THE POTENTIAL OF CANOLA PROTEIN FOR BIO-BASED WOOD ADHESIVES

by

KRISTEN HALE

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Biological and Agricultural Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

Major Professor
Donghai Wang

Abstract

Currently, the majority of adhesives used for wood veneer, plywood, and composite applications are formaldehyde-based. Formaldehyde is derived from petroleum and natural gas, making it non-renewable and toxic. Therefore, extensive research has been conducted to develop bio-based adhesives to replace formaldehyde-based adhesives. Soy protein has shown great potential to partially replace formaldehyde adhesives, and canola protein has similar properties to soy protein. However, little research has been conducted on the feasibility of using canola protein for wood adhesive applications. The objective of this research was to study the adhesion performance of canola protein. Canola protein was modified with different chemical modifiers including sodium dodecyl sulfate (SDS), calcium carbonate ($CaCO_3$), zinc sulfate ($ZnSO_4$), calcium chloride ($CaCl_2$), and 2-octen-1-ylsuccinic anhydride (OSA) as well as combined chemical modifications. The wet, dry, and soak shear strengths of the adhesive formulations were determined. Viscosity testing, differential scanning calorimetry, and TEM and SEM imaging were used to characterize protein properties.

Chemical modification with SDS (1%, 3%, and 5%), $CaCO_3$ (1%, 3%, and 5%), $ZnSO_4$ (1%), and OSA (2%, 3.5%, and 5%) improved the dry and soak shear strengths compared to unmodified canola protein. Canola protein modified with 3.5% OSA had improved wet, dry, and soak shear strengths. Combined chemical modification of canola protein did not show significant improvement on shear strength. Thermal modification of canola protein adhesives showed a trend of increasing shear strength with increasing press temperature. The data suggests that with further research, canola protein has potential to be used as a commercial adhesive or as an additive to formaldehyde-based adhesives to make them more environmentally-friendly.

Table of Contents

List of Figures

List of Tables

Acknowledgements

First and foremost, I wish to thank Dr. Donghai Wang, my advisor, for all of his help, guidance, and support. I would also like to thank Dr. Susan Sun and Dr. Lisa Wilken for being on my supervisory committee. I am very thankful for the friendship and support of my fellow graduate students and post-doctoral researchers in the BAE and Grain Science departments. I would especially like to thank Ningbo Li, Dr. Guanyan Qi, Dr. Yonghui Li, and Leidy Peña for helping me with research suggestions as well as for training me on the equipment in the lab.

I thank my fiancé, Tanner Yost, and my family for their love, encouragement, and support throughout the years. I am also very thankful for all of my friends who have encouraged me and been there for me throughout my college career.

1. Introduction

The United States uses approximately 20 billion pounds of adhesives annually, including 8 billion pounds of formaldehyde-based adhesives utilized by the wood industry (Sun 2011). The main type of wood adhesive used is formaldehyde-based, with primary types being phenol-formaldehyde, resorcinol-formaldehyde, urea-formaldehyde, melamine-formaldehyde, and isocyanate adhesives (Frihart 2005).

Though the wood adhesive industry is thriving, many concerns have arisen over the use of formaldehyde-based adhesives because they are derived from petroleum and natural gas, making the adhesives non-renewable. Therefore, producing formaldehyde-based adhesives depends on the use of unsustainable, finite resources. When the world supply of oil is depleted, formaldehyde-based adhesives will cease to exist. As the supply of oil decreases, the price of petroleum increases, raising the cost of wood adhesives as well. Another concern about formaldehyde-based adhesives is that formaldehyde is a toxic substance as well as a known carcinogen. Workers and homeowners are often exposed to harmful formaldehyde emissions through adhesives. In order to aid the environment, improve human health, and ease resource limitation, renewable sources that benefit both the environment and sustainable economic development must be used for adhesives.

Currently, bio-based adhesives are being researched and developed to replace formaldehyde adhesives. Bio-based protein adhesives such as soy and canola have benefits over formaldehyde adhesive because they are renewable, non-toxic, and can be produced from low-cost sources such as byproducts from oil extraction. To produce protein-based adhesives, oil is first extracted from plant seeds, leaving behind a protein rich meal. The meal is then further processed and the protein is separated from the rest of the meal and typically modified in order to increase performance. The resulting product is then utilized as an adhesive.

At present, most research focuses on soy protein adhesives which, when modified, have comparable performances to formaldehyde-based adhesives. Many different modifications and processing parameters for soy protein wood adhesives have been studied and reported (Huang and Sun 2000a; Mo et al. 2004; Qi et al. 2013). Recently, research on the use of modified canola protein for wood adhesives has been reported (Li et al. 2011; Li et al. 2012).

Canola is an abundant oilseed crop grown primarily in Canada and the United States. Canola protein is similar to soy protein, but limited research has been conducted in order to develop canola-based adhesives. Due to a high amount of protein, canola meal has potential to be used in various industrial products such as composites, plastics, and adhesives (Manamperi et al. 2007). While soy meal can be used in products for human consumption, the utilization of canola meal for human consumption is more difficult due to the presence of hazardous compounds in canola meal. Generating canola-protein-based adhesives, therefore, does not compete with the food industry. Canola protein shows great promise as a novel source for wood adhesive applications.

The objectives of this research were to evaluate the physical and chemical properties of modified canola protein and to increase the adhesion performance of canola protein through physical and chemical modifications. Previous research has utilized many chemical modifiers, including sodium dodecyl sulfate (SDS), calcium carbonate ($CaCO_3$), zinc sulfate ($ZnSO_4$), calcium chloride ($CaCl_2$), and 2-octen-1-ylsuccinic anhydride (OSA), to successfully modify soy protein in order to increase adhesion strength and water resistance of adhesives (Mo et al. 2004; Liu et al. 2010; Qi et al. 2013). In this research, canola protein was modified with SDS, $CaCO_3$, $ZnSO_4$, $CaCl_2$, and OSA to improve adhesive performance. Adhesion performance, rheological properties, thermal properties, and morphological properties were measured.

2. Literature Review

2.1 Canola

Canola is an oilseed from the *Brassica* family, originally bred from rapeseed. Rapeseed contains high levels of erucic acid and glucosinolates, making it unsuitable for human and animal consumption. In order to overcome nutritional problems associated with rapeseed, canola was bred from rapeseed in the 1970s. The name canola is derived from "Canadian oil, low in acid" due to canola being primarily grown and bred in Canada. By definition, a variety must contain less than two percent erucic acid and less than 30 micromoles of glucosinolates to be classified as canola (Canola Council of Canada 2011). Canola is the second largest feed meal and ranks third in the world of oilseed crops produced (USDA 2010).

2.1.1 Seed composition

Canola typically has an oil content of approximately 45-50% (Manamperi et al. 2007). To extract oil, canola seeds are first flaked, which ruptures cell walls, making oil extraction easier. The flaked seeds are screw-pressed at either room temperature or an elevated temperature to remove the oil (Canola Council of Canada 2011). The product remaining after oil extraction is canola meal. Canola oil is used to make edible oil and biodiesel, and the meal is often used as animal feed.

Canola meal contains 30-40% protein and is usually not used in human food applications due to the presence of glucosinolates, erucic acid, phytates, and phenolics (Manamperi et al. 2007). The meal is primarily used as animal feed, but this also has limitations due to the previously listed compounds. The effects of these compounds on food and feed applications are vast.

Glucosinolates are sulphonated oxime thioesters of glucose that, when hydrolyzed, yield an unstable aglucone that can be broken down into a variety of products, including isothiocyanates and nitriles (Uppström 1995). In excess, the resulting products can lead to reduced growth, thyroid function interference, and skeletal abnormalities in humans (Manamperi et al. 2007; Uppström 1995). Therefore, one of the reasons canola meal is not used in human applications is because of the associated risks with ingesting excess amounts of glucosinolates.

However, glucosinolate levels in canola remain low enough for the meal to be used in animal feed applications.

Erucic acid is a monosaturated omega-9 fatty acid found in rapeseed oil. Rapeseed oil is not suitable for human consumption because erucic acid can produce toxic effects in the heart. Canola oil is used instead because it eliminates the health risks associated with erucic acid.

Phytic acid exists as mixed salts (phytates) of calcium, magnesium, and potassium in *Brassica* varieties (Uppström 1995). Phytates are found inside protein bodies, and concentration is greatly affected by phosphorus availability in the soil in which the plant is grown. The function of phytates is to be a reserve for phosphorus, energy, and cations for the plant. However, the presence of phytates in food sources can reduce mineral availability, digestibility, amino acid availability, and starch hydrolysis, therefore limiting metabolism (Uppström 1995).

Phenolics are chemical compounds consisting of a hydroxyl group bonded to an aromatic hydrocarbon group. The presence of phenolics leads to the dark color, bitter taste, and astringency of canola meal (Uppström 1995).

2.1.2 Protein isolation

The most common protein extraction method used to extract canola protein from canola meal is alkaline extraction (Tan et al. 2011). A schematic flowchart for alkaline extraction can be found in Figure 1.

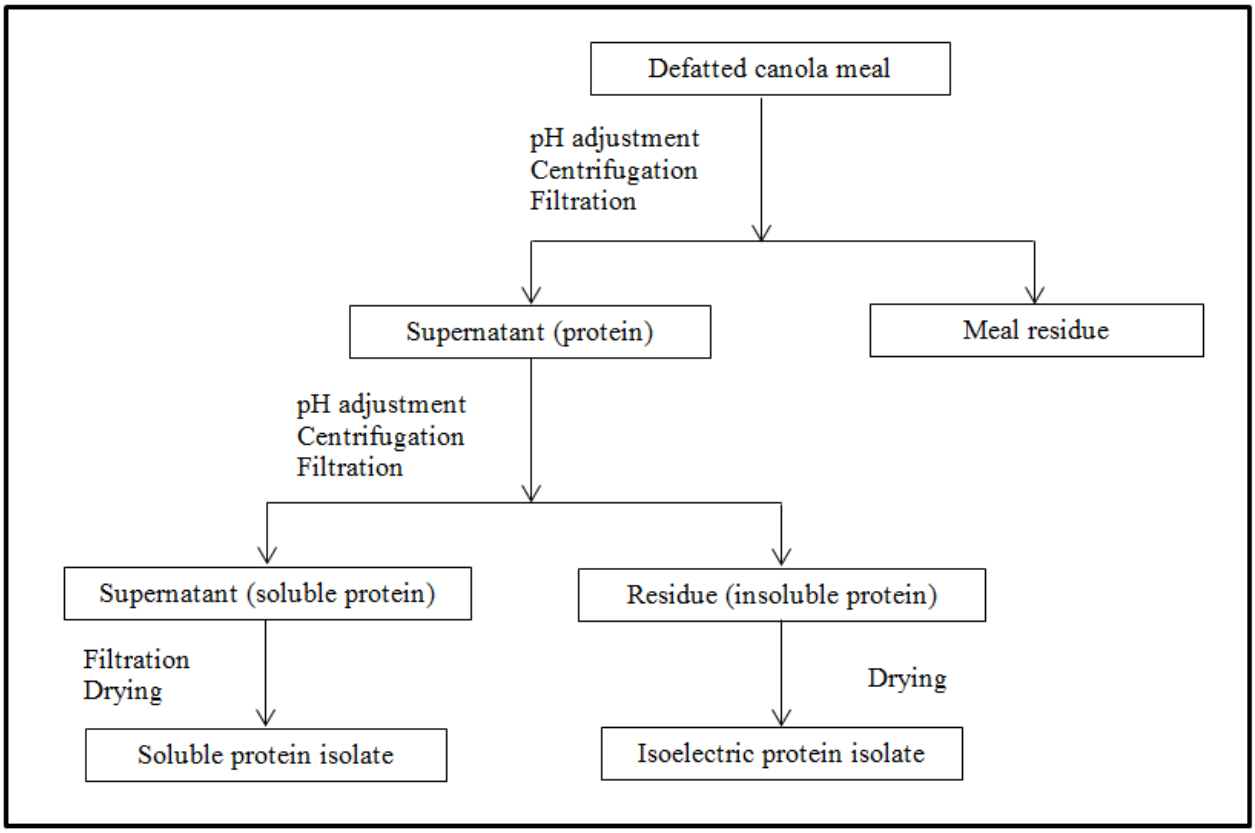

Figure 1. Alkaline extraction process (Modified from Tan et al. 2011)

Canola meal is first defatted with hexane to remove residual oil, and then sodium hydroxide is added to solubilize the protein by adjusting the pH to 11 or 12. (Manamperi et al. 2011). After the protein has been solubilized, the solution is centrifuged to remove the meal residue. A dilute acid, usually hydrochloric acid, is added to the remaining solution to precipitate the protein by adjusting pH between 3 and 5 (Tan et al. 2011). The pI is the pH where the protein has the lowest solubility and is commonly the pH value used to precipitate the protein. The solution is then centrifuged to separate the insoluble protein from the solution. Finally, the insoluble protein is freeze-dried or spray-dried. Optionally, the soluble proteins can be isolated by ultrafiltration and diafiltration and then dried to increase yield.

The pH used to solubilize the protein and the pH used to precipitate the protein greatly affect the protein yield from alkaline extraction. Different protein fractions are soluble at different pH values. The protein composition of canola is very complicated, containing protein with varying isoelectric points and molecular weights (Tzeng et al. 1990). Therefore, using one

pH value cannot solubilize all proteins or precipitate all proteins. Multiple pIs have been used in various studies so that different protein fractions can be isolated (Tan et al. 2011). Therefore, multiple centrifugation steps had to be completed in order to isolate the maximum amount of protein.

2.1.3 Protein composition

The major components of canola protein are cruciferin and napin. Cruciferin and napin are storage proteins that account for 60% and 20% of canola storage protein respectively (Wu and Muir 2008). Storage proteins can be categorized according to the Osborne method based on solubility in water (albumins), salt solutions (globulins), alcohol (prolamins), and alkali solutions (glutelins) (Manamperi et al. 2007). Protein properties can vary due to the isolation method used, as well as plant variety and environmental conditions.

Cruciferin is a 12S globulin with a total molecular mass of 300,000 (Rödin 1990). The structure of cruciferin is very complex, having an oligomeric complex composed of six subunits (Rödin 1990). Within the subunits, four different subunit pairs exist, with the majority being disulphide linked. Wu and Muir (2008) found the denaturation temperature of cruciferin to be 91°C, which is similar to other globulins. Cruciferin has high foaming capacity and acts as a gelling agent in its undenatured form (Schwenke 1994; Manamperi et al. 2007).

Napin is a 2S albumin with a total molecular mass of 12,500 to 14,500 (Manamperi et al. 2007). Napin consists of two polypeptides linked covalently by disulphide bonds (Uppström 1995). Wu and Muir (2008) found the denaturation temperature of napin to be 110°C, which is comparatively high due to the high thermal stability of the disulfide bonds.

Oleosin is another type of protein found in canola, accounting for 8% of the total canola protein. Oleosin is a structural protein associated with oil bodies. It is a low molecular weight protein.

The amino acid composition of canola varies by species and environmental conditions. Amino acid compositions of winter and summer *Brassica napus* are shown in Table 1.

Table 1. Amino acid composition of *Brassica napus* (Adapted from Uppström 1995**)**

Amino acid	Winter	Summer
Alanine	4.6	4.6
Arginine	6.6	6.8
Aspartic acid	7.7	8.0
Cystine	2.8	2.5
Glutamic acid	18.7	18.3
Glycine	5.2	5.5
Histidine	4.2	4.5
Isoleucine	4.5	4.5
Leucine	7.4	7.4
Lysine	6.3	5.9
Methionine	2.3	2.2
Phenylalanine	4.2	4.2
Proline	6.1	6.0
Serine	4.8	5.0
Threonine	4.8	4.9
Tryptophan	nd	nd
Tyrosine	3.3	3.1
Valine	5.5	5.5

Overall, canola contains substantial amounts of lysine and threonine, with high levels of sulfur amino acids, methionine and cysteine, compared to other cereal oilseeds (Uppström 1995). Canola also contains very high amounts of glutamic acid, aspartic acid, and leucine in both the winter and summer varieties.

2.2 Adhesion

Adhesion can be defined as the molecular attraction force within the contact area of two bodies that acts to hold them together (Dictionary.com). Adhesives have been used for centuries to bond materials together. Many different theories of adhesion have been proposed including mechanical interlocking theory, electronic theory, adsorption theory, diffusion theory, and chemical bonding theory. The following sections describe the history of adhesives, theories of adhesion, and wood adhesion.

