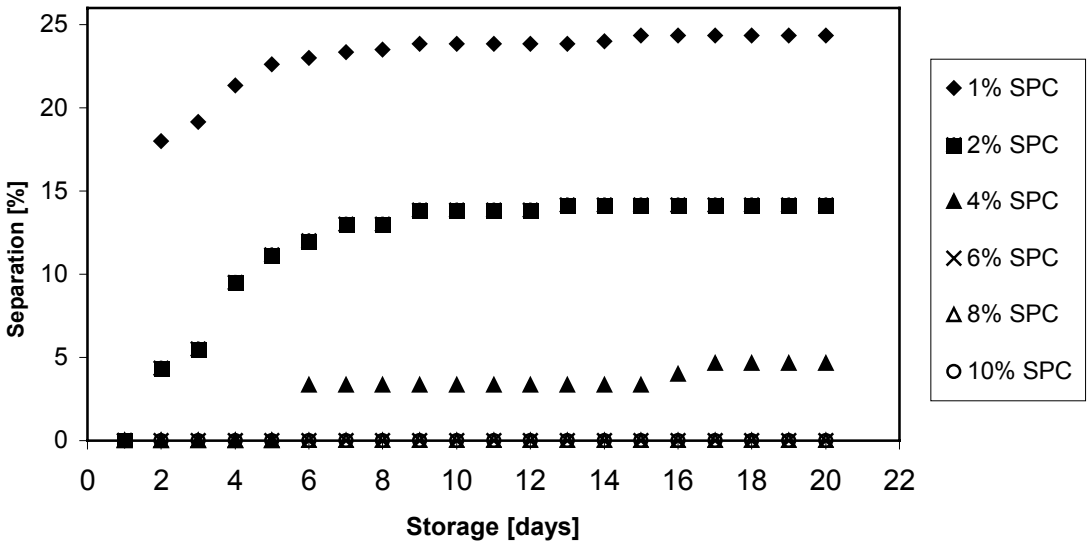

Figure 3.4: Stability upon storage at 4°C of 10% oil emulsions at various concentrations of SPC. Separation is indicated as % total height. Values are the averages of 3 replicate samples.

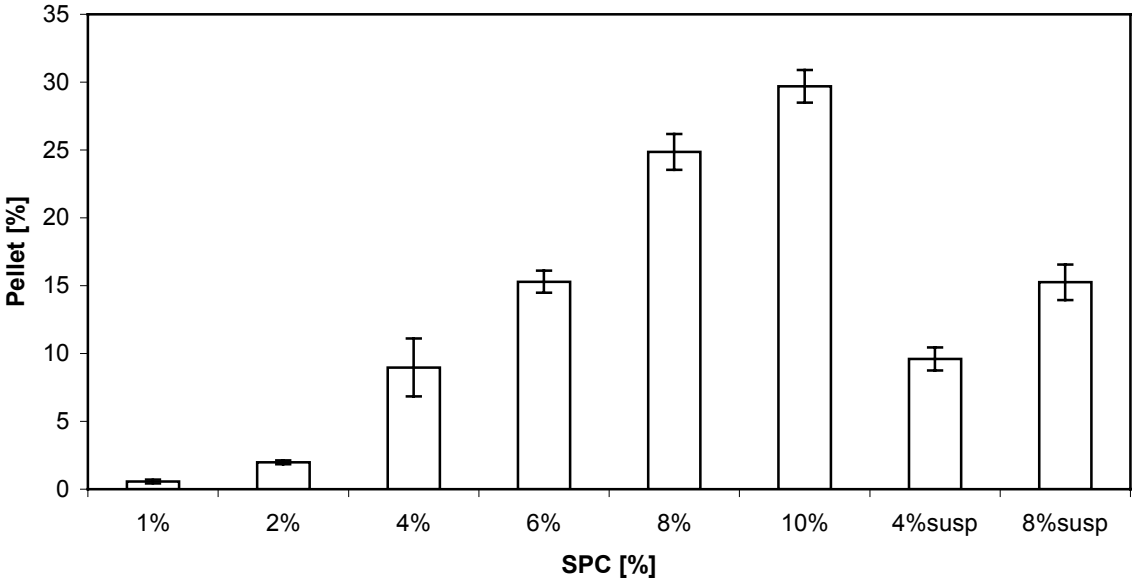

Figure 3.5: % (w/w total emulsion) of pellet after centrifugation of 10% soy oil emulsions. Values are the averages of 3 replicate samples.

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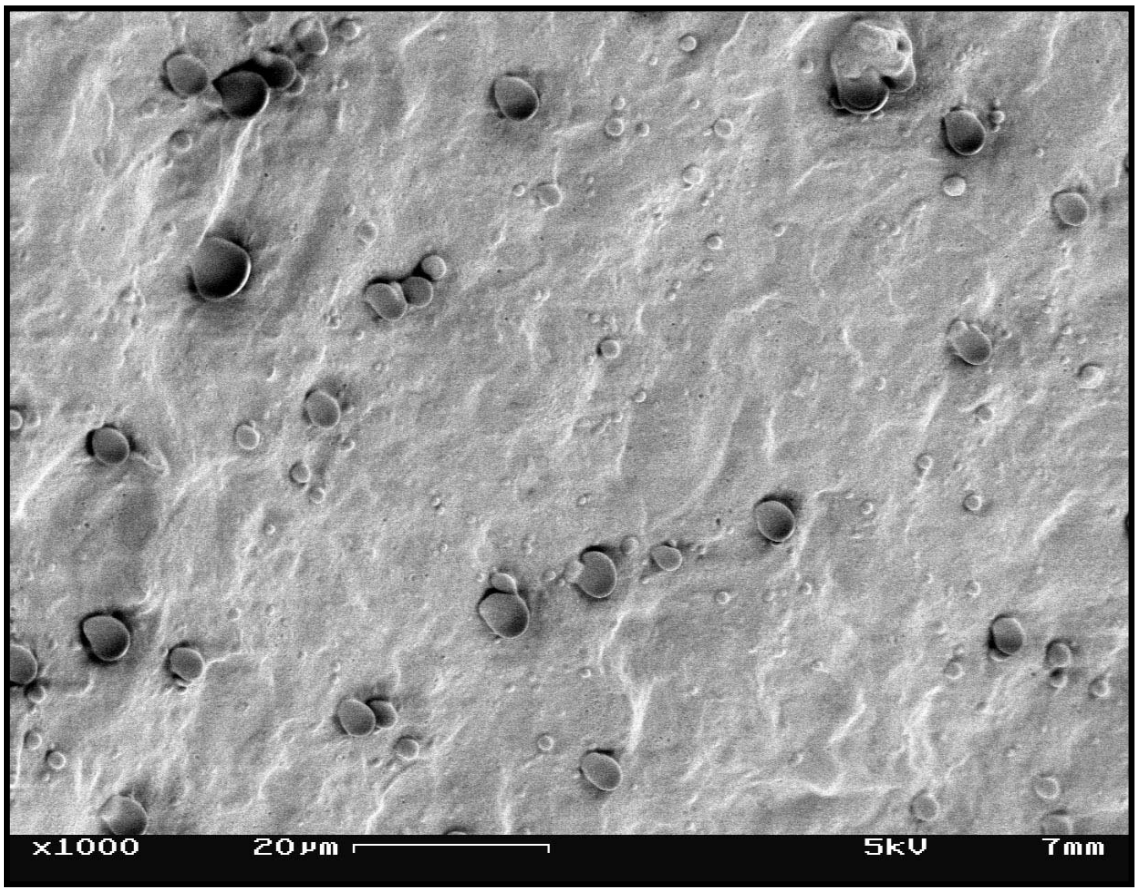

Figure 3.6: Scanning electron microscope image of insoluble pellet extracted after centrifugation from an emulsion containing 10% oil and 8% SPC. Pellet was freeze-dried before the analysis. Scale bar represents 20 μm.

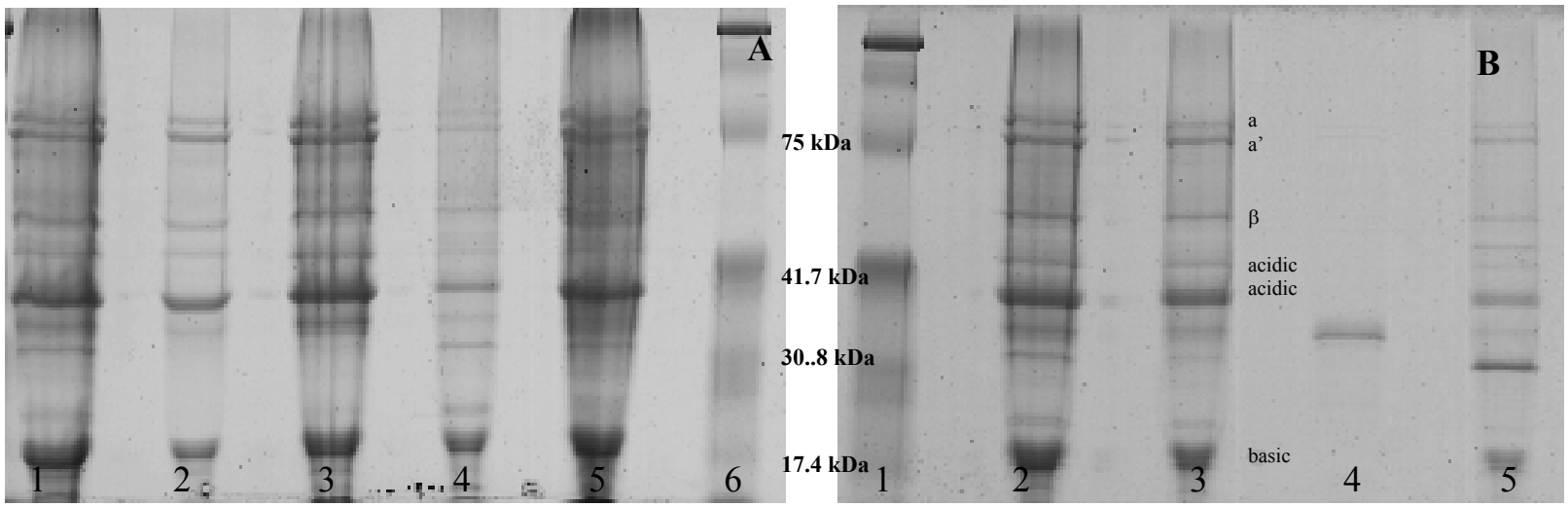

Figure 3.7: SDS-PAGE gel of 10% oil-in-water emulsions containing 6% (A) and 2% (B) SPC. Figure 7A: Lane 1: emulsion; Lane 2: cream phase; Lane 3: subnatant; Lane 4: insoluble pellet; Lane 5: 6% SPC suspension; Lane 6: molecular weight standards. Figure 7B: Lane 1: molecular weight standard; Lane 2: emulsion; Lane 3: cream phase; Lane 4: serum; Lane 5: insoluble pellet.

