# MODIFIED SOY PROTEIN BASED ADHESIVES AND THEIR PHYSICOCHEMICAL PROPERTIES

by

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B.S., Henan University of Technology, P. R. China, 2004
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AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

# ABSTRACT

Soy protein is one of the most promising bio-degradable adhesives, with great potential as alternatives synthetic petroleum based adhesives for wood composite industries. However, its intrinsic drawbacks such as low water resistance, high viscosity, and short shelf life still limit its broad application. In this research, soy protein was further modified and characterized to improve adhesion properties, flow-ability, water resistance, and long shelf life, which could facilitate the industrialization of soy protein based adhesives.

In this study, we exploited the in situ sodium bisulfite ($NaHSO_3$) modification on soy protein in soy flour-water extracts, and then the modified soy protein was obtained through acid precipitation. First, different concentrations of $NaHSO_3$ were used to modify soy flour slurry, then glycinin-rich and β-conglycinin-rich fractions were precipitated at pH 5.4 (SP 5.4) and pH 4.5 (SP 4.5), respectively. Unmodified sample SP 5.4 and SP 4.5 showed clay-like properties and viscoelastic properties, respectively; whereas with addition of $NaHSO_3$ in range of 2-8 g/L, both SP 5.4 and SP 4.5 had the viscous cohesive phase with good hand-ability and flow-ability. The overall adhesion performance of SP 4.5 was better than SP 5.4; the wet strength of these two fractions was in the range of 2.5-3.2 MPa compared to 1.6 MPa of control soy protein isolate.

Then soy protein with various β-conglycinin/glycinin (7S/11S) ratios were extracted from soy flour slurry and characterized for adhesion properties based on the different solubility of 7S and 11S globulins. Seven glycinin-rich soy protein fractions and six β-conglycinin-rich soy protein fractions were obtained. According to the morphology, viscosity, and particle size results, we proposed that proper protein-protein interaction, hydration capacity (glycinin-rich fractions), and certain 7S/11S ratios (β-conglycinin-rich fractions) in modified soy protein are crucial to continuous protein phase formation. The viscous cohesive samples were stable for up to several months without phase separation at room temperature, with the wet adhesion strength of 2.0-2.8 MPa.

The soy protein modified with $NaHSO_3$ showed good compatibility with commercial glues applied on plywood and paper labeling fields. The modified soy protein made some functional groups, carboxylic (-COOH), hydroxyl (-OH) and amino groups ($-NH_2$) available, which cross-linked with hydroxymethyl groups ($-CH_2$-OH) from urea formaldehyde (UF)

wood glue. The modified soy protein (MSP) with pH 4.8 also acted as an acidic catalyst for the self-polymerization of UF based resin. The wet adhesion strength of MSP/UF blends (40/60) was 6.4 MPa with 100% wood cohesive failure, as compared to 4.66 MPa of UF. As to the paper labeling application, peel strength of MSP on glass substrate increased rapidly, with curing time much shorter than commercial polyvinyl acetate based adhesives (PVAc). And the MSP/ PVAc blends showed shorter curing time, higher water resistance and lower viscosity than pure PVAc.

Chemical modification could also enhance the adhesion strength of MSP. 2-octen-1-ylsuccinic anhydride (OSA) was proved to be grafted on soy protein through reaction between amine, hydroxyl groups of protein and anhydride groups. The oily nature and hydrophobic long alkyl chains of OSA mainly contributed to the significant water resistance improvement of MSP.

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Major Professor
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# ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my major advisor, Dr. Xiuzhi Susan Sun for her constant guidance, valuable advice, kindness, tireless patience to my mistakes and continuous encouragement, to help me complete the PhD degree in Department of Grain Science and Industry. I feel so lucky to be recruited as Dr Sun's student; her enthusiastic and strict attitude to life and work deeply affects me; I learned how to become a qualified scientist and it will definitely benefit my whole career. Truly appreciation is showed to Dr. Donghai Wang, Dr. Hulya Dogan, Dr. Jon Faubion, and Dr. Takashi Ito for their interest in serving the supervisory committee and providing valuable suggestions.

I would like to thank Dr. David Wetzel for letting me use the FTIR instrument; Dr. Praveen Vadlani for generously letting me use the UV spectrometer in his lab, and Dr. Daniel L. Boyle for his excellent laboratory assistance of TEM imaging. Truly thanks Dr. Walker for his kindness and lots of help in my study and student life.

An expression of appreciation and thinks to all my fellows in Dr Sun's research group, Xin, Yonghui, Hongzhou, Tianjian, Kollbe, and Minjung for their help and friendship. Also my dear friends Dr. Shuping Yan and Dr. Xiaorong Wu, Shifeng Ruan, Feng Xu, Liming Cai and Liyan Chen, without their friendship, it would have been a tedious life in the last few years. Special thanks go to the postdocs in Biomaterial and Technology Lab, Dr. Amy Mo, Dr. Jeanne Shera and Dr. Karthik Venkateshan, for their unreserved support and help provided to my research work. Further thanks go to the staff and graduate students in Grain Science Department for their support.

At last, I deeply express my appreciation and gratitude to my parents Shizheng Qi and Huanqiao Hou, Sister Qingru Qi and brother in law Chunpeng Zhang and my fiancé Ningbo Li, who give me unconditional love, patience and emotional support. My life is wonderful because of them.

# Chapter 1 - INTRODUCTION

## 1.1. GENERAL BACKGROUND

Currently, the major adhesive resins used for wood composites are synthetic thermosetting resins: phenol formaldehyde, urea formaldehyde, melamine formaldehyde and resorcinol formaldehyde, which are derived from fossil fuel products, oil and natural gas (Koch et al., 1987). They account for 90% of the total wood panel adhesives in the market. These chemicals are always melted, dissolved in a solvent (water), or emulsified in a solvent prior to application; they could be combined for us as binders or adhesives for manufacturing of various wood composites. Synthetic petrochemical polymers are also the primary adhesives used in the labeling industries such as polyvinyl formal and acrylic ester etc (Wang et al 2006).

Date back to the end of the World War II, the low-cost; highly durable synthetic adhesives were developed dramatically from petrochemicals because they were no longer to support the war effort (Lambuth, 1989). Unlike the natural counterparts with pretty poor water resistance, these highly durable synthetic adhesives could be used as the exterior applications. In addition, durable synthetic adhesives allow efficient and economical utilization of diverse and changing wood resources, such as manufacture of useful products from residues and waste wood, reassembling of smaller forms of wood such as veneer, flakes, and fibers into efficiently engineered shapes and products etc (Wood: Adhesive, 2001). Efficiency in converting trees and waste wood to useful products will help to ensure the continual conservation of forest resources. Therefore, the production of bonded-wood products is stimulated prominently in the following decade's years. It is said that about 8 billion pounds petrochemical based adhesives are used annually for plywood, particleboard, and fiberboard and furniture industry (Sun, 2004).

However, with the development of society, people begin to realize that petroleum based adhesives can pose numerous environmental, health and safety issues. Because petroleum chemicals are non-biodegradable, they can contaminate the ground water and the soil, leading to the growing landfill disposal in a long-term way (CPC Aeroscience 2005). Formaldehyde is considered as one of the more common indoor air pollutants, causing

headaches, irritating eyes, damaging respiratory system, triggering asthma symptoms etc at concentration above 0.1 ppm in the air. In the United States, a bill was passed in congress on July 7, 2010 regarding the use of formaldehyde in hardwood plywood, particle board, and medium density fiberboard. The bill limited the allowable amount of formaldehyde emissions from these wood products to 0.09 ppm, a standard which companies will have to meet by January, 2013 (Open Congress 2010). Besides, as the raw material, crude oil demand is expected to grow 50% by 2025, while the oil reserves have been dropping for a decade; and the extensive drilling is being still undertaken worldwide. The dilemma between the depleting reserve and increasing demand may escalate the price on global market; even cause the dramatic political upheavals (Hirsch, 2005).

However, the expanding market for adhesive bonded wood products does not suspended at all because of the negative effects brought by formaldehyde based adhesives. And more than 70% of all wood products are still need to be bonded. Therefore, people are requested to exploit a new adhesive system with the following requirements: low cost, biodegradable, non-toxic, and excellent water resistance, alternative to the traditional petroleum based adhesives.

Bulk soybean flour is usually considered as the source of wood adhesives, containing about 44-50% protein. In the early '50s soybean meal became available as a low-cost, high protein feed ingredient, triggering an explosion in U.S. livestock and poultry production. Right now, farmers in over 30 states grow soybeans in United State, and they are the second largest crop in cash sales and number 1 value crop export (Soystats, 2010. Figure 1.1). In the world wide, soybeans accounted for 38% of the world soybean production with 91.48 million metric tons in 2009 (Soystats 2010. Figure 1.2).

Soy protein adhesive has shown the great potential as adhesives in the early 1900s, and its first public disclosure was in 1928 (Keimel, 1994). Nowadays, with the environmental concerns and regulations, soy protein adhesives are increasingly re-applied in many industrial fields such as paper coating, texture paints, cosmetics, printing inks, plastics, textiles, particleboard, fiberboard and plywood, and so on. Compared to the synthetic resins, the advantages of soy protein based adhesives are increasingly highlighted, such as renewable, bio-degradable, low price, environmentally friendly, cured by either hot or cold pressing

condition, and easily modified functional properties. However, its poor water resistance is always the biggest hindrance for soy protein as the wood adhesives.

Until now, most researches working on soy protein adhesives are to improve its water resistance, suitable for the exterior bonded wood products. First of all, understanding the protein structure is crucial to the successful modification of soy protein, since it is closely related to its functional properties such as solubility, viscosity, gelling properties and adhesion performance. Soy protein is characterized by a complex three dimensional structure of highly ordered, with hydrophobic groups buried inside and hydrophilic groups exposed outside in nature (Horton et al., 1996). This highly ordered structure caused insufficient contact area and limited functional groups available to the wood substrate, affecting the adhesion strength negatively. Therefore, in order to function as an excellent protein, the internal interactions among proteins must be destabilized, with consequent exposed functional groups, especially the hydrophobic groups; improving the contacted area and number of the amino groups and the adhesion strength. The disruptions of internal interactions are accomplished by adding many denaturation agents such as alkaline, urea, SDS, cationic detergent, $NaHSO_3$ etc (Hettiarachchy et al., 1995; Sun and Bian 1999; Zhang, 2008; Wang et al., 2005).

Besides, the protein cross-linking reaction induced by cross-linker reagent, have been also confirmed to greatly improve the adhesive performance, especially the water resistance, by forming a more compact protein complex during thermo setting, preventing the penetration of water molecules into the interface of protein and wood (Wang et al., 2007, Zhong et al., 2007a, Huang and Li, 2007). Another hot topic last few years is the adhesive blends, which is a short-term solution for people reducing dependence on petrochemicals. Extensive researches have been done to incorporate soy flour and soy protein in the following resins: phenol formaldehyde, urea formaldehyde, Polyamide-epichorohydrin (PAE) polyvinyl alcohol, polyvinyl acetate resin (Zhong et al., 2007a; Zhong et al., 2007b; Kumar et al., 2002); and the so called adhesive blends reduce the formaldehyde emission, and raw material cost, but improve the adhesion performance significantly.

Though, great improvement on the soy protein adhesive performance has been achieved, researchers still need to overcome the native limitations hindering soy protein as commercialized products: low water resistance, low solid content, high viscosity, short shelf

life and high cost. In Sun's group, an innovative soy protein system modified with reducing agents (i.e. $NaHSO_3$) is developed, having the viscous cohesive properties, with adhesive strength comparable to formaldehyde based glue (Sun et al., 2008). However, previous studies have revealed the insignificant or negative effects of $NaHSO_3$ modification on the adhesion properties of soy protein isolate, pure 7S and 11S globulin solutions (Kalapathy et al., 1996; Zhang and Sun, 2008; Zhang and Sun, 2010). Therefore, in this study we need to investigate how $NaHSO_3$ affect the physicochemical properties of soy protein fractions including 7S-rich soy protein, 11S-rich soy protein and soy protein fractions with various 7S/11S ratios through in situ chemical modification in soy flour-water extract. And then we further improved the adhesion performance of these $NaHSO_3$ modified soy protein fractions through physical and chemical treatments.

## 1.2. OBJECTIVES

The overall objective of this research was to overcome the intrinsic drawbacks of soy protein based adhesives, including poor water resistance, low solid content, high viscosity and short shelf life through chemical and physical modification; facilitating the commercialization of soy protein adhesive.

The specific objectives were to:

1. Investigate the effects of $NaHSO_3$ concentration on soy protein (7S rich fractions and 11S rich fractions) through in situ modification in soy flour-water extracts, and study the physicochemical properties of the extracted soy protein with potential for adhesive applications.
2. Study the effects of $NaHSO_3$ (6g/L) on physicochemical properties of soy protein fraction with various 7S/11S ratios extracted from soy flour-water extracts at different pH.
3. Study the compatibility of selected MSP with different kinds of commercial veneer glue, and characterize the physicochemical properties of adhesive blends including thermal, morphology, rheological properties and shear adhesion performance.
4. Characterize the peel adhesion properties of MSP and study its compatibility with polyvinyl acetate based glue, applied in the paper labeling area.
5. Investigate the effects of 2-octen-1-ylsuccinic anhydride (OSA) modification on the

adhesion performance of selected MSP used as wood adhesive.

**Figure 1.1. U.S. land use for crop production in 2009 (Soytats 2010).**

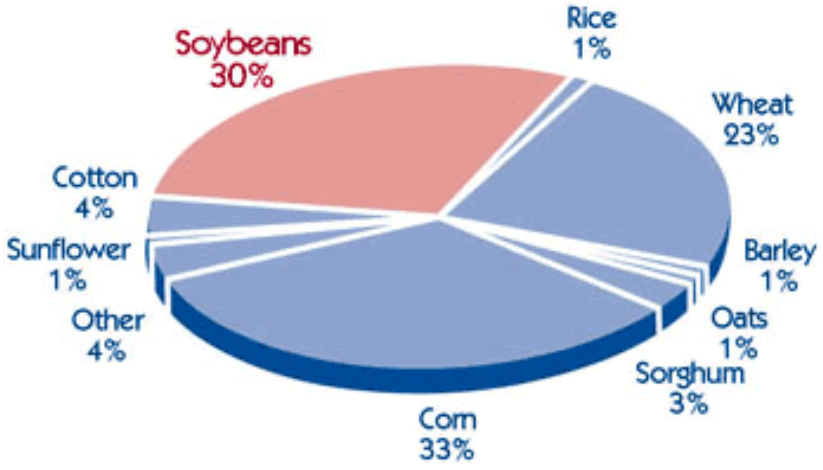
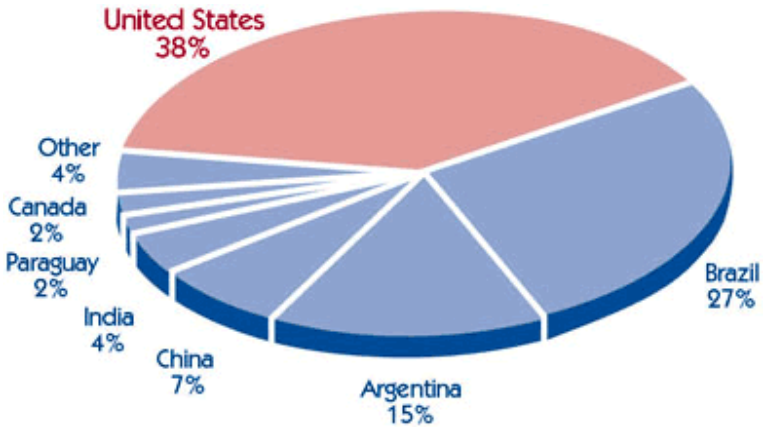


**Figure 1.2. World soybean production 2009 (Adapted from www.soystats.com).**



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# Chapter 2 - LITERATURE REVIEW

## 2.1. ADHESION MECHANISUM

Adhesion is the tendency of dissimilar particles and/or surfaces to cling to one another. Several adhesion theories have been proposed and five universal accepted ones include mechanical interlocking, interfaces adsorption, chemical bonding, diffusion, and electron transfer, (Schultz et al., 1999).

The mechanical bonding theory describes how adhesives spread and wet the surface of the substrate; penetrate into the fibers cells through the capillary path, acting like a mechanical anchor. The roughness of the substrate and the flow behavior of the adhesives are the two major factors determining the degree of mechanical interlocking among the substrate and adhesives. The adsorption theory is about any physical or electrostatic attraction between adhesive polymer and substrate through hydrogen bonding and van der Waals forces. Good wetting between adhesive and substrate should be established to obtain the good adhesive strength. Chemical bonding refers to the formation of covalent chemical bonds between adhesive and substrate; it is expected to be the strongest and most durable bonding. The diffusive forces are somewhat like mechanical tethering at the molecular level; it occurs when species from one surface penetrate into an adjacent surface while still being bound to the phase of their surface of origin. The electrostatic theory states that electrostatic bonds may occur between adhesive and adherend, which may pass electrons to form a difference in electrical charge at the join (Green, 2002; Wikipedia).

## 2.2. WOOD ADHESIVES

Adhesives for wood bonding are ranging from the natural products to the very durable synthetic resins from petrochemicals. Essentially all the adhesives used for wood bonding were of natural resources before World War II, such as mud, dung, and clay, along with mixtures of these substances. Then with the introduction of synthetic resins from petrochemicals, they were dominated in the wood composite market rapidly, and exceeded most of the older natural glues in importance for wood bonding.

There are two distinct categories from synthetic resin derived from petrochemicals: thermosetting and thermoplastic adhesives. During the polymerization and cross-linking reaction, the thermosetting adhesive undergoes the irreversible chemical and physical changes, converting to the insoluble and infusible materials after heating. While thermoplastic is known as the thermo softening plastic; there are no chemical cross-linking reaction during thermal curing, therefore, it remains in a reversible state and can readily be softened (heating) or solidified (cooling) repeatedly many times (Eckelman, 1997).

The most commonly commercial synthetic thermosetting resins include urea-formaldehyde (UF), melamine-formaldehyde (MF), phenol-formaldehyde (PF), phenol-resorcinol-formaldehyde (PRF). UF resins are synthesized by combining urea and formaldehyde in molar ratios that range from 1:1.1 to 1:2.0 to form a mixture of mono-, di-, tri- and tetra-methylol ureas in the first step. Then the condensation reaction of these methyloureas occurred to form cross-linked polymers networks under the acidic condition. UF resins are available in both liquid and powder, and cure at elevated temperatures (95-130 °C) with the acidic-cure catalyst such as ammonium chloride and ammonium sulfate (Dunky, 1998). The fillers and extenders are also incorporated to reduce the glue line costs and to control flow and viscosity of the UF resins as well (Eckelman, 1997). Compared to PF resins, they are low cost, ease of handling, fast curing and colorless. However, due to the low water resistance, UF resins are mainly used for manufacturing interior plywood, particleboard and medium density fiberboard.

Melamine has three amino groups that react with formaldehyde to form MF resins. MF resins are used primarily to improve the moisture resistance of urea-resin adhesives, but still inferior to the phenol-resin adhesives. The quite expensive cost and high cure temperature (at least 120 °C) make the application of MF for wood composites is not as board as UF and PF resins in the market (Koch, 1987; Eckelman, 1997).

PF resins are synthesized by a step-growth polymerization reaction of phenols and formaldehyde that can be either acid or base catalyst. Phenolic resins create strong, water resistance bonds but require long cure time and high cure temperature (120-150 °C). They are widely used for the softwood plywood under the severe storage conditions. Moreover, because of the dark reddish in color, phenolic resins are not used in applications where they are visible (Huang, 2007).

Resorcinol formaldehyde (RF) resin is derived from resorcinol and formaldehyde. Due to the second hydroxyl group in the metal position, it is more reactive with formaldehyde than phenol. RF resins provide the stronger bond for the wood composites because of the high degree of cross-linking. However, these glues are more expensive than the PF resins and are used primarily as special purpose adhesives such as glued laminated wood beams and I joists which must resist exposure to the severe weather and water. As a matter of fact, phenol is used to partially replace resorcinol to form the phenol-resorcinol formaldehyde (PRF) resins which are much more commonly used in the wood composite than RF resins because of the reduced cost (Houwink et al., 1965; Eckelman, 1997.).

Polyvinyl acetate (PVA) is the thermoplastic polymer prepared by polymerization of vinyl acetate monomer (the free radical polymerization of vinyl acetate). It is the common white glue, sold as the ready-to-use aqueous emulsion and used for the furniture assembly. PVAc resins can be cured at room temperature with low clamping pressure, even though the water resistance is inferior to the UF and PF resin, it has the high dry strength and invisible glueline, therefore it is desirable for the furniture manufacture (Eckelman, 1997).

Even though those synthetic adhesives have led to the technical advances in wood use and extension of the lumber supply, many researchers need to focus on reducing formaldehyde emissions while increasing cure rates from formaldehyde-containing binders.

Furthermore, in recent years, severe problems have been arisen by those synthetic adhesives such as limited raw resources, environmental pollution and healthy issues. Therefore, in order to meet the people's continuous expanding consumption of wood product bonded by adhesives, the bio-based adhesives from natural material re-attract people's attention for several decades, including animal glues, blood-based adhesives, casein-based adhesives, lignin adhesives, and various kinds of protein (wheat protein, zein, canola protein, soy protein etc) (Pizzi, 2006; Yang et al., 2006; Lin and Gunasekaran, 2010). Soy protein has been considered to be one of the most promising adhesives alternatives to the petroleum based adhesives, with the following advantages: abundant, inexpensive, renewable, easy to handle, and suitable for cold or hot press.

## 2.3. SOYBEAN AND SOY PROTEIN CHEMISTRY

### *2.3.1. Soybeans*

As early as 5000 years ago, Chinese farmers started to grew the soybeans (Glycine max), one of the most miracle crop and is the world's foremost provider of protein oil. Date back to 1800s, soybeans made their debut in America through a Yankee clipper ship, and then many America famers popularized this crop, mainly for food and feed. By the end of the World War II, soybeans become a major crop in the United States and became the world's leading soybean producer and exporter. Nowadays, 77.5 million acres were used for the soybeans plants in United States (Figure 2.1); producing 3.361 billion bushels of soybeans which accounted for 46% of the world's soybean trade (Soystats, 2010).

The mature soybean is mainly consisted of 38% protein, 30% carbohydrate, 18% oil and 14% moisture, ash and hull. During the processing, soybeans are normally cleaned, cracked, dehulled and rolled into soy flakes, during which the oil cell were ruptured for efficient oil extraction through hexane solvents (Sun, 2005). Soybean hulls are further processed for fiber additives for baking products, cereals, or snacks. The extracted oil is always considered as leading vegetable oil, used for cooking oil, margarine, and shortening. Lecthin, is an emulsifying agent, separated from soy oil, used for everything from pharmaceuticals to protective coatings. Once the oil removal, the remaining defatted soy flakes are the basis of a variety of soy protein products including soy flour, soy protein isolates, soy protein concentrates, and soy textured protein (WISHH). They are extensively used not only for human food and animal feed, but also for the industrial bio-based products such as adhesives, films, plastics, emulsions, inks, particleboard, and textile fibers etc.

### *2.3.2. Soy protein chemistry*

Around 90% of total proteins in soybeans are globulin and can be extractable with water or dilute salt solutions. Soy proteins consist of discrete groups of proteins which cover a broad range of molecular sizes, as shown by use of ultracentrifuge or by gel filtration. The water extractable proteins typically show four fractions designated as 2S, 7S, 11S, and 15S on the basis of the sedimentation rates (Table2.1); each fraction is a complex mixture of proteins (Wolf, 1970). The 2S fraction, which accounts for 20% of the extractable protein, is mainly composed of metabolic proteins like trypsin inhibitor. The 7S and 11S fractions represent the predominate proteins of soybeans, accounted for 37% and 30% of the total

extractable protein respectively; more than one-half of 7S fractions are composed of 7S globulin and 11S globulin makes up for the bulk of the 11S fractions (Peng, 1984). The amino acid composition of 11S and 7S component is shown in Table 2.2 (Utsumi et al., 1997). The 15S fraction accounting for about 10% of the total soybean proteins is considered as a dimmer of glycinin (Nielsen, 1974).

7S globulins are classified into three major fractions with different physicochemical properties, named as $\beta$-conglycinin, $\gamma$-conglycinin, and basic 7S globulin, of which $\beta$-conglycinin is the main components, with 30-50% of the total seeds protein (Hou and Chang, 2004). $\beta$-conglycinin is a trimeric glycoprotein of 140-170 kDa, consisting of three types of subunits: α' (MW 57-72 kDa), α (57-68 kDa), and β (42-52 kDa) (Than and Shibasaki, 1977). A subunit termed as γ of 42 kDa copurifies with β subunits (Petruccelli and Anon, 1995). $\beta$-conglycinin exhibits molecular heterogeneity and seven different types of oligomers are identified: $B_1$ ($\alpha'\beta_2$); $B_2$ ($\alpha\beta_2$); $B_3$ ($\alpha\alpha'\beta$); $B_4$ ($\alpha_2\beta$); $B_5$ ($\alpha_2\alpha'$); B6 ($\alpha_3$) (Thanh and Shibasaki, 1976), and $B_0$ ($\beta_3$) (Yamauchi et al., 1981; Sykes and Gayler, 1981). The β subunit was reported to have 4 components with isoelectric points between 5.8-6.2 ($\beta_1$-$\beta_4$), while α and α' subunits have the single components having isoelectric points of 5.2 and 5.3, respectively (Than and Shibasaki, 1977).

The α and α' subunits contain the extension regions in addition to the core regions common to all subunits, which are N-glycosylated (Maruyama et al., 1998). Because of the high hydrophobicity of the extension regions, it plays an important role in the solubility of α and α' subunits above pH5.5 and pH 6.0. All three subunits are rich in glutamate, aspirate, leucine, and arginine, but very low in methionine and cystine (Than and Shibasaki, 1978). The β subunit is reported to had higher contents of hydrophobic amino acids than α and α' (Than and Shibasaki, 1977). The $NH_2$-terminal valine for both α and α' subunits and leucine for β subunit were described previously (Coates, 1985; Thanh and Shibasaki, 1977; Hirano et al., 1987).

The formed trimeric structure is non-covalently associated through hydrophobic interaction, hydrogen bonding and electrostatic force without any disulfide bonds. Even though disulfide bond does not involve into the protein structure stabilization, β-conglycinin is proved to have small quantity of sulfhydryl groups in α', α and β subunits (Hoshi et al., 1982), which closely related to the polymerization of 7S globulin and other functional

properties. High molecular weight aggregates (120 kDa) could be formed by α' and α subunits but no β subunit of β-conglycinin through disulfide bonds were observed previously (Silvana and Maria, 1995, Petruccelli and Anon, 1995); Petruccelli (1995) reported that both ionic interactions and disulfide bonds participated in this aggregation.

11S globulin is a very heterogeneous oligomeric protein ranging from 340 to 375 kDa (Utsumi et al., 1981); it consists of six subunits formed into a hexamer structure. Each subunit is composed of an acidic polypeptide (37-45kDa; pI=4.2-4.8) and basic polypeptide (18-20 kDa; pI=8.0-8.5) and these polypeptides are joined by disulfide bonds (except for $A_4$), designed as the five AB subunits: $A_{1a}B_{1b}$, $A_2B_{1a}$, $A_{1b}B_2$, $A_5A_4B_3$, and $A_3B_4$ (Badley et al., 1975). Only one disulfide bond was found to be involved in linking the acidic and the basic polypeptides of each subunit, and they were in analogous positions (Lawrence et al., 1994). The acidic and basic polypeptides alternate in the hexagonal layer and are held together by hydrophobic and disulfide bonds, forming three subunits. And the two identical hexagonal layers are held together by electronic interaction and hydrogen bonding to form one glycinin molecule (Peng et al., 1984). The amino acid composition of 11S protein has been determined and the proportion of hydrophobic amino acids and hydrophilic amino acids are 23.5% and 46.7% (Takagi et al., 1979). Acidic subunits have higher contents of glutamic acid, proline, and half cystine than do basic subunits, while basic subunits have a higher hydrophobic amino acid content (Leu, Ala, Val, Tyr, and Phe) than do acidic subunits (Catsimpoolas et al., 1971). 11S protein were proved having a relatively low content of ordered structure (α helix and β structure, 5.2% and 34.8% respectively) and a high content of random coil structure (60%) (Koshiyama, 1972).

Researchers discovered that both pH and ionic strength of solution could induce the complicated association-dissociation phenomenon of soy protein subunits. At pH 7.6 and 0.5 ionic strength, the 7S protein exists as a monomer with a molecular weight of 180-210 kDa, whereas it dimerizes to 9S when the ionic strength changed to 0.1. At higher ionic strength of > 0.8, β-conglycinin dissociated to 5.6S and 2S (Ibuchi and Imahori, 1978). In case of 11S, at pH 7.6 and an ionic strength of 0.5, it is a hexamer complex (11S), while low pH and low ionic strength convert 11S protein into a slow sedimenting component (2S) as a result of dissociation of the protein into subunits (Peng et al 1984). At slightly alkaline pH values and low-ionic strength, 11S protein readily dissociates into subunits. For example, in a 0.03 M

tris-HCl buffer and at pH 8.6, 11S extensively dissociates into 2S and 7S components (Eldridge and Wolf, 1967).

## 2.4. FUNCTIONALITY OF 7S AND 11S

The native structural difference between 7S and 11S contribute to variations in their functional properties such as thermal stability, water holding capacity, adhesion strength, gelling properties and extrusion performance etc. Furthermore, the effects of 7S to 11S globulins ratios on these functionalities were under investigation as well.

11S is well known to be more thermal stable than 7S due to much compact structure of 11S stabilized by the disulfide bonds; their denaturnation temperature are around 90 and 73 ℃, respectively (Wang et al., 2007; Zhang and Hua, 2007). Because of the more ordered structure, 11S has the low degree of denaturation when treated by chemical modification (Mo et al., 2004).

Water imbibing capacity is of great importance for the quality of different products, particularly doughs, batters, and comminuted meat systems (Sorgentini et al., 1991). Yao et al (1988) studied the relationship between ratio 7S/11S and water imbibing capacity and found that when the molar ratio of 7S and 11S was 1 in the mixture, the minimal WIC value was obtained, indicating the strongest protein-protein interactions occurred at this point.

The gel-forming ability induced by heating proteins is one of the most important functional properties with respect to their usage in the food systems. 7S and 11S show different gel forming properties and their gelation mechanisms are different. The galation rate of 11S is faster than that of 7S and sulfhydryl disulfide interchange reaction is important in the formation and maintenance of the structural matrix of 11S gels, whereas hydrophobic interactions and hydrogen bonds mainly involved in the gel network of 7S (55). 11S gel has a higher water-holding capacity, higher tensile value and higher hardness and expends more on heating than 7S gel (Saio and Watanabe, 1978; Renkema et al., 2001). Utsumi et al (1997) also reported that 7S largely contributed to the elasticity of the gels, and 11S was related to the hardness and unfracturability of the gel. In the soy protein isolate gels studied by several researchers (German et al., 1982; Utsumi and Kinsella, 1985), there is a preferential electrostatically interaction between β subunits of 7S and the basic subunits of glycinin (11S).

Ning and Villota (1994) reported that 11S protein appeared to favor expansion and water holding capacity of the extruded product; when high levels of 7S incorporated had the negative effects on soy protein texturization. 7S and 11S globulins interacted with each other during extrusion processing; with the best textural characteristics of the finished product being obtained with a feed formulation having an 11S/7S ratio of 1.5. Non-covalent interactions, such as hydrophobic interactions and hydrogen bonding were found to be predominant in the texture formation; and disulfide bonds make a contribution to texture formation as well.

## 2.5. FRACTIONATION OF SOY PROTEIN

Soy proteins are normally presented as two types: soy protein concentrates and soy protein isoates; the traditional way to extract them from soy flour is the acid precipitation (isoelectric point = 4.5-4.8). Soy protein isolates are the most refined forms, containing 90% or more protein (Wolf, 1970). Defatted soy flakes or flours are extracted with water plus alkali at pH of 7 or 8.5, during which the insoluble polysaccharides, fiber, enzyme etc are centrifuged out, then pH is adjusted to about pH 4.5 to precipitate the proteins, and removed by centrifugation. The supernatant contains sugars, ash and minor 2S and 7S soluble proteins (Iwabuchi and Yamauchi, 1987).

It is of increasing interest to study the individual soy protein subunits because they have different functionality induced by the distinct native structure, so they could exert their utmost function in different applications. Thermal properties, solubility, hydrophobicity, water absorption properties, gelling properties, forming properties and adhesion performance of 7S and 11S have been studied extensively. Therefore, fractionation of these two components with high purity and yield also attracts researchers' attention. Thanh and Shibasaki (1976) developed a straightforward fractionation of 7S and 11S globulins at pH 6.1-6.6, based on their different solubility. Adjusting the pH of the extract of defatted soybean meal to 6.4 caused precipitation of the 11S globulin, while the 7S globulin was separated from whey protein by adjusting pH to 4.8. Factors affecting the precipitation (namely, pH, Tris concentration, protein concentration, and ionic strength) are also optimized for simultaneous fractionation of 7S and 11S globulins. However, the cross contamination of both proteins in globulin fractions is still a hindrance to purify them. The glycinin fraction

was 79% glycinin, 6% conglycinin, and 15% other components. The purity of β-conglycinin fraction was only 52%, 3% glycinin and 45% others. Gel filtration and affinity chromatography are required to purify these two fractions, which are costly and difficult to scale up. It is reported that reducing agent prevents co-precipitation of glycinin and β-conglycinin, improving the fraction's purity (Thiering et al., 2001; Wolf 1993). Suitable reducing agents for use as protein fractionation aids are any sulfite compound that yields $SO_2$ in solution such as $NaHSO_3$, glutathione, cysteine, and β-mercaptoethanol, which are believed to reduce disulfide bonds and avoid coprecipation (Deak et al., 2006).

In order to improve the purity of each globulin fraction, many researchers are investigating the three isoelectric point precipitations. O'Keefe et al (1991) modified the conventional method by replacing the two isoelectric precipitations at pH 6.4 and 4.8 with three precipitations at pH 6.4, 5.3 and 4.8. Nagano et al (1992) and Bogracheva (1994) also obtained three fractions at isoelectric pH 6.4, 5.0 (5.5) and 4.8 instead of two. Therefore, one more soy protein fractions composed of mixture of glycinin and conglycinin were obtained in the pH 5.5, 5.3 or 5.0. Nagano et al claimed the purities of crude glycinin and β-conglycinin were >90%, however at the expense of yield. Wu et al (1999) and Rickert et al (2004) utilized the three steps fractionation in the pilot scale and optimized the processing parameters (pH, temperature, water-to flake ratio etc) to yield high purity and yield conglycinin fractions; the purity of both globulin were very comparable to those of the laboratory-scale process.

## 2.6. PROTEIN STRUCTURE AND STABILIZATION

The protein structure is characterized in terms of four aspects, depending on the spatial arrangement of polypeptide chains. The primary structure is the amino acid sequence which is connected by the peptide bonds. The secondary structure denotes the extended or helically coiled conformation of polypeptide chains, principally α-helices, β-sheets and random coil structure. The tertiary structure refers to the spatial arrangement of entire polypeptide chains, wherein secondary structure segments into a compact three-dimensional folded form with hydrophilic groups located on the protein exterior surface and most of the hydrophobic groups buried inside to exclude water. The quaternary structure is the spatial arrangement of the subunits containing several polypeptide chains.

Soy protein has highly ordered structure, with most hydrophilic groups outside and most hydrophilic groups buried inside (Horton et al., 1996). The forces stabilizing the protein structure mainly include hydrogen bonding, electrostatic interactions, van der Waals forces and hydrophobic interactions (non-covalent bond) and covalent disulfide bonds. The protein functional properties are closely related to the protein structure, which could be altered through physical, chemical modification.

## 2.7. SOY PROTEIN ADHEIVES AND ITS MODIFICATION

Soy based adhesive is one of the most popular bio-degradable adhesives under investigation right now, because of their unique advantages including high gluing strength, biodegradable, renewable, easy handling, suitable for hot and cold press temperature. Soy protein have been studied for adhesion properties on wood in forms of soy protein isolate, pure 7S globulin and 11S globulin suspension, mixture of 7S and 11S with different ratio, or 7S subunits (α, α', and β) and 11S subunits (acidic, basic subunits). Glycinin is the main contributor to the adhesive bonding strength, especially the wet strength of soy protein isolate adhesive. Adhesives made from glycinin had higher adhesion strength and water resistance than those made from conglycinin protein (Mo et al., 2004; Wang et al., 2005). Moreover, the basic subunits from glycinin had higher water resistance than acidic subunits due to the considerably more hydrophobic amino acids in basic subunits (Mo et al., 2006a). The adhesive made from β subunit with high content of hydrophobic amino acids also showed greater water resistance than α α' and β-conglycinin adhesives (Mo et al., 2011).