2.2.1 History of adhesives

Wood adhesives have been used for bonding for centuries. Modern history of wood adhesives includes the use of blood and casein-based adhesives in the early 1900s. With the start of WWI, these blood and casein-based adhesives were further improved by chemical and heat modifications. Soybean glues were developed and used for construction, packaging, and transportation during WWII, but were soon replaced with adhesives derived from petroleum sources (Lambuth 1989). Petroleum-based adhesives were developed because of the high availability and low cost of petroleum. Today, petroleum-based adhesives are still the primary type of wood adhesive used; however, protein-based adhesives currently are heavily researched.

2.2.2 Theories of adhesion

Many different theories of adhesion have been proposed, including mechanical interlocking theory, electronic theory, adsorption theory, diffusion theory, and chemical bonding theory (Schultz and Nardin 1994). These theories are based on mechanical or chemical bonding between the substrate and the adhesive along with the assumption that adhesion requires a variety of mechanisms, depending on adhesive and substrate type. Mechanical properties can determine the force on the chemical bonds, and chemical structure and interactions can determine the mechanical properties of the adhesive (Frihart 2005).

The mechanical interlocking theory states that adhesive adheres to a substrate by interlocking with irregularities in the substrate surface (Kinloch 1987). Adhesion occurs due to adhesive seeping into pores of the substrate, consequently binding the substrates together. Generally, mechanical interlocking provides greater resistance to shear forces than normal forces (Frihart 2005). In order for the mechanical interlocking theory to be valid, the substrate surface must be adequately irregular to allow adhesive to penetrate into the pores. Abrasion can be used to roughen the substrate so there is adequate pore space into which the adhesive can penetrate.

The electronic theory is the result of work by Deryaguin and co-workers (1948) and is based on the difference in electric band structures between an adhesive and its substrate. An electron transfer mechanism is generated between the adhesive and the substrate during de-bonding, equalizing the Fermi levels and thus creating a double layer of electrical charge at the interface (Schultz and Nardin 1994). The resulting electronic forces occurring from the double layer of electrical charge contribute significantly to adhesive strength. The junction between the

adhesive and the substrate is considered a capacitor. When distance between two substrates increases during an interface rupture, so does potential difference until a discharge occurs. The adhesive strength is a result of attractive electrostatic forces across the double layer of electrical charge. However, the electronic theory of adhesion is controversial because it asserts that electronic forces are a cause, instead of a result, of high joint strength.

The adsorption theory is a thermodynamic model based on the work of Sharpe and Schonhorn (1963) and is a widely accepted approach in adhesion science (Schultz and Nardin 1994). The theory states that adhesion is based on interatomic and intermolecular forces established at the interface resulting from van der Waals and Lewis acid-base interactions, given that intimate contact is achieved. The strength of these forces is dependent on thermodynamic qualities, including, surface-free energies of the adhesive and substrate.

The diffusion theory claims that polymers from the adhesive and substrate mix to form a single commingled phase (Frihart 2005). In other words, the polymer's adhesion strength is dependent on mutual diffusion of molecules across the interface, therefore creating an interphase between the adhesive and the substrate (Schultz and Nardin 1994). In order for this theory to be valid, the macromolecular chains must be adequately mobile and mutually soluble. The strength of the bond is dependent on factors such as contact time, curing temperature, and characteristics of the polymers.

The chemical bonding theory states that adhesion is based on primary bonds formed between the adhesive and the substrate. Ionic, covalent, and metallic bonds are the three types of primary bonds that can be formed. Covalent bonds are generally the strongest and most desired bond relating to wood adhesion.

2.2.3 Wood adhesion

Wood adhesion is complex because wood is a non-homogeneous, porous, and cellular substrate. Therefore, multiple adhesion theories relate to wood bonding. The first step into generating a strong wood adhesion bond is wetting of the wood substrate. Wetting is the molecular interaction at the interface of liquids and solids in the adhesive (Gardner 2005). A low contact angle, or angle between the edge of a droplet and the surface plane of the material, is desired for wetting (Frihart 2006). In order for a strong bond to be formed, adhesive must flow

into the wood cell walls and lumens so that molecular level contact can be achieved (Frihart 2006).

After wetting and contact between the adhesive and substrate is established, setting or curing of the adhesive must take place. Setting is the conversion of an adhesive into a hardened state by physical or chemical methods (Frihart 2005). Setting can be achieved by polymerization or solidification by cooling. Polymerization is the cross-linking of polymers to other polymers and to the wood surface. Methods for activation of polymerization include heat, catalyst, change in pH, radiation, or addition of a second component (Frihart 2005). Heat polymerization is commonly used to set wood adhesives, but uniform heating is difficult since wood is a good insulator. Non-uniform and incomplete heating leads to poor bond strength. Adhesive formulas must have advanced polymerization to insure that desired reactions are achieved during heating (Frihart 2005).

2.3 Soy protein for adhesives

Soybean adhesives were first developed in the late 1920s by the I. F. Laucks Company (Keimel 1994). When synthetic adhesives evolved, development of soybean adhesives was phased out by the stronger synthetic adhesives. Currently, research has focused on extracting and modifying soy protein to improve adhesion strength and water resistance in order to develop more environmentally-friendly adhesives as compared to formaldehyde and other synthetic adhesives. Certain formulations of soy protein adhesives have been commercialized for consumer use. Protein modification, adhesive viscosity, and hot press processing conditions are three major parameters that must be considered when developing soy protein adhesives.

2.3.1 Protein modification

In order to improve water resistance and shear strength of soy protein, the protein is typically modified first. Chemical modification, enzymatic modification, and mixing with commercial adhesive are all processes that have been used to modify soy proteins.

2.3.1.1 Chemical modification

Chemical modification of soy protein helps to denature and unfold protein, thus increasing the interaction between protein and wood surface in order to form strong bonds. In native unmodified protein, most polar and apolar groups are unavailable for bonding due to

internal forces. When modification is performed, the polar and apolar groups become exposed and are able to interact with the wood surface, increasing shear bonding strength (Hettiarachchy et al. 1995). Chemical modification is a common modification type to unfold protein groups. Common chemical modifiers of soy protein include alkali, detergent, and chemicals containing amino groups.

Alkali modification of soy protein raises the pH of soy protein in order to induce protein unfolding. Hettiarachchy et al. (1995) found that alkali modification of soy protein improved adhesive shear strength and water resistance of soy protein adhesives. As the pH increased from 8 to 12, adhesive strength increased as well. Alkali modification is one modification type which can be used to improve soy protein adhesives.

Modification with detergent is another way to modify soy protein adhesives. Detergent, like alkali, also helps to unfold soy protein groups. When detergent interacts with protein groups, inner hydrophobic groups can be exposed, resulting in protein unfolding (Sun 2011). Sodium dodecyl sulfate (SDS) is one type of detergent that unfolds and denatures protein. Huang and Sun (2000a) found that soy protein modified with 0.5% and 1% SDS had higher shear strengths than unmodified protein. Protein modified with SDS also had better water resistance as measured by soak strength than unmodified protein. Soy protein modified with 3% SDS did not show improved strength due to a considerable extent of protein unfolding (Huang and Sun 2000a). When soy protein is modified with detergent at certain concentrations, shear strength and water resistance increase.

Chemicals that have amino groups, such as urea, can also be used to modify soy protein by unfolding protein groups. Huang and Sun (2011b) found that modifying soy protein with urea at certain concentrations increased the shear strength of adhesives. At high concentrations of urea (8 M), the protein became completely denatured, leading to decreased shear strength (Sun 2011). Lower concentrations of urea, such as 1 M and 3 M, partially unfolded soy protein. Since the protein was only partially unfolded, it still retained some secondary structure, leading to increased shear strength (Sun 2011). The partial protein unfolding increases shear strength because it allows more protein groups to be exposed for bonding and cross-linking. Chemicals containing amino groups help to improve shear strength of soy protein adhesives by partially unfolding the protein.

Overall, the purpose of chemical modification is to unfold protein so that the polar and apolar groups that are usually unavailable for wood interaction are exposed. The groups can then interact with the wood surface leading to enhanced shear strength and water resistance.

2.3.1.2 Enzymatic modification

Another protein modification type is enzymatic modification. Enzymes modify protein by changing the protein structure. A major advantage of enzymatic modification is the specificity of enzymes in cleaving bonds (Kumar et al. 2002). Proteases including pepsin, trypsin, chymotrypsin, papain, and pronase are commonly used to modify soy protein (Kumar et al. 2002). Proteases hydrolyze certain bonds depending on the specific enzyme used to modify the protein. When specific bonds are hydrolyzed within the protein, secondary structure is altered, leading to better bonding and interaction with the wood surface. In one study, soy protein was modified with trypsin and, consequently had increased adhesive strength (Kalapathy et al. 1995).

2.3.1.3 Modification by mixing with commercial adhesive

Protein modification can also be achieved by mixing soy protein with commercial adhesive, thus requiring less commercial adhesive and lessening their negative environmental impact. Though the complete discontinuation of commercial formaldehyde-based adhesives would be ideal, mixing commercial adhesive with protein is a short-term solution for limiting the amount of formaldehyde adhesives produced and used (Sun 2011).

In one study, soy protein was mixed with various synthetic commercial adhesives (Qi and Sun 2011). Of the formulations used, urea-formaldehyde-based resin (60%) mixed with modified soy protein (40%) demonstrated higher wet shear strength than all other commercial mixtures and the control. The results of the study indicated that modified soy protein is able to act as an acidic catalyst for urea-formaldehyde-based resins (Qi and Sun 2011).

In another study, soy protein was mixed with phenol-formaldehyde resin for plywood adhesives (Zhong and Sun 2007). Viscosity of the adhesive mixtures was increased with increasing amounts of phenol-formaldehyde resin. Adhesive strength was improved by adding phenol-formaldehyde at ratios of 100:20 and 100:40 (soy protein: phenol-formaldehyde), and the blends had higher shear strengths than the pure resin at a pH of 7.1 (Zhong and Sun 2007). The increase in adhesion strength was attributed to the phenol-formaldehyde reacting with the protein functional groups which were cross-linked, leading to stronger bonding with the wood.

Overall, mixing soy protein with commercial adhesives offers a short-term solution for limiting the amount of formaldehyde produced. Ideally, adhesive mixtures should be developed that do not utilize commercial adhesives or formaldehyde.

2.3.2 Adhesive viscosity

The viscosity of adhesives is an important parameter when regarding adhesive application and strength. Sufficient viscosity and uniformity are necessary for easy application and processing of soy protein adhesives as viscosity must be high enough to allow for easy application, but low enough for the wood surface to be wetted. Factors such as protein concentration and modification affect viscosity.

The concentration of soy protein within adhesive formulations greatly affects the viscosity of soy protein adhesives. Increasing the concentration of soy protein increases the viscosity because more molecules are suspended in solution and protein unfolds in solution. The unfolded proteins lead to increased intermolecular interactions due to covalent bonding and electrostatic interactions (Kumar et al. 2002).

Protein modification affects the viscosity of soy protein adhesives. At high concentrations of SDS modification, soy protein adhesives demonstrate increased viscosities (Zhong et al. 2001). Increased viscosity with increasing SDS concentration is due to protein unfolding, thus causing molecules to become swollen, leading to an increase in the effective volume, decreasing the space between the protein molecules, and, therefore, increasing viscosity (Zhong et al. 2001). Other detergents typically have an identical effect on viscosity: increasing viscosity with increasing detergent concentration. In addition, various protein modifications also have dissimilar effects on protein viscosity. Qi et al. (2013) found that 2-octen-1-ylsuccinic anhydride (OSA) has a decreasing effect on viscosity of soy protein adhesives. Viscosity decreased as concentration of OSA was increased likely due to pH being lowered, compacting and aggregating protein molecules. Modification type is important to shear strength, but viscosity of adhesive formulations must be optimized as well.

2.3.3 Hot press processing conditions

Processing conditions used to hot press soy protein adhesives affect the shear strength of the adhesives. The curing process makes soy protein harder as a result of cross-linking reactions

(Sun 2011). The assembly time, press temperature, press pressure, and press time are all important processing parameters that affect the adhesive strength of soy protein adhesives.

2.3.3.1 Assembly time

In order to achieve surface wetting of the wood substrate, assembly time is required for preparing soy protein adhesives. Assembly time is the time between adhesive application and pressing. Zhong et al. (2001) modified soy protein with SDS and found that increasing the assembly time from one minute to fifteen minutes increased the shear strength of fiberboard adhesives. The increase in strength was likely due to protein molecular chains penetrating the wood surface and water evaporation during assembly. When the samples were pressed, water continued to evaporate and the protein further interacted with the wood surface (Zhong et al. 2001). If assembly time is too short, the protein does not have enough time to interact with the porous structure in the wood; consequently, if assembly time is too long, excessive water can evaporate, leading to negative effects on shear strength. Assembly time is an important parameter and should be optimized for varying adhesive formulations.

2.3.3.2 Press temperature

After protein adhesives have had sufficient assembly time, the adhesive and wood samples are hot pressed. Without hot pressing, the adhesive does not sufficiently interact with the wood leading to poor adhesion strength. The pressing temperature of the wood affects the protein's interaction with the wood and therefore affects adhesion of the protein to the wood surface. Zhong et al. (2001) found that increasing the press temperature of SDS modified soy protein to fiberboard from 25°C to 120°C increased the adhesive strength as well. The adhesive strength improved as temperature increased because of the proximity to and surpassing of the protein's denaturation temperature. When the temperature exceeded the denaturation temperature, protein molecules were initially unfolded, but became folded upon curing, leading to increased adhesion strength (Zhong et al. 2001). The denaturation temperature of soy protein is lowered with increasing water content (Sun 2011). However, when assemblies are pressed, water content begins to decrease due to water evaporation. Therefore, the relationship between water content and protein denaturation temperature should be considered.

2.3.3.3 Press pressure

The amount of pressure applied to soy protein adhesive wood assemblies is crucial for sufficient bonding. Without enough pressure, the protein is not forced to interact sufficiently with the wood. When press pressure increases, more contact between the substrate surface and protein at the interface can be achieved (Sun 2011). Sufficient pressure must be applied to protein adhesives and wood in order to gain adequate contact and successful bonding for high shear strength.

2.3.3.4 Press time

The amount of time that the adhesive and wood is pressed is another important processing condition. Increasing press time encourages protein molecules to penetrate the wood surface and promote chemical interactions at the interface (Sun 2011). Zhong et al. (2001) found that increasing the press time of SDS modified soy protein adhesives increased the shear strength of the adhesives as well. Press time is related to press temperature in that longer pressing time is needed for lower press temperatures (Sun 2011). In general, increasing press time increases the shear strength of soy protein adhesives.

2.4 Research objectives

The overall objective of this research was to determine the potential of canola proteins for bio-based wood adhesives. The specific objectives of this research were:

1. To evaluate the adhesion performance of unmodified and modified canola protein.
2. To improve the wet shear strength of canola protein through chemical modification.
3. To determine the effects of chemical modification on canola protein structure, thermal properties, and rheological properties.

3. Materials and Methods

3.1 Materials

Hexane, hydrochloric acid (HCl), sodium hydroxide (NaOH), calcium chloride ($CaCl_2$), and zinc sulfate ($ZnSO_4$) were purchased from Fisher Scientific (Pittsburg, PA, USA). Sodium dodecyl sulfate (SDS), calcium carbonate ($CaCO_3$), and 2-octen-1-ylsuccinic anhydride (OSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cold screw-pressed canola meal with 11% moisture content (wet base) was purchased from Planet Natural (Bozeman, MT, USA). Cherry wood was purchased from The Home Depot (Manhattan, KS, USA). Veneers were prepared from the cherry wood with dimensions of 50 x 127 x 5 mm (width x length x thickness).

3.2 Methods

The methods used to prepare samples and to analyze samples are outlined in the sections below, including protein extraction, protein purity, protein modification, shear strength measurements, rheological properties, thermal properties, and morphological properties.

3.2.1 Protein extraction

Protein was isolated from canola meal using methods similar to Li et al. (2011) with modifications. Canola meal was first dried in an oven at 49°C for 24 hours. The meal was then milled into powder with a cyclone sample mill (Udy Corp., Fort Collins, CO) to ensure a particle size of <0.50 mm. The meal was then defatted with hexane at a solid to liquid ratio of 1:10 (w/v) for two hours at room temperature and was repeated for three cycles. The defatted canola meal was then dried in a fume hood overnight to remove excess hexane. Next, the protein was separated from the meal by first adding distilled water at a solid to liquid ratio of 1:12 (w/v) to the meal and mixing for one hour with a stir plate. The pH of the solution was then adjusted to 12 with 6M NaOH and mixed for two hours in order to solubilize the protein. The solution was then centrifuged at 7500 x g for 15 minutes and the supernatant was decanted through six layers of cheesecloth. The pH of the supernatant was adjusted to 3.5 with 2M HCl and stirred for 15 minutes to precipitate the protein. The solution was then centrifuged at 7500 x g for 15 minutes in order to obtain the proteins. The protein was washed with distilled water three times to remove residual salts and then freeze-dried.