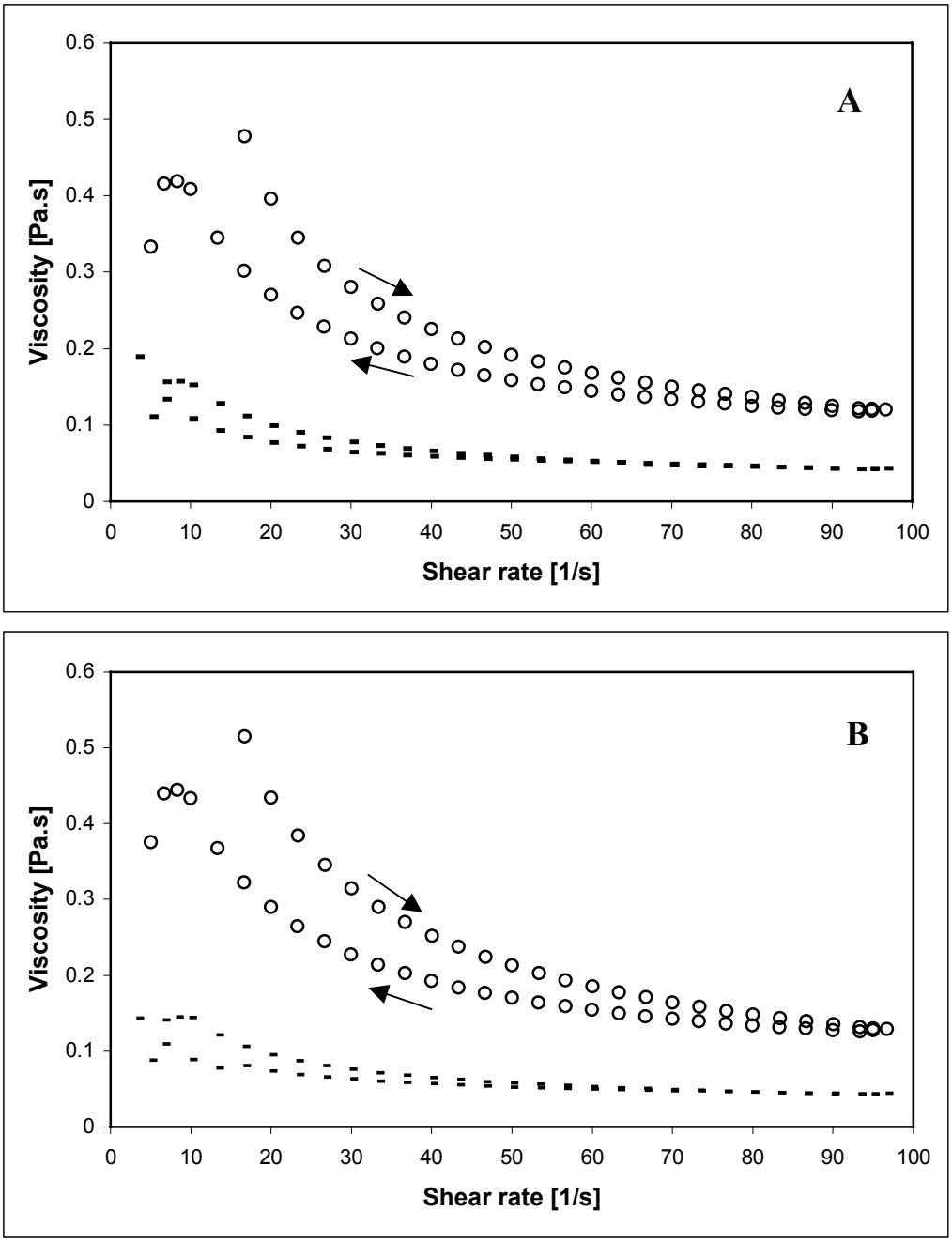

Figure 3.8: Effect of shear rate on viscosity for 10% soy oil emulsions containing different concentrations of SPC: (-) 6%; (○) 10%. Emulsions tested before (A) and after 10 days of storage at 4°C (B). Data shown is the average of three replicate experiments.

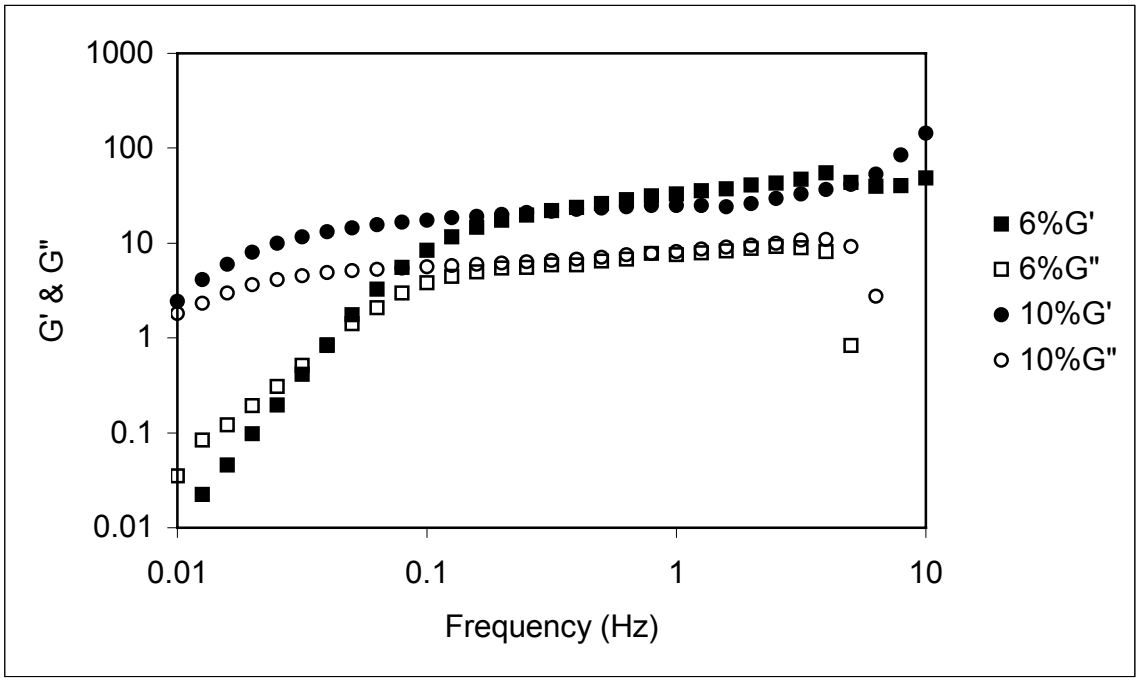

Figure 3.9: Average measurements of the of elastic (filled symbols) and viscous moduli (empty symbols) for 10% soy oil-in-water emulsions prepared with SPC as measured by controlled stress rheometry. Emulsions containing 6% SPC (■,□); 10% SPC (●,○).

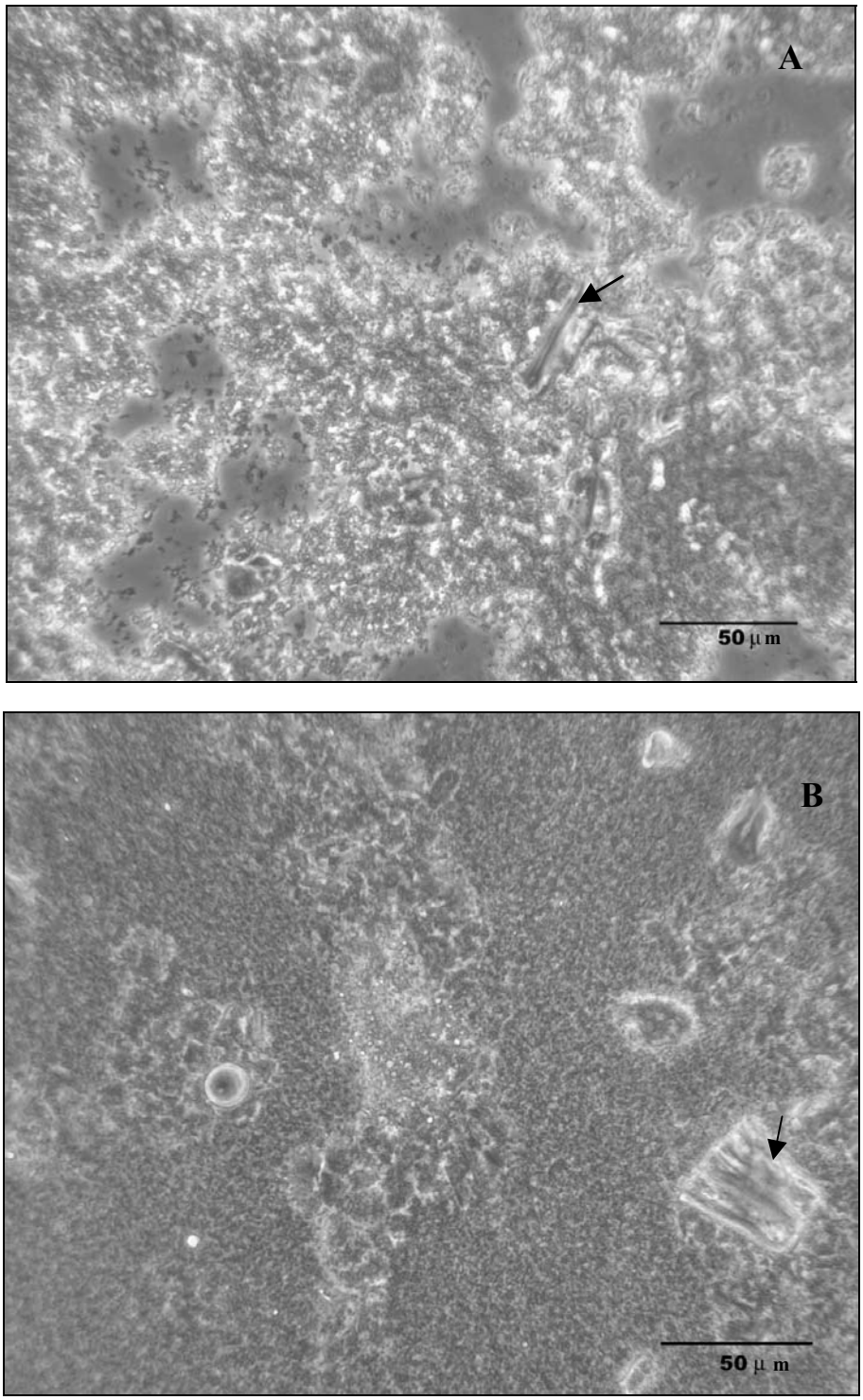

Figure 3.10: Phase contrast microphotographs of 10% oil emulsions prepared with 2% (A) and 6% (B) SPC. Arrows indicate insoluble fiber. Scale bar corresponds to 50 µm.

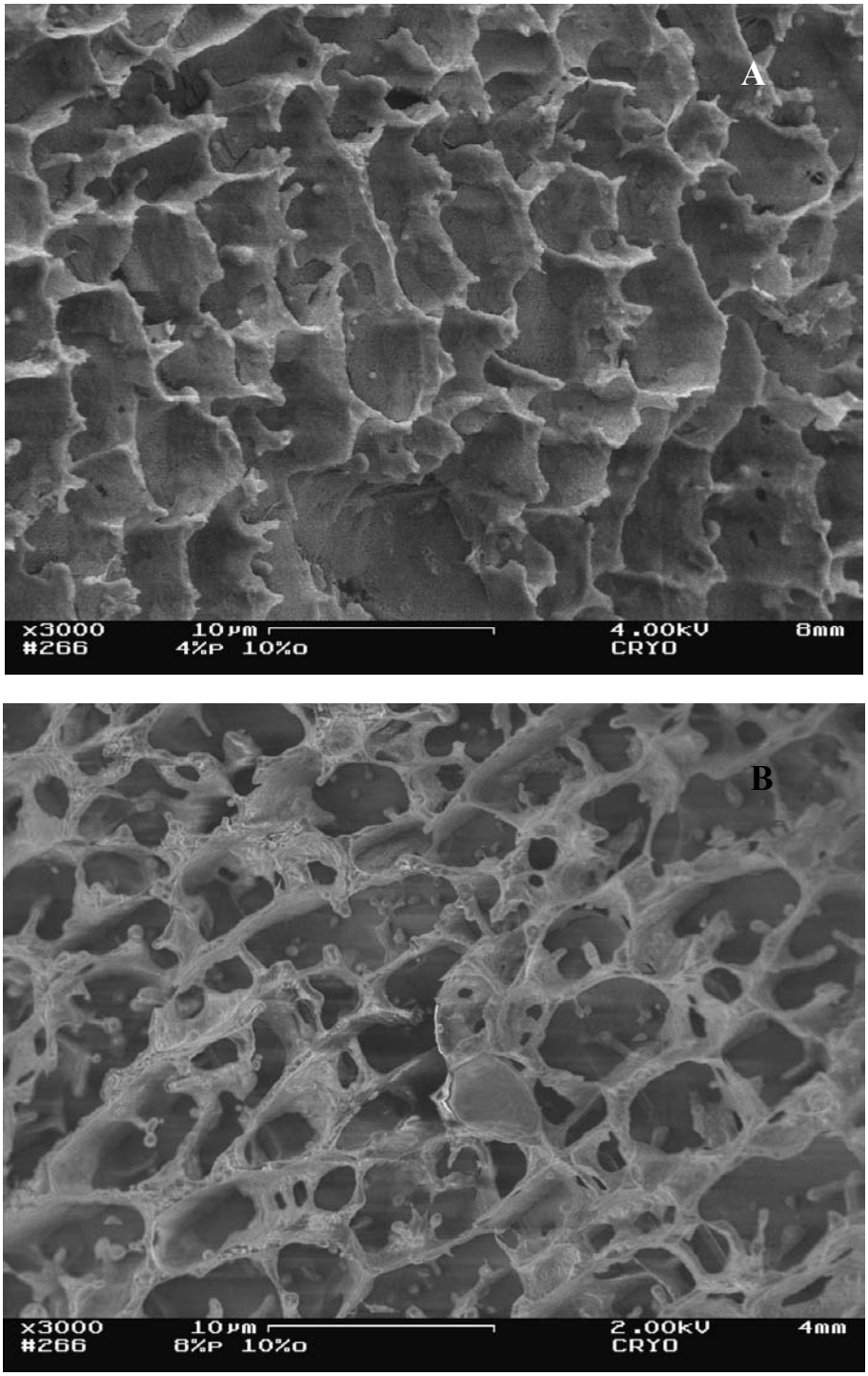

Figure 3.11: Scanning electron microscope images of emulsions prepared with 10% oil and 4% (A) or 8% (B) SPC. Samples were prepared using a cryogenic SEM unit. Scale bar denotes a length of 10 μm.