The adhesive strength of soy protein adhesives depends on its ability to disperse in water and the interactions of functional groups of protein with the substrate. The main reasons for low adhesive strength of soy protein is highly ordered native protein structures stabilized by internal bonds, like van der Waals forces, hydrogen bonds and hydrophobic interaction, which made the majority of polar and nonpolar groups unavailable for the wood panels; and the hydrophilic nature of the soy protein because of less contents of hydrophobic amino acids than hydrophilic amino acids in protein. Various physical and chemical modifications were investigated for improving the soy protein adhesion strength.

### *2.7.1. Chemical modification*

Chemical modification is the powerful tool to change protein properties and functionalities via changing the native protein conformation by some chemical reagents. The protein modification may induce alternations in structure and conformation of primary, secondary, tertiary and quaternary structure, through disrupting the covalent and non-covalent bonds stabilizing the protein structure. A number of functional groups of protein on amino acid side chains are available for the chemical reaction; its reactivity and accessibility to the chemical reagent depend not only on the native properties of the side chain and reagent, but also on the surrounding environment. Carboxyl, hydroxyl, amino, disulfide, imidazole, indole, phenolic, sulfhydryl groups can be modified by commonly used chemical agents. Minor changes in protein chemical structure could result in profound changes in their physical and biological properties (Feeney, 1977).

The principles of these chemical modifications of soy protein adhesives include 1) unfolding the protein compact structure using the detergents to expose as many as functional groups, especially the hydrophobic groups, available for the wood substrate. 2) Inducing cross-linking reactions among protein through cross-linker to form the protein complex having more entanglement during curing. 3) Introducing some specific groups to soy protein, contributing to the adhesion improvement.

**Unfolding detergent**

Various detergents have been investigated to improve the adhesion strength of soy protein. Alkali, such as sodium hydroxide has been the most common chemical used to unfold protein molecules and expose the functional groups. Hettiarachchy et al (1995) find that alkali treatment of soy protein could improve the water resistance of soy protein under moderate alkaline conditions (pH 10.0 and 50 ºC). Urea is another denaturation chemical, which could interact actively with hydroxyl groups of soy protein and then break down the hydrogen bonding, resulting in the unfolded protein structure. Sun and Bian (1999) reported that urea-modified soy protein adhesive had higher water resistance than the alkali-treated soy protein adhesives. Zhang and Hua (2007) investigated the wettability and adhesive properties of 7S and 11S modified by urea; results showed that wettability of these two components were improved and resulted in enhanced adhesion strength under 1M urea

modification. Another denaturing agent, guanidine hydrochloride can enhanced the protein hydrophobicity and adhesion strength (Huang and Sun, 2000a; Zhong et al., 2002). Sodium dodecyl sulfate (SDS) is the anionic detergent, which can dissociate the protein by disrupting the hydrophobic and electrostatic bonds; the reaction could move some inside hydrophobic side chains outward, where they could interact with the hydrophobic moieties of detergent molecules and form micelle like regions. These resultant protein structures induced by 0.5% SDS are proved to enhance the water resistance and adhesion strength of soy protein adhesives (Huang and Sun, 2000b; Mo et al., 2004).

**Reducing agents**

The presence of disulfide bonds in native protein molecules affects their structural integrity, protein stability, and unfolding properties. The glycinin component in soy protein has 18-20 both inter and intramolecular disulfide bonds, while only two disulfide bonds per mole exist in β-conglycinin (Koshiyama, 1972; Kella et al., 1986). Reducing agents, such as sulfites, bisulfites and sulfates, could cleave the inter- and intra-disulfide bonds in protein molecules, which leads to an increase in molecule flexibility, solubility, surface hydrophobicity, and an decrease in viscosity (Babajimopoulos, et al., 1983; Kella et al., 1986; Kalanathy et al., 1996). Kalanathy et al (1996) studied the effects of the disulfide bond cleavage induced by $Na_2SO_4$ and $Na_2SO_3$ on adhesion properties of soy protein isolate. Since there is the counteracting effects between increased hydrophobicity of soy protein and decreased effective wood-protein interfacial area due to the conversion of SH to $–SSO_3$, the shear adhesive and water resistance properties of soy protein adhesives were affected adversely. Zhang and Sun (2008, 2010) also reported that the negative effects of sodium bisulfite exerted the adhesiveness of pure glycinin and β-conglycinin globulin, respectively.

**Introducing specific functional groups**

Soy protein is known to contain about 20 amino acids, and they are attached to the side chain of the protein molecule through the functional groups. Theses functional groups include –OH, -NH2, -COOH, -SH, play an important role affecting the interactions with the wood substrates, and the resulted adhesion strength. Therefore, researchers raised a concept that introducing some specific groups which may contribute to the adhesion strength or

reducing the groups detrimental the adhesion performance. Mussel protein, served as a strong and water-resistant adhesive, contains a high amount of 3, 4-dihydroxyphenyl-alanine (DOPA) and mercapto (-SH) -containing cysteine. Liu and Li (2002) successfully grafted DOPA-like phenolic functional groups to soy protein could transform the soy protein to a strong and water-resistant adhesive. Increasing the free mercapto group content in soy protein could also greatly increase its gluing strength and water resistance (Liu and Li, 2004). Amino acid lysine has been reported to enhance adhesion strength (Yamamoto, 2000; Yu, 1998). In Zhu's study (2005), she reported that adhesive performance was reduced when the $–NH_2$ groups were substituted with –COOH (hydrophilic) groups at pH 7.6, while adhesion strength was improved when $–NH_2$ was replaced with $–CH_3$ (hydrophobic groups).

**Cross-linker**

Cross-linking of protein involves joining the two molecular components by a covalent bond achieved through the cross-linking agent. A compact protein complex would be formed, and induce more entanglements and cross-linking during thermal setting, which would maintain their structure better than the unmodified adhesive after water soaking. Rogers et al (2004) used 1,3-dichloro-2-propanol to cross-link soy protein through the reaction among the functional groups, and got enhanced adhesive performance. Zhong and Sun (2007) studied the interaction between Polyamide-epichlorohydrin (PAE) and soy protein and reported that the physical cross-linking reaction between them is reversible, and could be manipulated by ionic strength. This complexation formation contributed to the largely improved adhesive strength. Epoxies are active cross-linking agents for alkaline soy glues and improve the adhesive strength and durability (Lambuth., 1965; Huang, 2007). Wang et al (2007) reported that the wet strength of soy protein was improved by 115% at optimum concentration of glutaraldehyde (20mM). Efforts of cross-linking soy based adhesive with formaldehyde or its derivatives have long been reported. Liu and Li (2007) developed modified soy protein adhesives including two steps modification. Soy protein isolate (SPI) was first modified by maleic anhydride (MA) to form MA-grafted SPI (MPSI), then polyethylenimine (PEI) was used to modify MSPI. The optimum formula of the modified SPI was made from 20% PEI and 80% MSPI, which gave a dry strength 6.8% MPa and boiling strength of 1.5% MPa.

### *2.7.2. "Short cut method" adhesive blending*

Blending soy protein with synthetic resins is a short-term solution for reducing dependence on petrochemicals and lowering emissions of volatile organic compounds. The concept of using protein adhesive as copolymers with synthetic resins has been used successfully in the industrial fields. Blends of soy based protein with blood, casein, polyvinyl alcohol, polyvinyl acetate resin, or PF showed improved water resistance for wood applications (Kumar et al., 2002). Steele et al (1998) developed blends of soy protein and phenolic resins used for finger jointing of green lumber that cured rapidly at room temperature and had excellent water resistance and reduced formaldehyde emissions. As much as 70% of PF can be replaced by soy protein based adhesive with comparable physical properties for oriented and random strandboard (Hse et al., 2001; Wescott and Frihart, 2004). Medium density fiberboard bonded with copolymer of protein and phenolic resins (70/30) met the requirements for exterior or interior grades (Yang et al., 2006). In a study by Zhong and Sun (2007), blends adhesives (soy protein isolate/PF= 100/20) had the same level of adhesion strength as commercial PF adhesive alone, reduced formaldehyde usage, and had economic advantages. On the other hand, to promote reaction with synthetic polymers, soy protein must be unfolded to expose its functional groups. Various hydrolysis methods have been used to unfold soy proteins (Yang et al., 2006; Frihart and Wescoott, 2004], but these methods have not been able to overcome the hurdles of high viscosity, low concentration of soy protein, low water resistance, and short pot life.

### *2.7.3. Nano technology*

In recent several years, the nanoparticles reinforced adhesive has attracted researchers' attention. Several studies have confirmed that many mechanical properties of adhesive were significantly improved with addition of nano-scale filler (Chen et al., 2004; Gilbert et al., 2003), due to the large surface area of nano-scale filler and its ability to interlock mechanically with polymer (Hussain et al., 1996). Liu et al (2010) reported that the wettability, affinity, adhesion strength, and water resistance of the soy protein/$CaCO_3$ glue showed great advantages over pure soy protein adhesives; the hybrid adhesive showed stable wet adhesion strength more than 6 MPa. They attributed this great improvement to the compact rivets, interlocking links, and ion cross-linking reaction induced by calcium, carbonate and hydroxyl ions in the adhesives. In the study of Gao et al (2011), addition of 5-

10% nano-scale $CaCO_3$ can greatly improve the bond strength and bond durability of whey protein based adhesives. The environmentally friendly nanocomposite looks promising as a wood adhesive, and provides the new opportunity for the wood composite industry.

A rational design approach was used to successfully construct a functional protein nanomaterial adhesive in Mo's research (2006a, 2008). All protein nanomaterials contain a common hydrophobic corer flanked by charged amino acid sequences. The results suggested that the length of the hydrophobic core, the hydrophobicity, the amino-acid order, and the chemical composition are the important factors affecting adhesion. At protein molecular level, there are two requirements for adhesion: 1) protein nanomaterial needs to be in a beta sheet conformation where hydrogen bonds contribute to intermolecular assembly; and 2) requirement for a substantial number of hydrophobic amino acids with suitable side chain alkyl groups capable of forming van der Waals interactions.

**Figure 2.1. U.S. soybean area planted 1984-2009.**

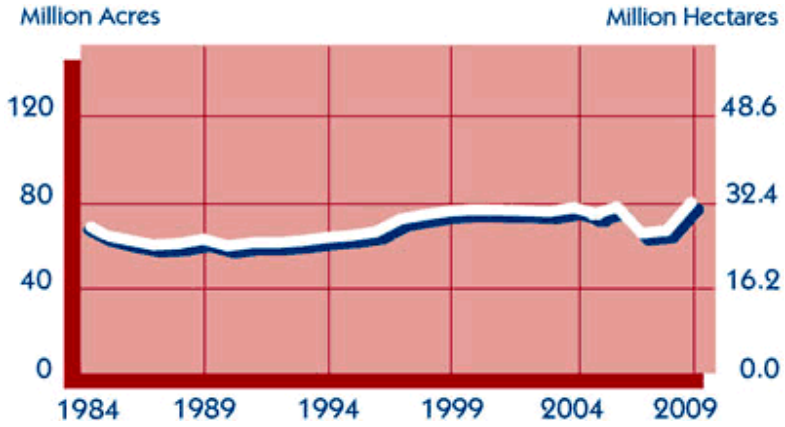

**Table 2.1. Amino acid compositions of 7S and 11S.**

| Amino acid | 7S | 11S |
|---|---|---|
| Ala | 7.49 | 5.17 |
| Arg | 7.29 | 6.32 |
| Asn | 5.18 | 7.35 |
| Asp | 0.14 | 4.31 |
| Cys | 8.86 | 1.48 |
| Gln | 13.15 | 9.89 |
| Glu | 4.84 | 8.74 |
| Gly | 2.32 | 7.27 |
| His | 5.72 | 1.97 |
| Ile | 8.72 | 4.56 |
| Leu | 6.13 | 6.90 |
| Lys | 0.34 | 4.31 |
| Met | 5.72 | 0.99 |
| Phe | 6.27 | 3.86 |
| Pro | 7.49 | 6.49 |
| Ser | 2.38 | 7.18 |
| Thr | 0.20 | 3.94 |
| Trp | 2.59 | 0.86 |
| Tyr | 5.18 | 2.55 |
| Val | 7.49 | 5.87 |

Adapted from Utsumi et al., 1997. Expressed as percentages

**Table 2.2. Amount and composition of ultracentrifuge fractions of water-extractable soybean proteins [a].**

| Fraction | Percentage (%) | Components | Molecular Weight (Da) | Isoelectric point |
|---|---|---|---|---|
| 2S | 22 | Trypsin inhibitors | 8000-21,500 | 4.2-4.5 |
| | | Cytochrome c | 12,000 | |
| 7S | 37 | Hemagglutinins | 110,000 | 6.00 |
| | | Lipoxygenase | 102,000 | 5.50 |
| | | β-Amylase | 61,700 | 5.85 |
| | | 7S Globulin | 180,000-210,000 | 4.80 |
| 11S | 31 | 11S Globulin | 350,000 | 6.40 |
| 15S | 11 | --- | 600,000 | |

[a] Adapted from Wolf, 1970 and Bogracheva et al 1996.

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# Chapter 3 - ADHESION AND PHYSOCOCHEMICAL PRPOPERTIES OF SOY PROTEIN ADHESIVES MODIFIED BY SODIUM BISULFITE

## 3.1 ABSTRACT

Soy protein adhesives with high solid content (30%-38%) were extracted from soy flour slurry modified with sodium bisulfite ($NaHSO_3$) at different concentrations. 11S dominated soy protein fractions (SP 5.4) and 7S dominated soy protein fractions (SP 4.5) were precipitated at pH 5.4 and pH 4.5, respectively. The objectives of this study were to investigate the effects of $NaHSO_3$ on the adhesion properties of soy proteins and characterize their physicochemical properties including SDS-PAGE, solubility and rheological, thermal and morphological properties. Unmodified sample SP 5.4 and SP 4.5 possessed clay-like properties and viscoelastic properties, respectively; whereas with addition of $NaHSO_3$ in range of 4-8 g/L, both SP 5.4 and SP 4.5 had the viscous cohesive phase with good hand-ability and flow-ability. The adhesion performance of SP 4.5 was better than SP 5.4; the wet strength of these two fractions was in the range of 2.5-3.2 MPa compared to 1.6 MPa of control soy protein isolate. $NaHSO_3$ had slightly negative effect on the adhesion properties of soy protein adhesives, but enhanced their rheological properties significantly. SDS-PAGE results indicated a certain reducing effects of $NaHSO_3$ on soy protein; the isoelectric point of soy protein was lowered obviously as $NaHSO_3$ increased due to the induced extra negative charges ($RS\text{-}SO_3^-$) on the protein surface. The balance between hydrophobic interaction and electrostatic force among proteins is critical to the continuous phase of SP 5.4, while the 7S/11S ratios and the high molecular weight protein aggregates connected by disulfide bonds are related closely to the continuous phase of SP 4.5.

## 3.2. INTRODUCTION

As one of the most promising bio-based adhesives, soy protein adhesives have been under intensive investigation for the last few decades; with the purpose of alleviating the severe problems aroused by the synthetic petrochemical adhesives such as environmental

pollution, detrimental to human health and limited petroleum resource. Even though many efforts have been done to improve the adhesion properties of soy protein used as wood adhesives (Hettiarachchy et al., 1995; Rogers et al., 2004; Li et al., 2004; Wang et al., 2007; Zhong et al., 2007; Qi and Sun, 2010), the overall performance remains inferior to the formaldehyde based adhesives, especially the water resistance.

The dominant storage protein in soybean is globulin, accounting for 50-90% of the total protein; glycinin (11S) and and β-conglycinin (7S) are its two major components. The relative proportion of 11S and 7S ranges from 1:3 to 3:1 depending on the cultivar and growing conditions (Wright, 1985). Glycinin is a large hexamer with molecular weight of about 350 kDa, consisting of five different types of subunits. Each subunit is composed of an acidic and a basic polypeptide linked by a disulfide linkage (Staswick et al., 1984). β-conglycinin is a trimer with molecular weight of 150-200 kDa; consisting of three subunits: α, α', and β. This trimer structure is mainly connected through non-covalent bonds: hydrophobic interaction and hydrogen bonding (Thanh and Shibasaki., 1978). In spite of a little cysteine content existing in α and α' subunits, it can form small amount of high molecular weight aggregates through disulfide bonds (Hoshi et al., 1982; Petruccelli and Anon, 1995). Qi et al (2011a) reported high quantity of these protein aggregates formed during the acid precipitation process, and they were highly related to the soy protein functionality. The inherent structure differences in 7S and 11S globulin are reflected on the different physicochemical functions in soy protein including thermal properties, water-binding capacity, solubility and adhesion performance etc (Thanh et al., 1976; Saio and Watanabe, 1978; Yao et al., 1988; Dias et al., 2003; Mo et al., 2004). Glycinin was found to improve wet adhesion strength (Mo et al., 2004; Wang et al., 2005). Mo and Sun (2006, 2011) also revealed that the basic subunits from 11S and β subunit from 7S showed greater water resistance than other subunits due to the considerably higher hydrophobic amino acids in basic and β subunit.

Soy protein and its subunits have been studied as wood adhesives in form of the protein suspension; the solid content is limited in range of 10-15% because of the high protein viscosity issues. In Sun's group, the new viscous cohesive soy protein adhesive system modified with $NaHSO_3$ was successfully exploited, with high solid content of 38%, good flow-ability, long shelf life and excellent water resistance comparable to formaldehyde

based adhesives (Sun et al., 2008; Qi et al 2011b). On the other hand, Zhang and Sun (2008, 2010) reported the insignificant or negative effects of $NaHSO_3$ modification on the adhesion properties of pure 7S and 11S suspension at 6.25% solid content. Therefore, in this study, we exploited the in situ $NaHSO_3$ modification on soy protein in the soy flour-water extracts, and then precipitated the modified soy protein with high solid content at pH 5.4 and pH 4.5 to get the 11S rich fractions and 7S rich fractions, respectively. The objectives of this study were to investigate the effects of $NaHSO_3$ concentration on adhesive properties of these two protein fractions, and to characterize their physicochemical properties such as electrophoresis profiles, solubility, rheological, thermal and morphological properties.

## 3.3. MATERIALS AND METHODS

### *3.3.1 Materials*

Defatted soy flour (Cargill, Cedar Rapids, IA) was used as the starting material. The soy flour contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium bisulfite ($NaHSO_3$) was obtained from Fisher Scientific (Fair Lawn, NJ). Cherry wood veneers with dimensions of 50 × 127 × 5 mm (width × length × thickness) were provided by Veneer One (Oceanside, NY).

### *3.3.2. Soy protein adhesive preparation*

The aqueous protein extract was prepared by mixing defatted soy flour in water at 6.25% solids content at pH 9.5 with 2 *N* NaOH. $NaHSO_3$ was added to the slurry at 0 g/L, 2 g/L, 4 g/L, 6 g/L, 8 g/L, 16 g/L, on the basis of water volume based on previous studies. The slurry was stirred for 2 h at room temperature, and then the carbohydrates were removed from the soy protein by Beckman Coulter Avanti J-25 centrifuge (Beckman Coulter, Inc. Brea, CA) at 12,000 *g*. The resulting supernatant was adjusted to pH at 5.4 using 2 *N* HCl, and centrifuged at 12,000 *g* to get the glycinin dominated soy protein fractions (labeled as SP 5.4). Then, the pH of each resulting supersaturate was adjusted to pH 4.5, and the slurries were centrifuged at 8,000 *g* to obtain β-conglycinin dominated soy protein fractions (labeled as SP 4.5). The soy protein yield is expressed as the gram of extracted wet soy protein per gram of dried soy flour.

### *3.3.3. SDS-Polyacrylamide Gel Electrophoresis*

SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system according to the method described by Laemmli (1970). Soy protein samples were mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue for the non-reducing gel. SDS-PAGE under reducing conditions was carried out with 2-mercaptoethonal added to the protein loading buffer. A total of 8 μg protein was applied to each sample slot. Electrophoresis was performed at 40 mA and 150 V for 120 min. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 40% methanol. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodak, Rochester, NY).

### *3.3.4. Determination of Solubility*

The pH-dependent solubility profile was determined by measuring absorbance of the supernatant of the centrifuged soy protein sample solutions with various pH values at 280 nm, as described by German et al (1982). Soy protein samples were diluted to 0.1% with deionized water. Diluted soy protein solutions were adjusted to desired pH values ranging from 3-10, stirred for 1 h, and centrifuged at 10,000 g for 15 min. Absorbance of the supernatant was measured at 280 nm by a spectrophotometer (Biomate 3, Thermo Electron Corporation, Madison, WI). All measurements were done in duplicate, and means were reported.

### *3.3.5. Rheological properties*

Apparent viscosity measurements of soy protein samples were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA). A parallel plate head was used with 20 mm plate diameter and 500 um gap. The apparent viscosity measurements were tested in the shear rate range of 0.1-50 $s^{-1}$. The testing temperature was 23 °C. A thin layer of silicone oil was spread over the circumference of the sample to prevent the sample from dehydrating during the test.

### *3.3.6. Thermal properties*

Thermal properties of soy protein samples were evaluated by a differential scanning calorimeter (DSC) (Q200, TA instrument, Schaumburg, IL) calibrated with indium and zinc. Fresh soy protein samples (20 mg) were hermetically sealed in Tzero aluminum hermetic pans. Each sample was held at 20 °C for 1 min, then scanned from 20 °C to 130 °C at a heating rate of 10 °C/min. Peak temperatures and denaturation enthalpies were calculated from thermograms by Universal Analysis 2000 software.

### *3.3.7. Morphological properties*

A Philips CM 100 (FEI Company, Hillsboro, OR) TEM was used to observe the microstructure of $NaHSO_3$-modified soy protein adhesive. Soy protein samples were diluted to 0.5% in deionized water and then sonicated for 10 min in an L & R320 ultrasonic stirrer (L & R Manufacturing Company, Keary, NJ). Diluted samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, FA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Burlington, VT) for 60 s at room temperature.

### *3.3.8. Wood specimen preparation*

Soy protein samples possessing flowable and brushable properties were selected to characterize their adhesion performance. Cherry wood veneers were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA) for 7 days at 23 °C under 50% relative humidity. The adhesives were brushed onto one end of the cherry wood piece with dimensions of 127 × 20 mm (length × width) until the entire area was completely wet. The amount of adhesive applied on each piece was about 0.3 g. Two brushed wood pieces were assembled immediately and conditioned for 20 min at room temperature. The assembled wood specimens were then pressed with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN) at 2.0 MPa and 170 °C for 10 min.

### *3.3.4. Shear strength measurements*

The wood assemblies glued with soy protein adhesives were cooled, conditioned at 23 °C under 50% relative humidity for 2 days, and cut into 5 pieces with dimensions of 80 × 20 mm (glued area of 20 × 20 mm). The cut wood specimens were conditioned for another 4

days before measurements were taken. Wood specimens were tested with an Instron Tester (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 at a crosshead speed of 1.6 mm/min. Shear adhesion strength at maximum load was recorded; reported values are the average of five specimen measurements.

Water resistance of the wood assemblies was measured following ASTM Standard Methods D1183-96 and D1151-00. The preconditioned specimens were soaked in tap water at 23 °C for 48 h, and the wet strength was tested immediately after soaking.

## 3.4. RESULTS ANDDISCUSSION

### *3.4.1. Soy protein fraction yield*

Two soy protein fractions SP 5.4 (11S dominated fraction) and SP 4.5 (7S dominated fraction) were extracted under various $NaHSO_3$ concentrations; their protein yield and water content (WC) are presented in Figure 3.1. Non-modified soy flour slurry yielded around 110% wet soy proteins at pH 5.4, whereas the protein yield decreased remarkably to about 86% when 2 g/L $NaHSO_3$ was added to the slurry. Then the insignificant decrease in protein yield occurred as $NaHSO_3$ concentration increased to 8 g/L. The WC of these soy proteins were approximate 69%, and the value increased slightly as $NaHSO_3$ increased. At 16 g/L $NaHSO_3$, only 69% wet SP 5.4 was extracted; and its water content increased in the further step (WC ~ 83%). It suggested that $NaHSO_3$ modification increased the water hydration ability of soy protein. In case of SP 4.5 fractions, the contrary protein yield trend to SP 5.4 was observed. The soy protein yield increased from 8% at 0 g/L $NaHSO_3$ to 42% at 16 g/L $NaHSO_3$, because more and more 11S was solubilized into the supernatants at pH 5.4, and then these 11S were incorporated to the 7S dominated protein fractions at pH 4.5. The WC (60%-64%) of SP 4.5 was also increased slightly as the amount of $NaHSO_3$ increased.

### *3.4.2. SDS-PAGE analysis*

The protein profile analyses of sample SP 5.4 and SP 4.5 modified with $NaHSO_3$ are shown in Figure 3.2 (Reducing condition) and Figure 3.3 (Non-reducing condition). The typical soy protein subunits for both fractions were identified as α', α, and β subunits from 7S; acidic (A) and basic (B) subunits from 11S. Unmodified sample SP 5.4 (Figure 3.2 A) contained around 20% 7S and 76% 11S (Table 3.1). Even though the density of 7S bands

were attenuated as $NaHSO_3$ concentration increased, the pure 11S samples were not obtained (Figure 3.2 B-E). Approximate 10% 7S was still existed in all $NaHSO_3$-modified sample SP 5.4. When soy protein treated with 16 g/L $NaHSO_3$ (Figure 3.2 E), because its product yield and solid contents were low, the bands appeared pretty weak. As to SP 4.5 fractions, the 7S accounted for almost 87 % at 0 g/L $NaHSO_3$. With the $NaHSO_3$ concentration increased from 0-16 g/L, the relative contents of 7S decreased from 87% to 16% and 11S increased from 3% to 83% (Figure 3.2 F-J, Table 3.1). In addition, the bands at 20 kDa and 15 kDa belonged to the 7S (Iwabuchi and Yamauchi, 1987) in sample SP 4.5 modified with lower $NaHSO_3$ concentration were faded when $NaHSO_3$ was above 6 g/L.

Non-reducing SDS-PAGE for SP 5.4 gave the major bands at 56 kDa (Figure 3.3 A-E), which corresponded to the glycinin A-B complexes composed of the acidic (38 kDa) and basic polypeptides (23 kDa) linked by disulfide bonds. The bands observed at about 32 kDa might be due to the non-covalently bonded acidic polypeptides ($A_4$) in the $G_4$ ($A_5A_4B_3$) glycinin subunits (Staswick et al., 1984). The faint bands at 38 kDa intensified as the $NaHSO_3$ increased, indicating the limited reducing effects of $NaHSO_3$ exerted on the 11S, even though the concentration was up to 16 g/L. Several weak bands occurred around 100 kDa, which was suggested as the result of the disulfide bond formation in aggregates induced by freeze-drying or thiol-disulfide exchange during the modification process (Wolf, 1993), also attenuated with the addition of $NaHSO_3$.

Furthermore, the weak high-molecular-weight band at around 120 kDa was observed in unmodified SP 5.4 (Figure 3.3 A); simultaneously, the density of bands related to α' and α subunits reduced remarkably in comparison of those under reducing SDS-PAGE. Besides, the 120 kDa bands faded gradually as $NaHSO_3$ concentration increased, during which the 7S content was reduced (Figure 3.3 B-E). Therefore, we assumed the 120 kDa protein aggregates were composed of α' and α subunits and connected with disulfide bonds. The band density of β subunit under non-reducing condition (Figure 3.3 A-E) was similar to those under reducing condition (Figure 3.2 A-E), suggesting that the β subunit did not participate in the aggregates formation.

In case of non-reducing SDS-PAGE of SP 4.5, 120 kDa protein aggregates accounted for large portion of the unmodified SP 4.5. And the largely reduced density of α' and α subunits compared to reducing SDS-PAGE further proved that these protein aggregates were

formed from α' and α subunits, and stabilized by disulfide bonds. The protein aggregates content faded gradually as $NaHSO_3$ concentration increased to 16 g/L (Figure 3.3 G-J). It is known that with the addition of $NaHSO_3$, it has the certain reducing effects exerted on protein, cleaving the disulfide bonds existed between the protein subunits. Some of the resulted sulfhydryls (R-SH) can be blocked as negative sulfonate groups ($RS\text{-}SO_3^-$) (Savolainen, 2004), then lowering the isoelectric point of 7S and 11S subunits. This reduced soy protein isoelectric point is the main reason that the protein composition (7S/11S ratios) altered when they were extracted from soy flour slurry at pH 4.5. In a similar way, the gradually faded 120 kDa band in $NaHSO_3$-modified SP 4.5 could be attributed to the disulfide bonds breakage among the protein aggregates. In addition, more and more 11S subunits were incorporated to the SP 4.5 fraction at higher $NaHSO_3$ concentration, which could interfere with the interaction between α' and α subunits to form protein aggregates in some degree. These high-molecular-weight protein aggregates formed during the extraction procedure played a very important role in the functional properties of soy protein samples, which will be discussed in the following section.

### *3.4.3. Solubility*

The typical U-shaped, solubility-pH profiles of SP 5.4 fractions are observed in Figure 3.4. As the pH approached isoelectric point, protein displayed lowest solubility because of the lowest electrostatic repulsion among protein molecules. Unmodified soy protein SP 5.4 had the minimum solubility in the region of 4.5 to 5.5, while $NaHSO_3$-modified SP 5.4 exhibited this insoluble region in a lower pH value. At 16 g/L $NaHSO_3$, isoelectric point of SP 5.4 was lowered to around pH 4.0 from pH 5.0 in unmodified sample. In addition, the protein solubility in all over the pH range was decreased significantly as $NaHSO_3$ concentration increased.

Protein solubility was manipulated by the interaction (electrostatic attractive, hydrophobic, van der Waals etc) and repulsion (electrostatic interaction) force among protein molecules. As mentioned previously, modifying soy protein with $NaHSO_3$ brought additional negative charge groups on protein molecules ($RS\text{-}SO_3^-$); as a result, the minimum protein solubility was shifted to a lower pH value. The largely decreased solubility all over the pH range in $NaHSO_3$ modified SP 5.4 was considered to be the salting-out process; increasing

the ionic strength could screen the charges on the protein surface, resulting in a lower electrostatic repulsion and lower solubility (Renkema et al., 2002). Also, as a reductant, $NaHSO_3$ released the some basic subunits from their associates with the acidic subunits in the oligomeric 11S (Figure 3.3 A-E). These dissociated basic subunits with high hydrophobic amino acids (Kella et al., 1986), tend to readily aggregate, which has been proved in many studies (Mo et al., 2006; German et al., 1982; Lakemond et al., 2000). The increased protein hydrophobic interaction might also contribute to the reduced solubility in $NaHSO_3$-modified SP 5.4.

### *3.4.4. Rheological properties*

The shear rate dependence of apparent viscosity of SP 5.4 and SP 4.5 fractions modified with $NaHSO_3$ are shown in Figure 3.5; all the soy protein samples exhibited as the shear thinning behavior. Addition of $NaHSO_3$ obviously decreased the viscosity of sample SP 5.4. Unmodified SP 5.4 had the viscous and clay-like properties, lacking of the continuous protein properties. When the $NaHSO_3$ concentration was in the range of 2-8 g/L, the viscosity of samples decreased and displayed as the viscose cohesive phase with good flow-ability. As the $NaHSO_3$ was up to 16 g/L, SP 5.4 exhibited the diluted state with pretty low viscosity. Apparent viscosity of soy protein is the result of protein intermolecular interaction such as disulfide bonds, electrostatic interaction and hydrophobic force etc. $NaHSO_3$ could break the protein disulfide linkage in some level, resulting in weakening protein-protein interaction and the reduced protein viscosity. In addition, the isoelectric point of soy protein was lowered by $NaHSO_3$, which indicated that the protein electrostatic repulsion was enhanced at extracted pH 5.4 in the $NaHSO_3$-modified SP 5.4 samples. This also caused the decreased apparent viscosity of SP 5.4 as $NaHSO_3$ increased.

Unmodified sample SP 4.5 possessed the viscoelastic properties and extremely high apparent viscosity. The viscoelastic properties were gradually disappeared and sharply decreased viscosity was occurred when soy protein was treated with $NaHSO_3$. The apparent viscosity of SP 4.5 modified with 2 g/L $NaHSO_3$ decreased by a factor of 9 from the unmodified one, and the lowest value was observed at 6 g/L $NaHSO_3$. Then its viscosity had the sudden increase at $NaHSO_3$ concentration above 6 g/L, but still lower than the one at 2 g/L. Viscose cohesive properties with good flow-ability was observed for SP 4.5 treated with

$NaHSO_3$ concentration in range of 4-8 g/L. Whereas soy flour slurry modified with 16 g/L $NaHSO_3$ yielded clay-like and non-cohesive protein samples. As shown in SDS-PAGE, unmodified sample SP 4.5 was 7S-rich protein and 120 kDa protein aggregates formed by α' and α accounted for a large amount. Therefore, we proposed that the high amount of protein aggregates played a vital role in the viscoelastic properties of soy protein. Along with the $NaHSO_3$ treatment, 120 kDa protein aggregates were dissociated by the disulfide bonds breakage; concomitantly, the protein had the largely reduced viscosity but still with the continuous protein phase. However, the protein aggregates was reduced completely when excessive amount of $NaHSO_3$ was used, and too much 11S subunits was incorporated to SP 4.5 fraction, resulting in the clay-like protein (16 g/L $NaHSO_3$).

### *3.4.5. Thermal properties*

As shown in Figure 3.6 A, the DSC thermogram of soy protein samples consisted with the SDS-PAGE results on the protein composition. The peak designated as the 7S globulin was exhibited as small shoulder in sample SP 5.4. The 11S globulin was characterized as the peak with denaturation temperature ($T_d$) around 98 °C in all SP 5.4 samples, indicating the reducing effects of $NaHSO_3$ played insignificant part on the thermal stability of soy protein. The denaturation enthalpy ($\Delta H_d$) of 11S increased from 8.43 J/g of unmodified SP 5.4 to 10.31 J/g at 2 g/L $NaHSO_3$, and then slightly decreased as the concentration increased (Table 3.2). $NaHSO_3$ is known to have the salt screening effects on the electrostatic forces of protein and strengthen the hydrophobic interactions, inducing more stable protein structure. However, $NaHSO_3$ was also proved to decrease the protein isoelectric point, leading to the increased protein electrostatic repulsion forces at pH 5.4 as $NaHSO_3$ increased, which could destabilize the protein structure. It is possible that the increased electrostatic repulsion dominated the salt screening effects of SP 5.4 at higher $NaHSO_3$ concentration, resulting in the reduced protein $\Delta H_d$.

One major endothermic transition peak with $T_d$ of 78 °C caused by 7S was observed in sample SP 4.5 at $NaHSO_3$ concentration $\leqslant$ 8 g/L; 11S denaturation peak at $T_d$ of 95 °C was appeared at $NaHSO_3$ concentration $\geqslant$ 8 g/L (Figure 3.6 B, Table 3.2). $NaHSO_3$ modification had no effects on the $T_d$ of both 7S and 11S in sample SP 4.5, whereas the $\Delta H_d$ of 7S was improved by about 1.0 J/g at 2 g/L $NaHSO_3$. And then further increasing the

$NaHSO_3$ concentration caused the reduced protein ΔH. Similar to the thermal behavior of sample SP 5.4, the salting screening effects of $NaHSO_3$ stabilize the protein structure, resulting in the increased ΔH of 7S in SP 4.5 at 2 g/L $NaHSO_3$. On the other hand, high amount of protein aggregates connected by disulfide bonds in unmodified SP 4.5 could have a more ordered protein structure that needs high energy to dissociate them. As the $NaHSO_3$ concentration increased above 4 g/L, the protein aggregates content in SP 4.5 largely decreased, therefore, the thermal stabilizing factor (salting screening effects) was overtaken by the thermal destabilizing factor (protein aggregates dissociation), as a result, the protein $\Delta H_d$ was reduced at higher $NaHSO_3$ concentration.

### *3.4.6. Morphological properties*

Unmodified SP 5.4 was mainly formed irregular shaped globular aggregates (Figure 3.7 A), whereas 6 g/L $NaHSO_3$ treatment induced significant changes in protein morphology appearance. The chain-like network built of large number of small protein clusters with diameters ranging from 15 nm to 30 nm, was observed (Figure 3.7 B). Increasing $NaHSO_3$ concentration to 16 g/L, the chain-like network was broken down again and dissociated into many individual protein clusters (Figure 3.7 C), which has the similar diameters to the protein cluster of SP 5.4 treated by 6 g/L $NaHSO_3$. Sun et al (2008) and Zhang and Sun (2008) also observed the similar chains of protein clusters on condition of hydrophobic interaction equals electrostatic force among protein molecules. $NaHSO_3$ is known not only to break down the disulfide linkages and but also alter the ionic strength in protein; inducing the changes in the hydrophobic interaction and electrostatic force among protein, which could manipulate the protein conformation. At 6 g/L $NaHSO_3$, the hydrophobic interaction and electrostatic force might reach balance, the small protein clusters were connected with each other to form the chain-like network structure. This chain-like network structure might play an important role in the formation of continuous protein phase with viscous cohesive properties. The further increased $NaHSO_3$ concentration (16 g/L) lowered the protein isoelectric point significantly; therefore, the increased the electrostatic repulsion at pH 5.4 could dominate the hydrophobic interactions in soy protein, resulting in the dissociation of the chain-like network into the small protein clusters.

