ISOLATION, CHARACTERIZATION AND ADHESION PERFORMANCE OF SORGHUM,

CANOLA AND CAMELINA PROTEINS

by

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B.S., Henan University of Technology, P. R. China, 2004
M.S., Henan University of Technology, P. R. China, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Biological and Agricultural Engineering
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Abstract

Sorghum distillers dried grains with solubles (DDGS), canola and camelina meals are the main co-products resulting from grain-based ethanol or oil production. The main objective of this research was to study physicochemical properties of proteins isolated from DDGS, canola and camelina meals and their adhesion performance. Acetic acid-extracted sorghum protein (PI) from DDGS had superior adhesion performance in terms of dry, wet and soak adhesion strength compared to acetic acid-extracted sorghum protein (PF) from sorghum flour and aqueous ethanol-extracted sorghum protein (PII) from DDGS. PI had a significantly higher wet strength (3.15 MPa) than PII (2.17 MPa), PF (2.59 MPa), and soy protein without modification (1.63 MPa). The high content of hydrophobic amino acids in PI (57%) was likely the key factor responsible for high water resistance.

Canola protein was extracted from canola meal and modified with different concentrations of $NaHSO_3$ (0 to 15 g/L) during protein isolation. Unmodified canola protein showed the highest wet shear strength of 3.97 MPa cured at 190 ℃. Adhesion strength of canola protein fractions extracted at pH 5.5 and pH 3.5 (3.9-4.1 MPa) was higher than the fraction extracted at pH 7.0. $NaHSO_3$ slightly weakened adhesion performance of canola protein; however, it improved handling and flow-ability due to breaking of disulfide bonds in proteins.

Albumin, globulin, and glutelins were isolated from camelina meal. Adhesion performance of globulin fraction behaved better than glutelin fraction. The greatest wet shear strength of globulin was 3.3 MPa at curing a temperature of 190 ℃. Glutelin had a more protein aggregation compared with globulin, as indicated by higher crystallinity and thermal stability, and dense protein aggregate. This compact structure of glutelins may partially contribute to lower adhesion strength as compared to globulin.

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Dedication

I would like to dedicate this thesis to my family.

Chapter 1 - Introduction

1.1 Background

Shoes, cars, airplanes, self-adhesive notes/envelopes, wood composites, and plasters are a few of the many products featuring adhesive. More than 20 billion pounds of adhesives and resins are consumed annually in the United States (DWN 200). Currently, a majority of adhesives are manufactured from petroleum-based materials such as phenol-formaldehyde, urea-formaldehyde, and melamine-formaldehyde. However, petroleum is non-renewable, and fossil fuels are subject to depletion. Growing concern also exists regarding the effects of increased petroleum usage on the environment and human health since petroleum-based adhesives are not biodegradable and pollute soil and groundwater when they are disposed (CPC Aeroscience, 2005). Formaldehyde emission from urea-formaldehyde adhesives also causes health problems for humans with common symptoms from acute exposure being irritation of the throat, nose, eyes, and skin. The International Agency for Research on Cancer (IARC) also reclassified formaldehyde from "probably carcinogenic to humans" to "carcinogenic to humans" (Environmental Health, 2008).

Continued development and adoption of protein-based adhesives, especially soy-based wood adhesives, have cost-saving advantages and increased environmental benefits for various lumber products, including wood panel products (plywood, veneer, oriented strand board, particleboard and medium-density fiberboard), engineered lumber, green framing lumber and wood pallets. Protein-based adhesives offer a safe alternative to formaldehyde in plywood adhesives.

Soy-based adhesive is one of the most popular bio-degradable adhesives currently under development because of its unique advantages, such as high gluing strength, biodegradability, renewability, ability to be easily handled, and suitability for hot and cold press temperatures. Soy protein fraction glycinin is proven to be the main contributor to adhesive bonding strength, especially wet strength of soy protein isolate adhesive. Adhesives with high amounts of glycinin showed higher dry and wet adhesion strengths because of a greater content of hydrophobic amino acids than other soy protein fractions (Mo et al, 2004; Wang et al, 2005). Adhesive made from β subunit with high content of hydrophobic amino acids also exhibited greater water resistance than α α' and β-conglycinin adhesives (Mo et al, 2011). The limitations of soy-based adhesive may include high viscosity and low water resistance. The high viscosity and low water

resistance of protein-based adhesives could be alternated through chemical modifications. The principles of these modifications include unfolding the protein compact structure using detergents to expose as many functional groups as possible, especially hydrophobic groups, available for the wood substrate; inducing cross-linking reactions among protein through cross-linker to form the protein complex having more entanglement during curing; introducing specific groups to soy protein, thus contributing to adhesion improvement.

Even though soy-based adhesives are advantageous and renewable, the price of soy bean largely depends on the price of raw material (e.g., soybean, soy flour, etc.); therefore, it is necessary to find alternatives for soy-based adhesives. The price of soybean fluctuates based on demand and weather. For example, United States' export bids of soybean prices, FOB Gulf, in August 2012 averaged $661 per ton, up $17 from July 2012. Due to the 2012 drought in the United States, strong demand and yield losses of soybean boosted prices to record highs (USDA 2012).

Compared to soy-based adhesives, little research has been conducted on sorghum, canola, and camelina protein-based adhesives. Grain sorghum is the third most important cereal crop in United States and fifth in the world, and the United States is the number-one producer and exporter of sorghum (Texas Tech University 2009). Approximately 10 million acres of sorghum are harvested and utilized mainly as animal feed in United States each year, and approximately 35% of the country's sorghum crop is used for ethanol production (U.S. Grain Council 2011). Distillers dried grains with solubles (DDGS) are a co-product of the distillation and dehydration process during ethanol production (Bonnardeaus 2009). DDGS contains approximately 30 to 40% of protein and serves as an inexpensive source of protein. More than 700 million lb of sorghum protein was available from DDGS in 2009, when sorghum production was 500 million bushels in the United States (Feed Outlook/FDS-10i/September 14, 2010). Sorghum protein is believed to be the most hydrophobic protein among vegetable proteins due to its unique amino acids profile.

Canola ranks as the third-largest oilseed crop produced worldwide after soy and palm. As an important edible and industrial oil source, canola production rapidly increased worldwide from 30.1 million metric tons (MT) in 2008-2009 to approximately 58.4 MT in 2010-2011 (USDA Oilseeds 2012). Canola meal, a byproduct of canola oil extraction, contains 30 to 50% protein on a dry basis among different hybrids. As a promising candidate for biobased products with

industrial applications, canola protein has been studied extensively for biodegradable thermal plastics (Wäsche et al 1998) and films (Manamperi et al 2010); however, little information is available regarding canola protein as an adhesive. As another oil seed, camelina is a new crop in the United States. Camelina meal, the by-product of camelina oil production, typically contains 40% crude protein, a maximum of 12% crude fiber, less than 15% residual oil, and a small portion of vitamins (Sampath 2009). Both canola and camelina proteins have considerable amounts of hydrophobic amino acids. The availability and hydrophobic property of sorghum, canola, and camelina proteins make them potential candidates for being used as adhesives. To better understand such potential, additional work on protein physicochemical properties and adhesion performance are needed. The investigation of adhesive potential for these three vegetable proteins is a pioneer work.

1.2 Research objectives

The overall objective of this research was to investigate the potential of sorghum, canola, and camelina proteins for use as adhesives. Specific objectives of this research were:

a) To compare adhesive performance of sorghum protein extracted using various methods and sources and to characterize physicochemical properties of proteins, including amino acid composition and rheological, and thermal and morphological properties.

b) To characterize physicochemical properties of canola protein and to evaluate adhesion performance of canola protein modified with sodium bisulfite.

c) To characterize physicochemical properties of canola protein fractions and to evaluate adhesion performance of canola protein fractions modified with sodium bisulfite.

d) To characterize extracting properties and physicochemical properties of camelina protein.

e) To evaluate adhesion performance of camelina proteins.

1.3 Related Current and Previous Research

1.3.1 Sorghum, canola and camelina proteins chemistry

Sorghum proteins: Protein functionality is derived from the protein's structure. Understanding structural and molecular properties of the sorghum protein may be of use in characterizing functional properties of isolated proteins.

Sorghum (Sorghum *bicolor L.* Moench) grain has protein content varying from 6 to 18% with an average of 11% (Lasztity 1996).Similar to other cereals proteins, such as wheat, corn, rice and soybean, sorghum grain proteins were fractionated into albumin (soluble in water), globulin (soluble in dilute salt solutions), glutelin (soluble in acids or bases) and prolamin (karfrins) (soluble in nonpolar solvents such as aqueous alcohols) proteins, respectively, based on their solubility with the classical work of Osborne. Prolamins usually account for 77-82% of the total protein in sorghum and are the major storage protein in sorghum (Shewry and Tatham 1990), while sorghum protein has approximately 30% of the other three protein fractions of albumin, globulin and glutelin (Belton et al 2006). In this research, our attention focused on the major protein fraction, kafirins proteins.

Based on differences in molecular weight (MW), extractability and structure, kafirins are further classified into four subunits: α-kafirins, β-kafirins, γ-kafirins, and δ-kafirins (Shull et al 1991). By comparing subunit structures of zein (70% ethanol soluble proteins in maize), MW 23 and 25 kDa karfirn poly-peptides were electrophoretically similar to α-zein while demonstrating similar extractability, thus requiring relatively high concentrations of alcohol to be solubilized (40-90%). Similarly, β-kafirins were with MW of 16, 18 and 20 kDa, and γ-kafirins showed MW of 28 kDa, respectively (Shull et al 1991). Wang et al (2009) studied karfirins in DDGS with Lab on a chip Electrophoresis extracted and found that α-kafirins were resolved by electrophoresis into two closely-spaced bands of approximately 25 kDa and 23 kDa (α1 and α2). Belton et al (2006) stated that α -kafirins comprised about 80－84% of the total fraction in vitreous sorghum endosperms and 66－71% in opaque endosperms. They are resolved by SDS-PAGE into two bands of MW approximately 25 kDa and 23 kDa. β-kafirins is one kind of protein with the MW range around 18 kDa. Wang et al (2009) found that β- kafirins have a lower molecular weight than α kafirins and were resolved at 18 kDa, as indicated by the first peak in the molecular weight analysis curve. Watterson et al (1993) reported that β-kafirins accounted for approximately 7－8% of total kafirins in vitreous endosperm tissue, but 10－13% in opaque endosperm tissue. However, the same group identified three major components of MW 15 kDa, 17 kDa and 18 kDa as β-zeins (Shull et al 1991). This contrasts with a recent report which indicated that only a single β-kafirins gene was present (Chamba et al 2005), agreeing with the presence of a single β-zein gene in maize (Coleman and Larkins 1999). The gene reported by Chamba et al (2005) encodes a mature protein of 172 amino acids (Mw 18,745), including 16

methionines (9.3 mol%) and 10 cysteines (5.8 mol%). The sequence shows a high level of identity with b-zein which has a similar content of methionine residues (11.4 mol%).

Wang et al (2009) reported that only faint bands for γ kafirins could be observed with a molecular weight of 28 kDa, and bands with a molecular weight of 46 kDa were kafirin dimer. The γ-kafirins are unique among prolamins in that they are readily soluble in water as reduced subunits; hence, the γ-zein of maize was initially described as reduced soluble protein by Wilson et al (1981). However, they are insoluble in the native state due to their presence in polymers stabilized by inter-chain disulphide bonds. Watterson et al (1993) have reported that γ-kafirins account for 9-12% of the total kafirins fraction in vitreous endosperms and 19-21% in opaque endosperms.

Because of the similarity of solubility property, it is commonly accepted that sorghum kafirin is similar to zeins obtained from corn in terms of structure (both are soluble in 70% ethanol). In corn grain, δ-zeins of maize comprise components of Mw approximately 14,400 and 21,100 which are encoded by single genes (Coleman and Larkins 1999). The δ -zeins can be detected by SDS-PAGE, and the corresponding δ-kafirin has not yet been identified at the protein level. In sorghum, there is no report regarding the detection of δ-kafirins, so karfirn obtained from sorghum may differ from karfirns extracted from corn.

Canola proteins: Canola, also known as rape, oilseed rape, rapa, or rapeseed, has 40% oil and 17-26% protein content and ranks third-largest, after soybeans and palm, for oilseed crop produced worldwide. Canola meal, which is a by-product of canola oil extraction, contains up to 50% of protein (dry basis). Worldwide production of canola increased rapidly from 30.1 million metric tons (MT) in 2008–2009 to approximately 58.4 MT in 2010–2011 (USDA Oilseeds). Even though canola proteins possess a well-balanced amino acid composition, the meal is not used in human food applications due to the presence of glucosinolates (which interfere with thyroid function, thus reducing growth), erucic acid (which has potential to produce toxic effects in the heart), phytates (which strongly bind polyvalent metal ions such as zinc and iron and make them unavailable for metabolism), and phenolics (which are bitter flavored and make protein products darker in color).

The four protein groups of albumins, prolamins, glutelins and globulins can be fractionated with the classical work of Osborne. Canola proteins are also differentiated into 12S, 7S, and 2S fractions according to corresponding sedimentation coefficient in Svedberg units (S) which

indicates speed of sedimentation of a macromolecule in a centrifugal field (Aider 2011). Two major storage canola proteins, napin (2S) and cruciferin (12S), constitute 20% and 60% of total protein in mature seeds, respectively (Hoglund et al 1992), and have very complicated protein compositions. Napin, which belongs to albumin storage proteins, is a highly basic protein with an isoelectric point of pH 11, exhibiting molecular weights from 12.5 to 14.5 KDa (Monsalve et al 1990). Disulfide bonds are the main force stabilizing the napin protein structure; they comprise two disulphide-linked polypeptide chains (Krzyzaniak et al 1998). Cruciferin belongs to globulin storage protein, has a hexamer structure comparable to soy glycinin protein (Berot et al 2005), and is a neutral protein with an isoelectric point around pH 7.2 and a molecular weight of around 300 KDa (Schwenke et al 1983). Both covalent (disulfide bonds) and non-covalent bonds dominate cruciferin protein structure (Wu and Muir 2008).

Camelina proteins: Compared to extensive research on sorghum and canola, very little research has been conducted on camelina, and its agronomic potential remains largely unknown. Camelina sativa, usually referred to as camelina, gold-of-pleasure, or false flax, and occasionally wild flax, linseed dodder, German sesame, and Siberian oilseed, is a flowering plant in the family of Brassicaceae, which includes mustard, cabbage, rapeseed, broccoli, cauliflower, kale, brussels sprouts. Camelina originated from Northern Europe and Central Asia more than 3000 years ago and currently can be found in states located in the northern of the United States, such as Montana and Wyoming.

1.3.2 Sorghum, canola and camelina proteins isolations

Sorghum protein fractions: Based on solubility properties, flour protein fractions that are albumin (soluble in water), globulins (soluble in dilute salt solutions), prolamins (kafirins) (soluble in alcohol), and glutelins (soluble in acids or bases) were extractable (Jamunathan et al 1975). Kafirins are the major fraction in sorghum protein, which account for 77-82% of the total protein in sorghum (Shewry and Tatham 1990). To develop methods for karfirins isolation is more beneficial. Intensive work has been conducted on karfirins extraction from sorghum with most extractants consisting of alcohols such as ethanol and tert-butanol or acetic acid (Taylor et al 2005). Emmambux and Taylor (2003) described a method widely used for karfirins extraction that involved milled sorghum first being mixed with a solution of 70% (w/w) absolute ethanol in distilled water and reducing agents such as sodium metabisulphite and sodium hydroxide. The

mixture was stirred at 70 ℃ for 1h. After centrifugation and solvent evaporation overnight in a fume cupboard, the protein suspension was acidified to approximately pH 5 with 1M HCl to precipitate the protein. The dry purified kafirins was obtained through defatting with hexane and freeze-dried. Da-Silva and Taylor (2004) extracted kafirins from sorghum bran, a co-product of sorghum dry milling, with 70% ethanol combined with reducing reagent such as sodium hydroxide and sodium metabisulfite at a ratio of 1:5 (w/w) bran to extractant with vigorous stirring at 70 ℃ for 1hr. The extraction rate of 15.9-26.7% of the total protein was achieved.

Because aqueous ethanol at elevated temperature is highly inflammable and not acceptable to certain religions and ter-butanol is considered slightly dangerous. Taylor et al (2005) developed a novel method to extract kafirins from sorghum with glacial acetic acid. Acetic acid appears to be effective on account of its low dialectic constant (6.1), which enables it to dissolve hydrophobic proteins such as kafirins. Sorghum flour was mixed with pure glacial acetic to solubilize the karfirins in sorghum and protein was precipitated by pH adjustment with saturated NaOH. Because the reaction was exothermic, this process was conducted in an ice water mixture (10℃) to ensure temperature of the mixture was held at 25°C. Wet karfirns were collected through centrifuge and the glacial acetic acid and resulting sodium acetate salt was removed by dialysis against distilled water. Recovery of kafirins could be up to 89.7%.

Wang et al (2009) extracted karfirins from sorghum DDGS with various extraction methods, including acetic acid, HCl-ethanol, and NaOH-ethanol under reducing conditions. Results showed that acetic acid and NaOH-ethanol produced protein with both higher yield and purity than karfirins isolated with HCl-ethanol protocol.

Canola protein fractions: Previous studies presented many canola protein isolation methods, such as the classical Osborne method, alkaline dissolution followed with acid precipitation method, membrane technology, and enzymatic hydrolysis followed by membrane filtration method, etc. (Tessier et al 2006; Ghodsvali et al 2005; Klocheman et al 1997; Manamperi et al 2008). According to previous studies, canola proteins are isolatable at a large range of pH values, indicating canola protein has more than one isoelectric points (pI) (Predroche et al 2004; Lönnerdal et al 1972). Therefore, canola protein can be fractionated according to both the Osborne method and multiple pIs. Manamperi et al (2008) extracted four protein fractions of albumins, globulins, prolamins, and glutelins with both conventional and modified Osborne sequences from canola meal flour with 45.0% protein content to indicate the

effect of different protein fractions isolating sequences on protein extracting and functional properties. In the conventional Osborne method, protein fractions were isolated according to the order of albumins, globulins, prolamins, and glutelins. When using modified Osborne method, isolation order was changed into globulins, albumins, prolamins, and glutelins. With the conventional Osborne procedure, 32.4% of albumins, 20.9% of globulins, 12.9% of glutelins and 5.3% of prolamins were isolated, respectively. When using modified Osborne method, albumins and glutelins yields decreased around 32%, and yield of globulins and prolamins increased by 22.7% and 65.7%, separately. It is worth noting that 27.8% and 36.1% of protein was left in the pellet after isolating, indicating that very large quantities of protein in canola meal were not successfully extracted.

Besides the Osborne method, another very common method of isolating canola protein is using alkaline solution to solubilize canola protein, followed by protein precipitation at the isoelectric point (PI) with HCl (Wäsche et al 1998). In this study (Wäsche et al 1998), canola meal was added to the alkaline solution with specific solid/liquid ratio and stirred for a period of time to solubilize the protein in canola meal. The mixture was centrifuged to separate solubilized protein and pellet. The pH of the supernatant was adjusted with dilute acids to precipitate the protein, and the protein was separated through centrifuging. Optimum pH values for maximizing canola protein recovery rate could vary from 3.5 to 5.5 among different canola varieties (Pedroche et al 2004; Tzeng et al 1988). In many studies, more than one PI of canola protein was reported. Manamperi et al (2010) also reported that canola protein fractions precipitated sequentially from pH 11 to 3 with increments of 1 pH unit, and that fractions behaved differently with respect to functional properties such as thermal, rheological, and mechanical properties. Lonnerdal and Janson (1972) found that the basic napin protein fraction was known to have a PI close to pH 11, and suggested that the PI of other canola protein fractions was located between pH 4 and 8. Predroche et al (2004) extracted two canola protein fractions from Brassica carinata defatted meal sequentially at pH 3.5 and pH 5.5.

Membrane technology was also applied for canola protein preparation. Generally, a built-in peristaltic pump drew the protein solution from a sample container and pumped it through the hollow fiber cartridge. The membrane was chosen based on molecular weight of the protein to be isolated, and pressure in the cartridge was controlled by a back-pressure valve at the outlet. The retentate was returned to the same container. The sample flowed continuously through

ultrafiltration unit until its volume decreased to 1:10 original volume. The concentrated sample was washed with distilled water and dried. Ghodsvali et al (2005) used the membrane technology (the membrane used had a nominal molecular weight cutoff of 10 kDa and an area of 0.1 m^2) to extract canola protein from defatted canola meals. The final protein isolates had protein content higher than 80% and accounted for approximately 50% of the nitrogen in the canola meals.

Among the methods mentioned previously, the procedure to use alkaline solution to solubilize canola protein followed by protein precipitation at the isoelectric point (PI) with HCl is most common because it is relatively simple and protein yield is high. This method was used for sorghum protein isolation in this research.

Camelina protein fractions: Compared with sorghum or canola, the volume of research on camelina protein is very small. However, the large volume of research on other genetically-related oil seeds proteins, such as canola, mustard, etc. (all are within the flowering plant family Brassicaceae) has served as a framework for studying the isolating property of camelina proteins. Research on canola protein is abundant and was previously demonstrated in the above section. Sadeghi and Bhagva (2009) extracted mustard protein in alkaline solution and performed detoxification with activated carbon treatment. Briefly, defatted mustard meal was mixed with 0.1 M NaCl in a ration 1:15 (w/v), and the pH of the mix was adjusted to pH 11 with 2N NaOH solution. The mixture was continuously shaken for 30 min at room temperature, followed by centrifugation to separate out the pellet. The protein was in the supernatant. It was precipitated by adjusting pH value to 7.0 and could be separated out by centrifugation. With this procedure, approximately 49-56% of the protein in original meal was isolated.

1.3.3 Adhesion performance of sorghum, canola and camelina proteins and protein modification

Protein based adhesives: Little to no research on sorghum, canola and camelina protein-based adhesives is available. However, extensive studies on soy-based adhesives provide clues as to the potential of sorghum, canola and camelina proteins as adhesives.

The mature soybean consists of 38% protein, 30% carbohydrate, 18% oil and 14% moisture, ash and hull (Sun 2005). In the early 1900s, soy protein, extracted from dehulled soybean flakes, showed great potential for adhesives, as shown in its first public disclosure in 1928 (Keimel 1994). Recently, environmental concerns and regulations have increasingly caused soy protein

adhesives to be re-applied in many industrial fields such as paper coating, texture paints, cosmetics, printing inks, plastics, textiles, particleboard, fiberboard, plywood, and many others.

Two predominant storage proteins account for 80% of the total protein content in soybean: glycinin (11S) and β-conglycinin (7S), (Peng et al 1984). 11S is a hexameric protein with a molecular weight of approximately 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, linked by a disulfide bridge. 11S has relatively high cysteine content with 18-20 intra- and intermolecular disulfide bonds (Kella et al 1986). β-Conglycinin (150-200 kDa) is a trimer, consisting of three subunits: α', α, and β. These subunits are non-covalently associated by hydrophobic interaction and hydrogen bonding without disulfide bonds (Thanh and Shibasaki 1978). Inherent differences in structure and molecular properties of 11S and 7S globulins result in varied functional properties of soy protein, such as solubility, thermal and morphological properties, and adhesion performance (Saio and Watanabe 1978; Ning and Villta 1994; Mo et al 2004; Zhang and Sun 2008; Zhang and Sun 2010). To date, soy protein has been studied for adhesion properties 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) (Qi et al 2011). 11S mainly contributes to adhesion strength, especially wet strength of soy protein isolate adhesive. Adhesives made from 11S had higher adhesion strength and water resistance than those made from 7S protein (Mo et al 2004; Wang et al 2005). In addition, due to larger amounts of hydrophobic amino acids in basic subunits from 11S protein, the basic subunits had higher water resistance than acidic subunits (Mo et al 2006). Adhesive made from β subunit with higher content of hydrophobic amino acids also displayed greater water resistance than α α' and β-conglycinin adhesives (Mo et al 2011).

Protein-based adhesives modification to improve water resistance: Much has been done to improve water resistance of soy protein adhesive, making it suitable for exterior-bonded wood products. First, understanding soy protein structure is crucial to the successful modification of soy protein since protein structure closely related to protein's functional properties such as solubility, viscosity, gelling properties, and adhesion performance. Soy protein is characterized by a complex three-dimensional structure of highly ordered amino acids, with hydrophobic groups buried inside and hydrophilic groups exposed outside in nature (Horton et al 1996). The protein structure is primarily stabilized by hydrogen bonding, electrostatic interactions, van der

Waals forces and hydrophobic interactions (non-covalent bond), and covalent disulfide bonds. This highly ordered structure could cause insufficient contact area and limited functional groups available to the wood substrate, especially hydrophobic groups, detrimental to protein adhesion strength. Therefore, in order to function as an excellent protein adhesive, internal interactions among proteins should be destabilized, exposing as much functional groups as possible, especially hydrophobic groups. Many unfolding agents and reducing agents were utilized to disrupt internation interactions among soy proteins, such as alkaline, urea, SDS, cationic detergent, and $NaHSO_3$.

Denaturation agents: Alkali, such as sodium hydroxide, is most commonly used to unfold and denature protein molecules. Water resistance of soy protein was improved by alkali treatment under moderate conditions (pH 10.0 and 50 °C) (Hettiarachchy et al 1995). Urea was also used to denature protein structure because it interacts actively with hydroxyl groups of soy protein and then breaks down hydrogen bonding, resulting in the unfolded protein structure. Zhang and Hua (2007) reported that wettability and adhesive properties of 7S and 11S were improved under 1 M urea modification. Similar to urea, guanidine hydrochloride was also proven to enhance protein hydrophobicity and adhesion strength (Huang and Sun 2000a; Zhong et al 2002). Sodium dodecyl sulfate (SDS) is the anionic detergent which dissociates the protein by disrupting hydrophobic and electrostatic bonds. The reaction possible could move various inside hydrophobic side chains outward where they could interact with hydrophobic moieties of detergent molecules and form micelle-like regions. Previous studies showed that 0.5% concentration of SDS could enhance water resistance and adhesion strength of soy protein adhesive (Huang and Sun 2000b; Mo et al 2004).

In addition, 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). The presence of disulfide bonds in glycinin protein molecules significantly contributes to the functional properties difference between glycinin and conglycinin, such as structural integrity, protein stability, or thermal properties. Reducing agents such as sulfites, bisulfites and sulfates, cleaved inter- and intra-disulfide bonds in protein molecules, thus increasing protein molecule flexibility, solubility, surface hydrophobicity, and decreasing viscosity (Babajimopoulos et al 1983; Kella et al 1986; Kalanathy et al 1996). Many previous studies have revealed insignificant or negative effects of Na_2SO_4, Na_2SO_3 and $NaHSO_3$

modification on adhesion properties of soy protein adhesive (Kalapathy et al 1996; Zhang and Sun 2008; Zhang and Sun 2010). Kalanathy et al (1996) stated that counteracting effects existed between increased hydrophobicity of soy protein and decreased effective wood-protein interfacial area because of the conversion of SH to $-SSO_3$ groups in soy protein. Therefore, adhesion strength and water resistance of soy protein was reduced. Zhang and Sun (2008 2010) also reported that negative effects of $NaHSO_3$ exerted adhesiveness of pure glycinin and β-conglycinin globulin, respectively. However, Qi et al (2011) investigated the effect of $NaHSO_3$ on 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 discovered that a viscous cohesive soy protein system with high solid content and good flowability was obtained through sodium bisulfite modification during the acid precipitation process. The modified soy protein adhesive had excellent adhesion strength and water resistance. In order to compete with commonly used synthetics adhesive resins (60-70% solid content) in the market, protein-based adhesive systems have to possess the following characteristics: high solid content, good flowability, and excellent mechanical strength. Low spread rate and reduced curing time can be achieved at high solid content of adhesives.

Crosslinking agent: Besides destabilizing the protein structure, another principle involved in soy protein adhesion strength improvement is the induction of more entanglement and the formation of a more compact protein complex during thermo setting through the cross-linking agent. This cured protein structure could maintain complex structure better than unmodified protein adhesives during water soaking by reducing penetration of water molecules into the interface of protein and wood. A number of functional groups of protein on amino acid side chains are available for the chemical/crosslinking reaction such as Carboxyl, hydroxyl, amino, disulfide, imidazole, indole, phenolic and sulfhydryl groups (Feeney 1977). Rogers et al (2004) reported that 1,3-dichloro-2-propanol could induce cross-linking of soy protein through the reaction among functional groups and improve soy protein adhesive performance. Zhong and Sun (2007) reported the physical cross-linking reaction between Polyamide-epichlorohydrin (PAE) and soy protein which was reversible and could be manipulated by environmental ionic strength. This complexation formation contributed to the largely improved adhesive strength. Epoxies are active cross-linking agents for alkaline soy adhesives and improve adhesive strength

and durability (Lambuth 1989; Huang 2007). Liu and Li (2007) developed modified soy protein adhesives along with 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 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. In addition, the crosslinking reaction between soy-based adhesive and formaldehyde or its derivatives has also been studied (Wang et al 2007; Qi and Sun 2010). Wang et al (2007) reported that wet strength of soy protein was improved by 115% at optimum concentration of glutaraldehyde (20mM).

Introducing specific functional groups: Another concept of soy protein adhesive modification is to introduce specific groups to soy protein molecules which may contribute to adhesion strength and water resistance and reduce the groups detrimental to adhesion performance. Soy protein is known to contain approximately 20 amino acids attached to the side chain of the protein molecule through the functional groups. These functional groups, such as OH, $-NH_2$, -COOH, -SH, can be used as "reaction bridges" connecting soy protein molecules and specific groups. Mussel protein, often 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 and the soy protein was proven to be transformed to a strong and water-resistant adhesive system. Increasing the free mercapto group content in soy protein also greatly increased adhesion strength and water resistance (Liu and Li 2004). Yamamoto (2000) and Yu (1998) reported that amino acid lysine could enhance adhesion strength of soy protein as well. In Zhu's study (2006), 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).

Adhesive blends: A short-term solution for researchers reducing dependence on petrochemicals and increasing adhesion performance of soy protein is adhesive blends. Extensive research has 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 and Sun 2007; Zhong et al 2007; Kumar et al 2002). The blend system had reduced raw material cost, formaldehyde emission, but enhanced adhesion performance. This concept has been used successfully in industrial fields. Steele et al (1998)

developed blends of soy protein and phenolic resins that cured rapidly at room temperature with excellent water resistance and reduced formaldehyde emissions which could be used for finger jointing green lumber. 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 and not only reduced formaldehyde usage, but also had economic advantages. Qi and Sun (2010) studied the reaction between soy protein adhesive and urea formaldehyde resin and found that blends of SP/UF at ratio of 60/40 had largely reduced apparent viscosity and improved flowability. Wet strength was also enhanced significantly as compared to pure UF resin. Conversely, in order to promote reaction with synthetic polymers, soy protein must be unfolded to expose its available functional groups for the reaction with other adhesive resin. 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 high viscosity, low concentration of soy protein, low water resistance, and short pot life.

This research focuses on destabilizing protein structure to expose functional groups as much as possible, especially hydrophobic groups. Decreasing protein adhesive's viscosity is another concern. All concerns considered, $NaHSO_3$ was selected as adhesive modifier for this research.

Chapter 2 - Adhesive performance of sorghum protein extracted from sorghum DDGS and flour[1]

2.1 Abstract

Distillers dried grains with solubles (DDGS) is the primary co-product from grain-based ethanol production. The objective of this research was to compare adhesive performance of three types of sorghum proteins: acetic acid-extracted sorghum protein from DDGS (PI), aqueous ethanol-extracted sorghum protein from DDGS (PII) and acetic acid-extracted sorghum protein from sorghum flour (PF). Physicochemical properties including amino acid composition and rheological, thermal and morphological properties also were characterized. Results showed that PI had the best adhesion performance in terms of dry, wet and soak adhesion strength, followed by PF and PII. Wet strength of PI at a concentration of 12% protein assembled at 150 ℃ was 3.15 MPa, compared to 2.17 MPa and 2.59 MPa for PII and PF, respectively. DSC thermograms indicated that PF protein isolates contained higher levels of carbohydrates than PI and PII; such non-protein contaminants in the PF could be the reason for its lower adhesion strength than PI. In addition, PI may have more hydrophobic amino acids aligned at the protein-wood interface than PII, which could explain the greater water resistance of PI. Optimum sorghum protein concentration and pressing temperature for maximum adhesion strength was 12% and 150 ℃. PI had a significantly higher wet strength (3.15 MPa) than unmodified soy protein (1.63 MPa for soy protein). The high percentage of hydrophobic amino acids in PI (57%) was likely a key factor in the increased water resistance of PI as compared to soy protein (36% hydrophobic amino acids). These results indicated that sorghum protein has huge potential as an alternative to petroleum-based adhesives.

2.2 Introduction

In the United States, approximately 20 billion pounds of adhesives and resins are used annually in plywood, particleboard, lamination, and various composites for construction,

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[1] Results have been published as a peer-reviewed paper. Li, N., Wang, Y., Tilley, M., Bean, S., Wu, X., Sun, S. X., & Wang, D. (2011). Adhesive performance of sorghum protein extracted from sorghum DDGS and flour. J. Polym. Environ. 19, 755-765.

packaging, furniture, etc. (DWN 2008). These adhesives are derived mostly from petroleum-based chemicals such as phenol-formaldehyde and urea-formaldehyde resins. Formaldehyde-based adhesives dominated the adhesive market in the mid-20th century due to long shelf lives and water resistance (Kumar et al 2008). However, due to finite petroleum resources, non-uniform distribution of these resources, volatile prices, and environmental concerns, the adhesive industry is increasingly interested in bio-based adhesives. Various natural resources, including animal glues, fish glues, casein and vegetable protein glues, starch glues, and blood albumen glues have been used in the wood industry. Several new technologies have been investigated to explore adhesive potential of various biomaterials, including tannins, lignins, carbohydrates, unsaturated oils, liquefied wood rice bran, soy protein, and organisms (Pizzi 2006; Smith and Callow 2006; Wang 2007). Soy protein isolates (SPI) gained much attention over the last century as bio-based and renewable materials. Much research also has been conducted on the use of soy proteins as adhesives in recent years (Wang 2006; Qi and Sun 2010), with a majority of this work focusing on the improvement of water resistance through increased hydrophobicity of soy proteins via chemical modification (Wu et al 2007).

Isolated sorghum proteins, another bio-based material, have been shown to produce biodegradable films and been used as extenders in plywood adhesives and other low-cost adhesives, wallboard and packaging materials (Buffo et al 1997; Ramos et al 1984; Rooney and Waniska 2000). Soy proteins containing a mixed content of hydrophilic and hydrophobic amino acids are less hydrophobic than sorghum kafirins (Icoz et al 2005). It is speculated that sorghum proteins may also have similar functions as soy proteins used as adhesives and, therefore, may provide better water resistance than soy protein when used as adhesive.

Grain sorghum is the third most important cereal crop in the United States and fifth in the world. The United States is the number-one producer and exporter of sorghum (Texas Tech University 2009). In the United States each year, approximately 10 million acres of sorghum are harvested and used mainly as animal feed, and approximately 25% of the sorghum crop is used for ethanol production (U.S. Grain Council 2010). DDGS) are a co-product of the distillation and dehydration process during ethanol production (Bonnardeaux 2009). DDGS contains approximately 30 to 40% of protein and serves as an inexpensive source of protein. More than 700 million lbs. of sorghum protein would have been available from DDGS in 2009 when sorghum production was 500 million bushels in the United States (Feed Outlook 2010).

Kafirins (prolamins) are the main components in sorghum protein, accounting for approximately 70 to 90% of total storage protein (Hamaker et al 1995). Several methods have been investigated for extracting kafirins from sorghum flour or bran for food and other uses. Based on solubility, kafirins can be extracted from sorghum or DDGS with alcohol (Wong et al 2009; El Nour et al 1998; Emmambux and Taylor 2009; Taylor et al 1984), acetic acid (Taylor et al 2005), or alkaline sodium borate/SDS buffers (Zhao et al 2008; Hamaker et al 2003; Park and Bean 2003). Wang et al. (2009) extracted sorghum protein from DDGS with NaOH-ethanol, acetic acid and HCl-ethanol, and found that using acetic acid and NaOH-ethanol as extraction solvents was more efficient for protein extraction than others tested. Building on this work, the objective of this study was to compare adhesive performance of sorghum protein extracted using different methods and to characterize physicochemical properties of proteins, including amino acid composition and rheological, thermal and morphological properties.

2.3 Materials and methods

2.3.1 Materials

Sorghum DDGS with 14.4% moisture content (wet base) was provided by White Energy (Russell, Kan.). Decorticated sorghum grains were provided by USDA-ARS Center for Grain and Animal Health Research (Manhattan, Kan.). Sodium metabisulfite, sodium sulfite, glacial acetic acid, ethyl alcohol and petroleum ether were purchased from Fisher Scientific (Pittsburgh, PA). Absolute ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, Ky.). Soy protein isolates (SPI) were provided by Bio-Materials and Technology Lab (Kansas State University, Manhattan, Kan.). Cherry wood samples with dimensions of 127 mm (length) $\times$ 50 mm (width) $\times$ 5 mm (thickness) were provided by Veneer One (Oceanside, NY).

2.3.2 Protein extraction using acetic acid

Acetic acid-extracted sorghum protein was prepared according to the method described by Taylor et al. (2005). Sorghum DDGS was milled with a cyclone sample mill (Udy Corp., Fort Collins, Colo.) into a powder with a particle size of <0.5 mm and then presoaked in four volumes of 0.5% (w/w) sodium metabisulfite for 16 h at 70 rpm agitation in a Gyromax 939 XL incubator shaker (Amerex Instruments, Inc., Lafayette, Calif.). After soaking, samples were centrifuged (Thermo IEC, Needham Heights, Mass.) at 3500 $\times$ g for 10 min and the supernatant discarded.

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Pellets remaining after centrifugation were then mixed with five volumes of glacial acetic acid and stirred for 1 h. The mixture was centrifuged at 3500 × g for 10 min and the supernatant was decanted through a six-layer cheesecloth to remove the topmost oil layer. The pH of the supernatant was slowly adjusted to 5.0 with 50% (w/v) NaOH in a beaker placed in an ice water bath. The mixture was kept overnight at 4 ℃ and then centrifuged at 3500 × g for 10 min. The precipitates were rinsed and washed with distilled water by centrifuging at 3500 × g for 10 min three times and then oven-dried at 49 ℃. The protein was defatted three times by mixing with a 5-fold weight of petroleum ether followed by shaking in the incubator shaker at 70 rpm for five min and centrifuging at 3500 × g for 10 min, then kept under a fume hood overnight to evaporate the solvent.