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CHAPTER 4

TEXTURE AND MICROSTRUCTURE OF EMULSIONS PREPARED WITH SOY PROTEIN CONCENTRATE BY HIGH PRESSURE HOMOGENIZATION[1]

[1]Roesch, R., Monagle, W., and Corredig, M. To be submitted to *Journal of Food Science.*

4. 1 Abstract

Oil-in-water emulsions containing a commercial fraction of soy protein concentrate (SPC) and 20% (w/w) soybean oil were the subject of this investigation. The effect of heating at 82^{o}C for 2 min before homogenization at 80 MPa on the rheological properties and microstructure of the emulsions was studied. The effect of varying concentration of SPC was determined by looking at the stability, particle size, surface adsorption, microstructure and rheological properties of the emulsions prepared. All emulsions showed a gel-like behavior, stability to creaming and did not separate upon quiescent storage for 20 days at 4^{o}C. All emulsions with a concentration of SPC > 2% were characterized by a creamy texture. SPC formed a continuous network around oil droplets, and the network structure appeared tighter upon increase in SPC concentration. No significant differences in microstructure and stability to creaming were shown between heated and unheated emulsions. However, heat treatment resulted in emulsions with higher elastic moduli (G') than those of unheated emulsions. In addition, heat treatment caused preferential adsorption of the basic subunits of 11S protein onto the oil-in-water interface. All emulsions contained a population of particles with size > 50 μm consisting of insoluble fiber, oil droplets and protein aggregates.

The unique properties described in this work have never been reported for emulsions in oil-in-water prepared with SPC. These findings will lead to the utilization of high-pressure homogenization of SPC in the development of novel soy-based foods.

4.2 Introduction

Application and study of dynamic high-pressure homogenization in the food industry has received considerable attention in the past several years. Different high-pressure technologies have been developed, with the first applications shown in biotechnology and in the pharmaceutical industry (Chandonnet et al., 1985). Dynamic high-pressure homogenization was introduced for the production of fine emulsions. This technology produces changes in food systems by the simultaneous force-induced phenomena of cavitation, shear, and turbulence (Wicker et al., 2000). Different types of equipment in this category exist in prototype or industrial scale, for example: Microfluidics™, jet homogenizers (Burgaud et al., 1990), and Emulsiflex™ from Avestin (Paquin, 1999). The latter, resembles a conventional valve homogenizer but it is characterized by a specific novel valve design.

It is known that the protein structure and interactions are sensitive to environmental conditions such as temperature, pH and ionic strength (Galazka et al., 2000). Intramolecular hydrophobic and electrostatic interactions are disrupted by the application of high pressure, with important consequences for the tertiary and quaternary structure of the proteins (Ker and Toledo, 1992; Subirade et al., 1998). Galazka et al. (2000) demonstrated that the quaternary structure of legume 11S globulin is disrupted by static high pressure and the interactions between the globulin and polysaccharides are altered. During high-pressure processing, the 11S globulins unfold and aggregate and protein-protein interactions increase (Galazka et al., 2000). In addition, Molina et al. (2001) studied the effect of static high pressure (200-600 MPa) on the emulsifying properties of soy protein isolates and demonstrated that while high pressure dissociated

7S subunits, the dissociation of 11S led to aggregation, by changing its surface hydrophobicity and as a consequence, its solubility.

High-pressure technology has been applied in many research areas of food science, and it has been shown that high pressure can affect not only fat globule size, but also macromolecules or colloids, and modify the structure and functional properties of biopolymers. For example, Wicker et al. (2000) studied the influence of high pressure throttling on acidified pectin-casein dispersions. Others demonstrated that ultra-high pressure homogenization produced irreversible degradation of methylcellulose molecules producing ingredients with weaker thickening properties (Floury at al., 2002), and that molecular weight and viscosity of pectins are also changed by dynamic high pressure (Corredig and Wicker, 2001).

Many studies have addressed the effect of high pressure on protein structure (Masson, 1992; Tanka et al., 1996; Subirade et al., 1998), functionality (Dickinson and Pawloswki, 1996), and enzymatic activity (Hamon et al., 1996; Degraeve et al., 1996). However, there is very little information available on the effect of dynamic high pressure on the emulsifying properties of proteins, and more in particular, on commercial soy protein ingredients. In spite of the increasing attention given to legume proteins and to soy proteins as substitutes for animal proteins as ingredients in emulsions, the understanding of the behavior of soy protein ingredients, such as soy protein concentrates (SPC) and soy protein isolates in oil-in-water emulsions is limited.

It has been reported that soy proteins aid in the formation of emulsions by decreasing the interfacial tension between water and oil (Molina et al., 2001). Soy proteins however, because of their globular aggregated structure, do not unfold and

adsorb at the interface, but rather form a thick interfacial layer, which acts as a physical barrier to coalescence (Dickinson and Stainsby, 1988; Molina et al., 2001).

Several studies have demonstrated that heat treatment strongly influences the interactions (protein-protein and protein-solvent) of soy proteins and their functional properties such as solubility, gelation, dispersability (Kinsella, 1976; Renkema et al., 2000; Renkema and Vliet, 2002). Heat treatment unfolds soy proteins, and sulphydryl groups and hydrophobic regions become exposed, resulting in protein-protein aggregation, network formation and gel stiffening (Kinsella, 1976; Renkema and Vliet, 2002). Heat denaturation has been considered the main factor in gel formation and the type of gel depends on heating and cooling conditions (Renkema et al., 2000). Temperature, pH and ionic strength influence the heat denaturation of β-conglycinin and glycinin (Puppo and Anon, 1998).

The protein denaturation occurring in a soy protein-based ingredient depends on its protein composition or its subunit variation (Damodaran et al., 1988; Renkema et al., 2001). It has been shown that the various soy proteins have a different behavior upon heating: β-conglycinin is less heat stable than glycinin, with an onset denaturation temperature of 70^{o}C for β-conglycinin and 80^{o}C for glycinin at neutral pH and non-salt added (Hermansson, 1986). At the same pH but with salt added (0.5M) the onset denaturation temperatures for β-conglycinin and glycinin are 63^{o}C and 80^{o}C, respectively (Renkema and Vliet, 2002).

During the preparation of SPC from defatted soy flour, physical-chemical changes affecting the functional properties of the protein occur (Cronakis, et al., 1995).

Commercial SPCs vary in their functional properties, not only because of compositional differences, but also because of their processing history.

The effect of high-pressure homogenization and heating on the behavior of oil-in-water emulsions prepared with SPC was the objective of the present study. To achieve this goal, emulsions containing 20% soybean oil and different concentrations of SPC were characterized for their physicochemical properties, stability, rheology and microstructure.

4.3 Methodology

4.3.1 Sample preparation

Soy protein concentrate (Promine™ SPC 3275) was obtained from Central Soya (Fort Wayne, IN). The SPC contained 19.4% dietary fiber (1.2% soluble and 18.2% insoluble fiber) and 70% protein (data provided by the Manufacturer). Suspensions were prepared by dispersing 2, 4, 6 and 8% (w/w) SPC in 50 mM sodium phosphate buffer, pH 7.5 containing 0.02% sodium azide (as an antimicrobial). Dispersions were stored overnight at 4°C to allow protein hydration. Emulsions were prepared by adding 20% (w/w) soybean oil to the SPC dispersions. The samples were pre-homogenized with a hand-held homogenizer (Polytron® PT 1200, Kinematica AG, Littau, Switzerland Littau) at 10,000 rpm for 1 min. Emulsions were then passed through a valve homogenizer (Emulsiflex C5, Avestin, Canada) at room temperature, in two stages, one at 5 MPa and the other at 70 MPa. Some emulsions were pasteurized at 82^{o}C for 2 min (come up time of 1.5 min), at constant stirring in a water bath and cooled to 40^{o}C and then homogenized as described above; the temperature was registered with a thermocouple. SPC suspensions were also heated and /or homogenized under the same conditions.

4.3.2 Particle size distribution

The particle size distribution and the volume-surface average particle diameter $d_{3,2}=(\Sigma n_i d_i^3/\Sigma n_i d_i^2)$ and $d_{4,3}=(\Sigma n_i d_i^4/\Sigma n_i d_i^3)$ of the heated and non-heated emulsions and soy suspensions were determined by integrated light scattering (Mastersizer S, Malvern Instruments, MA) using the standard presentation code for samples dissolved in water (1.5295, 0.1000 in 1.3300 for the relative particle refractive index, relative refractive index and dispersant refractive index, respectively). Measurements were also carried out after incubating the samples at room temperature for 30 min in a 1% (w/v) sodium dodecyl sulphate (SDS).

4.3.3 Creaming stability

Aliquots of emulsions were placed in duplicate in 10-mL graduated tubes (10 mm internal diameter) and stored quiescently at 4°C for 20 days. In addition, samples were centrifuged (Sorvall RC-5B, Fisher Sci.) at 19,000xg for 30 minutes at 25^{o}C to determine the presence of insoluble material or visible phase separation.

4.3.4 Viscoelastic properties

The viscoelastic properties were studied using a controlled stress rheometer (Model SR-5000, Rheometrics Scientific, Piscataway, NJ), measuring the frequency dependence of storage (G') and loss (G") moduli with a parallel plate geometry (40 mm plate) at 25^{o}C controlled by a peltier heating device with a gap of 0.8 mm. Differences in mechanical properties among emulsions were evaluated by fitting the values of G' and G" to a power law. The slope (n) indicated the variation in frequency dependence of G' and G" depending on the SPC concentration.

4.3.5 Microstructural Studies

Microstructural observations were carried out with a scanning electron microscope with a cold stage attachment (Leo 982 emission scanning electron microscope, Alto 2500 cryostage and cryoprep; Leo Electron Microscopy Inc., Thornwood, NY, USA). One drop of sample was placed on a specimen stub and frozen with liquid nitrogen. The sample was then introduced into the cryo-unit and fractured with a knife and coated with gold as previously described by Saio (1981).

Samples were also analyzed by light microscopy (Carl Zeiss Photomicroscope III, New York, NY, USA) in normal mode with a 40x objective and phase contrast mode with a Ph2 Neufluar 25x objective, inverted light microscope (Nikon eclipse TE300, Atlanta G.A) with a 20x Plan fluor objective in normal mode with a green filter. These techniques did not require any sample preparation.

Microstructural analysis was also carried out using a light microscope (Carl Zeiss Photomicroscope III, New York, NY, USA) in a differential interference contrast mode (DIC) and a multiphoton confocal scanning laser microscope (CSLM) (Leica TCS SP2/ Leica DMRXE) used in reflectance, transmittance and fluorescence mode after addition of a fluorescence dye specific for lipid fraction (BODIPY, C_{12} 558/568, Molecular probes). Microstructural analyses were carried out between 24 and 36 h from preparation, and unless otherwise indicated, observations were carried out at room temperature.