In case of SP 4.5, unmodified sample is mainly composed of the branch-like protein aggregates and large number of the surrounded small protein cluster lines (Figure 3.6 D). In SP 4.5 modified by 6 g/L $NaHSO_3$, it existed in form of numerous spherical protein aggregates with diameter of about 30 nm; and some of them grew into the bigger irregular protein clusters (Figure 3.6 E). As $NaHSO_3$ increased to 16 g/L, the branch-like aggregates and large number of protein cluster line were observed (Figure 3.6 F) again. As mentioned in SDS-PAGE results, unmodified SP 4.5 (87% 7S) had large portion of disulfide bonds linked protein aggregates formed from α' and α subunits. This might induce the highly aggregated branch-like morphology and fairly viscous soy protein samples with viscoelastic properties. With the addition of 6 g/L $NaHSO_3$, these protein aggregates contents reduced significantly, leading to the disappearance of the branch-like aggregates and appearance of the large numbers of small uniform spherical protein aggregates. When $NaHSO_3$ increased to 16 g/L, the protein aggregates were disappeared completely in SP 4.5, and the sample became to the 11S-rich proteins (83% of 11S) with the clay-like state. The more hydrophobic properties of 11S than 7S might contribute to the branch-like protein aggregates as well.

### *3.4.7. Mechanical properties*

$NaHSO_3$-modified soy protein adhesives with viscous cohesive properties were chosen to characterize their adhesion performance on cherry wood. All four SP 5.4 samples (2 g/L, 4 g/L, 6 g/L and 8 g/L) and three SP 4.5 samples (4 g/L, 6 g/L and 8 g/L) had great dry shear adhesion strength, exhibiting 100% wood cohesive failure (Table 3.3). $NaHSO_3$ had slightly negative effects on the dry strength of these soy protein adhesives. Their water resistance was reflected by the wet shear adhesion strength which was also decreased as $NaHSO_3$ concentration increased. Sample SP 5.4 modified with 2 g/L $NaHSO_3$ had wet strength 3.2 MPa, while it reduced to 2.5 MPa at $NaHSO_3$ concentration of 8 g/L. The wet strength of sample SP 4.5 was in the range of 2.9-3.3 MPa when 4-8 g/L $NaHSO_3$ was used, compared to 1.6 MPa for the native soy protein isolate adhesive. As shown in Table 3.3, the overall adhesion performance of SP 4.5 was greater than those of SP 5.4.

Several studies have revealed that $NaHSO_3$ modification had the negative or insignificant effects on the adhesion properties of soy protein adhesives (Kalapathy et al., 1996; Zhang and Sun, 2008; Zhang and Sun, 2010). Consistent with the previous study, two

different functions exerted on protein adhesives properties, which counteracted with each other, were also confirmed in this study. $NaHSO_3$ reduced the apparent viscosity of protein and enhanced the flow-ability and hand-ability on the wood surface, which is favorable for the protein adhesion performance. Moreover, the charge screening effects of $NaHSO_3$ on protein molecules' electrostatic forces can strengthen their hydrophobic interactions. This enhanced protein-protein interaction lead to more protein entanglement during curing process, and is beneficial to the adhesion strength, especially for the wet adhesion strength. On the other hand, the presence of $RS\text{-}SO_3^-$ groups converted from R-SH groups on the protein molecules could decrease the effective interfacial area and enhance electrostatic repulsion between protein and wood surface, resulting in the decreased adhesive strength (Kalapathy et al., 1996). As to the sample SP 5.4 and SP 4.5 in this study, it is possible that the negative effects of $NaHSO_3$ exceeded its positive effects on the adhesion strength, therefore, the dry and wet strength decreased slightly as $NaHSO_3$ increased.

To date, soy protein adhesives under general investigation were in form of the protein suspension (freeze dried soy protein powder mixed with water) with concentration in the range of 10%-15%. The limited protein concentration is attributed to the extreme high viscosity at high protein content. In this study, the wet soy protein samples were utilized as the wood adhesive upon the centrifuge, having high solid content (28%-39%) but with good follow-ability. High protein concentration is favorable for protein-protein entanglement and protein-wood surface interaction during curing, resulting in the enhanced mechanical interlocks and physical attractions, with the consequent improved adhesion strength. Therefore, sample SP 4.5 having protein content around 9% higher than SP 5.4 could contribute partially to the better adhesion performance than the latter.

## 3.5. CONCLUSIONS

Two soy protein fractions with high solid content and good flow-ability were extracted from soy flour slurry modified with $NaHSO_3$ at pH 5.4 and pH 4.5, named as 11S dominated protein (SP 5.4) and 7S dominated protein (SP 4.5), respectively. Even though $NaHSO_3$ had slightly negative effects on the adhesion properties of soy protein adhesives, it improved their hand-ability and flow-ability remarkably. The adhesion performance of $NaHSO_3$-modified SP 4.5 samples was better than the $NaHSO_3$-modified SP 5.4 samples; the

wet strength was in the range of 2.5-3.2 MPa compared to 1.6 MPa of control soy protein isolate. $NaHSO_3$ had a certain reducing effects on soy protein; and the isoelectric point of modified soy protein was lowered obviously because of the induced extra negative charges ($RS\text{-}SO_3^-$) on protein surface. The balance between hydrophobic interaction and electrostatic repulsion force among proteins is critical to the continuous phase of sample SP 5.4, while the 7S/11S ratios and the high molecular weight protein aggregates connected by disulfide bonds are related closely to the continuous phase of sample SP 4.5.

**Figure 3.1. The product yield of soy protein adhesives extracted from soy flour slurry modified with $NaHSO_3$ at different concentrations.**

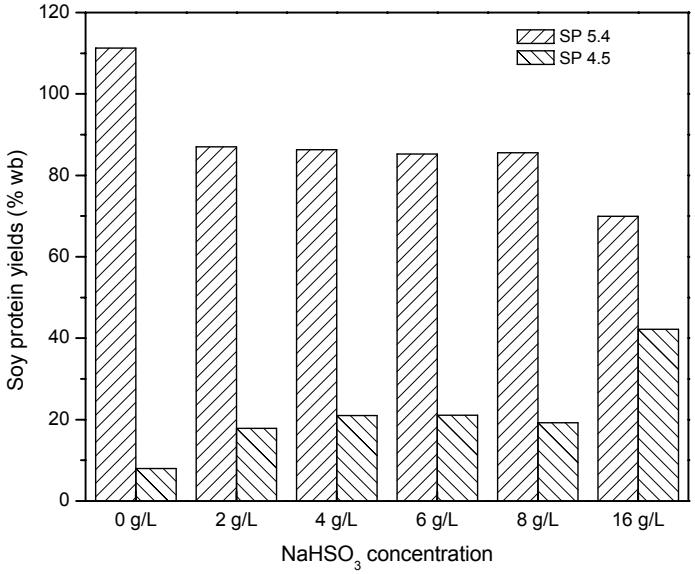


**Figure 3.2. Reducing SDS-PAGE of soy protein adhesives modified with $NaHSO_3$ at different concentrations. SP 5.4 (lane A-E); SP 4.5 (lane F-J). 0 g/L $NaHSO_3$ (lane A, F); 2 g/L $NaHSO_3$ (lane B, G); 6 g/L $NaHSO_3$ (lane C, H); 8 g/L $NaHSO_3$ (lane D, I); 16 g/L $NaHSO_3$ (lane E, J).**

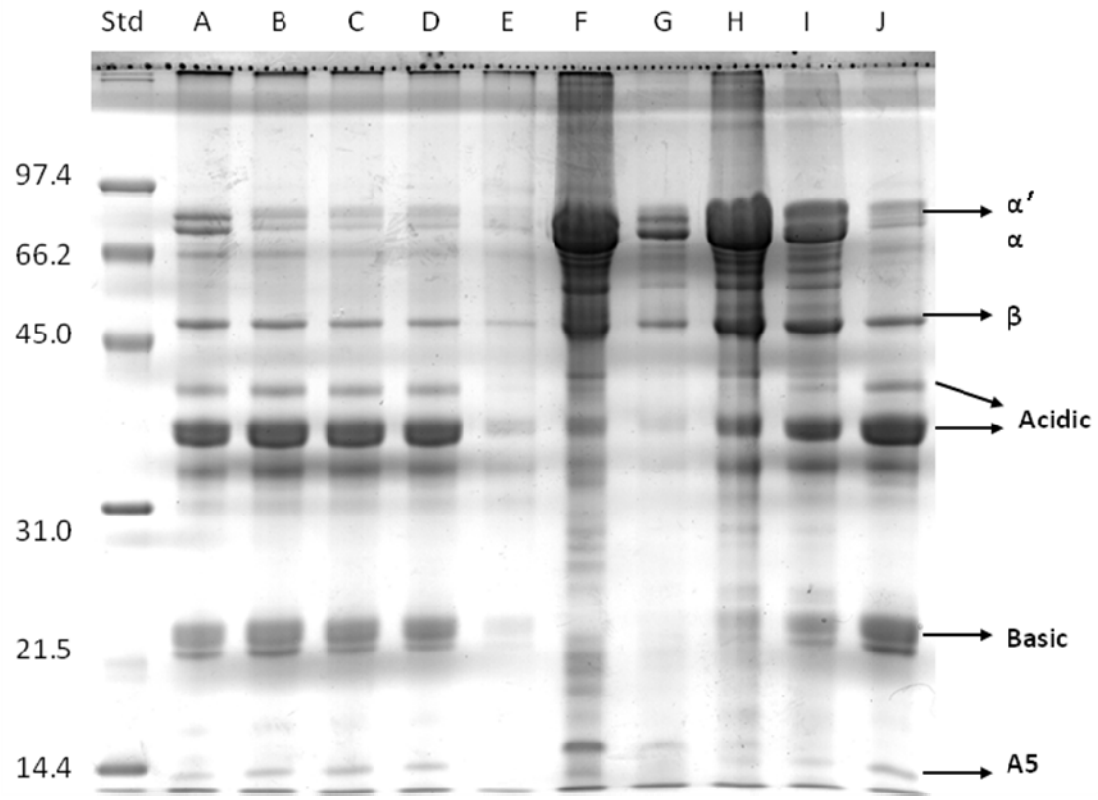


**Figure 3.3. Non-reducing SDS-PAGE of soy protein adhesives modified with $NaHSO_3$ at different concentrations. SP 5.4 (lane A-E); SP 4.5 (lane F-J). 0 g/L $NaHSO_3$ (lane A, F); 2 g/L $NaHSO_3$ (lane B, G); 6 g/L $NaHSO_3$ (lane C, H); 8 g/L $NaHSO_3$ (lane D, I); 16 g/L $NaHSO_3$ (lane E, J).**

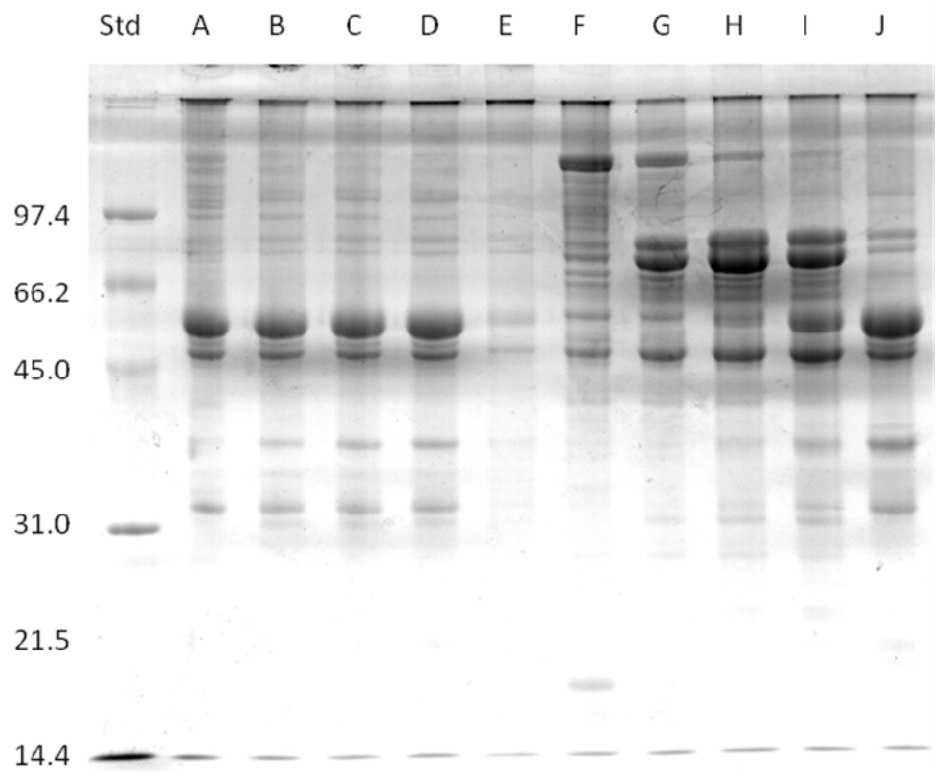


**Figure 3.4. pH solubility profile of soy protein adhesives (SP 5.4) modified with $NaHSO_3$ at different concentrations.**

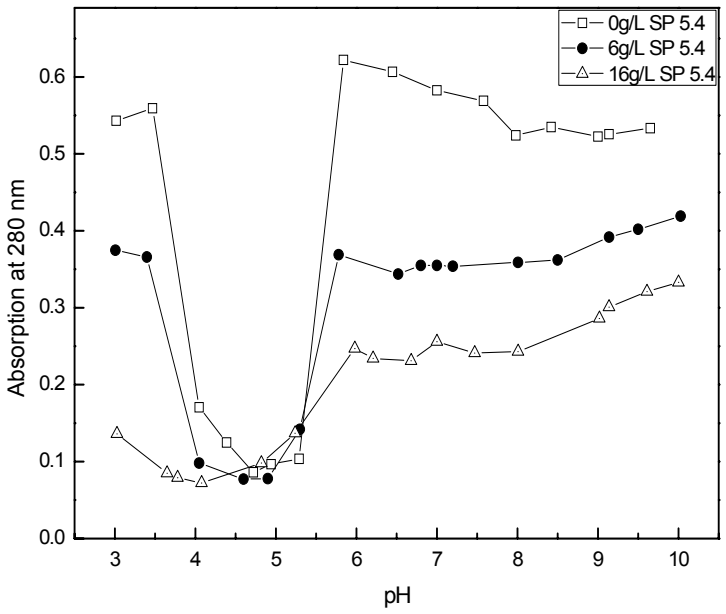


**Figure 3.5. Shear rate dependence of apparent viscosity of soy protein adhesives modified with $NaHSO_3$ at different concentrations. (A): SP 5.4; (B): SP 4.5.**

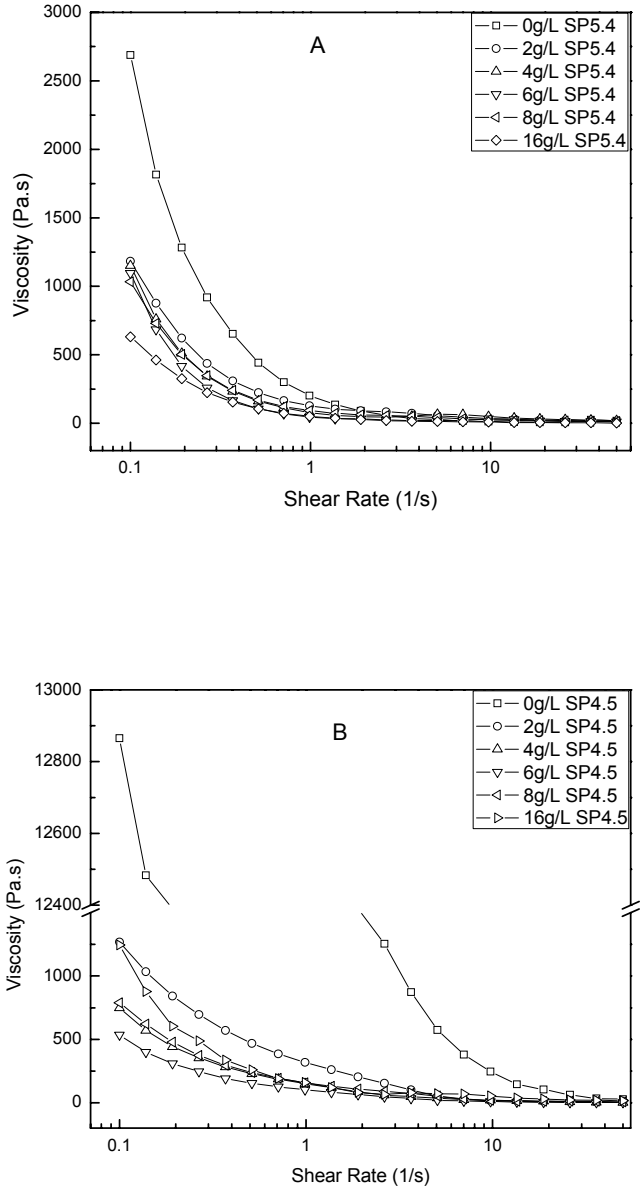
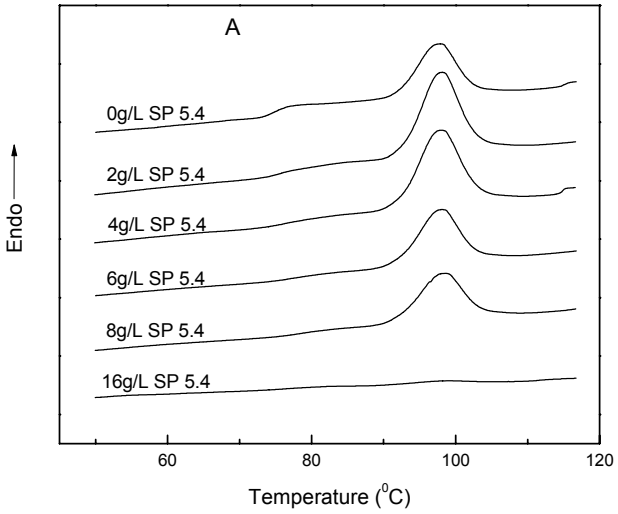


**Figure 3.6. DSC thermogram of soy protein adhesives modified with $NaHSO_3$ at different concentrations. (A): SP 5.4; (B): SP 4.5.**



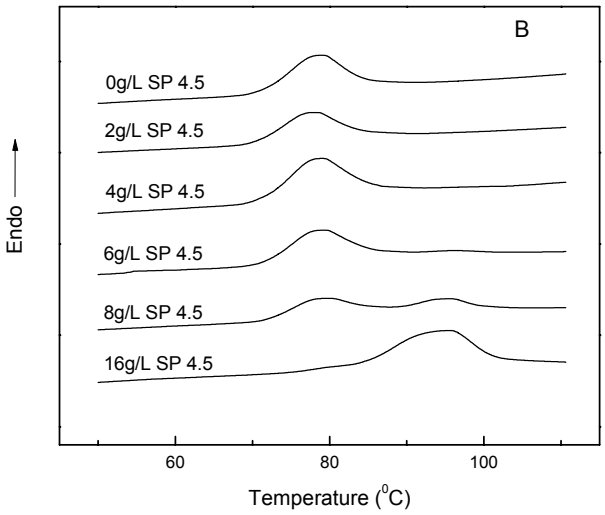
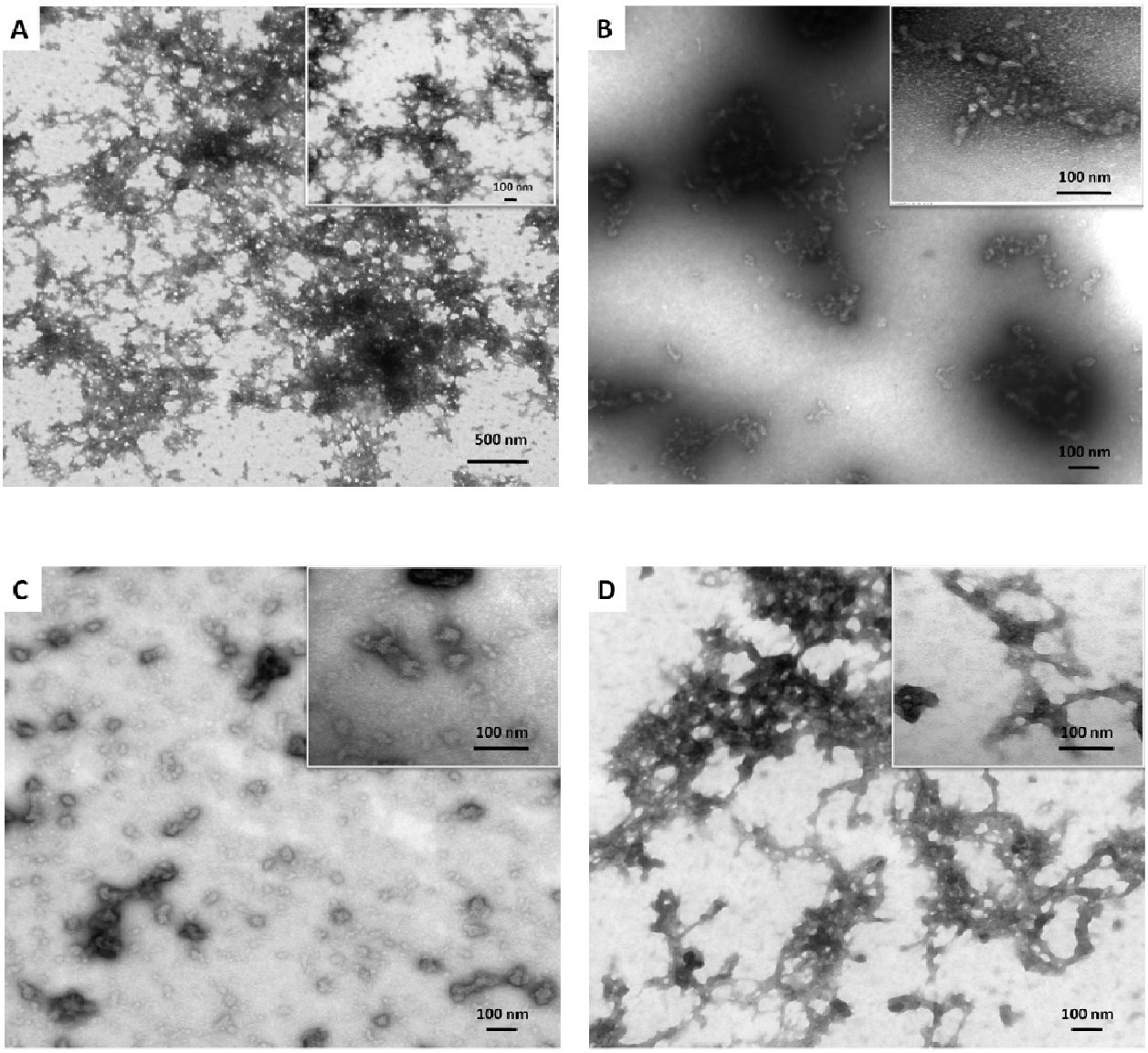



**Figure 3.7. TEM images of soy protein adhesives modified with $NaHSO_3$ at different concentrations. 0 g/L $NaHSO_3$ SP 5.4 (A); 6 g/L $NaHSO_3$ SP 5.4 (B); 16 g/L $NaHSO_3$ SP 5.4 (C); 0 g/L $NaHSO_3$ SP 4.5 (D); 6 g/L $NaHSO_3$ SP 4.5 (E); 16 g/L $NaHSO_3$ SP 4.5 (F).**



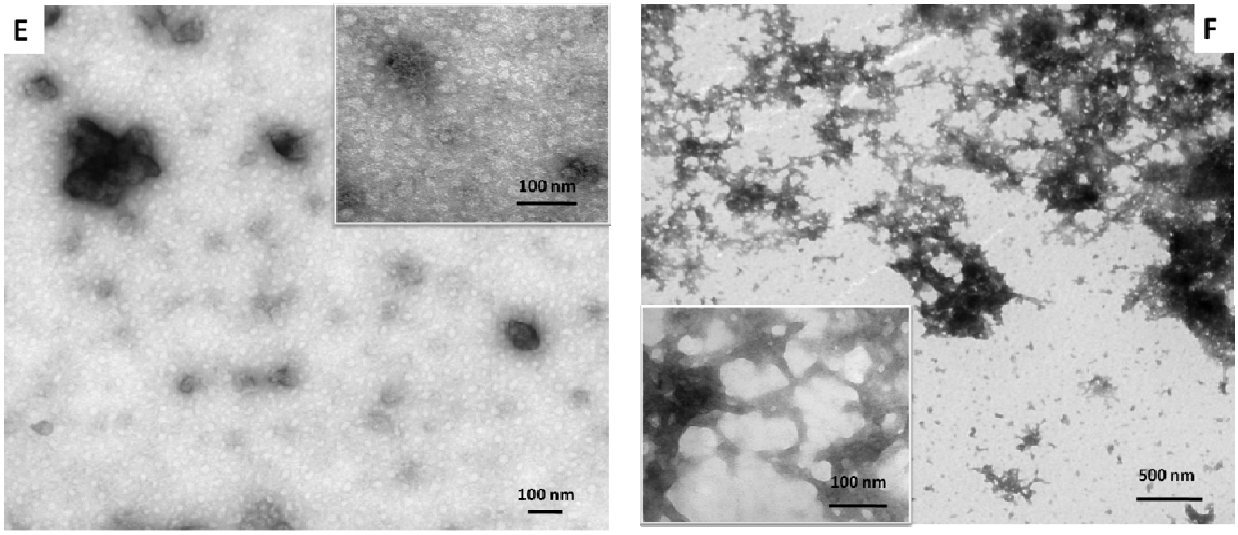

E
100 nm
100 nm
F
100 nm
500 nm

**Table 3.1. Estimated content of polypeptides of soy protein adhesives modified with $NaHSO_3$ at different concentrations.**

| Protein subunits | Soy protein fraction distribution (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SP 5.4 ( $NaHSO_3$ g/L) | | | | | SP 4.5 ( $NaHSO_3$ g/L) | | | | |
| | 0 | 2 | 6 | 8 | 16 | 0 | 2 | 6 | 8 | 16 |
| 7S | 19.7 | 11.8 | 11.2 | 10.2 | 13.7 | 87.5 | 66.8 | 57.7 | 39.5 | 15.8 |
| 11S | 75.9 | 85.7 | 85.8 | 87.7 | 76.3 | 3.0 | 23.5 | 34.2 | 55.9 | 82.9 |

**Table 3.2. Denaturation temperature ($T_d$) and total enthalpy of denaturation ($\Delta H_d$) of soy protein adhesives modified with $NaHSO_3$ at different concentrations.**

| $NaHSO_3$ concentration | 7S | | 11S | |
|---|---|---|---|---|
| | $T_d$ (°C) | $\Delta H$ (J/g) | $T_d$ (°C) | $\Delta H$ (J/g) |
| SP 5.4 | | | | |
| 0 g/L | 77.04 | 0.83 | 97.74 | 8.43 |
| 2 g/L | -- | -- | 97.87 | 10.31 |
| 4 g/L | -- | -- | 97.77 | 10.13 |
| 6 g/L | -- | -- | 97.80 | 9.78 |
| 8 g/L | -- | -- | 98.08 | 8.77 |
| 16 g/L | -- | -- | -- | -- |
| SP 4.5 | | | | |
| 0 g/L | 78.44 | 9.32 | -- | -- |
| 2 g/L | 78.09 | 10.29 | -- | -- |
| 4 g/L | 78.31 | 9.59 | -- | -- |
| 6 g/L | 78.44 | 7.72 | -- | -- |
| 8 g/L | 78.41 | 4.51 | 95.63 | 1.96 |
| 16 g/L | -- | -- | 95.13 | 10.82 |

**Table 3.3. Effects of extraction pH and concentration of $NaHSO_3$ on the adhesion properties of soy protein adhesives.**

| $NaHSO_3$ concentration | Dry strength (MPa) | Wood failure (%) | Wet strength (MPa) | Wood failure (%) |
|---|---|---|---|---|
| | | SP 5.4 | | |
| 2 g/L | 5.848±0.116 | 100% | 3.236±0.680 | 0% |
| 4 g/L | 5.508±0.125 | 100% | 2.725±0.375 | 0% |
| 6 g/L | 5.413±0.370 | 100% | 2.468±0.604 | 0% |
| 8 g/L | 5.007±0.826 | 100% | 2.456±0.236 | 0% |
| | | SP 4.5 | | |
| 4 g/L | 6.815±0.538 | 100% | 3.256±0.201 | 0% |
| 6 g/L | 6.340±0.256 | 100% | 3.177±0.247 | 0% |
| 8 g/L | 6.474±0.243 | 100% | 2.979±0.085 | 0% |

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# Chapter 4 - PHYSICOCHEMICAL CHARACTERIZATION OF SOY PROTEIN ADHESIVES OBTAINED BY IN SITU SODIUM BISULFITE MODIFICATION DURING ACID PRECIPITATION[1]

## 4.1. ABSTRACT

Successful industrial applications of soy protein adhesives require high adhesion strength and low viscosity at high solid protein concentration. This study examined the effects of 7S/11S mixtures modified by sodium bisulfite on adhesive properties and characterized the interactions and physiochemical properties of the modified 7S and 11S globulins. Soy protein adhesives with various 7S/11S ratios were extracted from soy flour slurry modified with sodium bisulfite using the acid precipitation method, which is based on the different solubilities of 7S and 11S globulins. Seven glycinin-rich soy protein fractions and six β-conglycinin-rich soy protein fractions were obtained. The external morphology of the samples changed from the viscous cohesive phase to the clay-like phase without cohesiveness. The viscous cohesive samples had good flow-ability and good water resistance with wet adhesion strength of 2.0 MPa to 2.8 MPa. They were stable for up to several months without phase separation at room temperature. Based on the observed morphology, viscosity, and particle size of the soy protein samples, we suggest that proper protein-protein interaction, hydration capacity (glycinin-rich soy protein fractions), and certain ratios of 7S and 11S (β-conglycinin rich soy protein fractions) in the soy protein sample are crucial to continuous protein phase formation. Hydrogen bonding, electrostatic forces, and hydrophobic interactions are involved in maintaining the protein viscous cohesive network, whereas disulfide bonds do not exert significant effects. This study describes a new way to investigate viscous cohesive soy protein systems with high solid protein content, thus alleviating the

[1] Results have been published. Qi, G., Li, N., Wang, D., Sun, X. S. 2011. Physicochemical properties of soy protein adhesives obtained by in situ sodium bisulfite modification during acid precipitation. J. Am. Oil Chem. Sci. DOI: 10.1007/s11746-011-1909-6 (reuse by permission of Springer).

disadvantages of traditional methods for studying the adhesive properties of soy protein isolates, which tend to have poor water resistance, high viscosity, and short storage life.

## 4.2. INTRODUCTION

Soy protein has been widely used in foods for its nutritional and functional properties. Biodegradable adhesives of soy protein also have been considered and extensively studied as potential alternatives to synthetic petrochemical adhesives. Two predominant storage proteins, glycinin (11S) and β-conglycinin (7S), constitute 80% of the total protein content in soybean, and they both have specific properties that contribute to the physicochemical properties of soy protein ingredients (Peng et al., 1984). Glycinin is a hexameric protein with a molecular weight of about 360 kDa composed of five different types of subunits: $A_{1a}B_{1b}$, $A_2B_{1a}$, $A_{1b}B_2$, $A_5A_4B_3$, and $A_3B_4$ (Staswick et al., 1984). Each subunit contains one acidic polypeptide and one basic polypeptide, which are linked by a disulfide bridge. Glycinin has relatively high cysteine content, with 18 to 20 intra- and intermolecular disulfide bonds (Kella et al., 1986). β-conglycinin (150 to 200 kDa) is a trimer, consisting of three subunits, α, α', and β. The subunits are non-covalently associated by hydrophobic interaction and hydrogen bonding, and do not contain disulfide bonds (Thanh and Shibasaki, 1978). Inherent differences in structure and molecular properties of the 11S and 7S globulins will lead to different functional properties of soy protein such as solubility, thermal and morphological properties, and adhesion performance (Saio and Watanabe, 1978; Ning and Villota, 1994; Mo et al., 2004; Zhang and Sun, 2008; Zhang and Sun, 2010).

Soy protein subunits have been studied for adhesion properties on wood. Several types of soy proteins have been investigated, including pure 7S globulin and 11S globulin suspensions (Zhang and Sun, 2008; Zhang and Sun, 2010; Wang et al., 2005), mixtures of 7S and 11S with various ratios (Mo et al., 2004), or 7S subunits (α, α', and β) and 11S subunits (acidic, basic subunits) (Mo et al., 2011; Mo et al., 2006). The β subunit showed greater water resistance than α, α', and β-conglycinin (Mo et al., 2011). Glycinin was found to improve wet adhesion strength (Mo et al., 2004; Wang et al., 2005). The basic subunits from glycinin had higher water resistance than acidic subunits due to the considerably more hydrophobic amino acids in the basic subunits (Mo et al., 2006). Previous studies also revealed that soy protein modified with sodium bisulfite had adhesive strength comparable to

formaldehyde-based adhesives (Qi and Sun 2010; Sun et al., 2008). However, Zhang and Sun (2008, 2010) reported that the adhesive performance of pure 11S and 7S globulin was not improved by sodium bisulfite modification. Therefore, we hypothesized that the interaction between 7S and 11S components in the soy protein adhesive system would dominate the adhesion performance. The ratio of unmodified 11S to 7S had shown significant influence on adhesion (Mo et al., 2004). In this study, we examined the interaction of 7S and 11S globulins at various ratios that were extracted directly from soy protein slurries modified with sodium bisulfite using the acid precipitation method. Because 7S and 11S have different solubilities under different pHs, we expected to obtain samples with various ratios of 7S and 11S by using different pHs during extraction. The objective of this study was to examine the effects of 7S/11S mixtures modified by sodium bisulfite on adhesive properties on wood, and to characterize the interactions and physiochemical properties of modified 7S and 11S such as electrophoresis profiles and rheological, thermal, and morphological properties.

## 4.3. MATERIALS AND METHODS

### *4.3.1. Materials*

Defatted soy flour (Cargill, Cedar Rapids, IA) was used as the starting material. The soy flour contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium bisulfite ($NaHSO_3$), Sodium Chloride (NaCl), and Urea were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium thiocyanate (NaSCN), 2-Mercaptoethanol (Me-SH), and propylene glycol (PG) were purchased from Sigma Aldrich (St. Louis. MO). Cherry wood veneers with dimensions of 50 × 127 × 5 mm (width × length × thickness) were provided by Veneer One (Oceanside, NY).

### *4.3.2. Soy protein adhesive preparation*

The aqueous protein extract was prepared by mixing defatted soy flour in water at 6.25% solids content at pH 9.5. $NaHSO_3$ was added to the slurry at 6 g/L on the basis of water volume based on previous studies (Qi and Sun, 2010; Sun et al., 2008). The slurry was stirred for 2 hr at room temperature, then the carbohydrates were removed from the soy protein by centrifuging at 12,000 × *g*. The resulting supernatant was divided into seven portions and each portion was adjusted to pH at 6.0, 5.8, 5.6, 5.4, 5.2, 5.0, and 4.8,

respectively, using 2 N HCl. The supernatants were centrifuged at 12,000 × *g* to get the glycinin-rich fractions (labeled as Gly pH6.0, Gly pH5.8, etc.). Then, the pH of each resulting supersaturate was adjusted to pH 4.8, and the slurries were centrifuged at 8,000 × *g* to obtain β-conglycinin-rich soy protein fractions (labeled as Cong pH6.0, Cong pH5.8, etc.). A flowchart is presented in Figure 4.1 to illustrate the procedure, using the pH 6.0 sample as an example. The product yield, water content, and external morphology of all samples are summarized in Table 4.1.

### *4.3.3. Electrophoresis (SDS-PAGE)*

SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system according to the method described by Laemmli (1970). $NaHSO_3$-modified soy protein samples were mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue for the non-reducing gel. SDS-PAGE under reducing conditions was carried out with 2-mercaptoethonal added to the protein loading buffer. A total of 8 μg protein was applied to each sample slot. Electrophoresis was performed at 40 mA and 150 V for 120 min. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 40% methanol. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodark, Rochester, NY).

### *4.3.4. Particle size analysis of protein precipitates aggregates*

The aqueous protein suspensions of pH (5.8, 5.4, 5.0, 4.8) and their resultant supersaturates after centrifugation at pH 4.8, as described in section 2.2, were used for the particle size distribution measurements. A LECOTRAC LTS-150 Particle Size Analyzer (LECO Corporation, Tampa, FL) was used for this study. Soy protein concentration was within the equipment recommendation, and ultrasonic was utilized to make the soy protein particles dispersed well in the sample cell. Two replicates were made on each sample. Mean diameter of volume distribution (mv) was recorded for each sample.