3.2.2 Protein purity

Protein content of isolated canola protein was measured using a PerkinElmer Model 2400 Series II CHNS/O Analyzer (Shelton, CT, USA). Nitrogen percentage was recorded and converted to protein percentage using a factor of 6.25. The samples tested were of the defatted canola meal, canola meal residue (from the extraction process), and the extracted canola protein.

3.2.3 Protein modification

In an attempt to improve the adhesion strength of canola protein, different protein modifications were used. The control was made by mixing 1.2g canola protein with 10mL distilled water, yielding a protein content of 12%. Chemical modification and combined chemical modification were used to improve adhesion strength. The modifications methods used are described in the following sections.

3.2.3.1 Chemical modification

The chemicals used for modification included SDS, $CaCO_3$, $ZnSO_4$, $CaCl_2$, and OSA. For the SDS modification, the methods used to prepare the samples were similar to those described by Huang and Sun (2000a). SDS solutions (0.5%, 1%, 3%, and 5%) were prepared at room temperature. Milled canola protein (1.2g) was suspended in each of the SDS solutions (10mL) and stirred for six hours.

The $CaCO_3$, $ZnSO_4$, and $CaCl_2$ modified samples were prepared using the same method as the SDS samples, but different concentrations of the chemicals were used; $CaCO_3$: 1%, 3%, and 5%, $ZnSO_4$: 0.1%, 0.5%, and 1%, and $CaCl_2$: 0.5%, 1%, 3%, and 5%. The $CaCO_3$, $ZnSO_4$, and $CaCl_2$ modified samples were mixed for one hour, unlike the SDS samples which were mixed for six hours. The OSA modified samples were prepared by first mixing canola protein (12%) with distilled water. OSA was added to the protein solution at concentrations of 2%, 3.5%, and 5% (w/v) and the solutions were mixed for two hours.

3.2.3.2 Combined chemical modification

For the combined chemical modification of canola protein, two different combinations were used: SDS and $CaCO_3$, and SDS and $ZnSO_4$. The SDS modification was done first by making SDS solutions of 1% and 3% in distilled water. Canola protein (12%) was added to the SDS solutions and stirred for two hours. Next, $CaCO_3$ (1%, 3%, or 5% w/v) or $ZnSO_4$ (0.1%,

0.5%, or 1% w/v) was added to the various SDS/canola protein solutions and mixed for an additional two hours. Overall, 12 different combinations (1% SDS with either 1% $CaCO_3$, 3% $CaCO_3$, 5% $CaCO_3$, 0.1% $ZnSO_4$, 0.5% $ZnSO_4$, or 1% $ZnSO_4$; 3% SDS with either 1% $CaCO_3$, 3% $CaCO_3$, 5% $CaCO_3$, 0.1% $ZnSO_4$, 0.5% $ZnSO_4$, or 1% $ZnSO_4$) were used.

3.2.4 Shear strength measurements

Shear strength was tested using the prepared adhesives and cherry wood veneers with dimensions of 50 x 127 x 5 mm (width x length x thickness). The wood veneers were conditioned for at least seven days at 25°C and 50% relative humidity (RH) in an environment chamber (Model 518, Electro-Tech Systems, Inc., Glenside, PA, USA). Prepared canola protein adhesives were applied to two pieces of wood with an application area of 127 x 20 mm (length x width). Approximately 500 µL of adhesive was brushed over the application area of each piece of wood until uniform and then allowed to set for 15 minutes.

The wood was assembled with adhesive areas combined as described by Mo et al. (2004). The assemblies were pressed in a Model 3890 Auto M hot press (Carver, Inc., Wabash, IN, USA) for 10 minutes at 2 MPa. The press temperature was 170°C, 180°C, or 190°C depending on the test. The pressed assemblies were then conditioned for two days at 25°C and 50% RH, and the conditioned assemblies were cut into five specimens, measuring 20 x 80 x 5 mm (width x length x thickness).

Shear strength was tested using an Instron Model 4465 (Canton, MA, USA) with a crosshead speed of 1.6mm/min. The shear strength at maximum load was recorded with reported values being the average of 3-8 different specimen measurements. Three different shear strength tests were performed on the samples: dry strength, wet strength, and soak strength. The dry strength was tested after conditioning the cut samples in the environment chamber at 25°C and 50% RH for five days according to the ASTM Standard Method D2339-98 (ASTM 2002). The wet strength was performed after soaking the cut samples in tap water for 48 hours and then testing immediately according to ASTM Standard Method D1183-96 (ASTM 2002). The soak strength was performed after soaking the cut samples in tap water for 48 hours, followed by conditioning them for seven days in the environment chamber at 25°C and 50% RH and then testing according to ASTM Standard Method D1151-00 (ASTM 2002).

3.2.5 Rheological properties

The apparent viscosity of the prepared samples was tested using a Bohlin C-VOR rheometer model CVO150 (Malvern Instruments, Westborough, MA, USA) with a 20-mm cone diameter. The distance between the cone and plate was set to 500µm for all measurements. All of the experiments were conducted in duplicate with the average values reported.

3.2.6 Thermal properties

The thermal transition properties of the samples were measured using Differential scanning calorimetry (DSC). Measurements were made with a DSC Q200 (TA Instruments, New Castle, DE, USA). Samples of modified freeze-dried protein adhesive weighing approximately 3mg were placed in DSC pans. The samples were first equilibrated to 20°C and then heated to 250°C at a heating rate of 10°C/min.

3.2.7 Morphological properties

The morphological properties of the canola protein adhesives were measured using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The program ImageJ was used for analysis of the images (http://rsb.info.nih.gov/ij/).

SEM was used to determine surface properties of canola protein adhesives. Samples were prepared by freeze-drying modified adhesive mixtures, then milling them into powder. The milled, modified canola protein was then affixed to an aluminum stub using two-sided carbon tape by gently dipping the stub into the protein powder. SEM images were taken with an FEI Nova NanoSEM 430 (Hillsboro, OR, USA) at an accelerating voltage of 5 kV with either a vCD (low-voltage high-contrast backscatter electron detector) detector or an EDS (energy dispersive spectroscopy) detector (x-max EDS 80mm^2 silicon drift detector).

TEM was used to determine protein structure and interaction. Samples were prepared by first mixing modified canola protein (1% weight) with distilled water. The diluted samples were then absorbed onto Formvar/carbon-coated 200-mesh copper grids for 60 seconds. Excess solution was wicked off with filter paper and the grids were suspended in 2% uranyl acetate for 60 seconds at room temperature. Excess uranyl acetate was removed from the grids, and the grids were stored for imaging. All TEM images were taken with an FEI CM 100 at an accelerating voltage of either 80 kV or 100kV (Hillsboro, OR, USA).

4. Results and Discussion

4.1 Percent protein composition

The total amount of defatted canola meal used for protein extraction was 1150g. The total amount of canola protein extracted from the defatted meal was 176.16g. After the protein was extracted, it was freeze-dried and then modified prior to application. Freeze-drying extracted proteins provides the advantage of not changing the protein structure while simultaneously increasing the shelf-life of the protein. If the protein is modified during the extraction process, often it must be assembled and pressed following extraction. The current method allows the user to store the protein for long periods of time and modify it directly prior to use.

The nitrogen content of the isolated canola protein was determined by elemental composition analysis and then converted to protein percent by multiplying the nitrogen percentage by a factor of 6.25. The protein content of the defatted canola meal was 51.61%, the protein content of the meal residue was 30.44%, and the protein content of the extracted protein was 83.88%, as shown in Table 2.

Table 2. Nitrogen and protein composition

Sample	% Nitrogen	% Protein
Defatted canola meal	8.26	51.61
Meal residue	4.87	30.44
Extracted protein	13.42	83.88

Overall, the pure protein extraction rate was 24.9% calculated from the equation:

$$Pure\ \ protein\ extraction\ rate\ (\%) = \frac{Total\ \ protein\ extracted}{Total\ \ protein\ in\ the\ meal} \times 100$$

The extraction rate was lower than reports using similar methods. Li et al. (2011) reported a protein recovery rate of 31.33% for unmodified canola protein extraction. Li et al. (2012) reported a protein recovery yield of 31.45% for unmodified canola protein extraction at a pH of 7.0. The differences in extraction rate between the previous reports and current work were

most likely due to slight differences in methods as well as equipment used for extraction. Also, nitrogen content was determined using dissimilar equipment, thus allowing for a possibility in the differences in numbers.

Canola contains protein fractions that are soluble in different solutions. Prolamins are the protein fraction soluble in ethanol. Ethanol was not used in the extraction and therefore, some of the protein fractions were not extracted from the meal. Klockeman et al. (1997) reported that prolamins in canola meal have 33.9% solubility in 60% (v/v) ethanol. If ethanol was used for the extraction, the protein extraction rate possibly could be higher than 24.9%.

Solubilization pH and precipitation pH are important factors affecting protein extraction. Manamperi et al. (2011) found that slightly alterations of solubilization pH and precipitation pH significantly affect the protein yield of canola meal. In order to increase protein yield in the current work, protein solubilization pH and precipitation pH could be optimized. Also, because many diverse protein fractions exist, multiple solubilization pHs and precipitation pHs could be used in order to extract the maximum amount of protein from canola meal.

4.2 Protein modification

Protein modification was performed in an attempt to improve the shear strength of canola protein adhesives. An image of the SDS modified canola protein samples are shown in Figure 2.

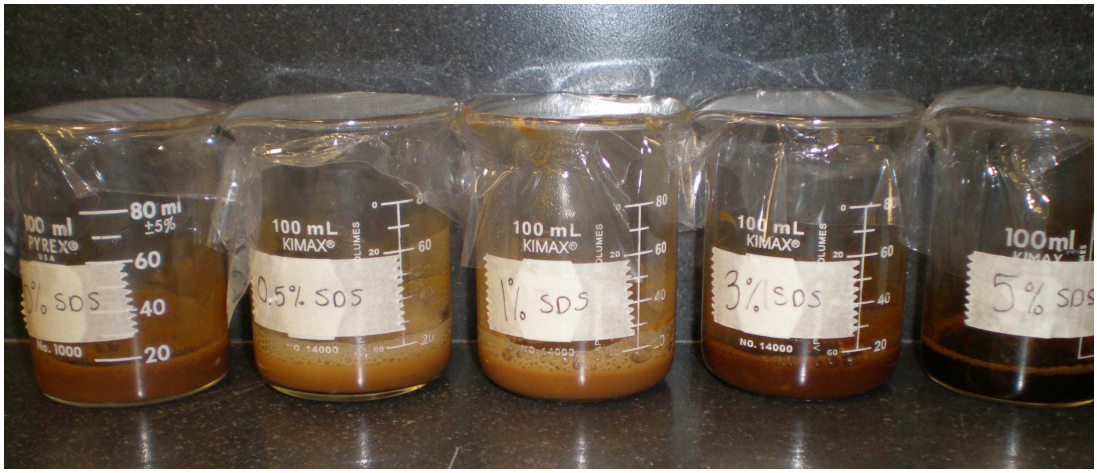

Figure 2. Image of SDS modified canola protein adhesives

As shown in Figure 2, SDS modification affected the color of canola protein adhesives. From left to right, the image shows the unmodified protein, 0.5% SDS modification, 1% SDS modification, 3% SDS modification, and 5% SDS modification, respectively. As the SDS concentration increased, the darkness of the samples increased, indicating more protein denaturation. In the 0%, 0.5%, and 1% SDS samples, the protein did not solubilize in solution and settled out of solution after approximately 15 minutes of no activity. In the 3% and 5% SDS samples, the protein did not settle out of solution, indicating that the protein was more unfolded and in a more denatured state. Overall, the color of the canola protein adhesives was fairly dark. A light to neutral colored adhesive is usually preferred for commercial applications.

For commercial adhesives, the consistency and uniformity of the adhesive are crucial. For canola protein adhesives to be commercially feasible, the protein needs to be solubilized and equally dispersed in solution. If the protein settles out of solution, it is very difficult to apply the adhesive to the substrate surface.

4.3 Adhesion performance of canola protein adhesives

The canola protein adhesives were tested for adhesion performance on dry, wet, and soak strengths. The results for adhesion performance of canola protein based on chemical modification, combined chemical modification, and temperature modification can be found in the following sections.

4.3.1 Effect of chemical modification on mechanical properties

SDS, $CaCO_3$, $ZnSO_4$, $CaCl_2$, and OSA were used as chemical modifiers. The effects of chemical modification on mechanical properties of canola protein adhesives are shown in Table 3. The samples were all pressed at 170°C with a press time of 10 minutes and a pressure of 2 MPa.

Table 3. Shear strength of chemically-modified canola protein

Adhesive Formulation	Dry Strength (MPa)	Wet Strength (MPa)	Soak Strength (MPa)
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
0.5% SDS	5.30±0.21	2.16±0.30	4.88±0.35
1% SDS	6.59±0.42	1.98±0.34	6.37±0.96
3% SDS	8.19±0.36	1.82±0.35	6.76±0.28
5% SDS	7.33±0.73	1.31±0.33	5.07±0.56
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
1% $CaCO_3$	6.39±0.88	1.16±0.25	5.77±0.22
3% $CaCO_3$	6.38±0.68	1.42±0.61	5.06±0.28
5% $CaCO_3$	7.41±1.20	0.82±0.33	5.84±0.76
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
0.1% $ZnSO_4$	5.56±0.44	1.80±0.21	5.13±0.51
0.5% $ZnSO_4$	5.39±0.96	1.32±0.37	5.22±0.43
1% $ZnSO_4$	6.10±0.42	1.33±0.39	5.37±0.64
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
0.5% $CaCl_2$	6.53±0.75	1.37±0.26	n/a
1%$CaCl_2$	5.90±1.05	1.74±0.43	n/a
3% $CaCl_2$	5.71±0.97	1.22±0.39	n/a
5% $CaCl_2$	5.37±0.78	1.03±0.41	n/a
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
2% OSA	5.91±0.76	1.96±0.49	6.14±0.96
3.5% OSA	5.89±0.92	2.72±0.74	5.19±0.78
5% OSA	5.99±0.42	1.70±0.63	5.02±0.80

For canola protein adhesives modified with SDS, dry strength increased as SDS concentration increased, up to 3% with 100% wood cohesive failure (WCF) for 0.5%, 1%, and 3% and partial WCF for 5%. Soak strength increased as SDS concentration increased up to 3%. Wet strength decreased as concentration of SDS increased. Protein unfolding and denaturation

may also have increased as SDS concentration increased. The decrease in wet strength as the concentration of SDS increases could be due to the SDS creating negative surface charges on the adhesive and therefore making the adhesive hydrophilic. Hydrophilic adhesive tends to absorb water, therefore disrupting the bond between adhesive and wood. Overall, the SDS modification increased dry shear strength and soak strength, but decreased the wet shear strength. The wet shear strength of this modification is too low for outdoor adhesive purposes but could possibly be used for indoor applications.

The canola protein adhesives modified with $CaCO_3$ had slightly higher dry and soak shear strength than the unmodified sample. $CaCO_3$ modification did not show any improvement on wet strength. $CaCO_3$ does not dissolve in water, therefore making a reaction with the protein impossible. The purpose of a modifier is to either unfold, denature, or crosslink protein, but $CaCO_3$ was not able to act as a modifier because it did not dissolve in solution with the protein. In the protein adhesive samples, the $CaCO_3$ settled below the protein in solution; therefore, the $CaCO_3$ was not able to react with the protein. The adhesive solution was not uniform, causing difficulty in achieving uniform application on the wood. Adding $CaCO_3$ did not significantly improve the wet, dry, or soak strengths.

The canola protein adhesives modified with $ZnSO_4$ had lower dry shear strengths than the unmodified protein samples. The wet strengths of the $ZnSO_4$ modified samples were lower than the unmodified protein but better than the $CaCO_3$ modified samples. The soak strengths of the $ZnSO_4$ modified samples were slightly higher than the unmodified protein but were not significantly greater. Overall, the canola protein modified with $ZnSO_4$ did not show significant improvement on shear strengths as compared to unmodified protein. When the $ZnSO_4$ was added to water, the $ZnSO_4$ dissolved in solution. However, the protein was not denatured and settled out of solution if the solution was not constantly mixed. Therefore, the $ZnSO_4$ did not act as a worthy modifier for canola protein and did not show any improvement on shear strength compared with the unmodified protein.