2.3.3 Protein extraction using ethanol

The ethanol extract method described by Emmambux et al. (2003) was used. Milled sorghum DDGS was mixed with 10-fold 70% ethanol, 0.35% NaOH (w/v) and 0.5% sodium metadisulfite (w/v). The mixture was placed in a water bath at 70 ℃, stirred for 1 h, and then centrifuged at 3500 × g for 10 min. Ethanol content in the supernatant was diluted to 40% and the suspension was put in a freezer at -20 ℃ overnight. The suspension was centrifuged at 3500 × g for 10 min and the precipitates were rinsed and washed three times with distilled water and oven-dried overnight at 49 ℃. The product was defatted following the procedure described previously and milled into powder.

2.3.4 Chemical analysis

Protein content was measured using nitrogen combustion via a LECO FP-2000 nitrogen determinator (St. Joseph, Mich.) according to AOAC method 990.03 (1995). Nitrogen was converted to protein using a factor of 6.25. Fat content was determined using the Soxhlet petroleum-ether extraction method according to AOAC method 920.39C for cereal fat and expressed as weight percentage on dry basis (1995). Crude fiber was determined according to AOCS-approved procedure Ba 6a-05 (1996).

2.3.5 Amino acid composition analysis

Samples were weighed and placed in approximately 0.5 ml of 6 N HCl solution along with the internal standard and hydrolyzed at 110 ℃ for 20 h. An aliquot, typically 10 or 20 μl,

was then diluted to 250 μl with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 μl of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 $\times$ 200 mm, 5 μm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, and 0.3% tetrahydrofuran, pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). Elution conditions went from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

2.3.6 Rheological properties

Rheological measurements of sorghum protein suspension with different concentrations were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, Mass.) with a CP 4/40 cone and plate fixture (4 ° cone angle, 40-mm cone diameter). Distance between cone and plate was set to 150 μm for all measurements. Experiments were conducted under steady shear flow at 23 ℃. Shear rates ranged from 10 to 240 s^{-1} at 10 s^{-1} increment. All experiments were done in duplicate, and average values were reported.

2.3.7 Morphological properties

A model CM 100 transmission electron microscopy (FEI Company, Hillsboro, Ore.) was operated at 100 kV. Protein samples (3% in acetic acid, w/w) were absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, Pa.) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, Vt.) for 60 s at room temperature before being viewed by transmission electron microscopy (TEM).

2.3.8 Differential Scanning Calorimetry (DSC)

Thermal transition properties of protein samples were measured with a TA Instruments DSC Q200 V24.4 instrument (TA Instruments, New Castle, Del.) that was calibrated with indium and zinc before making official measurements. Samples of sorghum proteins weighing approximately 10 to 15 mg were measured in a hermetic aluminum pan under a nitrogen

atmosphere with a gas flow rate of 50 mL/min. All samples were heated from 25 to 280 ℃ at a heating rate of 10 ℃/min in an inert environment. All experiments were performed in duplicate.

2.3.9 Wood specimen preparation

Cherry wood samples were preconditioned in a controlled-environment chamber (Model 518, Electro-tech systems, Inc., Glenside, Pa.) for 7 d at 25 ℃ and 50% relative humidity (RH). Sorghum proteins suspended in acetic acid at different concentrations and stirred with a magnetic stirrer for three hours were brushed separately along the edges of two pieces of cherry wood, with an application area of 127 mm $\times$ 20 mm, until the entire area was completely covered. Approximately 0.06 g of adhesive was applied on each piece and controlled using a pipette and consistent brushing procedure. The brushing and setting procedure followed the method described by Mo et al. (2004). The two pieces of suspension-brushed cherry wood were allowed to rest open at room temperature for 15 min, and then were assembled and pressed at a pressure of 3.57 MPa at 130 ℃, 150 ℃ or 170 ℃ for 10 min, respectively, using a hot press (Model 3890 Auto 'M', Carver Inc., Wabash, Ind.).

2.3.10 Mechanical properties

After pressing, the glued-wood assemblies were conditioned at 23 ℃ and 50% RH for two days and then cut into 5 127 mm (length) $\times$ 20 mm (width) $\times$ 5 mm (thickness) specimens. The cut specimens were conditioned for another five days at 23 ℃ and 50% RH before the dry test. Three adhesion strengths were tested: dry strength, soak strength, and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron (Model 4465, Canton, Mass.) according to ASTM Standard Method D2339-98 (2002). Crosshead speed of Instron for adhesion strength testing was 1.6 mm/min. Tensile strength at maximum load was recorded as adhesion strength. Reported results are an average of five samples.

Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002), respectively. Preconditioned specimens were soaked in tap water at 23 ℃ for 48 h and then tested immediately for wet strength. For the soak strength test, specimens were soaked in tap water at 23 ℃ for 48 h and then conditioned at 23 ℃ and 50% RH for an additional seven days before testing.

2.3.11 Statistical analysis

With the exception of the mechanical property evaluation which required an average of five samples, all experiments were carried out in duplicate. Data were analyzed using the analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level, according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, N.C.).

2.4 Results and discussions

2.4.1 Chemical composition of sorghum protein from DDGS and sorghum flour

Chemical composition of sorghum protein extracted under various methods is summarized in Table 1. The purity of ethanol-extracted protein (PII) (93.05%) was higher than that of acetic acid-extracted sorghum protein from DDGS (PI) (87.77%). When using acetic acid as buffer to extract protein from DDGS, NaOH solution was used to adjust the pH value to 5 in order to precipitate the protein. During this step, sodium acetate salt could be formed, which may explain why PI had lower purity than PII when the formed sodium acetate salt was not washed out completely (Taylor et al 2005). In addition, more protein was extracted from DDGS than directly from sorghum flour (PF) (71.82%), possibly due to a strong association between protein and non-protein components in sorghum flour, such as carbohydrate, as was the case for sorghum protein extraction conducted by Wang et al. (2009). However, protein purities were lower than values reported by Wang et al. (2009).

Table 2.2.1 Chemical composition of sorghum DDGS, sorghum flour, and sorghum proteins

Type of raw materials	Composition of extracted protein (%, dry basis)		
	Protein	Lipids	Crude fiber
DDGS	30.84	8.95	5.27
Sorghum flour	8.70	1.18	1.25
PI[1]	87.77b[4]	1.40b	0.79b
PII[2]	93.05a	2.60a	0.97a
PF[3]	71.82c	2.30a	0.84b

[1]PI=acetic acid-extracted sorghum protein from DDGS.

2.4.2 Amino acid composition

Results of amino acid analysis are shown in Table 2.2. Compared to results in Table 2.1, protein purities for the three proteins calculated based on accumulation of total amino acids were much lower than values obtained by the LECO FP-2000 nitrogen determinator (St. Joseph, Mich.) method. Lower accumulation of total amino acids could be attributed to the existence of non-protein nitrogen in DDGS or sorghum flour (Hamaker et al 1995; Yousif and El Tinay 2001), or destruction of tryptophan and cysteine by the liquid HCl hydrolysis assay during the amino acid composition test. Because the total amount of amino acids did not include tryptophan and cysteine, estimates of total protein and concentration would be slightly lower than the true number shown in Table 2.2. This difference in protein could also be due to the nitrogen conversion factor, 6.25, in approved methods, but some good data states the conversion factor for sorghum should be 5.8 (Mosse et al 1988).

Table 2.2 also shows that molar concentration of glutamic acid, leucine and asparagine in DDGS was decreased by 6.89%, 10.65%, and 29.79%, respectively, compared to sorghum flour. However, isoleucine, lysine, methionine, tyrosine, arginine, threonine, glycine, and histidine in DDGS increased by a greater extent, from 10.65% to 152.92%, among which histidine, lysine, methionine and threonine are essential amino acids. The increase of essential amino acids during the fermentation process improved sorghum protein's nutritional value and was partly due to the usage of yeast during fermentation (Yousif and El Tinay 2001; Cookman and Glatz 2009).

Table 2.2 Molar percentages of amino acids of different proteins

Amino acids (%)	PI[1]	PII[2]	PF[3]	DDGS	Sorghum	SPI[4]
Aspartic (ASX)	5.2b[5]	4.9b	4.4b	5.2b	5.6b	8.5a
Glutamine (GLX)	20.1a	18.4ab	17.7ab	11.7c	16.7b	16.2b
Serine (SER)	5.7b	6.2b	6.3ab	6.4ab	6.5ab	7.1a
Histidine (HIS)	1.2a	1.4ab	0.6ab	2.2a	1.4ab	2.0ab
Glycine (GLY)	2.3b	2.8b	2.1b	7.0a	6.1a	7.6a
Threonine (THR)	3.1c	3.0c	3.4bc	4.6a	3.7b	4.6a
Alanine (ALA)	16.6a	16.9a	16.9a	14.7b	14.6b	6.6c
Arginine (ARG)	1.5e	1.5e	2.3d	3.8b	2.9c	7.7a
Tyrosine (TYR)	3.4a	3.3a	3.5a	2.3b	0.9c	2.5b
Valine (VAL)	4.7c	4.8c	5.1b	5.7a	5.3b	5.0bc
Methionine (MET)	0.4d	0.5e	0.7d	1.4a	0.8c	1.2b
Phenylalanine (PHE)	4.9ab	4.9ab	5.1a	4.4c	4.4c	4.5bc
Isoleucine (ILE)	4.3bc	4.3bc	4.5b	4.5b	4.0c	4.9a
Leucine (LEU)	16.7a	16.8a	16.8a	13.4b	14.2b	8.4c
Lysine (LYS)	0.2de	0.0e	0.6d	3.0b	2.2c	6.9a
Proline (PRO)	9.7a	10.3a	10.0a	9.7a	10.8a	6.3b
T-AA (%, m/m)	100a	100a	100a	100a	100a	100a
T-protein (%, w/w)	67.2a	69.9a	52.1b	22.4c	6.20d	67.3a

[1]PI=acetic acid-extracted sorghum protein from DDGS.
[2]PII=ethanol-extracted sorghum protein from DDGS.
[3]PF=acetic acid-extracted sorghum protein from sorghum flour.
[4]SPI=soy protein isolates.
[5] Means in the same row followed by different letters are significantly different at $P<0.05$

Different amino acid compositions were detected in extracted sorghum kafirins as compared to DDGS and sorghum flour. Increases in glutamic acid, alanine, tyrosine and leucine were observed in extracted kafirins, while histidine, glycine, threonine, arginine, methionine and lysine decreased extensively compared with DDGS and sorghum flour. Similar results were also reported by Cookman et al. (2009). Four major classes of proteins in sorghum are glutelins, kafirins, albumins, and globulins (Youssef 1998), and each class has distinct amino acid profiles (Yousif and El Tinay 2001; Skoch et al 1970; Wu and Wall 1980). For instance, Yousif and Tinay reported that albumin and globulin proteins of sorghum had higher levels of lysine

compared to kafirins (Yousif and El Tinay 2001). This is most likely why isolated kafirins showed different amino acid content compared with sorghum flour and DDGS.

Amino acids can be classified into groups according to polarity, structure, nutritional requirements, and metabolic fate. Based on hydrophobicity, amino acids can be grouped into hydrophobic (non-polar amino acids) and hydrophilic (polar amino acids) types (Kyte and Doolittle 1982). Alanine, methinine, phenylananine, isoleucine, leucine, and proline belong to hydrophobic amino acids and accounted for 57.32% to 59.04% in three extracted sorghum protein kafirins (Table 2.3). These values were higher than numbers reported by Mokrane et al. (2010), who reported a range of hydrophobic amino acids from 45% to 50% in sorghum protein. This difference may be attributed to varying sorghum sources or measurement methods. Amino acid compositions can affect adhesive performance of protein-based adhesives (Liu and Li 2004; Zhao et al 2009; Kamino 2001), which will be discussed in detail in the next section.

Table 2.3 Hydrophilcity properties of amino acids in different proteins

Amino acids (% of total)	PI[1]	PII[2]	PF[3]	DDGS	Sorghum	SPI[4]
Hydrophobic[6]	57.32a[5]	58.49a	59.04a	53.79a	54.1a	36.89b
Hydrophilic[7]	42.68b	41.51b	40.96b	46.21b	45.9b	63.11a

[1]PI=acetic acid-extracted sorghum protein from DDGS.
[2]PII=ethanol-extracted sorghum protein from DDGS.
[3]PF=acetic acid-extracted sorghum protein from sorghum flour.
[4]SPI=soy protein isolates.
[5]Means in the same row followed by different letters are significantly different at P<0.05.
[6]Hydrophobic amino acid=alanine, methinine, phenylalanine, isoleucine, leucine and proline.
[7]Hydrophilic amino acid=lysine, tyrosine, arginine, threonine, glycine, histidine, serine, glutamine and asparagine.

2.4.3 Rheological properties

Rheological properties of extracted proteins are shown in Figure 2.1. The maximum viscosity was 1 Pa s, approximately 1,000x the viscosity of water, meaning that the sorghum protein suspensions had good flowability properties. Apparent viscosity increased as protein concentration increased, and viscosity decreased as shear rate increased, indicating that sorghum proteins in suspension showed shear thinning properties which can be expressed by the Herscher-Bulkley model: $\tau = \tau_0 + K\dot{\gamma}^n$ where τ_0 is the yield stress (N/m^2), τ is the shear stress

(N/m^2), $\dot{\gamma}$ is the shear rate (s^{-1}), and n and K are the flow behavior index and consistency index, respectively. The method of least squares was used to find the best-fitting equation: Estimate a τ_0 $(=\tau_{01})$ by extrapolating the plot τ vs $\dot{\gamma}$, plotting $\ln \tau$ vs $\ln \dot{\gamma}$, and getting K_1 and n_1 from linear regression by using Microsoft Excel. Then, K_1 and n_1 are reinserted into the equation and $\ln \tau$ vs $\ln \dot{\gamma}^n$ is plotted to get τ_{02} and K_2 from linear regression. Finally, τ_{01} and τ_{02} are compared until $\tau_{01} = \tau_{02}$, then τ_0 $(=\tau_{01})$, K $(= K_1 = K_2)$, and n $(= n_1)$ are obtained. Values of τ_0, n, and K are summarized in Table 2.4.

Table 2.4 Rheological parameters of sorghum proteins with different concentrations: yield stress (τ0, N/m2), flow behavior index (n), consistency index (K)

Rheological parameters	PI[1]				PII[2]				PF[3]			
	8%	10%	12%	16%	8%	10%	12%	16%	8%	10%	12%	16%
τ_0	0.20	0.10	0.10	0.20	0	0	0	0	0.01	0.10	0.40	4.00
n	0.55	0.49	0.60	0.61	0.63	0.53	0.69	0.64	0.49	0.57	0.61	0.83
K	0.38	0.65	0.96	0.87	0.08	0.09	0.27	0.25	0.83	0.96	0.96	1.21

[1]PI=acetic acid-extracted sorghum protein from DDGS.
[2]PII=ethanol-extracted sorghum protein from DDGS.
[3]PF=acetic acid-extracted sorghum protein from sorghum flour.
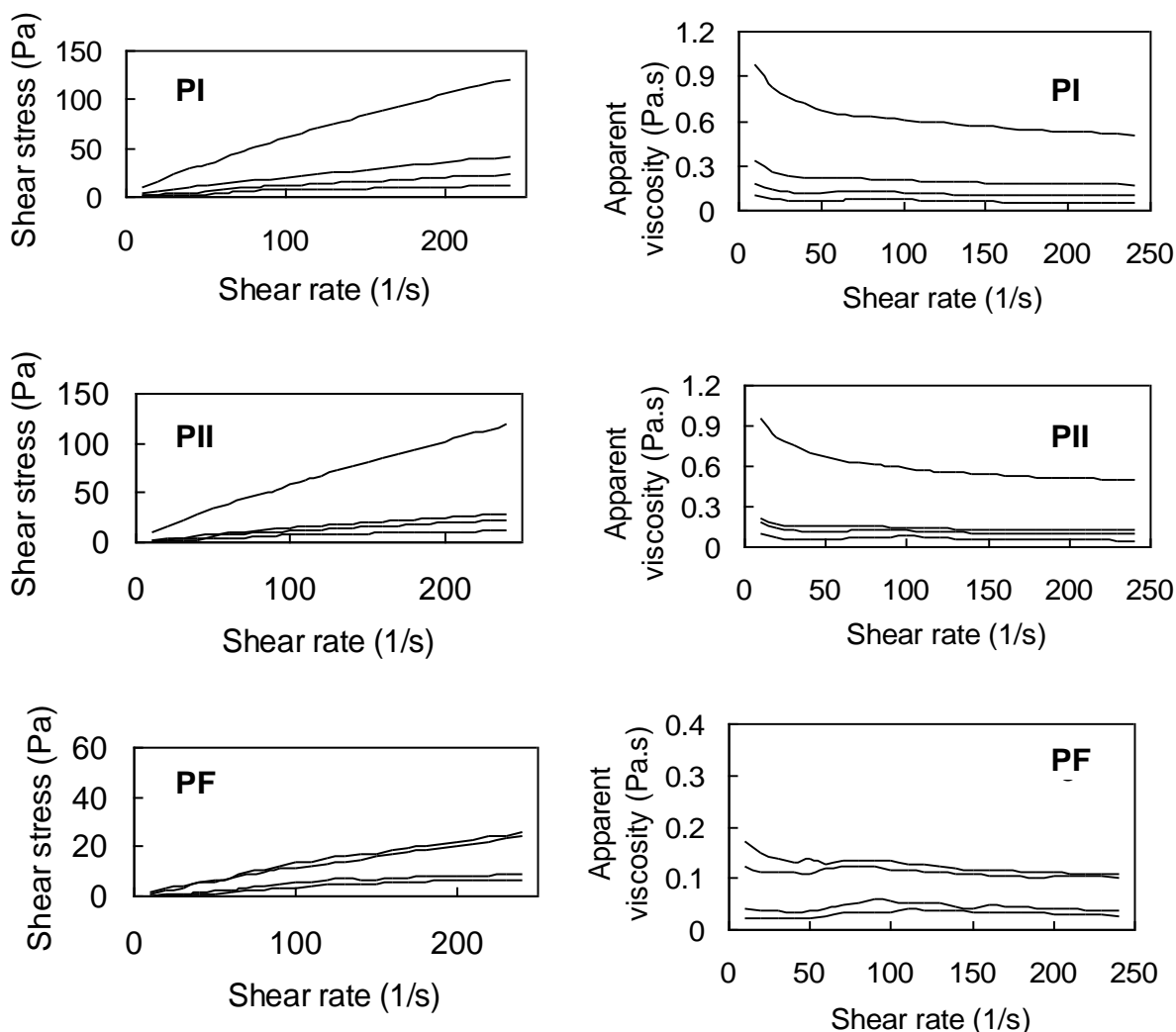
Figure 2.1 Shear behavior of sorghum proteins. Curves from top to bottom are sorghum adhesives with 8, 10, 12, and 16% protein concentrations, respectively. PI=acetic acid-extracted sorghum protein from DDGS, PII=ethanol-extracted sorghum protein from DDGS, and PF=acetic acid-extracted sorghum protein from sorghum flour.

2.4.5 Morphological properties

Microstructures of protein dispersions are displayed in Figure 2.2. Protein particles in suspension were evenly distributed, and PI and PF had larger particles than PII. The configuration of PI may differ from PII. PI may have more hydrophobic amino acids aligned at the interface than PII, and additional hydrophobic amino acids in PII may have been wrapped inside the particle. Some small particles surrounded the protein particle of the PF. Carbohydrate, including starch, was not fermented and remained in the sorghum flour. The strong association

between protein and non-protein could have resulted in some non-protein residue remaining in the protein extract, as indicated by small particles present in the protein suspension made from sorghum flour protein.

PI-1
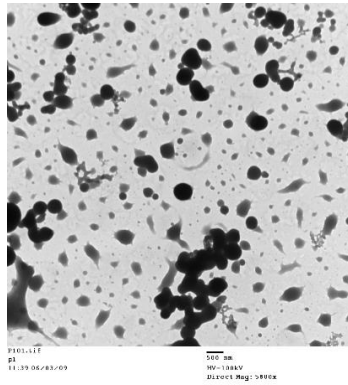
PI-2
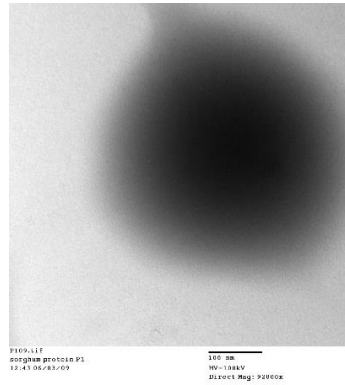
PII-1
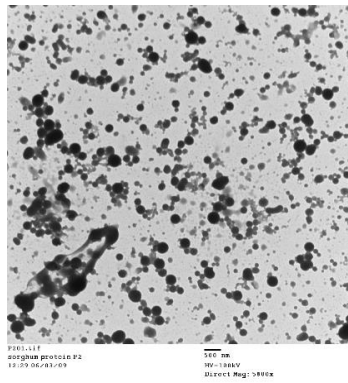
PII-2
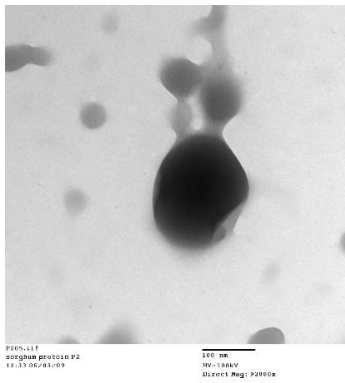
PF-1
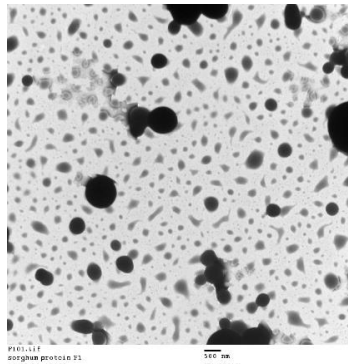
PF-2
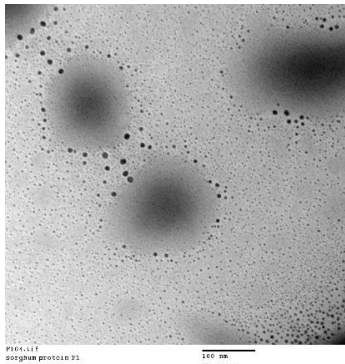
Figure 2.2 TEM micrographs of sorghum proteins at 3% solid content. Magnification: PI-1-PF-1, ×5800; PI-2-PF-2, ×92k. Scale bar represents 500 and 100 μm for ×5800 and ×92k magnifications, respectively.

2.4.6 DSC thermal transition properties

As shown in Figure 2.3, the DSC thermogram of PF exhibited extensive differences as compared to curves for PI and PII. A sharp and strong endothermic peak at approximately 57.9

℃ was observed for PF, while only trivial endothermic peaks around 63.8 ℃ and 62.5 ℃ were detected for PI and PII, respectively. The endothermic peak shown near this temperature range has been firmly related to starch gelatinization (Aboubacar and Hamaker 1999; Kelsall et al 1999; Zhan et al 2006). The strong signal at 57.9 ℃ observed in PF indicated more starch in the PF, whereas PI and PII have less starch content, as evidenced by tiny peaks; these results also can be reflected by lower protein purity of PF, shown in Table 1. PF also exhibited another endothermic peak with a temperature of 110.5 ℃, probably representingt protein denaturization. No peaks were observed around 110.5 ℃ in PI and PII, indicating that protein in sorghum DDGS already had been denatured during the fermentation process. Current results also confirmed previous results reported by Zhao et al. (2008) and Wang et al. (2009), who suggested that protein cross-linkage and denaturation occurred during fermentation. Strong exothermic peaks that occurred with the onset temperature around 176.3 ℃, 184.8 ℃, and 164.1 ℃ for PI ,PII, and PF, respectively, may be attributed to protein aggregation, as suggested by Mo et al. (2004). Protein aggregation may have a negative effect on adhesion performance when considering that mechanical strength decreased significantly at the assembling temperature of 170 ℃ as compared to 150 ℃ and 130 ℃ for PI and PF (Table 5). During protein aggregation, the exposed functional groups in sorghum protein-based adhesives that bonded with wood surface probably refolded again, resulting in weak bonding capacity between adhesives and wood surface. However, aggregation properties of sorghum protein need further study to determine conclusive results.
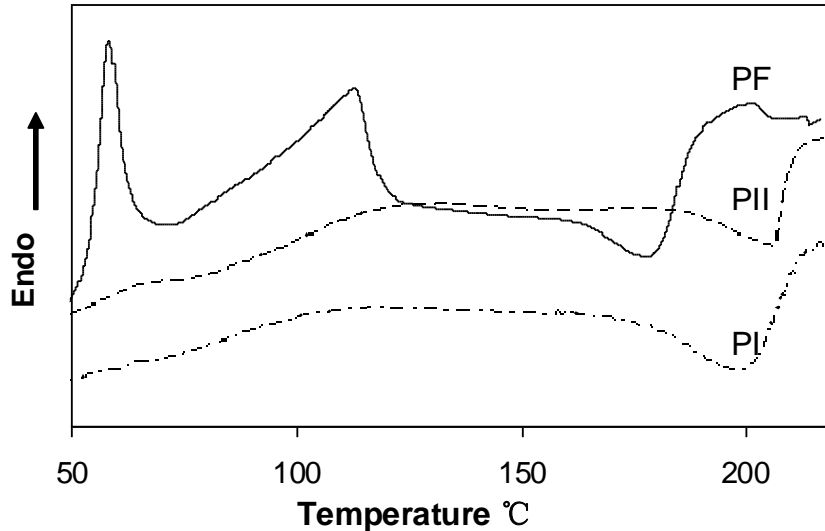
Figure 2.3 Thermograms of sorghum proteins; PI=acetic acid-extracted sorghum protein from DDGS, PII=ethanol-extracted sorghum protein from DDGS, and PF=acetic acid-extracted sorghum protein from sorghum flour.

2.4.7 Mechanical properties of sorghum protein

Sorghum protein concentration, pressing temperature, extraction methods and protein sources all had significant effects on adhesion properties of sorghum protein (Table 2.5). In general, wood failure occurred in almost all dry and soaked samples, indicating that bonding strengths between cherry wood and sorghum adhesives were stronger than the mechanical strength of cherry wood when resisting shear force. However, adhesives failure, not wood failure, was observed for wet samples. To data, adhesion strength of sorghum protein increased as sorghum protein concentration increased, and adhesion strength virtually leveled off when protein concentration increased to 16%. Increasing pressing temperature from 130 ℃ to 150 ℃ had a positive effect on protein adhesion strength, while increasing temperature to 170 ℃ had different effects on adhesion properties of three distinct sorghum protein isolates. PI and PF with 12% concentration exhibited a decrease in wet strength from 3.15 and 2.59 MPa to 1.92 and 1.19 MPa, respectively, as pressing temperature increased from 150 ℃ to 170 ℃. However, for PII with a concentration of 12%, wet strength increased to 2.51 from 2.17MPa as pressing temperature increased to 170 ℃ from 150 ℃. If further increasing the pressing temperature of PII to 190 ℃, its dry, soak and wet strength decreased to 4.21, 4.07 and 1.33 MPa, respectively

(Dates were not listed in Table 2.5). PI had better adhesion performance in terms of wet, dry and soak strength than that of the PII and PF, especially for wet strength. For example, wet strength for PI was 3.15 MPa at a pressing temperature of 150 ℃ and a concentration of 12%, compared with 2.17 MPa for PII and 2.59 MPa for PF.

Adhesion between proteins and wood surfaces occurs as the protein spreads, wets and penetrates the porous wood structure to achieve mechanical interlocking, physical attraction, and chemical bonding between wood and protein during the setting period, followed by entanglements and cross-link through physical attraction and chemical bonding between protein and wood and between protein and protein during thermal setting procedure (Wang et al 2006b). With increasing protein concentration, more protein is available to bond with wood and deliver greater strength. However, as protein concentration increases beyond optimum levels (>16%), protein-protein interaction could dominate the protein-wood interaction, resulting in uneven distribution of proteins or insufficient exposure of hydrophobic groups at the wood surface. As a result, limited contribution to adhesion performance improvement was observed when protein concentration reached 16%. Increasing press temperature had marked effects on solvent evaporation, immobilization of protein molecules, and possibility of chemical and physical interaction between proteins and wood surface. Conversely, when higher temperatures were applied to adhesives with high protein concentrations, more hydrophobic amino acids were available for hydrophobic interaction, leading to greater protein cohesion but possible detriment to the bonding ability between protein and wood (Wang et al 2007).

Lower protein purity (Figure 2.3, PF) and small non-protein particles surrounding the PF (Figure 2.2, PF-2) extracted from sorghum flour could explain why adhesive strengths were lower than PI extracted from DDGS. Smaller particles of PII make contact surface area between protein and protein not as great as PI and PF. Furthermore, as explained in the section on morphological properties, PI may have more hydrophobic amino acids aligned at the interface than PII, whereas more hydrophobic amino acids in PII may have been wrapped inside the particle, accounting for the increased water resistance of PI.

Table 2.5 Adhesion performance of sorghum protein adhesives

Extraction methods	Wet strength (MPa)			Dry strength (MPa)			Soaked strength (MPa)		
8% protein content	130 ℃	150 ℃	170 ℃	130 ℃	150 ℃	170 ℃	130 ℃	150 ℃	170 ℃
PI[1]	1.21±0.15	1.71±0.08	1.23±0.22	4.82±0.43	4.85±0.42	5.52±0.23	4.07±0.16	4.70±0.32	4.51±0.08
PII[2]	1.22±0.10	1.27±0.07	1.81±0.10	4.07±0.32	4.01±0.39	4.07±0.36	3.63±0.28	3.73±0.17	3.91±0.17
PF[3]	1.71±0.26	1.67±0.09	1.44±0.08	4.69±0.28	4.52±0.13	4.51±0.25	4.08±0.29	4.22±0.11	4.18±0.16
10% protein content									
PI[1]	1.89±0.09	2.32±0.16	1.44±0.08	5.05±0.27	5.49±0.30	5.25±0.19	4.93±0.18	4.90±0.21	4.32±0.08
PII[2]	1.32±0.14	1.70±0.15	1.85±0.18	5.18±0.07	4.62±0.08	4.85±0.19	4.79±0.08	4.54±0.32	4.61±0.26
PF[3]	1.63±0.14	1.88±0.13	1.19±0.07	4.68±0.21	4.42±0.29	4.17±0.10	4.14±0.05	4.32±0.21	4.06±0.10
12% protein content									
PI[1]	2.89±0.18	3.15±0.11	1.92±0.10	5.08±0.26	5.05±0.17	4.78±0.39	4.26±0.13	4.42±0.26	4.92±0.48
PII[2]	1.61±0.10	2.17±0.04	2.51±0.13	4.95±0.29	5.02±0.14	4.98±0.13	4.23±0.15	4.33±0.35	4.61±0.28
PF[3]	2.23±0.13	2.59±0.07	1.19±0.14	4.70±0.25	4.52±0.16	4.49±0.17	4.43±0.12	4.35±0.12	4.35±0.06
16% protein content									
PI[1]	3.01±0.10	3.29±0.19	1.32±0.10	5.16±0.20	5.08±0.30	4.85±0.30	4.95±0.17	4.71±0.29	4.50±0.14
PII[2]	1.49±0.10	1.92±0.09	2.42±0.14	4.95±0.19	5.13±0.13	4.85±0.10	4.90±0.08	5.10±0.15	5.44±0.17
PF[3]	2.36±0.22	2.45±0.10	1.13±0.04	5.00±0.14	5.23±0.08	4.36±0.12	4.90±0.20	4.89±0.27	4.22±0.24

[1]PI=acetic acid-extracted sorghum protein from DDGS, [2]PII=ethanol-extracted sorghum protein from DDGS, [3]PF=acetic acid-extracted sorghum protein from sorghum flour.

2.4.8 Comparison of sorghum protein and soy protein isolates (SPI) adhesives

To date, soy protein-based adhesives are considered the most promising bio-based adhesives to partially replace petroleum-based adhesives because of their excellent adhesion performance on wood and other materials, as reported by Wool and Sun (2005). Table 6 compares adhesion performance between soy protein isolates (SPI) and various sorghum kafirin proteins (PI, PII, PF) at a concentration of 12%. Sorghum protein showed excellent water resistance, which commonly has been the biggest challenge for soy protein adhesives. For instance, wet strength of PI adhesive (2.89, 3.15 and 1.92 MPa) was much higher than those of unmodified soy protein adhesives (1.61, 1.63 and 1.98 MPa) under pressing temperatures of 130 °C, 150 °C and 170 °C, respectively.

Amino acid composition and overall hydrophobicity of proteins are essential factors affecting protein adhesive performance. As shown in Tables 2 and 3, large differences in amino acid composition between sorghum proteins and soy protein were observed. In terms of total hydrophobic amino acids, sorghum protein was much higher (about 58 mol %) than soy protein (~37 mol %). Hydrophobic protein adhesives repelled water when assembled cherry wood samples were soaked, ensuring interaction between the wood boards and boundaries formed by protein adhesives. Wood surfaces remained intact. Conversely, hydrophilic protein adhesives may absorb water when applied on cherry wood board, thus destroying cohesion between adhesives and wood surface. Furthermore, cross-linking among kafirins may offer another reason why sorghum protein adhesives had better water resistance than soy protein. Kafirins are known to cross-link significantly when heated (Belton et al 2006; Shull et al 1991) and may have done so when the adhesives were heated.

Table 2.6 Comparison of adhesion performance of sorghum protein adhesives and soy protein isolates at 12% protein concentration

Hot press condition	Sorghum protein (MPa)			SPI[4] (MPa)
	PI[1]	PII[2]	PF[3]	
Wet strength				
130 ℃	2.89± 0.18	1.61± 0.10	2.23± 0.13	1.61
150 ℃	3.15± 0.11	2.17± 0.04	2.59± 0.07	1.63
170 ℃	1.92± 0.10	2.51± 0.13	1.19±0.14	1.98
Dry strength				
130 ℃	5.08± 0.26	4.95± 0.29	4.70± 0.25	4.55
150 ℃	5.05± 0.17	5.02± 0.14	4.52± 0.16	5.29
170 ℃	4.78± 0.39	4.98± 0.13	4.49± 0.17	4.88
Soaked strength				
130 ℃	4.26± 0.13	4.23± 0.15	4.43± 0.12	4.17
150 ℃	4.42± 0.26	4.33± 0.35	4.35± 0.12	4.35
170 ℃	4.92± 0.48	4.61± 0.28	4.35± 0.06	4.42

[1]PI=acetic acid-extracted sorghum protein from DDGS.
[2]PII=ethanol-extracted sorghum protein from DDGS.
[3]PF=acetic acid-extracted sorghum protein from sorghum flour.
[4]SPI=soy protein isolates.

Another advantage of sorghum protein used as adhesive compared to soy protein is that sorghum protein adhesives need less energy than soy protein adhesive when assembling the wood board using a hot press method. Results have shown that 150 ℃ for sorghum protein was the optimum temperature, whereas soy proteins typically need 170 ℃ or higher to achieve maximum strength (Table 2.6).

However, some challenges are notable for the use of sorghum protein isolates as adhesives. First, finding a low-cost solvent to dissolve isolated sorghum kafirins for adhesive use was

difficult; in contrast, uniform suspension of soy proteins can be obtained by mixing soy protein with water. In addition, the lower efficiency of sorghum protein recovery and complicated extraction procedures compared with soy protein are a concern. Further research is needed to improve extraction of sorghum proteins.

2.5 Conclusions

Sorghum protein extracted from sorghum DDGS and sorghum flour with different methods had distinct adhesion performances. Results showed that PI had the best adhesion performance, followed by PF and PII, especially for wet strength. The wet strength of PI at a 12% concentration assembled at 150 °C was 3.15 MPa, compared to 2.17 MPa for PII and 2.59 MPa for PF. Low protein purity caused by non-protein materials of PF may be the primary cause for lower adhesion strength than PI. In addition, PI may have more hydrophobic amino acids aligned at the interface than PII, thus explaining the greater water resistance of PI. Optimum sorghum protein concentration and pressing temperature for maximum adhesion strength is 12% and 150 °C. Compared with soy protein-based adhesives, PI had advantages such as significantly higher water resistance and lower energy input. These results indicate that sorghum protein displays huge potential as an alternative to petroleum-based adhesives.

Chapter 3 - Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite[2]

3.1 Abstract

The objective of this research was to study adhesion properties of sodium bisulfite ($NaHSO_3$)-modified canola protein. Protein was extracted from canola meal through alkali solubilization and acid precipitation methods, then modified with different concentrations of $NaHSO_3$ (0g/L to 15 g/L) during the isolation process. As $NaHSO_3$ concentration increased, canola protein purities decreased. Amino acid profiles showed that hydrophobic amino acids in canola protein constituted only 27% of total protein, indicating that canola protein is mostly hydrophilic. Reducing effects of $NaHSO_3$ were exerted on canola protein through the breaking of disulfide bonds in both its cruciferin and napin components, as reflected by the protein electrophoresis profile, DSC data, and morphological images. Wet protein isolates were used as adhesives. The greatest wet shear strength of canola protein adhesive without modification was 3.97 MPa with 100% wood cohesive failure (WCF), observed at a curing temperature of 190 ℃. $NaHSO_3$ had slight weakening effects on adhesion performance of canola protein. Canola protein modified with 3g/L $NaHSO_3$ exhibited wet shear strength similar to the control at 190 ℃ and higher strength at 150 ℃ and 170 ℃. The $NaHSO_3$ modification significantly improved handling and flowability of canola protein adhesives.

3.2 Introduction

In the current market, prominent commercial adhesives for wood composites are petroleum-based resins such as phenol-formaldehyde (PF), urea-formaldehyde (UF), melamine-formaldehyde (MF) and isocyanates. To alleviate issues with limited petroleum resources and environmental pollution, many efforts have been made to develop bio-based adhesives, including soybean protein adhesive, lignins, animal glue, and blood-based adhesives, of which soy protein

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[2] Results have been published as a peer-reviewed paper. Li, N., Qi, G., Sun, X. S., Stamm, M. J., & Wang, D. (2012). Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite. J. Am. Oil Chem. Soc. 89:897-908.

is one of the most promising and most investigated (Wool and Sun 2005). Another concern has been raised, however, about competition between bio-based products and human food and animal feed. Consequently, researchers should explore other renewable resources with adhesive potential.