### *4.3.5. Determination of protein solubility*

Two soy protein samples, Gly pH5.4 and Cong pH5.4, were selected to study their solubility in various reagents. One gram of freeze-dried soy protein powder was dispersed in

10 ml citric acid-$Na_2HPO_4$ buffer (pH 5.4 and 4.8, respectively) containing either 0.5 M NaCl, 0.5M NaSCN, 3 M Urea, 0.2 M Me-SH, or 20% PG to make a suspension. After 2 hr constant stirring at room temperature, each suspension was centrifuged for 15 min at 8,000 × *g*. The supernatant was discarded gently and the precipitated protein was freeze-dried for the protein solubility calculation.

### *4.3.6. Rheological properties*

Apparent viscosity measurements of $NaHSO_3$-modified soy protein samples were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) with a CP 4/40 cone and plate fixture (4 cone angle, 40mm cone diameter). The distance between the cone and the plate was set to 150 μm. The apparent viscosity measurements were tested in the shear rate range of 0.1 to 100 $s^{-1}$. The testing temperature was 23 °C. A thin layer of silicone oil was spread over the circumference of the sample to prevent the sample from dehydrating during the test.

### *4.3.7. Thermal properties*

Thermal properties of $NaHSO_3$-modified soy protein samples were evaluated by a differential scanning calorimeter (DSC) (Q200, TA instrument, Schaumburg, IL) calibrated with indium and zinc. Fresh soy protein samples (20 mg) were hermetically sealed in Tzero aluminum hermetic pans. Each sample was held at 20 °C for 1 min, then scanned from 20 °C to 130 °C at a heating rate of 10 °C/min. Peak temperatures and denaturation enthalpies were calculated from thermograms by Universal Analysis 2000 software.

### *4.3.8. Morphological properties*

A Philips CM 100 (FEI Company, Hillsboro, OR) TEM was used to observe the microstructure of $NaHSO_3$-modified soy protein adhesive. $NaHSO_3$-modified soy protein samples were diluted to 1% in deionized water and then sonicated for 10 min in an L&R320 ultrasonic stirrer (L&R Manufacturing Company, Keary, NJ). Diluted samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, FA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT) for 60 s at room temperature.

### *4.3.9. Wood specimen preparation*

$NaHSO_3$-modified soy protein possessing flowable and brushable properties were selected to characterize their adhesion performance. Cherry wood veneers were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA) for 7 days at 23 °C under 50% relative humidity. The adhesives were brushed onto one end of the cherry wood piece with dimensions of 127 × 20 mm (length × width) until the entire area was completely wet. The amount of adhesive applied on each piece was about 0.3 g. Two brushed wood pieces were assembled immediately and conditioned for 20 min at room temperature. Then the assembled wood specimens were pressed with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN) at 1.4 MPa and 150 °C for 10 min.

### *4.3.10. Shear strength measurements*

The wood assemblies glued with $NaHSO_3$-modified soy protein adhesive were cooled, conditioned at 23 °C under 50% relative humidity for 2 days, and cut into 5 pieces with dimensions of 80 × 20 mm (glued area of 20 × 20 mm). The cut wood specimens were conditioned for another 4 days before measurements were taken. Wood specimens were tested with an Instron Tester (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 at a crosshead speed of 1.6 mm/min. Shear adhesion strength at maximum load was recorded; reported values are the average of five specimen measurements.

Water resistance of the wood assemblies was measured following ASTM Standard Methods D1183-96 and D1151-00. The preconditioned specimens were soaked in tap water at 23 °C for 48 hr, and the wet strength was tested immediately after soaking.

## 4.4. RESULTS AND DISCUSSION

### *4.4.1. $NaHSO_3$-modified soy protein samples*

Thirteen $NaHSO_3$-modified soy protein samples were obtained upon centrifugation: seven glycinin-rich soy protein fractions and six β-conglycinin-rich soy protein fractions. The products yield (wet weight basis), water content, and external morphology are summarized in Table 4.1. The total protein yield of the glycinin-rich soy protein fractions were increased as the extraction pH decreased from 6.0 to 4.8, as more and more glycinin and β-conglycinin partitioned from the supernatant. Glycinin is known to precipitate in the pH range of 4.4 to

6.8, with the isoelectric point (IP) approximately at pH 5.4, while the β-conglycinin precipitates in the pH range of 4.0 to 5.6 with IP 4.8 (Thanh et al., 1976). However, as a reducing agent, $NaHSO_3$ introduced extra charges on the surface of soy proteins ($RS\text{-}SO_3^-$) and the minimum solubility range shifted to a lower pH value, which can be reflected by the very low extraction yield of Gly pH6.0 (9%). The glycinin-rich soy protein displayed viscous cohesive properties in the pH range of 5.4 to 5.8, while the soy protein became a clay-state phase of non-cohesiveness when the pH was lower than 5.4. The β-conglycinin-rich soy protein fractions exhibited viscous cohesive properties for samples Cong pH5.0, Cong pH5.2, and Cong pH5.4, whereas other sample fractionations obtained at pH>5.4 lost the continuous phase. In addition, due to the reducing effects of $NaHSO_3$, all samples had several months of storage life.

### *4.4.2. SDS-PAGE*

***Glycinin-rich soy protein fractions:*** Reducing SDS-PAGE was performed to study the subunits distribution in each soy protein sample. The electrophoresis profiles of glycinin-rich soy proteins are presented in Figure 4.2A. The predominant components were identified as α', α, and β subunits of β-conglycinin and acidic (A) and basic (B) polypeptides of glycinin. The bands observed at about 32 kDa might be due to the non-covalently bonded acidic polypeptides ($A_4$) in the $G_4$ ($A_5A_4B_3$) glycinin subunits (Staswick et al., 1984). The 30 kDa band belongs to the agglutinin subunits of 7S (Petruccelli and Anon, 1995), and its isoelectric point was at pH 6.0 (Bogracheva et al., 1996). Therefore, its intensity attenuated gradually as pH decreased from 6.0 to 5.0. These bands disappeared under non-reducing SDS-PAGE (Figure 4.2B), indicating that agglutinin was connected by the disulfide linkage (Petruccelli and Anon, 1995). A trace amount of the band at 18 kDa, corresponding to trypsin inhibitor of 7S, intensified as the pH decreased as well (Iwabuchi and Yamauchi, 1987). The percentages of 7S increased from 9% to 15%, whereas 11S accounted for about 70%, as pH decreased from 6.0 to 5.0 (Table 4.2), indicating a significant fractionation of 7S and 11S globulins in this pH range. A majority of 7S was precipitated at pH 4.8, accounting for about 30% of the total protein.

Moreover, trivial amounts of protein aggregates were observed on the top of the resolving gel, and the intensity decreased as the pH decreased. $NaHSO_3$ breaks the disulfide

linkage, releasing some acidic and basic polypeptides. The basic subunits were highly hydrophobic, which could facilitate the self-association and formation of protein aggregates (Lakemond et al., 2000). Furthermore, the IP of the basic subunits were reported to be at pH 8.0 to 8.5 or pH 4.5 to 8.0 from different soy flour species (Mo et al., 2006). Hence, we believe that those protein complexes were composed of basic polypeptides which interacted mainly through hydrophobic association, and the band intensity decreased as pH decreased.

Non-reducing SDS-PAGE profiles of glycinin-rich soy protein samples showed a major band at about 56 kDa, corresponding to the glycinin A-B complexes composed of the acidic and basic polypeptides linked by disulfide bonds (Figure 4.2B). The appearance of the faint bands at 38 kDa (acidic) and 23 kDa (basic) resulted from the reduction of disulfide bond-linked A-B complexes, suggesting that the reducing effects of $NaHSO_3$ on the soy protein were insignificant. The trace bands around 100 kDa could be the result of the disulfide bond formation in aggregates induced by freeze-drying or thiol-disulfide exchange during the modification process (Wolf, 1993). Some protein aggregates remained on the top of the resolving and staking gels. The major force involved in these aggregates could be the disulfide bond formation as evidenced by the near disappearance of these bands in the reducing electrophoresis.

***β-conglycinin-rich soy protein fractions:*** In the reducing SDS-PAGE, the subunits distribution of β-conglycinin exhibited significant differences among different pH fractions (Figure 4.2C). As shown in Table 4.3, the content of 7S and 11S globulins was reciprocal: 23.5% to 72% increase for 7S and 64.2% to 23.9% decrease for 11S, respectively.

Non-reducing electrophoresis profiles gave three types of protein aggregates linked by disulfide bonds (Figure 4.2D). The aggregates at approximately 100 kDa were formed by the glycinin subunits as mentioned in Figure 4.2B, and the bands faded as the glycinin content decreased. Another bands located at about 120 kDa were intensified as the β-conglycinin fraction increased in the soy protein samples. These bands could be formed by α and α' subunits connected by disulfide bonds, as suggested by Petruccelli and Anon (1995). The even higher molecular weight protein complex remained on the top of resolving and stacking gels. Individual polypeptides are known to be released from soy protein due to the reducing and unfolding effects of $NaHSO_3$. Some of those polypeptides might re-associate

into high molecular weight protein complexes through disulfide bond formation, as displayed in the gel.

### *4.4.3. Apparent viscosity*

The shear rate dependence of apparent viscosity of the soy protein samples are summarized in Figure 4.3. Apparent viscosity decreased as the shear rate increased in the range of 0.1 $s^{-1}$ to 100 $s^{-1}$, revealing the shear thinning behavior of the soy protein. Under the same shear condition, apparent viscosity of glycinin-rich soy protein fractions increased as the extraction pH decreased from 6.0 to 5.0; however, as the extraction pH reached 4.8, its viscosity decreased somewhat (Figure 4.3A). Simultaneously, the external morphology of the samples transformed from a diluted state to a very viscous clay state as the pH decreased from 6.0 to 5.0. Similar phenomena were observed from previous study (Liu et al., 2007). The protein samples formed a solid plug rather than a viscous liquid upon centrifugation in the low-pH level (pH 4.0 to 5.3), and their morphology images displayed the promoted protein aggregates with larger particle size. As the extraction pH approached the isoelectric point, strong protein-protein intermolecular forces were achieved, resulting in high-density, viscous protein aggregates.

The strong molecular forces could also be reflected by the particle size of protein precipitates as shown in Figure 4.4A. The acid precipitation of soy protein is generally thought to occur through the rapid formation of primary particles in the size range of 0.1 to 0.3 μm followed by aggregation of these particles via collision to aggregates 1 to 50 μm in size (Liu et al., 2007). The size of aggregates can be manipulated by pH, salt concentration, temperature, etc., which could control the intermolecular forces of protein molecules. Mean diameter of volume distribution (mv) of glycinin-rich soy protein fractions increased gradually, with pH decreases from 5.8 (1.56 μm) to 5.4 (6.74 μm), and a large extented protein agglomeration (58.8 μm) occurred at pH 5.0. In the case of pH 4.8 (40 μm), a larger amount of 7S of lower molecular weight was precipitated from the supernatant, which could reduce the particle size of the final product, and, to some extent, the apparent viscosity. The bimodal size distribution occurred at pH 5.0 and 4.8, which was also observed by Liu et al. (Liu et al., 2007). Furthermore, proteins at the isoelectric point exhibited minimum water hydration and swelling ability due to the zero net charge on the protein surface; therefore,

more water would be expelled out the protein structure, making the protein samples less cohesive (Gly pH5.2, pH5.0, pH4.8).

As to the β-conglycinin-rich soy protein fractions, sample Cong pH5.4 exhibited the highest apparent viscosity, while other samples possessed viscosity in a similar range (Figure 4.3B). However, only slight differences in particle size were observed (5.8μm to 5.0μm) among all the samples (Figure 4.4B). Interestingly, the ratio of 7S and 11S globulins in Cong pH5.4 was approximately 1 based on densitometry analysis, suggesting that specific and stronger intermolecular interactions might be induced at certain 7S/11S ratios. Additionally, when 7S component presented predominately in the protein fraction, the sample displayed viscous cohesive properties as well (Cong pH5.2, pH 5.0). In contrast, the glycinin-dominated soy protein system resulted in a non-continuous protein phase, which is reflected in the phase separation of water and protein for samples Cong pH6.0 and Cong pH5.8. This phenomenon also could be partially attributed to the fact that 11S had greater surface hydrophobicity than 7S, which could expel more water from the protein network (Riblett et al., 2001; Wool and Sun, 2005).

The soy protein structure was unfolded and the protein conformation was altered by $NaHSO_3$ modification through the ion pair shielding effect and reducing effects (Babajimopoulos et al., 1983). This re-associated protein conformation could be manipulated by pH (Glycinin-rich soy protein fractions) and protein composition (Conglycinin-rich soy protein fractions). We propose that the protein aggregates could be coalesced upon centrifugation to form a homogeneous and continuous protein phase, exhibited as viscous cohesive substances when the re-formed protein network had the ability to retain the proper amount of water. These high protein content phases were stable for up to several months without phase separation when stored at room temperature.

### *4.4.4. Solubility of soy protein in different reagents*

The effects of various reagents such as NaCl, NaSCN, Urea, Me-SH, and PG on the solubilities of protein samples could reflect the molecular forces contributing to the maintenance of the soy protein's viscous cohesive network. Protein samples Gly pH5.4 and Cong pH5.4 were dissolved in these reagents. The amount of protein solubilized from the sample was determined as shown in Table 4.4. Approximately 25% of these two proteins

dissolved into the citric acid-$Na_2HPO_4$ buffer. More protein was solubilized in the presence of 0.5 M NaCl or 0.5M NaSCN, indicating the salt in effect of the natural salts at low salt concentration. Urea increased the solubilities of Gly pH5.4 and Cong pH5.4 to 61% and 76%, respectively. Urea is known to destabilize the hydrophobic interactions and hydrogen bonding, which suggested that these forces are involved in maintaining the protein matrix. In the presence of Me-SH, about 35% of proteins were solubilized, indicating that the disulfide bond had limited effects in stabilizing the protein network. Propylene glycol could diminish the hydrophobic strength but enhance the hydrogen bonds and electrostatic interaction by lowering the dielectric constant (Utsumi and Kinsella, 1985). Thus, the lowest solubility of soy protein in PG indicated that increasing hydrogen bonding and electrostatic interactions could stabilize the protein network. Therefore, the electrostatic force, hydrogen bond, and hydrophobic interactions were involved in maintaining the protein's viscous cohesive network, whereas disulfide bonds did not play significant role.

### *4.4.5. Thermal properties*

The thermal denaturnation transition of proteins can be determined using differential scanning calorimetry by measuring the amount of energy absorbed or released from protein samples. This energy is usually detected as the endothermic peak in the DSC thermogram, in terms of the parameters of denaturnation temperature ($T_d$) and total denaturnation enthalpy ($\Delta H_d$). The typical DSC thermogram of glycinin-rich soy protein is shown in Figure 4.7A. The major peak at $T_d$ of 97 to 98 °C was designated as the glycinin globulin, and the tiny shoulder peak was the β-conglycinin at $T_d$ of 80 °C. Gly pH5.8 had the higher $T_d$ (98.84 °C) and $\Delta H_d$ (16.6 J/g) than other samples (Table 4.5). As pH decreased from 5.8 to 4.8, the denaturation temperature for both glycinin and β-conglycinin gradually shifted to a lower temperature, and the total $\Delta H_d$ decreased from 16.6J/g to 8.68 J/g. These $T_d$ results seem to conflict with the theory that protein at IP had the highest thermal stability. However, as showed in Figure 4.2A and 2B, high molecular weight protein aggregates were detected, and the intensity of those high molecular weight proteins decreased as the pH decreased from 6.0 to 5.0. Therefore, we believe these small amounts of protein aggregates play a vital role in the thermal stability of the samples. In addition, glycinin is well known to be much more thermal stable than β-conglycinin due to its existing disulfide linkage (Badley et al., 1975).

Although only a small amount of β-conglycinin was incorporated into soy protein extracted at lower pH, it could have decreased the unfolding enthalpy as pH decreased.

In the thermogram of β-conglycinin-rich soy protein (Figure 4.7B), $T_d$ of both glycinin and β-conglycinin increased slightly as the sample gradually become viscous cohesive phases, suggesting that more thermal stable states were formed (Table 4.5). Cong pH5.4 had the highest denaturing temperature, suggesting strong protein interactions that may have resulted from equal amounts of 7S and 11S components in the sample. The total $\Delta H_d$ of soy protein decreased by 3.3 J/g as fractionation pH decreased from 6.0 to 5.6. This might be the result of an increasing amount of less stable 7S globulin presented in the samples. While the $\Delta H_d$ of Cong pH5.4 improved suddenly to 8.07 J/g from 6.30 J/g in Cong pH5.6, and then it dropped around 0.6 J/g in samples Cong pH5.2 and Cong pH5.0, which agreed with the $T_d$ results.

### *4.4.6. Morphology properties*

As a salt, $NaHSO_3$ could enhance protein hydrophobic interaction through ion shielding effects, which would lead to favoring protein aggregation and cluster formation [Zhang and Sun, 2008]. However, as a reducing agent, $NaHSO_3$ disrupts the protein structure by breaking the hydrophobic and electrostatic interactions and hydrogen bonds. Consequently, individual polypeptides are formed. And the rearranged protein network and aggregates from those individual polypeptides would be depended on pH, ionic strength, and protein composition.

The microstructures of glycinin-rich and β-conglycinin-rich soy protein samples are displayed in Figure 4.6. Large numbers of spherical protein aggregates with similar diameters were observed in the Gly pH5.8 sample (Figure 4.6A). These aggregates connected with each other to form a uniform, continuous network. The thread-shaped protein peptides were enclosed inside the continuous network. As pH decreased to 5.4, the network was mainly composed of the thread-shaped polypeptides while the uniform chain-like network structures disappeared, and the spherical protein clusters with different diameters were observed (Figure 4.6B). When the separation pH decreased to 5.0, the continuous phase disappeared from the network structure and numerous individual spherical protein aggregates formed in similar size (Figure 4.6C). The continuous network in Gly pH5.8 might be able to hold the

proper amount of water inside the protein structure like pocket, which would be beneficial to the cohesive substance formation.

As to the β-conglycinin-rich protein, Cong pH5.4 was characterized by the chain-like networks composed of spherical protein clusters (Figure 4.6D), whereas Cong pH5.8 displayed disintegrated protein clusters as the number of short chains increased. This indicates much stronger protein-protein interactions in the Cong pH5.4 sample. Sun et al. (2008) also reported that the chain-like networks connected by protein aggregates would be formed when the electrostatic attraction force favors hydrophobic interaction in the cohesive soy protein samples with high solid protein content. Therefore, we assume that the chain-like protein aggregate structures favor the formation of viscous cohesive properties at high protein concentration, such as in the Cong pH5.4 sample.

### *4.4.7. Shear adhesion strength*

Several factors could affect the practicality of soy protein adhesives for industrial applications, including protein yield, solid protein content, and flowability. Six $NaHSO_3$-modified soy protein samples with viscous cohesive properties were chosen to study their tensile strength. Adhesion performance was characterized in terms of dry and wet shear adhesion strength (Table 4.6). All soy protein samples exhibited excellent dry strength with 100% wood cohesive failure, except the Gly pH5.8 sample (4.54 MPa). Similar trend was observed for the wet strength that viscous cohesive samples exhibited good water resistance with strength in the range of 1.9 to 2.8 MPa, but 1.2 MPa for Cong pH5.8, compared to 1.6 MPa for the control soy protein isolate adhesive under the same curing conditions. Results also showed that the dry and wet adhesion performance of β-conglycinin-rich soy protein was much better than glycinin-rich soy protein adhesives.

Many factors are involved in affecting the strength of protein adhesives: pH, composition and structure of the protein, solid protein contents, flowability, and the interactions of hydrophobic and hydrophilic groups with the wood surface. Soy protein adhesives prepared at or close to their isoelectric point (IP = 4.5) were proven to have the highest shear strength due to the strong protein-protein interactions during the curing process (Mo et al., 2006; Wang et al., 2009). These interactions might contribute to better adhesion performance of the β-conglycinin-rich soy protein (pH 4.8) than that of the glycinin-rich soy

fractions. And for the Gly pH5.8 sample, which is far away from its IP, the electrostatic repulsions between protein molecules occurred due to the redundant surface charges, which might, in turn, enhance protein-water interactions instead of protein-protein and protein-wood interactions, leading to the reduced adhesion strength. In addition, the β-conglycinin-rich fractions were 5% higher in protein concentration than that of glycinin-rich fractions (Table 4.1). This could also contribute to higher adhesion strength in the β-conglycinin-rich samples.

Furthermore, one of the hurdles for soy protein adhesives application is their high viscosity; hence, the solid content commonly used in the lab and industrial field is in the range of 10 to 15%. In our study, the solid content of viscous cohesive soy proteins was 33%, about 15% higher than that used in normal industrial applications. High protein concentration could favor the protein cross-link, entanglement, and protein-wood surface interactions in the curing process, leading to the reduced protein-water interactions and improved water resistance. The most important factor affecting adhesive strength is the chemical modification of soy protein, as discussed previously. $NaHSO_3$ could bring the hydrophobic amino groups outside of the protein molecules due to the breakage of inter-disulfide bonds, leading to improved surface hydrophobicity of the soy protein, which is beneficial for adhesion strength improvement, especially for water resistance.

## 4.5. CONCLUSIONS

A viscous cohesive soy protein system with good flow-ability could be produced by adjusting extraction pHs and sodium bisulfite modifications during the acid precipitation process. The viscous cohesive samples were stable for up to several months without phase separation when stored at room temperature. The wet adhesion strength of the viscous cohesive soy protein adhesives could reach to 2.8 MPa. Based on the morphology, viscosity, and particle size of the precipitates, we propose that proper protein-protein interaction, hydration capacity (glycinin-rich soy protein fractions), and certain ratios of 7S and 11S (β-conglycinin rich protein fractions) in protein systems are crucial to the continuous protein phase formation. Hydrogen bonding, electrostatic force, and hydrophobic interactions are involved in maintaining the protein latex network; however, the effect of disulfide bonds is insignificant.

**Figure 4.1. Procedures of $NaHSO_3$ modified soy protein adhesives extraction from soy flour.**

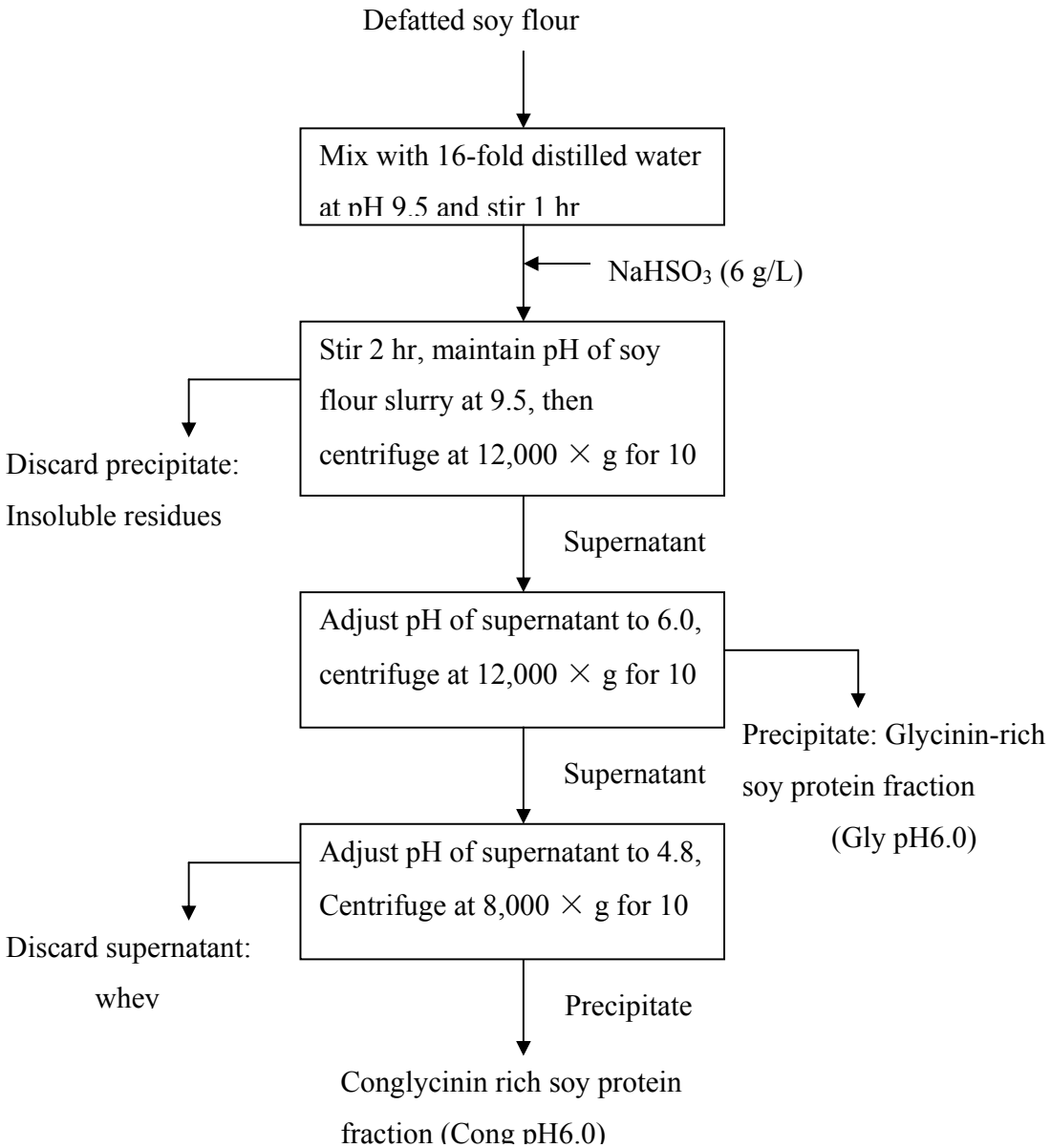
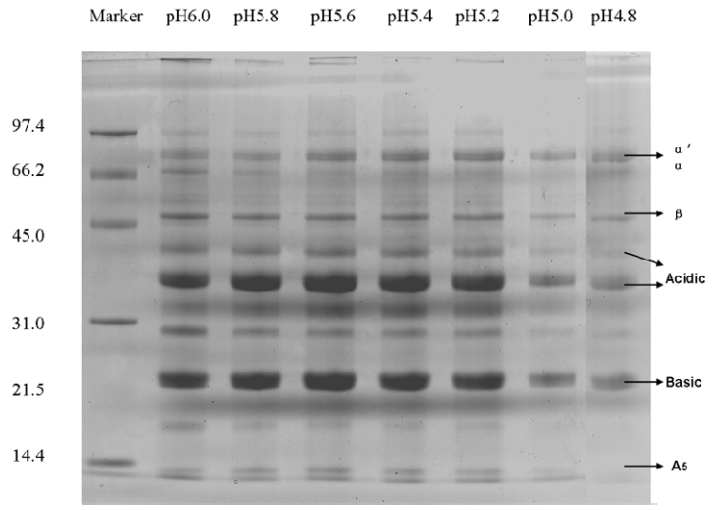
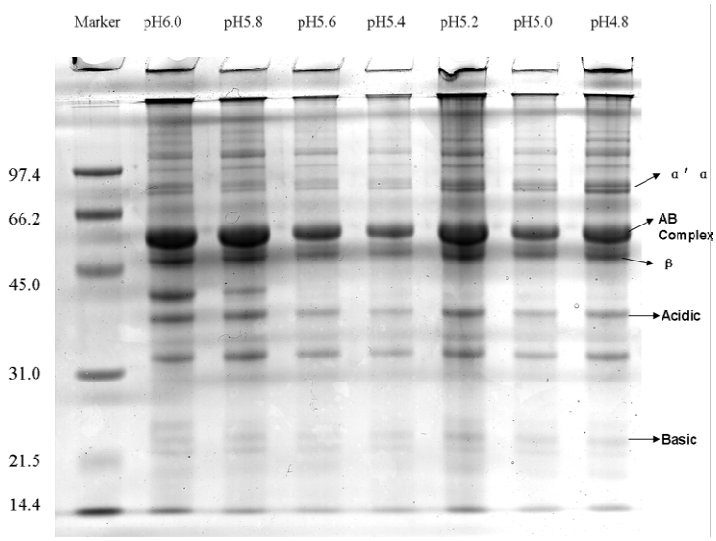
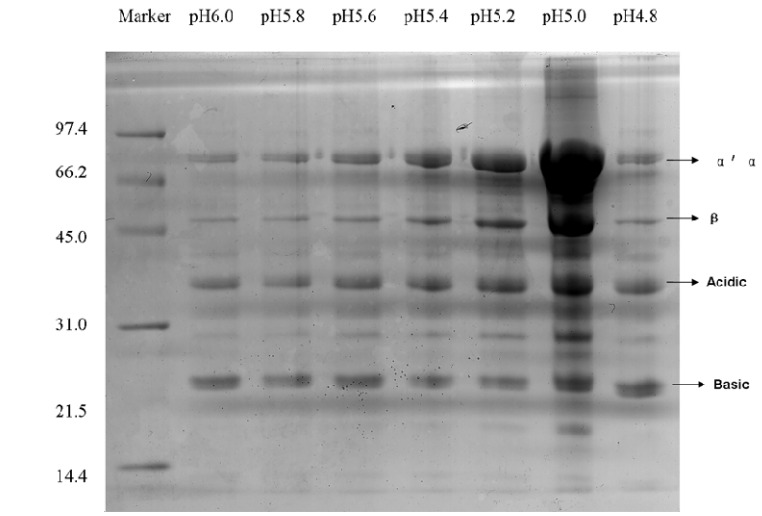
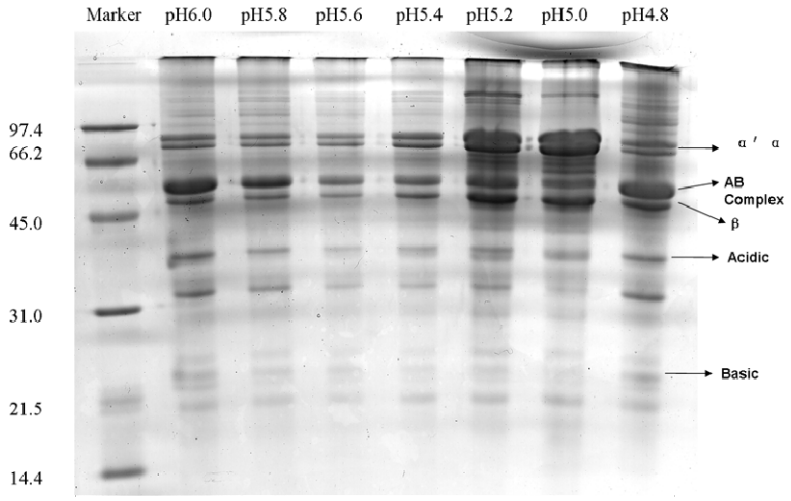


**Figure 4.2. Reducing and non-reducing SDS-PAGE patterns of $NaHSO_3$-modified soy protein adhesives. glycinin-rich fractions under reducing conditions (A); glycinin-rich fractions under non-reducing conditions (B); cong-rich fractions under reducing conditions (C); cong-rich fractions under non-reducing condition (D).**




Marker
pH6.0
pH5.8
pH5.6
pH5.4
pH5.2
pH5.0
pH4.8
97.4
66.2
45.0
31.0
21.5
14.4
α ′ α
β
Acidic
Basic


Marker
pH6.0
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pH5.6
pH5.4
pH5.2
pH5.0
pH4.8
97.4
66.2
45.0
31.0
21.5
14.4
α ′ α
AB
Complex
β
Acidic
Basic

**Figure 4.3. Shear rate dependence of apparent viscosity of $NaHSO_3$-modified soy protein adhesives: glycinin-rich soy protein fractions (A) and β-conglycinin-rich soy protein fractions (B).**

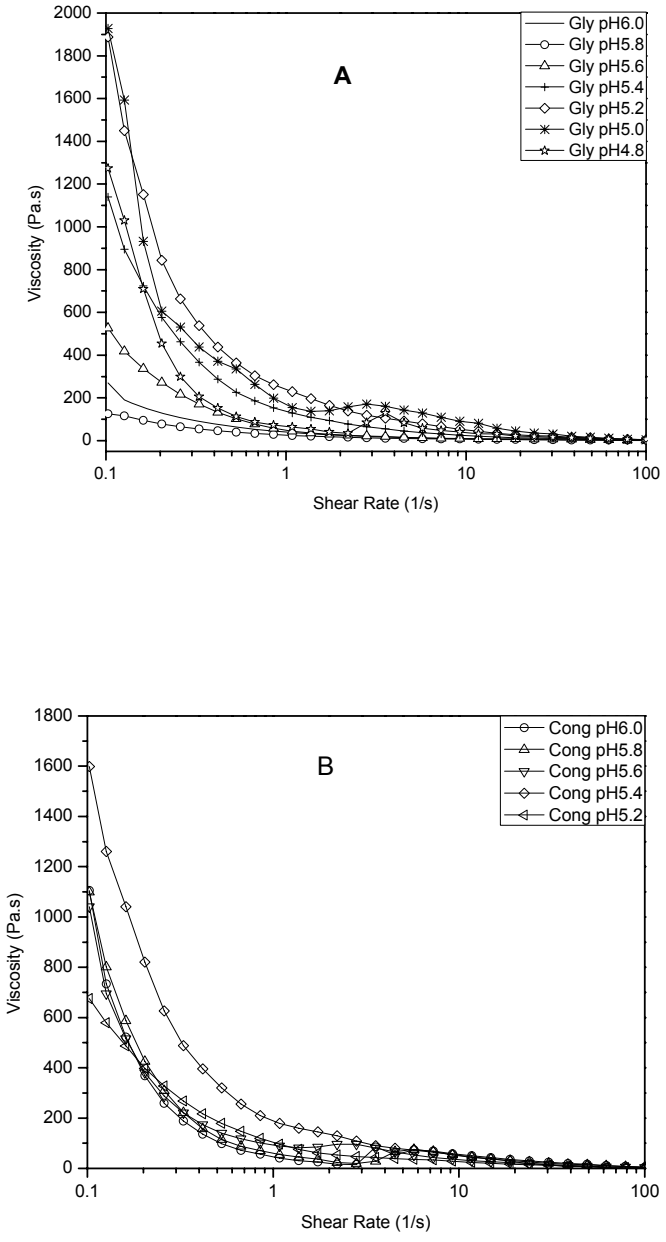
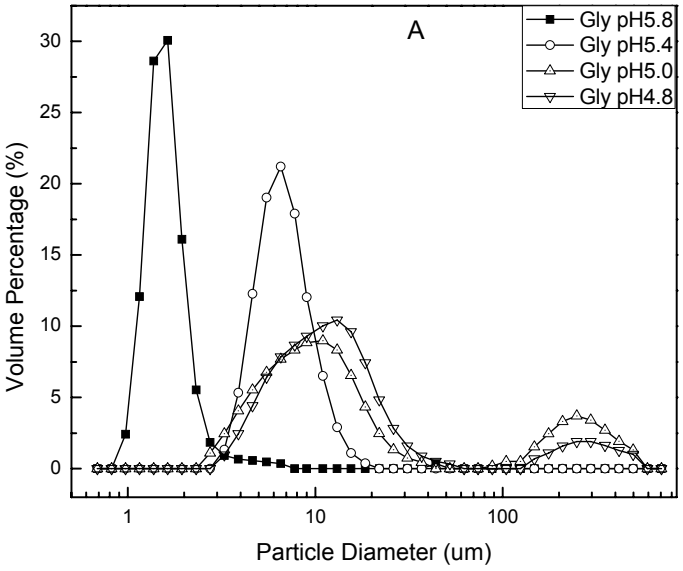


**Figure 4.4. Particles size distribution of the soy protein precipitates: glycinin-rich soy protein fractions (A) and β-conglycinin-rich soy protein fractions (B).**



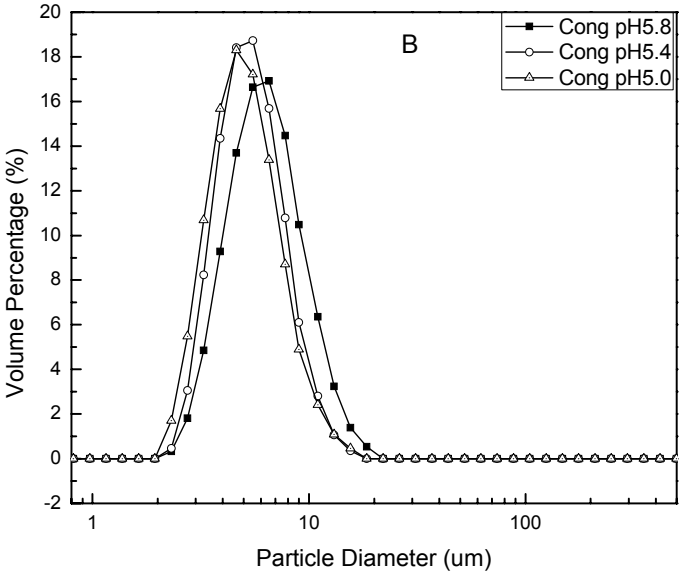



**Figure 4.5. DSC thermogram of $NaHSO_3$-modified soy protein adhesives: glycinin-rich soy protein fractions (A) and β-conglycinin-rich soy protein fractions (B).**