The canola protein adhesives modified with $CaCl_2$ had high dry strength, with 100% WCF for the 0.5, 1, and 3% modifications. The wet strength of the 1% $CaCl_2$ modification was relatively high, but was still not as high as the unmodified protein. Soak strength was not tested for the $CaCl_2$ samples because the wet strength was not increased with modification. The $CaCl_2$ dissolved in water when the samples were prepared; however, the protein was not modified or

denatured when added to the modified solution because the protein would settle down to the bottom. The adhesive was therefore not uniform and difficult to apply.

The canola protein adhesives modified with OSA had improved dry and soak strengths. The 3.5% OSA modification had a wet strength greater than the unmodified adhesive and caused partial wood failure. The 2% and 5% OSA modifications had wet shear strengths slightly lower than the unmodified protein. The increase in wet shear strength of the 3.5% OSA samples could be attributed to the fact that OSA is an oil-like substance, making it hydrophobic. The OSA could have made the protein groups hydrophobic as well, thus preventing water from penetrating between the adhesive and wood surface, leading to higher shear strength (Qi et al. 2013).

Overall, the 3.5% OSA chemical modification of canola protein had the greatest wet shear strength. All other chemical modifications decreased wet strength compared to unmodified canola protein. Other chemical modifications had increased dry and soak shear strengths at certain modifier percentages but lower wet shear strengths compared to unmodified protein (Table 3).

4.3.2 Effect of combined chemical modification on mechanical properties

The combined chemical modification of canola protein by SDS and $CaCO_3$ or $ZnSO_4$ was evaluated. The effects of combined chemical modification of canola protein adhesives on mechanical properties are shown in Table 4. All wood specimens were pressed at 170°C and 2 MPa for 10 minutes.

Table 4. Shear strength of canola protein with combined chemical modification

Adhesive Formulation	Dry Strength (MPa)	Wet Strength (MPa)	Soak Strength (MPa)
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
1%SDS 1% $CaCO_3$	4.39±0.56	1.38±0.28	4.31±1.26
1%SDS 3% $CaCO_3$	5.30±0.55	1.23±0.33	4.57±0.53
1%SDS 5% $CaCO_3$	5.30±1.15	0.84±0.34	4.37±0.99
3%SDS 1% $CaCO_3$	5.84±1.79	0.75±0.62	4.31±1.38
3%SDS 3% $CaCO_3$	4.88±0.63	0.17±0.29	2.71±0.95
3%SDS 5% $CaCO_3$	3.75±1.01	0.05±0.05	0.39±0.31
Unmodified	5.73±0.77	2.20±0.15	4.99±0.33
1%SDS 0.1% $ZnSO_4$	5.04±0.27	1.87±0.36	4.34±0.40
1%SDS 0.5% $ZnSO_4$	4.24±0.58	1.97±0.42	4.36±0.67
1%SDS 1% $ZnSO_4$	3.91±1.15	1.57±0.47	4.68±0.77
3%SDS 0.1% $ZnSO_4$	4.02±0.80	1.78±0.43	4.33±1.14
3%SDS 0.5% $ZnSO_4$	3.44±0.36	1.42±0.27	3.67±1.42
3%SDS 1% $ZnSO_4$	3.23±0.61	0.89±0.32	3.29±0.76

The canola protein adhesives modified with both SDS and $CaCO_3$ showed decreased wet strength compared to the unmodified protein samples. The samples with 3% SDS and various concentrations of $CaCO_3$ were so weak that the Instron equipment barely measured the strength, and the samples could be easily broken apart by hand. The poor strength is possible due to the 3% SDS solution denaturing the protein and the $CaCO_3$ not dissolving in solution. Overall, the SDS and $CaCO_3$ modified samples had decreased dry, wet, and soak strengths compared to unmodified canola protein.

Samples modified with SDS and $ZnSO_4$ showed little to no wood failure for dry strength and poor wet strength. For all modifications of SDS and $ZnSO_4$, the dry, wet, and soak strengths were lower than that of the unmodified protein. Samples that contained 3% SDS and $ZnSO_4$ had lower wet shear strengths than the samples with 1% SDS and $ZnSO_4$ likely due to SDS

excessively denaturing the protein. None of the samples had wood failure, indicating combined chemical modification did not improve the adhesion performance of canola protein adhesives.

4.3.3 Effect of temperature on mechanical properties

The effects of press temperature on mechanical properties of canola protein adhesives are shown in Table 5. Canola protein adhesives modified by SDS were used to determine the effect of temperature (170°C, 180°C, and 190°C) on shear strength. All samples were pressed at 2 MPa for 10 minutes.

Table 5. Shear strength of SDS modified canola protein at 170°C, 180°C, and 190°C

Adhesive Formulation	Dry Strength (MPa)	Wet Strength (MPa)	Soak Strength (MPa)
Unmodified 170°C	5.73±0.77	2.20±0.15	4.99±0.33
0.5% SDS 170°C	5.30±0.21	2.16±0.30	4.88±0.35
1% SDS 170°C	6.59±0.42	1.98±0.34	6.37±0.96
3% SDS 170°C	8.19±0.36	1.82±0.35	6.76±0.28
5% SDS 170°C	7.33±0.73	1.31±0.33	5.07±0.56
Unmodified 180°C	6.61±0.37	3.49±0.36	6.48±1.25
1% SDS 180°C	6.52±0.40	2.68±0.36	6.28±0.51
3% SDS 180°C	5.71±1.58	2.13±0.39	5.30±1.02
Unmodified 190°C	7.03±0.70	3.14±0.89	7.76±0.34
0.5% SDS 190°C	6.00±0.69	3.52±0.48	6.66±0.07
1% SDS 190°C	6.35±0.92	3.45±0.28	6.41±0.82
3% SDS 190°C	5.69±1.88	2.42±0.39	6.71±0.88
5% SDS 190°C	6.47±0.84	1.91±0.68	6.46±0.69

For unmodified canola protein, the general trend indicated that as the temperature increased, the dry, wet, and soak shear strengths of the samples increased as well. Increasing the press temperature increases protein cross-linking, therefore increasing the shear strength. At press temperatures of 180°C and 190°C, the wood was slightly darkened, which is not desirable for a commercial process. Therefore, the temperatures of 180°C and 190°C are too high for the

wood type used even though the shear strength was increased. Kumar et al. (2002) obtained similar results with soy protein adhesives; as press temperature increased, so did the shear strength of soy protein wood adhesives. The increase in strength is likely due to the temperature being increasingly above the denaturation temperature of the protein. As protein denaturation increases, interaction between the protein and wood surface is improved.

The samples modified with SDS and pressed at a temperature of 180°C, had similar dry and soak strengths to the 170°C SDS samples. However, the wet shear strength of the 180°C SDS samples was greater than the 170°C SDS samples. Wood with the 180°C SDS samples was slightly darkened due to the increased press temperature.

The samples modified with SDS and pressed at a temperature of 190°C instead of 170°C had increased strengths. However, the 190°C temperature had a negative darkening effect on the wood surface even though the adhesion strength was increased.

Figure 3 summarizes results from Table 5 by comparing shear strength vs. concentration of SDS for dry strength, wet strength, and soak strength.

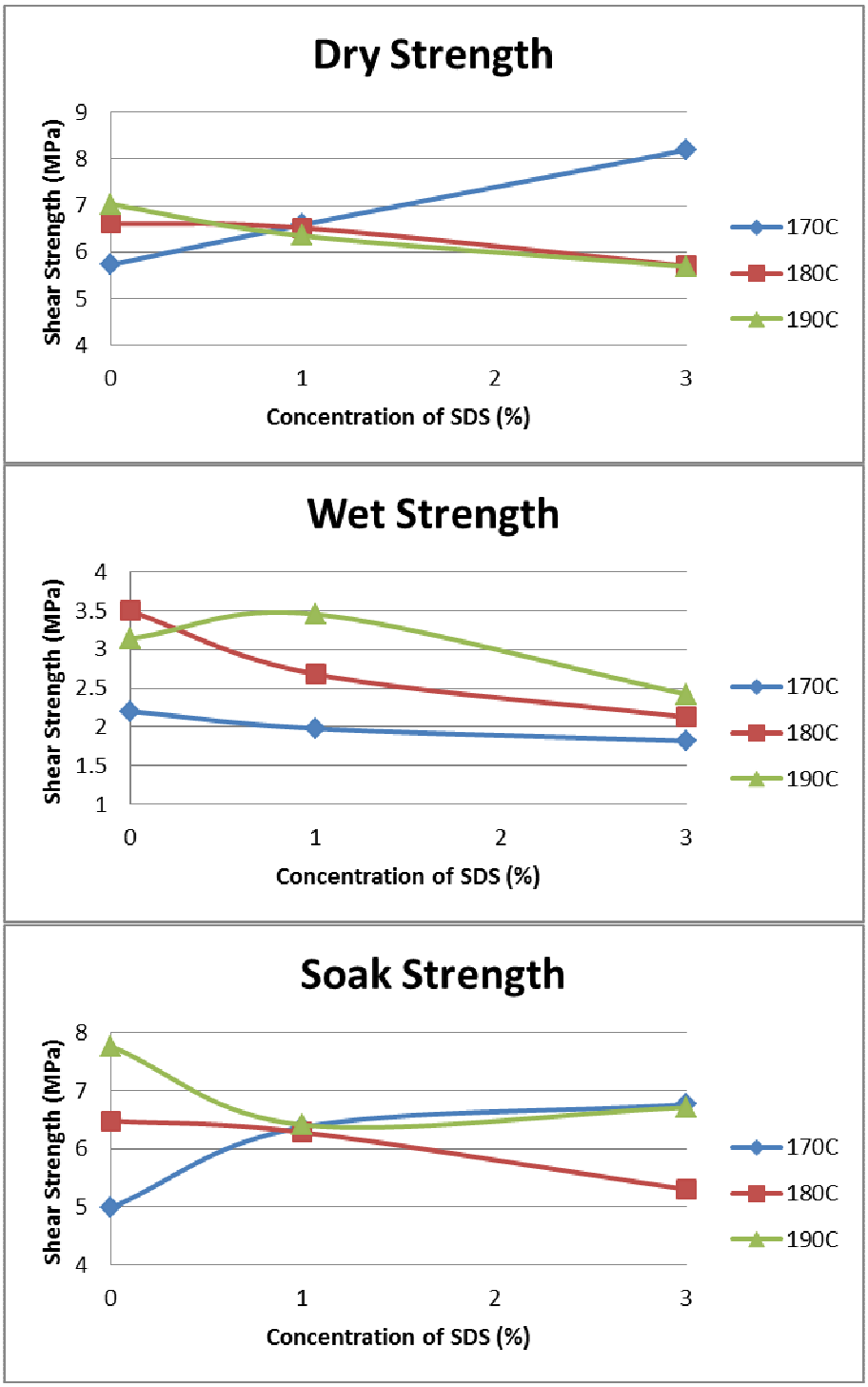

Figure 3. Shear strength vs. concentration of SDS at 170°C, 180°C, and 190°C

As illistrated in Figure 3, the dry, wet, and soak strengths of unmodified protein adhesive increased as the hot press temperature increased. However, for the wet strength, the 180°C samples had an average shear strength greater than the 190°C samples average.

The trends for wet, dry, and soak strength vary when comparing the 1% SDS modified samples. Hot press temperature did not have a significant effect on the dry and soak strength; all three temperatures registered approximately 6.5 MPa. However, hot press temperature significantly effected the wet strength, showing that wet strength increased as temperature increased. Increasing temperature also had a positive affect on wet shear strength.

Comparing the 3% SDS modified samples, the wet, dry, and soak strengths did not have similar trends. The dry strength was highest at 170°C and was approximately 5.7 MPa for the 180°C and 190°C temperatures. The wet strength of the 3% SDS modified samples increased as temperature increased. The soak strength was similar for 190°C and 170°C, with 180°C being the lowest. Overall, for the 3% SDS samples, no clear trends emerged for the dry, wet, or soak strength averages.

Figure 4 displays the shear strength vs. temperature of unmodififed canola protein adhesives for dry, wet, and soak strengths.

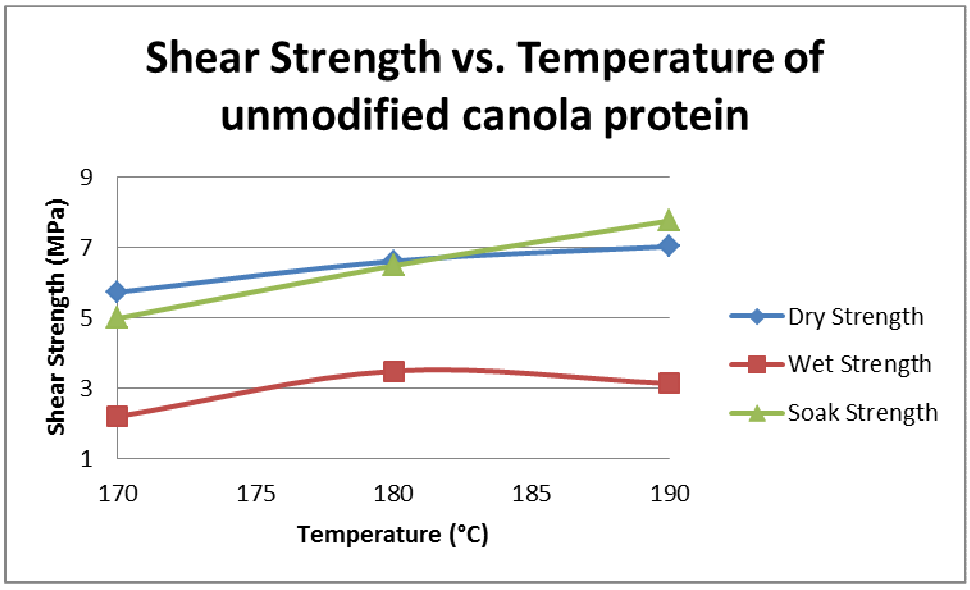

Figure 4. Shear strength vs. temperature of unmodified canola protein

As seen from Figure 4, the general trend for unmodified canola protein adhesives was that the shear strengths increased as press temperature increased. The only data point that does not follow this trend is the wet strength of the unmodified protein samples at 190°C. As press temperature increases, immobilization of the protein adhesive, as well as the possibilty of chemical reactions at the interface are enhanced (Sun 2011).

Overall Table 5 indicates, that the shear strength of canola protein adhesives increased as the press temperature increased. However, high temperature may damage the wood surface, leading to an unpleasing product appearance.

4.4 Rheological properties of canola protein adhesives

Viscosity is an important factor in adhesive handling and application. A relatively high apparent viscosity is desirable because it allows for easier adhesive application. The viscosity results for different chemical modifications can be found in the following sections.

4.4.1 Effect of chemical modification on rheological properties

The results for viscosity vs. shear rate for SDS modified canola protein can be found in Figure 5. All of the SDS modified adhesive samples exhibited shear thinning behavior.