4.3.6 Surface protein coverage

The cream was separated from the serum phase by centrifugation of emulsions at 19,000x g for 30 min at 25^{o}C in a refrigerated centrifuge (Sorvall RC-5B, Fisher Sci.). The cream phase was then redispersed in 50 mM sodium phosphate buffer, pH 7.5 to the

initial concentration (20% w/w), and centrifuged again under the same conditions. Creams were then dried on a Whatman no.1 filter paper as previously described by other authors (Hunt and Dalgleish, 1994). After the first centrifugation, the serum was carefully removed using a syringe and filtered through a 0.22 μm filter (Millex GP, Millipore Corp. Bedford, MA). Cream and serum were then dispersed in electrophoresis buffer (12.5% Tris-HCl pH 6.8, 20%(w/v) SDS, 1.25% bromophenol blue, 5% β-mercaptoethanol, 25% Glycerol) and denatured in a boiling water bath. Samples were then centrifuged with an Eppendorf centrifuge (Brinkmann Instruments, Westbury N.Y) at 15,000 g for 1.5 min and loaded onto a 12.5 Tris-HCl acrylamide gel (Biorad, Hercules CA). Gels were run, stained with Coomassie blue, destained according to manufacturer's instructions and air-dried. Gels were then scanned with an imaging densitometer (Biorad GS-7000) and profile analysis of the intensities and position of the protein bands was carried out (Biorad Molecular Analyst® software, Hercules, CA).

4.3.7 Statistical Analysis

Results presented are the average of at least two replicates. Significant differences were determined by the general linear model procedure and Duncan grouping (SAS version 6.12). Treatments were considered significant at $p<0.05$.

4.4 Results and Discussion

Figure 4.1 depicts the effect of heating and homogenization on the particle size distribution of 6% SPC suspensions. The 6% SPC suspensions before heating and homogenization were characterized by a wide distribution of particle sizes, with 90% of the particles > than 10 μm. After homogenization and/or heat treatment, suspensions were still characterized by a wide distribution of particles, but smaller than those of

untreated SPC. Heat treatment before homogenization seemed to produce a similar effect in particle size reduction than that caused by homogenization only.

High pressure homogenization of SPC with 20% soybean oil did not show reduction in particle size distribution, and a large volume of the emulsions was occupied by particles > than 5 μm (Figure 4.2). Emulsions at 6% or 8% SPC subjected to heat treatment before homogenization did not show significant differences in particle size distribution from non-heated emulsions. Only 2% SPC emulsions after heating showed a smaller particle size distribution than the unheated samples, with an average size at about 12 μm instead of 80 μm.

After homogenization both non-heated and heated emulsions and suspensions contained large particles. After addition of SDS, the particle size distribution of both heated and unheated emulsions was quite different from those shown in the Figure 4.2. However 6% SPC suspensions did not shown any change in particle size.

In unheated emulsions (Figure 4.3A), a bimodal distribution of sizes was shown in samples containing > 2% SPC, with one population of oil droplets with an average of about 0.5 μm and a second population of particles of size > 10 μm. When the amount of SPC present in the emulsion was not enough to cover the surface of the oil droplets (for example 2% SPC), the particle size distribution after treatment with SDS showed a much smaller population than that shown in Figure 4.2. In heated emulsions (figure 4.3B) also a bimodal distribution of particles was shown with a small population of particles >10 μm (compare Figure 4.3 A and B). The presence of a population containing small particles after the incubation with SDS suggests the presence of flocculated oil droplets. In addition, it was concluded that heating before homogenization improved the

functionality of SPC. The viscoelastic properties of the emulsions prepared with different concentrations of SPC are shown in Figure 4.4. Results obtained by analysis of G' and G'' as a function of the oscillatory frequency and SPC concentration demonstrated the gel-like behavior of all the samples analyzed. In general, very little frequency dependence of the G' and G'' emulsions was determined. Table 4.1 summarizes the results obtained by fitting a power law to results of Figure 4.4. The slope (n) of G' and G" as a function of frequency indicated little or no frequency dependence of the samples analyzed, as affected by SPC concentration and heating treatment. All emulsions showed similar n values for G', regardless of SPC concentration, suggesting that the samples were physical gels characterized by a similar structure (Gunasekaran and Mehment, 2000). G' and G" for emulsions heat- treated and non-heat treated containing 6% SPC, as well as non-heat treated emulsions containing 8% SPC showed same n values indicating the parallel nature of this dependence on frequency.

A significant effect of concentration on the average values of G' and G'' was shown for both heated and unheated emulsions (Table 4.2 and Figure 4.4). By increasing the SPC concentration the emulsion showed a higher G' indicating a stronger gel. In addition, heat treated emulsions showed higher G' and G" values than non-heat treated emulsions, suggesting that heating at 82°C for 2 min caused stronger network interactions, and perhaps additional protein incorporation in the network. This observation was in agreement with previous published work indicating that heating temperature strongly influences the functional properties of soy proteins (Kinsella, 1976; Kim et al, 2001). Renkema et al. (2000) hypothesized that high G' values are correlated with a low solubility after heating, with the precipitated proteins participating in network

formation. An increase in G' after the onset gelation of soy protein isolates was also described as caused by the incorporation of proteins in the network by fusion of protein aggregates in the gel strands (Renkema and Vliet, 2002).

All emulsions showed a "creamy" texture at concentrations higher than 2%. This is a unique property that has never been reported for SPC emulsions. These results may lead to the utilization of high-pressure homogenization of SPC to develop new soy-based products.

All emulsions, regardless of the amount of SPC present showed stability to creaming and no phase separation upon 20 days of storage at 4^{o}C. In addition heating the emulsions before homogenization did not have an effect on creaming stability. Emulsions containing > 2% SPC did not show phase separation even after centrifugation. These results were different from previously reported data on the stability of emulsions prepared with SPC and 10% oil (Roesch and Corredig, 2002). The lack of phase separation upon centrifugation was an indication of the increased water holding capacity of SPC under those conditions. Water holding capacity has been previously reported as one of the main characteristics of a real soy protein gel (Puppo et al., 1995). The results obtained during stability studies confirmed the data from rheological measurements and suggested the formation of a strong protein matrix upon homogenization of SPC with 20% oil.

The results obtained from particle size, rheological and stability studies where complemented with microstructural observations, in the effort to obtain a better picture of how the biopolymers present in the SPC were organized after high-pressure homogenization.

Laser confocal microscopy (Figure 4.5) conducted in reflectance and fluorescence mode (by the addition of a lipid sensitive dye) showed differences in the organization of the protein matrix, after the addition of oil and homogenization. Figure 4.5A illustrates a 6% SPC suspension after homogenization. In suspensions, the continuous phase was uniformly distributed and had a smooth appearance. On the other hand, in emulsions containing 20% oil and the same concentration of SPC (Figure 4.5B) large oil droplets were present as evidence of floculation and coalescence. Segregation of the oil droplets in the protein network could be observed. It was hypothesized that the viscoelastic properties of the emulsions were a result of this segregation of oil droplets, because of incompatibility between the protein and the oil phase.

Protein aggregation, large oil droplets, oil droplets segregation and the presence of non-soluble polymers were observed in the SPC emulsions prepared by high pressure homogenization (Figures 4.6 and 4.7). Light microscopy, by using phase contrast and differential interference contrast, clearly showed the distribution of large oil droplets in the midst of a continuous phase containing SPC. At low concentration (2% SPC, Figures 4.6A and 4.7A) aggregated flocs were shown in the continuous phase, however, this did not seem to have any consequences on their creaming behavior. Indeed, all emulsions analyzed showed stability to creaming and no phase separation. At higher concentrations of SPC a decrease in the space between aggregates was observed. It was concluded that particle movement was more limited at higher concentration of SPC; this caused the emulsions to be stable to creaming and not show phase separation upon centrifugation. These microscopy observations confirmed data collected during rheological analysis: SPC concentration increased the elastic and viscous moduli of the emulsions.

Scanning Electron Microscopy (SEM) observations of unheated and heated emulsions containing different amounts of SPC showed similar microstructure (Figure 4.8). Globular soy proteins and oil droplets were shown as well as the protein network formed by SPC. Increasing amounts of SPC tightened the network structure, showing a solid surface in emulsions containing 8% SPC. This network formed by unfolding of globular proteins, may be caused by the high-pressure homogenization or/and heat treatment applied. Observations at higher magnification showed details of the network formed (Figure 4.9) and the presence of fat droplets embedded in the protein matrix (Figure 4.10).

The soy fiber present in SPC seemed to play a role in the stability of the emulsions, by occupying space in the continuous phase, competing for water and binding the protein-covered oil droplets. Figure 4.11 depicts a detail of fiber observed by confocal microscopy. The fiber seemed to be interacting with the oil droplets. This may have further limited the movement of oil droplets, helping in increasing their stability to creaming. The presence of large particles as shown by integrated light scattering (Figures 4.1, 4.2, 4.3) could be at least partly attributed to such large soy fiber structures. According to microscopy observations these fiber particles showed an average size between 50 and 100 μm (Figure 4.12) and their size did not seem to be affected by heat treatment or by high-pressure homogenization (Figure 4.1).

When the protein present in the various phases of the heat-treated emulsions was analyzed by SDS-PAGE electrophoresis (Figure 4.13), in emulsions containing only 2% SPC, most of the protein was adsorbed onto the cream phase and no protein was present in the soluble phase. This indicated that not enough protein was present to cover the oil

water interface. When the protein present in emulsions, cream and serum phase of samples with SPC > 2% was analyzed by SDS-PAGE, a different protein migration pattern was shown between the protein present in the cream and serum phases (Figure 4.13, 4.14). Figure 4.13 clearly illustrates the preferential adsorption in the cream phase of the 11S basic subunits. The 11S basic subunits were mostly adsorbed onto the cream phase, while the acidic 1, 2 and 4 subunits showed a higher amount in the serum phase. On the other hand, the 7S subunits did not show preferential absorption of α, α' and β subunits; however, the serum phase showed the preferential adsorption of secondary subunits.

These results were not in agreement with the observations on SPC emulsions prepared with 10% oil and 20% oil with no heat applied before homogenization (Roesch and Corredig, 2002).

Molina et al. (2001) suggested that 11S solubility is greatly influenced by hydrostatic high-pressure treatment at pH 7.5. The same authors also hypothesized that pressure treatment negatively affects the solubility and the surface hydrophobicity of SPI. The combination of heat and high-pressure homogenization may have caused the disruption of the 11S structure and an increased adsorption at the interface of the basic subunits. An increased solubility and protein-protein interactions may also be the cause of the preferential adsorption of the basic 11S. These results were in disagreement with Liu et al. (1999) who demonstrated the higher emulsifying properties of acidic 11S subunits in a purified system. The data reported in this research, however, are result of the study of a commercial SPC product containing proteins with a distinct thermal and processing history.

SDS-PAGE electrophoresis analysis of the protein present in the serum phase of the emulsions also showed a band at about 58 kDa with a higher or same intensity than that of the β-subunits (Figure 4.14). This band was not present in the original SPC, and it could be result of subunit aggregation during heating in combination with high-pressure homogenization.

4.5 Conclusions

In general, heat-treated emulsions did not show major differences in stability and microstructure when compared to non-heated emulsions.

All emulsions showed a gel-like behavior and stability to creaming upon storage at 4º C for 20 days and good water holding capacity. High-pressure homogenization caused unfolding and network formation of SPC proteins. Aggregated protein, presence of aggregated oil droplets and fiber were the cause of the gel-like properties of these emulsions. Heat treatment before homogenization increased the gel strength producing gels with higher G' and G" than those of unheated emulsions.

Some very large particles (> 50 μm) were present in the emulsions and identified as insoluble fiber. Homogenization and/or heat treatment did not modify their size, and they were shown to interact with the oil droplets.

The unique properties of SPC in emulsions prepared by high-pressure homogenization are quite significant in light of the utilization of SPC as an ingredient in novel soy-based foods.

Table 4.1: Means comparison (as determined by general linear model at $p<0.05$) for the calculated slope of power law for the values of G' and G" versus frequency of emulsions prepared with different concentrations of SPC unheated and heated before homogenization. Different superscript letter shows significant difference, letters a,b are within row; c,d within column.