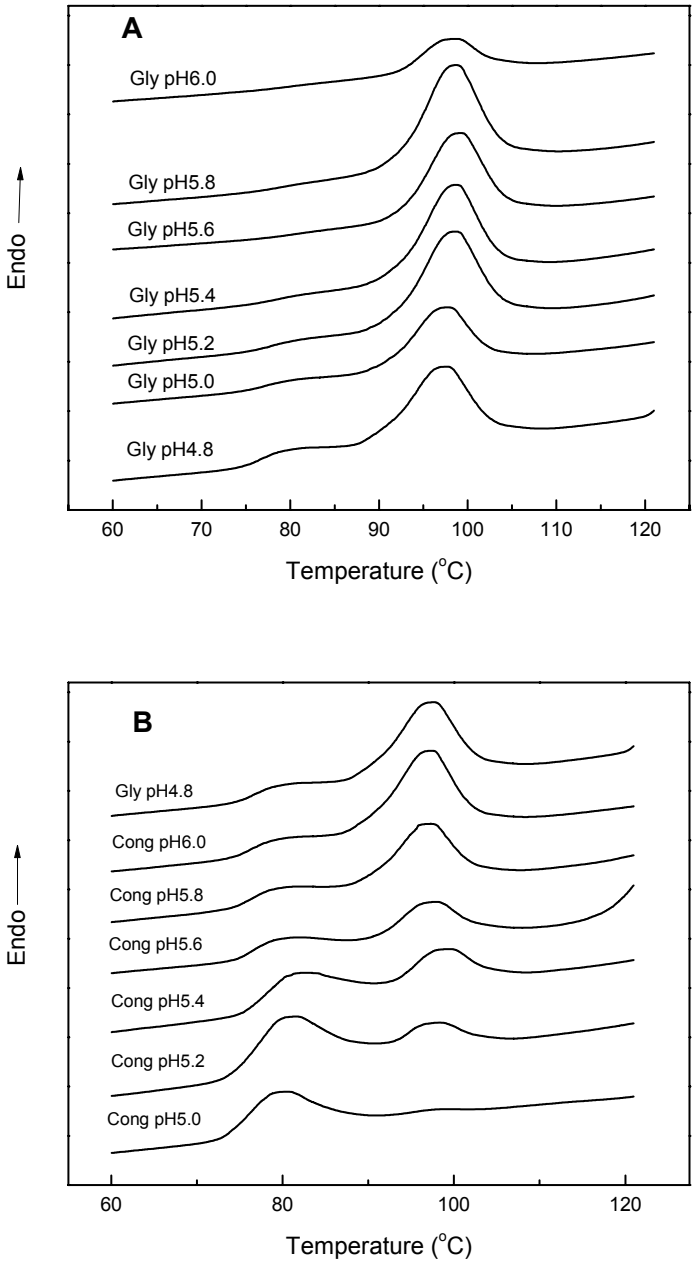
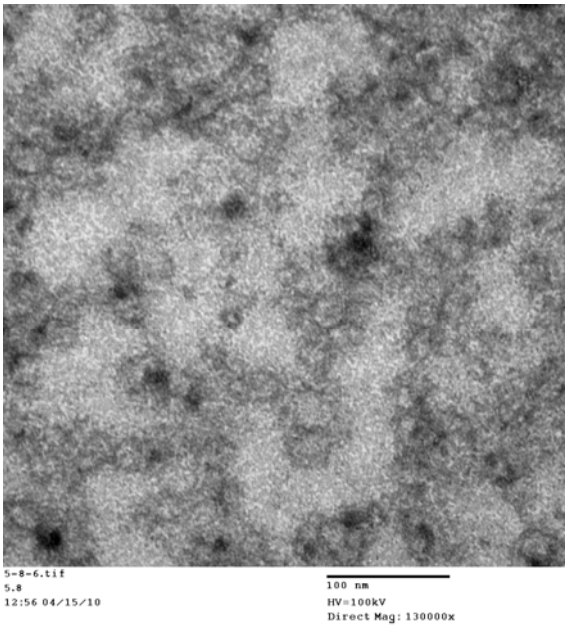
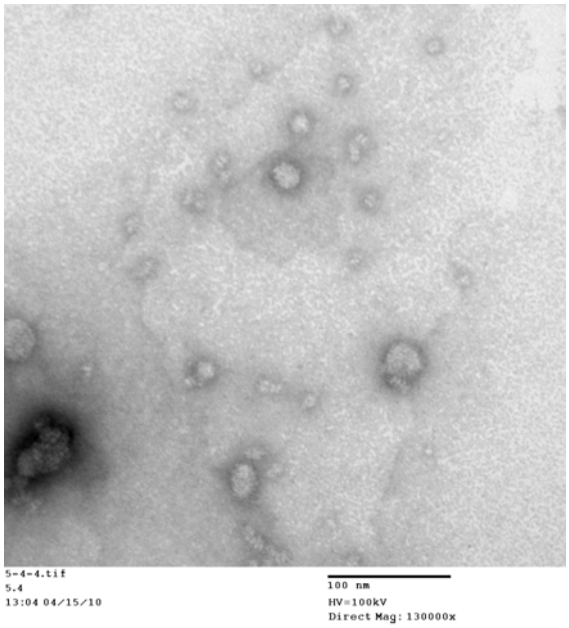


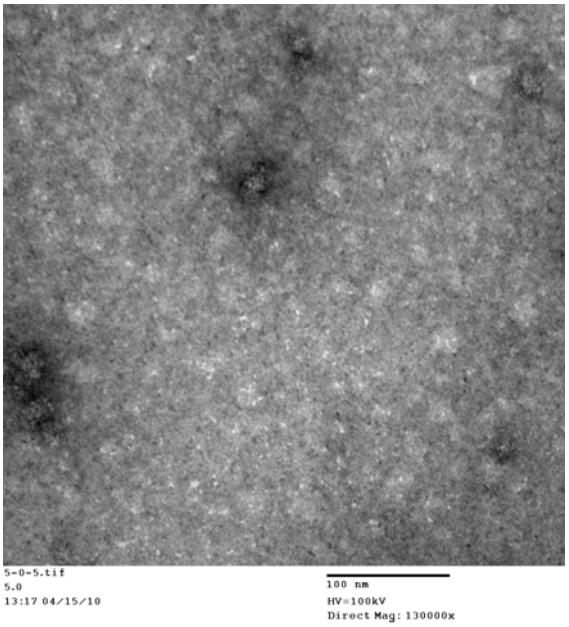
**Figure 4.6. TEM images of $NaHSO_3$ modified soy protein adhesives; glycinin-rich soy protein fractions. A: Gly pH5.8; B: Gly pH5.4; C: Gly pH5.0; and β-conglycinin-rich soy protein fractions. D: Cong pH5.8; E: Cong pH5.4.**



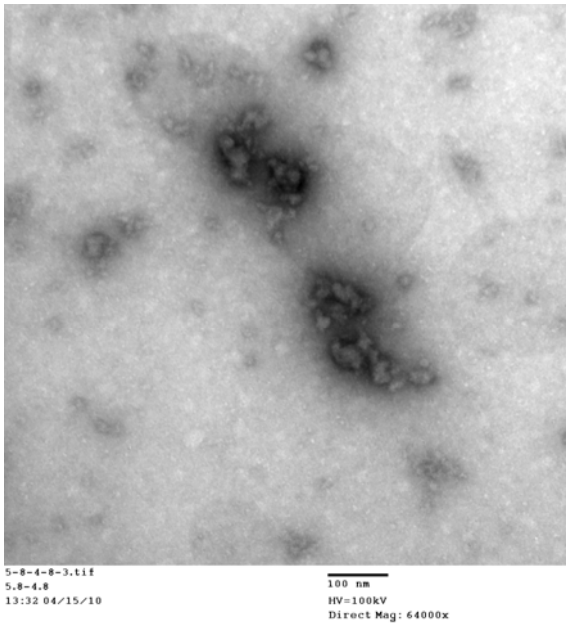

A



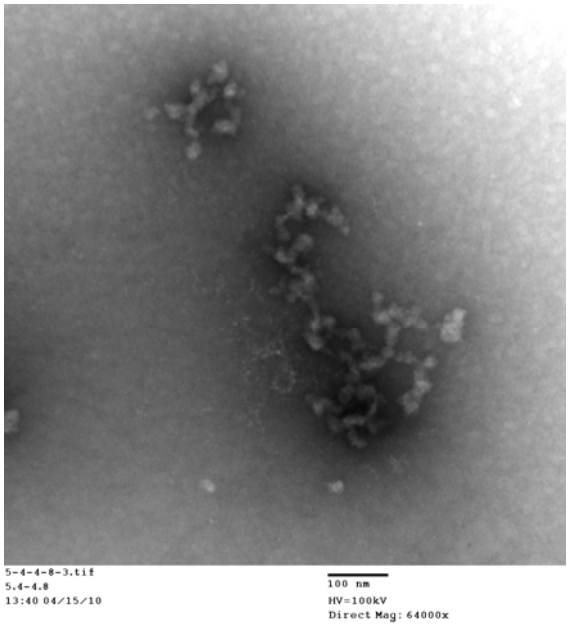

B




C




D




E

**Table 4.1. $NaHSO_3$-modified soy protein adhesive product yield, water content and external morphology.**

| Number | Soy products | Yields | Solid content | External morphology |
|---|---|---|---|---|
| Glycinin-rich soy protein adhesives | | | | |
| 1 | Gly pH6.0 | 9.1% | 22% | Diluted, no ahesion |
| 2 | Gly pH5.8 | 23.0% | 28% | Viscous cohesive |
| 3 | Gly pH5.6 | 43.8% | 27% | Viscous cohesive |
| 4 | Gly pH5.4 | 62.5% | 29% | Somewhat clay |
| 5 | Gly pH5.2 | 67.7% | 31% | Clay-state |
| 6 | Gly pH5.0 | 69.3% | 33% | Clay-state |
| 7 | Gly pH4.8 | 70.0% | 34% | Clay-state |
| β-conglycinin-rich soy protein adhesives | | | | |
| 8 | Cong pH6.0 | 64% | 34% | Clay-state |
| 9 | Cong pH5.8 | 56.9% | 33% | Clay-state |
| 10 | Cong pH5.6 | 39.2% | 32% | Somewhat clay |
| 11 | Cong pH5.4 | 14.9% | 33% | Viscous cohesive |
| 12 | Cong pH5.2 | 6.54% | 33% | Viscous cohesive |
| 13 | Cong pH5.0 | 1.7% | 34% | Viscous cohesive |

**Table 4.2. Estimated content of polypeptides of $NaHSO_3$-modified soy protein adheisves: glycinin-rich soy protein fractions.**

| Protein subunits | Soy protein fraction distribution (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | pH 6.0 | pH 5.8 | pH 5.6 | pH 5.4 | pH 5.2 | pH 5.0 | pH 4.8 |
| 7S ( α+ α′+ β) | 8.9% | 8.7% | 9.6% | 12.2% | 12.7% | 15.3% | 30.2% |
| 11S (acidic+basic) | 71.2% | 75.8% | 69.7% | 70.9% | 73.3% | 67.0% | 57.3% |

**Table 4.3. Estimated content of polypeptides of $NaHSO_3$-modified soy protein adhesives: β-conglycinin-rich soy protein fractions.**

| Protein subunits | Soy protein fraction distribution (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | pH6.0 | pH5.8 | pH5.6 | pH5.4 | pH5.2 | pH5.0 | pH4.8 |
| 7S ( α+ α′+ β) | 23.5% | 36.1% | 32.4% | 46.9% | 47.9% | 71.7% | 30.2% |
| 11s (acidic+basic) | 64.2% | 49.5% | 54.0% | 43.9% | 31.3% | 23.9% | 57.3% |

**Table 4.4. Solubilities of $NaHSO_3$-modified soy protein adhesives in various reagents.**

| Reagent | Gly pH5.4 | Cong pH5.4 |
|---|---|---|
| Buffer | 25.88[d1] | 22.10[d] |
| NaCl | 46.62[b] | 60.62[b] |
| NaSCN | 48.31[b] | 48.54[b] |
| Urea | 61.96[a] | 76.58[a] |
| Me-SH | 37.82[c] | 34.64[c] |
| PG | 16.48[e] | 21.03[d] |

[1]Means in the same column followed by different letters are significantly different at $p<0.05$.

**Table 4.5. Denaturation temperature ($T_d$) and total enthalpy of denaturation ($\Delta H_d$) of $NaHSO_3$-modified soy protein adhesives: glycinin-rich soy protein fractions and β-conglycinin-rich soy protein fractions.**

| Adhesive pH | $T_d$ (°C) | | Total ΔH |
|---|---|---|---|
| | 7S | 11S | (J/g) |
| Gly pH6.0 | -- | 97.90[c] | 6.842[hi] |
| Gly pH5.8 | -- | 98.84[a] | 16.600[a] |
| Gly pH5.6 | -- | 98.40[b] | 12.228[b] |
| Gly pH5.4 | 80.71[b1] | 98.15[bc] | 10.981[c] |
| Gly pH5.2 | 80.55[b] | 97.94[c] | 10.581[c] |
| Gly pH5.0 | 80.09[b] | 97.18[de] | 8.997[e] |
| Gly pH4.8 | 79.40[c] | 96.92[ef] | 8.681[e] |
| Cong pH6.0 | 79.30[c] | 96.92[ef] | 9.913[d] |
| Cong pH5.8 | 79.26[c] | 96.80[f] | 7.776[fg] |
| Cong pH5.6 | 79.39[c] | 97.36[d] | 6.303i |
| Cong pH5.4 | 81.24[a] | 98.86[a] | 8.070[f] |
| Cong pH5.2 | 80.38[b] | 98.37[b] | 7.368[gh] |
| Cong pH5.0 | 80.68[b] | 98.88[a] | 7.377[gh] |

[1]Means in the same column followed by different letters are significantly different at $p<0.05$.

**Table 4.6. Dry and wet shear adhesion strength of $NaHSO_3$-modified soy protein adhesives with viscous cohesive properties.**

| Adhesive fraction | Dry strength (MPa) | Wood failure (%) | Wet strength (MPa) | Wood failure (%) |
|---|---|---|---|---|
| Gly pH5.8 | 4.542±0.737 | 0% | 1.224±0.481 | 0% |
| Gly pH5.6 | 6.180±0.355 | 100% | 1.810±0.418 | 0% |
| Gly pH5.4 | 6.212±0.693 | 100% | 1.926±0.299 | 0% |
| Cong pH5.6 | 6.823±0.349 | 100% | 2.845±0.381 | 0% |
| Cong pH5.4 | 6.666±0.627 | 100% | 2.536±0.346 | 0% |
| Cong pH5.2 | 6.530±0.761 | 100% | 2.364±0.312 | 0% |

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# Chapter 5 - SOY PROTEIN ADHESIVE BLENDS WITH SYNTHETIC LATEX ON WOOD VENEER[2]

## 5.1. ABSTRACT

Environmental pollution has prompted an interest in and a need for bio-based wood adhesives. Modified soy protein (MSP) has shown adhesion properties similar to those of formaldehyde based adhesives. The objective of this research was to investigate the compatibility of a modified soy protein (MSP) with six commercial synthetic latex adhesives (SLAs). Four different blending ratios of MSP and SLAs were studied. Adhesion; structural change; and rheological, thermal, and morphological properties of the MSP/SLAs blends were characterized. Dry adhesion strength of MSP, SLAs and their blends were all similar with 100% wood cohesive failure. Water resistance of all six SLAs was improved by blending with MSP in terms of the wet adhesion strength. The wet adhesion strength of MSP/PBG (40/60) blends was 6.416 MPa, as compared to 4.66 MPa of pure PBG (Press Bond Glue, Urea formaldehyde based resin). Viscosity of MSP/SLAs blends was reduced significantly and reached the lowest value at 40% to 60% MSP. Infrared spectra, thermal properties, and morphological images indicated that chemical reactions occurred between soy protein and PBG molecules. The MSP provided some functional groups, such as carboxylic (-COOH), hydroxyl (-OH) and amino groups ($-NH_2$), that cross-linked with hydroxymethyl groups ($-CH_2$-OH) of PBG, and also acted as an acidic catalyst for the self-polymerization of urea formaldehyde based resin.

## 5.2. INTRODUCTION

Most of the currently available commercial adhesives for wood composites are petroleum based; these include thermosetting resins such as phenol-formaldehyde (PF), urea-formaldehyde (UF), and melamine-formaldehyde (MF) and isocyanates. Polyvinyl acetate

[2] Results have been published. Qi, G., Sun, X. S. 2011. Soy protein adhesive blends with synthetic latex on wood veneer. J. Am. Oil Chem. Soc. 88: 271-281. (Reuse by permission of Springer).

adhesive (PVA) is a thermoplastic resin used mainly in furniture assembling (Seller, 2005). A few bio based adhesives such as soybean adhesive, phenolic-containing lignins and tannins adhesives, animal glue, casein-based adhesive, and blood based adhesive are also currently used as wood adhesives. Bio based adhesives are inferior to petroleum based adhesives in terms of water resistance and cost. However, in recent years, limited petroleum resources and the pollution caused by formaldehyde-based adhesives have spurred many efforts to develop bio-based adhesives with good adhesion properties that can compete with synthetic petroleum based adhesives.

Soy-protein-based adhesives have shown great potential as bio-based adhesives since the 1930s; they are abundant, inexpensive, renewable, and easy to handle. Efforts to improve adhesion properties of these adhesives have included using denaturants, reducing agents, and cross-linking agents as well as enzyme hydrolysis (Hettiarachchy et al., 1995; Kalapathy et al., 1995; Kalapathy et al., 1996; Huang and Sun, 2000a; Huang and Sun, 2000b; Li et al., 2004). However, the low water resistance of soy protein based adhesives still limits their extensive application.

Blending soy protein with synthetic resins is a short-term solution for reducing dependence on petrochemicals and lowering emissions of volatile organic compounds. Blends of soy based protein with blood, casein, polyvinyl alcohol, polyvinyl acetate resin, or PF showed improved water resistance for wood applications (Kumar et al., 2002). Steele et al (1998) developed blends of soy protein and phenolic resins used for finger jointing of green lumber that cured rapidly at room temperature and had excellent water resistance and reduced formaldehyde emissions. As much as 70% of PF can be replaced by soy protein based adhesive with comparable physical properties for oriented and random strandboard (Hse et al., 2001; Wescott and Frihart, 2004). In a study by Zhong and Sun (2007), blends adhesives (soy protein isolate/PF= 100/20) had the same level of adhesion strength as commercial PF adhesive alone, reduced formaldehyde usage, and had economic advantages. Polyamide-epichorohydrin (PAE) is an excellent curing agent for soy protein; Zhong and Sun (2007) also showed that the complexation interaction for PAE-soy protein blends greatly enhanced their adhesion properties.

To promote reaction with synthetic polymers, soy protein must be unfolded to expose its functional groups. Various hydrolysis methods have been used to unfold soy proteins

(Yang et al., 2006; Frihart and Wescoott, 2004), but these methods have not been able to overcome the hurdles of high viscosity, low concentration of soy protein, low water resistance, and short pot life. Sun et al (2008) previously showed that a partially unfolded soy protein with 38% solids content had dry and wet adhesion strength comparable to formaldehyde-based adhesives. We hypothesized that unfolded soy protein may act as a copolymer, reacting with various synthetic resins to enhance adhesion performance and reduce emissions of volatile organic compounds. Therefore, the objectives of this study were to investigate the compatibility of modified soy protein (MSP) with commercial synthetic latex adhesives (SLAs) and characterize the adhesion; structural change; and rheological, thermal, and morphological properties of MSP/SLAs blends.

## 5.3. MATERIALS AND METHODS

### *5.3.1. Materials*

Defatted soy flour (Cargill, Cedar Rapids, IA) was used as the starting material. The soy flour contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium bisulfite ($NaHSO_3$) was obtained from Fisher Scientific (Fair Lawn, NJ). Cherry wood veneers with dimensions of 50 × 127 × 3 mm (width × length × thickness) were provided by Veneer One (Oceanside, NY). Six different commercial SLA were used. Press Bond Glue (PBG, UF based resin) and Veneer Glue (VG, Dichloromethane and toluene based resin) were purchased from Constantine's Wood Center (Fort Lauderdale, FL). Heat Lock Iron Veneer Glue (HLIG, PVA based resin) and Cold Press Veneer Glue (CPG) were purchased from Highland Woodworking Company (Atlanta, GA). Cold Press Light Veneer Glue (CPLG) and Flex-Pro Veneer Adhesive (FPV) were purchased from Veneer Supplies Company (Louisville, KY). The detailed MSDS sheet of each product is available online from the website of each company. For example, PBG is described online as "easy to use, very water resistant, does not creep minimize bleed-through on veneers. Suggested hot pressing pressure is 100-200psi, pressing temperature is 250 ℉, and pressing time is 1 to 3.5 min."

### *5.3.2. Adhesive preparation*

The MSP was prepared according to the method described by Sun et al [Sun et al., 2008]. Soy flour was dispersed in water at 6.25% solids content at pH 9.5, $NaHSO_3$ was added to the slurry at 6 g/L on the basis of water volume, and the slurry was stirred for 2 h at room temperature. The pH of the slurry was adjusted to 5.4 with 2N HCl to remove carbohydrates and some glycinin proteins by centrifuging at 12,000 × *g*. The pH of the supernatant was then adjusted to 4.8 with 2N HCl, and the slurry was centrifuged at 8,000 × *g* to obtain MSP with a solids content of 38%. MSP and SLAs were mixed manually until it become uniform; the ratios of MSP/PBG blends were: 20/80, 40/60, 60/40, and 80/20 (total weight basis). Following initial analyses, the MSP/PBG (40/60) blends was prepared for further analysis and characterized in detail.

### *5.3.4. Apparent viscosity*

Apparent viscosity measurements of fresh MSP/SLAs blends were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) with a CP 4/40 cone and plate fixture (4 cone angle, 40mm cone diameter). The distance between cone and plate was set as 150 μm for all measurements. Experiments were conducted under steady shear flow at 23 °C. Shear rate was set at 50 $s^{-1}$.

### *5.3.5. Spread rates*

Cherry wood was oven dried at 130 ℃ for 1 h, weighed, and then conditioned at 23 ℃ and 50% relative humidity for 2 d. The conditioned cherry veneer was coated with fresh MSP/SLAs (40/60) blends on one side uniformly, and was allowed to rest at room temperature for 10 min. The coated cherry veneer was then oven dried at 130 ℃ for 1 h and weighed. Spread rate of the adhesive was calculated as the dry adhesive weight per square meter of single glue line. Reported values are the average of three replications.

### *5.3.6. Wood specimen preparation*

Cherry wood veneers were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA) for 7 d at 23 ℃ and 50% relative humidity. The MSP/SLAs adhesives were brushed onto one end of a piece of cherry wood with dimensions of 127 × 20 mm (length × width) until the entire area was completely wet. The amount of adhesive on each piece was

controlled with a balance. Two brushed wood pieces were left at room conditions for 5 min then assembled and pressed with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN) at 2 MPa and 170 ℃ for 10 min (lab procedure). In the preliminary test, we compared the adhesion strength of each SLAs under its suggested bonding procedure with the one under the soy protein adhesive curing procedure in our lab, and the data showed that latter condition exhibited a much better adhesion performance (results were not shown). Therefore, in order to coincide with the curing condition, the lab curing procedure was chosen in this study.

### *5.3.7. Shear adhesion strength*

The wood assemblies glued with MSP/SLAs blends were cooled, conditioned at 23 ℃ and 50% relative humidity for 2 d, and cut into 5 pieces with dimensions of 80 × 20 mm (glued area of 20 × 20 mm). The cut wood specimens were conditioned for another 4 d before measurements were taken. Wood specimens were tested with an Instron Tester (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 at a crosshead speed of 1.6 mm/min. Shear adhesion strength at maximum load was recorded; reported values are the average of five specimen measurements.

### *5.3.8. Water resistance*

Water resistance of the wood assemblies was measured following ASTM Standard Methods D1183-96 and D1151-00. The preconditioned specimens were soaked in tap water at 23 ℃ for 48 h, and wet strength was tested immediately after soaking.

### *5.3.9. Infrared spectroscopy*

On the basis of the shear adhesion results, the MSP/PBG (40/60) blends was selected for further characterization. The MSP/HLIG (40/60) blends was used for comparison purposes as needed.

Fourier transform infrared (FTIR) spectroscopic data were collected in the region of 800 to 4000 $cm^{-1}$ with a PerkinElmer Spotlight 300 spectrometer (Shelton, CT). Fresh adhesive blends were dried for 24 h in a vacuum oven at 50 ℃ and then ground into powder. A very thin layer of adhesive samples (i.e., easy for light to penetrate) was put on low-e

Microscope slides for the test. The transmission spectra of 64 scans of each disk were collected at a resolution of 2 $cm^{-1}$.

### *5.3.10. Transmission electron microscopy*

A Philips CM 100 (FEI Company, Hillsboro, OR) TEM was used to observe the microstructure of selected blends at the 40/60 ratio. Fresh adhesive blends were diluted to 1% with deionized water for imaging and sonicated for 5 min in an L&R320 ultrasonic stirrer (L&R Manufacturing Company, Keary, NJ). Diluted samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, PA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT) for 60 s at room temperature.

### *5.3.11. Dynamic viscoelastic measurement*

Dynamic oscillatory shear testing of the fresh MSP/PBG blends was performed with a Bohlin CVOR 150 rheometer to characterize the gelling process. A parallel plate head with an 8 mm plate diameter and 500 μm gap was used. The measurements were performed at 50 ℃ with a small strain (5%) at frequency value of 1 Hz. The elastic modulus (G') and viscous modulus (G") were continuously registered.

### *5.3.12. Scanning electron microscopy*

A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of cured MSP/PBG blends. The cured MSP/PBG blends grounded powder were affixed to an aluminum stub with two-sided adhesive tape, and coated with an alloy of 60% gold and 40% palladium with a sputter coater (Desk II Sputter/Etch Unit, Moorestown, NJ). The SEM images of the blends were performed with operation conditions at an accelerating voltage of 5 kV.

### *5.3.13. Differential scanning calorimetry*

Thermal denaturation properties of the MSP/PBG blends were studied using a DSC (DSC7, Perkin-Elmer, Norwalk, CT) calibrated with indium and zinc. Fresh MSP/PBG blends (5 mg) were hermetically sealed in the large-volume stainless steel pan. All samples

were held at 20 ℃for 1 min and then scanned from 20 ℃ to 180 ℃ at a heating rate of 10 ℃/min. Peak temperatures and denaturation enthalpies were calculated from thermograms.

### *5.3.14. Thermogravimetric analysis*

Thermal degradation patterns of the cured MSP/PBG blends were studied using a TGA (TGA 7, Perkin-Elmer, Norwalk, CT) in a nitrogen atmosphere. Fresh MSP/PBG blends were vacuum dried at 50 ℃ for 24 h and ground. Then 15 mg of the ground powder was weighed into a platinum cup and scanned from 20 ℃ to 700 ℃ at a heating rate of 10 ℃/min. Maximum degradation rate was calculated as mass (%) at peak temperature divided by peak temperature.

## 5.4. RESULTS AND DISCUSSION

### *5.4.1. Apparent viscosity*

Viscosity is an important physical property that governs the adhesive behavior of wood adhesives. Low viscosity allows easy handling and good flowability on the wood surface. The apparent viscosity of HLIG and FPV decreased significantly when blended with 20% to 60% MSP, whereas adhesive blends with 80% MSP had apparent viscosity similar to that of pure MSP (Figure 5.1). The other three synthetic adhesives (CPLG, CPG, and VG) exhibited similar trends (results not shown). For PBG (Figure 5.1), apparent viscosity was reduced extensively in the range of 20% to 40% MSP (4.5 Pa.s and 3.1 Pa.s, respectively, compared with 12.7 Pa.s for pure PBG). However, viscosity of the PBG/MSP blends started to increase dramatically at 60% MSP and increased to 14.53 Pa.s at 80% MSP. For all MSP/SLAs blends, viscosity was lowest at a MSP/SLAs ratio of 40/60. In addition, all MSP/PBG blends gradually became a gel and finally a complete solid. The gelling process was from 3 to 48 h depending on MSP concentration; the higher the MSP concentration, the faster the gelling process.

### *5.4.2. Spread rates*

Spread rate is a characterization of the amount of adhesive usage per unit area. The spread rates of SLAs, MSP, and MSP/SLAs (40/60) blends are shown in Figure 5.2. Except for PBG, there were no obvious differences in spread rates between MSP/SLAs blends and

pure SLAs. As mentioned in section 3.1, the MSP/PBG (40/60) blends had the lowest apparent viscosity compared with pure PBG which led to good flow ability and easy spread ability, and subsequently having the largest spread rate reduction of all blends.

### *5.4.3. Shear adhesion strength*

Dry shear adhesion strength of the MSP, SLAs (except for VG), and their blends was similar; all exhibited 100% wood cohesive failure (Table 5.1). The dry shear adhesion strength of VG was low but significantly increased when VG was blended with MSP. The blends had 100% wood cohesive failure at 60% to 80% MSP.

Wet shear adhesion strength of all SLAs except PBG was low (0.7 to 1.8 MPa; Table 3.2) but improved to different levels when the SLAs were blended with MSP and increased as the percentage of MSP increased. For example, wet shear adhesion strength was 3.29 MPa for MSP/VG (60/40) and 1.8 MPa for pure VG. It was assumed that VG is a viscoelastic polymer with good cohesiveness properties and water resistance; while blending with MSP, VG could entrap the soy protein molecules more efficiently than others, thus leading to an obvious improved adhesion strength (no chemical reaction occurred between them, preliminary data not shown). The PBG was more hydrophobic, with wet shear adhesion strength of 4.66 MPa. Wet shear adhesion strength of PBG progressively improved from 20% to 40% MSP, reaching 6.42 MPa with 100% wood cohesive failure at 40% MSP, and then decreased at 60% and 80% MSP, Similar to other SLAs, PBG exhibited a significant reduction in apparent viscosity in the range of 20% to 40% MSP. As discussed in section 3.6, blends of PBG with 60% and 80% MSP had high viscosity because of the fast curing process which led to a poor diffusion ability and lower adhesion strength. Besides the physical synergistic effects, the interaction between soy protein and selected synthetic adhesives was further characterized in the following sections.

### *5.4.4. FTIR spectra*

On the basis of the initial analysis, PBG and HLIG were selected for further study of chemical interactions between SLA and MSP. Two typical characteristic absorption bands of protein (amide I and II) normally appear at about 1653 and 1567 $cm^{-1}$, respectively (Stuart, 1997), which are related to C=O stretching and N-H bending. As shown in Figure 5.3, amide I and II bands appeared at 1667 and 1543 $cm^{-1}$ in MSP. The bands related to $COO^-$, amide III

(-N-H in plane and C-N stretch), and –C-$NH_2$ bending were located at 1398, 1234, and 1079 $cm^{-1}$, respectively.

Because PBG is a type of amino resin, the main characteristic absorption bands that appeared in PBG at 1668 and 1534 $cm^{-1}$ were identified as amide I and II, respectively. The band at 1255 $cm^{-1}$ represents C-N and N-H stretching of tertiary amides. The bands at 1130 and 1035 $cm^{-1}$ are due to C-O stretching of aliphatic ether and C-N or N-C-N stretching of methylene linkage. All of the basic structural absorption peaks of amino resins still appeared in the spectra of MSP/PBG (40/60) blends, and the band intensities seemed to occupy an intermediate position between those of MSP and PBG. The amide I peak shifted to 1655 $cm^{-1}$, indicating that possible intra-hydrogen bonding was involved between two polymers, resulting in the C=O stretching frequency change. Soy protein contains many functional groups including carboxylic (-COOH), hydroxyl (-OH) and amino groups (-$NH_2$), which have potential to react with hydroxymethyl groups (-$CH_2$-OH) of PBG to form ester bonds (C-（C=O)-O-C), ether linkages (–$CH_2$-O-$CH_2$-), and amide linkages (–NH-CO-), respectively. The new small peak observed at 1738 $cm^{-1}$ in MSP/PGB blends is likely the C=O absorption from the new ester bond. The other absorption bands arising from C-O stretching vibration of esters consisted of C(C=O)-O and O-C-C and normally occurred in the region of 1300 to 1000 $cm^{-1}$ (Silverstein and Webster 1997). We believed these bands overlapped with amide III and –C-$NH_2$ bending in amide resin (Figure 5.3). The ether and amide linkages of MSP/PBG blends could also be formed and were overlapped with the large number of amide linkages in the amide resin. In addition, although MSP is a partially unfolded soy protein modified by reducing agent $NaHSO_3$, many functional groups available for the chemical reaction were still buried inside; therefore, the absorption peaks for the new bonds were not strong enough to be observed in the infrared spectra.

Figure 5.3 shows that HLIG, a PVA based adhesive, had two characteristic absorption bands at 1745 and 1253 $cm^{-1}$ corresponding to C=O and C-O stretching (Sivalingam et al., 2003). In the MSP/HLIG blends, specific absorption peaks from HLIG occurred in the same positions as those in pure HLIG. Characteristic bands related to soy protein (amide I and II) were present, and no new absorption bands were observed; these results indicated there were no chemical interactions between MSP and HLIG.

### *5.4.5. Transmission electron microscopy*

Morphological properties of the MSP/PBG (40/60) and MSP/HLIG (40/60) blends are shown in Figure 5.4. Pure MSP existed mainly in the form of globular aggregates with different sizes and a chain-like network (Figure 5.4 a). PBG is a chain-like network (Figure 5.4 b) connected by methylene and methylene ether bridges (Zorba et al., 2008). In the MSP/PBG (40/60) blends, some globular aggregates formed into the larger ones, accompanied with the many small ones (Figure 5.4 d), uniformly embedded in a much denser networks. This arrangement improves water resistance; the small aggregates facilitate the penetration of adhesive into the wood surface, and the dense structure reduces the penetration of water into the interfacial layer between wood and adhesives.

The HLIG mainly formed small, irregular aggregates (Figure 5.4 c); these aggregates surrounded a big, black, globular chunk. Similar to the MSP/PBG (40/60) blends, larger globular aggregates of protein formed and were embedded in a much denser structure in the MSP/HLIG (40/60) blends (Figure 5.4 e). The big, black, globular chunk was still retained in the blends. Soy protein aggregates and much denser structures were formed in MSP/PBG blends with chemical reaction and in MSP/HLIG blends without chemical reaction; this denser structure is beneficial for adhesive performance. The larger aggregates in the blends also could be one of the reasons for the reduced viscosity of adhesive blends. These aggregates reduce water-imbibing capacity, resulting in more free water in the adhesive blends system (Yao et al., 1988). Consequently, friction between protein molecules is reduced and a less viscous system is formed.

### *5.4.6. Dynamic viscoelasticity*

The gelling process of wood adhesives is important in terms of adhesion strength (Halasz et al., 2000). During the curing process, high molecular weight, three dimensional networks formed and resulted in a strong, tight bond. The MSP/PGB blends gradually became gel and finally became solid. The gelling time of blends decreased as the amount of MSP increased. Dynamic rheological properties of the MSP/PBG blends in the initial 30 min of the curing process are summarized in Figure 5.5. The elastic modulus (G') of MSP/PBG blends with 60% and 80% MSP increased rapidly, indicating the formation of a viscoelastic soy protein-UF network. When PBG was blended with 80% MSP, G' increased from 300 Pa

to 20000 Pa at the end of the oscillatory shear performance. At low MSP concentration (i.e., 20%), gelling time increased significantly and the final G' was only approximately 400 Pa. However, pure PBG and MSP were still in a liquid state after 30 min of oscillation, and G' remained almost the same as well.

Urea-formaldehyde resins are obtained from the reaction of urea and formaldehyde through a two-step process consisting of alkaline methylation followed by acid condensation. It is well known that the condensation polymerization reaction of UF occurs under acidic conditions; however, the initial pH of PBG resin is always adjusted to 7.0 to 8.0 for storage reasons (Gao et al., 2009). Pure MSP and PBG had pH 4.8 and 7.8, respectively, whereas the pH of MSP/PBG blends was in the range of 4.9 to 5.5 (Figure 5.6). Addition of MSP to PBG resin provides an acidic environment for the UF curing process that facilitates chain length growth and formation of cross-links between chains. A higher MSP concentration in blends led to a more acidic environment, which made UF resin cure faster. And as mentioned in the infrared spectrum section (3.4), there were chemical reactions between MSP and PBG. At the higher levels of MSP, more functional groups (-COOH, -OH, and $-NH_2$) were available for the cross-linking reaction with PBG. Therefore, MSP/PBG blends with high MSP content cured faster than blends with low MSP content. This fast curing might also explain why MSP/PBG blends with 60% and 80% MSP had higher viscosity. Overall, results indicated that MSP provided functional groups for the chemical reactions with PBG and also acted as an acidic catalyst.