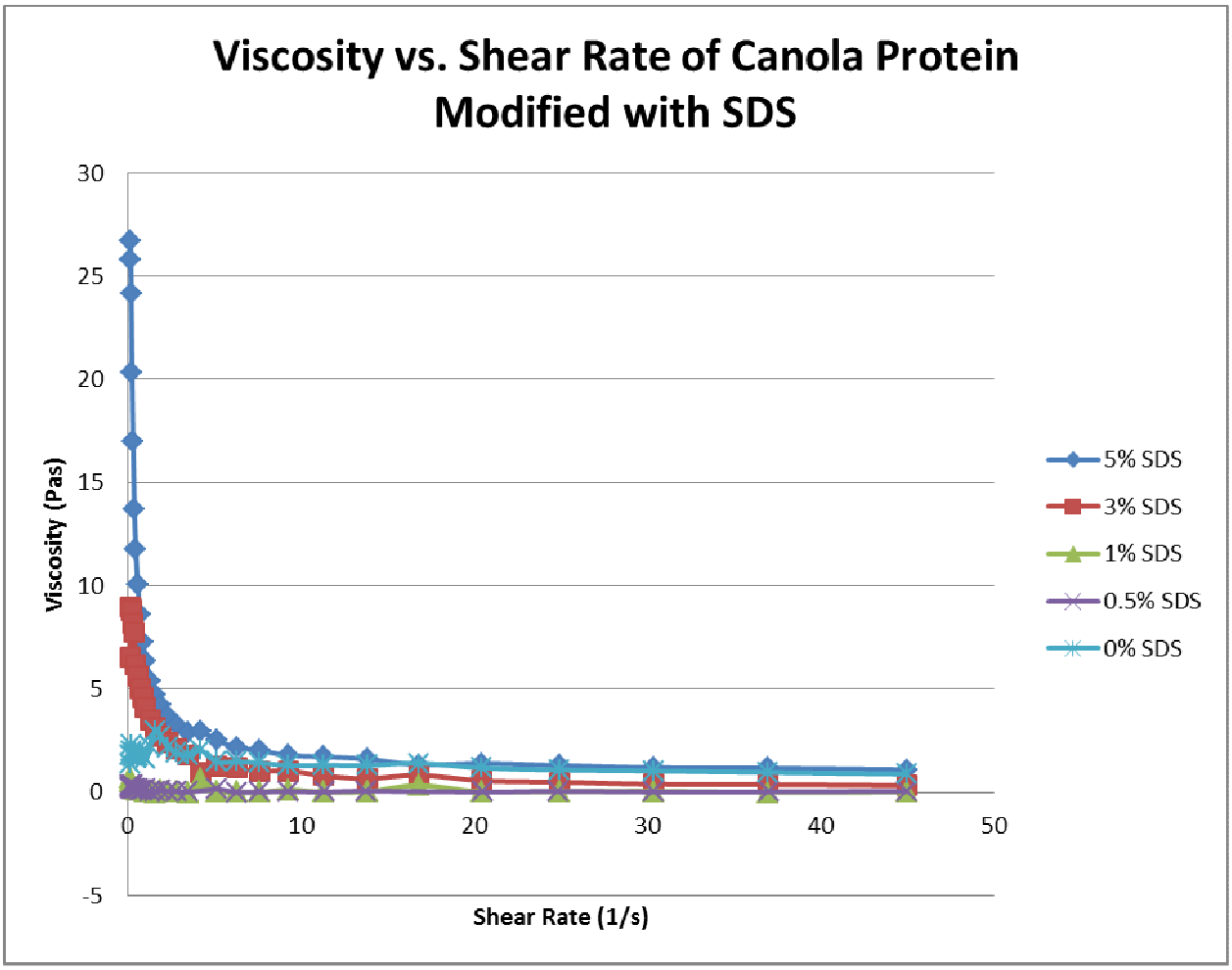

Figure 5. Viscosity vs. shear rate of canola protein modified with SDS

Apparent viscosity increased as SDS concentration increased. The 0.5% SDS modified adhesive sample had the lowest viscosity, followed by the 1% SDS sample, 3% SDS sample, and 5% SDS sample. The unmodified adhesive sample with 0% SDS had a viscosity between 3% SDS and 1% SDS. The color of the modified samples in Figure 2 corresponds to the viscosity of the samples in Figure 5. The 5% SDS solution was the darkest, then 3% SDS and unmodified, followed by 1% SDS and 0.5% SDS. Therefore, for the SDS modified samples, the darker color indicates the adhesive has a relatively high viscosity.

The samples modified with 5% SDS and 3% SDS were the most uniform and viscous of all the samples. The color of the 5% SDS and 3% SDS modified samples was darker than the other samples and the protein did not settle out of solution when not mixed. The uniformity and

color difference could be due to the protein being denatured. The 1% SDS and 0.5% SDS modified adhesives were not as uniform and the protein settled out.

The results for viscosity vs. shear rate of canola protein modified with OSA can be found in Figure 6.

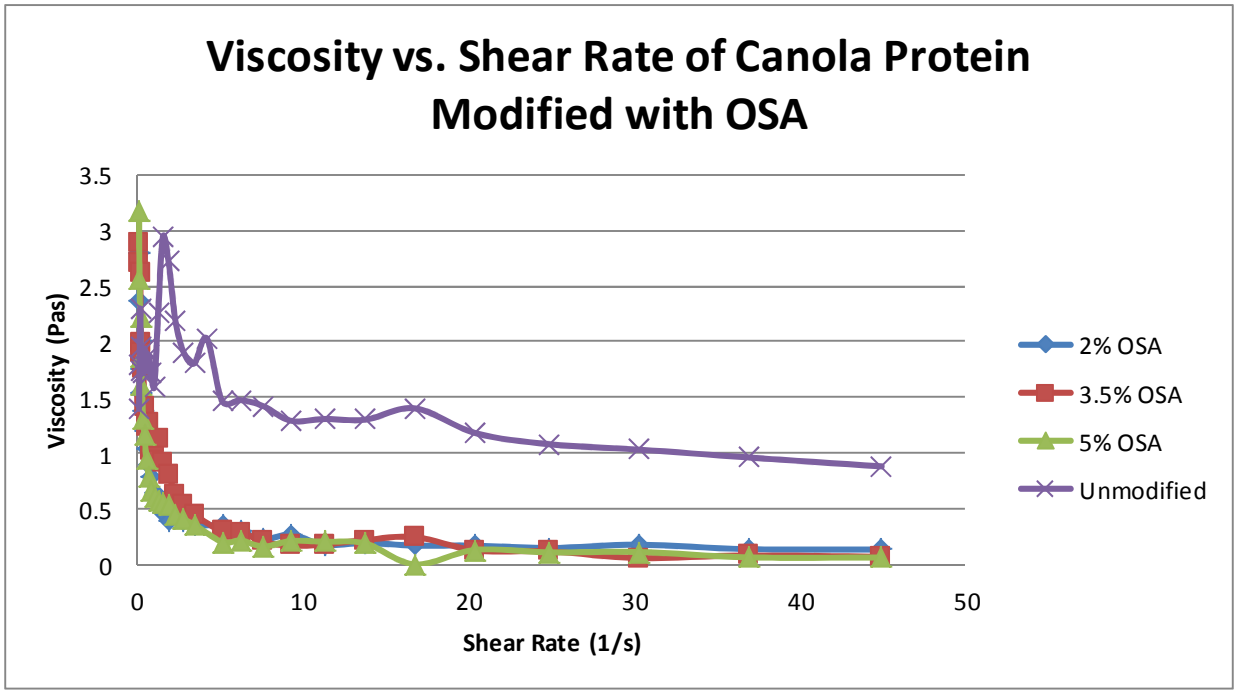

Figure 6. Viscosity vs. shear rate of canola protein modified with OSA

The viscosity of the OSA modified samples was lower than the unmodified canola protein (Figure 6), but all samples displayed shear thinning behavior. No significant difference was apparent in viscosity among the 2%, 3.5%, and 5% OSA modified samples. Qi and Sun (2013) found a similar trend for soy protein modified with OSA; the OSA modified samples had a lower viscosity than the unmodified soy protein sample. Increasing the concentration of OSA decreased the viscosity of the soy protein samples. The viscosity probably decreased due to the protein reaching its isoelectric point, therefore decreasing electrostatic repulsion and compacting the protein molecules in aggregate form (Qi and Sun 2013). However, for the OSA modified samples in this study (Figure 6), pH was not measured and therefore no conclusion can be reached regarding if the pH of the samples was near the protein isoelectric point. No clear distinction exists between the 2% OSA, 3.5% OSA, and 5% OSA modified samples, possibly

due to lack of uniformity of the samples. The OSA did not completely mix with the protein and water and, consequently, excess OSA remained at the top of the solution. The specific samples used for the viscosity testing could have not been completely uniform, leading to no trend for the various concentrations of OSA.

Overall, chemical modification affected the viscosity of the canola protein samples. Increased SDS concentration increased the viscosity of the samples. The color of the adhesives was an indicator of the viscosity, with the darker color indicating a higher viscosity. In general, OSA modification decreased the viscosity of the samples compared to the unmodified samples. Chemical modification of canola protein affects the viscosity of adhesive samples.

4.4.2 Effect of combined chemical modification on rheological properties

The viscosity vs. shear rate of canola protein modified with SDS and $ZnSO_4$ is shown in Figure 7.

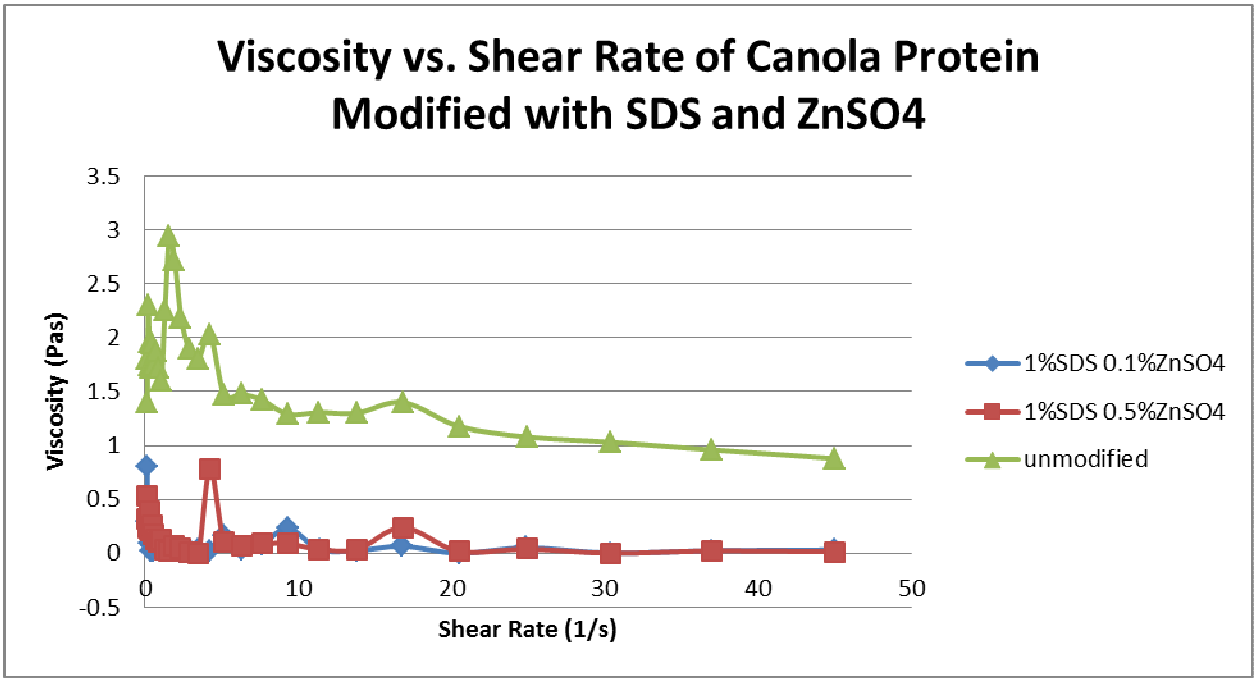

Figure 7. Viscosity vs. shear rate of canola protein modified with SDS and $ZnSO_4$

The viscosity results for the protein modified with SDS and $ZnSO_4$ are shown in Figure 7. Both samples were not uniform and therefore gave poor viscosity results. Changing the $ZnSO_4$

concentration from 0.1% to 0.5% had little effect on the viscosity. Both samples were not uniform and the protein settled out shortly after mixing. The overall pattern of the two modified adhesive samples shows a shear-thinning behavior, but the lowness of the viscosity causes difficulty in determining the effect of $ZnSO_4$ concentration on viscosity.

The results of viscosity vs. shear rate of canola protein modified with SDS and $CaCO_3$ are demonstrated in Figure 8.

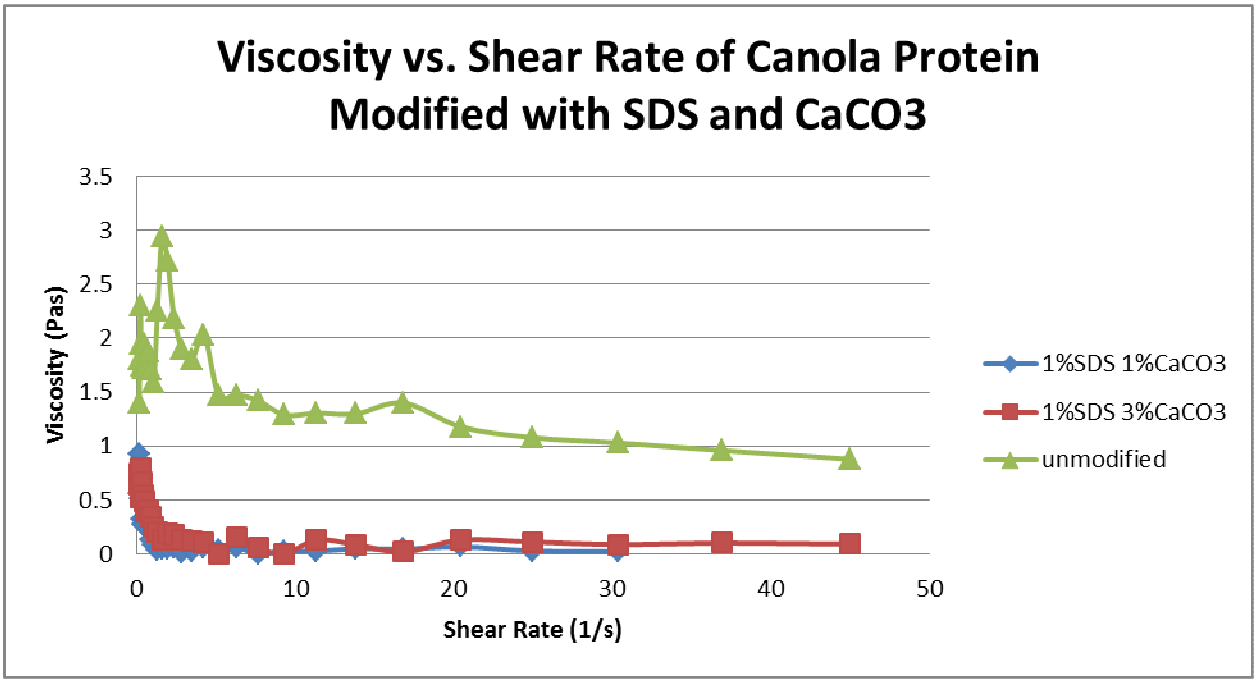

Figure 8. Viscosity vs. shear rate of canola protein modified with SDS and $CaCO_3$

Viscosity results for the protein modified with SDS and $CaCO_3$ are shown in Figure 8. Increasing the $CaCO_3$ concentration slightly increased the viscosity of the adhesive samples. Results show that the adhesives are shear-thinning; however, the samples were not uniform and separated when not mixed due to the protein not being denatured and/or sufficiently cross-linked. The viscosity of the samples was very low.

Overall, combined chemical modification with SDS and $ZnSO_4$ or $CaCO_3$ decreased the viscosity of canola protein adhesives compared to unmodified canola protein adhesives. The shear strength of the combined chemical modifications was very low and could partially be attributed to the low viscosities of the samples.

4.5 Thermal properties of canola protein adhesives

Thermal properties of canola protein adhesives were measured using Differential Scanning Calorimetry (DSC). Denaturation temperature (T_d) and enthalpy of denaturation (ΔH) were determined from DSC thermograms. DSC was performed with the unmodified protein, SDS modified protein, and combined-chemical modified protein.

The thermal stability of protein can be affected by many structural factors, including protein-protein interaction, binding of groups and metals, internal linkages, amino acid composition, and environmental factors (Wu and Muir 2008).

4.5.1 Effect of chemical modification on thermal properties

The DSC thermograms of SDS modified canola protein are demonstrated in Figure 9.