Unheated, SPC conc.	G' n	G" n
2%	$0.085^{a\ c}$	$0.045^{b\ c}$
4%	$0.083^{a\ c}$	$0.049^{b\ c}$
6%	$0.082^{a\ c}$	$0.063^{a\ c}$
8%	$0.087^{a\ c}$	$0.095^{a\ d}$

Heated, SPC conc.	G' n	G" n
2%	$0.104^{a\ c}$	$0.039^{b\ c}$
4%	$0.093^{a\ c}$	$0.058^{b\ c}$
6%	$0.096^{a\ c}$	$0.115^{a\ d}$
8%	$0.092^{a\ c}$	$0.144^{b\ d}$

Table 4.2: Means comparison of the values of elastic (G') and viscous (G'') moduli at 1 Hz of emulsions made with 20% oil and different SPC concentrations. When comparing values of G' or G" within the same row with the same superscript letter are not significant ($p<0.05$).

	2%		4%		6%		8%	
	G' (Pa)	G"(Pa)	G'(Pa)	G"(Pa)	G'(Pa)	G" (Pa)	G'(Pa)	G"(Pa)
Unheated	256.0^{c}	32.1^{d}	675.6^{b}	95.8^{d}	1027.2^{b}	180.6^{d}	1576.7^{a}	334.0^{d}
Heated	1952.7^{a}	252.6^{c}	4135.9^{b}	637.5^{c}	9242.7^{b}	2116.1^{ce}	13281.7^{b}	3668.6^{e}

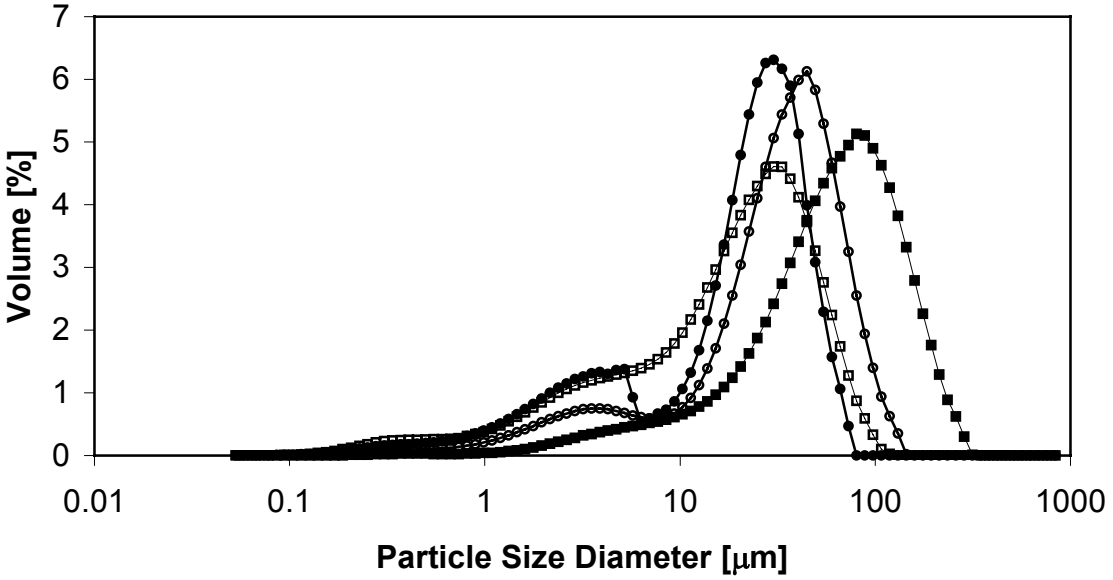


Figure 4.1: Particle size distribution of SPC suspensions as determined by integrated light scattering. 6% SPC [■], 6% SPC after homogenization [□], 6% SPC after heating at 82°C for 2min [●], 6% SPC after heating and homogenization [o].

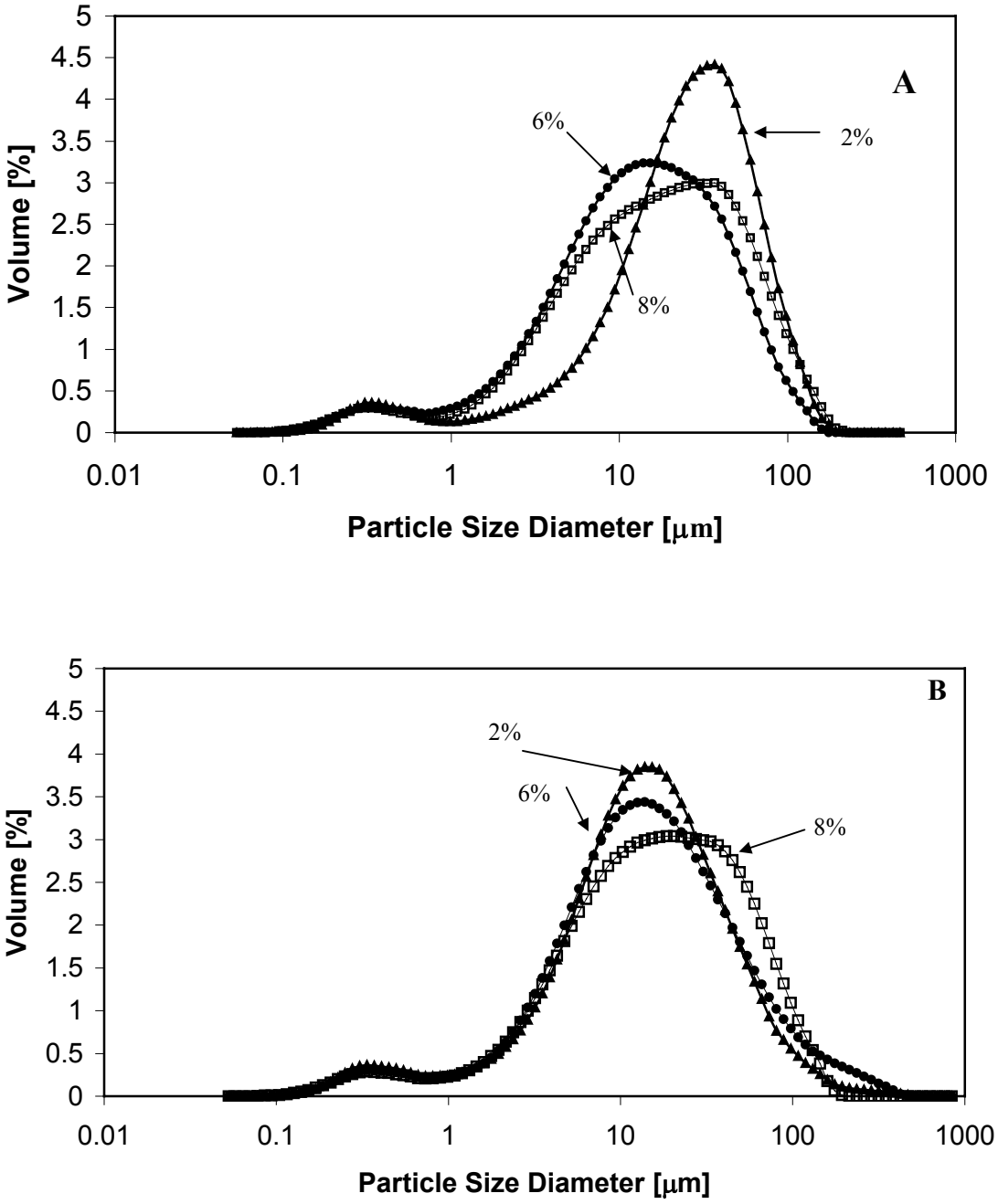


Figure 4.2: Particle size distribution of 20% soy oil emulsions prepared with SPC as determined by integrated light scattering. 2% SPC [▲]; 6% SPC [●]; 8% SPC [□]; homogenized (A) and homogenized after heating at 82°C for 2 min (B). Distributions are the average of 3 replicate emulsions.

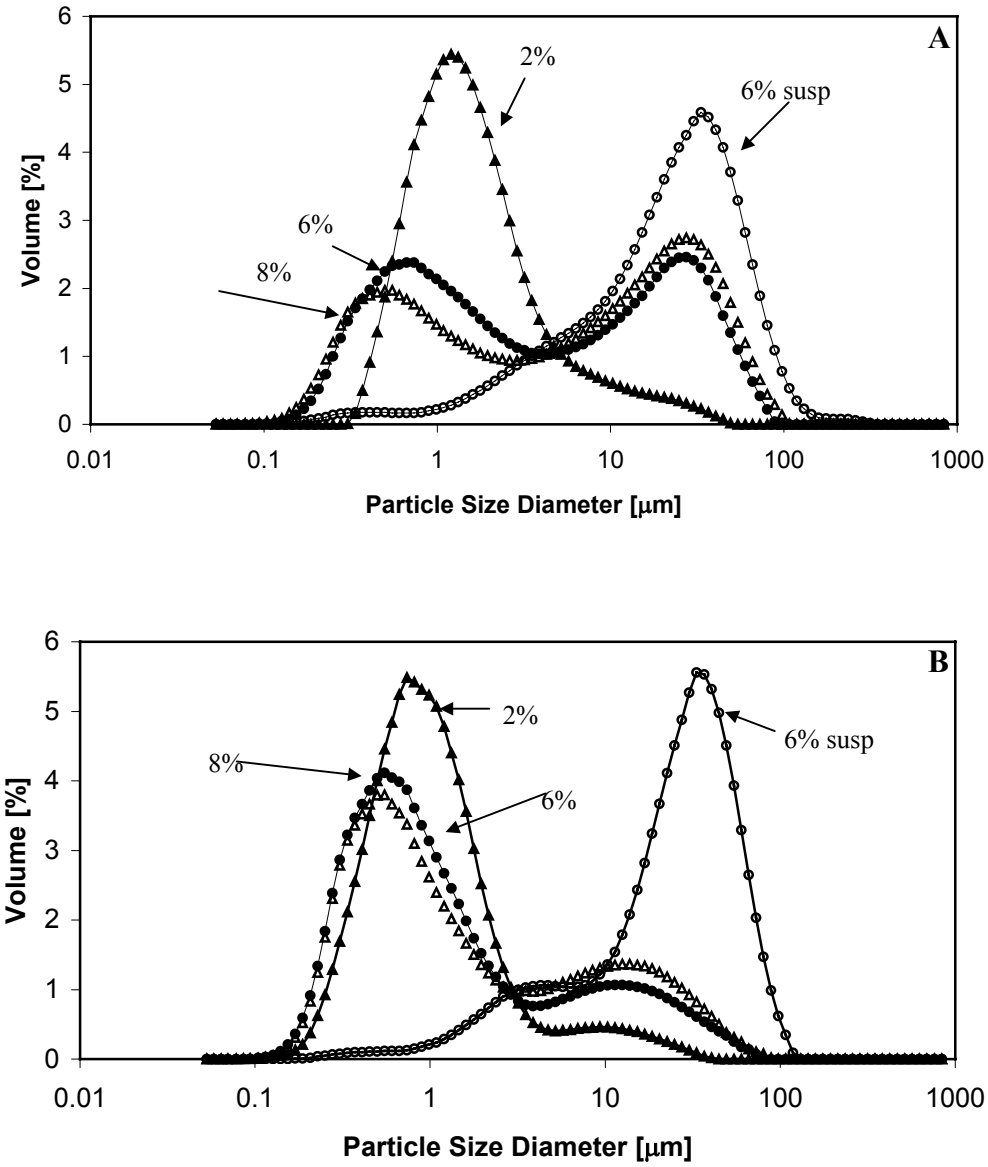


Figure 4.3: Particle size distribution of 20% soy oil emulsions prepared with different concentrations of SPC as determined by integrated light scattering after dilution in 1% SDS. 6% SPC suspension [o], 2% SPC [▲] 6% SPC [●], 8% SPC [Δ]; homogenized (A), heat and homogenized (B). Distributions are the average of 3 independent samples.