### *5.4.7. Scanning electron microscopy*

The microstructures of cured MSP/PBG blends are presented in Figure 5.7. Both MSP and PBG showed irregular particles with different sizes and smooth surfaces. Some small particles were stuck to the surfaces of the larger particles (Figure 5.7 A, F). At 80% MSP, many small, rough particles with porous morphology were attached to large individual particles (Figure 5.7 B). At MSP loading levels lower than 80% (Figure 5.7 C-E), these small particles became more compacted and tightly coated the surface of reacted soy protein and UF polymers. In addition, the amount of these coatings decreased as MSP content decreased. As described in section 3.6, a higher MSP content would lead to more cross-linking reactions with PBG and, subsequently, more reacted rough coatings. Soy proteins are highly complex

ordered macromolecules with many functional groups buried inside; this structure limits the degree of cross-linking with PBG. As the reaction proceeded, more compact particles were produced and attached to PBG and the soy protein surface, preventing further interaction between these two polymers. Also, because MSP is a partially unfolded protein, there are not enough functional groups available for a complete reaction with PBG; this results in a limited coating substance on the surface of these two polymers.

### *5.4.8. Denaturation properties*

The typical DSC thermogram of MSP showed two endothermic transitions ($T_d$) at about 79.5 ℃and 96.6 ℃ caused by conglycinin (7S) and glycinin (11S) denaturation and had a total denaturation enthalpy ($\Delta H_d$) of 8.12 J/g (Table 5.3). In this study, the MSP modified with the reducing agent $NaHSO_3$ was more thermally stable than the 10% soy protein isolate suspension with $T_d$ values of 74.9 ℃ and 88.8 ℃ for 7S and 11S reported by Huang and Sun (2000a). The salt's screening effects of $NaHSO_3$ on the protein's electrostatic forces resulted in greater hydrophobic interactions, which could compensate for the negative destabilization effect of $NaHSO_3$ as a reducing agent.

The two endothermic transitions caused by 7S and 11S denaturation were also observed in all MSP/PBG blends (Figure 5.8). In terms of overall denaturation trends of subunits in MSP, both $T_d$ and $\Delta H_d$ increased with PBG concentration (Table 5.3). The denaturation temperature increased to approximately 90 ℃ from 80 ℃ for 7s and to 100 ℃ from 96 ℃ for 11S, which could be a result of their different native structures. Chemical reactions and inter-hydrogen bonding between the two polymers could form macro complexes with more compact structures, which would make soy protein chains less mobile and resulted in higher thermal stability. However, the limited reactions between MSP and PBG observed in SEM images indicated there might be a threshold for the cross-linking reaction at a certain PBG concentration (possibly, lower than 20%PBG). It is well known that 7S globulin exists as trimetric complexes through non-covalent bonds such as hydrogen and electrostatic force, whereas 11S has a hexametric form connected by disulfide bonds (Badley et al., 1975). Although MSP was modified by reducing agent $NaHSO_3$, complete reduction of disulfide bonds could not be achieved in glycinin (Zhang and Sun 2008). The disulfide bonds make 11S more thermally stable than 7S (Renkema et al., 2002). The total enthalpy of soy

protein decreased to 5.12 from 8.12 J/g when the soy protein was blended with 20% PBG (Table 5.3). Protein aggregates formed when soy protein was mixed with PBG (Figure 5.2 d); this is an exothermal process and could compensate for the energy absorbed by the cross-linking reaction, resulting in lower ΔH. Furthermore, PBG resin is a mixture of polymer and mono free formaldehyde. More PBG resin would provide more hydroxymethyl groups and free formaldehyde cross-linked with soy protein, resulting in increasing total denaturation enthalpy with increasing amount of PBG.

### *5.4.9. Thermogravimetric analysis*

The TGA and differential TGA (dTG) curves of cured MSP/PBG blends (ground powder in an $N_2$ atmosphere) are shown in Figure 5.9. The MSP degraded in three weight loss stages (Figure 5.9 b). In the first stage, from 25 ℃ to 160 ℃, 10% weight loss for MSP was due to evaporation of residual moisture. Weight loss in the second stage, between 208 ℃ and 400 ℃, was due to the degradation of soy protein involving broken intermolecular and intramolecular hydrogen bonds, electrostatic bonds, and cleavage of the covalent bonding between the peptide bonds of amino acid residues (Das et al., 2008). Further heating caused breakage of S-S, O-N, and O-O linkages, and finally protein backbone peptide bonds were decomposed, producing various gases such as CO, $CO_2$, $NH_3$, and $H_2S$. The first weight loss stage (5%) of PBG, from 50 ℃ to 170 ℃, was due to the residue water and the water that resulted from the condensation reaction. The exothermic peak due to PBG curing overlapped with the endothermic effects of water evaporation (Siimer et al., 2003). Almost 60% weight loss occurred in the second stage with a peak temperature of 320 ℃; it was caused by decomposition of the most stable unit in the PBG resin-methylenediurea (Camino et al., 1983). The small peak that appeared in the temperature of 201℃ to 228 ℃ was the formaldehyde emitted from the PBG resin.

During the heating process, the decomposition rate varied from 90% to 20% (Figure 5.9 a). As MSP content increased in MSP/PBG blends, the weight-temperature slopes became smaller. Similar trends were observed for the final residues weights corresponding to the temperature. Variations in weight loss of different blends were not observed until approximately 245 ℃ (Figure 5.9 b), which is probably due to the cross-linking reaction between soy protein and formaldehyde emissions from pure PBG (small peak between 201℃

to 228 ℃). New peaks appeared in dTG curves of MSP/PBG blends in the range of 270 ℃ to 281 ℃, which is further evidence that chemical bonds formed between these two polymers. The main decomposition temperature of blends with different ratios decreased slightly, compared with either of the neat polymers. Soy protein powder is granular and has long protein chains compactly arranged in α-helix and β-sheet structures; this formation leads to a high initial decomposition temperature (Li et al., 2008). As shown in the SEM image, part of the PBG resin reacted with soy protein molecules. We assumed that the branched PBG structure could interrupt the regularity of soy protein macromolecule chains to a certain extent, leading to decreased thermal stability. Simultaneously, un-reacted MSP dispersed in the PBG matrix, which could reduce such interaction.

## 5.5. CONCLUSIONS

Soy protein can be modified into functional copolymers that interact and react with commercial synthetic adhesives to enhance adhesion performance. Apparent viscosity of MSP/SLAs blends was reduced significantly at 20% to 60% MSP, which improved flowability and spread rate. The modified MSP reacted with UF based resin to form a gel within 3 to 48 h depending on soy protein concentration. The blends of MSP with 60% UF based resin (PBG) had the wet adhesion strength of 6.4 MPa with 100% wood cohesive failure; pure PBG had the wet adhesion strength of 4.7 MPa. Results indicated that MSP acts as an acidic catalyst for UF based resins such as PBG.

**Figure 5.1. Effects of MSP concentration on apparent viscosity of MSP and MSP/SLAs blends.**

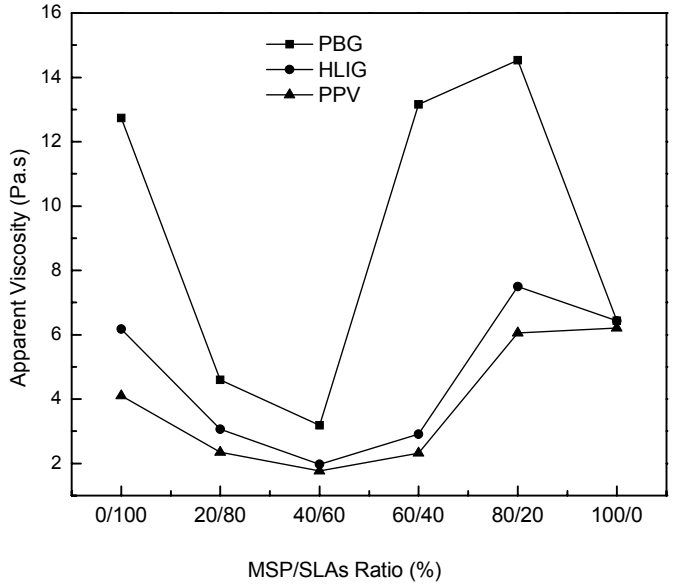


**Figure 5.2. Effects of MSP concentration on spread rate of MSP and MSP/SLAs blends.**

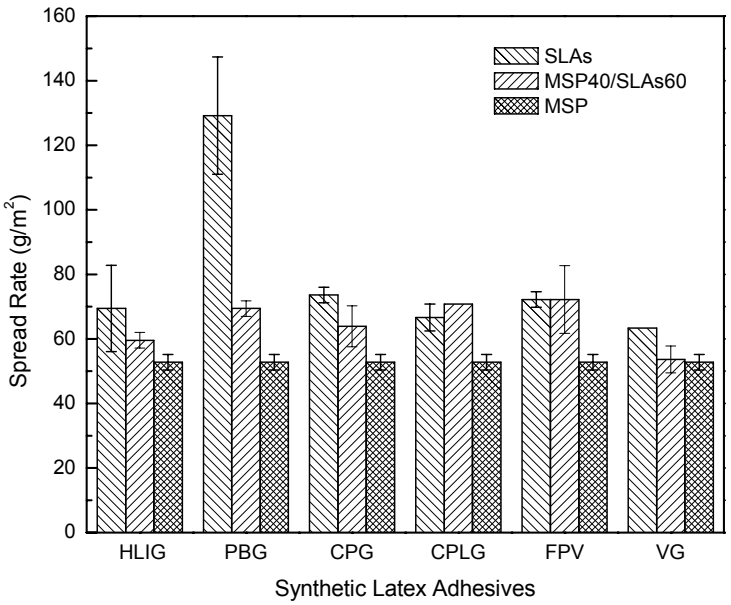


**Figure 5.3. FTIR spectra of MSP, MSP/PBG, and MSP/HLIG blends.**

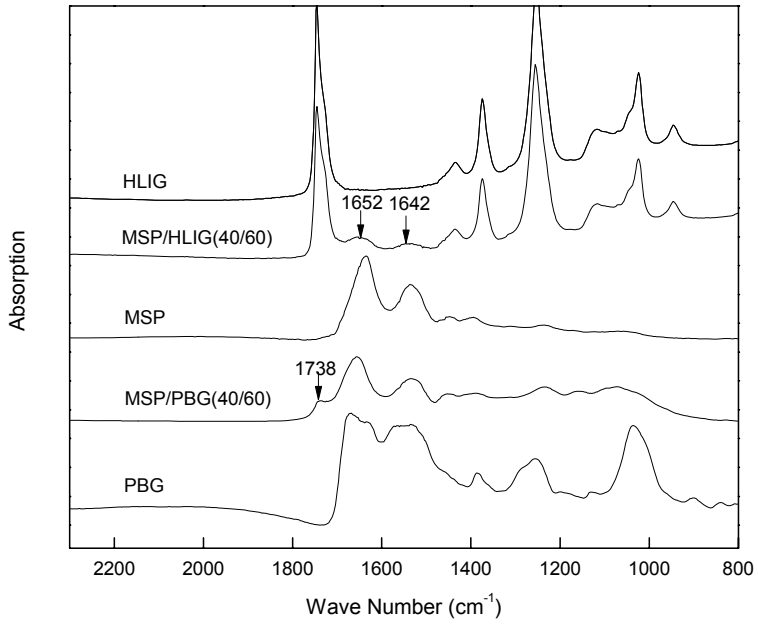
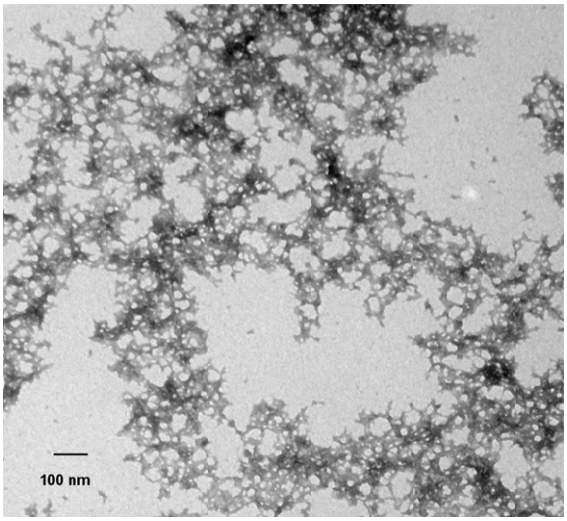
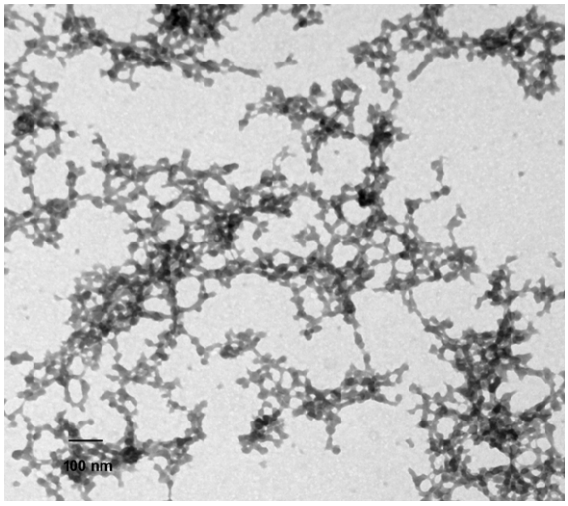


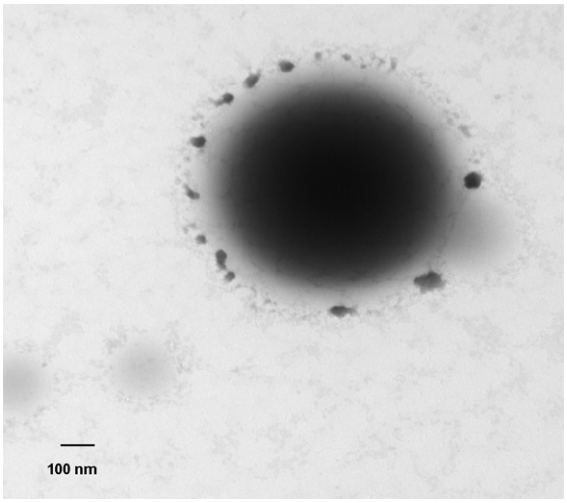
**Figure 5.4. TEM image of MSP/SLAs blends: soy protein modified by sodium bisulfite, MSP (a), PBG resin (b), HLIG resin (c), MSP/PBG (40/60) (d), and MSP/HLIG (40/60) (e).**



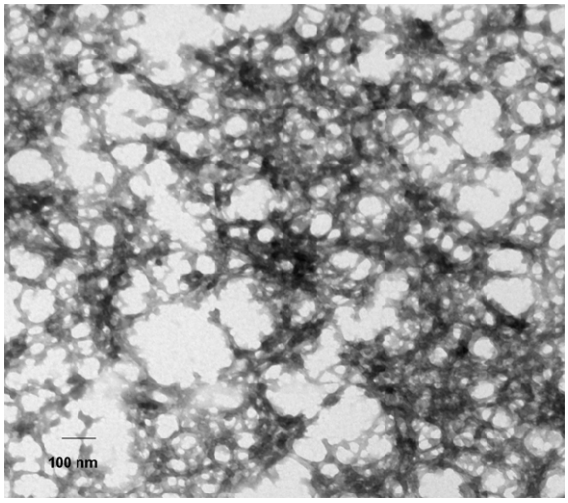

A



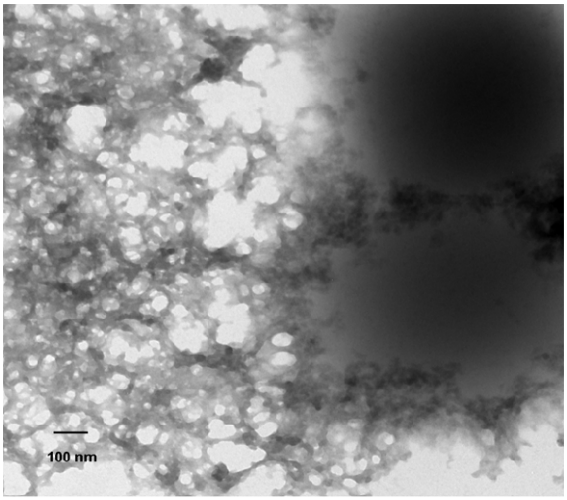

B




C




D




E

**Figure 5.5. Effects of MSP concentration on elastic modulus (G') of MSP/PBG blends.**

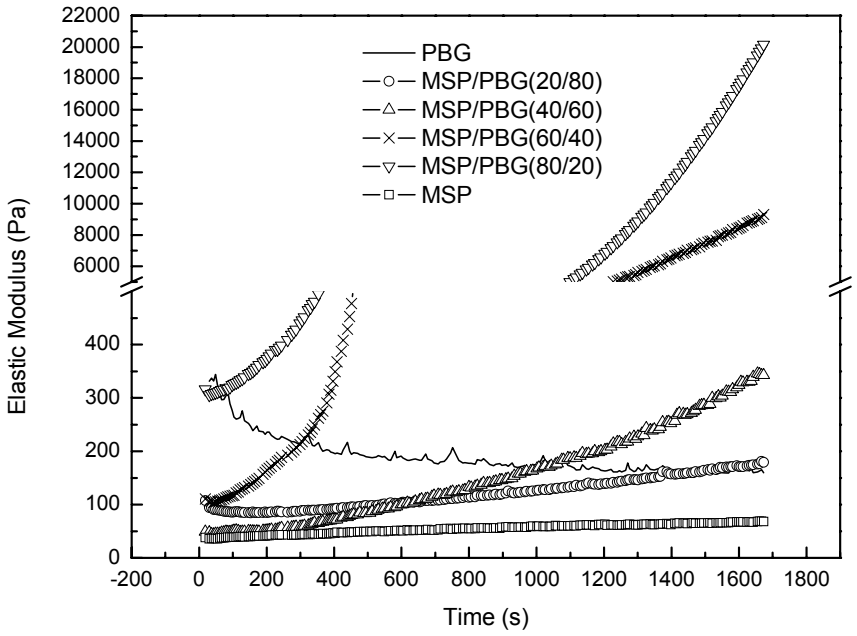


**Figure 5.6. Effects of MSP concentration on pH values of MSP/PBG blends.**

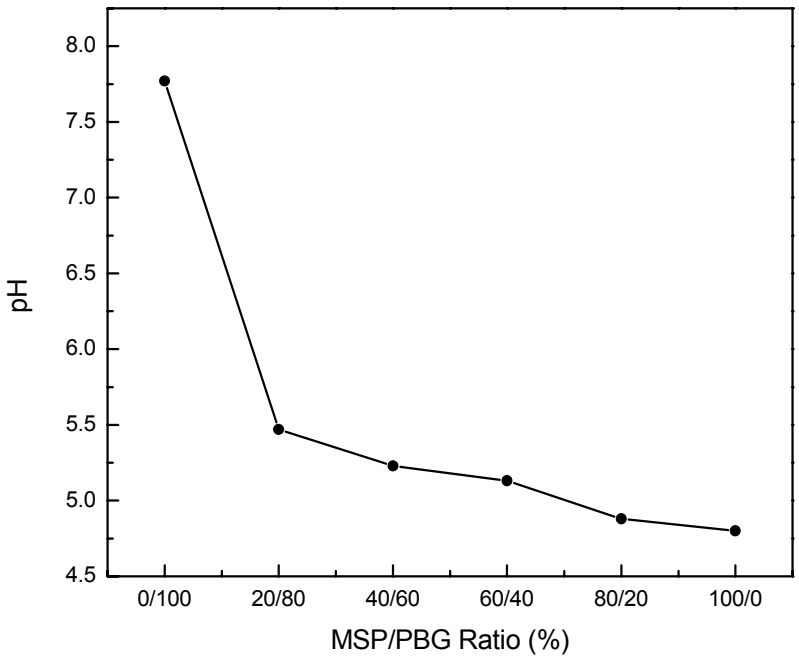
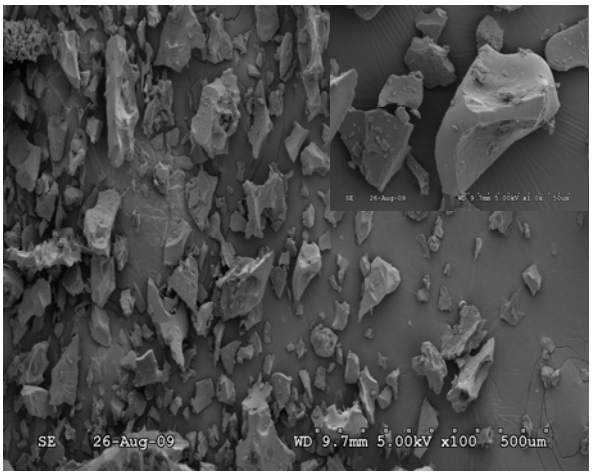
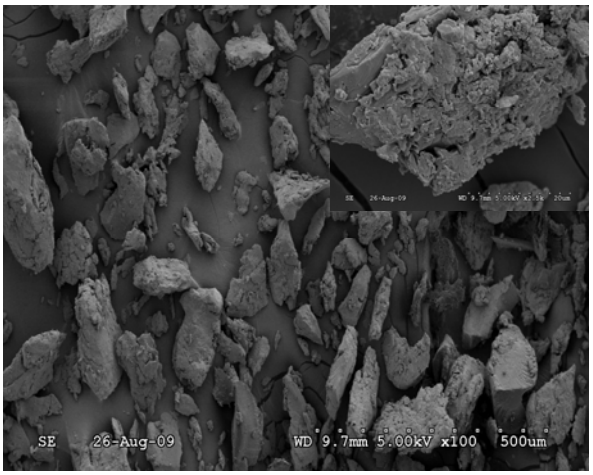


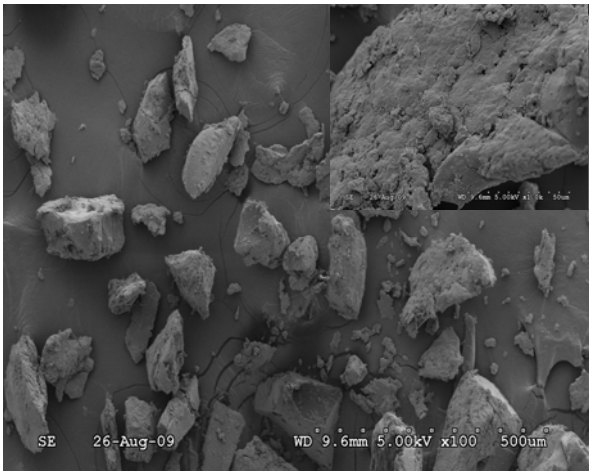
**Figure 5.7. SEM image of MSP/PBG blends: MSP (A, a), MSP/PBG (80/20) (B, b), MSP/PBG (60/40) (C, c), MSP/PBG (40/60) (D, d), MSP/PBG (20/80) (E, e), and PBG (F, f).**



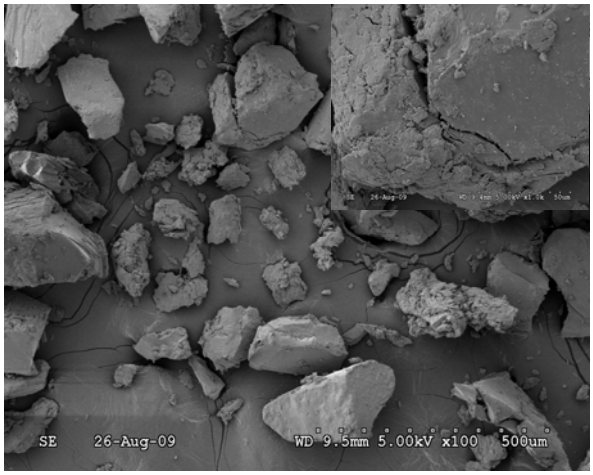

A




B




C

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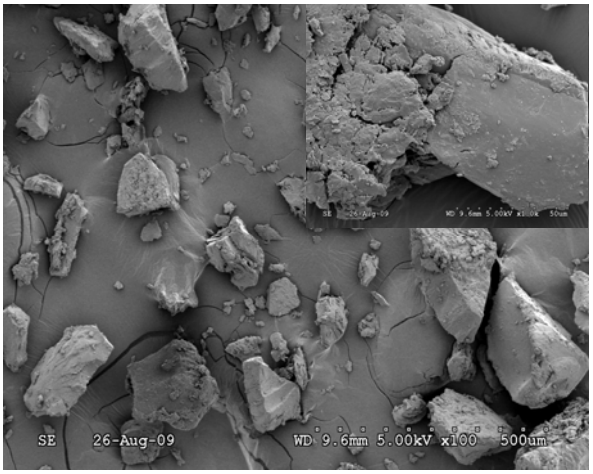
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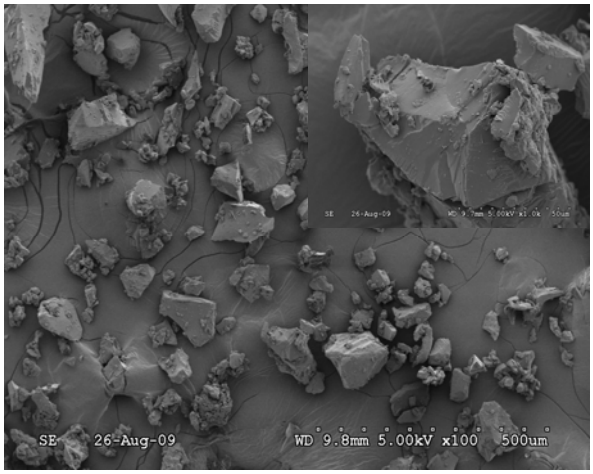
E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F

**Figure 5.8. DSC thermogram of MSP and MSP/PBG blends.**

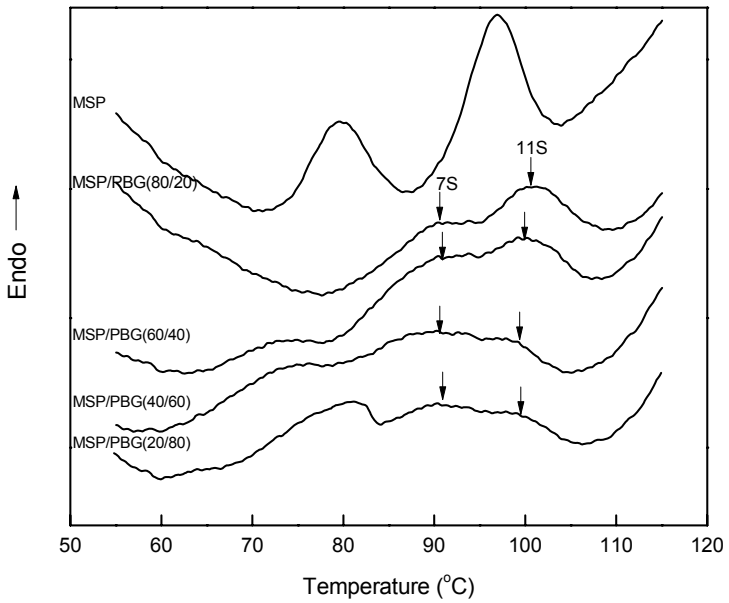

**Figure 5.9. Thermogravimetric (A) and derivative thermogravimetric (B) curves for MSP/PBG blends.**

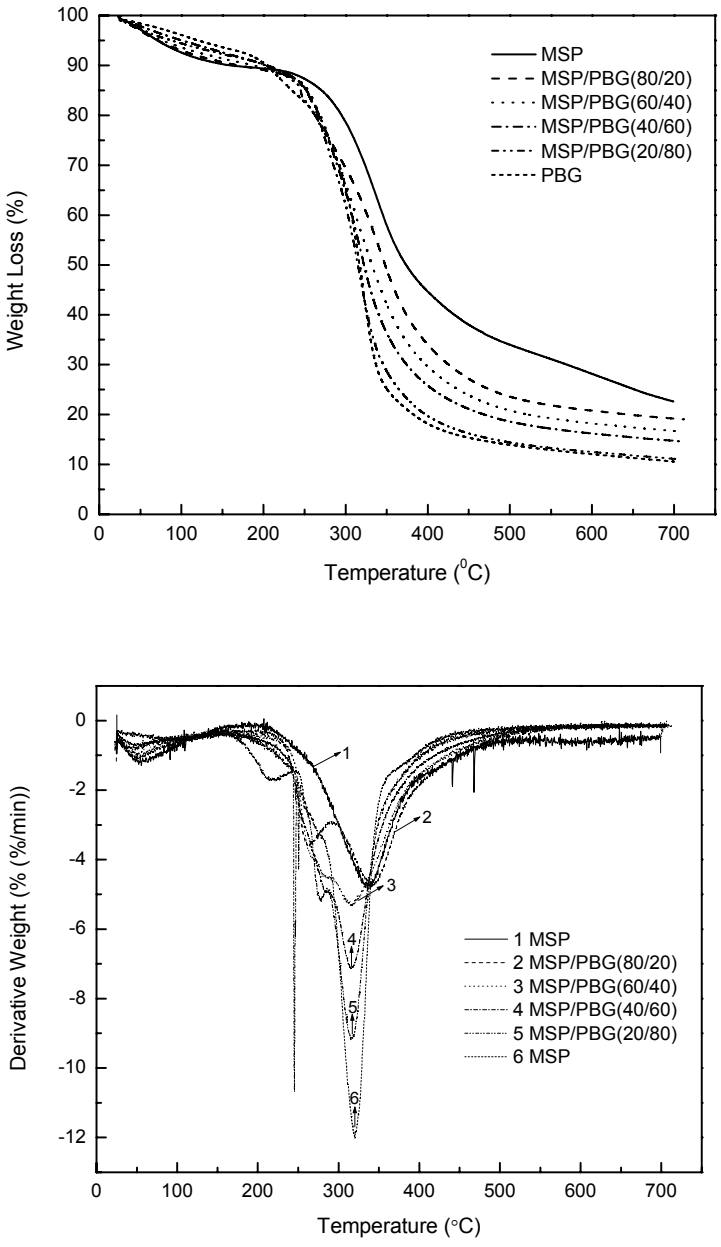

**Table 5.1. Dry shear adhesion strength of MSP and MSP/SLAs blends.**

| Adhesive Blends | Dry Shear Strength (MPa) | | | | | |
|---|---|---|---|---|---|---|
| | PBG | HLIG | CPLG | CPG | FPV | VG |
| 0% MSP | 5.60±0.31 cwf | 7.51±0.21 cwf | 7.79±1.17 cwf | 5.61±0.51 cwf | 5.73±0.91 cwf | 3.50±0.40 |
| 20% MSP | 5.41±0.68 cwf | 7.51±0.52 cwf | 6.10±0.49 cwf | 5.85±1.01 cwf | 6.53±1.38 cwf | 4.25±0.59 |
| 40% MSP | 6.13±0.28 cwf | 7.26±0.32 cwf | 6.72±0.26 cwf | 5.75±0.50 cwf | 6.72±0.51 cwf | 4.45±0.23 |
| 60% MSP | 5.37±0.29 cwf | 5.72±0.24 cwf | 5.92±0.89 cwf | 6.72±0.49 cwf | 5.94±0.76 cwf | 4.28±0.28 cwf |
| 80% MSP | 5.53±0.44 cwf | 4.49±0.48 cwf | 5.74±0.24 cwf | 4.72±0.63 cwf | 6.21±0.47 cwf | 5.46±1.53 cwf |
| 100% MSP | 5.58±0.41 cwf | 5.58±0.41 cwf | 5.58±0.41 cwf | 5.58±0.41 cwf | 5.58±0.41 cwf | 5.58±0.41 cwf |

cwf: 100% cohesive wood failure.

**Table 5.2. Wet shear strength of MSP and MSP/SLAs blends.**

| Adhesive Blends | Wet Shear Strength (MPa) | | | | | |
|---|---|---|---|---|---|---|
| | PBG | HLIG | CPLG | CPG | FPV | VG |
| 0% MSP | 4.66±0.09 cwf | 1.43±0.30 | 0.74±0.40 | 1.61±0.12 | 1.18±0.18 | 1.81±0.21 |
| 20% MSP | 5.91±0.52 cwf | 1.65±0.23 | 0.83±0.29 | 1.36±0.31 | 0.90±0.37 | 1.81±0.09 |
| 40% MSP | 6.42±0.49 cwf | 2.01±0.39 | 1.11±0.16 | 1.55±0.22 | 1.20±0.17 | 2.93±0.18 |
| 60% MSP | 4.37±0.58 cwf | 1.90±0.20 | 1.23±0.22 | 2.12±0.28 | 1.51±0.34 | 3.29±0.44 |
| 80% MSP | 4.08±0.24 cwf | 2.35±0.45 | 2.32±0.46 | 1.86±0.18 | 2.82±0.47 | 3.74±0.04 |
| 100% MSP | 3.60±0.42 | 3.60±0.42 | 3.60±0.42 | 3.60±0.42 | 3.60±0.42 | 3.60±0.42 |

cwf: 100% cohesive wood failure

**Table 5.3. Denaturation temperature ($T_d$) and enthalpy of denaturation (ΔH) of MSP in MSP/PBG blends.**

| MSP/PBG Blends | Td (℃) 7S | Td (℃) 11S | Total (ΔHd) (J/g) |
|---|---|---|---|
| MSP | 79.49 | 96.75 | 8.12 |
| MSP/PBG (80/20) | 89.83 | 101.18 | 5.85 |
| MSP/PBG (60/40) | 89.15 | 99.22 | 10.57 |
| MSP/PBG (40/60) | 90.43 | 98.55 | 9.84 |
| MSP/PBG (20/80) | 90.30 | 99.19 | 13.80 |

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# Chapter 6 - PEEL ADHESION PROPERTIES OF MODIFIED SOY PROTEIN ADHESIVE ONGLASS PANEL[3]

## 6.1. ABSTRACT

Soybean proteins have shown great potential for use as renewable, environmentally friendly adhesives. In this research, peel adhesion properties of $NaHSO_3$-modified soy protein adhesives (MSP) between a paper label and glass substrate were studied. Soy protein isolates (SPI), soy flour (SF) suspension, and commercial polyvinyl acetate (PVAc) based adhesive were used as comparison. With a 30 g/m$^2$ coating cured at 24°C, peel strength of MSP on a glass substrate increased rapidly with curing time and resulted in paper cohesive failure at about 150 s of curing time. Paper cohesive failure occurred at about 180 s for SPI and SF suspension and at 10 min for PVAc. At a higher curing temperature, less curing time for MSP was needed to reach the same peel strength. MSP had better water resistance than SPI, SF suspension, and PVAc in terms of "sweating" peel strength. When blended with MSP, PVAc showed shorter curing time, higher water resistance, and lower viscosity. Thermal and morphological studies showed that no chemical reaction occurred between soy protein and PVAc.

## 6.2. INTRODUCTION

Recently, concerns about environmental pollution, resource scarcity, and related health issues have pushed scientists to replace synthetic petrochemical polymers, which are used extensively in construction, packaging, and labeling industries, with bio-based adhesives. Synthetic latex-based adhesives are currently used for most glass bottles and jars for beer, beverages, food, and pharmaceutical products. Major adhesives currently used in the beer bottle industry include polyvinyl formal (PVF), acrylic ester, modified corn and potato starch, and casein (Wang et al., 2006). Each adhesive has its own limitations: PVF can cause health problems; acrylic ester is expensive, and its water resistance is too good to permit

[3] Results have been published. Qi, G., Sun, X.S. 2010. Peel adhesion properties of modified soy protein adhesive on a glass panel. 32: 208-212. (reuse of by permission of Elsevier)

recycling of the beer bottle; modified starch has poor water resistance; and casein is expensive. Klimets et al. (2004) showed that high quality label adhesives should have the following characteristics: high reliability of the adhesive joint in the initial stage of drying, short setting time, and pseudoplastic flow behavior.

Soybean protein adhesives have shown great potential for use as bio-based adhesives since the 1930s and are primarily used for plywood. Soy protein is also widely known for its peptizing and protective colloid action; it is compatible with most other binders and components used in coating formulations (Garey, 1997). Major challenges of soy protein as labeling adhesives are its low tacky property and poor water resistance. We hypothesized that exposure of polar and nonpolar groups would improve tacky strength and water resistance of soy protein adhesives. However, most of these groups were not available as a result of internal bonds due to van der Walls forces, hydrogen bonds, and hydrophobic interaction (Kumar et al., 2004).

Soy proteins have been modified by chemical alkali (Hettiarachchy et al., 1995), urea (Huang and Sun, 2000a), and sodium dodecyl sulfate (Huang and Sun, 2000b). However, most of these modifications resulted in soy protein suspensions with about 85% or higher water content, which caused lower tacky strength and required a longer curing time. The aim of this research was to improve tacky strength, curing time, and water resistance by modifying soy protein with a reducing agent and evaluate the resulting adhesive's potential for use on paper labels for glass bottles. The modified soy protein adhesives (MSP) were evaluated for peel adhesion and compared with adhesives made from soy protein isolate (SPI) and soy flour (SF). Polyvinyl acetate (PVAc) is a rubbery synthetic polymer, and PVAc emulsions are widely used as adhesives for wood, paper coating, paint, and other industrial coatings. However, the water resistance and gap-filling properties of PVAc are poor. Therefore, commercial PVAc-based latex adhesive was also used for comparison and the compatibility of MSP with PVAc was studied as well.

