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# Comparison of soy-flour hydrolysates for wood adhesive systems

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# Comparison of soy-flour hydrolysates for wood adhesive systems

by

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## LIST OF ABBREVIATIONS

Alkaline - soy flour hydrolysate produced in 8% sodium hydroxide solution

ASTM - American Society for Testing and Methods

Carbohydrase - soy flour hydrolysate produced using a carbohydrase enzymes

CarbPro - soy flour hydrolysate produced using carbohydrase enzymes followed by a protease treatment

cP - centipoise

DOPA - 3,4-dihydroxyphenyl-alanine

IB - internal bond strength

MDI - methylene diphenyl diisocyanate

MOE - modulus of elasticity

MOR - modulus of rupture

Protease - soy flour hydrolysate produced using a single protease treatment

ProCarb - soy flour hydrolysate produced using a protease enzyme followed by a treatment with carbohydrase enzymes

PF - phenol-formaldehyde (petroleum-based synthetic resin)

PFC - phenol-formaldehyde resin made and used commercially

PFL - lab synthesized phenol-formaldehyde resin

SPI - soy protein isolate

TS-2B - thickness swell after 2-h boiling test

TS-24S - thickness swell after 24-h soaking test

UF - urea formaldehyde

UTS – ultimate testing system

# ABSTRACT

Soy-protein-based adhesives have not been used extensively in wood products since the 1960's because of poor performance, stability, and water-resistance issues compared to petroleum-based adhesives. The early soy-protein adhesives were made from defatted flours and were dispersed in alkaline solutions to denature proteins and make more polar amino acid groups available for adhesion bonding. Recent research has focused on soy flour adhesives due to increased phenol prices and environmental concerns related to the use and exposure to formaldehyde. Soy protein isolates can be modified in highly caustic alkaline solutions to reduce viscosity and improve adhesive properties, but the price of isolates is cost prohibitive for most industry applications. Soy flour hydrolysates produced with enzymes were evaluated in adhesive formulations. Soy hydrolysate and phenol-formaldehyde (PF) resin blends made from four types of enzymatic hydolysates were tested for the time required for polymerization, water-resistance, and their performance in medium-density fiberboard applications. Results show that for all of the aforementioned analyses that as the level of soy hydrolysate increased, properties of hydrolysate-PF blends decreased. Testing also showed distinguishable differences between alkaline and enzymatic hydrolysates, as well as differences among enzymatic treatments when used in resin blends.

# CHAPTER 1

## General Introduction

### Thesis Organization

This thesis includes 2 primary chapters. Chapters 2 and 3 are manuscripts, which will be submitted for publication in the Journal of the American Oil Chemists Society. Chapter 2 is a study comparing alkaline hydrolyzed soy flour to three enzymatic soy hydrolysates, blended with two types of phenol-formaldehyde resin, for the properties of polymerization time and water susceptibility. Chapter 3 is a related study in which soy flour hydrolysates were blended with a lab synthesized phenol-formaldehyde resin and used to produce medium-density fiberboard. ASTM tests were used to characterize the mechanical and water-resistance properties of medium-density fiberboards bonded with the resin blends. Each of these studies provides insight into the use of soy flour hydrolysates as an ingredient in wood adhesive formulations. Summary conclusions are discussed in Chapter 4 along with ideas for future work that could be done based on the results of these two studies.