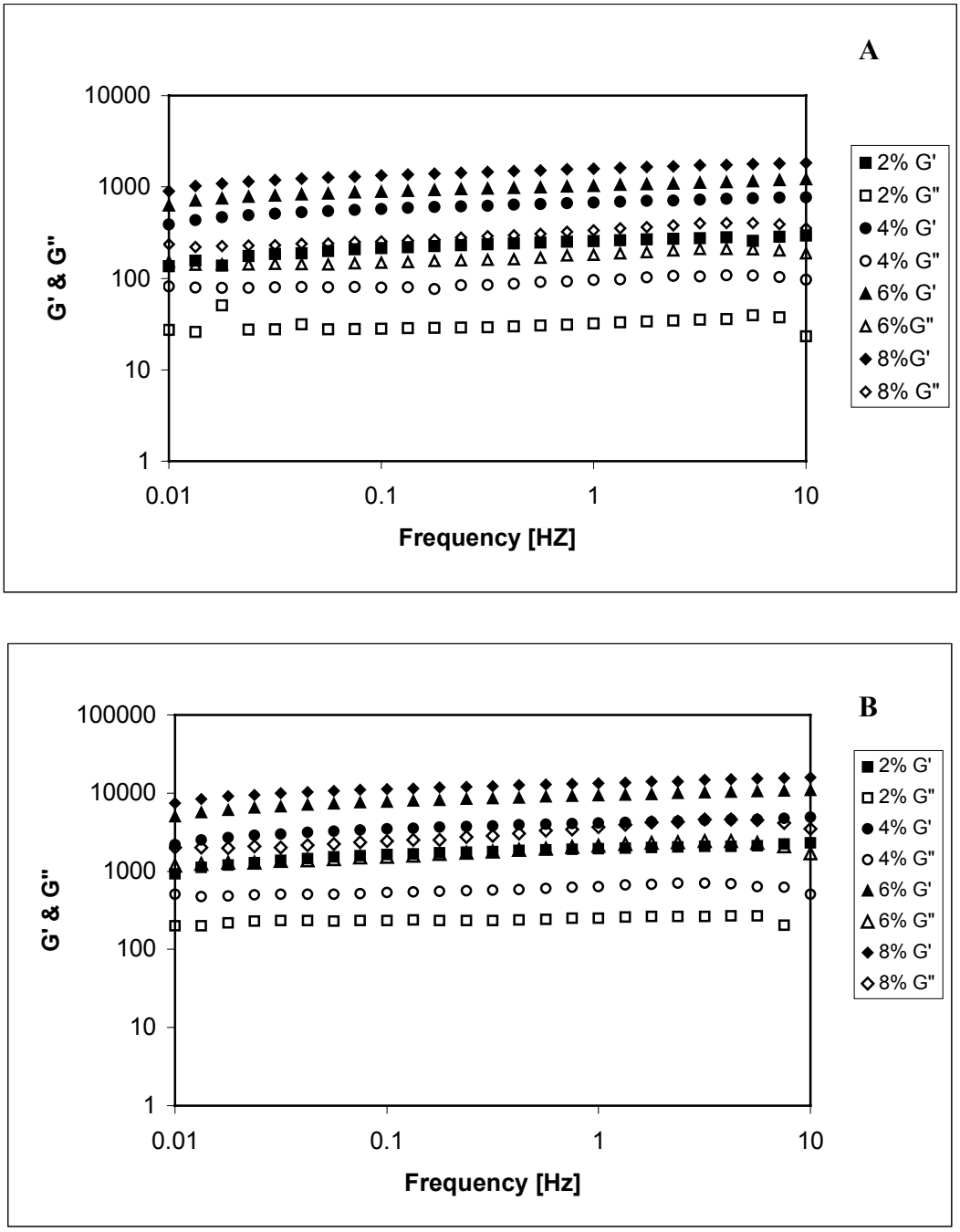


Figure 4.4: Average measurements of the evolution of storage (filled symbols) and loss moduli (empty symbols) for 20% soy oil-in-water emulsions prepared with SPC, as measured by controlled stress rheometry. Emulsions containing 2% SPC (■,□); 4% SPC (●,○); 6% SPC (▲,Δ); 8% SPC (♦,◊) unheated (A) and heated (B) before homogenization. Results are the average of three independent experiments.

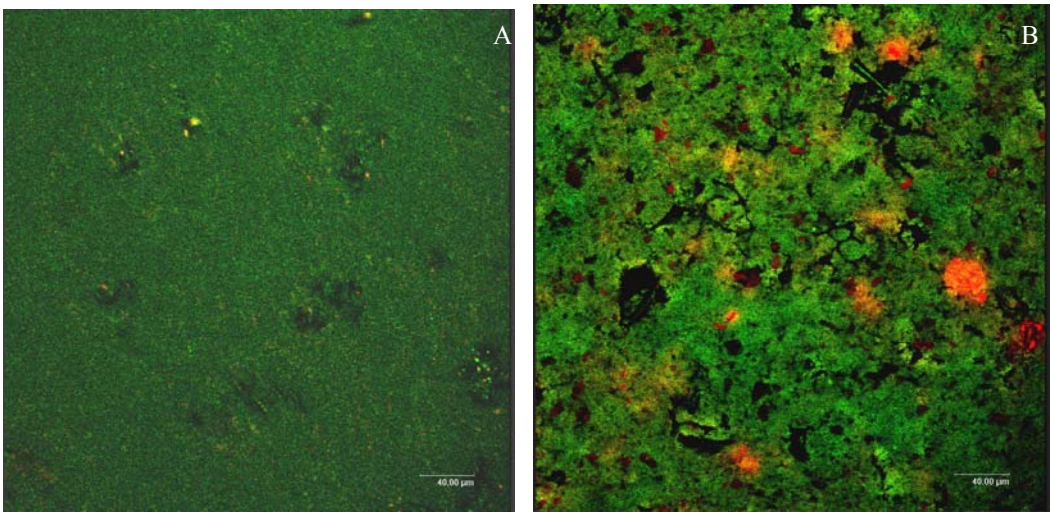


Figure 4.5: Images collected with a multiphoton confocal laser scanning microscope of a suspension (A) and emulsion (B) containing 6% SPC. Dark areas correspond to the protein signal in reflection mode and lighter areas correspond to the signal of the fluorescent dye Biodipy. The scale bars denote a length of 40 μm.

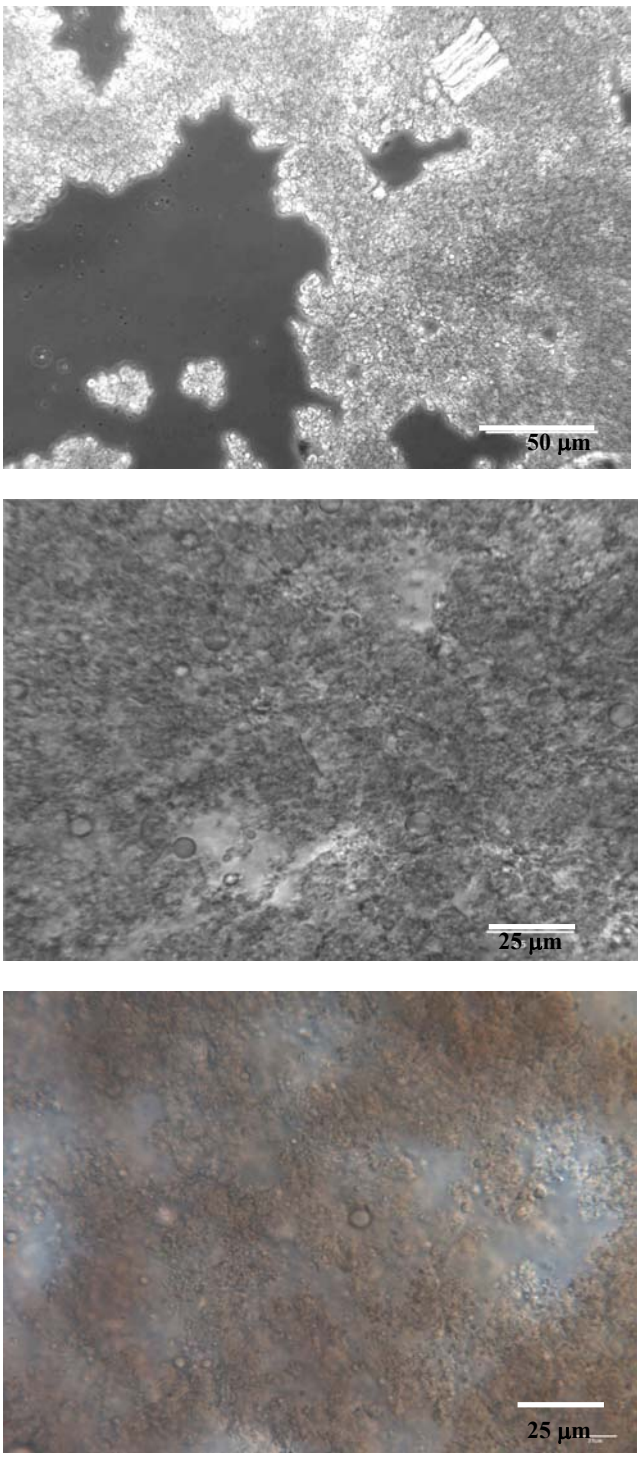


Figure 4.6: Images of 20% soy oil emulsions collected with a light microscope in phase contrast mode (250x) and normal light mode (400x). (A) 2% SPC emulsion, phase contrast (scale bar 50 µm); (B) 6% SPC and (C) 8% SPC, light microscopy (scale bar 25 µm).

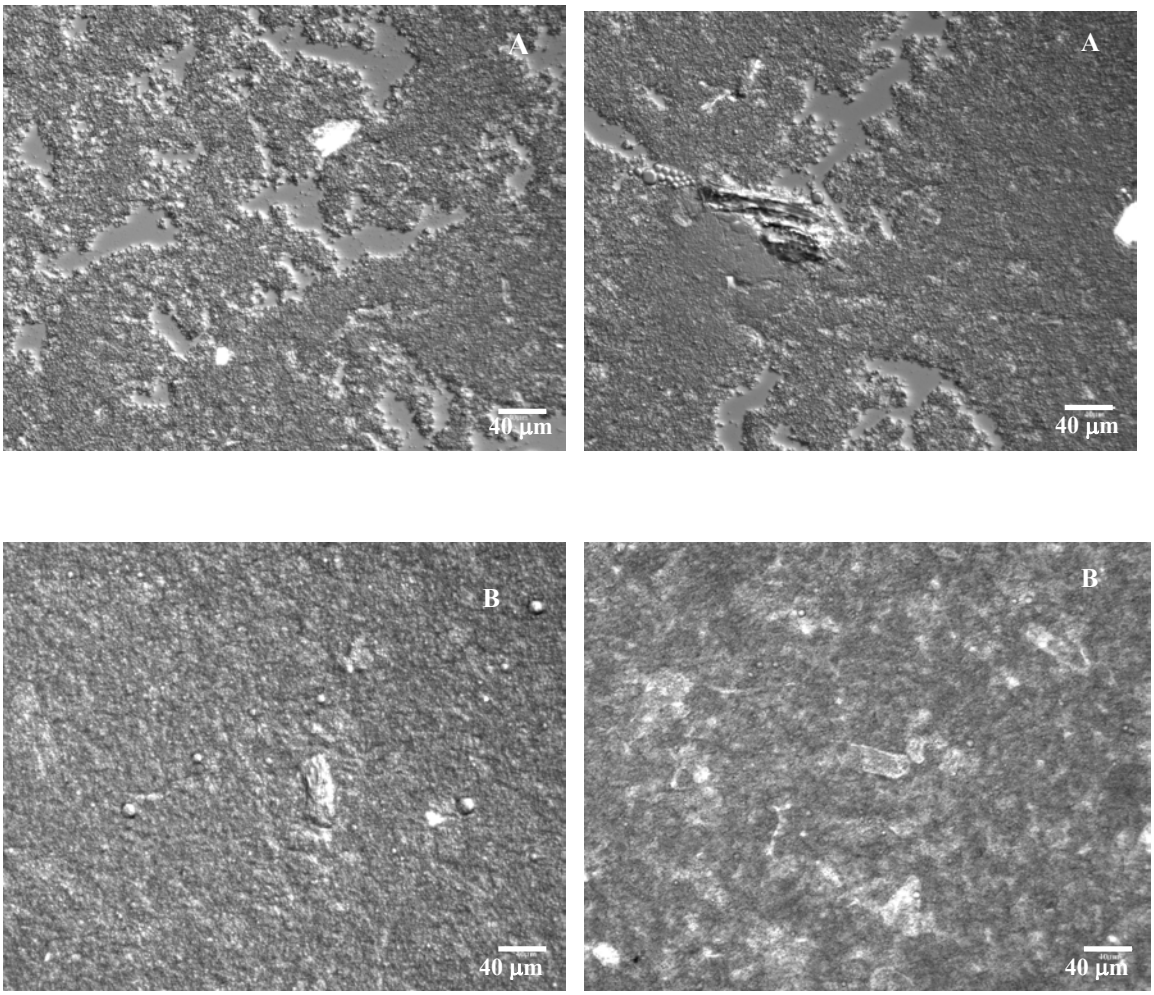


Figure 4.7: Images of 20% soy oil emulsions collected by light microscopy (225x) by differential interference contrast mode (DIC) (left) and normal light mode using with a green filter (right). Emulsions were heated at 82°C for 2 min before homogenization and contained 2% SPC (A) and 8% SPC (B). Scale bar denotes a length of 40 µm.