## 6.3. MATERIALS AND METHODS

### *6.3.1. Materials*

Defatted SF obtained from Cargill (Cedar Rapids, IA) was used as the starting material. The SF contained about 50% protein and 10% moisture with a dispersion index of

90. Soy protein isolates were isolated from defatted SF by isoelectric point precipitation at pH 4.2 and then adjusted to neutral pH. The precipitate was freeze-dried (Model 62,111-0495 Freeze Dryer, Virtis, Gardiner, NY) and then milled (Cyclone Sample Mill, UD, Fort Collins) into powder. Sodium bisulfite ($NaHSO_3$; Fisher Scientific, Fair Lawn, NJ) was used as the reducing agent. Washable School Glue (a PVAc-based adhesive) was bought from Elmer's products, Inc. (Columbus, OH).

### *6.3.2. Soy protein adhesive preparation*

MSP was prepared according to the method described by Sun et al. (2008). Soy flour was dispersed in water at 6.25% solid content at pH 9.5, $NaHSO_3$ was added to the slurry at 6 g/L based on water volume, and the slurry was stirred for 2 h at room temperature. The pH of the slurry was adjusted to 5.4 with 2N HCl to remove carbohydrates and some glycinin proteins by centrifuging at 12,000 × *g*. The pH of the supernatant was then adjusted to 4.8 with 2N HCl, and the supernatant was centrifuged at 8,000 × *g* to obtain MSP with a solid content of 38%. The SPI and SF suspensions were obtained by dissolving SPI and SF in distilled water at 10% solid content at neutral pH and stirring (magnetic stirrer) for 2 h at room temperature. PVAc was blended with MSP at 0%, 20%, 40%, 60%, 80%, and 100% (total weight basis), to study the compatibility between these two adhesives.

### *6.3.3. Paper-labeled glass specimen preparation*

Paper-labeled glass specimens were prepared according to ASTM method D3330/D3330M-04 (ASTM, 2004). A glass panel made by a local glass company in Manhattan, KS, was cut into a 50 × 125 mm panel. The panel was cleaned with ethanol and then allowed to dry in oven at 105°C for 10 min. Gloss copy paper (International Paper Inc.) was cut into 24 × 170 mm strips. MSP was applied on strips at 30, 40, 50, and 60 g/m$^2$ to study the effects of coating amount on peel adhesion. Three strips were used for each treatment. Twenty millimeters at one end of each strip were left untreated, and MSP was brushed uniformly on the remaining area. The coating amount applied to each strip was controlled with a balance. The end of the strip with adhesive was gently laid on the glass panel, and the other part of the strip was positioned loosely above the panel. A rubber roller with a weight of 2040 g was rolled by hand twice in each lengthwise direction of the glass

panel to ensure the strips adhered to the glass panel. The same procedure was used to prepare paper-labeled glass specimens for SPI, SF suspension, PVAc, and MSP/PVAc blends.

The effect of storage time (shelf life) of MSP and MSP/PVAc blends on paper-glass peel adhesion was also evaluated. These adhesives were stored at room temperature and evaluated for peel adhesion at 10 days, 20 days and 3 months following the same specimen preparation and testing procedures used for the fresh adhesive samples.

### *6.3.4. Apparent viscosity*

Apparent viscosity of the adhesive samples were measured with a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) with a CP 4/40 cone and plate fixture (4 cone angle, 40 mm cone diameter). The distance between cone and plate was set to 150 μm for all measurements. Experiments were conducted under steady shear flow at 23°C, and shear rate was set at 70 $s^{-1}$.

### *6.3.5. Differential scanning calorimeter (DSC)*

Thermal properties of soy protein samples were studied using a DSC (DSC7, Perkin-Elmer, Norwalk, CT) calibrated with indium and zinc. Protein solutions (20 mg) were hermetically sealed in the large-volume stainless steel pan. All samples were held at 20°C for 1 min and then scanned from 20°C to 150°C at a heating rate of 10°C/min. Peak temperatures and denaturation enthalpies were calculated from thermograms.

### *6.3.6. Morphology properties*

A Philips CM 100 (FEI Company, Hillsboro, OR) transmission electron microscope (TEM) was used to investigate the microstructure of MSP, PVAc, and MSP/PVAc (40/60) blends. All samples were diluted to 1% with deionized water and sonicated for 5 min in an L&R320 ultrasonic stirrer (L&R Manufacturing Company, Keary, NJ). Samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, PA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT). Microstructure of the sample was observed with operation conditions at an accelerating voltage of 100 kV.

### *6.3.7. Peel adhesion test*

The 90° peel adhesion test was conducted according to ASTM method D3330/D3330M-04 (ASTM, 2004). The glass specimen was placed on a fixture clamped to the moving steel panel of the peel adhesion tester (Vertical Motorized Test Stand, MV-110-S). The non-adhesive-coated end of each strip was doubled back at a 90° angle and clamped into the moving jaw to ensure a peel angle of 90° during peel testing. Peel adhesion tests were performed every 30 s of curing time for each paper-labeled specimen until the paper cohesive failure happened (adhesive adhesion strength is much stronger than the strength of paper substrates). The speed of the moving jaw for the peel test was 5.0 ± 0.2 mm/s. Data were collected after first 25 mm of tape was peeled, and average peel adhesion strength was obtained for peeling the rest of the tape. Reported values are the average of the three replications for each treatment.

### *6.3.8. Water resistance*

Water resistance was evaluated by determining "sweating" peel strength. The glass panels with paper labels were stored in a refrigerator at 4ºC for 24 h and then set at room temperature for 3 min to allow water to condense on the glass substrate. Then, sweating peel strength was obtained by following the same procedures used for the peel adhesion test.

## 6.4. RESULTS AND DISCUSSION

### *6.4.1. Effects of coating amount of MSP and curing temperature on peel strength*

Peel strength of MSP on the glass substrate increased quickly as curing time increased (Table 6.1). When soy protein adhesive is applied on a glass panel, the polar and apolar groups of protein molecules interact with the glass surface by physical and/or chemical forces such as hydrogen bonding and van der Walls forces. Application of an extra press (roller with weight of 2040 g) to the paper on the glass enhanced the interaction between the protein molecules and the glass substrate. For adhesive coatings of 30-50 g/m$^2$ cured at 24°C, there was no significant difference in peel strength, and paper cohesive failure occurred at 150 s. When adhesive coating amount increased to 60 g/m$^2$, peel strength was reduced, and paper cohesive failure occurred at 210 s because of the excessive adhesive layer between the paper

label and the glass substrate. There was no water penetration into paper at any coating amount level. A coating amount of 30 g/m$^2$ was selected for the following study.

Curing temperature had marked effects on water evaporation, immobilization of protein molecules, and physical interaction between adhesive and the glass substrate. At a higher curing temperature, a shorter curing time was required to reach the same peel strength. When curing temperature increased to 50°C, peel strength of MSP at 30 s of curing time increased to 0.68 N/cm from 0.325 N/cm at 24°C curing temperature, and paper cohesive failure occurred at 90 s.

### *6.4.2. Comparison of MSP with SPI, SF suspension, and PVAc on peel strength*

For MSP coating amount of 30 g/m$^2$ cured at room temperature, peel strength increased rapidly with curing time, and paper cohesive failure occurred at about 150 s (Table 6.2). Under the same curing conditions, peel strength of SPI and SF suspension at 10% concentration also increased rapidly with curing time, and paper cohesive failure occurred at 180 s. Peel strength of SPI and SF suspension at 30 and 60 s were not collected because the paper was wet as a result of rapid water penetration. Curing strength and rate are related to several factors such as available functional groups, coating amount, temperature, and water evaporation. MSP cured faster than SPI and SF suspension because it had less water content and more functional groups available because of the chemical modification with $NaHSO_3$. MSP also showed initial tackiness to the glass substrate. Peel strength of PVAc increased much more slowly in the initial curing stage, and paper cohesive failure took about 10 min.

### *6.4.3. Peel adhesion properties of MSP/PVAc blends*

Many studies have shown that adhesives with enhanced performance can be obtained by blending soy protein adhesive with other protein or synthetic adhesives. Weakley et al. (1972) reported that SF and SPI blends with dialdehyde starch used for paper coating applications showed improved wax pick and wet-rub resistance. In our study, peel adhesion properties of MSP blended with PVAc at 0%, 20%, 40%, 60%, 80%, and 100% were studied. Peel strength of the blends (Figure 6.1) confirmed that addition of MSP enhanced the peel strength of PVAc between the paper label and glass substrate. At the same curing time, peel strength of the blends increased as MSP content increased. Blended with 40% MSP, paper

cohesive failure occurred at 180 s, which is significantly shorter than the 10 min for pure PVAc.

As observed from the DSC data shown in Figure 6.2, no chemical reaction occurred between soy protein and PVAc. The thermogram of MSP showed denaturation peaks attributed to conglycinin (7S) and glycinin (11S) subunits. The denaturation temperatures ($T_d$) were 78.97°C and 96.04°C for 7S and 11S, respectively, with a total denaturation enthalpy ($\Delta H_d$) of 9.00 J/g. MSP with 60% PVAc had similar denaturation behaviors in terms of $T_d$ and $\Delta H_d$, which indicated no structural/conformational changes in soy protein in MSP/PVAc blends. Thus, the improvement in peel adhesion properties of PVAc resulted from synergistic effects of the physical interaction between PVAc and soy protein polymers.

### *6.4.4. Water resistance evaluation*

Water resistance is an important property that determines glue durability. Labels should not fall off when condensation forms on glass bottles as a result of temperature change or soaking in ice. The sweating peel strength was 0.85, 0.76, and 0.26 N/cm for SPI, SF suspension, and PVAc respectively, but paper cohesive failure was observed for MSP. Water resistance of PVAc was improved greatly by blending with MSP, and paper cohesive failure was observed for the blends with 40% MSP. An observation experiment was also performed on glass bottles full of water. Glass bottles with paper labels were stored in a refrigerator for 24 h and then placed at room temperature until they sweated. Labels with SPI, SF suspension, and PVAc were easy to remove, whereas the labels with MSP and MSP/PVAc blends stuck tightly to the sweating surface of the bottle.

When specimens were removed from the refrigerator, water molecules resulting from glass panel sweating could penetrate into the glue area and interact with adhesive molecules, resulting in reduced interaction between the adhesive and glass substrate. Sweating peel strength indicated that MSP and MSP/PVAc blends had better water resistance than SPI and SF suspension. The better water resistance of MSP is due to the $NaHSO_3$ (reducing agent) modification, which breaks disulfite bonds and brings hydrophobic groups to the protein surface.

### *6.4.5. Shelf life evaluation*

MSP stored for 3 months still showed good peel strength between the paper label and glass substrate (results not shown). MSP/PVAc blends stored for 20 and 30 days were used for the peel adhesion test. Peel strength was consistent with data obtained immediately after blending (results not shown), which indicated that MSP/PVAc blends had good adhesion sustainability. The SPI, SF suspension, and their blends with PVAc became moldy after several days of storage because of the high water content.

### *6.4.6. Rheological properties of MSP and MSP/PVAc blends*

Viscosity is an important physical property that governs the flow behavior of adhesives. Storage time significantly affected rheological properties of MSP adhesive (Figure 6.3). Apparent viscosity of MSP decreased as storage time increased; it decreased by almost 60% as storage time increased from 1 d to 30 d. Soy protein molecules could form aggregates during storage, and aggregated proteins have low protein hydration potential. Wagner et al. (1992) showed that loss of water retention capacity could lead to a decrease in hydrodynamic volume and, consequently, viscosity of soy protein. Another possibility is that protein polymers continue unfolding, which would result in less entanglement and hence, reduce viscosity; this effect requires further study.

Apparent viscosity of MSP was largely reduced by blending with PVAc at 40% or more (Figure 6.4). For MSP/PVAc (80/20) blends, apparent viscosity was reduced only a little bit compared with that of pure MSP. The apparent viscosity of soy protein is a result of intermolecular interactions, such as electrostatic interaction and disulfide bonding between protein molecules. Some PVAc molecules could interpose to the soy protein molecules, parting protein molecules and reducing protein-protein interaction. Therefore, apparent viscosity of MSP/PVAc blends decreased at 40% PVAc and above. In addition, similar to pure MSP, apparent viscosity of MSP/PVAc (80/20) blends decreased as storage time increased, but storage time had little effect on apparent viscosity of blends with higher percentages of PVAc.

### *6.4.7. Morphology of MSP and MSP/PVAc blends*

The TEM images of MSP, PVAc, and MSP/PVAc blends are shown in Figure 6.5 A-C. MSP was composed of many globular protein aggregates with uniform diameters (Figure

6.5 A). Several large aggregates were formed and many small ones in different sizes were observed in PVAc (Figure 6.5 B). The density of aggregates in MSP/PVAc (40/60) blends (Figure 6.5 C) was higher than that in PVAc. These aggregates are believed to be the mixture of soy protein and PVAc. The larger, bright aggregates (dark arrow) are from PVAc resin, whereas the smaller, less bright ones (white arrow) are the soy protein molecules. The individual morphological properties of both soy protein and PVAc were retained in the blends. The image results, combined with thermal properties shown in Figure 6.2, further indicated that no chemical reaction occurred between soy protein and PVAc.

## 6.5. CONCLUSIONS

Compared with SPI and SF suspension, MSP had a higher curing rate, better water resistance, and longer shelf life for paper-glass labels. Curing temperature had significant effects on the curing rate of MSP; a higher curing temperature was needed to reduce curing time. PVAc had a much slower curing rate and inferior water resistance compared with all soy protein based adhesives used in this study.

MSP showed good compatibility with PVAc. Blending with MSP could shorten the curing time, enhance the water resistance, and reduce the apparent viscosity of PVAc. Thermal and morphology studies indicated that no chemical reactions occurred between soy protein molecules and PVAc.

**Figure 6.1. Effects of PVAc level and curing time on peel strength of MSP on a glass substrate (N/cm).**

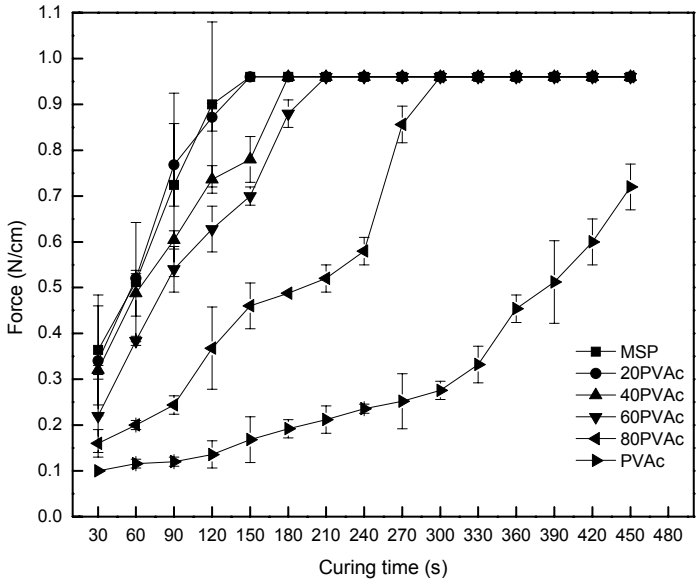


**Figure 6.2. DSC thermogram of MSP and MSP/PVAc (40/60) showing denaturation temperature ($T_d$) and enthalpy of denaturation (ΔH).**

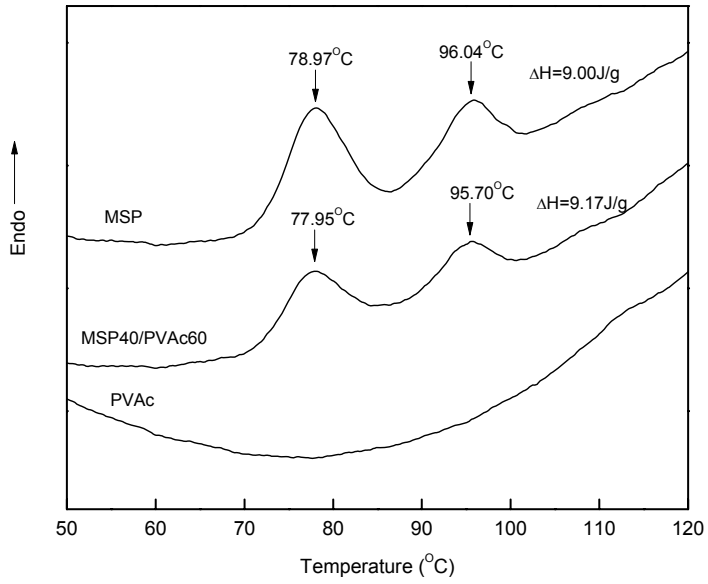


**Figure 6.3. Effects of storage time on apparent viscosity of MSP.**

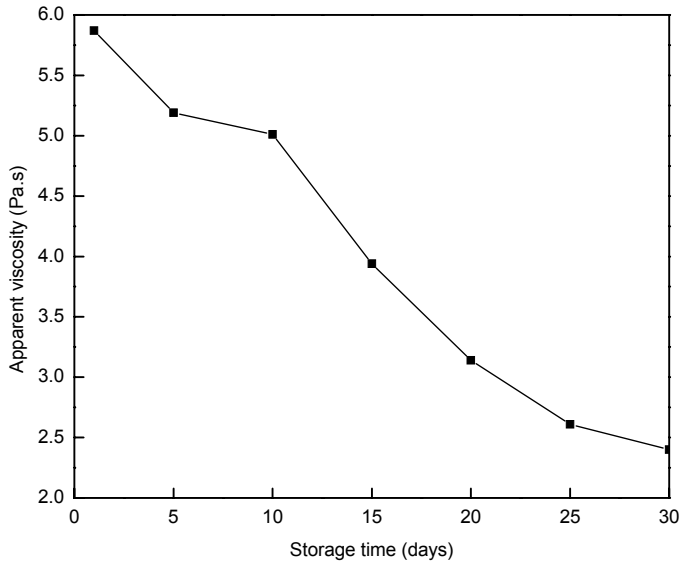


**Figure 6.4. Effects of PVAc level and storage time on apparent viscosity of MSP.**

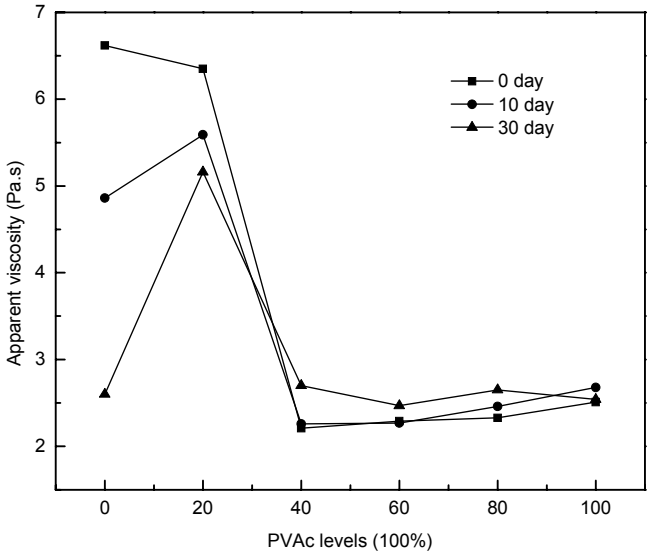
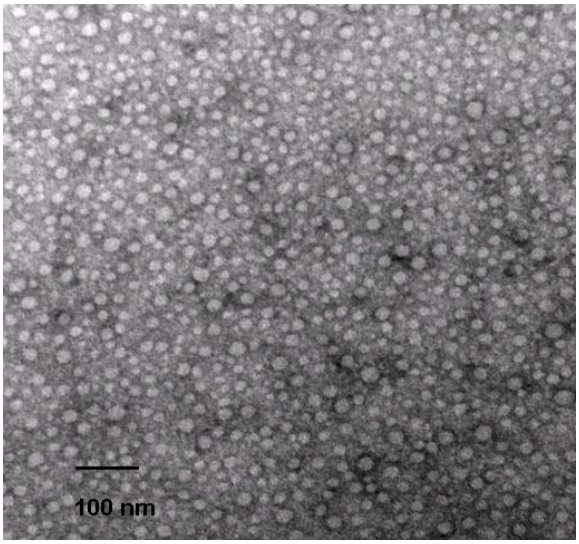
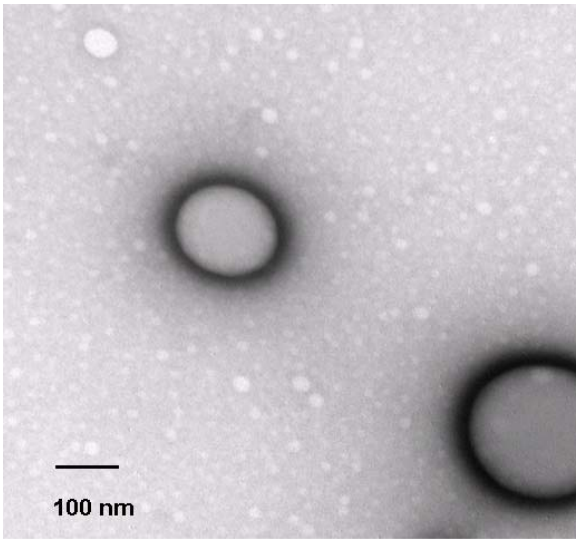


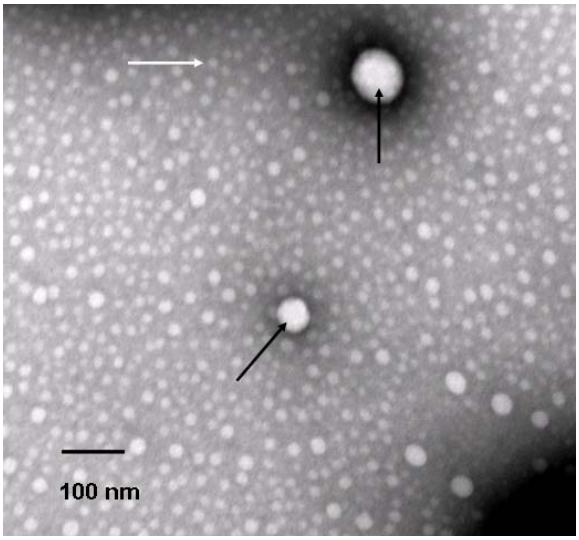
**Figure 6.5. TEM images of MSP (A), PVAc (B), and MSP/PVAc (40/60) blends (C).**




A




B




C

**Table 6.1. Effects of adhesive amount on peel strength of MSP on a glass substrate (N/cm).**

| Adhesive coating amount ($g/m^2$) | Curing time(s) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 210 |
| 30 | 0.352 ±0.06 | 0.512 ±0.06 | 0.724 ±0.11 | 0.864 ±0.15 | Failure | | |
| 40 | 0.335 ±0.09 | 0.489 ±0.18 | 0.717 ±0.24 | 0.845 ±0.08 | Failure | | |
| 50 | 0.324 ±0.05 | 0.537 ±0.10 | 0.615 ±0.09 | 0.767 ±0.11 | Failure | | |
| 60 | 0.241 ±0.01 | 0.384 ±0.04 | 0.503 ±0.08 | 0.556 ±0.30 | 0.703 ±0.12 | 0.763 ±0.10 | Failure |

Failure: paper cohesive failure.

**Table 6.2. Comparison of peel strength of MSP, SPI, SF suspension, and PVAc on a glass substrate (N/cm).**

| Adhesive type | Curing time (s) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 |
| MSP | 0.352 ±0.06 | 0.512 ±0.06 | 0.724 ±0.11 | 0.864 ±0.15 | Failure | |
| SPI suspension | -- | -- | 0.536 ±0.05 | 0.604 ±0.02 | 0.78 ±0.16 | Failure |
| SF suspension | -- | -- | 0.520 ±0.04 | 0.624 ±0.10 | 0.76 ±0.15 | Failure |
| PVAc | 0.10 ±0 | 0.116 ±0.01 | 0.12 ±0.01 | 0.136 ±0.03 | 0.168 ±0.05 | 0.192 ±0.02 |

Failure: paper cohesive failure.

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# Chapter 7 - PHYSICOCHEMICAL PROPERTIES OF SOY PROTEIN ADHESIVS MODIFIED BY 2-OCTEN-1-YLSUCCINIC ANHYDRIDE

## 7.1. ABSTRACT

Sodium bisulfite modified soy protein adhesives (MSP) with high solid content display great potential as alternatives to petroleum-based adhesives. This study investigated the adhesive properties of SPA as modified by 2-octen-1-ylsuccinic anhydride (OSA) at different concentrations. Physicochemical properties including electrophoresis profile and turbidity and thermal and rheological properties also were characterized in detail. OSA was grafted to some soy protein molecules through a reaction between amine, hydroxyl groups of protein, and anhydride groups as confirmed by Fourier transform infrared spectroscopy (FTIR). The conformation of OSA-modified MSP was unfolded as indicated by the absence of high molecular weight protein bands in reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and by the decrease in thermal stability detected by Differential Scanning Calorimetry (DSC). The turbidity of OSA-modified MSP decreased at pH basic to isoelectrical point (IP) but increased at pH acidic to IP. The wet strength of MSP applied on two ply plywood increased to 3.2 MPa at 3.5% OSA concentration compared to 1.8 MPa for the control; then the strength leveled off as OSA concentration increased further. MSP modified with 3.5% OSA worked better on maple wood veneer than yellow poplar wood veneer when three ply plywood was made. Wood cohesive failure (WCF) was observed for both soaked maple and yellow poplar plywood specimens: 60% WCF for the former and 5% WCF for the latter. The oily nature and hydrophobic long alkyl chains are the main reasons to improve the adhesion performance of MSP.

## 7.2. INTRODUCTION

As bio-based resources, soybean proteins have shown great potential as adhesives in the wood composite, packaging, and labeling industries (Zhong et al., 2001, Qi and Sun, 2010). The dominant storage proteins in soybean are globulins (50-90%), which comprise

glycinin (11S) and **β**-conglycinin globulins (7S). The two components are composed of a combination of 20 different amino acids (Peng et al., 1984). Each amino acid is known to have functional groups attached to the side polypeptide chains of the protein molecule. These functional groups, including OH, COOH, $NH_3$, are available for various chemical modifications, such as succinylation, alkylation, acetylation, and esterfication that could alter the microstructure of soybean protein and affect its functionalities (chemical and mechanical properties) to a large extent (Kim and Kinsella, 1986; Howell, 1996; Zhu, 2005). For the last few decades, substantial research has been conducted to improve the water resistance of soy protein through chemical modification (Huang and Sun, 2000; Rogers et al., 2004; Wang et al., 2005; Wang et al., 2007; Huang, 2007; Zhong et al., 2007; Qi and Sun, 2011).

Succinic anhydride is the most frequently used chemical agent for protein derivatization. It is used primarily to improve functional properties of protein in various food products by increasing protein solubility and emulsification properties and a lower isoelectric point (Franzen and Kinsella, 1976; Achouri and Zhang, 2001; Lawal and Adebowale, 2004). The principle reaction mainly involves N-acylation: succinic anhydride reacts with the **ε**-amino group of lysine in protein, converting it to a negatively charged residue (Beuchat, 1977). Little information is available about the effects of anhydride on adhesion performance of soy protein adhesives. Zhu et al (2005) utilized solid succinic anhydride to modify soy protein isolate through succinylation reaction and reported a decrease in adhesion strength due to hydrophilic carboxyl groups. Liu and Li (2007) also found maleic anhydride to have negative effects on adhesion performance of soy protein. In this research, liquid 2-octen-1-ylsuccinic anhydride (OSA), which had an oily nature and possessed long hydrophobic alkyl chains, was used to modify soy protein adhesives. We assumed that the oily nature and long alkyl chain introduced to soy protein accompanied by the succinylation reaction would improve protein adhesion strength. The proposed scheme of reactions between soy protein and OSA is described in Figure 1.

Our preliminary studies successfully exploited a new viscous cohesive soy protein adhesive modified by sodium bisulfide (MSP), with high solid content of 27%, good flow-ability, long shelf life, and good water resistance (Qi et al., 2011). The objective of this research was to study the adhesive properties of MSP as modified by OSA at various

concentrations, and to characterize physicochemical properties of OSA-modified MSP, such as turbidity, thermal, rheological, and morphological properties.

## 7.3. MATERIALS AND METHODS

### *7.3.1. Materials*

Defatted soy flour (Cargill, Cedar Rapids, IA) was used as the starting material. The soy flour contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium bisulfite ($NaHSO_3$) was obtained from Fisher Scientific (Fair Lawn, NJ). OSA was purchased from Sigma Aldrich (St. Louis, MO). Cherry wood veneers with dimensions of 50 × 127 × 5 mm (width × length × thickness) were provided by Veneer One (Oceanside, NY). Yellow poplar veneer and maple wood veneer with dimensions of 300 × 300 × 3.5 mm (width × length × thickness) were provided by Ashland Company (Covington, KY).

### *7.3.2. Adhesive preparation and modification*

MSP was prepared based on previous research (Qi et al., 2011). The aqueous protein extract was prepared by mixing defatted soy flour in water at 6.25% solid content at pH 9.5. $NaHSO_3$ was added to the slurry at 6 g/L on the basis of water volume, and the slurry was stirred for 2 h at room temperature. Then the carbohydrate was removed from soy protein by centrifuging at 12,000× *g*. The pH of the supernatant was adjusted to 5.6 with 2 N HCl, centrifuged at 12,000× *g*, and the precipitation with 27% solid content was used as the control MSP. The product yield was approximately 45% (wet protein/soy flour), and soy protein was predominantly glycinin. OSA was added to MSP at concentrations of 2%, 3.5%, 5%, and 6.5% (dry weight basis), then stirred for 2 h before use as an adhesive.

### *7.3.3. Infrared spectroscopy*

Fourier transform infrared (FTIR) spectroscopic data were collected in the region of 800-4000 $cm^{-1}$ using a PerkinElmer Spectrum 100 FTIR spectrometer (Waltham, MA). OSA-modified MSP samples were freeze-dried and ground for FTIR analysis. Then the samples were made into a disk under the pressure of 30. Each disk was scanned 16 times at a resolution of 2 $cm^{-1}$ and transmission spectra were recorded.

### *7.3.4. Electrophoresis (SDS-PAGE)*

Reducing SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system according to the method described by Laemmli (1970). OSA-modified MSP samples were mixed with a sample buffer containing 5% 2-mercaptoethonal, 2% SDS, 25% glycerol, and 0.01% bromphenol blue. A total of 8 µg of protein was applied to sample slots. Molecular weight standards were run with the samples. Electrophoresis was performed at 40 mA and 150 V for 120 min. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 40% methanol.

### *7.3.5. Rheology properties*

Apparent viscosity measurements of OSA-modified MSP samples were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) with a parallel plate (PP20, 20 mm plate diameter and 500 um gap). The shear rate dependence of apparent viscosity measurements were tested in the shear rate range of 0.1 to 50 $s^{-1}$. The testing temperature was 23 °C. A thin layer of silicone oil was spread over the circumference of the sample to prevent sample dehydration during the test.

### *7.3.6. Differential Scanning Calorimetry*

Thermal denaturation properties of OSA-modified MSP samples were evaluated by a differential scanning calorimeter (DSC) (Q200, TA instrument, Schaumburg, IL) calibrated with indium and zinc. Modified soy protein samples (20 mg) were hermetically sealed in Tzero aluminum hermetic pans. Each sample was held at 20 °C for 1 min then scanned from 20 °C to 130 °C at a heating rate of 10 °C/min. Peak temperatures and denaturation enthalpies were calculated from thermograms by Universal Analysis 2000 software.

### *7.3.7. Turbidity*

The turbidity of OSA-modified MSP samples were determined by spectrometer (UV-1650PC, Shimadzu Scientific Instruments, Columbia, MD). Samples were diluted to 0.1% with deionized water and the solution was adjusted to various pH values. The absorbance of protein solutions was measured at 600 nm after 30 min stirring. All measurements were done in duplicate and the average was reported.

### *7.3.8. Scanning Electron Microscopy*

AA Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of OSA-modified MSP samples. The freeze-dried samples were ground into fine powder, then affixed to an aluminum stub with two-sided adhesive tape and coated with an alloy of 60% gold and 40% palladium with a sputter coater (Desk II Sputter/Etch Unit, Moorestown, NJ). The SEM images of the samples were performed with an accelerating voltage of 5 kV.

### *7.3.9. Two ply plywood samples*

***Two ply plywood specimen preparation:*** Cherry wood veneers with dimensions of 50 × 127 × 5 mm were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA) for 7 d at 23 °C and 50% relative humidity. The adhesives were brushed onto one end of a piece of cherry wood with dimensions of 127 × 20 mm (length × width) until the entire area was completely wet. The amount of adhesive on each piece was controlled with a balance. Two brushed wood pieces were assembled immediately and conditioned for 15 min at room temperature. Then the assembled wood specimens were pressed with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN) at 1.4 MPa and 150 °C for 10 min.

***Shear strength measurement:*** The wood assemblies wood samples were cooled, conditioned at 23 °C and 50% relative humidity for 48 h, and cut into 5 pieces with dimensions of 80 × 20 mm (glued area of 20 × 20 mm). The cut wood specimens were conditioned for another 4 d before measurements were taken. Wood specimens were tested with an Instron Tester (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 at a crosshead speed of 1.6 mm/min. Shear adhesion strength at maximum load was recorded; reported values are the average of five specimen measurements. Water resistance of the wood assemblies was measured following ASTM Standard Methods D1183-96 and D1151-00. The preconditioned specimens were soaked in tap water at 23 °C for 48 h, and wet strength was tested immediately after soaking.

### *7.3.10. Three ply plywood samples*

***Three ply plywood specimen preparation:*** Maple veneer and yellow poplar veneer with dimension of 300 × 300 × 3.5 mm were pre-conditioned in the 27 °C and 30% relative humidity chamber for 7 d prior to the panel assemble. The adhesive was applied to the

bottom and top of the center ply only by a brush with spread rate 20-22 g/ft$^2$, on a wet weight basis. Veneers were oriented in the typical layup, in which the grain of the middle panel is perpendicular to the grain of the top and bottom panels. The assembled three ply veneers were conditioned for 15min at room temperature, and then hot pressed at 150 psi (1.03 MPa) and 150 °C for 10 min.

***Shear strength measurement:*** The bonded wood samples were conditioned at 23 °C and 50% relative humidity for 48 h before cutting. From each panel, 10 specimens (4 for dry strength and 6 for wet strength) with dimensions of 82.6 × 25.4 mm were obtained according to ASTM Standard Method D 906-98, as shown in Figure 7.2. The cut specimens were conditioned for 48 h, and tested with the same Instron tester at a crosshead speed of 1.6 mm/min. Dry shear adhesion strength at maximum load was recorded; reported values are the average of four specimen measurements.

Water resistance of three ply plywood samples was evaluated in terms of wet shear strength and three-cycle soak test. Six preconditioned specimens (82.6 × 25.4 mm) were soaked in water at 23 °C for 24 h, and wet strength was tested immediately after soaking. Another 4 specimens with 50 × 127 mm cut from each panel were for three-cycle soak test in accord with the American National Standard for Hardwood and Decorative Plywood (ANSI/HPVA HP-1-2004). Four specimens were soaked in water at 23 °C for 4 h, and then dried in chamber at 50 °C with good air circulation for 19 h. The soaking/drying cycle was repeated until three cycles were completed. All specimens were inspected to see whether they were delaminated after each cycle. Each bond line from three cycle soak score is rated on a 0-10 scale. 0 equates to no visible delamination, 5 is the maximum allowable delamination to pass per the ANSI/HPVA procedure (50 mm continuous delamination), and 10 equates to complete veneer separation. Any individual value higher than 5 is considered as a failed bond line. The pass rate is recorded as the ratio of the number of specimens pass through each soak test and the total specimens.

## 7.4. RESULTS AND DISCUSSION

### *7.4.1. FT-IR spectroscopy*

The FTIR spectrums of the OSA-modified MSP samples are presented in Figure 7.3. Both control and modified protein samples are typically characterized by amide I (1633 cm$^{-1}$)

and amide II (1515 $cm^{-1}$) absorption peaks, related to C=O stretching and N-H bending (Stuart 1997). Compared to the control MSP, a tiny shoulder was observed at 1731 $cm^{-1}$ for MSP modified with 3.5% and 6.5% OSA, indicating the ester formation induced by hydroxyl groups in protein and anhydride groups in OSA as proposed in scheme 1. Except for the C=O absorption bands (1731 $cm^{-1}$), the increase in the band at 1165 $cm^{-1}$ is indicative of the C-O asymmetrical stretching vibration of the ester: C(C=O)-O and O-C-C (Silverstein and Webster, 1997). In addition, the evidence of OSA grafted on some soy protein functional groups also could appear as the bands at 966 $cm^{-1}$ contributed by the –COOH absorption.

### *7.4.2. SDS-PAGE analysis*

T The reducing electrophoresis profiles of OSA-modified MSP samples are displayed in Figure 7.4. Unmodified MSP gave main bands corresponding to acidic (38 kDa) and basic (23 kDa) subunits of glycinin component, whereas the intensity of the bands related to **β**-conglycinin polypeptide (**α'**, **α**, **β**) were fairly low (Staswick et al., 1984). Based on the densitometry data, around 10% **β**-conglycinin and 70% glycinin accounted for the total protein. Another characteristic band in unmodified MSP was observed at the top of the resolving gel; this protein aggregate complex was believed to be composed of basic polypeptides mainly through hydrophobic association, as studied in previous research (Sun et al., 2008; Qi et al., 2011). OSA-modified MSP showed similar SDS-PAGE patterns to the control, except the high molecular weight band. The intensity of this band decreased as OSA concentration increased, which suggested the protein aggregates were dissociated to some extent as a result of different levels of succinylation reaction.