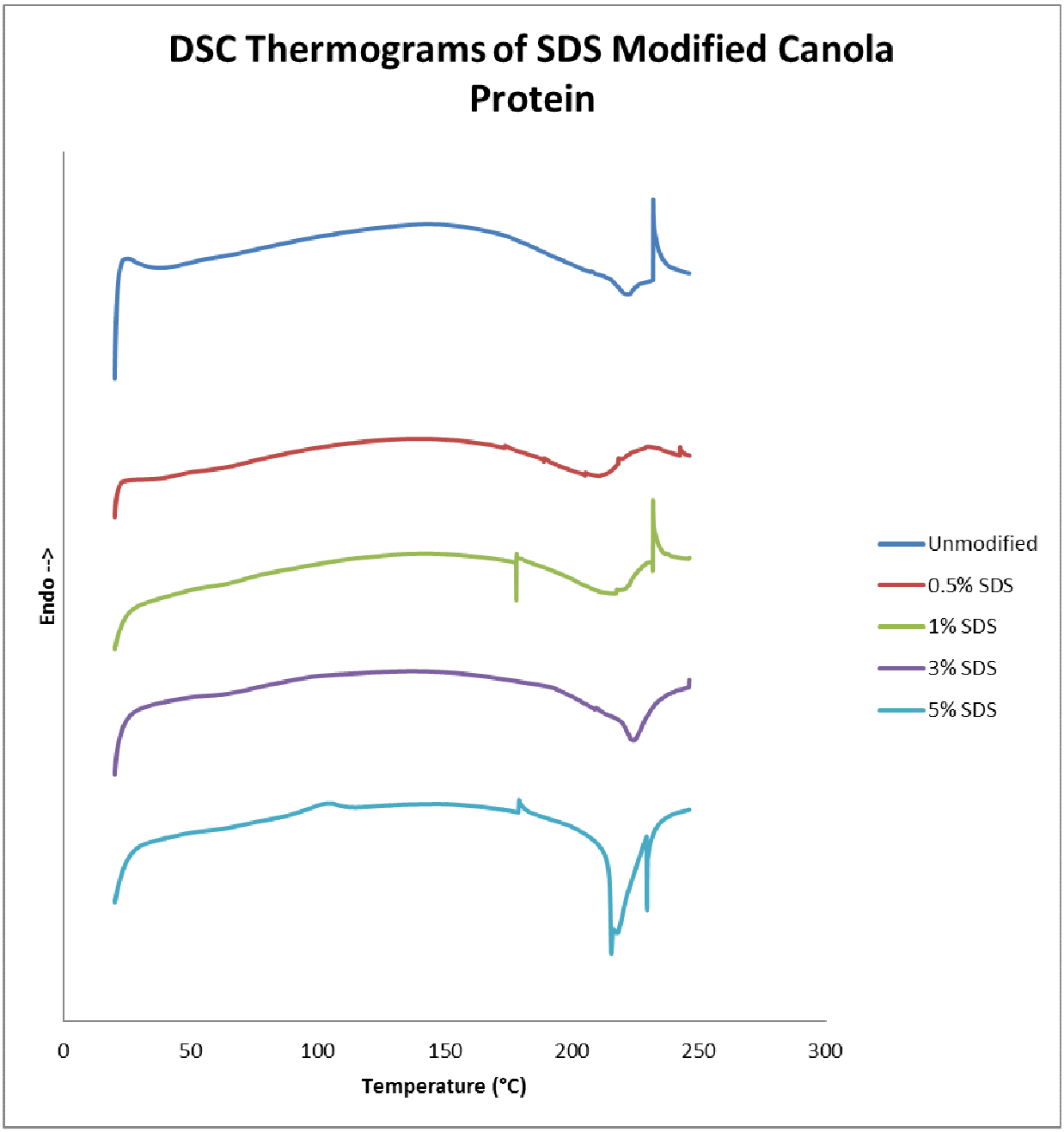

Figure 9. DSC thermograms of SDS modified canola protein

The SDS modified samples and the control had a broad peak between 100°C and 170°C, indicating the denaturation temperature, T_d (Figure 9). The broad peak could be attributed to the denaturation of two major protein components, cruciferin and napin, overlapping one another. The denaturation temperatures and enthalpies of denaturation for the SDS modified samples are shown in Table 6.

Table 6. Denaturation (T_d) temperatures and enthalpy of denaturation (ΔH) of SDS modified canola protein

Modification	T_d (°C)	ΔH (J/g)
unmodified	148.69	184.5
0.5% SDS	145.13	154.0
1% SDS	144.16	146.4
3% SDS	139.14	131.4
5% SDS	149.87	134.4

In general, protein modification with SDS decreased the denaturation temperature and enthalpy of denaturation. This trend is consistent with other studies using soy protein modified with OSA (Qi et al. 2013) and canola protein modified with sodium bisulfite (Li et al. 2012). However, the trend of the current study varied slightly from previous studies in that 5% SDS modified canola protein had a higher denaturation temperature than the unmodified canola protein. One reason the data does not follow a consistent trend could be variations in water content since the water content of each sample was not measured. For soy protein, the denaturation temperature decreases with increasing water content (Sun 2011). Canola protein could behave similarly to soy protein and, therefore, the water content of the 5% SDS modified sample could have been lower, yielding a higher denaturation temperature than other samples.

The denaturation temperatures of SDS modified samples in Table 6 are relatively high compared to the denaturation temperatures of canola protein found in previous works. In one study, Wu and Muir (2008) found the denaturation temperature of extracted canola protein isolate to be 83.9°C. Mu and Muir (2008) also found the denaturation temperature of the cruciferin portion of protein to be 90.7°C and napin to be 109.9°C. The extraction methods and sample type used in Wu and Muir's study were different than extraction methods used in the current work. Another reason for reported high temperatures could be the presence of non-protein components. Purity of the extracted canola protein was 83.88% (Table 2); therefore, samples tested in Figure 9 were not 100% pure protein samples. Li et al. (2012) also reported temperatures higher than those reported by Wu and Muir (2008). However, denaturation temperatures reported in the current study were approximately 30°C higher than those reported by Li et al. (2012).

4.5.2 Effect of combined chemical modification on thermal properties

DSC thermograms of canola protein modified with SDS and $CaCO_3$ and SDS and $ZnSO_4$ are illustrated in Figure 10.

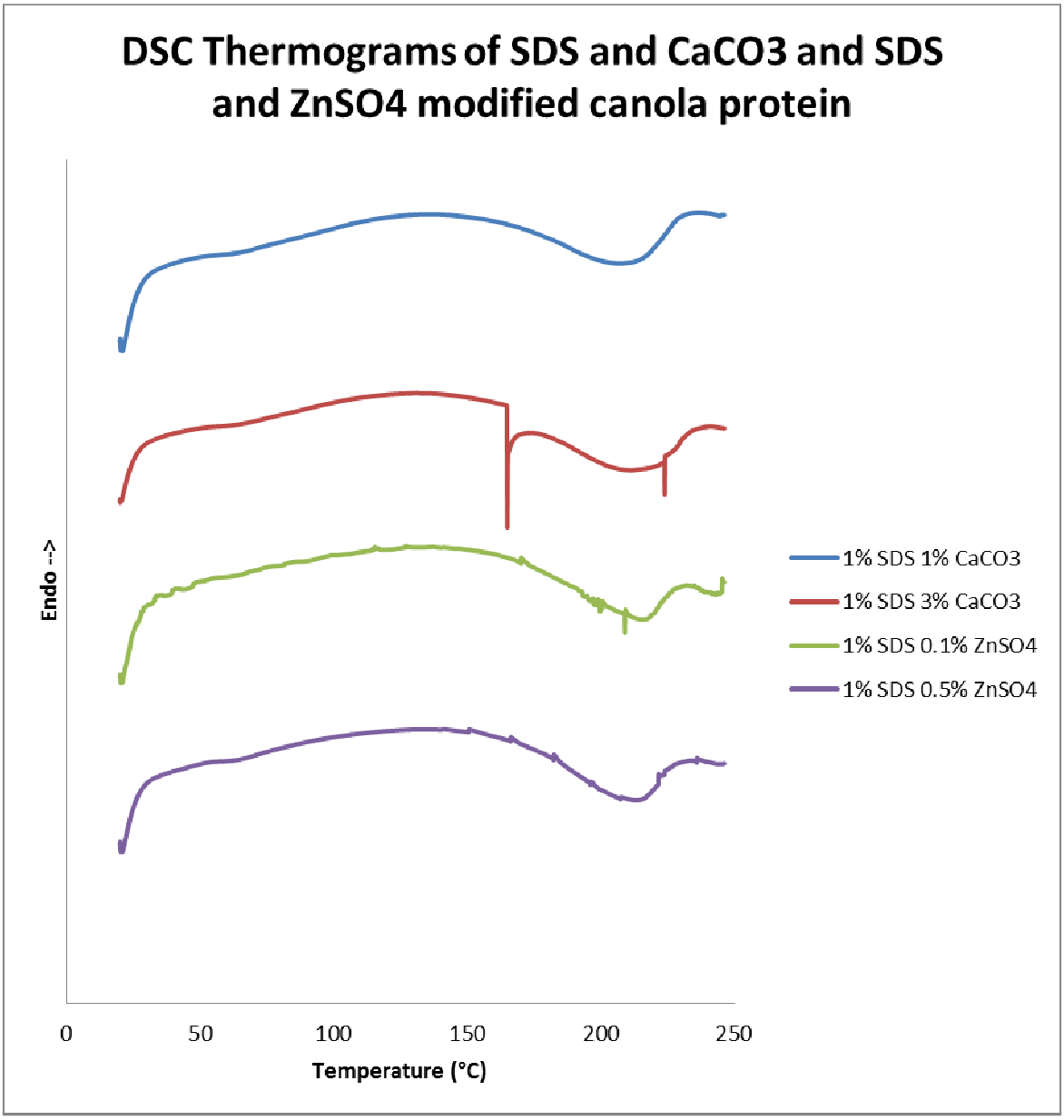

Figure 10. DSC thermograms of SDS and $CaCO_3$ or $ZnSO_4$ modified canola protein

Increasing the concentration of either $CaCO_3$ or $ZnSO_4$ decreased the denaturation temperature of canola protein samples. All of the samples contained broad peaks between 120°C

and 170°C with denaturation temperatures around 130°C. The denaturation temperatures and enthalpies of denaturation are shown in Table 7.

Table 7. Denaturation (T_d) temperatures and enthalpy of denaturation (ΔH) of SDS and $CaCO_3$ or $ZnSO_4$ modified canola protein

Modification	T_d (°C)	ΔH (J/g)
unmodified	148.69	184.5
1% SDS 1% $CaCO_3$	136.97	130.7
1% SDS 3% $CaCO_3$	131.29	119.4
1% SDS 0.1% $ZnSO_4$	137.22	138.0
1% SDS 0.5% $ZnSO_4$	128.71	115.8

As modifier concentration increased, the denaturation temperature decreased along with the enthalpy of denaturation (Table 7). This trend is consistent with results from Huang and Sun (2000a) for modified soy protein. The denaturation temperature and enthalpy decreased due to higher degree of protein unfolding that occurs with increased modifier concentration. The denaturation temperature of the samples is higher than reported averages of approximately 100°C (Wu and Muir 2008), possibly due to the difference in isolation technique as well as the specific variety of canola used.

Overall, the high denaturation temperature of canola protein could be attributed to low shear strength for various samples in the current study. The denaturation temperature was approximately 130-150°C and the samples were pressed at 170°C. However, when SDS samples were pressed at 190°C the strengths increased greatly. Increasing the press temperature improved the shear strength of samples due to greater protein cross-linking (Sun 2011). The denaturation temperature of canola protein is a challenge for commercializing canola protein adhesives.

4.6 Morphological properties of canola protein adhesives

SEM and TEM imaging were used to determine morphological properties of canola protein adhesives. Unmodified protein and SDS modified protein (0.5%, 1%, 3%, and 5%) were examined with SEM and TEM imaging. The results for morphological properties of canola protein adhesives are described in the following sections.

4.6.1 SEM results of chemically-modified canola protein adhesives

Figure 11 shows SEM images of unmodified milled canola protein at magnifications of 3000x and 10741x.

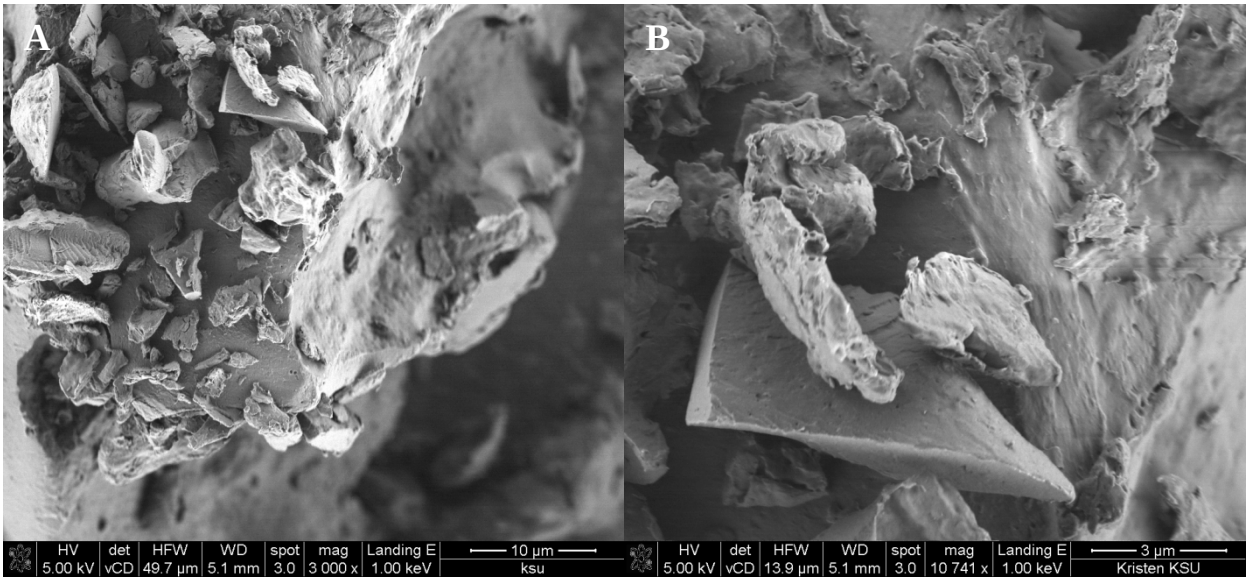

Figure 11. SEM of unmodified canola protein: 3000x (A) and 10741x (B)

The unmodified canola protein had irregular particle sizes with diverse surface structures (Figure 11) and small, rough particles attached to large particles. The irregularity of particle size could be due to the freeze-drying and milling process. A slight contrast in small particles, most likely due to charging from the electron beam of the microscope, is evident in image A and the large particle in the bottom left of image B is much smoother than the smaller particles. The protein is not uniform and has a variety of textures and sizes within the aggregates.

Figure 12 shows canola protein modified with SDS at concentrations of 0.5% and 1% with magnifications of 500x and 2000x. ImageJ was used to improve brightness and contrast.

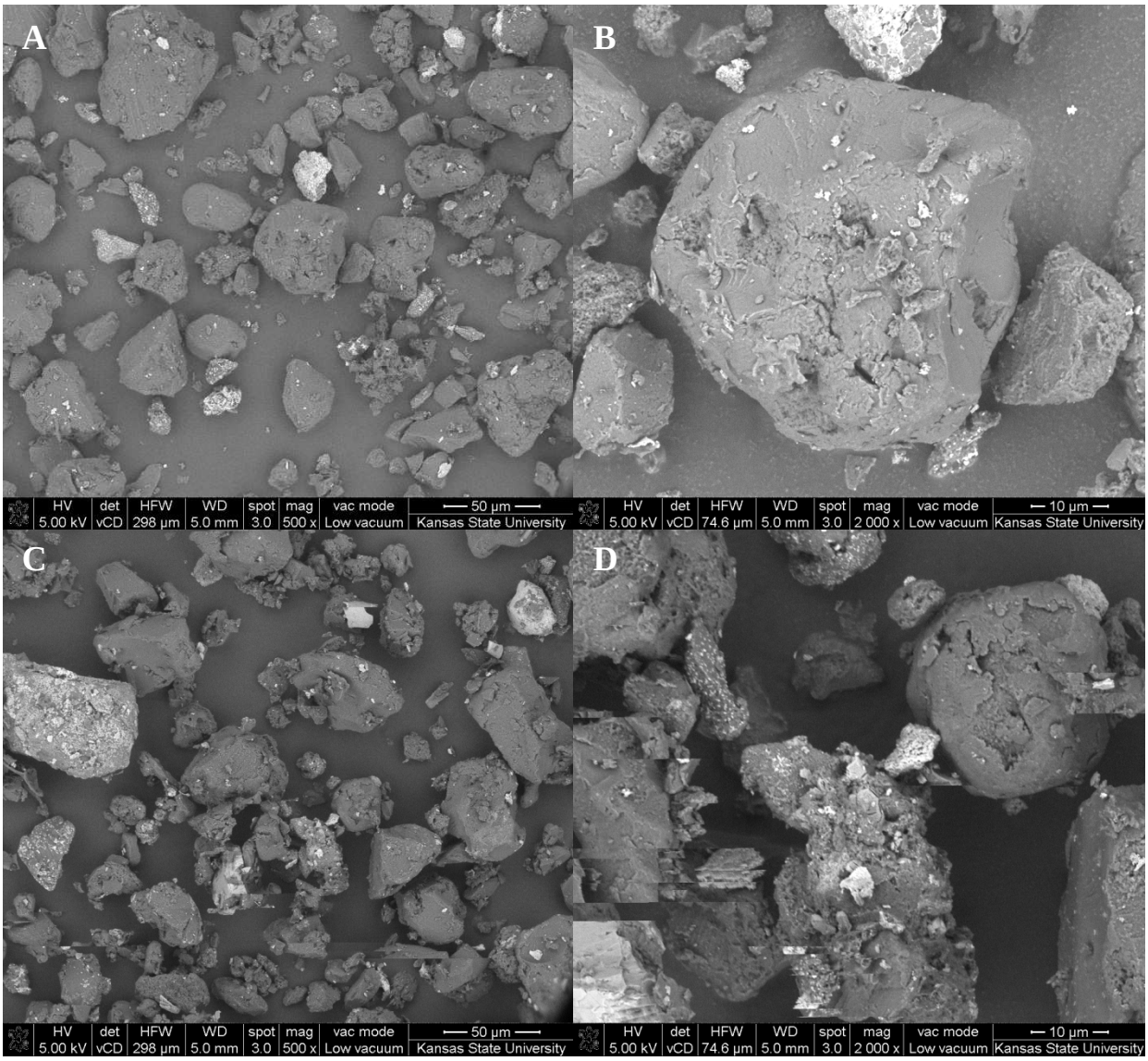

Figure 12. SEM image of modified canola protein: 0.5% SDS 500x (A), 0.5% SDS 2000x (B), 1%SDS 500x (C), and 1% SDS 2000x (D)

The images in Figure 12 show similar irregular particles, in the form of large clumps with smaller attached particles, similar to Figure 11. The large particles are fairly smooth, whereas the small particles are rough and irregular. In all four images, various particles are much lighter in color than the surrounding material due to differences in material density and composition. The light color cannot be attributed to charging because the images were taken in low vacuum mode and the back scattered electrons give elemental analysis. For 0.5% SDS modified protein, images A and B, the large particles have an approximate diameter of 20-50µm (measured with ImageJ).