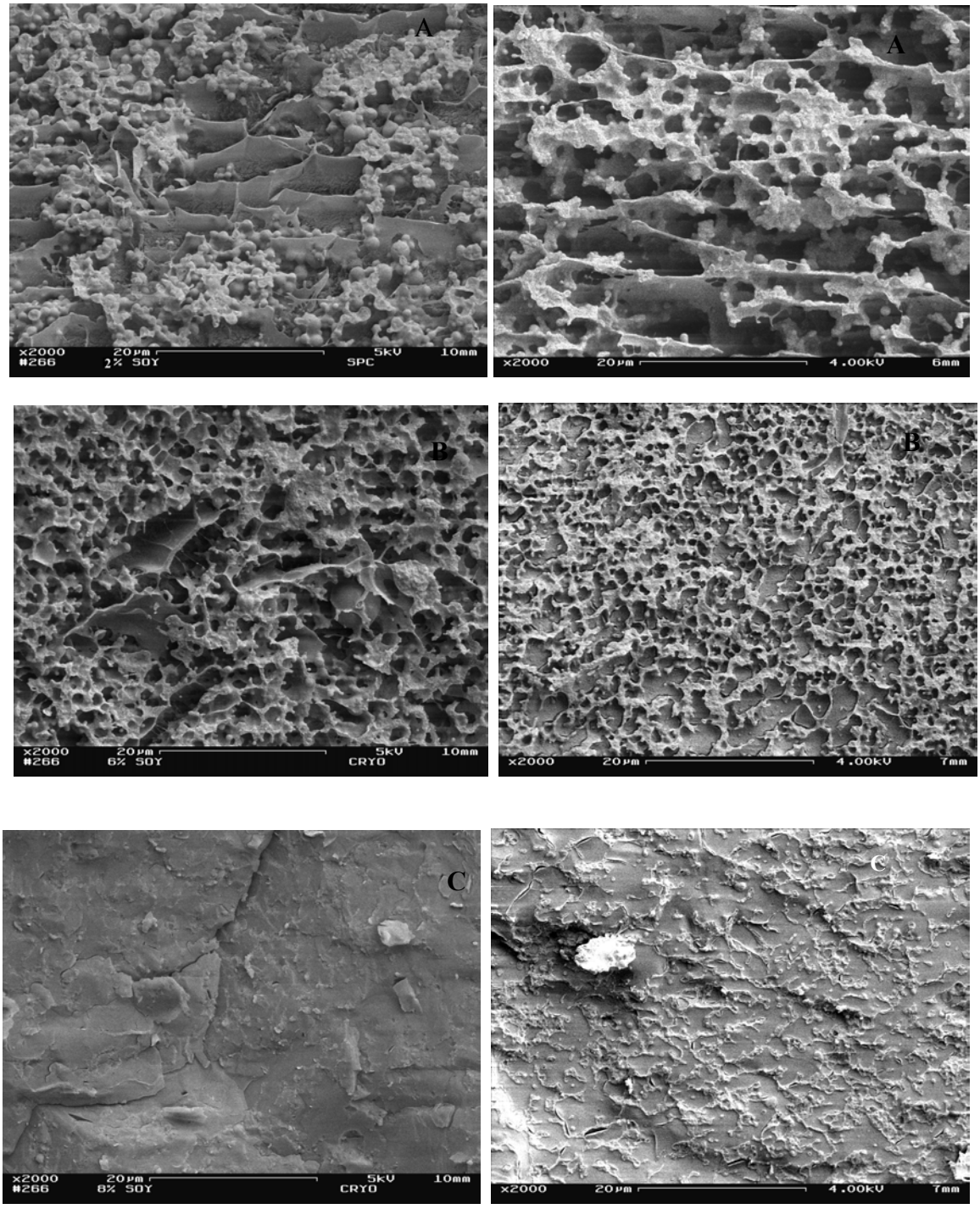


Figure 4.8: Effect of heat treatment on microstructure of 20% soy oil emulsions. Scanning electron microscopy images (2,000x) of unheated (left) and heated (right) emulsions prepared with 2% SPC (A), 6% SPC (B) and 8% SPC (C). Samples were prepared using a cryogenic SEM unit (see methods). Scale bars represent 20 μm.

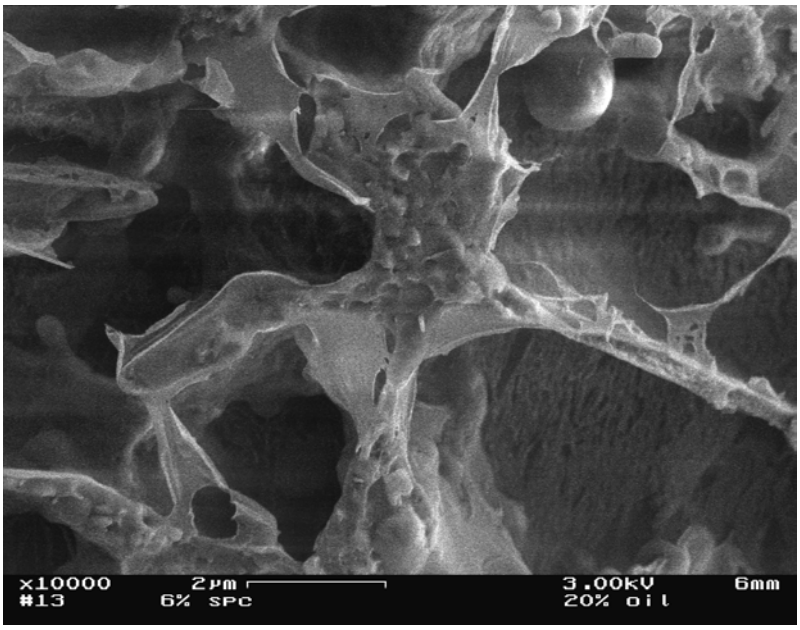


Figure 4.9: Scanning electron microscopy image (10,000x) of the SPC network of an emulsion prepared with 6% SPC and 20% oil. The scale bar denotes a length of 2 μm.

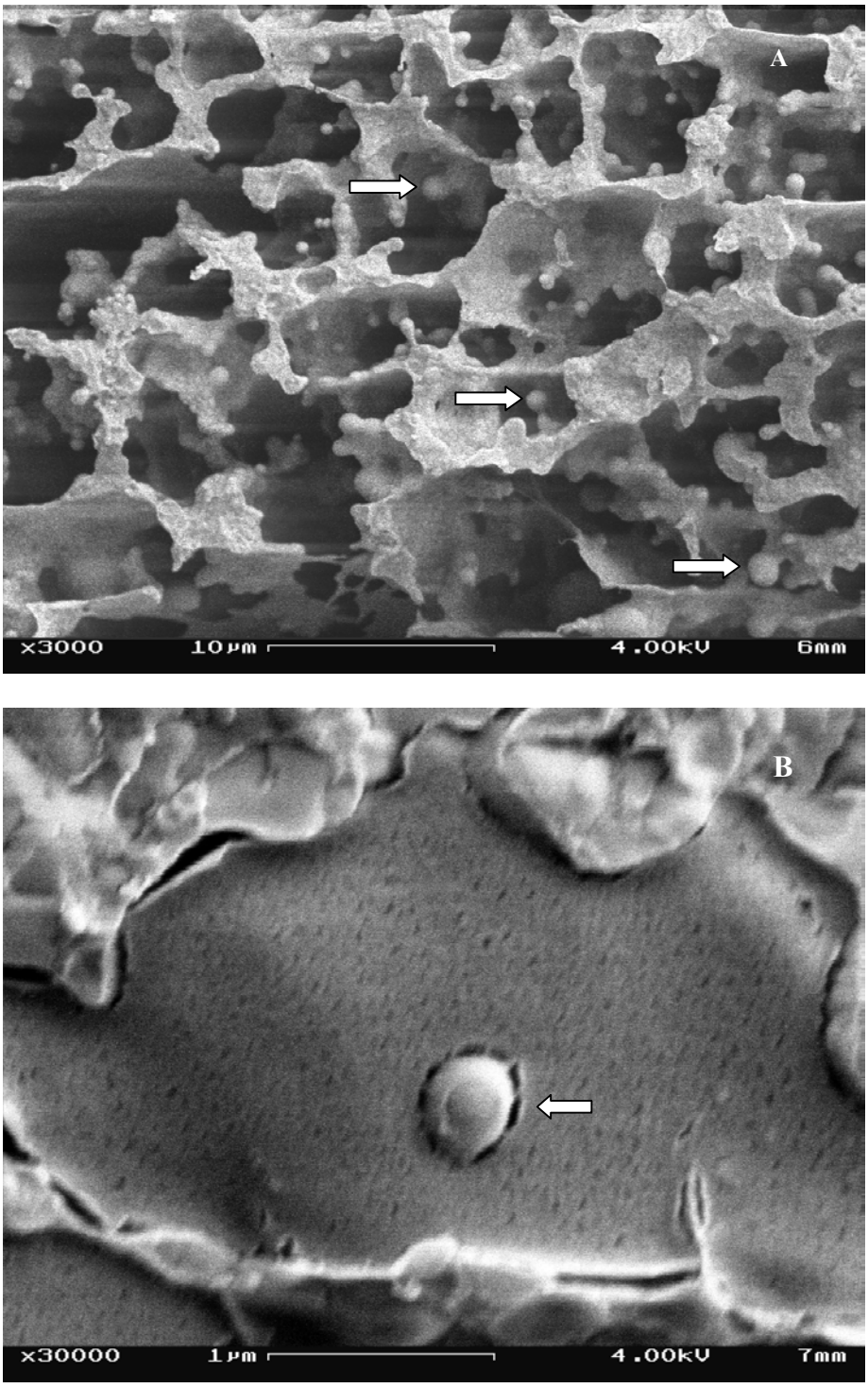


Figure 4.10: Particular of oil droplets (indicated by arrows) in the SEM micrographs. Emulsions prepared with 6% SPC and 20% oil and heated at 82°C for 2 min before homogenization. 3,000x (A) and 30,000x (B). The scale bar denotes lengths of 10 and 1 μm, respectively.

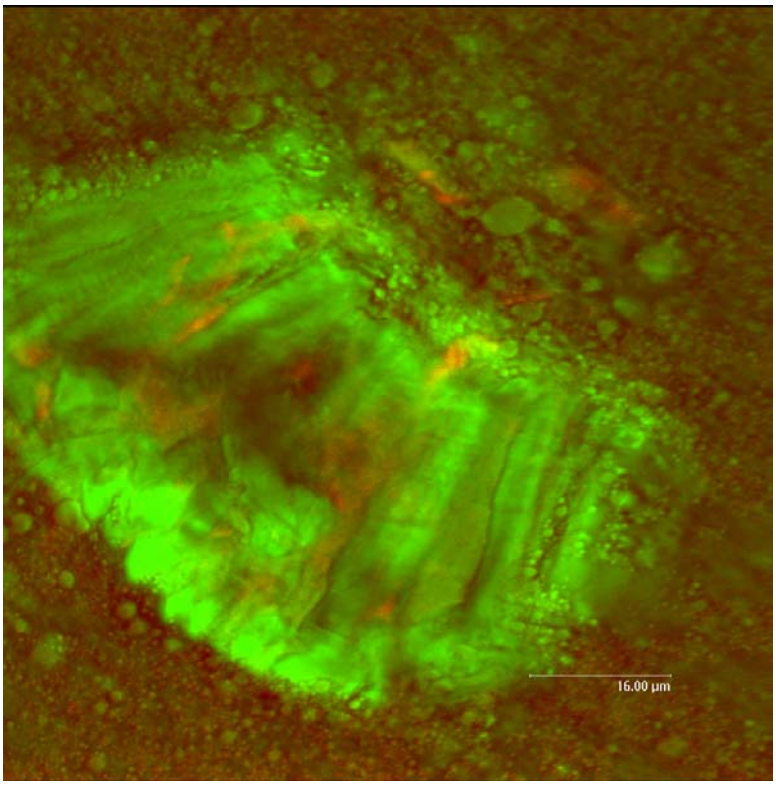


Figure 4.11: Confocal microscopy image collected in transmittance mode of a 6% SPC and 20% soy oil emulsion. Detail of insoluble fiber (scale bar of 16 µm).