Canola, also known as rape, oilseed rape, rapa, or rapeseed, has 40% oil and 17-26% protein content and ranks as the third-largest oilseed crop produced worldwide after soybeans and palm. Worldwide production of canola increased rapidly from 30.1 million metric tons (MT) in 2008–2009 to approximately 58.4 MT in 2010–2011 (USDA 2011). Although canola proteins possess a well-balanced amino acid composition, the meal is not used in human food applications due to the presence of glucosinolates (which interfere with thyroid function, thus reducing growth), erucic acid (which has potential to produce toxic effects in the heart), phytates (which strongly bind polyvalent metal ions such as zinc and iron and make them unavailable for metabolism), and phenolics (which are bitter flavored and make protein products darker in color). Napin (2S), cruciferin (12S), and oleosin are major components in canola protein and constitute 20%, 60%, and 8%, respectively, of the total protein in mature seeds (Hoglund et al 1992). High-value use of canola protein has been studied. Isolated canola proteins, another bio-based material, have been shown to be capable of producing biodegradable materials such as films (Sung-Ae et al 2010) and thermal plastics (Manamperi et al 2010), but reports on application of canola protein for biodegradable adhesives are limited. Narayanamurti et al. (1943) studied rapeseed (canola) protein-formaldehyde dispersions as plywood adhesives and found that optimum curing conditions for canola protein-based adhesives require a temperature of 120 ℃, pressure of 1.38 MPa, and time of 10-15 min. Optimum data for shear strength of canola protein adhesives was 1.92 MPa with wood failure rate of 30% in dry shear strength and 1.38 MPa with 0% wood failure in wet shear strength.

Technical challenges for protein-based adhesives are high viscosity, low water resistance, and short pot life. Studies on soy protein adhesives focus on improving water resistance by using modifiers, such as denaturants, cross-linking agents, and reducing agents. In the study by Qi and Sun (2011), soy protein adhesive with high solid content and excellent water resistance was extracted directly from soy flour slurries and modified with sodium bisulfite ($NaHSO_3$) using acid precipitation. Sodium bisulfite ($NaHSO_3$) functions as a reducing agent to modify protein-based adhesives by cleaving disulfide bonds in protein. It also is capable of lowering viscosity of

wet protein isolates, as well as improving soy protein's properties of flowability, solubility, and hydrophobicity (Zhang and Sun 2011). Similar to soy protein, cruciferin and napin canola protein fractions contain disulfide linkages among protein polypeptides, and the napin protein structure is stabilized mainly by disulfide bonds; therefore, it is speculated that canola protein may also have functions similar to soy protein adhesives and adhesion performance could be improved by $NaHSO_3$ treatment. Preliminary results revealed that when sodium bisulfate concentration was over 20 g/L, adhesion strength decreased significantly. Therefore, $NaHSO_3$ concentrations of 0, 3, 6, 9, or 15 g/L were evaluated in this research.

A limitation of previous studies on canola protein adhesive was that purity of the protein isolate was as low as 47.5% (Narayanamurti et al 1943). The objective of this research was to study adhesion property of high purity canola protein extracted from canola meal and modified with different concentrations of $NaHSO_3$, as well as characterizing physicochemical properties of the protein, such as amino acid composition and electrophoresis profile, and thermal, rheological, and morphological characteristics.

3.3 Materials and Methods

3.3.1 Materials

Canola meal with 11.0% moisture content (wb) was purchased from Planet Natural (Bozeman, Mont.). Sodium bisulfite ($NaHSO_3$), hexanes, hydrochloric acid (HCl), 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 $50 \times 127 \times 5$ mm (width $\times$ length $\times$ thickness) were provided by Veneer One (Oceanside, N.Y.).

3.3.2 Canola Protein Extraction

Protein was isolated from canola meal as described by Manamperi et al (2010), with modifications. Canola meal was first dried overnight in an oven at 49 ℃. Dried canola meal was milled with a cyclone sample mill (Udy Corp., Fort Collins, Colo.) into powder to ensure that particle size of the meal was <0.25 mm. Then, dried and milled canola meal was defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 h at room temperature in three cycles. The defatted meal was placed in a fume hood overnight to remove residual hexane. The defatted and dried canola meal was mixed with distilled water at the solid/liquid ratio of 1:12 (w/v), and the

slurry was presoaked for 1 h with stirring. Then, $NaHSO_3$ was added to the slurry in concentrations of 0, 3, 6, 9, or 15 g/L separately on the basis of water volume, and the pH value of the slurry was adjusted to 12 with 6 mol/L NaOH solution. The slurry was stirred for another 2 h at room temperature to solubilize the protein in canola meal. The slurry mixture was centrifuged at 12,000 × g for 15 min, and the supernatant was decanted through a six-layer cheesecloth to remove impurities on top of the supernatant. The pH value of the supernatant was then slowly adjusted to 3.5 with 2 mol/L HCl solution to precipitate the protein. The mixture was centrifuged again at 12,000 × g for 15 min to isolate wet canola protein. A portion of the wet protein was used as adhesive and the remainder was freeze-dried and ready for physicochemical analysis.

3.3.3 Chemical Analysis

Protein content was measured by combustion via a LECO FP-2000 nitrogen determinator (St. Joseph, Mich.) according to AOAC method 990.03 (1995). Nitrogen was converted to protein using a factor of 6.25. Fat content was determined through the Soxhlet petroleum-ether extraction method according to AOAC method 920.39C for cereal fat and expressed as weight percentage on a dry basis (1995). Crude fiber was determined according to AOCS-approved procedure Ba 6a-05 (1996).

3.3.4 Electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system as described by Laemmli (1970). A canola protein sample was mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To study disulfide bonds in canola protein, SDS-PAGE under both reducing (2-mercaptoethonal) and non-reducing conditions were carried out. A total of 8 μg of protein was applied to sample wells. Molecular weight standards (14.4-97.4 kDa) 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. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodak, Rochester, N.Y.).

3.3.5 Amino Acid Composition Analysis

Amino acid composition was analyzed following the procedure described by Li et al (2011). Approximately 100 mg for each sample were weighed, placed in 0.5 mL of 6 N HCl solution along with the internal standard, and hydrolyzed at 110 °C for 20 h. An aliquot, typically 10 or 20 μl, was then made up to 250 μL with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 μL of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 × 200 mm, 5 μm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, 0.3% tetrahydrofuran, and pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). Elution conditions progressed from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

3.3.6 Rheological Properties

Apparent viscosities of isolated canola proteins with 30% solid content (wb) were determined using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, Mass.) with a CP 4/40 cone and plate fixture (4° cone angle, 40-mm cone diameter). 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 rates ranged from 10-240 s^{-1} in increments of 10 s^{-1}. All experiments were done in duplicate, and average values were reported.

3.3.7 Transmission Electron Microscopy (TEM)

A model CM 100 TEM (FEI Company, Hillsboro, Ore.) was operated at 100 kV. Canola protein isolates were first dissolved in distilled water with a solid concentration of 0.01% (W/W). The pH value of the mixture was adjusted to 12 to dissolve the protein isolates, and then was lowered to 3.5 to precipitate the protein isolate by imitating protein isolation conditions. Prepared protein samples were absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort

Washington, Pa.) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, Vt.) for 60 s at room temperature before being viewed by TEM.

3.3.8 Scanning Electron Microscopy (SEM)

A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of dried canola protein isolate powder. The ground protein powder was 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, N.J.). SEM images of the protein isolates were performed with operation conditions at an accelerating voltage of 5 kV.

3.3.9 Differential Scanning Calorimetry (DSC)

Thermal transition properties of protein samples were measured with a DSC Q200 V24.4 instrument (TA Instruments, New Castle, Del.) that was calibrated with indium and zinc before making official measurements. Samples of dry canola proteins weighing approximately 7–10 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL/min. All samples were heated from 25 ℃ to 280 ℃ at a heating rate of 10 ℃/min in an inert environment. All experiments were performed in duplicate.

3.3.10 Wood specimen preparation

Cherry wood samples were preconditioned in a controlled-environment chamber (Model 518, Electro-tech systems, Inc., Glenside, Pa.) for 7 d at 25 ℃ and 50% relative humidity (RH). Canola protein adhesives, isolated with different methods, were brushed separately along the edges of two pieces of cherry wood with an application area of 127 mm $\times$ 20 mm until the entire area was completely covered. The adhesive amount applied on each piece was approximately 0.06 g (dry basis). The brushing and setting procedure followed the method described by Mo et al. (2004). Brushed areas of the two pieces were assembled together at room temperature for 15 min, then pressed at a pressure of 3.57 MPa at 150 ℃, 170 ℃, or 190 ℃ for 10 min using a hot press (Model 3890 Auto 'M', Carver Inc., Wabash, Ind.).

3.3.11 Mechanical Properties

After pressing, the glued-wood assemblies were conditioned at 23 °C and 50% RH for two days, then cut into five specimens, each measuring 127 mm (length) $\times$ 20 mm (width) $\times$ 5 mm (thickness). The cut specimens were conditioned for an additional five days at 23 °C and 50% RH before the dry test. Three adhesion strengths were tested: dry strength, soak strength, and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron (Model 4465, Canton, Mass.) according to ASTM Standard Method D2339-98 (2002). The crosshead speed of Instron for adhesion strength testing was 1.6 mm/min, and adhesion strength was recorded as tensile strength at the maximum load. Reported results are an average of five samples.

Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002), respectively. Preconditioned specimens were soaked in tap water at 23 °C for 48 h and then tested immediately for wet strength. For the soak strength test, specimens were soaked in tap water at 23 °C for 48 h and then conditioned at 23 °C and 50% RH for an additional seven days before testing.

3.3.12 Statistical Analysis

Data from the mechanical property evaluation were taken from an average of five samples. Data from experiments carried out in duplicate were analyzed through analysis of variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, N.C.).

3.4 Results and Discussion

3.4.1 Chemical Composition of Canola Protein and Canola Meal

$NaHSO_3$-modified canola protein recovery rate and partial proximate composition are summarized in Table 3.1. The protein recovery rate from canola meal increased slightly from 31.33% to 34.37% as the $NaHSO_3$ concentration increased from 0 g/L to 15 g/L. Moisture content of extracted canola protein isolates after centrifuging increased as $NaHSO_3$ concentration increased, implying that the water bonding capacity of protein isolates increased due to the existence of $NaHSO_3$. Water bonding capacity in isolated canola protein could affect adhesion performance, which will be discussed in the Mechanical Properties section.

Defatted canola meal contained approximately 49.3% protein. A higher purity of dried canola protein isolates was found in the control canola protein (CP-0, 87.30%). When $NaHSO_3$ concentration increased from 0 g/L to 15 g/L, isolated canola protein purity decreased from 87.30% to 84.47%. Impurities, such as lipids and crude fibers, were detected in small amounts in all isolated proteins. Other impurities are speculated to be phenolic compounds or glucosinolates which cannot be removed with regular extraction methods (Frank and Kazimierz 1984).

Table 3.1 Recovery rate and partial proximate composition of canola meal and protein isolates produced by modification with various concentrations of $NaHSO_3$.

Canola protein samples	Protein recovery (%, db)	Moisture content (%, db)	Composition of protein sources (%, db)		
			Protein	Lipids	Fiber
D-CM[1]	--		49.26c	0.89a	8.62a
CP[2]-0	31.33	72.64b[3]	87.30a	0.22b	0.05b
CP-3	34.37	72.85b	86.46a	0.22b	0.03b
CP-6	33.71	77.8a	83.90b	0.33b	0.01b
CP-9	32.89	76.61a	84.84b	0.29b	0.02b
CP-15	34.06	77.78a	84.47b	0.24b	0.01b

[1]D-CM: Defatted canola meal
[2]CP: Canola protein modified by 0, 3, 6, 9, 15 g/L $NaHSO_3$
[3]Means in the same column followed by different letters are significantly different at $P<0.05$

3.4.2 Amino Acid Composition

The amino acid composition in $NaHSO_3$-modified canola protein is shown in Table 2. Canola protein purities based on the total sum of amino acids were much lower than values obtained by the nitrogen combustion method. The difference could be attributed to non-protein nitrogen in the canola meal and protein isolates. Krishnamoorthy reported that the concentration of non-protein nitrogen in isolated canola protein could be up to 26.8% (Krishnamoorthy et al 1982). Another reason for the difference may be the destruction of tryptophan and cysteine by liquid HCl hydrolysis assay during the amino acid composition analysis. Because the total amount of amino acids did not include tryptophan and cysteine, estimates of total protein and concentration would be slightly lower than the true number, as shown in Table 2.

Amino acid composition (Table 3.2) is comparable to published data (Slominski et al 1999); however, relatively huge differences in glutamate, serine, and glycine content were noticed, possibly because of varied canola varieties. Table 2 also shows that amounts of aspartate, tyrosine, methionine, and phenylalanine in isolated canola protein were greater than those in canola meal, whereas glutamate and lysine content were lower. The decrease in lysine content during canola protein extraction was also observed by Shahidi et al. (1992) and attributed to formation of lysinoalanine in the alkaline extractant. Four classes of proteins in canola meal are extractable: glutelins, prolamins, albumins, and globulins (Manamperi et al 2007). Each class may have different amino acid profiles. Only portions of albumins, glutelins, and globulins are extractable with alkaline extraction (Manamperi et al 2007) which is most likely why isolated canola proteins showed distinct amino acid content than canola meal.

Amino acids can be classified into groups according to polarity, structure, nutritional requirements, and metabolic fate. Based on hydrophobicity, amino acids can also be grouped into hydrophobic (non-polar) and hydrophilic (polar) types. Alanine, methionine, phenylananine, isoleucine, leucine, and proline belong to the hydrophobic group and account for 27.68% to 27.92% of canola protein isolates (Table 3.2). a previous study has demonstrated that amino acid compositions can affect adhesive performance of protein-based adhesives (Li et al 2011). Further discussion will occur in detail in the next section.

Table 3.2 Amino acid compositions of canola meal and canola protein isolates modified with NaHSO$_3$ at various concentrations.

Amino acid (Mol %)	D-CM[1]	CP[2] modified with NaHSO$_3$				
		CP-0	CP-3	CP-6	CP-9	CP-15
Aspartate	8.44	10.54	10.42	10.27	10.48	10.22
Glutamate	22.37	20.79	20.48	20.52	21.12	20.91
Serine	5.47	5.42	5.47	5.44	5.40	5.39
Histidine	3.32	3.03	3.06	3.11	3.03	3.04
Glycine	6.07	6.25	6.24	6.19	6.23	6.18
Threonine	5.45	5.00	4.97	4.92	4.72	4.78
Alanine	5.40	5.26	5.35	5.31	5.20	5.27
Arginine	6.95	7.01	6.87	7.02	6.91	7.05
Tyrosine	3.23	3.81	3.85	3.91	3.80	3.87
Valine	6.05	5.97	5.94	5.87	5.83	5.82
Methionine	1.74	1.97	2.10	2.17	2.15	2.12
Phenylalanine	5.00	5.62	5.54	5.54	5.58	5.54
Isoleucine	5.80	5.77	5.78	5.76	5.70	5.78
Leucine	8.86	9.11	9.15	9.14	9.06	9.06
Lysine	5.85	4.46	4.77	4.83	4.79	4.97
T-AA (%)	100	100	100	100	100	100
T-protein (%)	34.84	65.76	65.45	63.67	63.67	63.45
Hydrophobic[3]	26.80	27.72	27.92	27.92	27.68	27.76
Hydrophilic[4]	73.20	72.28	72.08	72.08	72.32	72.24

[1]D-CM: Defatted canola meal
[2]CP: Canola protein modified by 0, 3, 6, 9, 15 g/L NaHSO$_3$
[3]Hydrophobic: Alanine, methinine, phenylalanine, isoleucine, leucine and proline
[4]Hydrophilic: Lysine, tyrosine, arginine, threonine, glycine, histidine, serine, glutamine and asparagine

3.4.3 Rheological Properties

Viscosity is an important physical property that governs behavior of wood adhesives. Low viscosity allows for easy handling and good flowability on wood surfaces. Apparent viscosity decreased as shear rate increased (Figure 3.1), indicating that canola protein isolates showed shear thinning properties. Unmodified canola protein adhesive exhibited maximum viscosity around 9,000 Pa.s. Apparent viscosities of modified proteins decreased notably as $NaHSO_3$ concentration increased, thus improving handling and flowability of canola protein. Minimum apparent viscosity of approximately 1,200 Pa.s was observed at $NaHSO_3$ concentration of 15 g/L (Figure 3.1). The apparent viscosity of protein is manipulated by intermolecular interaction, such as electrostatic interactions and disulfide bonds (Cheng 2004). As a reducing agent, $NaHSO_3$ could break disulfide bonds among polypeptides, dissociating canola protein into smaller subunits. As a result, weakening protein-protein interactions reduced the apparent viscosity of $NaHSO_3$-canola protein adhesives.
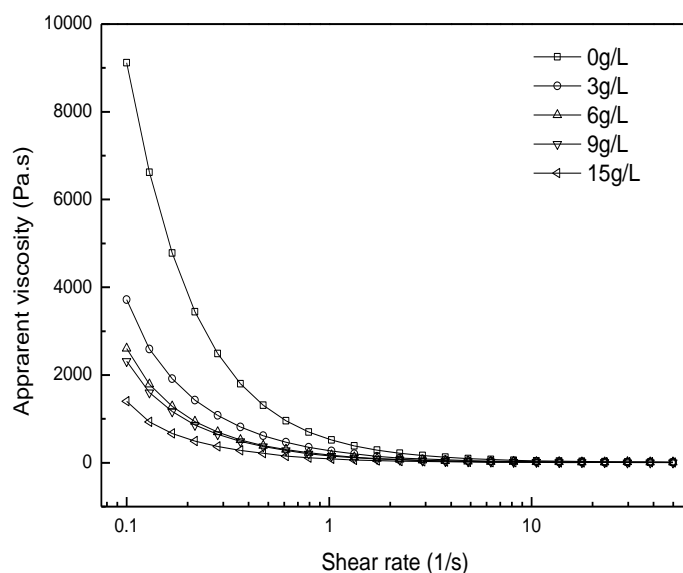
Figure 3.1 Shear rate dependence of apparent viscosity of canola protein adhesives with 30% solid content (wb) modified with NaHSO3 at various concentrations.

3.4.4 Morphological Properties

TEM images of $NaHSO_3$-canola proteins are displayed in Figure 3.2. In unmodified canola protein, irregular, highly dense protein clusters were observed as a mixture of spherical and rod-shaped clusters with diameters from 30 to 250 nm (Figure 3.2 A). The protein clusters were dissociated into smaller protein aggregates when canola protein was treated with $NaHSO_3$. Their diameters decreased as $NaHSO_3$ concentration increased (Figure 3.2 B-E), with the exception of 6 g/L $NaHSO_3$ (Figure 3.2 C). In this sample, branch-like materials dispersed among the small protein particles probably were non-protein substance, which may also explain the lower purity than other samples (Table 3.1).
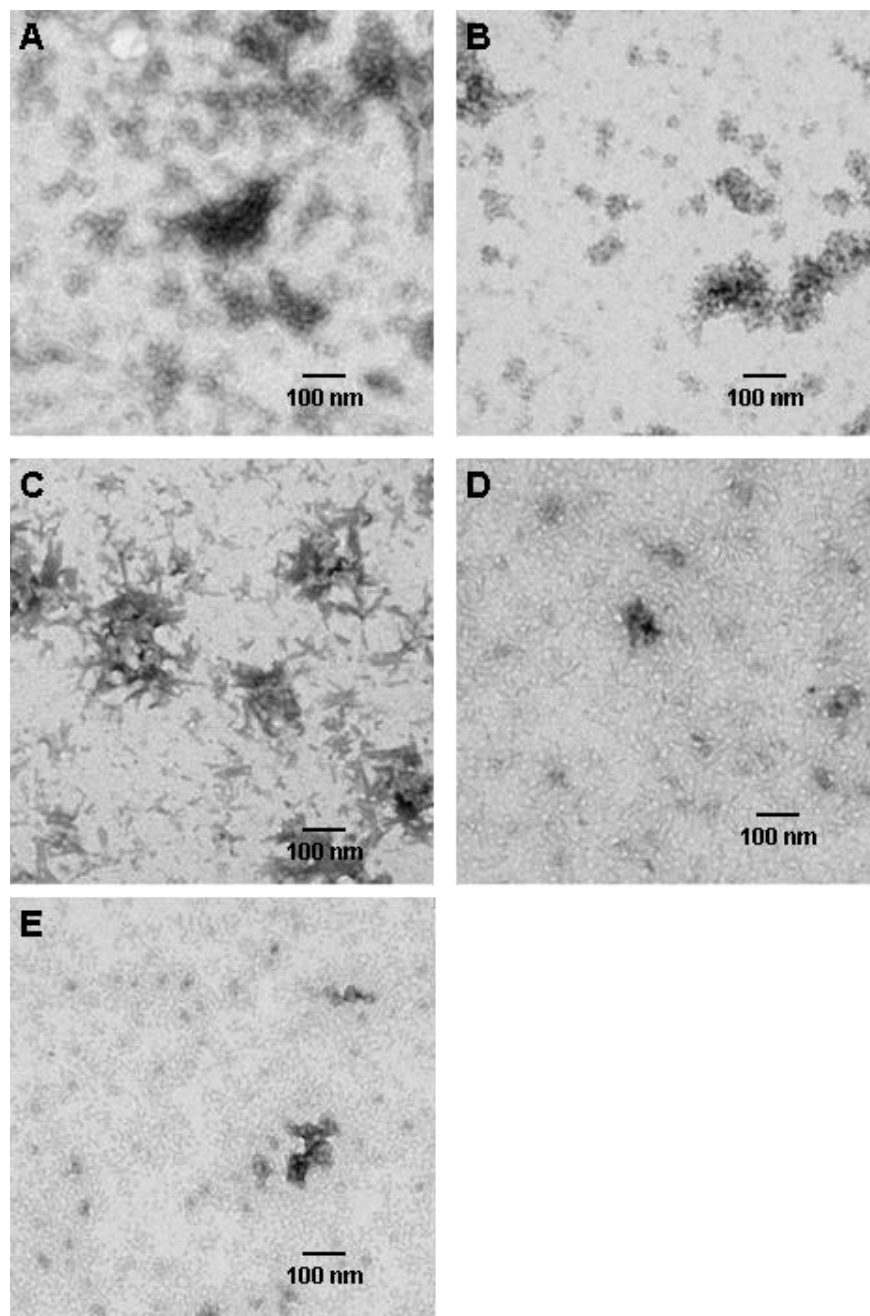
Figure 3.2 TEM images of canola meal and canola protein adhesives modified with $NaHSO_3$ at various concentrations: 0g/L $NaHSO_3$ (A); 3g/L NaHSO3 (B); 6g/L $NaHSO_3$ (C); 9g/L $NaHSO_3$ (D); 15g/L $NaHSO_3$ (E). Magnification: $\times 64k$. Scale bar represents 100 nm.

The highly dense protein clusters in the control canola protein (Figure 3.2 A) indicated that strong protein aggregation occurred in the sample. Most of the surface of protein aggregates is considered hydrophilic because the hydrophobic portion is buried through protein aggregation

(Sun et al 2008). Reduced protein aggregation was achieved in $NaHSO_3$-modified canola protein by breaking disulfide bonds between proteins and introducing negatively charged groups (SO_3^-) to protein molecules (Sun et al 2008). As a result, some hydrophobic groups could be exposed on the canola protein surface, followed by the aggregates dissociation process. Exposing more hydrophobic groups can be beneficial for adhesion performance of canola protein.

SEM images (Figure 3.3) showed that, in defatted canola meal (top left), protein granules with a diameter of approximately 12 μm were wrapped with fibers or another non-protein component. For the unmodified canola protein sample (Figure 3.3 A), a loosely cross-linked protein network with non-uniform size pores was observed; this fairly rough surface can probably be attributed to strong protein aggregation among canola protein molecules. In contrast, $NaHSO_3$-modified canola protein exhibited a regular and smooth surface due to less extensive protein aggregation (Figure 3.3 B-E).
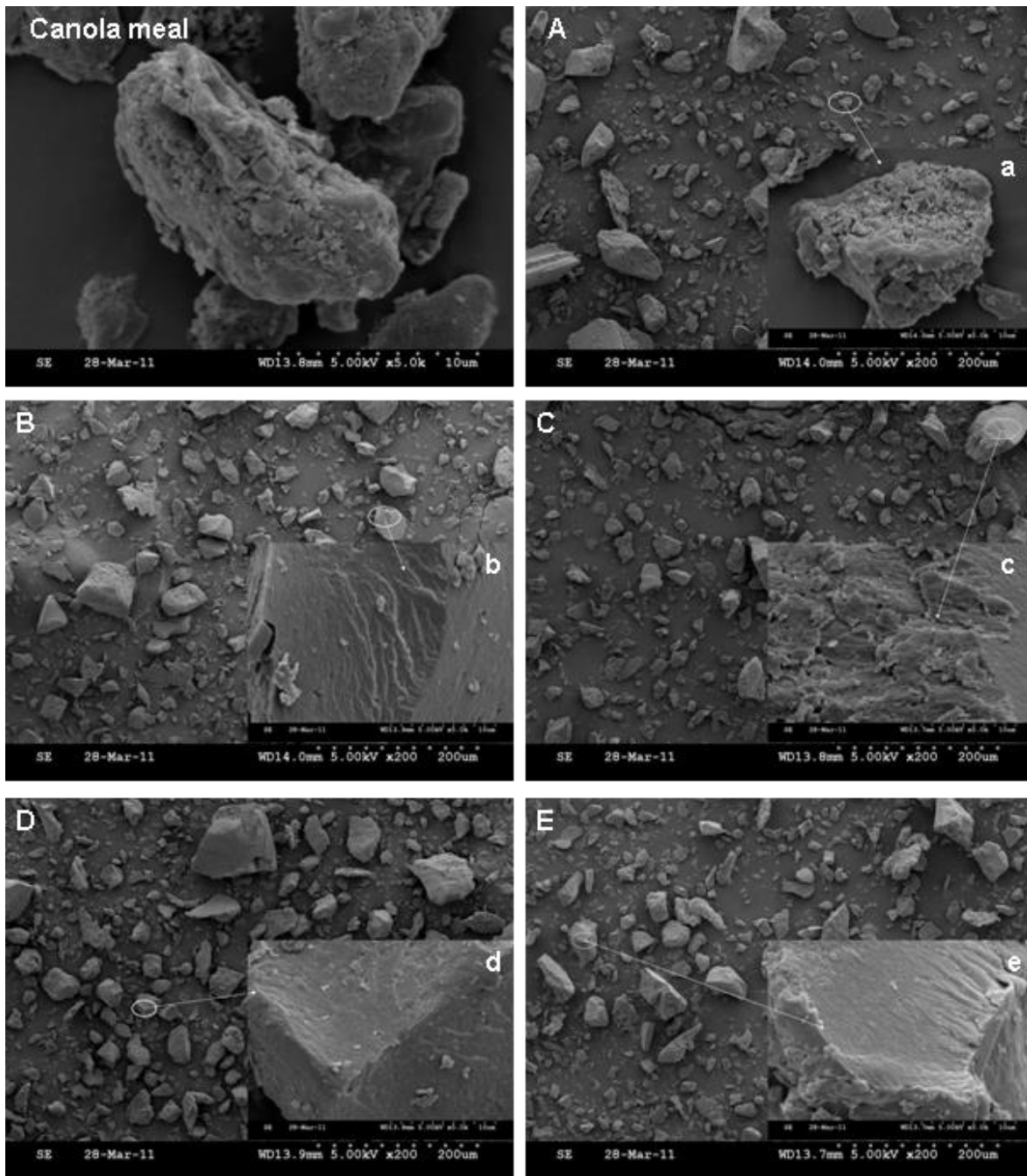
Figure 3.3 SEM images of canola meal and canola protein adhesives modified with NaHSO$_3$ at various concentrations: Canola meal, ×5000; 0g/L NaHSO3 (A,×200; a, ×5000); 3g/L NaHSO3 (B,×200; b, ×5000); 6g/L NaHSO3 (C,×200; c, ×5000); 9g/L NaHSO3 (D,×200; d, ×5000); 15g/L NaHSO3 (E,×200; e, ×5000).

3.4.5 SDS-PAGE

In the absence of ME (Figure 3.4 A-E), two major components in native canola protein, cruciferin (12S) and napin (2S), were observed with polypeptides' molecular weights ranging from 17 to 55 kDa. Cruciferin subunits were composed of bands with MW above 20 kDa, and napin subunits had 17 and 12 kDa bands (Wu and Muir 2008). In modified canola protein samples, intensity of polypeptide bands at 17 kDa changed insignificantly at $NaHSO_3$ concentrations in the range of 3-9 g/L (Figure 4 B-D); however, bands almost disappeared when the concentration of $NaHSO_3$ was 15 g/L (Figure 3.4 E), and density of smaller polypeptides at 9 kDa increased concomitantly, indicating the presence of disulfide bonds in napin protein molecules. Reducing effects of $NaHSO_3$ on protein can break disulfide linkage and dissociate the protein into smaller polypeptide chains. A concentration of 15 g/L was apparently needed to completely break disulfide bonds in napin. Reducing effects of $NaHSO_3$ could also be observed by attenuated bands at 55 kDa, belonging to cruciferin subunits stabilized by disulfide bonds, and intense bands at 30 kDa, belonging to disassociated polypeptides (Uruakpa and Arntfield 2006).

In addition, high molecular weight canola protein aggregates were observed on top of the resolving gel in the absence of ME. Some bands remained in the presence of ME (Figure 3.4 F-J), suggesting that these protein aggregates were stabilized by other covalent bands in addition to disulfide bonds.
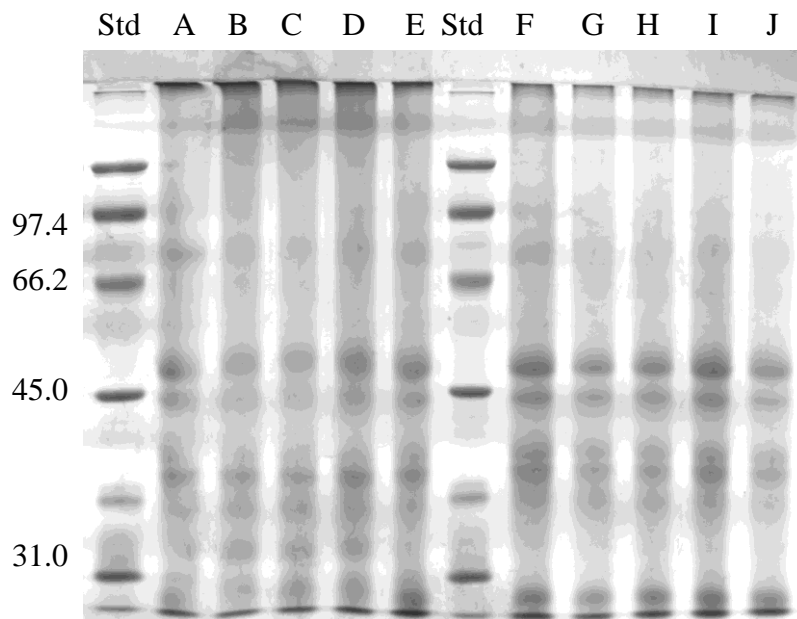
Figure 3.4 Non-reducing SDS-PAGE pattern of NaHSO$_3$-modified canola protein in the absence of 2-mercaptoethanol (lane A-E) and reducing SDS-PAGE pattern in the presence of 2-mercaptoethanol (lane F-J): 0g/L NaHSO$_3$ (lane A, F); 3g/L NaHSO$_3$ (lane B, G); 6g/L NaHSO$_3$ (lane C, H); 9g/L NaHSO$_3$ (lane D, I); 15g/L NaHSO$_3$ (lane E, J).

3.4.6 Differential Scanning Calorimetry (DSC)

In DSC thermograms of NaHSO$_3$-modified canola proteins (Figure 3.5), the first endothermic peak at approximately 65 ℃ was ascribed to water evaporation. The broad endothermic peak was attributed to thermal denaturation of two major canola proteins: cruciferin and napin (Manamperi et al 2010). As shown in Table 3.3, both denaturation temperature (T$_d$) and enthalpy (ΔH) of canola protein were obviously affected by NaHSO$_3$. The T$_d$ decreased gradually as the NaHSO$_3$ concentration increased, from 124 ℃ for unmodified CP to 115 ℃ for CP treated with 15 g/L NaHSO$_3$. Meanwhile, enthalpy of CP decreased by 30% after modification with 15 g/L NaHSO$_3$. Destabilized canola protein indicated that the reducing properties of NaHSO$_3$ were exerted on protein polypeptide chains connected by disulfide bonds; NaHSO$_3$ could dissociate compact canola cruciferin and napin to a less stable protein structure/conformation. Results were consistent with CP SDS-PAGE data, demonstrating that disulfide bonds were cleaved in modified CP.
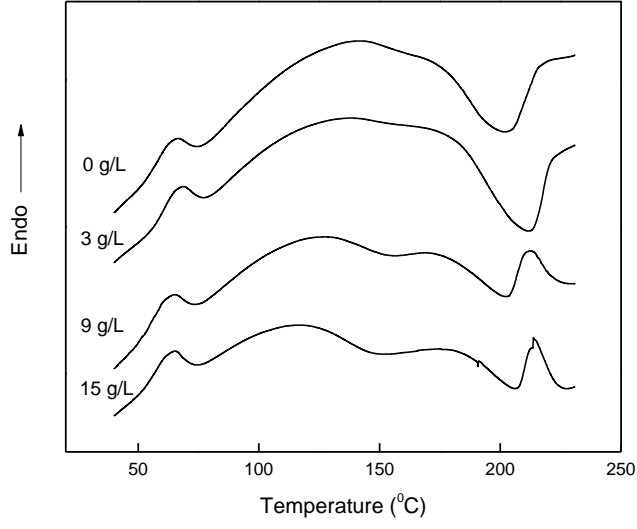
Figure 3.5 DSC thermograms of canola protein modified with $NaHSO_3$ at various concentrations.

Table 3.3 Thermal transition characterization of canola proteins modified with $NaHSO_3$ at various concentrations.

Canola protein samples	Endothermic peak		Exothermic peak	
	T_d (°C)	ΔH (J/g)	T (°C)	ΔH (J/g)
CP[1]-0	124.45a[2]	22.26a	201.81a	19.02a
CP-3	119.85a	17.44b	211.10a	21.59a
CP-9	117.70a	16.73b	201.22a	7.74b
CP-15	115.49a	14.95b	206.24a	9.07b

[1]CP: Canola protein modified by 0, 3, 9, 15 g/L $NaHSO_3$
[2]Means in the same column followed by different letters are significantly different at $P<0.05$

The DSC thermogram was also characterized by an exothermic peak above 200 ℃, which was considered to be the aggregation of exposed canola protein subunits. Li et al. (2011) reported that the exothermic peak with an onset temperature of 170 ℃ was probably related to protein aggregation and significantly decreased adhesion strength of sorghum protein at a pressing temperature of 170 ℃. In this research, the exothermic peak was above 200 ℃ and, considering that the ignition point of wood is approximately 240 ℃, the plywood was not assembled at pressing temperatures above 200 ℃. Furthermore, when CP was treated with $NaHSO_3$ above a concentration of 9 g/L, one more exothermic peak appeared at temperatures higher than 220 ℃ (results not shown). $NaHSO_3$ could release smaller subunits by breaking existing disulfide bonds in cruciferin and napin. These subunits may have a different protein thermal aggregation process than unmodified canola protein adhesive.

3.4.7 Mechanical Properties of Canola Protein Adhesives

Both $NaHSO_3$ concentration and curing temperature had significant effects on wet adhesion properties of canola protein adhesives (Table 3.4). In general, 100% wood cohesive failure (WCF) was observed with all dry and soaked plywood specimens at curing temperatures of 150 ℃ to 190 ℃, indicating that bonding strength between wood and canola protein adhesives was stronger than mechanical strength of the wood at dry and soaked conditions. Only partial WCF happened to the wet assembled plywood samples (Table 3.4), however, and the percentage of WCF for samples varied greatly with $NaHSO_3$ concentration and curing temperature.

As shown in Table 3.4, increasing the curing temperature from 150 ℃ to 190 ℃ improved wet adhesion performance of the canola protein, but had almost no effect on soak or dry strength. Optimum wet strength occurred at 190 ℃ for all unmodified and $NaHSO_3$-modified canola protein adhesives. Curing temperatures higher than 190 ℃ were not applied because the wood was overheating and darkening at this curing temperature. Also, higher temperature consumes more energy, thus increasing mill production costs. Wet adhesion strength of canola protein adhesives improved as curing temperature increased, as reflected by wet shear strength and WCF rate. At a curing temperature of 170 ℃, wet shear strength of unmodified canola adhesive was 2.23 MPa, an increase of 14% compared with the curing temperature of 150 ℃. The increase was small compared with the drastic improvement at a curing temperature of

55

190 ℃, which demonstrated wet shear strength of 3.79 MPa, an increase of 78%. Canola protein modified with NaHSO$_3$ at various concentrations showed a similar trend. In addition, 0% WCF was observed in assembled plywood specimens when the curing temperature was 150 ℃ and 170 ℃. Different levels of WCF, from 40-100%, existed in both unmodified and NaHSO$_3$-modified canola protein adhesives at a curing temperature of 190 ℃.

NaHSO$_3$ had a negative effect on adhesion performance of canola protein adhesives under a curing temperature of 190 ℃ and almost no effect when cured at 150 or 170 ℃. Wet shear strength decreased slightly as concentration of NaHSO$_3$ increased from 0g/L to 6 g/L, then increased slightly as NaHSO$_3$ concentration rose from 9 g/L to 15 g/L. Wet shear strength remained lower than that of unmodified canola protein adhesives. Optimum wet shear strength of canola protein adhesive (3.97 MPa) is higher than commercial urea-formaldehyde-based veneer adhesive (3.46 MPa) (Zhong et al 2007). The WCF rate decreased from 100% to 40% as NaHSO$_3$ concentration increased from 0 g/L to 15 g/L, further reflected by insignificant negative effects of NaHSO$_3$ on the water resistance of canola protein adhesives. Furthermore, at curing temperatures of 150 ℃ and 170 ℃, adhesion improvement applies only at levels of NaHSO$_3$ from 0 and 3% g/L and all other levels of modification show little differences in mean values obtained, which can be attributed to reduced viscosity and improved flowability of NaHSO$_3$-modified canola proteins, as has been previously detailed in the section on rheological properties.

The weakening effect of NaHSO$_3$ on adhesion performance was also observed on soy protein adhesives (Zhang and Sun 2010). NaHSO$_3$ can cleave disulfide bonds in soy proteins to form R-SH groups. During the reducing reaction, some sulfhydryls resulting from deoxidization are blocked as a sulfonate group (RS-SO$_3^-$); therefore, negative effects of NaHSO$_3$ can be attributed to this extra negative RS-SO$_3^-$ group that bonded with water through formation of a chemical bond in the protein adhesive, stimulating hydrophilic behavior and causing the RS-SO$_3^-$ group to absorb more water and disrupt the continuous adhesive matrix, an action that is detrimental to its wet shear strength. Cruciferinciferin and napin are known to have disulfide bonds, especially in the polypeptide chains of napin fraction, which are mainly held together by disulfide bridges (Schwenke 1994). Consequently, the induced extra negative RS-SO$_3^-$ group is one of the reasons that NaHSO$_3$ decreases adhesion strength of canola protein adhesives.