The 1% SDS modified protein samples, images C and D, also have diameters of approximately 20-50µm. Images C and D have distortion lines due to unknown interference in the building where the images were taken. Images B (0.5% SDS) and D (1% SDS) were similar, indicating that different concentrations of SDS did not significantly affect protein structure in the freeze-dried samples.

To determine if the small white particles in Figure 12 were different from the other darker material, EDS was conducted and a copper grid was used for calibration.

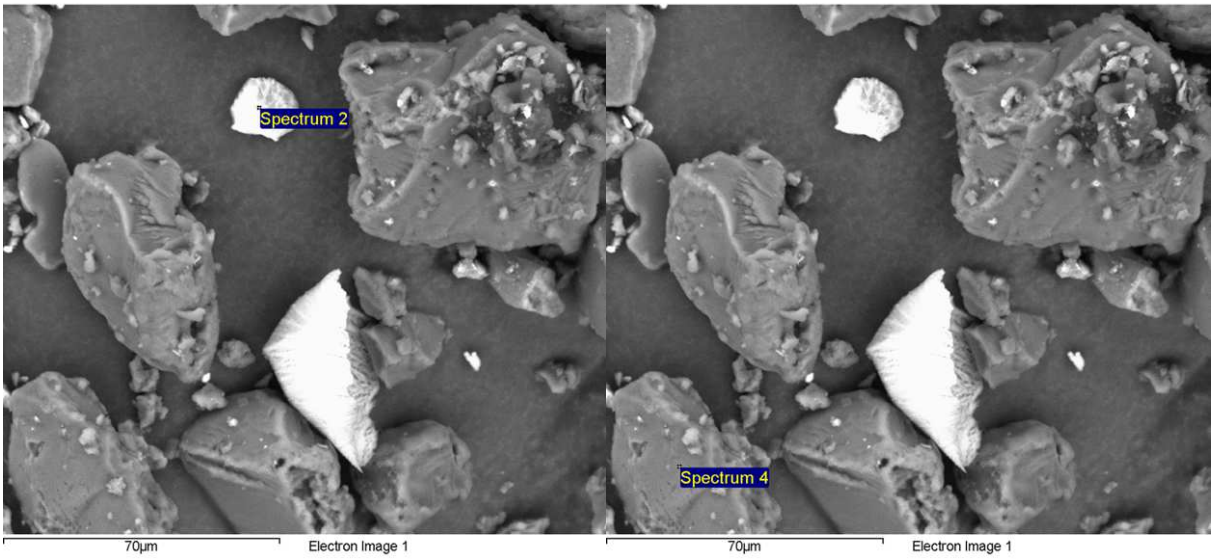

Figure 13. EDS images of 0.5% SDS modified canola protein

The EDS spectrum was analyzed with INCA to determine the elemental composition of various spectrums. From the analysis, Spectrum 2 was found to have 30.94% O, 20.26% Na, 5.85% S, 34.08% Cl, and 2.93% K. Spectrum 2 contains no carbon, indicating that it is not protein but rather a type of salt or other non-protein component. Spectrum 4 was found to have 66.19% C, 26.00% O, 0.80% P, 3.43% S, 2.02% Cl, and 1.57% Zn. Spectrum 4 is thought to be protein and has very different composition percentages from Spectrum 2.

Figure 14 shows canola protein modified with SDS at concentrations of 3% and 5% with magnifications of 500x and 2000x. ImageJ was used to improve brightness and contrast.

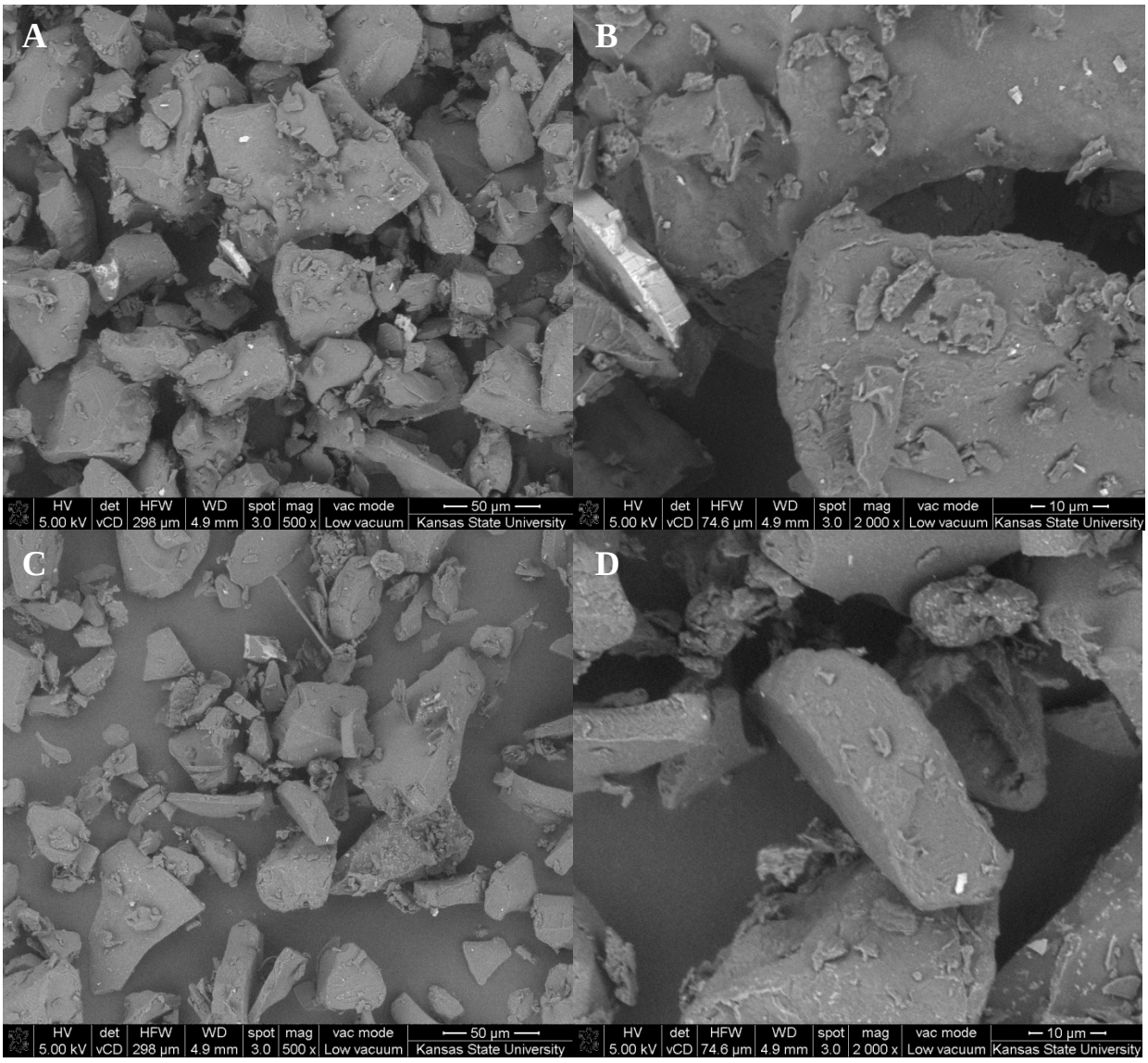

Figure 14. SEM image of modified canola protein: 3% SDS 500x (A), 3% SDS 2000x (B), 5% SDS 500x (C), and 5% SDS 2000x (D)

The images in Figure 14 show similar structure to Figure 11 and 12; however, images in Figure 14 show slightly larger chunks with fewer smaller particles on top. The large particles in images A (3% SDS) and C (5% SDS) are smoother than the images at the same magnification in Figure 12 of the protein modified with 0.5% SDS and 1% SDS. The images in Figure 14 also showed fewer white particles than the images in Figure 12. Images A and B of Figure 14 show the large particles have an approximate diameter of 20-100µm. While smaller diameter particles remain, more large particles are present than in images from Figure 12. In image C, large

particles have a diameter of approximately 20-80µm. Overall, more large particles are present in Figure 14 than in Figure 12, indicating that the increase in SDS concentration could have an effect on particle size.

4.6.1.1 SEM discussion

All of the SEM images show similar morphology: large particles of protein with smaller particles attached. The protein most likely has this shape due to the freeze-drying and milling process. During the freeze-drying process, the protein dries in the same configuration it is in when suspended in water. The milling process changes the particle size of protein particles and is likely the reason for variation in particle size as well as very small particles. A more accurate demonstration of how protein forms in water could be seen if the milling step eliminated. Relation of the SEM images to adhesion strength is difficult because the protein is not suspended in water.

Figure 11 exhibits various particles that are very light in color due to area charging of the microscope. Figures 12, 13, and 14 have light-colored particles as well, but color cannot be attributed to charging because they were taken in low vacuum mode using back-scattered electrons. Back-scattered electrons give elemental information and, therefore, the areas of lighter color were due to differences in material density. When light areas were compared to darker areas using EDS, the light areas did not contain carbon. The lighter areas were likely a type of salt possibly resulting from protein extraction or some type of non-protein component. Figure 12 has more light-colored particles than Figure 14 perhaps because of how SDS affects protein and salts.

Overall, particle sizes in Figure 14 (3% and 5% SDS) are slightly larger than particle sizes in Figure 12 (0.5% and 1% SDS). Figure 14 also demonstrates slightly smoother surfaces than Figure 12, but particle size and structure are difficult to relate to adhesion strength because the adhesive was applied in a wet form, not a dry form, and the protein was milled before imaging. Results affirm that SDS modification does alter protein structure.

Li et al. (2012) found similar SEM images when canola protein was modified with sodium bisulfate. The unmodified protein had a more rigid, rough surface structure, whereas when the modification was increased, the surface structure became smoother. The increase in surface smoothness as the modification increased could be attributed to weaker protein-protein interaction during milling (Li et al. 2012).

4.6.2 TEM results of chemically-modified canola protein adhesives

An FEI CM 100 was used to take all TEM images and was set at an accelerating voltage of either 80kV or 100kV.

Figure 15 shows two images of unmodified canola protein at magnifications of 13500x and 130000x.

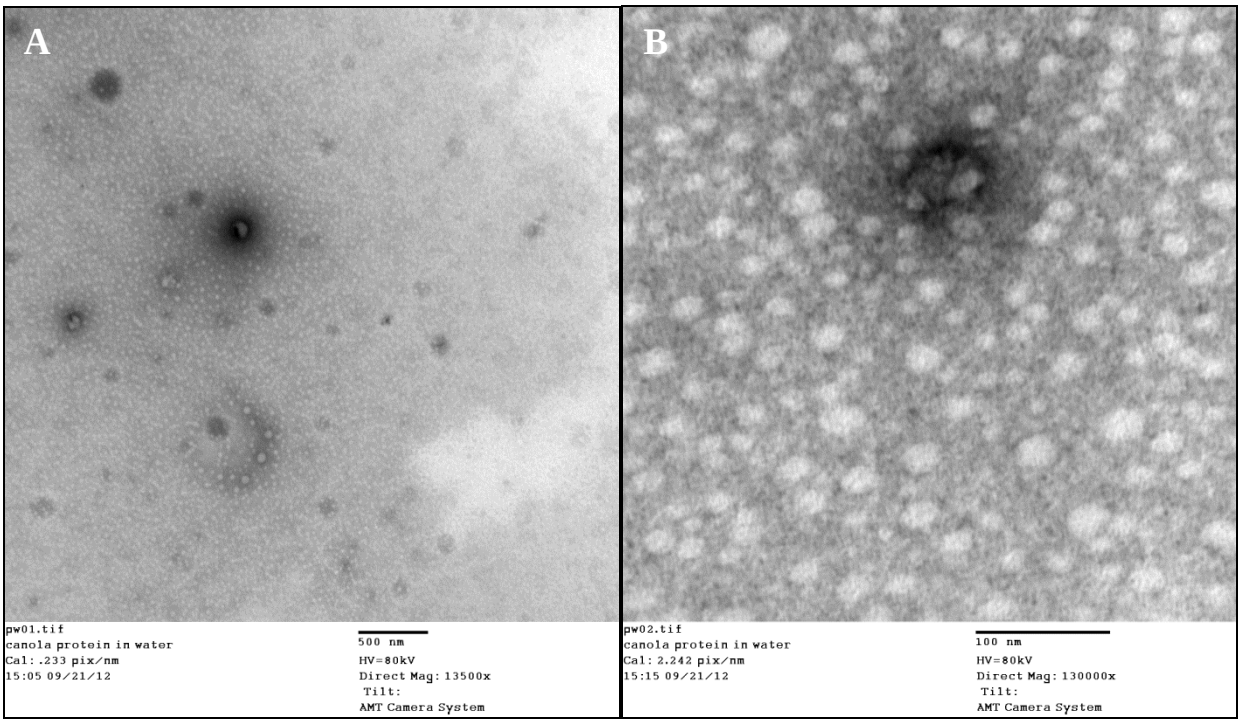

Figure 15. TEM image of unmodified canola protein: 13500x (A) and 130000x (B)

In image A, many small white circular units that have correlating stain are observed. The protein layer is thicker in lighter areas and the stain collected in pools and crevices around the globular protein. The primary structure of the protein included small white subunits, whereas the quaternary structure was shown by the larger globular circles. The white circles of globular protein in image B were approximately 20-30nm in diameter.

Figure 16 shows two images of 3% SDS modified canola protein at magnifications of 46000x and 130000x.

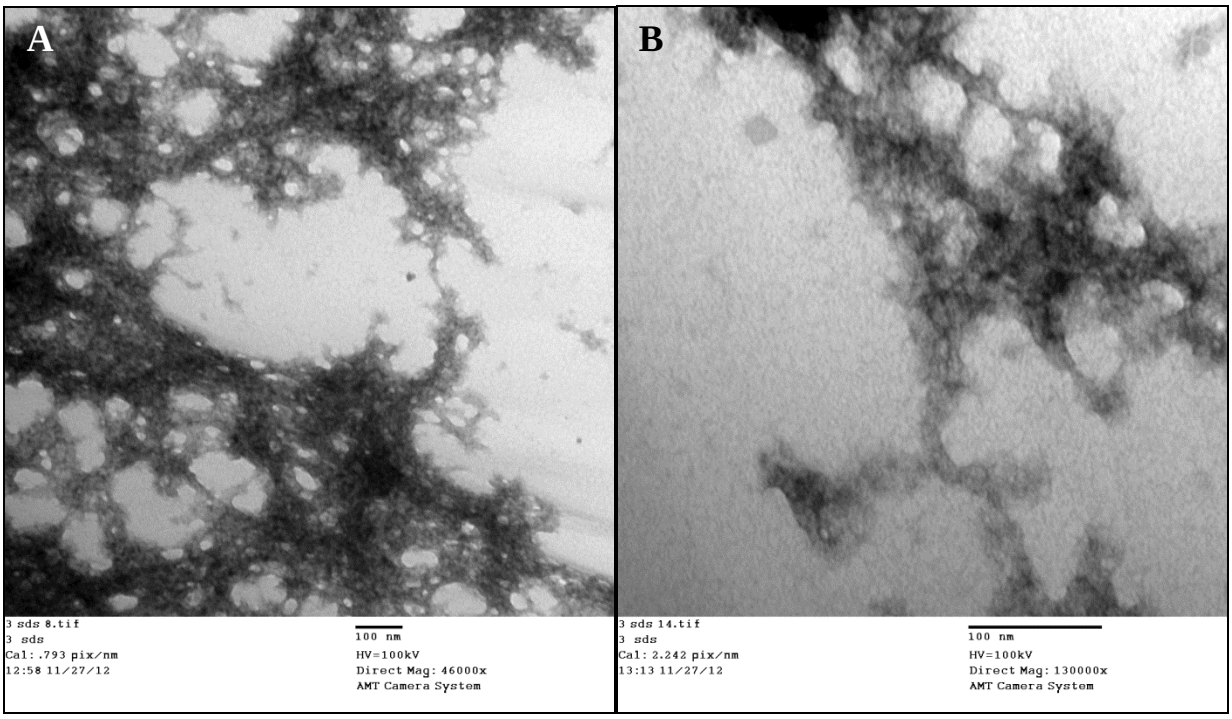

Figure 16. TEM image of 3% SDS modified canola protein: 46000x (A) and 130000x (B)

In image A, the protein forms large areas of aggregation in the light-colored areas while the dark areas demonstrate the presence of stain collected in pools. The images are very dissimilar from the unmodified protein in Figure 15 because the darker areas are congregated together and spread out in web-like figures. As shown in image B, the darker areas still contain smaller circular subunits found in the unmodified protein, which can also be seen at the top of the image, where more protein has collected. The subunits in image B have diameters of 10-15nm instead of 20-30nm like the unmodified protein. The difference is most likely due to SDS impacting the protein structure.