### *7.4.3. Turbidity*

Turbidity can reflect the protein association and dissociation induced by pH change, chemical modification, etc. The turbidity of OSA-modified MSP in the pH range from acidic to basic is summarized in Figure 7.4. Above the isoelectric point of MSP (pH = 4.5), the turbidity decreased as OSA concentration increased, indicating dissociation of the protein complex. Protein succinylation induced by OSA converted the positively charged amino groups to negatively charged carboxyl residues (Achouri et al., 1998); thus, the high net charge on the protein surface enhanced the electrostatic repulsion force with subsequent

unfolding of polypeptide chains and dissociation of the protein quaternary structure. This process decreases protein aggregation and increases solubility.

While below the isoelectric point, protein had a positive surface charge; as a result, the introduced negative groups by OSA neutralized the electrostatic repulsions, resulting in protein association with decreased solubility. A higher degree of succcinylation consumed higher levels of cationic amino groups, which contributed to the higher extent of protein aggregation. Furthermore, the hydrophobic force also might be enhanced through the interactions between the grafted alkyl chains and hydrophobic groups in soy protein. Therefore, the protein's resolubilization ability at pH values acidic to isoelectric point decreased as OSA concentration increased (Figure 7.5).

### *7.4.4. Thermal denaturation properties*

Denaturation temperature ($T_d$) and enthalpy ($\Delta H_d$) of OSA-modified MSP samples were affected slightly by OSA at a concentration range of 2% to 6.5% (Table 7.1). The thermogram of unmodified MSP was characterized by a major peak at 99.14 °C assigned as glycinin globulin, whereas a tiny shoulder detected at 80 °C was caused by small amount of **β**-conglycinin globulin (Figure 7.6). As OSA concentration increased, $T_d$ and $\Delta H_d$ of glycinin component shifted to lower value, and the shoulder peak corresponding to **β**-conglycinin disappeared. As explained in section 3.3, the chemical interaction among the reactive groups between protein and OSA could unfold the polypeptide chains and dissociate the protein aggregates, resulting in a less thermal-stable protein conformation. Apart from the protein dissociation induced by succinylation, re-association of polypeptides occurred simultaneously as pH of MSP lowered from 5.6 to 4.5, which is close to the protein isoelectric point, by addition of acidic chemical OSA. Besides, the induced alkyl chains could enhance the hydrophobic interactions among protein. Therefore, the competition between the protein association and dissociation contributed to the slight changes of $T_d$ and $\Delta H_d$ as shown in Table 1.

### *7.4.5. Rheological properties*

The shear rate dependence of apparent viscosity of OSA-modified MSP samples is summarized in Figure 7.7. Viscosity of all samples decreased as the shear rate increased in the range of 0.1 $s^{-1}$ to 100 $s^{-1}$, indicating their shear thinning behavior. Under the same shear

condition, apparent viscosity of MSP modified by OSA in the range of 2% to 6.5% decreased considerably to a similar range. As mentioned in section 3.4, when MSP was modified by OSA, its pH decreased from pH 5.6 to around isoelectric point (pH 4.5). At isoelectric point, protein has minimal electrostatic repulsion among protein molecules and the compact protein molecules precipitated in aggregate form. In addition, protein hydration capacity reached the lowest at isoelectric point; consequently, the decrease in hydrodynamic volume of protein micelles (Tschapek and Wasowski, 1979; Monkos, 2005) enhanced the distance and decreased the friction between protein molecules, which was reflected as the low apparent viscosity of the OSA-modified MSP.

### *7.4.6. Scanning electron microscopy*

The microstructures of OSA-modified MSP samples at various OSA concentrations are presented in Figure 7.8. Pure MSP displayed irregular compact disks with a fairly smooth surface where a few tiny particles were attached (Figure 7.8 A). The particle size of the OSA-modified samples increased as OSA concentration increased from 2% to 6.5%, and the protein particle surface became more coarse and fluctuant, exhibiting a rough appearance (Figure 7.8 B-E). The tiny particles attached to the protein surface in the control became embedded inside the protein body in the OSA-modified MSP, and a large number of big bumps formed at 5% and 6.5% OSA concentration (Figure 7.8 D and E). As explained previously, OSA could unfold protein structure by reacting with amino groups within protein molecules, resulting in more exposed protein surface area. Meanwhile, high OSA levels of modification introduced a larger number of long alkyl chains, which could disturb the integrity of protein structure; therefore, the bigger protein chunk and a tough surface developed.

In addition, fewer cavities were observed on the protein particles of the OSA-modified MSP samples. OSA increased the hydrophobicity of protein due to the added alkyl chains and their oily nature. High hydrophobic interactions within protein expelled water outside the hydrophobic clusters; therefore, when the sample was lyophilized, some cavities and holes formed on the surface.

### *7.4.7. Shear adhesion strength*

Dry and wet shear adhesion strength of OSA-modified MSP samples are summarized in Table 7.2. The dry shear strength of soy protein was not significantly affected by OSA; all samples displayed 100% WCF. The wet strength of MSP was considerably improved at OSA concentration up to 3.5%, from 1.8 MPa to 3.1 MPa, then leveled off as OSA content increased further. Partial WCF was also observed in terms of that the fiber was pulled out from the glued wood surface at OSA concentration higher than 2% OSA could be grafted onto some soy protein functional groups, such as $NH_2$ and OH, through succinylation reaction, thus inducing protein conformation changes that would increase contact surface area of protein with wood substrate, which should be beneficial to the protein adhesion strength. Meanwhile, succinylation not only generated newly formed hydrophilic carboxyl groups, but also introduced the hydrophobic alkyl long chains to the protein complex, which enhanced the hydrophobic interactions through reacting with hydrophobic groups within the protein. Therefore, it is possible that the increased hydrophilic carboxyl groups' detriment to water resistance might be negligible. In addition, OSA is an oil-like material in nature; it cannot dissolve in water. When the assembled wood sample was soaked in water, the hydrophilic groups of protein dissolved into the water and cavities were generated between the protein and wood surface, thus weakening the adhesive strength. On the other hand, when MSP was modified with OSA, the introduced hydrophobic groups to protein molecules and the hydrophobic nature of OSA prevented the water from penetrating the interfacial surface of adhesive and wood surface, thus hindering the formation of hollow cavities. This could be the main reason for significant improvement of water resistance of MSP samples.

The OSA-modified MSP samples lost flowability (continuous protein phase) when OSA concentration was higher than 3.5%. At this point, the maximum electrostatic interaction around the isoelectric point and enhanced hydrophobic force could promote the formation of larger-size protein aggregates; as a result, the wetting ability between protein and wood surface would decrease. Poor wetting ability counteracted increased protein-protein interactions, leading to limited improvement in wet strength as OSA increased from 3.5% to 6.5%.

Control MSP and MSP modified with 3.5% OSA were selected to assemble both maple and yellow poplar wood veneer in terms of three ply plywood. As shown in Table 7.3

and Table 7.4, the wet strength of control MSP on yellow poplar wood veneer is too low to be accurately determined by Instron, and all 4 specimens (50 × 127 mm) delaminated in the third cycle soak test. When maple wood veneer was used, 5% WCF occurred during the MSP wet strength test, and all specimens passed through the three cycle soak test; the evaluation score for each cycle was lower than 3. It indicated that soy protein based adhesives worked better on the cherry wood veneer than yellow poplar wood veneer. As to the 3.5% OSA-modified MSP, all the assembled plywood passed through the three cycle soak test for these two types of veneer. The soaked yellow poplar veneer specimens exhibited only 10% WCF, while the soaked maple veneer specimens were observed 60% WCF.

## 7.5. CONCLUSIONS

OSA was grafted onto soy protein molecules through a reaction between amine, hydroxyl groups of protein, and anhydride groups of OSA. Soy protein polypeptides were disassociated and unfolded to some extent through a succinylation reaction. The wet strength of the MSP sample was greatly improved by OSA modification; soy protein based adhesives worked better on maple wood veneer than the yellow poplar wood veneer. The oily nature and introduced hydrophobic long alkyl chains of OSA are the main reason for soy protein adhesion performance improvement.

**Figure 7.1. Schematic illustration of the reaction mechanism involved in soy protein and OSA where the functional groups of amine-anhydride and hydroxyl-anhydride are illustrated.**

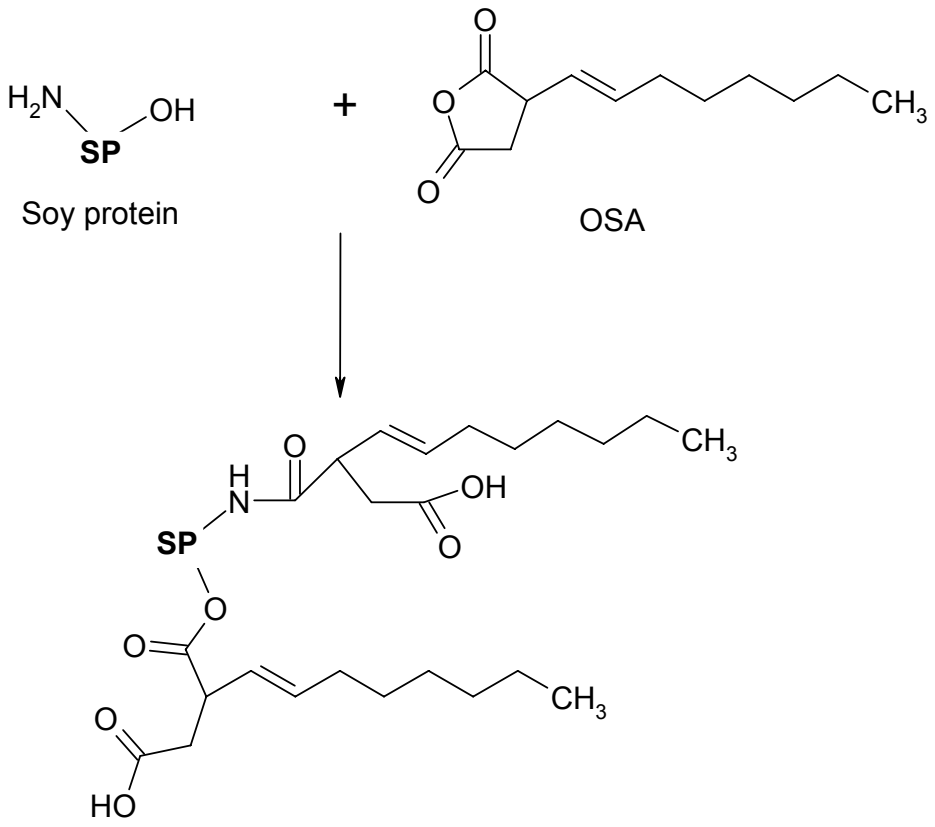


**Figure 7.2. Schematic diagram of three ply plywood specimen for shear strength test.**

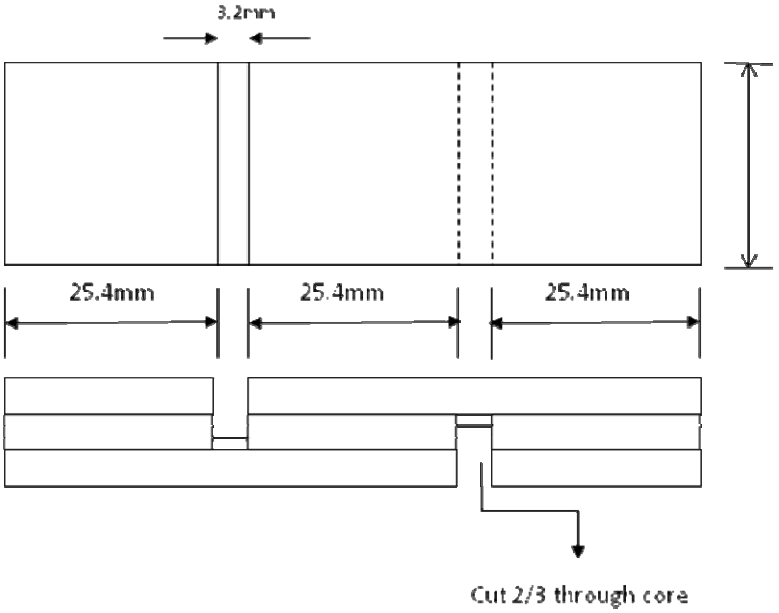


**Figure 7.3. FTIR spectra of MSP modified with OSA concentrations of 0%, 3.5%, 6.5%.**

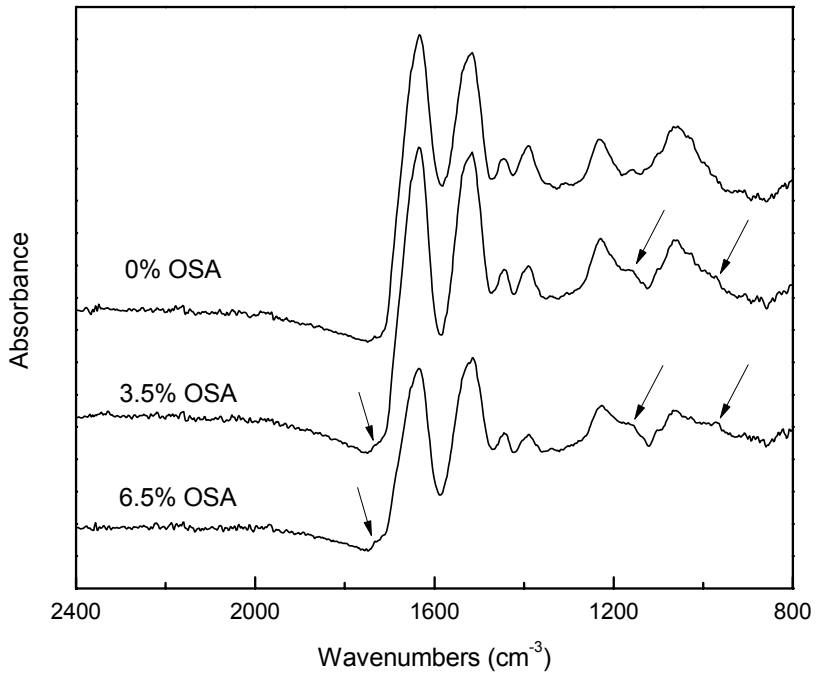


**Figure 7.4. Reducing SDS-PAGE pattern of MSP modified with OSA concentrations of 0%, 2%, 3.5%, 5%, 6.5%.**

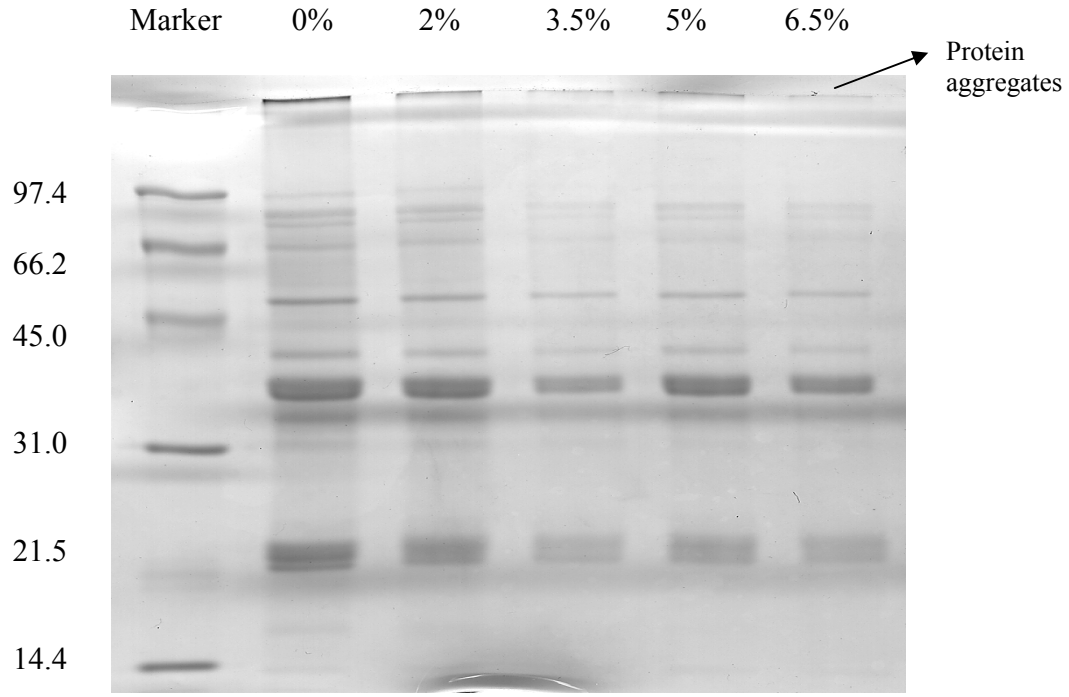


**Figure 7.5. Turbidity of MSP modified with OSA concentrations of 0%, 2%, 5%, 6.5%.**

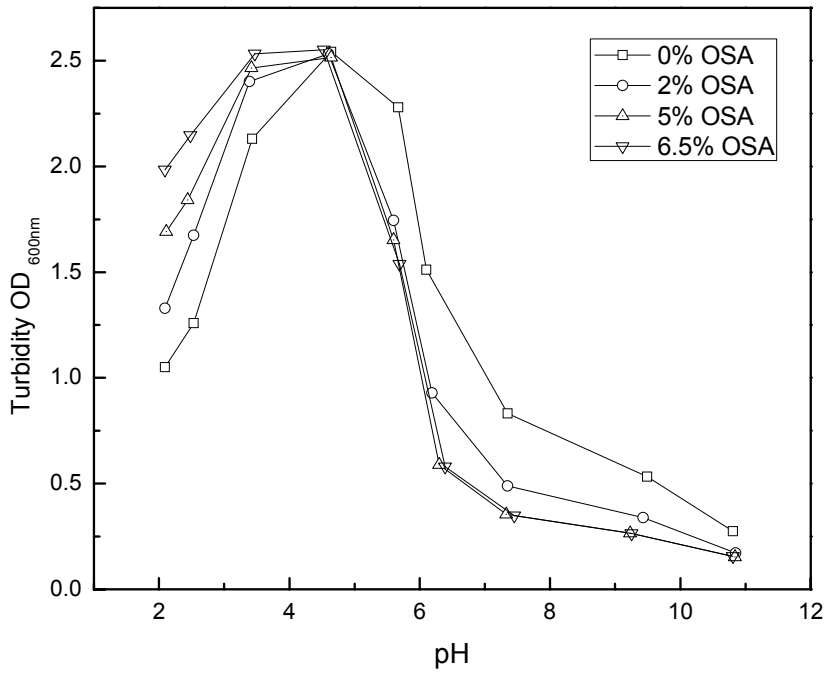


**Figure 7.6. DSC thermogram of MSP modified with OSA concentrations of 0%, 2%, 3.5%, 5%, 6.5%.**

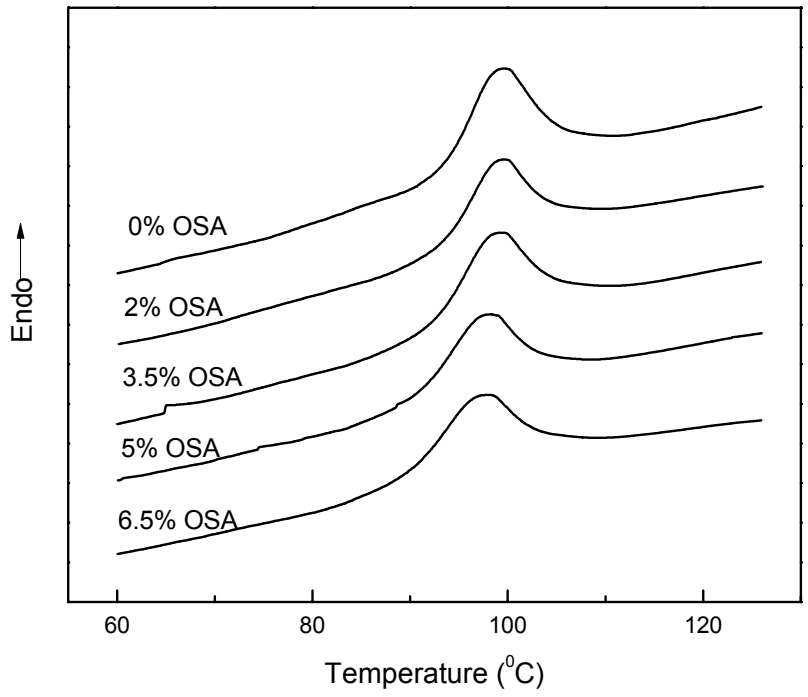


**Figure 7.7. Shear rate dependence of apparent viscosity of MSP modified with OSA concentrations of 0%, 2%, 3.5%, 5%, 6.5%.**

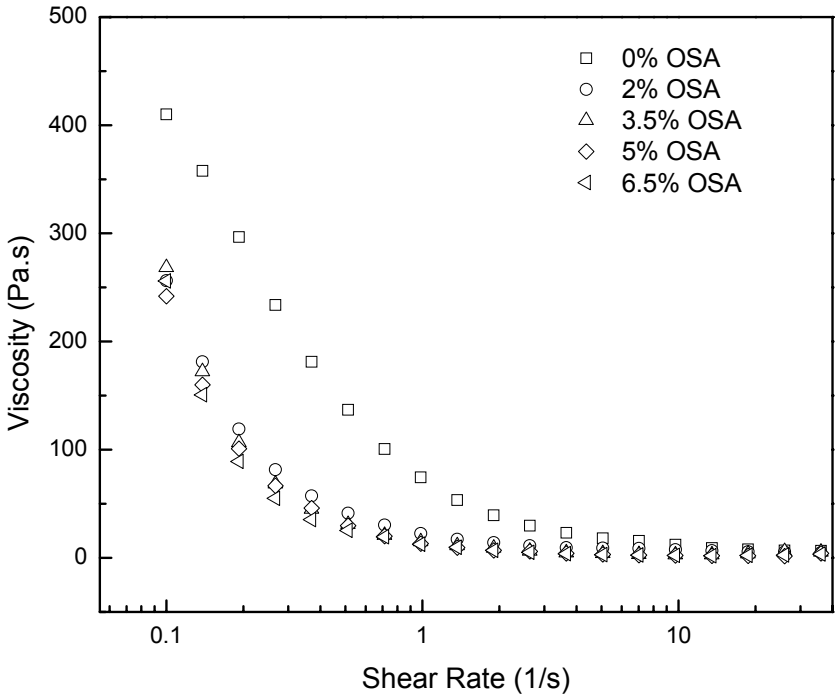
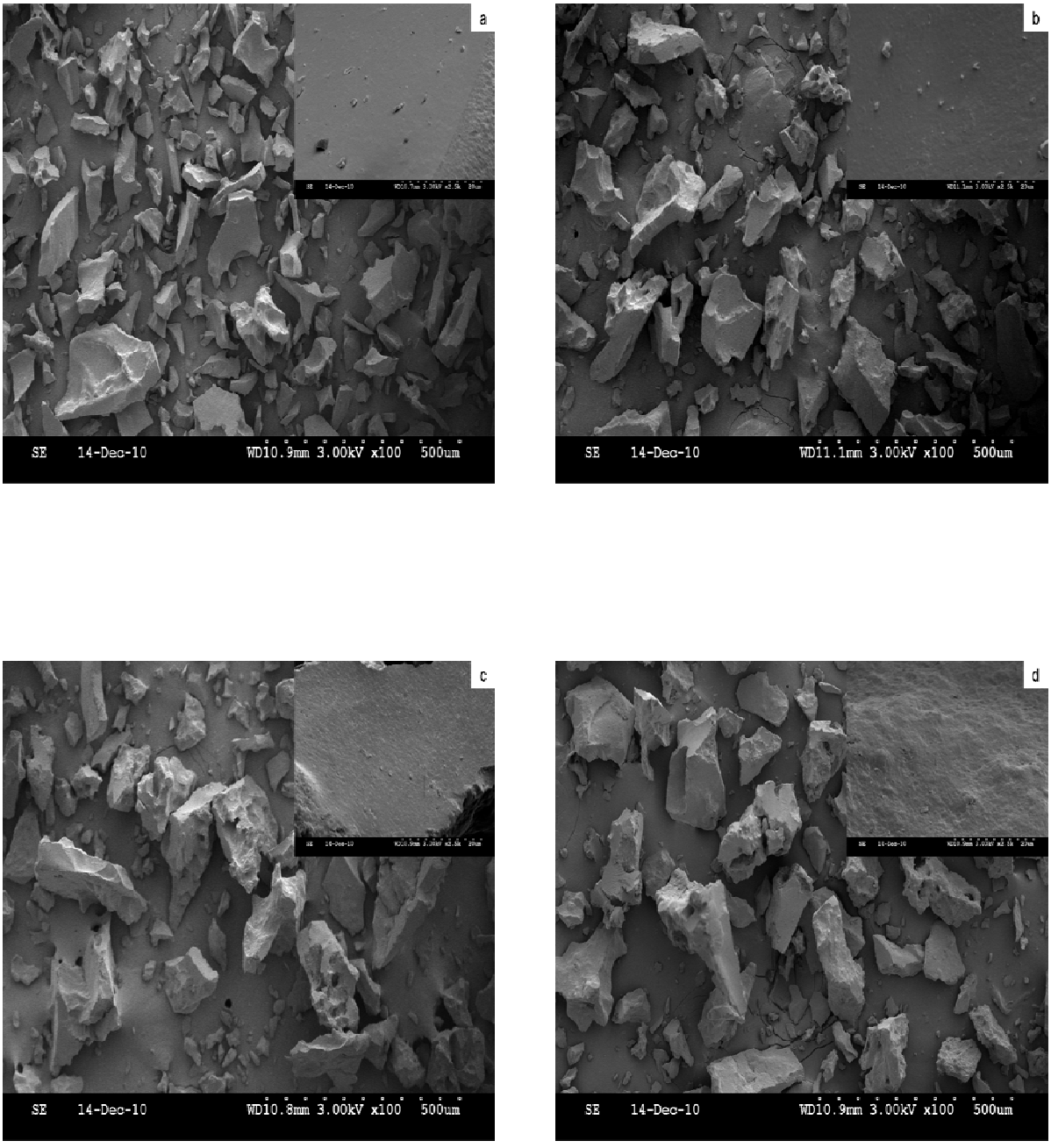


**Figure 7.8. SEM images of OSA modified MSP: control MSP (A); 2% OSA (B); 3.5% OSA (C); 5% OSA (D); 6.5% OSA (E).**



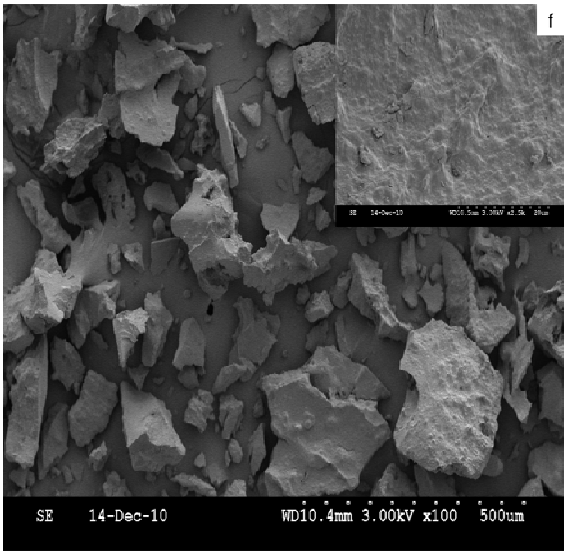

f
SE 14-Dec-10 WD10.4mm 3.00kV x100 500um

**Table 7.1. Denaturation temperature ($T_d$) and enthalpy of denaturation ($\Delta H_d$) of MSP modified with OSA at concentrations of 0%, 2%, 3.5%, 5%, 6.5%.**

| OSA (%) | $T_d$ (°C) | $\Delta H_d$ (J/g) |
|---|---|---|
| 0 | 99.14 | 2.49 |
| 2 | 99.05 | 2.53 |
| 3.5 | 98.64 | 2.07 |
| 5 | 97.66 | 2.30 |
| 6.5 | 96.78 | 1.92 |

**Table 7.2. Dry and wet shear adhesion strength of MSP modified with OSA at concentrations of 0%, 2%, 3.5%, 5%, 6.5%.**

| Adhesive formulation | Dry strength (MPa) | WCF | Wet strength (MPa) | WCF |
|---|---|---|---|---|
| 0% | 5.676±0.463 | 100% | 1.810±0.418 | 0% |
| 2% | 5.527±0.580 | 100% | 2.684±0.650 | 0% |
| 3.5% | 5.823±0.869 | 100% | 3.132±0.276 | Fiber pulled out |
| 5% | 5.917±0.389 | 100% | 3.074±0.252 | Fiber pulled out |
| 6.5% | 5.398±0.610 | 100% | 3.169±0.285 | Fiber pulled out |

WCF: Wood cohesive failure

**Table 7.3. Dry and wet shear adhesion strength of MSP modified with 0% and 3.5% OSA, evaluated on three ply plywood specimens.**

| Adhesive/wood veneer | Dry strength (MPa) | WCF | Wet strength (MPa) | WCF |
|---|---|---|---|---|
| Yellow poplar veneer | | | | |
| 0% | 0.902±0.109 | 5% | / | 0% |
| 3.5% | 0.944±0.090 | 100% | 0.289±0.114 | 5% |
| Maple veneer | | | | |
| 0% | 1.560±0.293 | 5% | 1.274±0.330 | 5% |
| 3.5% | 2.332±0.210 | 80% | 2.266±0.349 | 60% |

WCF: Wood cohesive failure

**Table 7.4. Three cycle soak test evaluation score of MSP modified with 0% and 3.5% OSA, evaluated on three ply plywood specimens.**

| Adhesive/wood veneer | Cycle 1 | | Cycle 2 | | Cycle 3 | |
|---|---|---|---|---|---|---|
| Yellow poplar veneer | Score | Pass (%) | Score | Pass (%) | Score | Pass (%) |
| 0% | 2.3 | 100 | 4.3 | 100 | 9.3 | 0 |
| 3.5% | 0.8 | 100 | 1.5 | 100 | 3.3 | 100 |
| Cherry veneer | Score | Pass (%) | Score | Pass (%) | Score | Pass (%) |
| 0% | 1.5 | 100 | 2.3 | 100 | 2.8 | 100 |
| 3.5% | 1.2 | 100 | 2.0 | 100 | 2.8 | 100 |

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# Chapter 8 - CONCLUSION AND RECOMMENDATION

## 8.1 CONCLUSION

The newly modified soy protein (MSP) alleviates the barriers of traditional methods for studying soy protein isolate adhesives, which tend to be poor water resistance, high viscosity, and short shelf life. These viscous cohesive soy protein adhesives could be obtained by in situ sodium bisulfite modification on soy protein in soy flour-water extracts. Physical and chemical treatment could further improve the adhesion performance of MSP.

Two soy protein fractions with high solid content and good flow-ability were extracted from soy flour slurry modified with $NaHSO_3$ in the concentration range of 2g/L-16g/L at pH 5.4 (11S dominated protein) and pH 4.5 (7S dominated protein), respectively. Even though $NaHSO_3$ had slightly negative effects on the adhesion properties of soy protein adhesives, it remarkably improved their hand-ability and flow-ability. The adhesion performance of MSP at pH 4.5 was better than that at pH 5.4; the wet strength was in the range of 2.5-3.2 MPa (170 °C curing temperature). $NaHSO_3$ had a certain reducing effects on soy protein; and the isoelectric point of MSP was lowered obviously because of the induced extra negative charges ($RS\text{-}SO_3^-$) on protein surface. The balance between hydrophobic interaction and electrostatic force among proteins is critical to the continuous phase of MSP at pH 5.4, while the 7S/11S ratios and the high molecular weight protein aggregates connected by disulfide bonds are related closely to the continuous phase of MSP at pH 4.5.

6g/L of $NaHSO_3$ concentration was selected to treat the soy flour-water extract; and MSP with various 7S/11S ratios were precipitated at different pH based on their different solubility. Based on the morphology, viscosity, and particle size of the protein precipitates, we proposed that protein-protein interaction, hydration capacity (glycinin-rich soy protein fractions), and certain ratios of 7S and 11S (β-conglycinin rich protein fractions) in protein systems are crucial to the continuous protein phase formation. The viscous cohesive samples were stable for up to several months without phase separation when stored at room temperature. The wet adhesion strength of the viscous cohesive soy protein adhesives could reach to 2.8 MPa (150 °C curing temperature).

MSP (pH 4.8) has good compatibility with six commercial wood glues. Water resistance of commercial adhesives was improved by blending with MSP to a different extent.

For example, the wet adhesion strength of MSP/urea formaldehyde (UF) blends (40/60) was 6.4 MPa with 100% wood cohesive failure, as compared to 4.66 MPa of UF (170 °C curing temperature). Viscosity of adhesive blends was reduced significantly and reached the lowest value at 40% to 60% MSP. Infrared spectra, thermal properties, and morphological images indicated that chemical reactions only occurred between soy protein and UF molecules. Soy protein provided some functional groups, carboxylic (-COOH), hydroxyl (-OH) and amino groups ($-NH_2$), that cross-linked with hydroxymethyl groups ($-CH_2$-OH) of UF, and also acted as an acidic catalyst for the self-polymerization of UF based resin.

Peel strength of MSP (pH 4.8) on glass substrate increased rapidly with curing time and resulted in paper cohesive failure at about 150s of curing time, compared with 180s for soy protein isolate (SPI) and soy flour (SF) suspension and 10 min for polyvinyl acetate based adhesives (PVAc). Curing time of MSP, SPI, and SF was all reduced at a higher curing temperature to reach the same peel strength. MSP had the best water resistance in terms of "sweating" peel strength. The blends of MSP and PVAc showed shorter curing time, higher water resistance and lower viscosity. Thermal and morphological studies showed that no chemical reaction occurred between soy proteins and PVA.

2-octen-1-ylsuccinic anhydride (OSA) were used to further chemically modify the MSP (pH 5.6) (OSA-MSP), improving its adhesion performance. OSA was grafted on soy protein through reaction between anhydride groups and amine, hydroxyl groups of soy protein. The conformation of OSA-MSP was unfolded as indicated by the absence of high molecular weight protein bands in reducing SDS-PAGE and by the decrease in thermal stability detected by DSC. The turbidity of OSA-modified MSP decreased at pH basic to isoelectrical point (IP) but increased at pH acidic to IP. The wet strength of MSP applied on two ply plywood increased to 3.2 MPa at 3.5% OSA concentration compared to 1.8 MPa for the control (150 °C curing temperature); then the strength leveled off as OSA concentration increased further. MSP modified with 3.5% OSA worked better on maple wood veneer than yellow poplar wood veneer when three ply plywood was made. Wood cohesive failure (WCF) was observed for both soaked maple and yellow poplar plywood specimens: 60% WCF for the former and 5% WCF for the latter. The significant water resistance enhancement was mainly attributed to the oily nature and hydrophobic long alkyl chains of OSA.

## 8.2 RECOMMENDATION

Before acid precipitation of $NaHSO_3$ modified soy protein adhesives, the pH of soy protein slurry was adjusted to around 9.5 to solubilize and denature soy protein. We proposed that NaOH could hydrolyze and extensively unfold the protein, resulting in better reducing effects of $NaHSO_3$ exerted on soy protein molecules. The reduction of disulfite bond in soy protein would improve the polypeptide flexibility and reduce the viscosity of soy protein adhesives. In addition, some of sulfhydryl residues (-SH) were converted to sulfonate group ($R–SSO_3^-$) during reducing. Therefore, it would interrupt the balance between the protein molecules attractive and repulsive force, resulting in the series changes of physicochemical properties of the modified soy protein. Therefore, it is necessary to study the effects of alkaline pH (i.e. 8-13) of soy flour slurry on the physicochemical properties of soy protein obtained during acid precipitation including solubility, surface hydrophobicity, thermal, rheological and morphological properties.

Cross-linking of protein would form a compact protein complex, and induce more entanglements and cross-linking during thermal setting, which would maintain their structure better than the unmodified adhesive after water soaking. It is the most efficient way to improve the adhesion properties of soy protein adhesives. Therefore, we could introduce the cross-linking agent (i.e. glutaraldehyde) into soy protein during the in situ chemical modification, then extract soy protein adhesive through acid precipitation, studying its adhesion performance and other physicochemical properties including solubility, surface hydrophobicity, rheological, thermal, and morphological properties.

Based on the research, the 7S components is the main contributor to the viscous and excellent adhesion properties of soy protein adhesives extracted at pH 4.5. But in this study, the soy flour we used give fairly low soy protein extraction yields at pH 4.5 (15% wb), which limited its real application as wood adhesives. So, the further research could utilize the soy flour with high proportion of 7S components, obtaining the high soy protein yields at pH 4.5 in the first place, and then study its adhesion performance.