# Literature Review

## Soy Protein

### *Soy Protein Products and Characterization*

Soybean plants are legumes that originated in eastern Asia (1). Soybeans are typically processed by cracking the seed, dehulling, drying, flaking, extracting oil, redrying, and milling the co-products into useful fractions (2). Co-products of the milling process include soy flours, from which soy concentrates and soy protein isolates can by produced – all of which vary in protein content (3). Soy flours are the least refined co-product and are produced by simply grinding defatted soybeans. They are comprised of 45-50% protein, 30% carbohydrates, and 20-25% fiber, ash, and water (4). On a dry basis, soy protein concentrates contain over 65% protein. Protein concentrates are made by leaching the water or alcohol soluble components. Compared to soy flour, concentrates have improved flavor characteristics and possess greater water- and fat-binding properties (3). Soy protein isolates are the most refined coproduct and represent the highest overall protein concentration. Soy protein isolates contain at least 90% protein on a dry basis (5). Soy protein products vary not only in protein concentration, but depending on the processing conditions, can vary based on particle size, solubility, water absorption, color, nutritional quality, viscosity, and adhesive quality (3).

Although carbohydrates play roles in water-binding and viscosity, proteins are the primary functional component in soy co-products (6). In general terms, protein is a polymer of $\alpha$-amino carboxylic acids linked by peptide bonds. All proteins contain a peptide backbone, but the distinguishing characteristics of proteins are the sequence of side groups disseminating from the peptide backbone. Soybeans commonly have eighteen different amino acids with different functional side groups. These side groups include hydrocarbons, amines, carboxylic acid, hydroxyl, sulfurs, and phenolic groups (7). Side groups on the peptide chain represent the reactive sites of proteins, and each side group has various reactions that it will undergo, reacting with other amino acids or other reactants.

Proteins have primary, secondary, tertiary, and quaternary structures. Primary structure is the amino acid sequence linked by peptide bonds (5). Secondary structure is correlated to the position of amino acids within a polypeptide chain, which form helices and sheets. Tertiary protein structure is related to the position of a portion of the peptide chain with respect to other parts of the same peptide chain, and quaternary structure is the arrangement in space of one peptide chain with other peptide chain(s) (8). Primary and secondary structures are the relation of amino acids groups, whereas tertiary and quaternary structure refer to peptide folding, bonding, and interactions with itself and other protein subunits.

Proteins fit into two broad macromolecular classes. The first is fibrous proteins which are insoluble in water. The second are globular proteins. Globular proteins are soluble in aqueous solutions of acids or bases (7). Globular protein structure is stabilized by several molecular forces including van der Waals' forces, hydrophobic interactions, ionic bonds, hydrogen bonds, and disulfide bonds. Soy proteins are globular proteins with numerous polypeptide chains folded into compact, interconnected units. Protein structure not only determines solubility, but it also affects the functional properties (6).

As mentioned previously, the carbohydrate fraction in soy flours is approximately 30%. Soy concentrates contain about 20% carbohydrate, whereas isolates have only 1% carbohydrate (9). Carbohydrate fractions in soy co-products are held in place either by covalent bonds to the polypeptide chain or van der Waals' forces (5). Carbohydrate fractions have various levels of impact in soy co-products related to the amount of carbohydrate present and the specified use of co-products. Residual lipids, fiber, and ash also exist in varying amounts, however, they have limited effect on protein physical and functional properties (6).

### *Soy Protein Properties*

Water solubility is considered one of the primary physical characteristics of soy proteins (10). The major soy proteins are globulins, which are insoluble at their isoelectric point (pI). They are, however, soluble in water or salt solutions above or below their pI (11). Soy proteins have limited solubility between pH 3.75 and 5.25. The maximum solubility was observed at pH 1.5-2.5 and pH > 6.3 (1). Insolubility of protein between pH 3.75 and 5.25 is a result of soy proteins pI of 4.5. A range of solubilities can be obtained for soybean proteins by using different heat or chemical treatments. Likewise, soy proteins can be made soluble near the pI by hydrolysis of the native protein state into smaller peptide chains (6). Hydrolysis of the native state alters the charge distribution of the protein creating a product which would be insoluble at a different pH (7).

Protein interactions with water are important to viscosity and general dispersibility. The viscosity of protein dispersions is influenced primarily by intrinsic factors. Likewise, intrinsic factors can be altered by environmental factors such as pH and exposure to heat, which in turn alter the intrinsic structure and interactions in proteins (6). Along with other factors, these two properties directly influence the utilization of soy proteins in industrial applications.