Amino acid composition and hydrophobic properties of proteins are also essential factors in protein adhesive performance (Li et al 2011). Hydrophobic protein adhesives may have

repelled water when the assembled cherry wood samples were soaked, thus ensuring that interaction between the wood boards and boundaries formed by protein adhesives and wood surfaces remained intact. Conversely, hydrophilic protein adhesives may absorb water when applied on cherry wood board, potentially destroying cohesion between adhesives and the wood surface. Theoretically, proteins with more hydrophobic amino acids are good candidates for bio-adhesives; however, canola protein adhesives with relatively low amounts of hydrophobic amino acids (~27%) (Table 3.2) had excellent water resistance (3.97 MPa) compared with sorghum protein (~58%, 3.15 MPa) and soy protein (~37%, 1.63MPa) (Li et al 2011). This is probably due to alteration of amino acids' hydrophobic property at high temperatures. Weak, dispersed canola protein adhesive was cured into a rigid, solid state when heat was applied. Its hydrophobic property may have changed during this process; however, mechanism of the change should be investigated.

Table 3.4 Effect of NaHSO$_3$ concentrations and curing temperatures on adhesion properties of canola protein adhesives.

Canola protein samples	Wet strength (MPa)			Soak strength (MPa)			Dry strength (MPa)		
	150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃
CP-0	1.95±1.07 0% WCF[2]	2.23±0.45 0% WCF	3.97±0.53 100% WCF	4.76±0.36 100% WCF	5.45±0.23 100% WCF	5.24±0.21 100% WCF	4.63±0.57 100% WCF	5.15±0.33 100% WCF	5.44±0.12 100% WCF
CP-3	2.34±0.14 0% WCF	2.86±0.10 0% WCF	3.92±0.05 70% WCF	5.43±0.07 100% WCF	5.80±0.44 100% WCF	5.74±0.15 100% WCF	5.78±0.32 100% WCF	5.08±0.35 100% WCF	5.40±0.37 100% WCF
CP-6	2.37±0.06 0% WCF	2.78±0.12 0% WCF	3.33±0.11 60% WCF	5.22±0.16 100% WCF	5.53±0.35 100% WCF	5.35±0.17 100% WCF	5.06±0.21 100% WCF	5.15±0.21 100% WCF	5.15±0.26 100% WCF
CP-9	2.39±0.19 0% WCF	2.85±0.13 0% WCF	3.39±0.15 40% WCF	4.98±0.17 100% WCF	5.44±0.48 100% WCF	5.56±0.08 100% WCF	5.17±0.13 100% WCF	5.40±0.12 100% WCF	4.98±0.39 100% WCF
CP-15	2.76±0.16 0% WCF	2.77±0.11 0% WCF	3.79±0.08 40% WCF	5.33±0.29 100% WCF	5.21±0.15 100% WCF	5.21±0.27 100% WCF	5.40±0.51 100% WCF	5.21±0.41 100% WCF	5.02±0.08 100% WCF

[1]CP: Canola protein modified by 0, 3, 9, 15 g/L NaHSO$_3$
[2]WCF: Wood cohesive failure

3.5 Conclusions

Canola protein was extracted from defatted meal slurry modified with varying $NaHSO_3$ concentrations and by using the alkali solubilization-acid precipitation method. As concentration of $NaHSO_3$ increased, canola protein recovery increased but canola protein purities decreased. Even though canola protein had a low content of hydrophobic amino acids, water resistance was excellent when used as a wood adhesive. $NaHSO_3$ had slight weakening effects on adhesion performance of canola protein, resulting from counterbalanced effects on adhesion performance: positive effects of good flowability and negative effects of induced extra charges ($RS-SO_3^-$) in $NaHSO_3$-modified samples. Canola protein modified with 3g/L $NaHSO_3$ had wet shear strength comparable to the control but significantly improved flowability and handling.

Chapter 4 - Effects of Sodium Bisulfite on Physicochemical and Adhesion Properties of Canola Protein Fractions[3]

4.1 Abstract

This study focused on the potential of using canola protein fractions as bio-degradable wood adhesives. Native and sodium bisulfite ($NaHSO_3$)-modified canola protein fractions isolated successively at different pH values (7.0, 5.5, and 3.5) were used as adhesives. Wood specimens were assembled with adhesives at a pressure of 2 MPa at 150 ℃, 170 ℃, or 190 ℃ for 10 min. Adhesion performance of adhesives were evaluated by wet, soak, and dry shear strength. Physicochemical properties such as extractability, electrophoresis profiles, thermal, rheological, and morphological properties were also characterized. Results showed that canola protein had the highest protein yield and purity at pH 5.5. Electrophoresis profile proved that $NaHSO_3$ cleaved disulfide bonds in canola protein. The $NaHSO_3$ could induce extra charges ($RS\text{-}SO_3^-$) on the protein surface leading to reduced apparent viscosity. Thermal analysis implied that the thermal transition temperature of canola protein decreased with modification of $NaHSO_3$. Canola protein adhesives showed excellent dry and soak shear strength with 100% wood cohesive failure in all curing temperatures. Wet adhesion strength of native and modified canola protein fraction adhesives at pH 5.5 and pH 3.5 (3.9-4.1 MPa) was higher than fractions at pH 7.0. $NaHSO_3$ had insignificant effects on adhesion performance of canola protein adhesives but notably improved handling and flow-ability properties of canola protein adhesives.

4.2 Introduction

Since the last few decades, biobased adhesives have been popular research topics due to deepening deficiencies of petroleum resources as well as environmental pollution and health concerns caused by synthetic petroleum resins. Natural resources, including soy, casein, starch, sorghum, and wheat gluten, are extensively investigated as alternative adhesives (Wool and Sun

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[3] Results have been published as a peer-reviewed paper. Li, N., Qi, G., Sun, X. S., & Wang, D. (2012). Effects of sodium bisulfite on the physicochemical properties and adhesion performance of canola protein fractions. J. Polym. Environ. 20:905-915.

2005). Canola ranks as the third-largest oilseed crop produced worldwide after soy and palm. Canola meal, a byproduct of canola oil extraction, contains 30 to 50% protein on a dry basis among different hybrids. Studies show that canola protein is used mainly for animal feed instead of human food due to anti-nutritional factors in canola meal (Aider and Barbana 2010).

Two major storage canola proteins, napin and cruciferin, constitute 20% and 60% of total protein in mature seeds, respectively (Hoglund et al 1992), and have very complicated protein compositions. Napin, which belongs to albumin storage proteins, is a highly basic protein with an isoelectric point of pH 11, and it exhibits molecular weights from 12.5 to 14.5 KDa [5]. Disulfide bonds are the main force stabilizing napin protein structure by comprising two disulphide-linked polypeptide chains (Krzyzaniak et al 1998). Cruciferin belongs to globulin storage protein, has a hexamer structure like soy glycinin protein (Berot et al 2005), and is a neutral protein with an isoelectric point around pH 7.2 and a molecular weight around 300 KDa (Schwenke et al 1983). Both covalent (disulfide bonds) and non-covalent bonds dominate cruciferin protein structure (Wu and Muir 2008).

The most common method of isolating canola protein is the use of alkaline solution to solubilize canola protein, followed by protein precipitation at the isoelectric point (PI) with HCl (Wäsche et al 1998). Optimum pH values for maximizing the canola protein recovery rate varies from 3.5 to 5.5 among canola varieties (Pedroche et al 2004; Tzeng et al 1988). In many studies, more than one PI of canola protein was reported. Lonnerdal and Janson (1972) found that the basic napin protein fraction was known to have a PI close to pH 11, and they suggested that the PI of other canola protein fractions was located between pH 4 and 8. Predroche et al. (2004) extracted two protein fractions from *Brassica carinata* defatted meal, related to rapeseed, sequentially at its two PI: pH 3.5 and pH 5.5. Manamperi et al (2010) also reported that canola protein fractions precipitated sequentially from pH 11 to 3 with increments of 1 pH unit and that fractions behaved differently with respect to functional properties such as thermal, rheological, and mechanical properties.

A promising candidate for biobased products with industrial applications, canola protein has been studied extensively for uses such as biodegradable thermal plastics (Manamperi et al 2010), films (Wäsche et al 1998), and surfactants; however, little information is available regarding canola protein as an adhesive. Previous research revealed that canola protein and $NaHSO_3$-modified canola protein extracted at pH 3.5 displayed excellent adhesion properties

important to adhesion, as high as 3.97 MPa with 100% wood cohesive failure for wet shear strength at optimum curing temperature (Li et al 2011). Because canola protein fractions are isolatable within a large range of pH values, these fractions may have different properties. The objective of this study was to investigate adhesive behavior of native canola protein fractions and $NaHSO_3$-modified canola protein fractions isolated sequentially at pH values of 7.0, 5.5, and 3.5, and then characterize their physicochemical properties, such as chemical and amino acid composition; protein electrophoresis profiles; and thermal, rheological, and morphological properties. Extractability and physicochemical properties of canola protein fractions at pH values higher than 7.0 or lower than 3.5 were not studied in this research because only trace amounts of canola protein can be precipitated in these ranges according to preliminary studies. Based on previous study, 0 and 6 g/L $NaHSO_3$ were applied to modify protein adhesive fractions in this research because 6 g/L is optimum to improve handling property without notable damage to adhesion properties of canola protein adhesive (Li et al 2011).

4.3 Materials and Methods

4.3.1 Materials

Canola meal with 11.0% moisture content, produced after canola seed was cold screw pressed, was purchased from Planet Natural (Bozeman, Mont.). Sodium bisulfite ($NaHSO_3$), hexanes, hydrochloric acid (HCl), sodium hydroxide (NaOH), 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 $50 \times 127 \times 5$ mm (width $\times$ length $\times$ thickness) were provided by Veneer One (Oceanside, N.Y.).

4.3.2 Canola Protein Fraction Preparation

The canola protein fraction was isolated from canola meal using the method described by Manamperi et al (2010) with various modifications. Canola meal was first milled into powder with a cyclone sample mill (Udy Corp., Fort Collins, Colo.) to ensure a particle size of <0.25 mm and then dried and milled canola meal was defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 h at room temperature in three cycles. The defatted meal was placed in a fume hood overnight to remove residual hexane. The defatted and dried canola meal was mixed with

distilled water at the solid/liquid ratio of 1:12 (w/v), and the slurry was presoaked for 1 h with stirring. Then, $NaHSO_3$ was added to the slurry in concentrations of 0 g/L and 6 g/L separately on the basis of water volume. The pH value of the slurry was adjusted to 12 with 6 mol/L NaOH solution and the slurry was stirred for an additional 2 h at room temperature to solubilize the protein in canola meal. The slurry mixture was centrifuged at 12000 $\times$ g for 15 min, and the supernatant was decanted through a six-layer cheesecloth to remove impurities on top of the supernatant. The pH value of the supernatant was then slowly adjusted to 7.0 with 2 mol/L HCl solution to precipitate the protein fraction at pH 7.0. The mixture was centrifuged at 12000 $\times$ g for 15 min to isolate the wet protein fraction. After centrifugation, the pH value of the supernatant was adjusted to 5.5 with the same HCl solution, followed by centrifugation in the same condition to isolate wet protein fraction at pH 5.5. Wet protein fraction at pH 3.5 was isolated by adjusting the pH value of the supernatant to 3.5 with the same HCl solution, followed by centrifugation at 12000 $\times$ g for 15 min. A portion of the wet protein was used as adhesive and for rheological property study and the rest of the wet protein was freeze-dried for physicochemical analysis.

4.3.3 Chemical Analysis

Protein content was measured by combustion via a LECO FP-2000 nitrogen determinator (St. Joseph, Mich.) according to AOAC method 990.03 (1995). Nitrogen was converted to protein using a factor of 6.25. Fat content was determined through the Soxhlet petroleum-ether extraction method according to AOAC method 920.39C for cereal fat and was expressed as weight percentage on dry basis (1995). Crude fiber was determined according to AOCS-approved procedure Ba 6a-05 (1996).

4.3.4 Amino Acid Composition Analysis

Approximately 100 mg of each sample was weighed and placed in 0.5 ml of 6 N HCl solution along with the internal standard and hydrolyzed at 110 °C for 20 h. An aliquot, typically 10 or 20 µl, was then made up to 250 µl with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 µl of this diluent was injected into an HPLC system with a C18

column (Hypersil AA-ODS, 2.1 × 200 mm, 5 μm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, and 0.3% tetrahydrofuran, and pH was adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). Elution conditions went from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

4.3.5 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). A canola protein sample was mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To study disulfide bonds in canola protein, SDS-PAGE was carried out under reducing (2- mercaptoethanol) and non-reducing conditions. A total of 8 μg of protein was applied to sample wells and molecular weight standards (14.4-97.4 kDa) 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. Densitometry was obtained by analyzing the gel image using Kodak 1D Image Analysis software, version 4.6 (Kodark, Rochester, N.Y.).

4.3.6 Differential Scanning Calorimetry (DSC)

Thermal transition properties of protein samples were measured with a TA Instruments DSC Q200 V24.4 instrument (TA Instruments, New Castle, Del.) calibrated with indium and zinc before making official measurements. Samples of dry canola proteins weighing approximately 7 to 10 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL/min. All samples were heated from 25 to 280 ℃ at a heating rate of 10 ℃/min in an inert environment. All experiments were performed in duplicate.

4.3.7 Rheological Properties

Apparent viscosities on isolated canola proteins were performed with a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, Mass.) with a CP 4/40 cone and plate fixture (4 ° cone angle, 40-mm cone diameter). Distance between cone and plate was set to 150 μm for all measurements and experiments were conducted under steady shear flow at 23 ℃. Shear rates ranged from 10 to 240 s^{-1} in increments of 10 s^{-1}. All experiments were done in duplicate, and average values were reported.

4.3.8 Scanning Electron Microscopy

A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of dried canola protein isolate powder. Ground protein powder was 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, N.J.). SEM images of protein isolates were performed with operating conditions at an accelerating voltage of 5 kV.

4.3.9 Wood Specimen Preparation

Cherry wood samples were preconditioned in a controlled-environment chamber (Model 518, Electro-tech systems, Inc., Glenside, Pa.) for 7 d at 25 ℃ and 50% relative humidity (RH). Canola protein adhesives isolated with different methods were brushed separately along the edges of two pieces of cherry wood, with an application area of 127 mm × 20 mm, until the entire area was completely covered. The adhesive amount applied to each piece was approximately 0.06 g (dry basis). The brushing and setting procedure followed the method described by Mo et al. (2004). The brushed areas of the two pieces were assembled together at room temperature for 15 min, then pressed at a pressure of 2 MPa at 150 ℃, 170 ℃, or 190 ℃ for 10 min using a hot press (Model 3890 Auto 'M', Carver Inc., Wabash, Ind.).

4.3.10 Mechanical Properties

After pressing, the glued-wood assemblies were conditioned at 23 ℃ and 50% RH for 2 d, then cut into five specimens, each measuring 127 mm (length) × 20 mm (width) × 5 mm

(thickness) specimens. The cut specimens were conditioned again for another 5 d at 23 ℃ and 50% RH before the dry test. Three adhesion strengths were tested: dry strength, soak strength, and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron (Model 4465, Canton, Mass.) according to ASTM Standard Method D2339-98 (2002). Crosshead speed of Instron for adhesion strength testing was 1.6 mm/min. Tensile strength at the maximum load was recorded as adhesion strength; reported results are an average of five specimen measurements.

Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002), respectively. Preconditioned specimens were soaked in tap water at 23 ℃ for 48 h and then tested immediately for wet strength. For the soak strength test, specimens were soaked in tap water at 23 ℃ for 48 h and then conditioned at 23 ℃ and 50% RH for an additional 7 d before testing.

4.3.11 Statistical Analysis

Data for the mechanical property evaluation were an average of five samples. Data of experiments carried out in duplicate were analyzed using the analysis of variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, NC).

4.4 Results and discussion

4.4.1 Chemical Composition of Canola Protein Fraction Adhesives

Partial proximate compositions and recovery rates of native and $NaHSO_3$-modified canola protein fraction adhesives isolated at different pH values are summarized in Table 4.1. Protein content in defatted canola meal was approximately 49.3%, which coincides with a previous report (Aider and Barbana 2011). Total native protein recovery rate from canola meal was 31.45%, which increased slightly to 33.10% when the canola protein was modified with 6 g/L $NaHSO_3$, perhaps because the protein matrix was broken down by $NaHSO_3$ and became easier to extract (Choi et al 2008).

For native canola protein isolates, the largest fraction (52.1%, based on total extracted protein) was precipitated at a pH of approximately 5.5, followed by pH 7.0 (36.7%) and pH 3.5 (11.23%). The presence of $NaHSO_3$ significantly rearranged weight distribution of isolated canola protein fractions. Only 6.4% canola protein was precipitated at pH 7.0. The most protein fraction was still extracted at pH 5.5, but protein fraction recovery rate increased from 52.1% to 78.1%. Canola protein yield isolated at pH 3.5 also increased by 4% compared to the native protein. $NaHSO_3$ could bring extra negative groups on the protein surface ($RS\text{-}SO_3^-$), as suggested by Kalapathy et al. (1996); consequently, the isoelectric point (pI) of canola protein decreased, pH 7.0 was pushed farther away from the isoelectric point when $NaHSO_3$ was used, and a smaller amount of canola protein was extracted.

Purity of $NaHSO_3$-modified canola protein fractions was lower than native fractions, thus agreeing with Blaicher et al (1983). The highest purity for both native and $NaHSO_3$-modified canola protein fractions was obtained at pH 5.5, which is close to the isoelectric point of canola protein (Manamperi et al 2010), followed by fractions isolated at pH 3.5 and pH 7.0. Impurities such as lipids and crude fibers were detected in small amounts in all extracted protein fractions. Other impurities were speculated to be phenolic compounds or glucosinolates which can bond with canola protein and be extracted with the protein during acid precipitation (Blaicher et al 1983). Glucosinolates may account for up to 10% of the total isolated rapeseed protein (Blaicher et al 1983; Sosulski and Dabrowski 1984). In this research, most phenolic compounds were precipitated out at pH 7.0, which is partially attributed to the lowest purity of canola protein at pH 7.0.

In addition, canola proteins isolated with 6 g/L $NaHSO_3$ exhibited higher moisture content than native protein fractions (Figure 4.1), implying that water bonding capacity increased due to the presence of $NaHSO_3$.

Table 4.1 Recovery rate and partial proximate composition of canola meal and protein fractions isolated at different pH values and modified with various concentration of $NaHSO_3$.

Canola protein samples	pH	Chemical composition of protein extracted at different pH (%, db)			Protein fraction recovery (%,db)	Protein recovery (%, db)
		Protein	Lipids	Fiber		
D-CM[1]		49.26b[2]	0.89a	8.62a		
CP-0[5]	7.0	84.91a	0.15b	0.04b	36.66[3]	
	5.5	94.59a	0.25b	0.01b	52.11	31.45[4]
	3.5	89.09a	0.20b	0.01b	11.23	
CP-6[6]	7.0	79.71a	0.35b	0.09b	6.40	
	5.5	86.14a	0.18b	0.02b	78.16	33.10
	3.5	84.22a	0.16b	0.07b	15.44	

[1]Defatted canola meal.
[2]Means in the same column followed by different letters are significantly different at $p<0.05$.
[3]Based on total extracted canola protein.
[4]Based on total protein in canola meal.
[5]Native canola protein fractions.
[6]$NaHSO_3$-modified canola protein fractions at 6g/L.
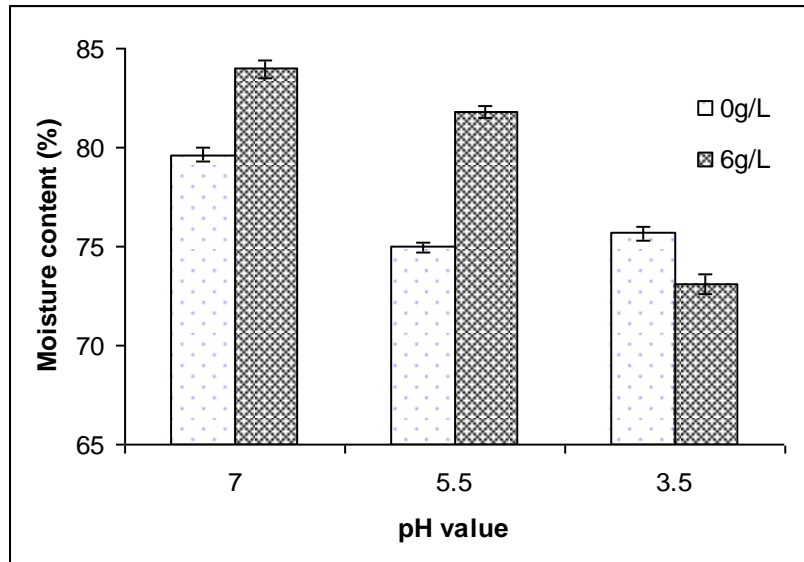
Figure 4.1 Moisture content (wet basis) of isolated wet canola protein modified with NaHSO3 at various concentrations.

4.4.2 Amino Acid Composition of Canola Protein Fraction Adhesives

Amino acid profiles of native and $NaHSO_3$-modified canola protein fractions are summarized in Table 4.2. Canola protein exhibited a different amino acid profile than canola meal, which can be attributed to the protein extraction method used in this study. Four kinds of proteins present in canola protein are glutelins, prolamins, alumins, and globulins, each of which may have a distinct amino acid profile; however, with alkaline solubilization and acid precipitation methods, only parts of the total protein, such as glutelins, were extractable (Manamperi et al 2007).

Amino acid profiles of canola proteins extracted at different pH values also varied significantly. Canola protein fractions at pH 3.5 had higher glutamate content compared with fractions at pH 5.5 and 7.0, but the fraction at extraction pH 7.0 had higher amounts of aspartete, threonine, alanine, arginine, tyrosine, valine, phenylalanine, isolecucine, and lysine than the other two fractions. Amino acid profiles of canola proteins at pH 5.5 were characterized as an intermediate position between pH 7.0 and 3.5. Based on hydrophobicity, amino acids can be grouped into hydrophobic (non-polar amino acid) and hydrophilic (polar amino acid) types. Alanine, methinine, phenylananine, isoleucine, leucine, and proline belong to hydrophobic

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amino acids, and they accounted for 25.69% to 28.17% of canola protein fractions. A previous study demonstrated that amino acid compositions could affect adhesive performance of protein-based adhesives (Li et al 2011), which will be discussed in detail in the next section.

Compared to Table 4.1, net canola protein (sum of amino acid content) was lower than values obtained through the LECO FP-2000 nitrogen determinator (St. Joseph, Mich.) method. The first possible explanation for this is that some non-protein nitrogen was in the protein isolates; one report noted that non-protein nitrogen could be up to 26.8% of canola protein (Krishnamoorthy et al 1982). A second possibility is that not all amino acids were detectable because tryptophan and cysteine could be destroyed by the liquid HCl hydrolysis assay during the amino acid composition test (Albin et al 2000). Thirdly, the nitrogen conversion factor could be a value other than 6.25. Some reports have suggested that the conversion factor for oilseed protein, such as flaxseed, should be 5.41 (Oomah and Mazza 1995).

Table 4.2 Amino acid compositions of native and NaHSO$_3$-modified canola protein adhesives extracted at different pH.

Amino acid (% of total)	Native CP[1] fraction extracted at different pH			CP[1] fraction modified with NaHSO$_3$ (6g/L) at different pH			D-CM[2]
	pH 7.0	pH 5.5	pH 3.5	pH 7.0	pH 5.5	pH 3.5	
Aspartate	9.21	8.78	8.15	9.88	9.79	8.24	8.44
Glutamate	22.14	24.01	26.97	20.02	21.60	25.73	22.37
Serine	5.35	5.27	5.32	5.56	5.25	5.06	5.47
Histidine	3.30	3.35	3.48	3.24	3.15	3.41	3.32
Glycine	6.05	5.99	5.95	6.04	5.99	6.33	6.07
Threonine	4.79	4.50	4.37	5.18	4.76	4.19	5.45
Alanine	5.27	5.08	4.86	5.46	5.16	4.78	5.40
Arginine	6.95	6.73	6.67	7.19	6.97	7.30	6.95
Tyrosine	3.77	3.41	2.75	4.10	3.69	2.89	3.23
Valine	5.73	5.67	5.52	5.85	5.94	5.58	6.05
Methionine	2.07	2.12	2.14	2.23	2.21	2.19	1.74
Phenylalanine	5.48	5.34	4.94	5.45	5.53	5.04	5.00
Isoleucine	5.62	5.54	5.27	5.75	5.82	5.37	5.80
Leucine	9.15	8.92	8.58	9.28	8.97	8.31	8.86
Lysine	5.13	5.30	5.04	4.76	5.19	5.59	5.85
T-AA (%)	100	100	100	100	100	100	100
T-protein (%)	65.19	70.09	69.57	64.16	66.92	60.32	34.84
Hydrophobic[3]	27.59	26.99	25.78	28.17	27.69	25.69	26.80
Hydrophilic[4]	72.41	73.01	74.22	71.83	72.31	74.31	73.20

[1]Canola protein.
[2]Defatted canola meal.
[3]Alanine, methinine, phenylalanine, isoleucine, leucine and proline.
[4]Lysine, tyrosine, arginine, threonine, glycine, histidine, serine, glutamine and asparagine.

4.4.3 Electrophoresis (SDS-PAGE)

Figure 4.2 shows the polypeptides composition of native (A-C) and NaHSO₃-modified (D-F) canola proteins extracted at three distinct pH values (7.0, 5.5, and 3.5) under non-reducing conditions. For native canola protein fractions, two major protein fractions were detected: cruciferin (12S), with a molecular weight above 20 kDa, and napin (2S), with molecular weights of 12 and 17 kDa (Wäsche et al 1998; Lonnerdal and Janson 1972). The bands corresponding to these two protein fractions intensified as the extraction pH decreased from 7.0 to 3.5. Meanwhile, bands observed on top of the stacking and resolving gel, considered to the high molecular weight protein aggregates, faded as extraction pH decreased. In the presence of ME, these high molecular weight bands gradually attenuated and eventually disappeared in a sample with pH 3.5, indicating disulfide bonds involved in stabilizing the protein polypeptides chain structure (Figure 3). On the other hand, remaining bands in pH 7.0 on top of the reducing electrophoresis were assumed to be either protein aggregates stabilized by other covalent bonds or contaminates that precipitated simultaneously with the protein extractions, as mentioned in Table 4.1.

The 55 kDa protein bands of 12S globulin disappeared in NaHSO₃-modified canola protein, indicating that the reducing effects of NaHSO₃ were exerted on polypeptides linked by disulfide bonds. Also, densities of napin bands at 14 kDa that were connected by disulfide bonds decreased when the canola protein was treated by NaHSO₃ (Figure 4.2). In the reducing SDS-PAGE of native and NaHSO₃-modified canola protein (Figure 4.3), all polypeptides stabilized by disulfide bonds in cruciferin and napin were dissociated into smaller polypeptide chains, as previously mentioned.
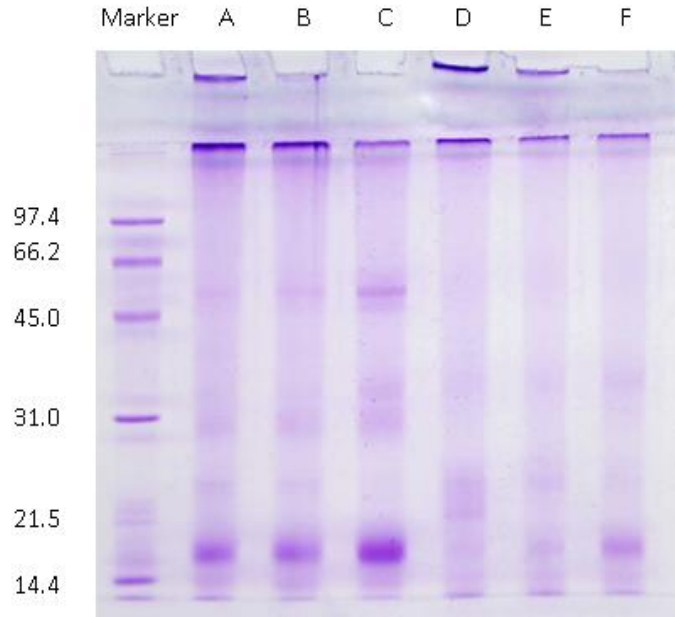
Figure 4.2 Non-reducing SDS-PAGE pattern of native and NaHSO$_3$-modified canola protein extracted at different pH. Native canola protein: pH 7.0 (lane A); pH 5.5 (lane B); pH 3.5 (lane C); 6g/L NaHSO3-modified canola protein: pH 7.0 (lane D); pH 5.5 (lane E); pH 3.5 (lane F).
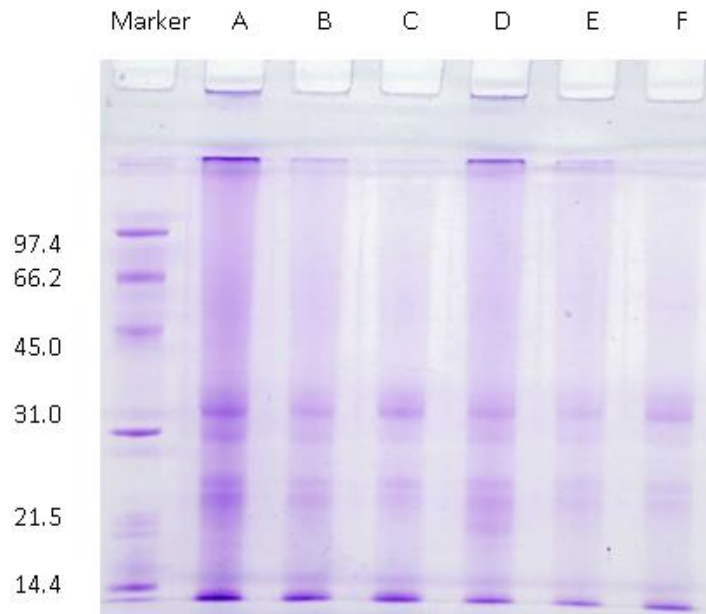
Figure 4.3 Reducing SDS-PAGE pattern of native and NaHSO$_3$-modified canola protein extracted at different pH. Native canola protein: pH 7.0 (lane A); pH 5.5 (lane B); pH 3.5 (lane C); 6g/L NaHSO$_3$-modified canola protein: pH 7.0 (lane D); pH 5.5 (lane E); pH 3.5 (lane F).

4.4.4 Differential Scanning Calorimetry (DSC)

DSC thermograms of native and NaHSO$_3$-modified canola protein fractions are presented in Figure 4.4. Two major broad endothermic peaks occurred for both native and modified canola proteins isolated at pH 7.0 and 5.5, whereas samples at pH 3.5 were characterized by endothermic and exothermic peaks. The first endothermic peak was due to canola protein thermal denaturation; denaturation temperature (T$_d$) and total enthalpy (Peak area, ΔH) increased as pH decreased from 7.5 to 5.5 and 3.5 (Table 3.3). Results were attributed to the fact that more compact and thermally stable protein structure exists close to the isoelectric point. The reducing effect of NaHSO$_3$ caused thermal stability of canola protein to decrease, as reflected by the decreased T$_d$ in modified canola protein fractions; however, only slight change occurred in the ΔH, indicating that conformation of modified protein fraction was insignificantly altered by 6 g/L NaHSO$_3$ modification.
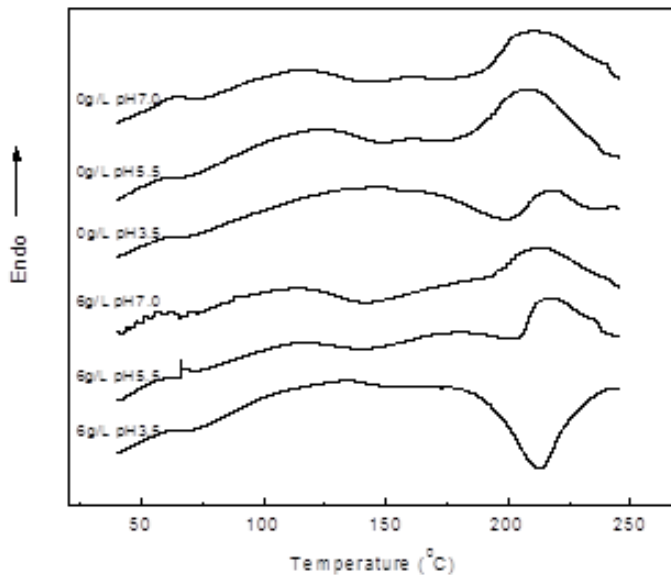
Figure 4.4 DSC thermograms of native and NaHSO$_3$-modified canola protein adhesives extracted at different pH.

The second endothermic peak in the samples isolated at pH 7.5 and 5.5 was accompanied by the exothermic heat effect of protein aggregation, which was also reported by Krzyzaniak et al. [6], making precise calculation of endothermic transition enthalpy difficult. As shown in SDS-PAGE results, high molecular weight of protein aggregates or non-protein contaminates were observed

in protein samples extracted at pH 7.5 and pH 5.5. Because of this result, it is assumed that the endothermic peak was to break down protein aggregates or non-protein materials. As for native and $NaHSO_3$-modified canola protein at pH 3.5, the exothermic peak that appeared around 200 ℃ was assumed to be an aggregation of exposed canola protein subunits. Similar exothermic peaks were also reported in soy protein and sorghum protein (Li et al 2011; Mo et al 1999). The subunits released in modified canola protein through breaking disulfide bonds may have different protein aggregation processes than native canola protein, as shown in Figure 4.4.

Table 4.3 Thermal transition characterization of native and $NaHSO_3$-modified canola protein adhesives extracted at different pH.

CP[1] modified with NaHSO$_3$ (g/L)	Endothermic peak		Exothermic peak	
	T_d (℃)	ΔH (J/g)	T_a (℃)	ΔH (J/g)
0g/L				
pH-7.0	113.00a[2]	16.22a		
pH-5.5	117.17a	22.16a		
pH-3.5	116.58a	20.81a	198.89a	18.91b
6g/L				
pH 7.0	110.97a	17.14a		
pH 5.5	113.13a	19.28a		
pH 3.5	112.62a	22.93a	213.50a	47.84a

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

4.4.5 Rheological Properties

Viscosity of protein-based adhesives is an important physical property that governs handling and flow-ability properties as well as adhesive behavior. Apparent viscosity of native and NaHSO$_3$-modified canola protein fraction adhesives isolated at different pH values is presented in Figure 4.5. All canola protein adhesives exhibited shear-thinning behavior. Apparent viscosity of all canola protein fractions is much lower than that of the total canola protein accumulated at pH 3.5. Although viscosity of native canola protein fraction isolated at pH 7.0 is slightly lower than the protein fraction isolated at pH 5.5, when the pH value decreased to 3.5, viscosity decreased significantly. Most canola protein extracted at pH 5.5 showed the highest apparent viscosity close to the isoelectric point, where intermolecular interaction was the strongest.

As explained previously, the isoelectric point of the NaHSO$_3$-modified canola protein shifted to a lower value. When extracted at pH 7, the product yield decreased from 36% to only 6%, and the sample displayed a diluted state (high moisture content) with fairly low viscosity (weak protein-protein interaction). The highest viscosity was observed at pH 5.5 (strongest protein-protein interaction), indicating that the reduced isoelectric point was still close to pH 5.5. A similar phenomenon was observed in soy protein. The soy protein sample became more and more solid and plug-like when it was extracted around its isoelectric point, rather than assuming the liquid viscous state when soy protein was precipitated away from its isoelectric point (Liu et al 2007). In addition, apparent viscosity of modified canola protein at pH 5.5 was much lower than native samples at pH 5.5 because of disulfide bond breakage induced by NaHSO$_3$, resulting in weak protein intermolecular interaction.
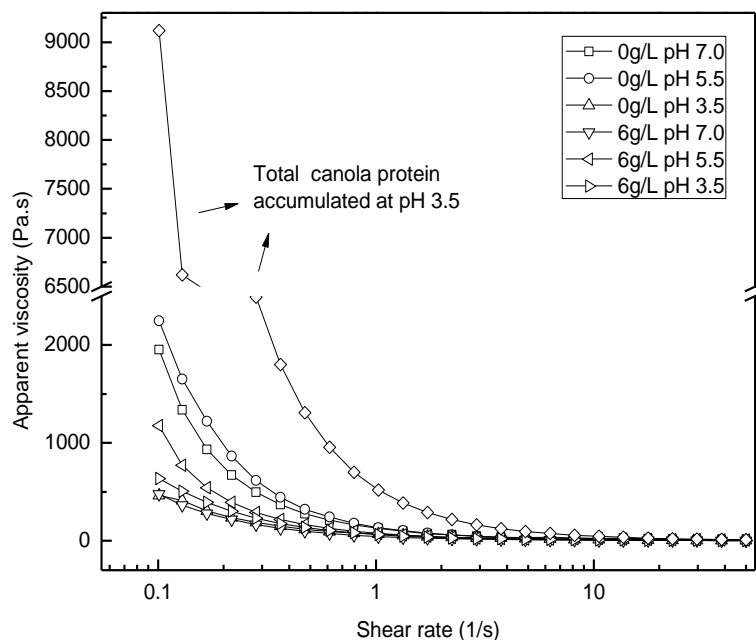
Figure 4.5 Shear rate dependence of apparent viscosity of native and NaHSO$_3$-modified canola protein adhesives extracted at different pH.

4.4.6 Morphological Properties

Microstructure images of both native and NaHSO$_3$-modified canola protein fraction adhesives are shown in Figure 6. Native canola protein isolated at pH 7.5 exhibited a coarse surface with many holes (marked with arrows) on protein particles (Figure 6 A). In protein fractions isolated at pH 5.5 and 3.5, more compact and smoother particle surfaces were observed (Figure 6 B, C). The holes observed in canola proteins at pH 7.5 may be the water sublimating during freezing, indicating that at this pH, canola protein had higher water hydration properties than other protein fractions. The rough surface suggested a weaker protein-protein interaction under the grinding procedure. At pH 5.5 and 3.5, which are closer to the isoelectric point of canola protein, canola protein had the lowest water hydration ability and the strongest protein-protein interaction, resulting in compact protein particles, as shown in Figure 6 C.