Figure 17 shows images of 5% SDS modified canola protein magnifications of 46000x and 130000x.

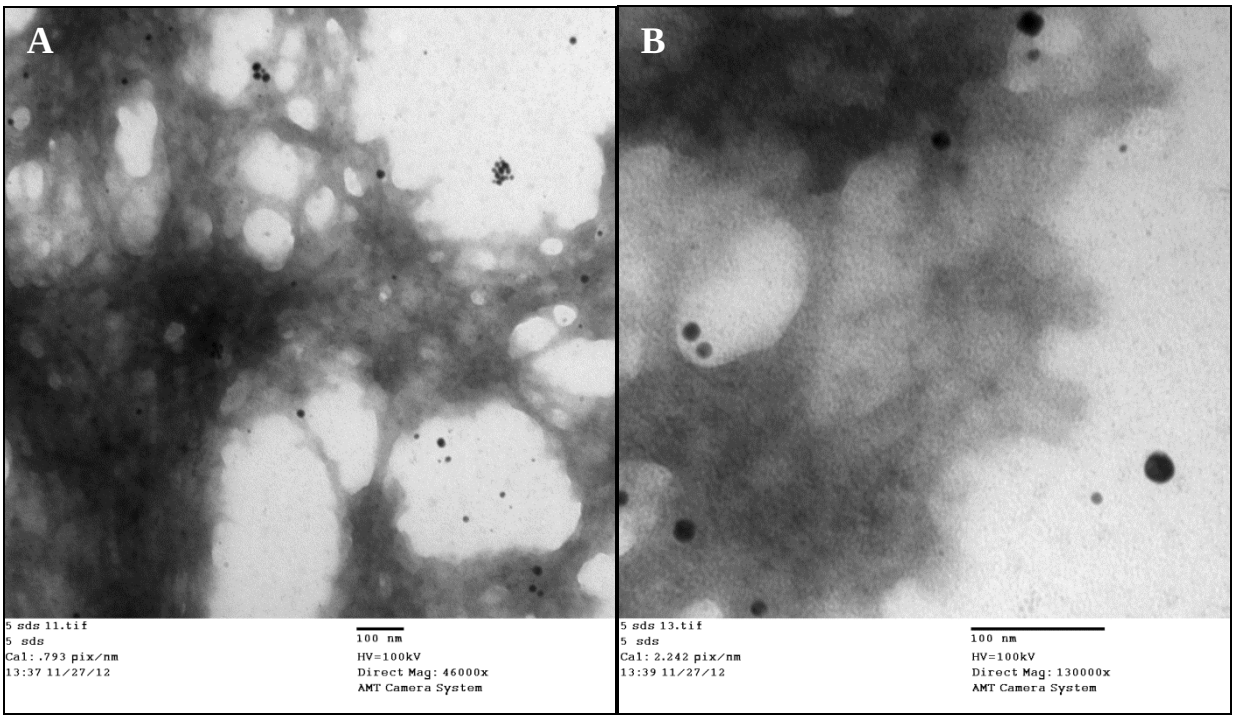

Figure 17. TEM image of 5% SDS modified canola protein: 46000x (A) and 130000x (B)

The web-like structure formed by the dark areas is similar to the structure shown in Figure 16. The darker areas verify where stain has collected and pooled. The top portion of the image reveals denser clumps of protein. The very small dark circles in the two images are contamination, not protein. The form of the small white subunits is shown in image B; however, the subunits were not clear enough in the picture to clearly discerned and measured, possibly due to resolution difficulty. The dark dense circles in the images are not protein but likely a type of contamination.

4.6.2.1 TEM discussion

The TEM images all show similar globular white protein structures which are not exactly spherical but have variation within the same sample as well as in different samples. The variation in size could be due to protein concentration, solubility, hydrophobicity, and denaturation conditions. The small white circles are composed of peptides, but the magnification and resolution were not high enough to clearly identify the individual peptide units.

The material in Figures 16 and 17 appears in a web-like structure. The proteins have different thicknesses, therefore causing stain to collect in darker areas. Figures 16 and 17 are

very different from Figure 15 in overall appearance, likely due to the presence of SDS. The difference in appearance could also be attributed to a difference in protein concentration in the imaging area. The 3% SDS protein (Figure 16) has a tighter structure with more chain-like structures than the 5% SDS protein (Figure 17), possibly due to the greater extent of protein unfolding and denaturation in the 5% SDS modified protein. The web-like structure of protein in Figures 16 and 17could be favorable for dry adhesion strength of the protein, but a negative effect on the wet shear strength of the protein adhesives was observed. The decrease in wet strength is likely due to SDS creating negative surface charges on the surface of the adhesive, making the adhesive hydrophilic, thus causing the adhesive to take up water and disrupt the bond between adhesive and wood surface.

Figure 17 contains artifacts in the form of dark circles that are not protein. The dark circles are fairly uniform with dark, dense spots in the middle. The contamination is probably a virus that contaminated the sample and/or grid before imaging.

Li et al. (2011) found similar TEM imaging results for canola protein modified with sodium bisulfite. The protein also formed clusters with smaller aggregates, and the diameter of globular protein decreased when chemical modification was performed (Li et al. 2011).

5. Conclusions and Recommendations

5.1 Conclusions

Depending on chemical and concentration, modifying canola protein improved the shear strength of canola protein wood adhesives. Chemical modification with SDS (1%, 3%, and 5%), $CaCO_3$ (1%, 3%, and 5%), $ZnSO_4$ (1%), and OSA (2%, 3.5%, and 5%) improved the dry and soak strengths compared to unmodified canola protein. These modifications may have potential for interior applications but not exterior due to the low wet shear strengths. The viscosity of the SDS-modified canola protein adhesives increased as the SDS concentration increased, due to the greater extent of protein unfolding and crosslinking. TEM images show the protein arrangement as well as protein denaturation due to chemical modification. In general, SDS lowered the denaturation temperature of canola protein. The 3.5% OSA chemically-modified canola protein adhesive was the only modification that had improved wet, dry, and soak shear strengths. The increase in wet shear strength is possibly due to the hydrophobic nature of the OSA imparting hydrophobic behavior on the protein, therefore preventing water to disrupt the bond between adhesive and wood surface. The viscosities of the OSA-modified samples were lower than that of unmodified canola protein. The 3.5% OSA modified canola protein adhesive had improved properties compared to the other modifications.

The combined chemical modifications of canola protein using SDS and $CaCO_3$ and SDS and $ZnSO_4$ did not improve the shear strength of the canola protein adhesives. Combined chemical modification decreased the denaturation temperature and shear strength of the modified samples because of too much protein denaturation through SDS and excess undissolved chemicals in the adhesive formulation.

Results showed that press temperature had a significant effect on shear strength of canola protein adhesives. The shear strength increased as press temperature increased likely due to press temperature being increasingly distinct from denaturation temperature, allowing for more chemical interactions at the interface, increasing the adhesion. However, increasing the press temperature is not economically feasible due to high energy inputs required and the darkening effect on wood.

Overall, canola protein was modified with different types of chemicals and combinations of chemicals to produce wood adhesives. Of the chemical modifications used, the 3.5% OSA

modification had wet, dry, and soak shear strengths greater than unmodified canola protein, making it the most successful chemical modification. As the press temperature of adhesives increased, the shear strengths of unmodified canola protein increased as well as SDS-modified canola protein.

5.2 Recommendations

Based on the results of this study, future research on canola protein adhesives should focus on the following:

1. Optimizing the protein extraction process to increase the extraction rate as well as improve protein purity.
2. Separating different protein fractions within canola protein to test adhesion strength of individual types of canola protein.
3. Chemically modifying canola protein simultaneously with extraction instead of after the extraction process.
4. Testing other chemical and enzymatic modifications on canola protein, as well as varying the concentration of protein used.
5. Mixing canola protein with various types of commercial adhesives to increase the amount of bio-based material within the adhesive mixtures.

Overall, canola protein has potential to be utilized as a commercial wood adhesive; however, many obstacles must be overcome and optimizations must be performed before commercialization. Process optimization is a critical first step to increase the extraction rate of canola protein and to increase the purity of the extracted protein. After the extraction process is optimized, other modification techniques should be researched to further improve adhesion strength and adhesive uniformity. With more research, canola protein adhesive has the potential to be an environmentally-friendly alternative or additive to formaldehyde-based adhesive.

References

ASTM Standards. 2002. D1151-00: Effect of moisture and temperature on adhesive bonds. West Conshohocken, Pennsylvania: ASTM International.

ASTM Standards. 2002. D1183-03: Resistance of adhesive to cyclic laboratory aging conditions. West Conshohocken, Pennsylvania: ASTM International.

ASTM Standards. 2002. D2339-98: Strength properties of adhesives in two-ply wood construction in shear by tension loading. West Conshohocken, Pennsylvania: ASTM International.

Canola Council of Canada. 2011. Overview of Canada's Canola Industry. Canola Council of Canada. Available at: www.canolacouncil.org. Accessed 21 May 2012.

Dictionary.com. Adhesion. Dictionary.com Unabridged. Random House, Inc. Available at: http://dictionary.reference.com. Accessed 19 March 2013.

Frihart, C. R. 2005. Chapter 9: Wood adhesion and adhesives. In *Handbook of Wood Chemistry and Wood Composites*, 215-278. Boca Raton, Florida: CRC Press.

Frihart, C. R. 2006. Chapter 18: Wood structure and adhesive bond strength. In *Characterization of the Cellulosic Cell Wall*, 241-253. Ames, Iowa: Blackwell Publishing.

Gardner, D. J. 2005. Chapter 19: Adhesion mechanisms of durable wood adhesive bonds. In *Characterization of the Cellulosic Cell Wall*, 254-265. Ames, Iowa: Blackwell Publishing.

Hettiarachchy, N. S., U. Kalapathy, and D. J. Myers. 1995. Alkali-modified soy protein with improved adhesive and hydrophobic properties. *Journal of the American Oil Chemists' Society* 72(12): 1461-1464.

Huang, W. and X. Sun. 2000a. Adhesive properties of soy proteins modified by sodium dodecyl sulfate and sodium dodecyl benzene sulfonate. *Journal of the American Oil Chemists' Society* 77(7): 705-708.

Huang, W. and X. Sun. 2000b. Adhesive properties of soy proteins modified by urea and guanidine hydrochloride. *Journal of the American Oil Chemists' Society* 77(1): 101-104.

Kalapathy, U., N. S. Hettiarachchy, D. Myers, and M. A. Hanna. 1995. Modification of soy proteins and their adhesive properties on woods. *Journal of the American Oil Chemists' Society* 72(5): 507-510.

Keimel, F. A. 1994. Chapter 1: Historical development of adhesives and adhesive bonding. In *Handbook of Adhesive Technology*, 3-15. A. Pizzi and K. L. Mittal, Eds. New York, New York: Marcel Dekker, Inc.

Kinloch, A. J. 1987. *Adhesion and Adhesives: Science and Technology*. New York, New York: Chapman and Hall.

Klockeman, D. M., R. Toledo, and K. A. Sims. 1997. *Journal of Agricultural and Food Chemistry* 45: 3867-3870.

Kumar, R., V. Choudhary, S. Misha, I. K. Varma, and B. Mattiason. 2002. Adhesives and plastics based on soy protein products. *Industrial Crops and Products* 16: 155-172.

Lambuth, 1989. Adhesives from renewable 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, D.C.: American Chemical Society.

Li, N., G. Qi, X. S. Sun, M. J. Stamm, and D. Wang. 2011. Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite. *Journal of the American Oil Chemists' Society*.

Li, N., G. Qi, X. S. Sun, and D. Wang. 2012. Effects of sodium bisulfite on the physiochemical and adhesion properties of canola protein fractions. *Journal of Polymers and the Environment*.

Liu, D., H. Chen, P. R. Chang, Q. Wu, K. Li, and L. Guan. 2010. Biomimetic soy protein nanocomposites with calcium carbonate crystalline arrays for use as wood adhesive. *Bioresource Technology* 101: 6235-6241.

Manamperi, W. A. R., D. P. Wiesenborn, S. K. C. Chang, and S. W. Pryor. 2011. Effects of protein separation conditions on the functional and thermal properties of canola protein isolates. *Journal of Food Science* 76(3): 266-273.

Manamperi, W. A. R., S. W. Pryor, and S. K. C. Chang. 2007. Separation and evaluation of canola meal and protein for industrial bioproducts. ASABE Paper No. RRV07116. Fargo, North Dakota: ASAE.

Mo, X., X. Sun, and D. Wang. 2004. Thermal properties and adhesion strength of modified soybean storage proteins. *Journal of the American Oil Chemists' Society* 81(4): 395-400.

Qi, G. and X. S. Sun. 2011. Soy protein adhesive blends with synthetic latex on wood veneer. *Journal of the American Oil Chemists' Society* 88: 271-281.

Qi, G., N. Li, D. Wang, and S. Sun. 2013. Physicochemical properties of soy protein adhesives modified by 2-octen-1-ylsuccinic anhydride. *Industrial Crops and Products* 46: 165-172.

Rödin, J. 1990. Studies on the Structure and Expression of Cruciferin, the 12S Storage Globulin from Brassica Napus (Oilseed Rape). PhD diss. Uppsala, Sweden: Swedish University of Agricultural Sciences, Department of Cell Research.

Schultz, J. and M. Nardin. 1994. Theories and mechanisms of adhesion. In *Handbook of Adhesive Technology*, 19-33. A. Pizzi and K.L. Mittal, Eds. New York, New York: Marcel Dekker, Inc.

Schwenke, K. D. 1994. Rapeseed Protein. In *New and Developing Sources of Food Proteins*, 281-306. B.J.F. Hudson, ed. London, England: Chapman and Hall.

Sun, X. S. 2011. Soy protein polymers and adhesion properties. *Journal of Biobased Materials and Bioenergy* 5:409-423.

Tan, S. H., R. J. Mailer, C. L. Blanchard, and S. O. Agboola. 2011. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of Food Science* 76(1): 16-28.

Tzeng, Y. M., L. L. Diosady, and L. J. Rubin. 1990. Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. *Journal of Food Science* 55(4): 1147-1151.

Uppström, B. 1995. Seed Chemistry. In *Brassica Oilseeds: Production and Utilization*, 217-242. D. S. Kimber and D. I. McGregor, eds. Wallingford, England: CAB International.

USDA. 2010. Soybeans and Oil Crops: Canola. Economic Research Service: United States Department of Agriculture. Available at: www.ers.usda.gov. Accessed 24 May 2012.

Wu, J. and A. D. Muir. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of Food Science* 73(3): 210-216.

Zhong, Z., and X. S. Sun. 2007. Plywood adhesives by blending soy protein polymer with phenol-formaldehyde resin. *Journal of Biobased Materials and Bioenergy* 1(3): 380-387.

Zhong, Z., X. S. Sun, X. Fang, and J. A. Ratto. 2001. Adhesion strength of sodium dodecyl sulfate-modified soy protein to fiberboard. *Journal of Adhesion Science and Technology* 15(12): 1417-1427.