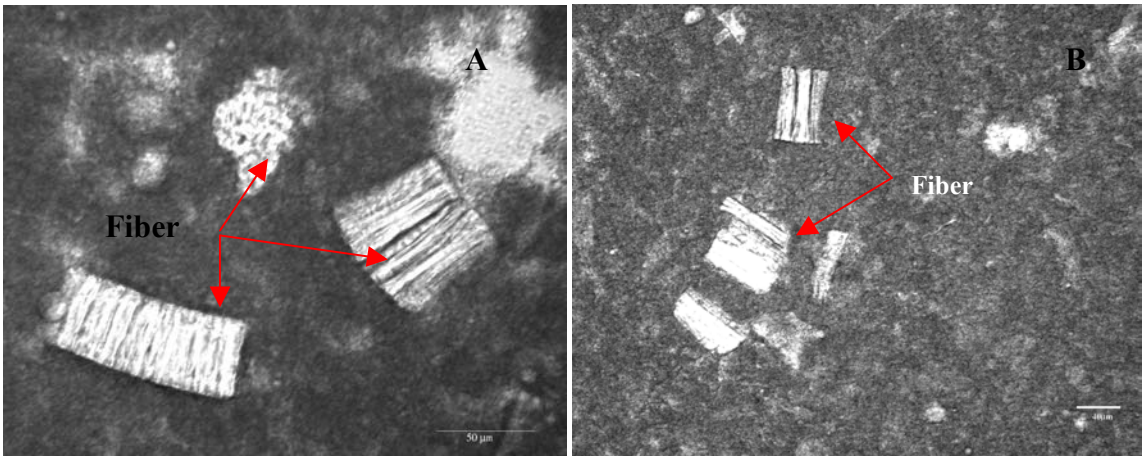


Figure 4.12: Effect of heat treatment on the soy fiber fragments present in emulsions prepared with 20% soy oil and 6% SPC. Phase contrast images of unheated (left) and heated (right) emulsions. The dark areas represent the continuous phase containing protein (scale bar 50 (A) and 40 (B) µm).

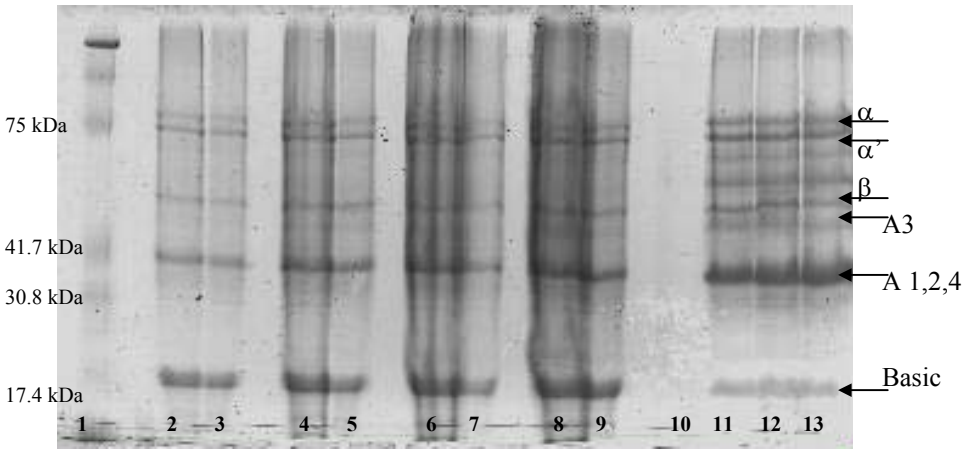
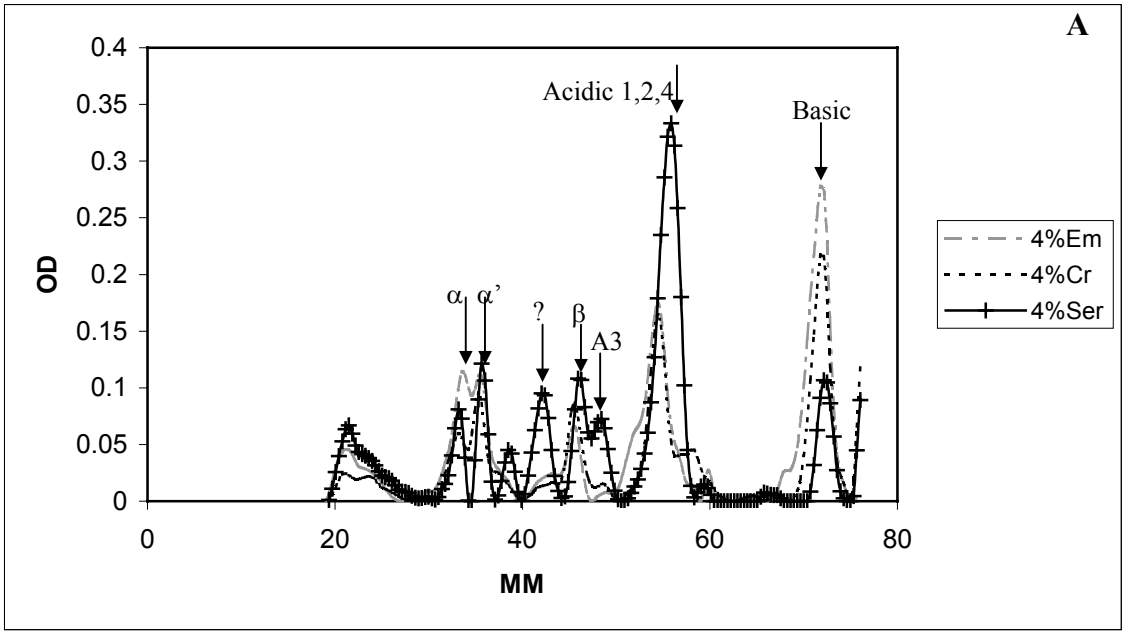


Figure 4.13: SDS-PAGE of heated oil-in-water emulsions prepared with various concentrations of SPC. Lane 1: molecular weight standard; lane 2: 2% SPC emulsion; lane 3: 2% SPC cream; lane 4: 4% SPC emulsion; lane 5: 4% SPC cream; lane 6: 6% SPC emulsion; lane 7: 6% SPC cream; lane 8: 8% SPC emulsion; lane 9: 8% SPC cream; lane 10: 2% SPC serum; lane 11: 4% SPC serum; lane 12: 6% SPC serum and lane 13: 8% SPC serum.



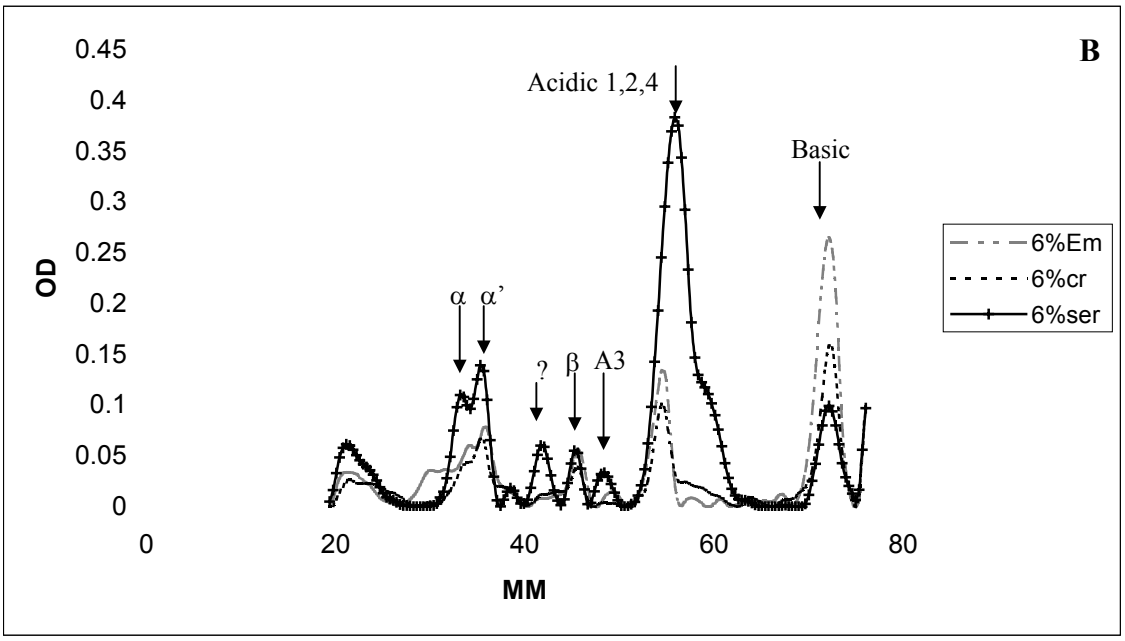


Figure 4.14: Protein migration as measured by scanning densitometry of samples from oil-in-water emulsions prepared by separating phases by centrifugation: emulsion, cream and serum phases, containing 4% SPC (A) and 6% SPC (B).

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CHAPTER 5

CONCLUSIONS

The increment of volume fraction of soybean oil from 0.1 to 0.2 resulted in emulsions with very little similarities demonstrating that the structure and viscoelastic properties of SPC emulsions can be modified according to a specific texture needs by changing the concentration of SPC or oil.

The partially denatured state of the proteins present in SPC, caused by their processing history, and the presence of the insoluble fiber, make SPC a unique ingredient among soy ingredients.

The first objective of this research was to study the effects of dynamic high-pressure homogenization. Upon homogenization disruption of the protein quaternary structure, unfolding and protein network formation occurred. Regardless of the SPC and oil concentration, high-pressure or/and heat at 82°C for 2 min caused a reduction in particle size distribution in both emulsions and suspensions. The presence of large particles (>50 μm) could be attributed to insoluble fiber, aggregated proteins and oil droplets.

In 10% oil emulsions prepared with SPC >4% no phase separation was detected, the oil droplets were entrapped in a structured protein network and showed no changes over time of storage. Viscosity increased with increasing concentration of SPC. Most emulsions showed shear thinning behavior, but only at 8 and 10% SPC emulsions also showed hysteresis during flow measurements. This and their dynamic viscoelastic

behavior were an indication of increase in flocculated oil droplets and aggregated protein in the dispersed phase, at these high SPC concentrations.

All emulsions containing 20% oil showed not only creamy texture (as for 10% emulsions) but also a gel-like behavior, good water holding capacity and stability to creaming upon storage for 20 days at 4°C. Heating before homogenization of 20% oil emulsions did not show differences in stability and microstructure from unheated emulsions. However, heating increased the gel strength showing higher G' and G'' than those determined in non-heated emulsions. In addition, unheated emulsions containing SPC did not show preferential adsorption of proteins at the oil droplet interface, while emulsions containing 20% oil after heating showed preferential adsorption of 11S basic subunits.

The second objective was to understand the role played by the different components of SPC in oil-in-water emulsions. The distribution of oil droplets within the protein matrix was clearly shown by microscopy, and seemed to explain the stability and rheological properties of the emulsions. Microstructural observations showed the presence of large particles of insoluble fiber interacting with oil droplets.

These results, at times in disagreement with previous literature results, confirmed the need for a better understanding of the functionality and behavior of commercial soy protein concentrates in mixed systems. The textural properties and stability of the emulsions prepared with SPC confirmed our hypothesis that SPC is an ingredient with functional characteristics that differentiate it from any other soy ingredient. Our findings will increase SPC utilization in novel food formulations containing soy.

In addition, this study has shown that processing history is of fundamental importance in determining the functional properties of soy ingredients. The role played by the different components of SPC in oil-in-water emulsions, especially the role of the insoluble fiber is still to be understood; however, our findings indicated the importance of each component in affecting the final microstructure and rheological property of the oil-in-water emulsions.