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The NaHSO$_3$-modified canola protein isolated at pH 7.0 showed a rougher, more fluctuating surface as well as several larger pores than native samples isolated at the same pH value. As mentioned previously, the isoelectric point of NaHSO$_3$-modified canola protein fraction shifted to lower pH values; therefore, the pH value of 7.0 was even farther from the canola protein isoelectric point with the presence of NaHSO$_3$, resulting in abundant negative charges on the surface of protein isolates. These negative charges led to strong electrostatic repulsion among protein particles, reflected by the porousness of protein particles. A similar trend was observed in native canola protein fractions isolated at the same pH value. As the isolation pH value decreased to 5.5 and 3.5, which were closer to the protein isoelectric point, extracted canola protein fractions exhibited much smoother surfaces and more compact structure as a result of stronger protein-protein interaction.
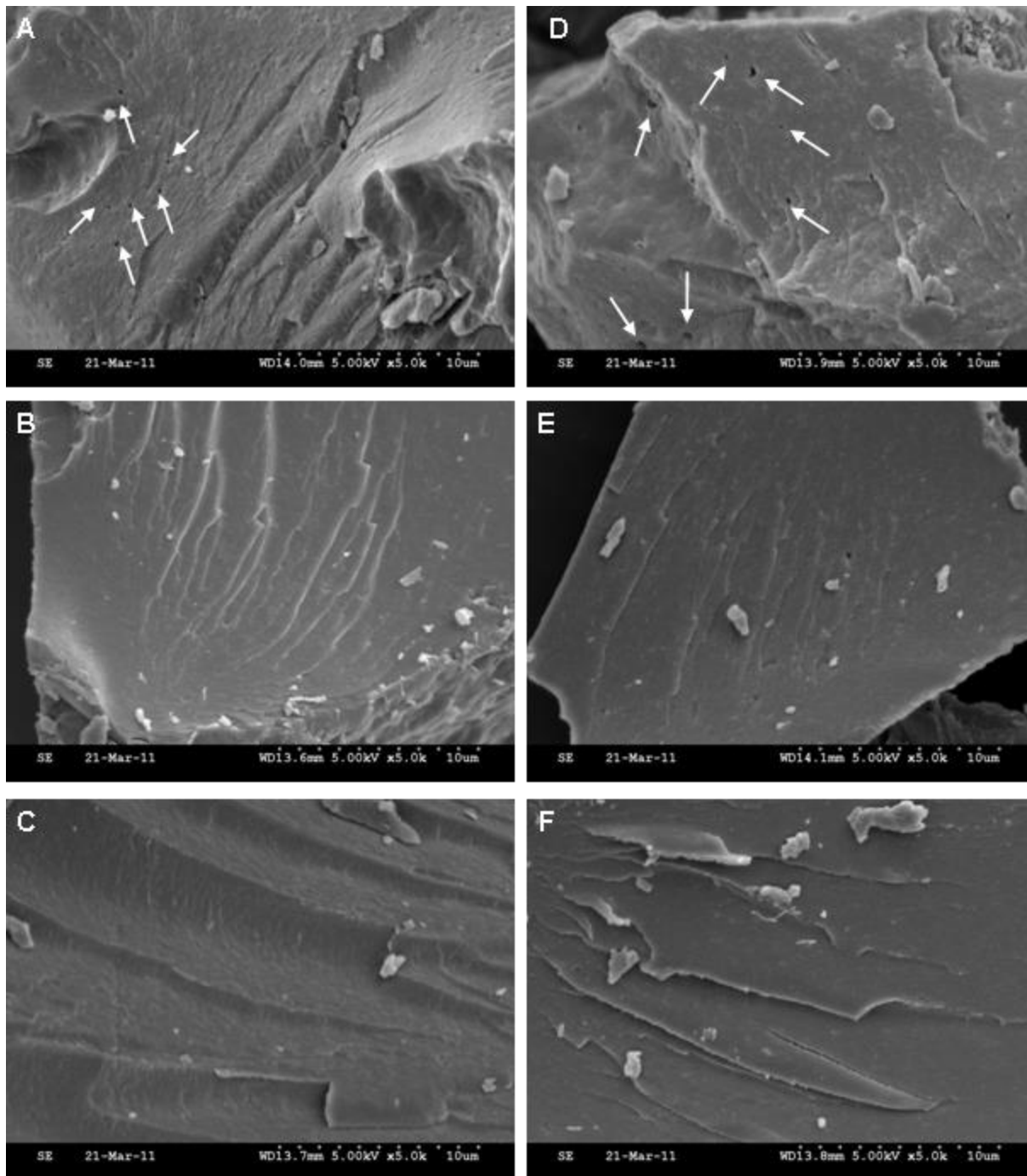
Figure 4.6 SEM images of native and NaHSO3-modified canola protein fraction adhesives extracted at different pH. Native canola protein fractions: pH 7.0 (A, × 5000); pH 5.5 (B, × 5000); pH 3.5 (C, × 5000). 6g/L NaHSO3-modified canola protein fractions: pH 7.0 (D, × 5000); pH 5.5 (E, × 5000); pH 3.5 (F, × 5000).

4.4.7 Adhesion Properties of Canola Protein Fraction Adhesives

Mechanical shear adhesion strengths of native and $NaHSO_3$-modified canola protein fraction adhesives isolated at different pH values are summarized in Table 4.4. Curing temperature, extraction pH value, and $NaHSO_3$ concentration had significant effects on adhesion properties of canola protein. In general, 100% wood cohesive failure (WCF) was observed in all dry and soaked plywood specimens at curing temperatures from 150 °C to 190 °C. Wet strength of canola protein adhesives decreased significantly and only partial WCF was observed for specimens cured at 170 °C and 190 °C (Table 4.4).

Increasing curing temperature is known to have marked effects on solvent evaporation, immobilization of protein molecules, and the possibility of chemical and physical interaction between proteins and the wood surface. Wet shear strength of canola protein adhesive improved as curing temperature increased, and the best water resistance of canola protein adhesives occurred at 190 °C in all samples. Canola protein fractions isolated at different pH values exhibited varied adhesion performance, as well. Under a curing temperature of 190 °C, native canola protein fractions isolated at pH 7.0 had an insignificantly reduced wet strength of 3.83 MPa, but a lower degree of WCF. $NaHSO_3$-modified canola protein fraction adhesives at different pH values displayed similar trends. The isoelectric point of canola protein was proven to have an approximate pH of 5.5, at which point protein fractions had the strongest protein-protein interaction, resulting in higher adhesion strength during the curing process. On the contrary, at pH 7.0, the protein had redundant charges on its surface because this pH point is far from the isoelectic point; therefore, the protein-water interaction was preferred over the protein-protein interactions during the water-soaking process and the adhesive had lower wet strength.

$NaHSO_3$ could break disulfide bonds in the canola protein (napin and cruciferin), lowering apparent viscosity and improving flow-ability as well as handle-ability of canola protein fraction adhesives, thus improving adhesion performance. Conversely, accompanying the disulfide bonds cleavage are SO_3^- groups that are induced on the protein surface as sulfonate groups ($RS\text{-}SO_3^-$) to prevent sulfhydryls (R-SH) formation. The formed $RS\text{-}SO_3^-$ groups could enhance protein interaction with water and decrease the effective wood-protein interfacial area due to the presence of ions (Kalapathy et al 1996). Hence, 6 g/L $NaHSO_3$-modified canola

protein fraction adhesives had insignificantly lower wet strength (approximately 3.8 MPa, 100% WCF) compared with native protein fractions because of counterbalance between the two effects at a curing temperature of 190 ℃.

In a previous study (Li et al 2011a), wet strength of total native canola protein accumulated at pH 3.5 was 3.9 MPa at a curing temperature of 190 ℃, but the protein adhesive also exhibited extremely high viscosity and poor flow-ability. As for canola protein fraction adhesives isolated at pH 5.5 and 3.5 at curing temperature of 170 ℃, wet strength was 3.1 and 3.4 MPa, respectively, and was further increased to 3.7 MPa for 6 g/L NaHSO$_3$-modified canola protein fraction isolated at pH 5.5. These results were much higher than adhesion performance for total accumulated canola protein when cured at the same temperature (2.2 MPa, 0% WCF) (Li et al 2011a). Also, their flow-ability was significantly better than total accumulated canola protein. These results demonstrated the remarkable improvement in adhesion performance of canola protein fractions curing at 170 ℃.

Both amino acid composition and hydrophobicity of proteins are essential factors affecting adhesive performance of proteins. Research has reported that a higher hydrophobic amino acid content was beneficial to improved water resistance of soy protein/sorghum protein (Li et al 2011b). However, in this study, a canola protein adhesive with relatively low hydrophobic amino acid content (~27%) (Table 2) also exhibited excellent water resistance (4.04 MPa, 100% WCF) compared with soy protein (~37%, 1.63 MPa, 0% WCF) and sorghum protein (~58%, 3.15 MPa, 0% WCF) (Li et al 2011b).

4.5 Conclusions

Canola protein-based adhesives were developed and showed great potential as bio-based adhesives. Dry and soak wood specimens had 100% wood failure, indicating good adhesion performance. Wet specimens showed partial wood specimens only at high curing temperatures of 170 and 190 ℃. Modifier NaHSO$_3$ did not improve adhesive properties of canola protein but greatly improved flow-ability and handle-ability. NaHSO$_3$ functionalized canola protein adhesive as the reducing agent and decrease of molecular weight, thermal transition temperature, and apparent viscosity of canola protein directly proved its reducing effect. Future work can focus on

lowering canola protein adhesive's curing temperature to make this adhesive more environmentally friendly.

Table 4.4 Effect of NaHSO3 concentration and curing temperature on adhesion properties of native and $NaHSO_3$-modified canola protein adhesives extracted at different pH.

CP[1] fraction modified with NaHSO$_3$ (g/L)	pH	Wet strength (MPa)			Soak strength (MPa)			Dry strength (MPa)		
		150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃
0 (g/L)	7.0	2.15±0.14 0%WCF[2]	2.87±0.15 0%WCF	3.83±0.17 50%WCF	5.44±0.50 100%WCF	5.55±0.43 100%WCF	5.57±0.31 100%WCF	5.23±0.33 100%WCF	5.75±0.23 100%WCF	5.40±0.16 100%WCF
	5.5	2.22±0.20 0%WCF	3.11±0.09 0%WCF	4.04±0.15 100%WCF (severe)	5.57±0.22 100%WCF	5.08±0.51 100%WCF	5.64±0.15 100%WCF	5.38±0.54 100%WCF	5.87±0.14 100%WCF	5.70±0.07 100%WCF
	3.5	2.39±0.16 0%WCF	3.41±0.21 0%WCF	4.07±0.16 100%WCF (severe)	5.32±0.12 100%WCF	5.64±0.23 100%WCF	5.43±0.28 100%WCF	5.60±0.18 100%WCF	5.19±0.35 100%WCF	5.28±0.47 100%WCF
6 (g/L)	7.0	2.25±0.06 0%WCF	3.52±0.18 0%WCF	3.54±0.14 40%WCF	5.28±0.38 100%WCF	5.48±0.24 100%WCF	5.36±0.22 100%WCF	5.33±0.25 100%WCF	5.22±0.43 100%WCF	5.18±0.28 100%WCF
	5.5	2.42±0.22 0%WCF	3.74±0.13 40%WCF	3.89±0.08 100%WCF	5.42±0.19 100%WCF	5.89±0.38 100%WCF	5.49±0.35 100%WCF	5.66±0.30 100%WCF	5.76±0.09 100%WCF	5.56±0.20 100%WCF
	3.5	2.55±0.15 0%WCF	3.03±0.12 0%WCF	3.87±0.09 100%WCF	5.63±0.17 100%WCF	5.22±0.51 100%WCF	5.12±0.24 100%WCF	5.34±0.13 100%WCF	5.38±0.29 100%WCF	5.54±0.33 100%WCF

[1]CP: Canola protein. [2]WCF: wood cohesive failure.

Chapter 5 - Isolation and characterization of protein fractions isolated from camelina meal

5.1 Abstract

Camelina is a new oil crop in North America. Camelina meal, a by-product of the camelina oil extraction process, typically contains 10-15% residual oil and 40% crude protein. As camelina oil demand increaseS, utilization of camelina protein for value-added products is critical to food and biotechnology industries; however, few researches have been conducted on camelina proteins. In this study, camelina protein fractions — albumin, globulin, and glutelins — were isolated from camelina meal using three different methods: the Osborne sequence (S2), a modified Osbrone sequence plus a degumming step (S1), and the isolation method without a degumming step (S0). Proteins' physicochemical properties, including solubility, amino acids profiles, molecular weight (MW), thermal and morphological properties, were also characterized. Results showed that S1 (88.20%) harvested more protein than S0 (84.05%) and S2 (76.52%). Glutelin was the major fraction (64.64%), followed by globulin (17.67%), and albumin (10.54%) in camelina. Essential amino acids accounted for approximately 40% of the total amino acids in camelina protein. High molecular weight aggregates stabilized by covalent bonds in glutelin and albumin fractions, as shown in SEC, are closely related to larger size protein aggregates observed in TEM images.

5.2 Introduction

Camelina sativa, also known as camelina, gold-of-pleasure, false flax, wild flax, linseed dodder, or German sesame, is an important and ancient oil plant originated from Germany around 600 B.C. (Budin et al 1995). In North America, camelina is a new oil crop possibly introduced as a weed in flax. Camelina is an annual summer or wintering plant with a short mature period (85-100 days) (Budin et al 1995; Sampath 2009).

In general, camelina contains 29.9-38.3% oil, 23-30% protein, 10% carbohydrates, and 6.6% ash, depending on the variety and variations of soil composition and environment (Budin et al 1995; Sampath 2009). Camelina oil contains up to 90% unsaturated fatty acid, of which

approximately 33.6% is α-linolenic acid (18:3, omega-3), which is lower than flaxseed (45.1%) but far exceeds canola (6.6%), soybean (7.2%), and sunflower (0%) (Budin et al 1995). High omega-3 content in camelina offers an opportunity to supply the growing demand for good-quality edible oils. Camelina oil also shows great potential as a source of biodiesel; in particular, it can be used to produce jet fuels that, compared to petroleum-based jet fuel, reduces greenhouse gas emissions up to 80% (Shonnard et al 2010). Camelina meal is a by-product of the oil extraction process from camelina seed that typically contains 10-15% residual oil, 40% crude protein, 5% minerals, 10-12% crude fiber, and a small portion of vitamins (Sampath 2009). As edible oil demands and biodiesel production increase, utilization of camelina protein for value-added products is critical to food and biotechnology industries.

Compared with other oilseeds such as canola, flaxseed, or soybean, camelina is less investigated for its protein. However, protein isolation technologies applied to other oil seeds provided a framework for recovering proteins from camelina. According to previous studies, oilseeds usually contain mixed or heterogeneous proteins comprising different protein fractions (Manamperi et al 2008; Ayad 2010). Manamperi et al (2008) used the Osborne method to isolate and characterize four protein fractions from canola meal: albumins (water-soluble), globulins (5% NaCl-soluble), prolamins (70% ethanol-soluble), and glutelins (0.1 N NaOH-soluble). Results indicated a protein recovery rate of 78.6%, among which albumins were the major fraction (38.7%), followed by globulins (22.0%), glutelins (10.3%), and prolamins (7.6%). These protein fractions were characterized with varied functional properties. Prolamins showed higher fat absorption property, whereas globulins were characterized by better emulsifying activity. Ayad (2010) also used the Osborne method to isolate flaxseed protein fractions from defatted flaxseed meal, and 38.1% of albumin, 27.9% of globulin, and 22.45% of glutelin were extracted.

This preliminary study showed that camelina proteins are a mixture of protein fractions of albumins, globulins, and glutelins with varied solubility. Therefore, the objective of this research was to study camelina protein fractions isolation processes and to characterize their physicochemical properties, including solubility, morphological characteristics, and thermal properties as well as amino acid profiles.

5.3 Materials and methods

5.3.1 Materials

Camelina meal (CM) with 15% lipid (db), 32.4% crude protein (db), and 11.0% moisture content (wb) was purchased from Montana Gluten Free Processors (Belgrade, Mont.). Meal pellets emerged from a screw oil press at approximately 80 ^{o}C. Hexanes, Bradford assay kit, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fair Lawn, N.J.).

5.3.2 Camelina meal defatting

Camelina meal with particle size <0.5 mm was obtained by using a cyclone sample mill (Udy Corp., Fort Collins, Colo.). Camelina meal was then defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 hr at room temperature in three cycles. The defatted camelina meal (DCM) was placed in a fume hood with a very thin layer (around 2 mm) for 24 hr to evaporate residual hexane.

5.3.3 Maximum solubility pH of glutelin

A standard curve was created first. Standard protein solutions were prepared using 0, 0.10, 0.25, 0.50, 0.75, and 1.00 mg protein/mL bovine serum albumin (BSA), and absorption readings of the solutions were measured with a BioMate 3 UV-Vis Spectrophotometer (Madison, Wis.) at 595 nm. The readings and known protein concentrations were interpolated in the calibration curve, as shown in Figure 5.1, and the standard curve was used to determine protein concentration in solutions tested in this study by spectrophotometer readings. The standard curve in Figure 5.1 was reliable only in the range from 0.0 to 1.0 mg/mL of protein, and protein contents of the samples studied in this part of the experiment remained in this range.
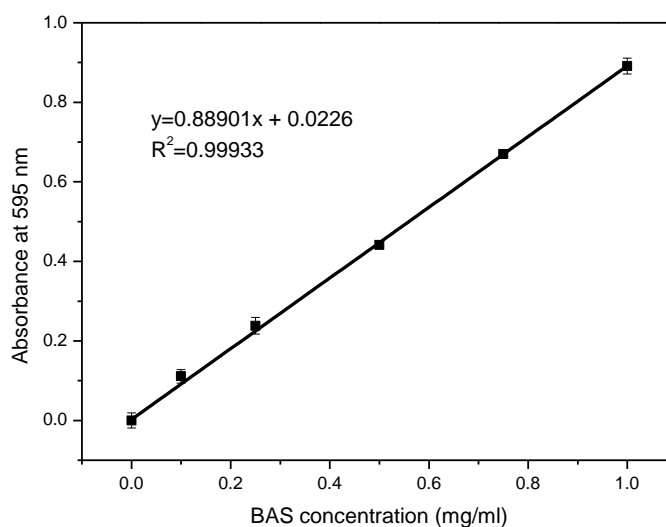
Figure 5.1 Standard curve of net absorbance versus BSA concentration.

Based on a preliminary test to determine at which pH camelina glutelin protein has maximum solubility, 10 g DCM was mixed in 1000 ml distilled water for 1 h, then adjusted to a pH of 10 using 2 N NaOH and stirred for 2 h. 5 mL of the slurry was collected and centrifuged it at 12,000 × g for 15 min, then decanted the supernatant through a six-layer cheesecloth to remove impurities. All centrifugation conditions mentioned in this paper were identical unless otherwise indicated. The pH of the remaining slurry samples was adjusted to 10.5, 11.0, 11.5, 12.0, 12.5, and 13.0 with 2 N of NaOH, then stirred for 2 h. Slurry was collected and centrifuged from each pH point. At the specified pH points, 100 uL of each supernatant was mixed with 3 mL Bradford reagent for 10 min at room temperature; then the sample was ready for testing by a BioMate 3 UV-Vis Spectrophotometer (Madison, Wis.) at 595 nm. Each sample was measured in triplicate.

5.3.4 Solubilities of protein fractions in camelina meal

5.3.4.1. Glutelin

10 g of DCM was mixed with 1000 g distilled water, adjusted pH to 12 with 2 N NaOH, stirred for 2 h, and centrifuged the slurry. The pH of the supernatant dropped from 12 to 1.0 in

increments of 0.5. 5 mL of the mixture was collected at each pH point, centrifuged the sample, and used the supernatants to measure protein content.

5.3.4.2. Albumin

10 g of DCM was mixed with 500 g distilled water for 2 h, with stirring followed by centrifugation. The pH of the supernatant initially dropped to 6.0, then from 6.0 to 1.0 with 2 N HCl in increments of 0.5. 5 mL of the mixture was collected at each pH point, centrifuged the sample, and measured protein content of the supernatants.

5.3.4.3. Globulin

10 g DCM was mixed with 500 g of 5% NaCl solution for 2 h and stirred and centrifuged the slurry. The pH of the supernatant initially dropped to 6.0, then from 6 to 1.0 with 2 N HCl in increments of 0.5. 5 mL of the mixture was collected at each pH point, centrifuged the sample, and measured protein content of the supernatants.

5.3.5 Camelina protein fractions isolation process

5.3.5.1. Osborne method

This extraction method is named sequence 2 (S2) (Figure 5.2A). Camelina protein fractions were isolated from DCM according to the Osborne fractionation scheme (Osborne 1924). Based on preliminary testing, three protein fractions, albumin, globulin, and glutelin, can be isolated with distilled water, 5% NaCl solution, and NaOH solution, respectively. Prolamin was not studied because only trace amounts exist in DCM.

DCM was mixed with distilled water at the solid/liquid ratio of 1:30 (w/v) for 2 h, then samples were centrifuged and residues were collected for further camelina protein fractions isolation. Both water-soluble gum and albumin were in the supernatant, so this was designated this as the degumming step. Because partial gum could be precipitated out with albumin due to the sticky nature, investigation of the degumming steps from albumin with the centrifuging technique could not be conducted. The pH of the supernatant was slowly adjusted to 3.0 with 2 N HCl, then centrifuged to precipitate the albumin fraction. Residues mentioned above were re-suspended in 5% NaCl at a solid/liquid ratio of 1:30 (w/v). The slurry was adjusted to pH 8.0 using 2 N NaOH and stirred for 2 h. After centrifuging, resulting residues were collected for further glutelin fraction extraction. The supernatant was adjusted to pH 3.0 with 2 N HCl and

centrifuged to produce globulin 2. The residues were re-suspended in water at the solid/liquid ratio of 1:30 (w/v) and the pH was adjusted to 12 using 2N NaOH and continuous stirring for 2 h. Then the slurry was centrifuged and the supernatant adjusted to pH 4.5 to precipitate glutelin 2 proteins separated by centrifugation. All camelina protein fractions were washed with distilled water for three cycles, freeze-dried, and ground into powder for further analysis.

5.3.5.2. Modified Osborne method

This extraction method is named sequence 1 (S1) (Figure 5.2B). The main difference from the Osborne sequence was that the glutelin fraction was isolated after albumin but before globulin. Protein isolates extracted with this method were named globulin 1 and glutelin 1, respectively.

5.3.5.3. Camelina protein extraction without degumming

This extraction method is named sequence 0 (S0). Protein isolation schemes of S0 are presented in detail in Figure 5.2C and D and produced globulin 0 and glutelin 0, respectively.

Figure 5.2 A

Figure 5.2 B

```
┌─────────────────────────┐
│  Defatted camelina meal │
│         flour           │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐                              ┌──────────────────────────┐
│ Mixed with 30-fold      │                              │ Degumming removed        │
│ distilled water and     │                              │ soluble polysaccharide   │
│ stirred 2 h, then       │───────┐      ┌────────────┐  └──────────────────────────┘
│ centrifuged at 12,000 × │       └─────▶│ Supernatant│           ▲
│ g for 15 min            │              └────────────┘           │
└─────────────────────────┘                    │                 │
             │                            ┌────────────┐          │
             ▼                            │  Albumin   │          │
┌─────────────────────────┐              └────────────┘          │
│ Residues: mixed with 30-│                    │                 │
│ fold distilled water,   │              ┌──────────────┐        │
│ adjusted pH to 12 with  │              │ Lyophilization│◀──────┘
│ 2 N NaOH, stirred 2 h,  │              └──────────────┘
│ then centrifuged at     │◀─┐
│ 12,000 × g for 15 min   │  │
└─────────────────────────┘  │
             │               │
             ▼               │
┌─────────────────────────┐  │
│ Supernatant: adjusted   │──┘
│ pH to 4.5, and then     │
│ centrifuged at          │
│ 12,000× g for 15 min    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│ Precipitate: Glutelin 1 │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│     Lyophilization      │
└─────────────────────────┘

┌─────────────────────────┐                              ┌──────────────────────────┐
│ Residues: mixed with 30-│                              │ Precipitate: non-        │
│ fold 5% NaCl solution , │─────────────────────────────▶│ protein impurities       │
│ adjusted pH to 8, stirred│                             └──────────────────────────┘
│ 2 h, then centrifuged at│
│ 12,000 × g for 15 min   │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│ Supernatant: adjusted   │
│ pH to 3.0, and          │
│ centrifuged at          │
│ 12,000× g for 15 min    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│ Precipitates: Globulin 1│
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│     Lyophilization      │
└─────────────────────────┘
```

Figure 5.2 Flowchart of camelina protein fractions extraction procedure. A: Osborne method. B: Modified Osborne method. C: Camelina glutelin fraction extraction without degumming. D: Camelina globulin fraction without degumming.

5.3.6 Chemical analysis

Moisture content was measured with the V30 Compact Volumetric KF Titrator (Columbus, Ohio). Carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content were measured with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, Conn.). Nitrogen was converted to protein using a factor of 6.25. All tests were performed in duplicate.

5.3.7 Amino acid composition analysis

Amino acids profiles in camelina proteins were measured by the method described by Li et al (2011 a). Approximately 100 mg of each sample was weighed and placed in 0.5 mL of 6 N HCl along with the internal standard and hydrolyzed at 110 ℃ for 20 h. An aliquot, typically 10 or 20 μl, was then made up to 250 μl with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 μl of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1×200 mm, 5 μm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, 0.3% tetrahydrofuran, and pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). Elution conditions progressed from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

5.3.8 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) data were collected in the region of 400 to 4000 cm^{-1} with a PerkinElmer Spectrum 400 FT-IR/FT-NIR spectrophotometer (Shelton, Conn.). Transmission spectra of 32 scans of each sample were collected at a resolution of 1 cm^{-1} in the reflectance mode. All samples were tested with duplications. Information on fat, carbohydrates, and protein in samples was given by absorptions. Because the objective of using FTIR was also to determine relative amounts of α-helix and β-sheet secondary structure protein, a band shape of each peak resolved by deconvolution needed to be produced that would allow peak area determination as a method of quantitative analysis. Fourier self-peak deconvolution (FSD), the most widely used tool, was used to identify the α-helix and β-sheet in protein amide I region,

and modeling by Peak Fitting Wizard tool with Gaussian function with OriginLab-Origin 8.0 data analysis and graphing software was also used (Northampton, Mass.) to obtain areas of individual protein forms. The α-helix and β-sheet content ratio was described as the ratio of peak areas (Wetzel et al 2003; Yu et al 2005).

5.3.9 Transmission electron microscopy

Transmission electron microscopy (TEM) was obtained using a model CM 100 TEM (FEI Company, Hillsboro, Ore.) operated at 100 kV. Camelina protein isolates were first dissolved in distilled water with a solid concentration of 0.05% (w/w). Prepared protein samples were absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, Pa.) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, Vt) for 60 s at room temperature before being viewed by TEM.

5.3.10. Thermal gravimetric analysis

Thermal Gravimetric Analysis (TGA) of camelina proteins was conducted with a TGA instrument (TGA 7, Perkin-Elmer, Norwalk, Conn.) in a nitrogen atmosphere. Approximately 10 mg of ground powder was weighed into a platinum cup and scanned from 25 to 900 ℃ at a heating rate of 10 ℃ /min. Maximum degradation rate was calculated as mass (%) at peak temperature divided by peak temperature.

5.3.11 Size exclusion chromatography

Size exclusion chromatography (SEC) analysis was conducted as described by Bean et al (2006). In specific, a high-performance liquid chromatography (HPLC) system (1100, Agilent, PaloAlto, Calif.) with a 300mm × 7.8mm BioSep-4000 column and security guard columns (Phenomonex, Torrance, Calif.) was used. The mobile phase was a pH 7 sodium phosphate buffer (50 mM) with 1% SDS added. Column temperature was maintained at 25 ℃, and flow rate was 1 mL/min. Samples for SEC analysis were re-dissolved in a pH 7 sodium borate buffer (12.5 mM) plus 1% SDS with or without 2% β-ME at a constant final protein concentration of 5 mg/L. Standard proteins BSA (66kDa), carbonic anhydrase (29kDa), and glutathione (307.3 Da) were analyzed to estimate molecular weight distribution of camelina protein fractions separated by SEC.

5.3.12 Statistical analysis

Data from experiments carried out in duplicate were analyzed through analysis of variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, N.C.).

5.4. Results and discussion

5.4.1 Camelina protein solubilities and precipitation properties

According to Figure 5.2, net absorbance at 595 nm showed a linear relationship with bovine serum albumin (BSA) at concentration from 0.0 to 1.0 mg/ml. The linear relationship is expressed by equation (1) with R^2 of 0.999:

$$Y = 0.88901X + 0.0226 \qquad (1)$$

where Y is absorbance at 595 nm and X is protein concentration (mg/ml). As shown in Figure 5.3, solubility of glutelin displayed high sensitivity to pH values. Solubility of glutelin increased slightly from pH 10.0 to pH 11.0, then reached its highest value around pH 12.0; therefore, pH 12 was recommended for camelina glutelin solubilization.
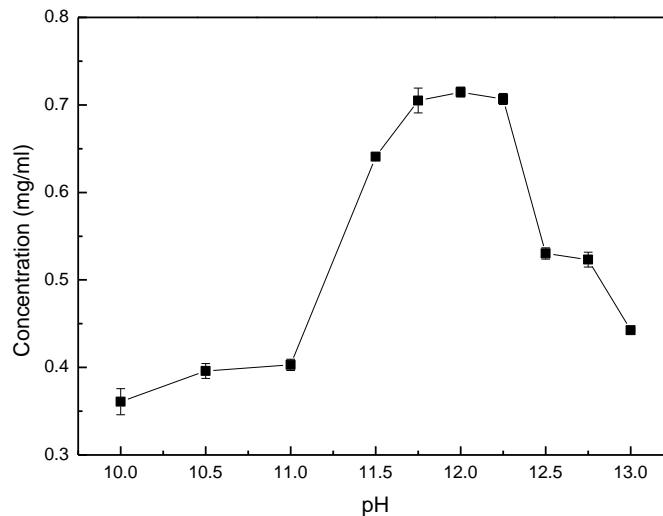
Figure 5.3 Effect of pH on glutelin solubility.

The purpose of characterizing precipitation properties of camelina proteins was to identify the protein's minimum solubility pH (MS-pH). As shown in Figure 5.4, albumin and

glutelin fractions exhibited a typical U-shaped solubility profile, whereas the globulin fraction showed a step shape. Lowest protein solubility was observed in the pH range from 2.5 to 3.0 for albumin fraction and pH 4.0-5.0 for glutelin fraction. In the globulin fraction, protein concentration decreased significantly from pH 6.93 to pH 4.0, then leveled off from pH 1.0 to 4.0. In this case, the MS-pH s of camelina protein fractions were considered to be pH 3.0, 4.0-5.0, and 3.0 for albumin, glutelin, and globulin, respectively.
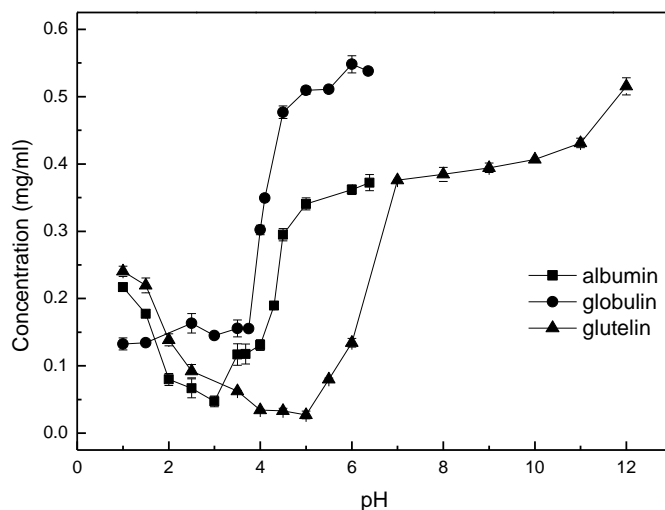
Figure 5.4 Effect of pH on the precipitation property of camelina protein fractions.

5.4.2 Partial proximate analysis and elemental composition of camelina proteins

Table 5.1 shows the partial proximate and elemental compositions of camelina fractions. Residual protein that was not successfully extracted was also indicated. The DCM consisted of approximately 38.12% crude protein. In all three isolation methods, glutelin (48.32 to 64.64%) was the major fraction in protein, followed by globulins (13.03-17.67%) and albumins (10.54%).

More protein isolates were recovered with S0 (48.67%) than with S1 (41.54%) and S2 (36.86%); however, protein purity with S0 (57%-70%) was far lower than that of S1 (81-87%) and S2 (83-86%), which could be attributed to the presence of gum in S0. Similarly, albumin fraction showed lower protein purity (56.57%) than globulin and glutelin fractions (81-87%) due to gum, which was extracted along with the albumin protein and consequently led to low protein purity. In addition, more pure globulin 2 and glutelin 1 fractions were extracted than globulin 0 and glutelin 0, indicating that the presence of gum negatively affected not only protein purity but also protein yield. The DCM-water slurry was very thick and sticky when those gums presented, possibly causing inefficient solublilization of protein in the slurry and resulting in lower protein extraction yield. More glutelin was extracted in S1 than in S2, which can be attributed to the effect of NaCl. As described in similar studies, NaCl could negatively affect protein solubility in specific pH ranges. Carbonaro et al (1997) found that lower solubility of fava bean, lentil, and

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chickpea proteins (all proteins had a MS-pH around pH 4.0) in NaCl at pH above 7.5 or with pH from 1.0 to 3.0 could be ascribed to increased hydrophobic interaction. Hydrophobic interactions are the driving force for protein-protein aggregation, leading to protein insolubilization. Markkar et al (2008) also reported that recovery of Jatropha protein decreased with the presence of NaCl at pH 10 or 11 due to an increase in ionic strength and the resulting increase in hydrophobic interaction, or, perhaps, due to the "salting out" effect of NaCl on protein resulting from competition between charged proteins and salt ions for necessary water for solvation (Badifu and Akubor 2001). In contrast, Carbonaro et al (1997) indicated that the shielding of charged groups of dry bean by NaCl resulted in increased electrostatic repulsive force that reduced protein aggregation and therefore improved solubility. In this research, camelina glutelin 2 was solubilized at pH 12, which is far away from its MS-pH (pH 4.0), with the presence of NaCl, resulting in glutelin 2's lower recovery rate due to strong ionic strength and protein aggregations driven from increased hydrophobic forces, as described by Markkar et al (2008).

Some protein remained in the residues: 3.45% for S1 and 5.86% for S2. Furthermore, the sum of isolated pure proteins (%) and un-extracted proteins in residues (%) was not 100%; instead, the sum was 97.24% for S1 and 91.89% for S2, implying that part of the protein was lost during the extraction process.

Elemental compositions of camelina proteins varied from different protein fractions (Table 5.1). Glutelin and albumin fractions contained higher C (42.33-49.22%), H (6.30-7.21%), and S (2.03-2.34%) contents than globulins (31.08-39.51%, 4.55-5.78%, and 1.62-1.75%, respectively). The sulfur in protein is known from the side-chains of amino acids methionine and cysteine (Brosnan and Brosnan 2006). Higher sulfur content in albumin and glutelins indicated higher methionine or cysteine content in amino acids profiles of camelina protein (Table 2). A carbon content of 31.45% is preferred for protein stability (Rajasekaran et al 2011). Li et al (2009) stated that carbon content in protein was negatively correlated to protein abundance, and amino acids with a high number of carbon atoms in their side-chains consumed more energy for synthesis. Hydrogen contributes protein structure, stability, and molecular recognition as the form of hydrogen bonds. Hydrogen bonding and the hydrophobic effect were believed to be the two main forces stabilizing proteins (Pace 2009). The core of most protein structures is composed of secondary structures such as alpha α and β sheet. This satisfies hydrogen-bonding

potential between main chain carbonyl oxygen and amide nitrogen buried in the hydrophobic core of the protein (Hubbard and Kamran Haider 2010).

Table 5.1 Partial proximate and elemental compositions of camelina meal and protein isolates fractions produced by different isolation sequences.

Camelina protein samples	Moisture content (%, db)	Weight of isolates compared to DCM (%,db)	Protein content (%, db)	Weight of protein compared to DCM (%, db)	Weight of protein compared to TP[a] in DCM (%, db)	Elemental composition of protein sources (%, db)			
						C	H	N	S
DCM[b]	9.35 a[c]		38.12			45.31 b	6.92 a	6.10a f	2.12 c
Albumin	7.30 e	7.10 h	56.57	4.02	10.54	42.33 c	6.30 b	9.05 e	2.27 ab
Globulin 0	7.20 e	10.66 g	57.56	6.14	16.10	31.08 e	4.55 e	9.21 e	1.75 d
Glutelin 0	8.73 c	30.91 d	70.81	21.89	57.42	45.92 b	6.71 ab	11.33 d	2.22 b
Globulin 1	6.57 f	6.13 i	81.00	4.97	13.03	37.96 d	5.54 cd	12.96 c	1.62 e
Glutelin 1	6.87 g	28.31 e	87.04	24.64	64.64	49.22 a	7.12 a	13.93 a	2.34 a
Globulin 2	6.14 h	7.75 h	86.93	6.74	17.67	39.51 d	5.78 c	13.91 a	1.70 de
Glutelin 2	7.54 d	22.01 f	83.68	18.42	48.32	46.03 b	6.71 ab	13.39 b	2.03 c
Residue 1	9.03 b	40.30 b	8.55	3.45	9.04	27.76 f	3.91 f	1.37 h	0.94 g
Residue 2	9.03 b	48.11 a	12.18	5.86	15.37	39.15 d	5.35 d	1.95 g	1.26 f
Sum of S0		48.67 a		32.04	84.05				
Sum of S1		41.54 b		33.62	88.20				
Sum of S2		36.86 c		29.17	76.52				

[a] Total protein.
[b] Defatted camelina meal.
[c] Means in the same column followed by different letters are significantly different at *P*<0.05.

5.4.3 Amino Acid Composition

Sixteen kinds of amino acids were detected and quantified in camelina proteins (Table 5.2). Tryptophan and cysteine were not detected because they were destroyed by the liquid HCl hydrolysis assay during the test, which may be one reason why the total sum of amino acids was lower than the protein content obtained by the nitrogen combustion method (Table 5.1). Tryptophan and cysteine in camelina amino acids accounted for only 1.15% and 2.12%, respectively, so it should not be the only explanation (Zubr 2002). Another reason may be the presence of non-protein nitrogen (NPN) in camelina. Although no published data are available on NPN content in camelina, NPN is very common in oilseeds. NPN content in soy, rape, and sunflower is up to 12.3%, 29.0%, and 15.4%, respectively, using 1% trichloroacetic acid as the extracting buffer (Bhatty and Finlayson 1973).

Camelina proteins are characterized by high glutamate (18.46-19.23%), aspartate (9.68-11.83%), leuicne (8.14-9.17%), arginine (7.60-8.57%), and phenylalanine (5.08-6.84%) content, but low ornithine (0%), methionine (1.68-2.46%), histidine (2.84-3.04%), and tyrosine (3.58-4.02%) content. Globulins showed lower levels of lysine, methionine, threonine, alanine, and glycine and higher levels of leucine, phenylalanine, valine, and aspartate amino acids. Notably, methionine, a sulfur-containing amino acid, is higher in glutelins than globulins, attributing to high sulfur content in glutelins. Compared with globulins, albumin had lower levels of isoleucine, leucine, phenylalanine, valine, and lysine, but amino acids of lysine, threonine, alanine, serine, and glycine in albumin exceed globulins. Furthermore, albumin exhibited lower levels of histidine, leucine, methionine, glutamate, and arginine and higher lysine, threonine, and aspartate amino acids than glutelins.

Amino acids were classified into groups according to physical, chemical, and structural properties. Nutritionally, camelina proteins contained approximately 40% of essential amino acids which cannot be synthesized by human and many farm animals, and approximately 60% of nonessential amino acids which can be produced in humans and animals. The percentage of essential amino acids in camelina is lower than in canola protein (42%) (Li et al., 2011 a), sorghum protein (48%) (Li et al 2011 b), and soy protein (49%) (Khorasani et al 1990). Amino acids are critical for metabolism and growth of humans and animals. Dietary requirement is the amount of protein or its constituent amino acids that must be supplied in the diet to satisfy

metabolic demand and achieve nitrogen equilibrium. In most cases, dietary requirements are greater than metabolic demand because of factors that influence efficiency of protein use; i.e., net protein utilization, digestion, and absorption. Lysine (4.12-5.88%) in all camelina protein fractions and phenylalanine (5.08-6.50%) in DCM, albumin, and glutelins are lower than World Health Organization (WHO) standards for children at 0.5 years old (lysine, 6.4%; phenylalanine, 5.9%), but all essential amino acid contents in camelina protein meet or exceed WHO amino acid requirement standards for children over one year old and adults (WHO Technical Report Series 935 2007).

Based on hydrophobicity, amino acids can be grouped into hydrophobic (non-polar) and hydrophilic (polar) types. Hydrophobic amino acids have side-chains that do not prefer an aqueous environment. Betts and Russell (2003) reported that these amino acids are generally buried within the hydrophobic core of the protein or within the lipid portion of the membrane. Among detected amino acids, alanine, methionine, phenylananine, isoleucine, and leucine belong to the hydrophobic group and account for approximately 26.34 to 28.40% of camelina protein fractions (Table 5.2). Albumin showed the lowest hydrophobic property, whereas globulin 2 was the most hydrophobic. Hydrophobic properties of camelina proteins are comparable to canola protein (26%), but lower than soy protein (37%) and sorghum protein (57%) (Li et al 2011a).

Table 5.2 Amino acid compositions of DCM, canola meal, and camelina protein isolates fractions.

	Amino acid (% of total)	albumin	globulin 0	glutelin 0	globulin 1	glutelin 1	globulin 2	glutelin 2	DCM[a]	Canola meal[b]
Essential	Histidine	2.84	2.97	3.02	3.04	3.04	3.00	3.02	2.90	3.32
	Isoleucine*	4.70	4.99	4.85	4.96	5.01	4.93	5.16	4.94	5.80
	Leucine*	8.14	9.21	8.66	9.14	8.93	9.37	9.12	8.32	8.86
	Lysine	5.88	4.42	5.41	4.36	5.05	4.12	5.93	5.93	5.85
	Methionine*	1.85	1.83	2.14	1.68	2.27	1.73	2.46	1.70	1.74
	Phenylalanine*	5.77	6.50	5.60	6.64	5.63	6.84	5.41	5.08	5.00
	Threonine	5.35	4.74	5.14	4.78	5.05	4.73	5.81	5.26	5.45
	Valine	5.75	6.09	5.71	6.09	5.77	6.11	5.84	5.63	6.05
	T[c]-essential	40.29	40.75	40.54	40.69	40.75	40.85	42.74	39.75	42.07
Non-essential	Alanine*	5.87	5.36	5.78	5.29	5.62	5.22	6.26	5.68	5.40
	Aspartate	11.45	11.56	9.97	11.19	9.70	11.83	9.68	9.84	8.44
	Glutamate	18.46	19.23	19.11	19.03	19.24	18.92	15.65	19.94	22.37
	Serine	6.41	5.98	6.12	5.91	6.02	5.92	6.44	6.09	5.47
	Arginine	7.71	7.60	8.42	8.16	8.53	7.77	8.09	8.57	6.95
	Glycine	6.24	5.88	6.13	5.95	6.12	5.79	6.46	6.40	6.07
	Tyrosine	3.58	3.63	3.93	3.79	4.02	3.71	4.69	3.73	3.23
	Ornithine	0	0	0	0	0	0	0	0	0
	T[c]-nonessential	59.71	59.24	59.46	59.31	59.25	59.15	57.26	60.25	57.93
	T[c]-AA	100	100	100	100	100	100	100	100	100
	T[c]-protein	46.35	51.05	61.84	67.38	68.91	76.10	72.07	34.13	34.84

* Hydrophilic amino acids.
[a] Defatted camelina meal. [b] Li, et al., 2011 a. [c] Total.

5.4.4 Size exclusion chromatography (SEC)

Molecular weight (MW) distributions of camelina protein fractions were characterized with SEC in the presence and absence of reducing agents (Figure 5.5 A). Under non-reducing conditions for all protein samples, three major peaks were detected at 8 min, 8.75 min, and 9.5 min. The major peak was around 66 kDa, except in glutelin 2 fraction which exhibited a major peak at approximately 9.5 min with MW of less than 29 kDa. Globulin 0 and globulin 2 showed stronger peak intensity than globulin 1 at MW around 66 kDa, indicating more protein subunits with larger MW presented in globulin 0 and 1. This difference in MW among globulins may be ascribed to effects of the initial protein isolating conditions and sequences. As stated in the protein isolation steps, globulin 0 and globulin 2 were extracted with 5% NaCl solution, whereas globulin 1 was extracted from the pellets already treated with NaOH solution. Some NaOH residue could have remained when performing globulin 1 isolation, so the globulin 1 fraction was extracted as a result of the combined action of both NaCl and NaOH. In addition, both albumin and glutelin fractions had a small peak around 5 min, indicating the presence of the high MW protein subunits. However, this peak was barely detected in globulin fraction except globulin 1, which also could be attributed to the combined action of both NaCl and NaOH in the globulin 1 fraction.

In the presence of a reducing agent (Figure 5.5 B), intensity of the peak around 66 kDa decreased significantly and shifted to MW lower than 29 kDa, indicating that disulfide-bonded cross-linked subunits were presented in all protein fractions. The high intensity of the peak at 66 kDa in globulin 0 and 2 fractions proved they contained more disulfide linkages; however, according to Tables 5.1 and 5.2, more sulfur content and methionine amino acids were detected in glutelin fraction, which should have translated into a larger number of disulfide bonds than the globulin fraction. Many studies have shown that glutelin from corn and wheat is composed of a high level of subunits linked by disulfide bonds (Nielsen et al 1970; Masci et al 1998). The reason for this may be that NaOH could destroy cystine and cleave disulfide bonds during extraction (Nielsen et al 1970; Tecson et al 1971), thus leading to reduced disulfide bonds in glutelin fraction. Furthermore, large MW peaks around 5 min were still detected under the reducing condition, indicating that protein aggregates were stabilized by covalent bonds other than disulfide bonds in those protein fractions.
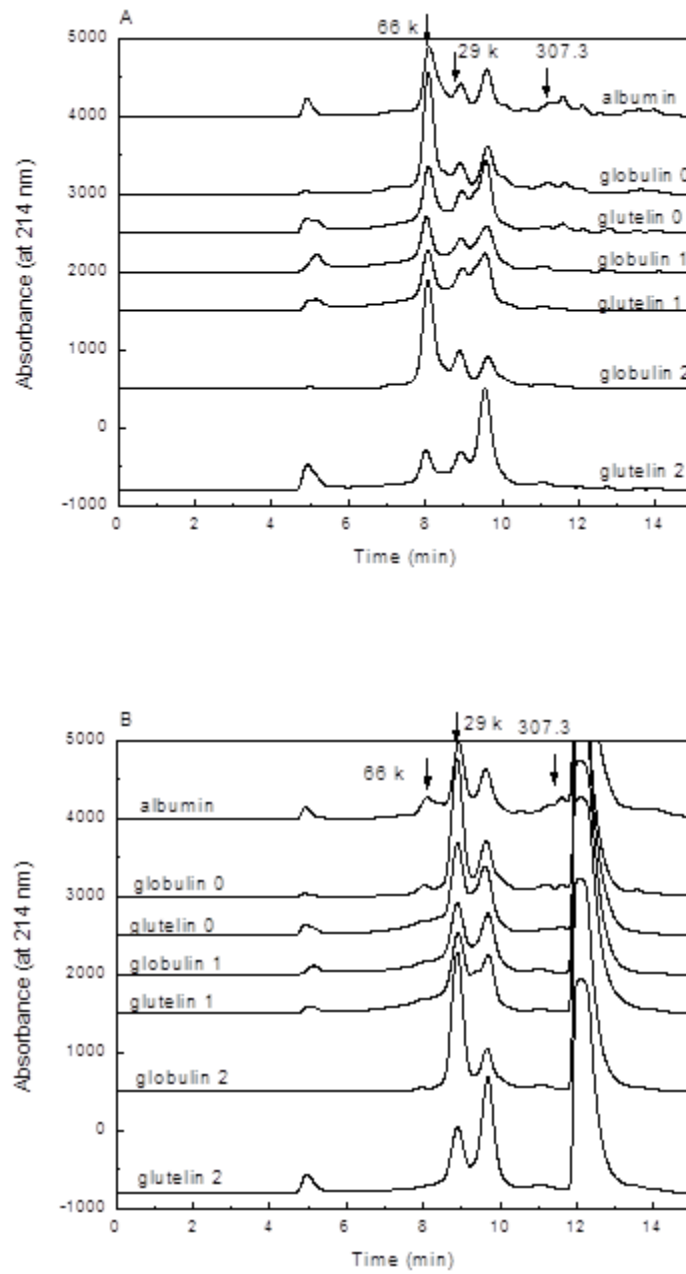
Figure 5.5 Size exclusion chromatography separations of camelina proteins at non-reducing (A) and reducing conditions (B).

5.4.5 Fourier transform infrared spectroscopy

As shown in Figure 5.6A, typical oil absorption bands localized at 1710 and 1745 cm^{-1} (C=O stretching) and at 2853, 2924 and 3006 cm^{-1} (C-H stretching) were detected in the spectra of DCM (Guillén and Cabo 1997). The significant diminishment or disappearance of these bands indicated only trace to low oil content in the isolated protein fractions. Absorptions in the range of 900 to 1250 cm^{-1} related to the C-O stretching vibrations in polysaccharides were detected for all samples with different intensities. Consistent with previous results, albumin and proteins extracted with S0 with low protein purities (Table 5.1) had higher peak intensity at 1050 cm^{-1}, indicating absorption of polysaccharides, than other fractions. Camelina seed was reported to contain polysaccharides, and polysaccharides in camelina seeds showed good water-binding capacity and were capable of aiding seed germination in dry environments (Grady and Nleya 2010).

Protein units give rise to nine characteristic absorption bands, namely, aimde A, B, and I-VII, among which the amide I and II bands are the most prominent vibrational bands of the protein backbone (Kong and Yu 2007). The peptide bond of protein is unique in containing C=O, C-N, and N-H. The amide I absorption contains contributions from primarily C=O stretching vibration (80%) with a minor C-N stretching vibration, whereas the amide II absorption appears to arise from N-H bending vibrations (60%) coupled with C-N stretching vibrations (40%) (Jackson and Mantsch 1995). Peaks at 1630 cm^{-1} and 1520 cm^{-1} are dominated by camelina protein secondary structures amide I and amide II, respectively (Yu et al 2005). After deconvolution, the α-helix in amide I was shown at frequency 1650 cm^{-1} for all protein samples (Figure 5.6 B). Absorptions of β-sheet were in the frequency range of 1626 to 1637 cm^{-1}. Interestingly, absorption peaks for globulin fractions were at higher frequencies than those of glutelin and albumin fractions and DCM. The ratio of α-helix and β-sheet in amide I was quantified by the peak area (Table 5.3). DCM showed higher α-helix and β-sheet ratio (1.12) than other samples, and the lowest ratio existed in albumin fraction (0.84). Glutelin exhibited a higher α-helix and β-sheet ratio (1.03-1.05) than globulin (0.91-1.00), indicating higher α-helix portion in the glutelin fraction.
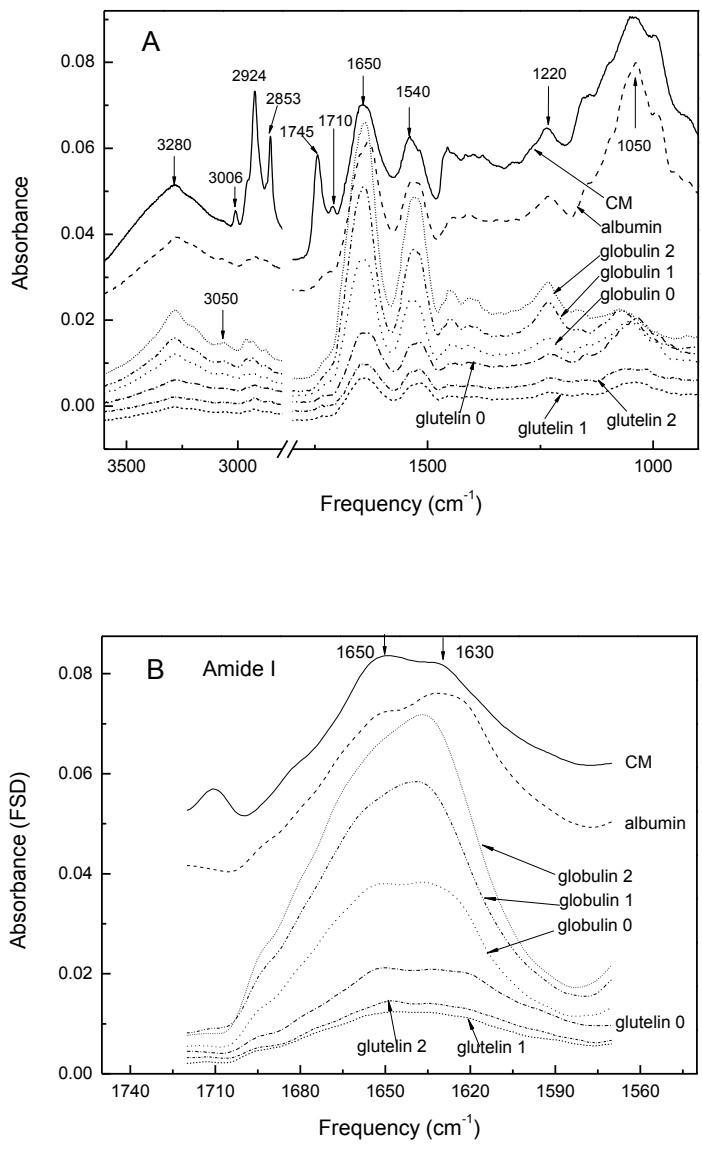
Figure 5.6 FTIR analysis of camelina meal and proteins (A) and α-helix, β-sheet identification in amide I (B).

Table 5.3 Fourier self-peak deconvolution of amide I.

Camelina protein samples	Amide I				
	α-helix		β-sheet		
	Frequency (cm-1)	area	Frequency (cm-1)	area	area ratio of α-helix / β-sheet
CM[a]	1650	1.16	1630	1.04	1.12
Albumin	1650	1.09	1630	1.29	0.84
Globulin 0	1650	1.12	1634	1.12	1.00
Glutelin 0	1650	0.66	1628	0.64	1.03
Globulin 1	1650	1.54	1637	1.70	0.91
Glutelin 1	1650	0.39	1626	0.39	1.00
Globulin 2	1650	1.98	1635	2.18	0.91
Glutelin 2	1650	0.42	1630	0.40	1.05

[a] Camelina meal.

5.4.6 Morphological properties

TEM images of camelina protein fractions are presented in Figure 5.7 at 130,000x magnification. Albumin showed a spherical shape with diameters from 10 nm to 85 nm (Figure 5.7 A), which is bigger than globulins (Figures 5.7B, D, and F) and glutelins (Figures 5.7 C, E, and G) on average. All globulins isolated with various methods exhibited a spherical shape with similar diameters of around 10 nm (Figures 5.7 B, D, F, and distributed uniformly. In the glutelins (Figures 5.7 C, E, and G), irregular and highly dense protein clusters were observed that comprised a mixture of spherical and rod-shaped clusters with diameters from less than 10 nm to several hundred nanometers. In short, bigger protein aggregates were exhibited in albumin and glutelin fractions compared with globulin fractions.

Generally, aggregates of proteins may arise from several mechanisms and may be classified in numerous ways, including soluble/insoluble, covalent/non-covalent, reversible/irreversible, and native/denatured, thus influencing the amount of aggregate produced during the cell culture and purification process. As shown in the SEC section, high MW subunits stabilized by covalent bonds were observed in all glutelin fractions, which may contribute to the larger size of protein aggregates. Cromwell et al (2006) concluded that more disulfide bonds played an important role in protein aggregates and resulting compact protein structures, but glutelin fractions contained fewer disulfide bond-linked subunits than globulin fractions (Figure 5.5A), indicating that disulfide bonds were insignificant in these larger aggregate formations. Moreover, oxidation of tyrosine may also result in covalent aggregation through the formation of bityrosine (Cromwell et al 2006). In the amino acid profiles of camelina protein (Table 5.2), glutelins contained more tyrosine than globulins, leading to the possibility that more covalent bonds could be formed through oxidation. Albumin fraction contained less tyrosine than globulins, suggesting that oxidation of tyrosine may play an insignificant role in protein aggregation.

Protein aggregation is known to affect physiological and pharmacokinetic properties. Some protein deposition diseases such as body myositis, light-chain deposition disease and cataracts, and Alzheimer's disease are related to protein aggregation (Fink 1998). Protein aggregation and, ultimately, precipitation — which results from environments such as

shear/shaking, temperature, pH, and protein concentration during drug production, storage and delivery processes — are also concerns with protein-based drugs (Frokjaer and Otzen 2005).
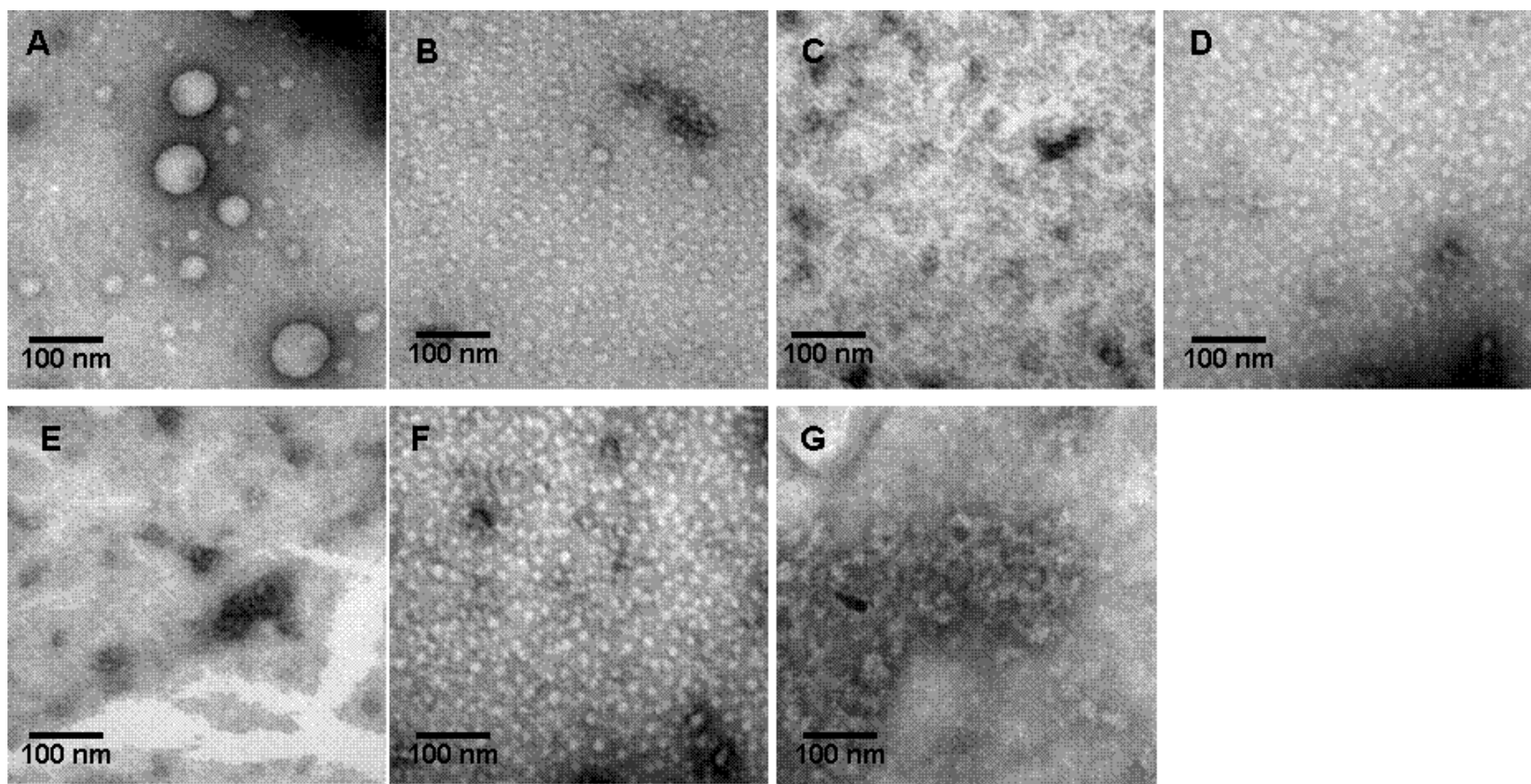
Figure 5.7 TEM images of DCM and camelina proteins: albumin (A, ×130,000); globulin 0 (B, ×130,000); glutelin 0 (C, ×130,000); globulin 1 (D, ×130,000); glutelin 1 (E, ×130,000); globulin 2 (F, ×130,000); glutelin 2 (G, ×130,000).

5.4.7 Thermal gravimetric analysis

TGA and derivative thermogravimetry (DTG) curves are presented in Figure 5.8 as the weight loss (%) and derivative weight loss rate (%/min), respectively, and as a function of sample temperature in range of 25 ℃ to 900 ℃. The degradation of camelina protein fractions underwent two to four stages with different final mass of retention residues. Noncombustible residues are inorganic materials. Albumin showed four stages followed by globulin 0 and glutelin 0, 1, and 2 with three stages and globulin 1 and 2 with two stages, indicating that albumin had more complicated compositions.

In the first stage, the mass of protein samples decreased from 3% to 7.5% from 25 ℃ to around 150 ℃, which could be ascribed to evaporation of both the free water and physically absorbed water in samples (Figure 5.8A). In this stage, maximum mass loss occurred to albumin, indicating that albumin had the highest water absorption ability, possibly because among camelina protein fractions, albumin fraction had the most hydrophilic nature (about 73% of hydrophilic amino acids) (Table 5.2). Hydrophilic amino acids side-chains are known to be either charged or polar, and both are capable of attracting water molecules involved in the formation of hydrogen bonds. They are also predominantly found on the exterior surfaces proteins (King 2011). The second peak at 206.4 ℃ in albumin was probably ascribed to the degradation of water-soluble gum which coexisted with albumin, as mentioned previously. Similarly, peaks with similar temperature were also observed in globulin 0 and glutelin 0 fractions extracted without the degumming procedure. In addition, the larger mass loss at 206.4 ℃ in globulin 0 indicated a high content of gum, which is consistent with the low protein purity of globulin 0 shown in Table 5.1. Notably, peaks were detected around 280-290 ℃ in all glutelin reactions, probably due to thermal breakage of weak non-covalent or covalent bonds.

The major peaks observed around 356 ℃ in all the protein fractions are believed to be protein degradation (Figure 5.8B), a process that involves broken intermolecular and intramolecular hydrogen bonds and electrostatic bonds, decomposition of protein side-chains, and rupture of weak bonds such as C-N, C (O)-NH, C (O)-NH$_2$, and NH$_2$ (Mo et al 2011). Glutelins had a lower degradation peak (around 348 ℃) than globulins (around 352 ℃), with the exception of glutelin 2 (356 ℃). As explained previously, NaCl increased ionic strength and resulted in enhanced hydrophobic interactions, thus improving thermal stability of globulins and

glutelin 2 in terms of the higher degradation peak. Besides, molecular conformation can also affect the protein degradation rate. Again, the globulin fraction had higher mass retention at approximately 350 ℃ (64.66-66.42%), which also suggested higher thermal stability.
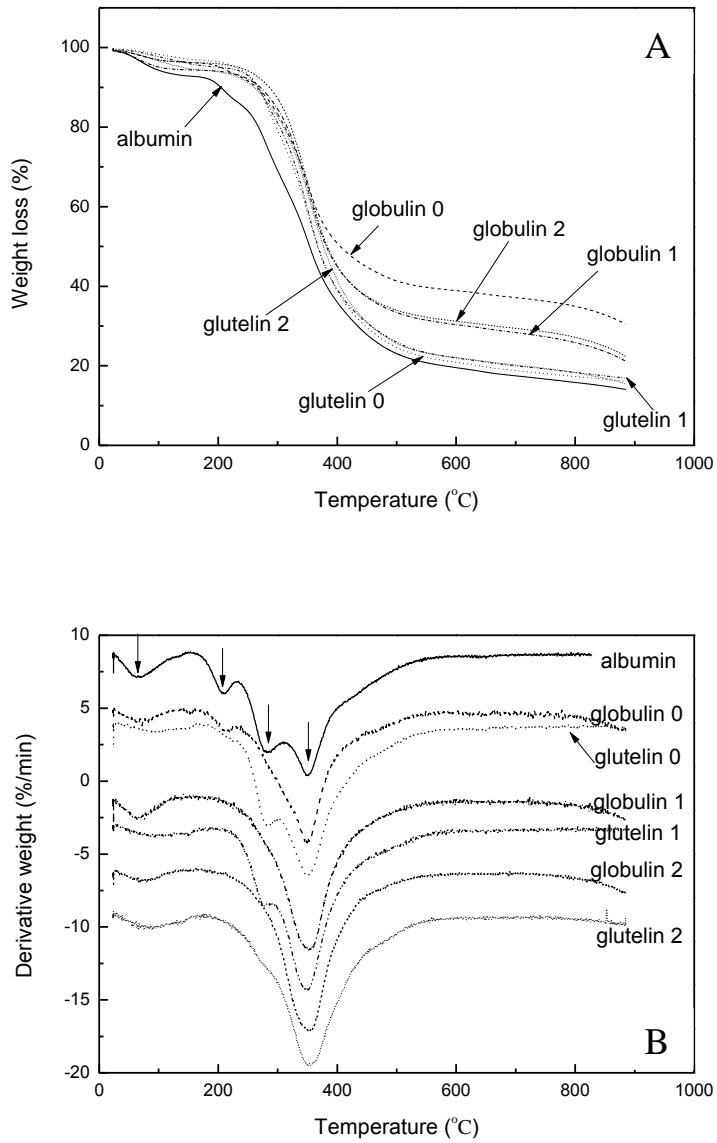
Figure 5.8 Thermogravimetric (A) and derivative thermogravimetric (B) curves of camelina proteins.

5.5. Conclusions

Physicochemical properties of camelina protein fractions, including solubility and precipitation abilities, amino acid profiles, molecular weight distributions, secondary structures, morphological properties, and thermal properties, varied in different protein fractions. The MS-pH of albumin, globulin, and glutelin were found at pH 3.0, 3.0, and 4.5-5.0, respectively. S0 extracted the highest amounts of protein isolates but the lowest protein purities due to the gum. S1 was more effective than S0 and S2 in terms of protein recovery and purities. Essential amino acids accounted for approximately 40% of total amino acids, and essential amino acid profiles met or exceeded WHO standards for children over one year old and adults. Camelina proteins had 26-28% hydrophobic amino acids, which is lower than canola, soy, and sorghum proteins. Glutelins exhibited higher α-helix and β-sheet ratios (1.03-1.05) than globulin fractions (0.91-1.00) and albumin (0.84).

Studying the camelina protein isolation process and its physicochemical properties is vital to understanding its unique functionality, and thus exploring its applications in food and industrial areas such as biodegradable adhesives, plastics, or films.

Chapter 6 - Adhesion property of camelina protein fractions isolated with different sequences

6.1 Abstract

The objective of this study was to investigate adhesion performance of different camelina protein fractions as affected by protein extraction methods. Physicochemical properties including electrophoresis profile, rheological, thermal and morphological properties, and crystallization were also studied. Two camelina protein fractions, globulin and glutelins, were isolated from defatted camelina meal using three different methods (S0, S1, S2). Results showed that the gums negatively affected both the protein yield and adhesion performance and needed to be removed through degumming step. Dry adhesion strength of camelina protein adhesives exhibited nearly 100% wood cohesive failure at the curing temperature from 150 to 190 ℃ except glutelin 2 and globulin 0. The overall adhesion performance of globulin fraction behaved better than glutelin fraction. The greatest wet shear strength of globulin 1 and 2 was around 3.3 MPa, curing at 190 ℃. The wet shear strength of glutulin 2 was inferior to glutelin 1 due to negative effects from NaCl. Glutelin had higher protein aggregation than globulin, as indicated by higher crystallinity, higher thermal stability, and dense protein aggregate. This compact structure of glutelins may partially contribute to lower adhesion strength than globulin.

6.2 Introduction

Camelina, a member of the mustard family and a distant relative to canola, is known in North America primarily as a weed, but as "gold of pleasure" to ancient European agriculturists. Camelina monocultures occurred in the Rhine River Valley as early as 600 BC. The crop was widely grown in Eastern Europe and Russia until the early 1940's with some production lasting up to the 1950's. Today, camelina is produced in Slovenia, Ukraine, China, Finland, Germany, Austria, and the United States (Montana and Wyoming). (http://www.montanaglutenfree.com/omegamontana-camelina-oil.html). Camelina contains

30-38% oil. Current interests of camelina mainly focus on application of camelina oil. Camelina has high levels of polyunsaturated fatty acids (90%), among which 38% are linoleic acids (18:3, omega-3) (Putnam 1993), suggesting camelina is a good candidate for high quality edible oils. Camelina oil also shows great potential as sources of biodiesel. Camelina-derived synthetic fuel has been used to power a variety of military and commercial aircrafts (http://www.gizmag.com/f-22-raptor-biofuel-flight/18218/). Increased interest in camelina oil-based biodiesel or fuel will trigger the spread of intentional planting of camelina, leading to greater availability of the byproducts such as camelina meal which is co-product from the camelina oil extraction process.

Camelina meal typically contains 40% crude protein, a maximum of 12% crude fiber, less than 15% residual oil, and a small portion of vitamins (Sampath 2009). Currently, camelina meal is mainly used as additives for animal feed as protein or omega-3 supplement resource (Ryhanen et al 2007, Rokka et al 2002). Besides animal feed, utilization of camelina protein as value-added products is critical to food and industrial application. Preliminary data indicated that the amino acid profile of camelina protein is similar to canola protein which displayed potential as an alternative to conventional petroleum-based adhesives (Li et al 2011a, Li et al 2011b). Therefore, the hypothesis is made that camelina protein may also have comparable adhesion properties to canola protein. In addition, soybean (oil seed) is one of the most promising and investigated bio-based adhesives over the last few decades (Qi et al 2011, Qi et al 2012, Mo et al 2011), reinforcing the worldwide conflict between bio-based products and the human food.

According to previous research, three protein fractions were extractable from camelina mel: albumin, globulin, and glutelin, using classical or modified Osborne protein fractionation method (Osborne 1924). The objective of the research was to study adhesion performance of camelina protein fractions isolated with different methods. Albumin fraction was not studied because it is water soluble and has strong swallowing properties, resulting in

poor adhesion performance. The protein electrophoresis profile, rheological, thermal and morphological properties, and crystallization were also investigated.

6.3 Materials and Methods

6.3.1 Materials

Camelina meal (CM) with 15% lipid (db), 32.4% crude protein (db), and 11.0% moisture content (db) was purchased from Montana Gluten Free Processors (Belgrade, Mont.). Meal pellets was produced with a screw oil press at a temperature around 170 °F. Hexanes, Bradford Assay, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fair Lawn, N.J.). Cherry wood veneers with dimensions of $50 \times 127 \times 5$ mm (width $\times$ length $\times$ thickness) were provided by Veneer One (Oceanside, N.Y.).

6.3.2 Camelina Protein Fractions Isolation Process

Osborne Method: This extraction method is named as sequence 2 (S2). Camelina protein fractions were isolated from defatted camelina meal (DCM) according to Osborne fractionation scheme (Osborne 1924). Camelina meal with particle size <0.5 mm was obtained by using a cyclone sample mill (Udy Corp., Fort Collins, Colo.). Then, camelina meal was defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 hr at room temperature in three cycles. The DCM was placed in a fume hood with a very thin layer (around 2 mm) for 24 hr to evaporate residual hexane.

Figure 5.2 A illustrates the Osborne fractionation scheme. The DCM was mixed with distilled water at the solid/liquid ratio of 1:30 (w/v). The slurry was stirred for 2 hr, and then centrifuged at $12,000 \times g$ for 15 min. Residues were collected for further camelina protein fractions isolation, and the supernatant containing water soluble protein or polysaccharides was discarded. Both the water soluble gum and albumin could be removed from this procedure, so this step was named the degumming step. Residues were re-suspended in 5% NaCl at the solid/liquid ratio of 1:30 (w/v). The slurry was adjusted to pH 8.0 using 2 N

NaOH and stirred for 2 hr. After centrifuging at 12,000 $\times$ g for 15 min, resulting residues were collected for further glutelin fraction extraction. The supernatant was adjusted to pH 3.0 with 2 N HCl and then centrifuged at 12,000 $\times$ g for 15 min to produce globulin 2. Residues were re-suspended in water with volume ratio 1:30 and adjusted pH to 12 using 2 N NaOH. After continuous stirring for 2 hr, the slurry was centrifuged (12,000 $\times$ g, 15 min) and the supernatant was adjusted to pH 4.5 to precipitate glutelin 2 proteins and collected by centrifuge (12,000 $\times$ g, 15 min). Partial protein isolates were directly used as adhesives and the rest were freeze-dried and grinded for further study.

Modified Osborne Method: This extraction method is named as sequence 1 (S1). Camelina protein fractions were isolated from DCM according to modified Osborne fractionation scheme (Osborne 1924). The main difference compared to Osborne sequence was that glutelin fraction was isolated preceding globulin. Protein isolates extracted with this method were named as globulin 1 and glutelin 1, respectively. This isolation sequence was presented in a flow chart (Figure 5.2 B).

Camelina Protein Extraction without Degumming: This extraction method is named as sequence 0 (S 0). Two batches of DCM with particle size less than 0.5 mm were mixed with 5 % NaCl solution at pH 8 and NaOH solution at pH 12, respectively, to solubilize these two protein fractions. Protein isolates extracted with this method were named globulin 0 and glutelin 0, respectively. Flow charts in Figure 5.2 C and D are presented to illustrate the procedure.

6.3.3 Chemical Analysis

Moisture content was measured using the V30 Compact Volumetric KF Titrator (Columbus, Ohio). Nitrogen (N) content was measured via a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, Conn.). Nitrogen was converted to protein using a factor of 6.25. All tests were performed with duplications.

6.3.4 SDS-PAGE Gel Electrophoresis

SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system, as described by Laemmli (1970). A camelina protein sample was mixed with a buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To determine the disulfide bonds in camelina protein, SDS-PAGE was carried out under both reducing (2-mercaptoethonal) and non-reducing conditions. A total of 8 μg of protein was applied to sample wells. Molecular weight standards (14.4-97.4 kDa) 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.

6.3.5 Rheological Properties

Apparent viscosities on isolated camelina proteins were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, Mass.) with a CP 4/40 cone and plate fixture (4 ° cone angle, 40-mm cone diameter). Distance between cone and plate was set to 150 μm for all measurements. Experiments were conducted under steady shear flow at 23 ℃. Shear rates ranged from 10-240 s^{-1} in increments of 10 s^{-1}. All experiments were done in duplicate, and average values were reported.

6.3.6 Differential Scanning Calorimetry (DSC)

Thermal transition properties of protein samples were measured with a DSC Q200 V24.4 instrument (TA Instruments, New Castle, Del.) calibrated with indium and zinc before making official measurements. Samples of dry camelina proteins weighing approximately 7–10 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL/min. All samples were heated from 25 ℃ to 280 ℃ at a heating rate of 10 ℃/min in an inert environment. All experiments were performed in duplicate.

6.3.7 Degree of crystallinity of camelina protein

Degree of crystallinity of dry camelina protein adhesives was studied with wide-angle X-ray diffraction (WAXD) technique. WAXD experiments were carried out at the advanced polymers beamline (X27 C), National Synchrotron Light Source, Brookhaven National Laboratory, in Upton, N.Y. Details of the experimental setup at the X27 C beamline have been reported elsewhere (Chu and Hsiao 2001). The wavelength used was 0.13714 nm. A 2D MAR-CCD (MAR USA, Inc.) X-ray detector was used for data collection. Data was collected from diffraction angle 5.0 to 35.0 ° (2θ). Crystallinity was estimated from an integrated diffraction intensity profile as the ratio of total crystal peak diffraction intensity to total diffraction intensity. A peak-fitting process was employed with Igor Pro 6.20 (WaveMetrics Inc. Lake Oswego, Ore.). The d-spacing between crystal lattice planes was estimated with Bragg equation (1):

$$2d \sin \Theta = \lambda \qquad (1)$$

Where d is the space between crystal lattice planes, λ is the wavelength, and 2Θ is the diffraction angle.

6.3.8 Scanning Electron Microscopy (SEM)

A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) SEM was used to observe the microstructure of dried camelina protein isolates powder. Ground protein powder was 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, N.J.). SEM images of protein isolates were performed with operation conditions at an accelerating voltage of 5 kV.

6.3.9 Wood specimen preparation

Cherry wood samples were preconditioned in a controlled-environment chamber (Model 518, Electro-tech systems, Inc., Glenside, Pa.) for 7 d at 25 ℃ and 50% relative humidity (RH). Canola protein adhesives, isolated with different methods, were brushed

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separately along the edges of two pieces of cherry wood with an application area of 127 mm × 20 mm until the entire area was completely covered. The adhesive amount applied on each piece was approximately 0.06 g (dry basis). The brushing and setting procedure followed the method described by Mo et al (2004). The brushed areas of the two pieces were assembled together at room temperature for 15 min, then pressed at a pressure of 2.0 MPa at 150 ℃, 170 ℃, or 190 °C for 10 min using a hot press (Model 3890 Auto 'M', Carver Inc., Wabash, Ind.).

6.3.10 Mechanical Properties

After pressing, the glued-wood assemblies were conditioned at 23 ℃ and 50% RH for 2 days, then cut into five specimens, each measuring 127 mm (length) × 20 mm (width) × 5 mm (thickness). The cut specimens were conditioned for another five days at 23 ℃ and 50% RH before the dry test. Three adhesion strengths were tested: dry strength, soak strength, and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron (Model 4465, Canton, Mass.) according to ASTM Standard Method D2339-98 (2002). The crosshead speed of Instron for adhesion strength testing was 1.6 mm/min, and adhesion strength was recorded as tensile strength at the maximum load. Reported results are an average of five samples.

Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 (2002) and D1151-00 (2002), respectively. Preconditioned specimens were soaked in tap water at 23 ℃ for 48 h and then tested immediately for wet strength. For the soak strength test, specimens were soaked in tap water at 23 ℃ for 48 h and then conditioned at 23℃ and 50% RH for an additional seven days before testing.

6.3.11 Statistical Analysis

Data from the mechanical property evaluation were taken from an average of five samples. Data from experiments carried out in duplicate were analyzed through analysis of

variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, N.C.).

6.4 Results and Discussion

6.4.1 Camelina protein yield and protein content

As shown in Table 6.1, S0 extracted more dry protein isolates (41.57% of globulin and glutelin) than those of S1 (34.44%) and S2 (29.76), which could be attributed to the existence of water soluble gums contamination in S0. Noted that S0 was without degumming steps, and the camelina meal-water slurry was very thick and sticky when those gums presented. Partial gums could possibly be co-extracted with proteins, resulting in higher protein yield as well as lower protein purity in S0. In the case of S1 and S2, no significant difference was observed on protein purity (P<0.05). However, adhesive yield (dry) varied greatly based on isolation methods. S1 yielded less globulin (6.13%) than that of S2 (7.75%), which may be because that partial globulin 1 had already been co-isolated with glutelin 1, leading to low globulin 1 yield in S1. Preliminary tests indicated that camelina globulin fraction was partial soluble at high pH (above pH 11). Similar phenomena was also reported by Sun regarding phaseolus globulin (Sun, 1975). On the other hand, S1 extracted more glutlein (glutelin 1, 28.31%) than that of S2 (glutelin 2, 22.01%), which could be ascribed to the negative effect of NaCl on protein solubility. Glutelin 2 was isolated from camelina meal pellets where it had been previously treated with NaCl for globulin extraction; therefore, glutelin 2 was unavoidably affected by the remaining NaCl. Makkar et al (2008) reported that NaCl negatively affected protein solubility by increasing the ionic strength in solutions, resulting in increased hydrophobic interaction among protein molecules and decreased protein solubility.

Table 6.1 Camelina protein adhesive yield and protein purity.

Camelina protein samples	Wet adhesive yield (db %)	Solid content (db %)	Dry adhesive yield (db %)	Protein purity (db %)
Globulin 0	130.00 b[1]	8.20 b	10.66	57.56 e

Glutelin 0	150.78 a	20.50 a	30.91	70.81 d
Globulin 1	81.19 d	7.55 b	6.13	81.00 c
Glutelin 1	149.00 a	19.00 a	28.31	87.01 a
Globulin 2	107.64 c	7.20 b	7.75	86.93 a
Glutelin 2	117.7 bc	18.70 a	22.01	83.68 b

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

6.4.2 SDS-PAGE

Reducing and non-reducing SDS-PAGE was performed to study subunit distribution and disulfide bonds in each camelina protein fractions. Figure 6.1 presents the electrophoresis of camelina protein under non-reducing condition. Dominant protein components in glutelin fraction (Figure 6.1 A, C, and E) were distributed at MW around 50, 30, 22 and 15 KDa. While major protein subunits in globulin (Figure 6.1 B, D, and F) were detected at MW around 50, 30, and 25 KDa, the faint band appeared at 15 KDa. Bands at 50 KDa were almost faded by the addition of reducing agent, as shown in Figure 6.2, indicating that the formation of disulfide bonds was one contributor for camelina protein aggregation. The intensified bands at 25 kDa under reducing condition were considered to result from the reduction of those aggregations (Figure 6.2). In addition, low MW bands at 15 kDa disappeared under the reducing condition and concomitant intensified bands are lower than 10 kDa, suggesting again the presence of disulfide bonds in camelina protein. Globulin had higher density of bands at 50 kDa and lower density of bands at 15 kDa than glutelin fraction, illustrating that disulfide bonds play an important role in stabilizing high molecular weight protein aggregates. It is reported that NaOH could destroy cystine and cleave disulfide bonds during glutelin extraction from corn or rice (Nielsen et al 1970; Tecson et al 1971). Therefore, glutelin extracted from camelina meal may also reduce disulfide bonds due to NaOH effects. Similar to canola protein, molecular weight distribution of camelina protein fractions under reducing condition was in the range of 6 kDa to 30 kDa (Manamperi et al 2008), which is much smaller than soy proteins (23kDa to 85 kDa) (Qi et al 2011).

In addition, small amounts of high molecular weight protein aggregates on top of the resolving gel were also observed under non-reducing condition (Figure 6.1). In the presence of reducing agent, those aggregates were faded completely in globulin fractions (Figure 2 B, D, and F), while trivial amounts still remained in glutelin fraction, suggesting that those glutelin protein aggregates were stabilized by other covalent bonds besides disulfide bonds. Similar protein aggregates were also detected in canola protein glutelin fraction and soy protein under the reducing condition (Manamperi et al 2008; Qi et al 2011). Those high molecular weight aggregates may affect physicochemical properties of camelina protein which will be further discussed.
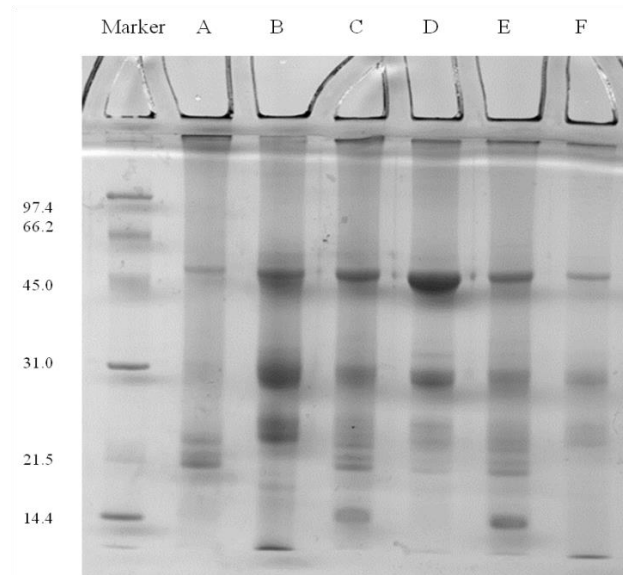
Figure 6.1 Non-reducing and reducing SDS-PAGE patterns of camelina protein fractions. A: Glutelin 2. B: Globulin 2. C: Glutelin 1. D: Globulin 1. E: Glutelin 0. F: Globulin 0.
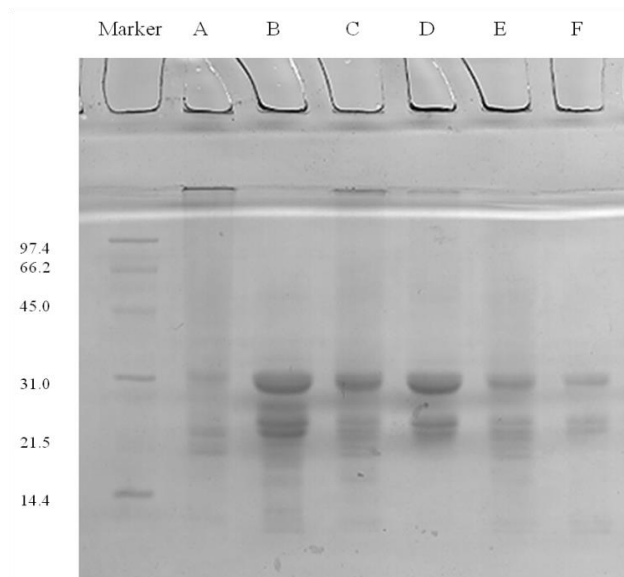
Figure 6.2 Reducing SDS-PAGE of camelina protein fractions. A: Glutelin 2. B: Globulin 2. C: Glutelin 1. D: Globulin 1. E: Glutelin 0. F: Globulin 0.

6.4.3 Rheological Properties

Rheological properties, which are related to adhesives' flowability and wetting properties, of camelina protein adhesives were presented in Figure 6.3 as apparent viscosity. Apparent viscosity decreased as shear rate increased, indicating shear thinning behavior to camelina protein adhesives. In general, glutelins (2500-8200 Pa.s) exhibited higher apparent viscosity than those of globulins (300-1600 Pa.s) because stronger aggregation, which could be illustrated in SDS patterns (Figure 6.1 2) and SEM images (Figure 6.6), in glutelins. Glutelin 2 fraction showed highest apparent viscosity due to maximum protein aggregation (Figure 6.1 and 6.2). Positive effects of protein aggregation on apparent viscosity was well documented. Kumar (2002) stated that high viscosity soybean proteins resulted from increased intermolecular interactions due to unfolded (aggregated) protein molecules. Rullier et al (2008) and Le Floch-Fouéré et al (2009) also indicated that interfacial rheology of protein mainly depends on the aggregation state or strength of protein-protein interactions. In addition, both glutelin 0 and globulin 0 showed lower apparent viscosities than glutelins and globulins, respectively, which could be ascribed to the presence of gums in these two fractions.

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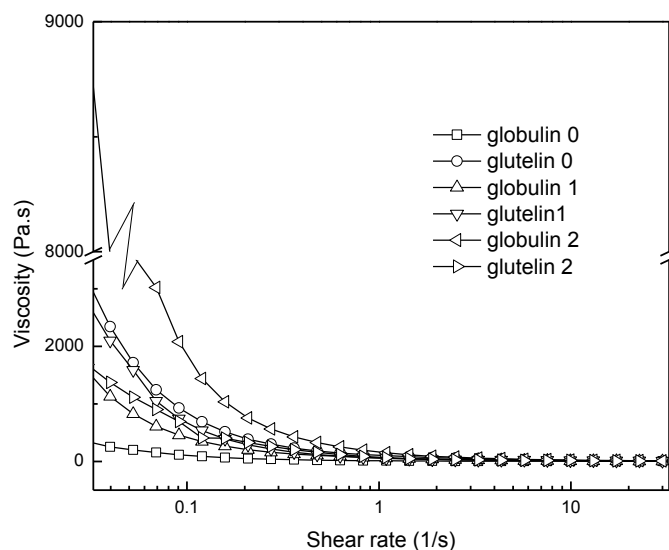
Figure 6.3 Apparent viscosity of camelina protein adhesives.

6.4.4 Thermal properties

Figure 6.4 shows thermal properties of camelina globulin and glutelin fractions. All samples presented a board denaturation endothermic peak and a following large exothermic peak which is related to protein aggregation. Camelina glutelin had the denaturation temperature (T_d) in the range of 131 ℃ - 141 ℃, while the globulin fraction denatured at 115 ℃ – 140 ℃ under different extraction conditions, suggesting glutelins were more thermal stable than globulins in camelina. Glutelin 2 had T_d around 141 ℃, possibly due to the salt effect with increased hydrophobic interaction and protein thermal stability (Fitzsimons et al 2007). This speculation was also in agreement with SDS-PAGE patterns (Figure 6.1 and 6.2), where glutelin 2 contained large amounts of high molecular weight protein aggregates, thus intensifying protein's stability.

Protein denaturation during thermal treatment involves the breakage of intramolecular bonds (covalent and non-covalent) by absorbing heat. Conversely, denatured (unfolded) protein molecules also favored the aggregation/association among each other

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through formation of new intermolecular bonds, giving rise to an exothermic process in DSC thermogram, as shown in Figure 6.5. Similar protein aggregation exotherm peak was also observed in other proteins such soy protein, sorghum, canola protein, whey protein and pea storage protein (Marshall and Zarins 1989; Mo et al 2004; Li et al 2011a; Li et al 2011b; Fitzsimons et al 2007; Bacon et al 1989). Without the degumming step, globulin 0 and glutelin 0 began to aggregate around 150 ℃, which may be related to their contamination (gum) with the exothermic peak around 150 ℃ (result not shown). The onset temperature of protein aggregation in globulin 1 and 2 (158 ℃ -178 ℃) was much lower than the glutelin 1 (199 ℃). However, glutelin 2 had the reduced onset protein aggregation temperature around 166 ℃. Results were consistent with previous studies regarding denaturation and aggregation process of whey protein (Fitzsimons et al 2007). Fitzsimons reported that aggregation temperature of whey proteins was moved to progressively lower temperature as salt concentration increased from 80 to 100 Mm, confirming that salt promotes aggregation of denatured protein.
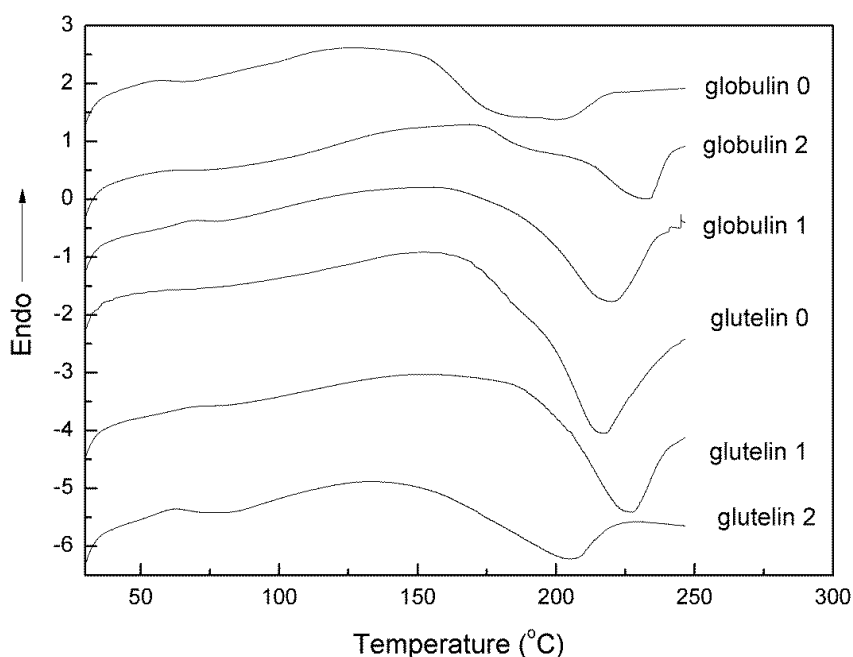
Figure 6.4 DSC thermogram of dry camelina protein adhesives.

6.4.5 Crystallinity of camelina protein adhesives

The WAXD pattern and crystallinity of camelina proteins were shown in Figure 6.5 and Table 6.1, respectively. Sharp peaks observed at diffraction angles of 24.4 ° and 28.2 ° (2θ) in all globulins and glutelin 2 fractions could be ascribed to crystallization of NaCl (Sulyanov et al 2003) because partial NaCl would be co-extracted with globulins and glutelin 2 during the isolation process. Two peaks observed around 7.9-9.01 ° (peak 1) and 17.5 ° (peak 2) (2θ) with corresponding d-spacing of 7.0-9.01Å and 4.43-4.54Å, respectively, were related to protein crystallization (Table 6.2). Interestingly, diffraction angles for the two peaks of glutelins were lower than globulins, indicating that glutelins had shorter d-spacing between crystal lattice planes (Table 6.2). Similar, but not identical, diffraction patterns were also observed on soy protein or silk protein, where diffraction angles were 8.7-8.8 ° and 19-19.6 ° (2θ) (Ki et al 2007; Zhou et al 2007), respectively. The discrepancy may be ascribed to different protein species or varied X-ray test conditions (λ). Interpretations on the two peaks are still in controversy. Sets of papers stated that both peak 1 (2θ=8.7-9.0 ° corresponding to

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d=9.7-10.1 Å) and peak 2 (2θ=19.6-20.6° corresponding to d=4.3-4.5 Å) were identical to β-sheet crystalline (Ki Chang 2007; Kim et al 2004; Valluzzi and Jin 2004); while, in contrast, Drummy et al (2007) ascribed the first peak (2θ=9.0° corresponding to d=9.5 Å) and peak 2 (d=4.5 Å) to α-helix and β-sheet, respectively. Elshemey et al (Elshemey et al 2010) studied the correlation to nine different proteins conformation of WAXD parameters and concluded that the ratio of intensities of 10 Å peak to the 4.5 Å peak (I_1/I_2) was positively sensitive towards α-helix content in proteins with a high correlation of 0.75, meanwhile showing a negative correlation (-0.71) to β-sheet structure. This result indicated that WAXD peak 1 (d=10 Å) was more related to α-helix, while peak 2 (d=4.5 Å) was more identical to β-sheet in protein.

Crystallinity (%) of camelina protein adhesives varied upon isolation methods. Glutelins (34-35.3%) exhibited higher crystallinity than that of globulins (27.4-32%), indicating more protein in glutelins was in the ordered state instead of amorphous state as compared to that of globulins. Higher crystallinity made glutelins have more compact and stable structures and, consequently, influence adhesion performance.
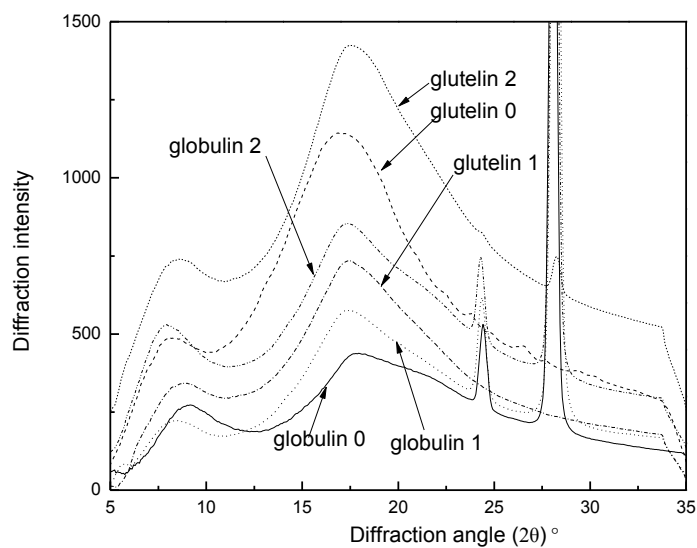
Figure 6.5 Crystallinity of dry camelina protein adhesives

Table 6.2 Diffraction angle (2Θ) and crystallinity of dry camelina protein adhesive.

Camelina protein samples	Peak 1		Peak 2		Crystallinity (%)
	Diffraction angle (2Θ) °	d-spacing (Å)	Diffraction angle (2Θ) °	d-spacing (Å)	
Globulin 0	7.93	9.91	17.45	4.52	32.00
Glutelin 0	8.89	8.84	17.82	4.43	34.00
Globulin 1	8.48	9.27	17.52	4.50	27.80
Glutelin 1	9.01	8.73	17.59	4.48	35.30
Globulin 2	7.90	9.95	17.38	4.54	27.40
Glutelin 2	8.56	9.19	17.59	4.48	34.20

6.4.6 Morphological properties

Morphological properties of camelina protein adhesives are represented in Figure 6.6 as SEM images. Globulins mainly existed as spherical granules with uniform diameter (Figure 6.6 A, C, and E) and had a looser protein network compared to glutelins in which protein granules were tightly clung together with a fairly compact and rigid texture (Figure 6.6 B, D, and F). This observation indicated stronger protein aggregation in glutelins than globulins. Formation of covalent bonds in protein was considered to be one pathway for protein aggregation (Cromwell et al 2006). Covalent bonds could be formed by both disulfide bonds and formation of bityrosine through oxidation of tyrosine amino acid. In amino acid profiles of camelina protein, glutelins contained more tyrosine than globulins (Table 5.2), possibly leading to more covalent bonds formation through oxidation.

No obvious morphological difference was observed among globulins fractions, with the exception of globulin 0. The considerable amount of thin film with both relative big and small holes and filament materials were observed among globulins 0 granules or on the granule surface, which could be water soluble gums co-extracted with globulin-0 (Figure 6.6 A). Compared with globulin 0, glutelin 0 contained less amount of gum (Figure 6.6 B). These observations were in agreement with results in Table 1, where globulin 0 showed the lowest protein purity (57.56%) followed by glutelin 0 (70.81%).
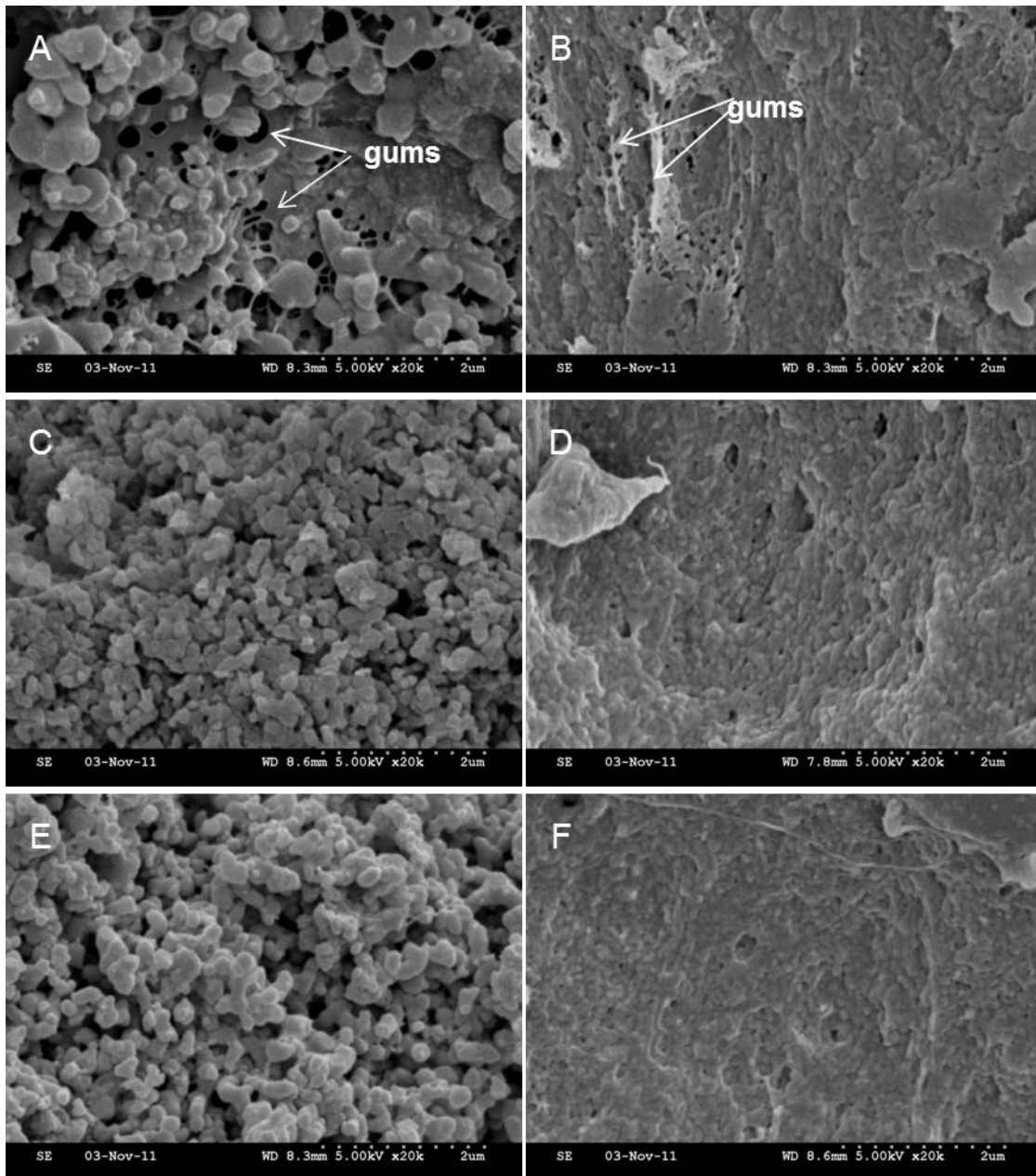
Figure 6.6 SEM images of dry camelina adhesives. Globulin 0 (A, X 20,000); Glutelin 0 (B, X 20,000); Globulin 1 (C, X 20,000); glutelin 1 (D, X 20,000); Globulin 2 (E, X 20,000); and Glutelin 2 (F, X 20,000)

6.4.7 Mechanical properties of camelina protein adhesives

Mechanical properties of camelina protein-based adhesives are summarized in Table 6.3 as wet, soak, and dry shear strength (MPa). The wood cohesive failure (WCF), an indicator that bonding strength between wood and camelina protein adhesives was stronger than mechanical strength of the wood itself, was only partially observed under soaked and dry conditions. Both camelina protein fractions isolated with varying methods and curing temperature significantly affected adhesion properties (Table 6.3). As curing temperature increased from 150 ℃ to 190 ℃, not only did interactions among protein molecular improve, but also chemical reactions at the interface between protein adhesives and wood were also enhanced (Mo et al 2006; Zhong et al 2001), resulting in improved adhesion performance of camelina protein fractions. However, higher curing temperatures did not always result in optimum adhesion performance to protein-based adhesives. Li et al (2011a) reported that wet adhesion strength of sorghum protein-based adhesives increased as curing temperature increased from 130 ℃ to 150 ℃, but decreased significantly from 3.15 MPa to 1.92 MPa at temperature of 190 ℃ because protein decomposition occured. This phenomenon was not observed for camelina protein adhesives, indicating camelina protein is more thermal stable than sorghum protein.

Adhesion performance of camelina protein fractions were also affected greatly by various protein isolation methods. Generally, protein adhesive isolated without degumming step (S0) had lower adhesion strength than ones with the degumming procedure (S1 and S2), indicating degumming is critical for both camelina protein isolations and protein applications. The excellent water absorption and swelling properties of camelina gums could damage wood-protein interactions by interfering in physical or chemical bonds, leading to poor mechanical strength of globulin 0 and glutelin 0.

Without considering the effect of gums, camelina globulins fraction adhesives (globulin 1, 2) exhibited better adhesion performance than glutelin fractions (glutelin 1, 2). According to the SEM image (Figure 6.6), the native loose and porous morphology of globulins provided them with larger surface area and more exposing polar and apolar groups which, in turn, form strong mechanical interlocking chemically or physically between wood and protein adhesives during the curing process, thereby leading to an improvement in adhesive strength and improved water resistance (Lambuth 1977). No significant difference on adhesion performance was observed among globulins, while glutelin 1 behaved better than glutelin 2. Results showed that glutelin 1

exhibited higher shear strength (4.68-5.88 MPa, WCF 100%) than glutelin 2 (3.13-4.51 MPa, WCF 0%) for soak and dry samples (Table 6.3). As mentioned in the protein isolation section, glutelin 1 was extracted from defatted camelina meal directly, while glutelin 2 was isolated from residue after the globulin 2 isolation. Therefore, some NaCl still remained in the glutelin 2 as residues which affected adhesion properties of glutelin 2. Effects of NaCl on functional properties of proteins, such as adhesive strength, solubility, solution viscosity, and water binding have been well documented. Kalapathy et al (1996) stated that viscosity and adhesive strength of soy protein at 14% solids concentration significantly decreased as NaCl increased from 0 to 0.2 M, which may result from weakened interaction of the polar groups of proteins with polar groups of woods. The negative effect of NaCl on protein adhesion could also enhance protein aggregation. Carbonaro et al (1997) reported that protein aggregation occurred when protein isolations were conducted on faba bean, lentil, and chickpea proteins with the presence of NaCl due to increased hydrophobic interaction, believed to be the driving force to protein aggregation. Glutelin 2 exhibited greater protein aggregation than that of glutlein 1 (Figures 6.1, 6.2, and 6.6), resulting in poorer adhesion performance.

6.4.8 Comparison of Camelina Protein and Other Protein-Based Adhesives

Mechanical strengths of camelina, canola and soy protein-based adhesives obtained at optimum conditions are presented in Table 6.4. All adhesives exhibited comparable dry and soak adhesion performance. However, the most significant difference was observed on wet shear strength, and wood failure was only observed with canola protein adhesive at curing temperature of 190 ℃. Optimum wet shear strength was observed at 190 ℃ for all adhesives excepting sorghum protein (at 150 ℃). Optimum adhesion performance of protein-based adhesive evolves in protein structure unfolding, melting, functional groups crossing linking among protein adhesive or between adhesive and wood surface, and re-crystallization processes at optimum curing conditions such as curing temperature, pressure, and pressing time. Those processes typically occurred at temperature for protein complete denaturation, varied with different protein sources, and could be roughly predicted by thermal property studies such as DSC (Li et al 2011a; Wang et al 2007). The temperature for protein complete denaturation was around 190 ℃ for camelina, canola, and soy protein, and 125 ℃ for sorghum protein (Li et al 2011a; Wang et al

2007). These results may explain why optimum adhesion performance was observed at lower temperature in sorghum protein than canola, camelina and soy proteins.

Even though camelina globulins showed better adhesion performance than glutelins, globulins only accounted for 15% (db) of the total camelina protein. Glutelins fraction accounts for 65% (db)   in total camelina protein, but had a weak water resistance (Table 6.4). Therefore, further work may focus on enhancing the water resistance of camelina glutelins by grafting specific hydrophobic groups, inducing the crosslinking agent, or using denaturants to disrupt the highly aggregated protein structure, and exploring the potential application of glutelin as bio-based wood adhesives.

Table 6.3 Adhesion properties of camelina protein adhesives.

Sample ID	Wet Strength (Mpa)			Soak Strength (Mpa)			Dry Strength (Mpa)		
	150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃	150 ℃	170 ℃	190 ℃
globulin 0	0.51±0.03 0% WCF[1]	0.58±0.07 0% WCF	1.27±0.11 0% WCF	3.21±0.16 0% WCF	3.51±0.23 0% WCF	4.24±0.13 0% WCF	3.57±0.55 0% WCF	3.78±0.41 0% WCF	4.43±0.37 0% WCF
globulin 1	1.31±0.12 0% WCF	2.10±0.17 0% WCF	3.23±0.14 0% WCF (fiber out)	4.32±0.07 30 % WCF	5.02±0.19 100 % WCF	4.96±0.56 100 % WCF	4.79±0.38 100 % WCF	5.28±0.21 100 % WCF	5.23±0.12 100 % WCF severe
globulin 2	1.26±0.10 0% WCF	1.99±0.41 0% WCF	3.32±0.15 0% WCF (fiber out)	4.96±0.69 60 % WCF	5.01±0.09 100 % WCF	5.07±0.30 100 % WCF	5.23±0.29 20 % WCF	5.40±0.32 100 % WCF	5.45±0.41 100 % WCF severe
glutelin 0	0.47±0.08 0% WCF	0.64±0.05 0% WCF	1.30±0.03 0% WCF	4.55±0.35 50 % WCF	4.68±0.25 60 % WCF	4.94±0.53 100 % WCF	4.85±0.56 100 % WCF	5.10±0.11 100 % WCF	5.01±0.50 100 % WCF
glutelin 1	0.35±0.02 0% WCF	0.72±0.02 0% WCF	2.10±0.26 0% WCF	4.68±0.27 40 % WCF	5.06±0.11 100 % WCF	5.41±0.19 100 % WCF	4.83±0.63 100 % WCF	5.11±0.28 100 % WCF	5.88±0.68 100 % WCF severe
glutelin 2	0.50±0.10 0% WCF	0.67±0.13 0% WCF	2.15±0.24 0% WCF	3.13±0.32 0 % WCF	4.39±0.29 0 % WCF	4.34±0.07 0 % WCF	3.45±0.15 0 % WCF	3.64±0.25 0 % WCF	4.51±0.36 0 % WCF

[1]WCF: Wood cohesive failure

Table 6.4 Comparison of camelina protein and other protein-based adhesives

Curing condition	Camelina proteins (MPa)		Canola protein[2] (MPa)	Sorghum protein[3] (MPa)	Soy protein[3] (MPa)
	Globulin-1	Glutelin-1			
Wet strength					
130 ℃				2.36± 0.22(0)	1.61(0)
150 ℃	1.31± 0.12(0[1])	0.35± 0.02(0)	1.95±1.07(0)	2.45± 0.10(0)	1.63(0)
170 ℃	2.10± 0.17(0)	0.72± 0.02(0)	2.23 ±0.45(0)	1.13± 0.04(0)	1.98(0)
190 ℃	3.23± 0.14(0)	2.10± 0.26(0)	3.97±0.53(100)		2.68[4]
Dry strength					
130 ℃				5.00± 0.14(100)	4.55(100)
150 ℃	4.79± 0.38(100)	4.83± 0.63(100)	4.63± 0.57(100)	5.23± 0.08(100)	5.29(100)
170 ℃	5.28± 0.21(100)	5.11± 0.28(100)	5.15± 0.33(100)	4.36± 0.12(100)	4.88(100)
190 ℃	5.23± 0.12(100)	5.88± 0.68(100)	5.44± 0.32(100)		5.80[4]
Soaked strength					
130 ℃				4.90± 0.20(100)	4.17(100)
150 ℃	4.32± 0.07(100)	4.68± 0.27(100)	4.76± 0.36(100)	4.89± 0.27(100)	4.35(100)
170 ℃	5.12± 0.19(100)	5.06± 0.11(100)	5.45± 0.23(100)	4.22± 0.24(100)	4.42(100)
190 ℃	4.96± 0.56(100)	5.41± 0.19(100)	5.24± 0.21(100)		5.73[4]

[1] Wood cohesive failure (%);
[2] Li N, 2011(a);
[3] Li N, 2011(b);
[4] Wang Y, 2007

6.5 Conclusions

Physicochemical properties and adhesion performance of camelina protein fractions, globulins and glutelins, isolated from camelina meal were characterized. Protein isolation sequence 1 (S1) was more effective than that of S0 and S2 in terms of both protein yield and purity. Glutelin (65% db) was the major fraction in total camelina protein, followed by globulin (15%). Glutelin had compacter protein structure and higher aggregation than globulin, which may contribute to lower adhesion strength to glutelin.

In general, camelina displayed comparable water resistance at optimum curing temperature with sorghum protein or soy protein, but was inferior to canola protein. Future work may involve grafting specific hydrophobic groups, inducing the crosslinking agent, or using denaturants to disrupt the highly aggregated glutelin protein structure in order to improve wet adhesion strength for bio-based wood adhesives application.

Chapter 7 - Conclusion and future work

7.1 Conclusion

Sorghum protein extracted from sorghum DDGS and sorghum flour with different methods affected adhesion performances. Results showed that PI had the best adhesion performance, followed by PF and PII, especially for wet strength. Wet strength of PI at 12% protein concentration assembled at 150 ℃ was 3.15 MPa, which is significantly higher than PII (2.17 MPa0 and PF (2.59 MPa). Low protein purity caused by non-protein materials of PF may be the primary reason for lower adhesion strength as compared to PI. In addition, PI may have more hydrophobic amino acids aligned at the interface than PII, which could explain the better water resistance of PI. Optimum sorghum protein concentration and pressing temperature for maximum adhesion strength is 12% and 150 ℃. Compared with soy protein-based adhesives, PI had advantages such as significantly higher water resistance and lower energy input. These results indicate that sorghum protein displays huge potential as an alternative to petroleum-based adhesives.

Canola protein was extracted from defatted meal slurry modified with varying $NaHSO_3$ concentrations and precipitated by using alkali solubilization-acid precipitation method. Canola protein recovery increased as concentration of $NaHSO_3$ increased, but canola protein purities decreased. Even though canola protein had a low content of hydrophobic amino acids, its water resistance was excellent when used as a wood adhesive. $NaHSO_3$ had slight weakening effects on adhesion performance of canola protein, resulting from counterbalanced effects on adhesion performance: positive effects of good flow-ability and negative effects of induced extra charges $(RS\text{-}SO_3^-)$ in $NaHSO_3$-modified samples. Canola protein modified with 3g/L $NaHSO_3$ had wet shear strength comparable to the control, but had significantly improved flow-ability and handling properties.

Canola protein-based adhesives were developed and showed great potential as bio-based adhesives. Dry and soak wood specimens had 100% wood failure. Wet specimens showed partial wood failure only at high curing temperature of 170 and 190 ℃. Modifier $NaHSO_3$ did not improve adhesive properties of canola protein, but it greatly improved flow-ability and handling properties. $NaHSO_3$ functionalized canola protein adhesive as the reducing agent, and the

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decrease of molecular weight, thermal transition temperature, and apparent viscosity of canola protein directly proved its reducing effect.

Physicochemical properties of camelina protein fractions, including solubility and precipitation abilities, amino acid profiles, molecular weight distributions, secondary structures, morphological properties, and thermal properties, varied in different protein fractions. The MS-pH of albumin, globulin, and glutelin were found at pH 3.0, 3.0, and 4.5-5.0, respectively. S0 extracted the highest amounts of protein isolates but with the lowest protein purities due to the gum. S1 was more effective than S0 and S2 in terms of protein recovery and purities. Essential amino acids accounted for approximately 40% of the total amino acids, and essential amino acid profiles met or exceeded WHO standards for children over one year old and adults. Camelina proteins had 26-28% hydrophobic amino acids, which is lower than canola, soy, and sorghum proteins. Glutelins exhibited higher α-helix and β-sheet ratios (1.03-1.05) than globulin fractions (0.91-1.00) and albumin (0.84).

Physicochemical properties and adhesion performance of camelina protein fractions, globulins and glutelins, isolated from camelina meal were characterized. Protein isolation sequence 1 (S1) was more effective than that of S0 and S2 in terms of both protein yield and purity. Glutelin (65% db) was the major fraction in total camelina protein, followed by globulin (15%). Glutelin had compacter protein structure and higher aggregation than globulin, which may contribute to lower adhesion strength compared to glutelin.

7.2 Future work

Some challenges for the use of sorghum protein isolates as adhesives are notable. First, finding a low-cost solvent to dissolve isolated sorghum kafirins for use as an adhesive was difficult; in contrast, uniform suspension of soy proteins can be obtained by mixing soy protein with water. In addition, lower efficiency of sorghum protein recovery and complicated extraction procedures compared with soy protein are a concern. Further research is needed to improve the extraction of sorghum proteins.

For canola protein, low protein extraction rate is a challenge. In this research, the highest extraction rate is only 30%. In addition, future work may focus on lowering canola protein adhesive's curing temperature to increase the final product quality.

In general, camelina protein displayed comparable water resistance at optimum curing temperature with sorghum protein or soy protein, but was inferior to canola protein. Future work may involve grafting specific hydrophobic groups, inducing the crosslinking agent, or using denaturants to disrupt the highly aggregated glutelin protein structure, to improve wet adhesion strength. Studying the camelina protein isolation process and physicochemical properties is vital to understanding its unique functionality and further exploring its applications in food and other industrial applications such as plastics and films.

References

Aboubacar, A., & Hamaker, B. R. (1999). Physicochemical properties of flours that relate to sorghum couscous quality. Cereal Chem. 79:308-311.

Aider, M., & Barbana, C. (2011). Canola proteins: composition, extraction, functional properties, bioacitivity, applications as a food ingredient and allergenicity-A practical and critical review. Trends. Food Sci. Tech. 22:21-29.

Albin, D. M., Wubben, J. E., & Gabert, V. M. (2000). Effect of hydrolysis time on the determination of amino acids in samples of soybean products with ion-exchange chromatography or precolumn derivatization with phenyl isothiocyanate. J. Agric. Food Chem. 48:1684-1691.

AOAC International (1995). Crude protein combustion method. Official Methods of Analysis of AOAC International, 15th ed. AOAC International. Method 990.03. Gaithersburg, MD

AOAC International (1995). Fat(crude) or ether extracts in food and animal feed-Soxhlet method. Official Methods of Analysis of AOAC International, 15th ed. AOAC International. Method 920.39C. Gaithersburg, MD

AOCS (1996). Approved method Ba 6a-05: Crude fiber analysis in feeds by filter bag technique. Official Method and Recommended Practices, 4th ed. American Oil Chemists' Society: Champaign, IL

ASTM D1151-00: Standard test methods for effect of moisture and temperature on adhesive bonds, in: Annual Book of ASTM Standards (American Society for Testing and Materials, West Conshohocken, PA, 2002), pp. 67-69

ASTM D1183-96: Standard test methods for resistant of adhesives to cyclic laboratory aging condition, in: Annual Book of ASTM Standards (American Society for Testing and Materials, West Conshohocken, PA, 2002), pp. 70-73

ASTM D2339-98: Standard test method for strength properties of adhesives in two-ply wood construction in shear by tension loading, in: Annual Book of ASTM Standards (American Society for Testing and Materials, West Conshohocken, PA, 2002), pp. 158-160

Ayad, A. (2010). Characterization and properties of flaxseed protein fractions. Doctoral dissertation. McGill University, Department of Food Science and Agricultural Chemistry

Babajimopoulos, M., Damodaran, S., Rizvi, Syed S. H., & Kinsella, J. E. (1983). Effects of various anions on the rheological and gelling behavior of soy proteins: Thermodynamic observations. J. Agric. Food Chem. 31: 1270-1275.

Bacon, J. R., Noel, T. R., & Wright, D. J. (1989). Studies on the thermal behaviour of pea (Pisum sativum) vicilin. J. Sci. Food Agr. 49, 335–345.

Badifu, G. & Akubor, P. (2001). Influence of pH and sodium chloride on selected functional and physical properties of African breadfruit (Treculia africana Decne) kernel flour. Plant. Foods. Hum. Nutr. 56, 105-115.

Bean, S. R., Ioerger, B. P., Park, S. H., & Singh, H. (2006). Interaction between sorghum protein extraction and precipitation conditions on yield, purity, and composition of purified protein fractions. Cereal Chem. 83(1), 99-107.

Belton, P. S., Delgadillo, I., Halford. N. G., & Shewry. P. R. (2006). Kafirin structure and functionality. J. Cereal Sci. 44:272-286.

Berot, S., Compoint, J.P., Larre, C., Malabat, C., & Gueguen, J. (2005). Large scale purification of rapeseed proteins (Brassica napus L.). J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 818(1): 35-42.

Betts, M., & Russell, R. (2003). Amino acid properties and consequences of substitutions. In: Barnes, M., Gray I. (Eds), Bioinformatics for Geneticists. John Wiley & Sons, New Yorkpp 290-316.

Bhatty, R., & Finlayson, A. (1973). Extraction of nonprotein nitrogen from oilseed meals with different solvents. Cereal Chem. 50, 329-335.

Blaicher, F. M., Elstner, F., Stein, W., & Mukherjee, K.D. (1983). Rapeseed Protein Isolates: Effect of Processing on Yield and Composition of Protein. J. Agric. Food Chem. 31(2):358-362.

Bonnardeaux, J. (2009). Potential uses for distillers grains. http://www. agric.wa.gov.au/content/sust/biofuel/potentialusesgrains042007.pdf. Accessed 20 Nov 2010

Brosnan, J., & Brosnan, M. (2006). The sulfur-containing amino acids: an overview. J. Nutr. 136, 1636s-1640s.

Budin, J., Breene, W., & Putnam, D. (1995). Some compositional properties of camelina (Camelina sativa l. Crantz) seeds and oils. J. Am. Oil Chem. Soc. 72, 309-315.

Buffo, R. A., Weller, C. A., & Gennadios, A. (1997). Films from laboratory extracted sorghum kafirin. Cereal Chem. 74:473-475.

Carbonaro, M., Cappelloni, M., Nicoli, S., & Lucarini, M. (1997). Carnovale, E. Solubility-digestibility relationship of legume proteins. J. Agric. Food Chem. 45, 3387-3394.

Chamba, E.B., Halford, N.G., Forsyth, J., Wilkinson, M., & Shewry, P.R. (2005). Molecular cloning of b-kafirin, a methionine-rich protein of sorghum grain. J. of Cereal Sci. 41, 381-383.

Cheng, E. (2004). Adhesion mechanism of soybean protein adhesives with cellulosic materials. Ph.D. dissertation. Kansas State University, Department of Grain Science and Industry

Choi, S. J., Woo, H. D., Ko, S. H., & Moon, T. W. (2008). Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on in vitro digestibility of waxy sorghum flour. Cereal Chem. 85(1):65-69.

Chu, B., & Hsiao, B. (2001). Small-angle x-ray scattering of polymers. Chem. Rev. 101(6), 1727-1762.

Coleman, C.E., & Larkins, B.A. (1999). Prolamins of maize. In: Shewry, P.R., Casey, R. (Eds.), Seed Proteins. Kluwer Academic Publishers, The Netherlands, pp. 109-139.

Cookman, D. J., & Glatz, C. E. (2009). Extraction of protein from Distiller's grain. Bioresour. Technol. 100:2012-2017.

CPC Aeroscience, Inc. (2005). The history and future of Soy-based cleaning solvents. Available at: cpcaerosols.com/soylution/soy_history.html.

Cromwell, M., Hilario, E., & Jacobson, F. (2006) Protein aggregation and bioprocessing. The AAPS Journal. 8(3): Article 66.

Da-Silva, L. S., & Taylor, J. R. N. (2004). Sorghum bran as a potential source of kafirin. Cereal Chem. 81, 322-327.

Drummy, L., Farmer, B., & Naik, R. (2007). Correlation of the β-sheet crystal size in silk fibers with the protein amino acid sequence. Soft Matter. 3, 877-882.

DWM (2008) Door & Window Manufacturer Magazine, 9, 6. http://www.usglassmag. com/Door_and_Window_Maker/Backissues/2008/June/sealitup.htm. Accessed 20 Nov 2010

El Nour, I. N. A., Peruffo, A. D. B., & Curioni, A. (1998). Characterisation of sorghum kafirins in relation to their cross-linking behaviour. J. Cereal Sci. 28:197-207.

Elshemey, W., Elfiky, A., & Gawad, W. (2010). Correlation to protein conformation of wide-angle X-ray scatter parameters. Protein J. 29, 545-550.

Emmambux, N. M., & Taylor, J. R. N. (2003). Sorghum kafirin interaction with various phenolic compounds. J. Sci. Food Agr. 83:402-407.

Emmambux, N. M., & Taylor, J. R. N. (2009). Properties of heat-treated sorghum and maize meal and their prolamin proteins. J. Sci. Food Agr. 57:1045-1050.

Environmental Health (2008). Formaldehyde Exposure in Homes:A Reference for State Officials to Use in Decision-making.

Feed Outlook/FDS-10i/September 14 (2010). Economic Research Service, USDA. http://usda.mannlib.cornell.edu/usda/ers/FDS/2010s/2010/FDS-09-14-2010.pdf. Accessed 14 Sept 2010

Feeney, R. E., & Whitaker, J. R. (1985). Chemical and enzymatic modification of plant proteins. In New Food Proteins, Vol. 5, 181-219. A. M.Altschul and H. L. Wilcke eds. New York: Academic Press.

Fink, A. (1998). Protein aggregation: folding aggregates, inclusion bodies and amyloid. Folding & Design. 3, R9-R23.

Fitzsimons, S. M., Mulvihill, D. M., & Morris, E. R. (2007). Denaturnation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. Food Hydrocolloids. 21: 638-644.

Frank, W. S., & Kazimierz, J. D. (1984). Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. J. Agric. Food Chem. 32 (5):1172–1175.

Frihart, C. R., & Wescoott, J. M. (2004). Improved water resistance of bio-based adhesives for wood bonding. In: Proceedings of 1st International conference on Environmentally-Compatible Forest Products. 22-24 September. Oporto, Portugal. pp 293-302.

Frokjaer, S., & Otzen, D. (2005). Protein drug stability: a formulation challenge. Nat. Rev. Drug Discov. 4, 298-306.

Ghodsvali, A., Khodaparast, M. H. H., Vosoughi, M., & Diosady, L. L. (2005). Preparation of canola protein materials using membrane technology and evaluation of meals functional properties. Food Research International, 38(2): 223-231.

Grady, K., & Nleya, T.(2010). Camelina production. Extension Extra 8167. South Dakota State University, College of Agriculture & Biological Sciences/USDA

Guillén, M., & Cabo, N. (1997). Characterization of oils and lard by Fourier Transform Infrared Spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. J. Am. Oil Chem. Soc. 74, 1281-1286.

Hamaker, B. R., Bugusu, B. A., % Afripro (2003). Workshop on the proteins of sorghum and millets: Enhancing nutritional and functional properties for Africa, eds. P.S. Belton, J.R.N. Taylor, paper 08. http://www.afripro.org.uk/. Accessed 20 Nov 2010

Hamaker, B. R., Mohamed, A. A., Habben, J. E., Huang, C. P., & Larkins, B. A. (1995). Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. J. Cereal Chem. 72:583-588.

Hettiarachchy, N. S., Kalapathy, U., & Myers, D. J. (1995). Alkali-modified soy protein with improved adhesive and hydrophobic properties. J. Am. Oil Chem. Soc. 72: 1461-1464.

Hoglund, A. S., Rodin, J., Larsson, E, & Rask, L. (1992). Distribution of napin and cruciferin in developing rape seed embryos. Plant Physiol. 98(2):509-515.

Horton, H. R., Moran, L. A., Ochs, R. S., Rawn, J. D., & Scrimgeour, L.G. (1996). Protein: Three-Dimensional structure and function. In Principles of Biochemistry, Prentice Hall, Upper Saddle River, NJ, pp. 79-117.

Hse, C. Y., Fu. F., & Bryant, B. S. (2001). Development of formaldehyde-based wood adhesives with co-reacted phenol/soybean flour. In: Proceedings of the Wood Adhesives 2000 Conference. 22-23 June. South Lake Tahoe, NV. pp 13-19.

Huang, J. (2007). Development and characterization of new formaldehyde-free soy flour-based adhesives for marking interior plywood. PhD diss. Corvallis, OR.: Oregon State University, Department of Wood Science.

Huang, W. N., & Sun, X. (2000a). Adhesive properties of soy proteins modified by urea and guanidine hydrochloride. J. Am. Oil Chem. Soc. 77: 101-104.

Huang, W. N., & Sun, X. (2000b). Adhesive properties of soy proteins modified by sodium dodecyl sulfate and sodium dodecylbenzene sulfonate. J. Am. Oil Chem. Soc. 77: 705-708.

Hubbard, R. E., & Kamran Haider, M. (2010). Hydrogen Bonds in Proteins: Role and Strength. eLS.

Icoz, D. Z., Dogan, H., & Kokini, J. L. (2005). Session 51, Food Chemistry: Proteins and enzymes. 2005 IFT Annual Meeting, July 15-20. New Orleans, LA

Jackson, M., & Mantsch, H. (1995). The use and misuse of FTIR spectroscopy in the determination of protein structure. Crit. Rev. Biochem. Mol. Biol. 30(2), 95-120.

Jambunathan, R., Mertz, E.T., & Axtell, J.D. (1975). Fractionation of soluble proteins of high lysine and normal sorghum grain. Cereal Chem. 52:119-121.

Kalapathy, U., Hettiarachchy, N., Myers, D., & Rhee, K. (1996). Alkali-modified soy proteins: Effect of salts and disulfide bond cleavage on adhesion and viscosity. J. Am. Oil Chem. Soc. 73:1063-1066.

Kamino, K. (2001). Novel barnacle underwater adhesive protein is a charged amino acid-rich protein constituted by a Cys-rich repetitive sequence. Biochem. J. 356:503-507.

Keimel, F. A. (1994). Historical development of adhesives and adhesive bonding. In Handbook of Adhesive Technology, 3-15. A. Pizzi and K. L. Mittal, eds. New York: Marcel Dekker, Inc.

Kella, N. K. D., Barbeau, W. E., & Kinsella, J. E. (1986) Effect of oxidative sulfitolysis of disulfide bonds of glycinin on solubility, surface hydrophobicity and in vitro digestibility. J. Agric. Food Chem. 34:251-256

Kelsall, D. R., & Lyons, T. P. (1999). Grain dry milling and cooking for alcohol production: designing for 23% ethanol and maximum yield. in: The Alcohol Textbook, eds. K.

Jacques, T.P. Lyons, and D.R. Kelsall (Nottingham University Press, Nottingham, UK), pp. 7–23

Khorasani, G., Sauer, W., Ozimek, L., & Kennelly, J. (1990). Digestion of soybean meal and canola meal protein and amino acids in the digestive tract of yound ruminants. J. Anim. Sci. 68, 3421-3428.

Ki, C., Kim, J., Oh, H., Lee, K., & Park, Y. (2007). The effect of residual silk sericin on the structure and mechanical property of regenerated silk filament. Int. J. Biol. Macromol. 41, 346-353.

Kim, U., Park, J., Li, C., Jin, H., Valluzzi, R., & Kaplan, D. (2004). Structure and properties of silk hydrogels. Biomacromolecules. 5, 786-792.

King, M.W. (2011). Chemical nature of the amino acids. http://themedicalbiochemistrypage.org/amino-acids.html. Accessed by 01/20/2012

Klockeman, D. M., Toledo, R., & Sims, K. A. (1997). Isolation and characterization of defatted canola meal protein. J. Agric. Food Chem. 45(10): 3867-3870.

Kong, J., & Yu, S. (2007). Fourier Transform Infrared Spectroscopic Analysis of protein secondary structures. Acta. Bioch. Bioph. Sin. 39(8), 549-559.

Koshiyama, I. (1972). Comparison of acid-induced conformation changes between 7S and 11 S globulins in soybean seeds. J. Sci. Food Agric. 34: 853-859.

Krishnamoorthy, U., Muscato, T. V., Sniffen, C. J., & Van Soest, P. J. (1982). Nitrogen fractions in selected feed stuffs. J. Dairy. Sci. 65:217-225.

Krzyzaniak, A., Burova, T., & Barciszewski, J. (1998). The structure and properties of Napin-seed storage protein from rape (Brassica napus L.). Nahrung 42:201-204.

Kumar, R., Choudhary, V., Mishra, S., Varma, I. K., & Mattiason, B. (2002). Adhesives and plasticsbased on soy protein products. Ind. Crop Prod. 16: 155-172.

Kumar, R., Liu, D., & Zhang, L. (2008). Advances in proteinous biomaterials. J. Biobased Mater. Bio. 2:1-24.

Kyte, J., & Doolittle, R. F. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-132.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227:680-685.

Lambuth, A. (1977). Soybean glues. In: Skeist, I. (ED.), Handbook of Adhesive, 2nd ed. Van Nostrand, New York, pp. 172-180.

Lambuth, A. L. (1989). Adhesives from renwwable resources: Historical Perspective and Wood Industry Needs. In Adhesives from Renewable Resources, 1-10. R. W. Hemingway, A.H. Conner and S.J. Branham, eds. Washington, DC: American Chemical Society.

Lasztity, R. (1996). Sorghum Proteins. In: Lasztity R, editor. The Chemistry of Cereal Proteins. 2nd ed. Boca Raton, Florida: CRC Press. p 227-248.

Le Floch-Fouéré, C., Pezennec, S., Lechevalier, V., Beaufils, S., Desbat, B., Pézolet, M., & Renault, A. (2009). Synergy between ovalbumin and lysozyme leads to non-additive interfacial and foaming properties of mixtures. Food Hydrocolloids 23, 352-365.

Li, N., Lv, J., & Niu, D. (2009). Low contents of carbon and nitrogen in highly abundant proteins: evidence of selection for the economy of atomic composition. J. Mol. Evol. 68, 248-255.

Li, N., Qi, G., Sun, X. S., Stamm, M. J., & Wang, D. (2012). Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite. J. Am. Oil Chem. Soc. 89:897-908.

Li, N., Wang, Y., Tilley, M., Bean, S., Wu, X., Sun, S. X., & Wang, D. (2011). Adhesive performance of sorghum protein extracted from sorghum DDGS and flour. J. Polym. Environ. 19, 755-765.

Liu, D. Y. M., Litster, J. D., & White, E. T. (2007). Precipitation of soy proteins: particle formation and protein separation. American Institute of Chemical Engineers 53:514-522.

Liu, Y., & Li, K. (2002). Chemical modification of soy protein for wood adhesives. Macromol. Rapid Commun. 23: 739-742.

Liu, Y., & Li, K. (2004). Modification of soy protein for wood adhesives using mussel protein as a model: the influence of a mercapto group. Macromol. Rapid Comm. 25:1835-1838.

Liu, Y., & Li, K. (2007). Development and characterization of adhesives from soy protein for bonding wood. Int. J. Adhes. Adhes. 27: 59-67.

Lönnerdal, B., & Janson, J. C. (1972). Studies on Brassica seed proteins. I. The low molecular weight proteins in rapeseed. Isolation and characterization. Biochim. Biophys. Acta. 278(1):175-183

Makkar, H., Grancis, G., & Becker, K. (2008). Protein concentrate from jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. J. Sci. food Agric. 88, 1542-1548.

Manamperi, W. A. R., Chang, S. K. C., Chad, A. U., & Pryor, S. W. (2010). Plastics from an improved canola protein isolate: preparation and properties. J. Am. Oil Chem. Soc. 87:909–915.

Manamperi, W. A., Pryor, S. W., & Chang, S. K. C. (2007). Separation and evaluation of canola meal and protein for industrial bioproducts. ASABE/CSBE 2007 North Central Intersectional Conference, Fargo, ND. Paper Number: RRV-07116

Manamperi, W. A. R., Chang. S. K. C, Wiesenborn, D., & Pryor, S. (2008). Alteration of Osborn sequence extraction for isolation of canola proteins. An ASABE Meeting presentation. Paper Number: 083924

Marshall, W. E., & Zarins, I. M. (1989). Exothermic transitions of glycinin determined by different scanning colorimetry. J. Agric. Food Chem. 37(4), 869-873.

Masci, S., D'Ovidio, R., Lafiandra, D., & Kasarda, D. D. (1998). Characterization of a Low-Molecular-Weight Glutenin Subunit Gene from Bread Wheat and the Corresponding Protein That Represents a Major Subunit of the Glutenin Polymer. Plant Physio. 118, 1147-1158.

Mo, X., Sun, X. S., & Wang, D. (2004). Thermal properties and adhesion strength of modified soybean storage proteins. J. Am. Oil Chem. Soc. 81: 395-400.

Mo, X., Sun, X. S., & Wang, Y. (1999). Effects of molding temperature and pressure on properties of soy protein polymers. J. Appl. Polym. Sci. 73: 2595-2602.

Mo, X., Wang, D., & Sun, X. S. (2011). Physicochemical Properties of β and α′α Subunits Isolated from Soybean β-Conglycinin. J. Agric. Food Chem. 59: 1217-1222.

Mo, X., Zhong, Z., Wang, D., & Sun, X. S. (2006). Soybean glycinin subunits: Characterization of physicochemical and adhesion properties. J. Agric. Food Chem. 54: 7589-7593.

Mokrane, H., Amoura, H., Belhaneche-Bensemra, N., Courin, C. M., Delcour, J.A., & Nadjemi, B. (2010). Assessment of algerian sorghum protein quality [sorghum bicolor (L.) moench] using amino acid analysis and in vitro pepsin digestibility. Food Chemistry 121:719-723.

Monsalve, R.I., & Rodriguez, R. (1990). Purification and characterization of proteins from the 2S fraction from seeds of the Brassicaceae family. J Expt. Bot. 41:89-94

Mosse, J., Huet, J. C., Bauedt, J. (1988). The amino acid composition of whole sorghum grain in relation to its nitrogen content. Cereal Chem. 65:271-277.

Narayanamurti, D., Ranganathan, V., Roy, D. C. (1943) Rapeseed protien-formaldehyde dispersions as plywood adhesives. Forest Research Institute Dehra Dun. Indian forest leaflet No. 58

Nielsen, H. C., Paulis, J. W., James, C., & Wall, J. S. (1970). Extraction and Structure Studies on Corn Glutelin Proteins. Cereal Chem. 47, 501-512.

Ning, L., & Villota, R. (1994). Influence of 7S and 11S globulins on the extrusion performance of soy protein concentrates. J. Food Process Preserv. 18: 421-436.

Oomah, B. D., Mazza, G. (1995). Functional properties, uses of flaxseed protein. INFORM, 6(11):1246-1252.

Osborne, T. (1924). The vegetable proteins. 2nd edition, Longmans, London.

Pace, C. (2009). Energetics of protein hydrogen bonds. Nat. Struct. Mol. Biol. 16, 681-682.

Park, S. H., Bean, S. R. (2003). Investigation and optimization of the factors influencing sorghum protein extraction. J. Agric. Food Chem. 51:7050-7054.

Patil, P., Gude, V., Camacho, L., & Deng, S. (2010). Microwave-assisted catalytic transesterification of camelina sativa oil. Energy Fuels 24, 1298-1304.

Pedroche, J., Yust, M. M., Lqari, H., Giron-Calle, J., Alaiz, M., Vioque, J., & Millan, F. (2004). Brassicacarinata protein isolates: chemical composition, protein characterization and improvement of functional properties by protein hydrolysis. Food Chem 88(3):337–346.

Peng, I. C., Quass, D. W., Dayton, W. R., & Allen, C. E. (1984). The physicochemical and functional properties of soybean 11s globulin-A review. Cereal Chem. 61: 480-490.

Pilgeram, A. (2009). Camelina for biodiesel - EPAC 2009. http://www.ethanolmt.org/presentations/2009/pilgeram.pdf

Pizzi, A. (2006). Recent developments in eco-efficient bio-based adhesives for wood bonding:opportunities and issues. J. Adhesion Sci. Technology 20:829-846.

Putnam, D., Budin, J., Field, L., & Breene, W. (1993). Camelina: a promising low-input oilseed. In: Hanick J, Simon J (eds) New crops. Wiley, New York, pp 314-322.

Qi, G. & Sun, X. S. (2010). Soy protein adhesive blends with synthetic latex on wood veneer. J. Am. Oil Chem. Soc. 88, 271-281.

Qi, G., Li, N., Wang, D., & Sun, X. (2012). Physicochemical properties of soy protein adhesives obtained by in situ sodium bisulfite modification during acid precipitation. J. Am. Oil Chem. Soc. 89, 301-312.

Rajasekaran, E., Akila, K., & Vennila, J. (2011). Carbon contents of H1N1 proteins. 2011 International Conference on Bioscience, Biochemistry and Bioinformatics IPCBEE. 5, 27-29.

Ramos, J., Cabral, R., & Chan, F. (1984), Utilization of sorghum flour as extender for plywood adhesive. NSTA Technol. J. July-Sept:29

Rogers, J., Geng, X., & Li, K. (2004). Soy-based adhesives with 1, 3- Dichloro-2-propanol as a curing agent. Wood Fiber Sci. 36: 186-194.

Rokka, T., Alen, K., Valaja, J., & Ryhanen, E. L. (2002). The effect of a Camelina sativa enriched diet on the composition and sensory quality of hen eggs. Food Res. Int. 35,253-256.

Rooney, L.W., & Waniska, R.D. (2000). in; Sorghum: Origin, History, Technology, and Production, eds. C.W. Smith and R.A. Frederiksen (John Wiley & Sons, Inc., New York,), pp. 689-729.

Rullier, B., Novales, B., & Axelos, M. A. V. (2008). Effect of protein aggregates on foaming properties of β-lactoglobulin. Colloids Surf., A. 330, 96-102

Ryhanen, E. L., Perttila, S., Tupasela, T., Valaja, J., Eriksson, C. & Larkka, K. (2007). Effect of Camelina sativa expeller cake on performance and meat quality of broilers. J. Sci. Food Agric. 87:1489–1494.

Sadeghi, M.A, & Bhagya, S. (2009). Effect of recovery method on different property of mustard protein. World Journal of Dairy & Food Sciences. 4(2), 100-106.

Saio, K., & Watanabe, T. (1978). Differences in functional properties of 7S and 11S soy bean proteins. J. Texture Stud. 9: 135-157.

Sampath, A. (2009). Chemical characterization of camelina seed oil. Master dissertation. The State University of New Jersey, Graduate School-New Brunswick Rutgers.

Schwenke, K. D. (1994). Rapeseed protein. In: Hudson JF (ed) New and developing sources of food proteins. Chapman and Hall, London, p 281–306.

Schwenke, K. D., Raab, B., Plietz, P., & Damaschun, G. (1983). The structure of the 12 S globulin from rapeseed (Brassica napus L.). Food/Nahrung 1983, 27, 165-175.

Shahidi, F., Naczk, M., Hall, D., & Synowiecki, J. (1992). Insensitivity of the amino acids of canola and rapeseed to methanol-ammonia extraction and commercial processing. Food Chem. 44(4):283-285.

Shewry, P. R., & Tatham, A. S. (1990). The prolamin storage proteins of cereal seeds: structure and evolution. Biochem. J. 267:1-12.

Shonnard, D., Williams, L., & Kalnes, T. (2010). Camelina-derived jet fuel and diesel: sustainable advanced biofuels. Environ. Prog. Sustain. Energy.2 9(3), 382-392.

Shull, J. M., Watterson, J. J. & Kirleis, A. W. (1991). Proposed nomenclature for the alcohol-soluble proteins (kafirins) of Sorghum bicolor (L. Moench) based on molecular weight, solubility and structure. J. Agric. Food Chem. 39, 83-87.

Skoch, L. V., Deyoe, C. W., Shoup, F. K., Bathurst, J., & Liang, D. (1970). Protein fractionation of sorghum grain. Cereal Chem. 47:472-482.

Slominski, B. A., Simbaya, J., Campbell, L. D., Rakow, G., & Guenter, W. (1999). Natritive value for broilers of meals derived from newly developed varieties of yellow seeded canola. Anim. Feed Sci. Techono. 78(3):249-262.

Smith, A. M., & Callow, J. A. (2006). Preface in Biological Adhesives. Springer: Heidelberg, Germany

Sosulski, F. W., & Dabrowski, K. J. (1984). Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. J. Agric. Food Chem. 32 (5):1172-1175.

Staswick, P. E., Hermodson, M. A., & Nielsen, N. C. (1984) Identification of the cystines which link the acidic and basic components of the glycinin subunits. J. Biol. Chem. 259:3431-3435.

Steele, P. H., Kreibich, R. E., Steynberg, P. J., & Hemingway, R. W. (1998). Finger jointing green southern yellow pine with a soy-based adhesive. Adhes. Age.10: 49–54.

Sulyanov, S., Kheiker, D., Vainshtein, D., & Hartog, H. (2003). Characterization of Na precipitates in electron irradiated NaCl crystals by wide angle X-ray scattering (WAXS). Solid State Commun. 128, 419-423.

Sun, S., & Hall, T. (1975). Solubility characteristics of globulins from phaseolus seeds in regard to their isolation and characterization. J. Agr. Food Chem. 23(2), 184-189.

Sun, X. S. (2005). Isolation and processing of plant materials. Pages 33-55 in: Bio-based polymers and composite Richard Wool and Xiuzhi Susan Sun eds. Elsevier Academic Press: Amsterdam.

Sun, X. S., Wang, D., Zhang, L., Mo, X., Zhu, L., & Bolye, D. (2008). Morphology and phase separation of hydrophobic clusters of soy globular protein polymers. Macromol. Biosci. 8:295-303.

Sung-Ae, J., Geum-OK, L., & Kyung, B. S. (2010). Preparation and mechanical properties of edible rapeseed protein films. J. of Food Sci. 76(2): C218–C223.

Taylor, J. R. N., Schüssler, L., & Van der Walt, W. H. (1984). Fractionation of proteins from low-tannin sorghum grain. J. Agric. Food Chem. 32:149-154.

Taylor, J., Taylor, J. R. N., Dutton, M. F., & Kock, S. (2005). Glacial acetic acid-a novel food-compatible solvent for kafirins extraction.Cereal Chem. 82:485-487

Tecson, E., Esmama, B., Lontok, L., & Juliano, B. (1971). Studies on the Extraction and Composition of Rice Endosperm Glutelin and Prolamin. Cereal Chem. 48, 168-181.

Tessier, B., Harscoat-Schiavo, C., & Marc, I. (2006). Selective separation of peptides contained in a rapeseed (Brassica campestris L.) protein hydrolysate using UF/NF membranes. J. Agric. Food Chem. 54(10): 3578-3584.

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Texas Tech University (2009). http://www.depts.ttu.edu/agriculturalsciences/ impacts08/fed/sorghum.php. Accessed 20 Nov 2010

Thanh, V. H., & Shibasaki, K. (1978). Major proteins of soybean seeds. subunit structure of beta-conglycinin. J. Agric. Food Chem. 26: 692-695.

Tzeng, Y. M., Diosady, L. L., & Rubin, L. J. (1988). Preparation of rapeseed protein isolate by sodium hexametaphosphate extraction, ultrafiltration, diafiltration, and ion-exchange. J. Food Sci. 53(5):1537-1542.

U.S. Grain Council. Sorghum (2009). http://www.grains.org/page.ww?section =Barley%2C+Corn+%26+Sorghum&name=Sorghum. Accessed 20 Nov 2010

Uruakpa, F. O., Arntfield, S. D. (2006). Impact of urea on the microstructure of commercial canola protein-carrageenan network: A research note. Int. J. Biiol. Macromol. 38:115–1119.

USDA 2011 Oilseeds: world markets and trade. http://www.fas.usda.gov/oilseeds/circular/2011/March/oilseeds.pdf. Accessed 26 May 2011.

USDA. 2012. Oilseeds: world markets and trade. Circular Series FOP 02-12 September 2012.

Valluzzi, R., & Jin, H. (2004). X-ray evidence for a "super"-secondary structure in silk fibers. Biomacromolecules 5, 696-703.

Wang, Y. (2006). Adhesive performance of soy protein isolate enhanced by chemical modification and physical treatment. Dissertation, Kansas State University

Wang, Y., Mo, X., Sun, X. S., & Wang, D. (2007). Soy protein adhesion enhanced by glutaraldehyde crosslink. J. Appl. Polymer. Sci. 104: 130-136.

Wang, Y., Sun, X. S, & Wang, D. (2006b). Performance of soy protein adhesive enhanced by esterification. ASABE 49:713-719.

Wang, Y., Sun, X., & Wang, D. (2007). Effects of preheating treatment on thermal property and adhesion performance of soy protein isolates. J. Adhesion Sci. Technol. 21(15), 1469-1481.

Wang, Y., Tilley, M., Bean, S., Sun, X. S., & Wang, D. (2009). Comparison of methods for extracting kafirin proteins from sorghum distillers dried grains with solubles. J. Agric. Food Chem. 57:8366-8372.

Wang, Y., Wang, D., & Sun, X. S. (2005). Thermal properties and adhesiveness of soy protein modified with cationic detergent. J. Am. Oil Chem. Soc. 82: 357-363.

Wäsche, A., Wurst, S., Borcherding, A., & Luck, T. (1998). Film forming properties of rape-seed protein after structural modification. Nahrung 42:269-271.

Watterson, J.J., Shull, J.M., & Kirleis, A.W. (1993). Quantitation of α, β, and γ - kafirins in vitreous and opaque endosperm of sorghum bicolor. Cereal Chem. 70:452-457.

Wescott, J. M., & Frihart, C. R. (2004). Competitive soybean flour/phenol-formaldehyde adhesives for oriented strandboard. In: Proceedings of 38th International Wood Composites Symposium. 6-9 April. Pullman, Washington. pp 199-206.

Wetzel, D., Srivarin, P., & Finney, J. (2003). Revealing protein infrared spectral detail in a heterogeneous matrix dominated by starch. Vib. Spectrosc. 31, 109-114.

WHO Technical Report Series 935. (2007). Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU expert consultation.

Wilson, C. M., Shewry, P. R., & Miflin, B. J. (1981). Maize endosperm proteins compared by sodium dodecyl sulphate gel electrophoresis and isoelectric focusing. Cereal Chemistry 58, 275-281.

Wong, J. H., Lau, T., Cai, N., Singh, J., Pedersen, J. F., Vensel, W.H., Hurkman, W. J., Wilson, J. D., Lemaux, P. G., & Buchanan, B. B. (2009). Digestibility of protein and starch from sorghum (Sorghum bicolor) is linked to biochemical and structural features of grain endosperm. J. Cereal Sci. 49:73-82.

Wool, R., & Sun, X. S. (2005). Soy protein adhesives. In: Bio-based Polymers and Composites, 1st edn. Elsevier Academic Press, Burlington, MA pp:327–368.

Wu, J., & Muir, A. D. (2008). Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. J. Food Sci. 73:210–216.

Wu, X., Zhao, R., Bean, S, R., Seib, P.A., McLauren, J. S., Madl, R. L., Tuinstra, M. R., Lenz, M. C., & Wang, D. (2007). Factors impacting ethanol production from sorghum. Cereal Chem. 84:130-136.

Wu, Y. V., & Wall, J. S. (1980). Lysine content of protein increased by germination of normal and high lysine sorghum. J. Agric. Food Chem. 28:455-458.

Yamamoto, H., Sakai, Y., & Ohkawa, K. (2000). Synthesis and wettability characteristics of model adhesive protein sequences inspired by amarine mussel. Biomacromolecules. 1: 543-51.

Yang, I., Kuo, M., Myers, D. J., & Pu, A. (2006). Comparision of protein-based adhesive resins for composites. J. Wood Sci. 52: 503-508.

Yousif, N. E., & El Tinay, A. H. (2001). Effect of fermentation on sorghum protein fractions and in vitro protein digestibility. Plant Food Hum. Nutr. 56:175-182.

Youssef, A. M. (1998). Extractability, fractionation and nutritional value of low and high tannin sorghum proteins. Food Chem. 63:325-329.

Yu, M., & Deming, T. J. (1998). Synthetic polypeptide mimics of marine adhesives, Macromolecules. 31: 4739-4745.

Yu, P., Mckinnon, J. J., Soita, H. W., Christensen, C. R., & Christensen, D. A. (2005). Use of synchrotron-based FTIR microspectroscopy to determine protein secondary structures of raw and heat-treated brown and golden flax seeds: A novel approach. Can. J. Anim Sci. 85, 437-448.

Zhan, X., Wang, D., Bean, S. R., Mo, X., Sun, X. S., & Boyle, D. (2006). Ethanol production from supercritical-fluid-extrusion cooked sorghum. Ind. Crop Prod. 23:304-310.

Zhang, L., & Sun, X. S. (2008). Effect of sodium bisulfite on properties of soybean glycinin. J. Agric. Food Chem. 56: 11192-11197.

Zhang, L., & Sun, X. S. (2010). Sodium bisufite induced changes in the physicochemical, surface and adhesive properties of soy β-conglycinin. J. Am. Oil Chem. 87: 583-590.

Zhang, Z., & Hua, Y. (2007). Urea-modified soy globulin proteins (7S and 11S): Effect of wettability and secondary structure on adhesion. J. Am. Oil Chem. Soc. 84: 853-857.

Zhao, H., Sagert, J., Hwang, D. S., & Waite, J. H. (2009). Glycosylated hydroxytryptophan in a mussel adhesive protein from perna viridis. J. Biol. Chem. 284:23344-52

Zhao, R., Bean, S. R., & Wang, D. (2008). Sorghum protein extraction by sonication and its relationship to ethanol fermentation. Cereal Chem. 85:837-842.

Zhong, Z., & Sun, X. (2007). Plywood adhesives by blending soy protein polymer with phenol-formaldehyde resin. J. Biobased Mater. Bio. 1: 380-387.

Zhong, Z., Sun, S.X., & Wang, D. (2007). Isoelectric pH of polyamide-epichlorohydrin modified soy protein improved water resistance and adhesion properties. J. Appl. Polym. Sci. 103:2261–2270.

Zhong, Z., Sun, X. S., Fang, X., & Ratto, J. A. (2002). Adhesive strength of guanidine hydrocholoride-modified soy protein for fiberboard application. Int. J. Adhes. Adhes. 22: 267-272.

Zhong, Z., Sun, X., Fang, X., & Ratto, J. (2001). Adhesion properties of soy protein with fiber cardboard. J. Am. Oil Chem. Soc. 78, 37-41.

Zhou, Z., Zheng, H., Wei, M., Huang, J., & Chen, Y. (2007). Structure and mechanical properties of cellulose derivatives/soy protein isolate blends. J. Appl. Polym Sci. 107, 3267-3274.

Zhu, L. (2006). Studies of soy protein adhesive performance on the effects of pH, amino acid group, and temperature. PhD diss. Manhattan, KS.: Kansas State University, Department of Grain Science and Industry.

Zubr, J. (2002). Dietary fatty acids and amino acids of Camelina sativa seed. J. Food Qual. 26(6), 451-462.