## Soy Protein Modifications

Soy proteins are used in a variety of food and non-food applications. A variety of research efforts continue to look at novel industrial application of soybean proteins. Soybean proteins have many functional properties that have been demonstrated in a variety of food applications (5, 6). Functional properties are any physico-chemical properties that change the processing or behavior, and quality of protein in food or non-food applications (12). The required functional properties for soy proteins change with the type of application, but the large variety of side groups present in soybeans leads to many diverse functional capabilities.

In wood adhesive applications, the desired functional properties include adhesion, water solubility, water-resistance, and viscosity. Unmodified soy proteins cannot meet all functional properties needed for adhesive applications (13). Functional properties can be improved by modification of protein structure or properties such as disulfide bonds, molecular size, and net charge (6). Soy proteins can be made to have varied functional properties through physical, chemical, or enzymatic modifications (14).

### *Denaturation*

Soy proteins are compact molecules, folded in on themselves in numerous locations (6). Denaturation of proteins refers to any modifications which change the quaternary, tertiary, or secondary structure, but do not alter the primary amino acid sequence. Denaturation is also commonly known as protein unfolding and occurs by breaking the hydrogen and disulfide bonds within higher orders of protein structure (15). Denaturation therefore leads to increased availability of amino acid side groups that were previously hidden within the internal structure of proteins (16).

Alkaline solutions to denature soy proteins have been used extensively. The early soy adhesives were alkaline dispersions and later work has shown such treatments improve solubility, adhesive properties, and decrease viscosity (2, 13, 17, 18). Sun, *et al* (19) showed alkaline dispersions of soy protein increase protein unfolding, resulting in increased exposure of internal hydrophobic protein groups. Strong alkaline dispersions are necessary to increase the solubility and adhesive properties, but high concentrations of alkali also cause staining on wood surfaces that is unacceptable for products with visible surfaces (13, 20).

Urea can also be used to denature protein. Urea interacts with hydroxyl groups and decreases the hydrogen bonding within the protein structure (21). Wolf (5) reported the dissociation and unfolding of soybean proteins in the presence of urea, and Nir, *et al* (22) showed the viscosity of soy protein dispersions decreased with increasing urea concentrations. In addition to lower viscosity, urea-modified soy proteins were shown to have higher shear strength and water-resistance than unmodified soy proteins in wood adhesive applications (19).

Heating soy protein dispersions above 60°C leads to dissociation of subunits and unfolding of protein structure (2). When proteins are heated hydrophobic interactions breakdown and subunits dissociate. The increase in availability of hydrophobic groups increases surface reactivity and improves the adhesive strength of protein materials (22). Heat denaturation, however, can lead to decreased solubility as well. Excessive denaturation due to heat will expose too many hydrophobic groups and decrease solubility due to the aggregation of hydrophobic groups (23).

Sulfites and thiols have also been used extensively to cleave disulfide bonds in proteins (6, 24). Unmodified soy proteins and those modified with sodium sulfite were compared by Kalapathy, *et al*. (25). Sulfite modified proteins had decreased viscosity and increased adhesive strength due to less protein molecular interaction. The use of sodium hydroxide and sodium sulfite were therefore used to control viscosity and adhesive strength of modified proteins for adhesive utilization.

### *Hydrolysis*

Hydrolysis breaks the peptide bonds of the protein backbone; causing cleavage of both primary and secondary structure. Hydrolysis of proteins leads to increased amine and carboxylic functional groups (16). Protein hydrolysis decreases peptide chain length and reduces both viscosity and molecular size (25). Short peptide chains have more functional groups exposed, which is believed to enhance the reaction of soy protein in adhesive formulations. If hydrolysis products are too small however, all functional properties can be lost (26).

Acid hydrolysis is used for quantitatively breaking protein into constituent amino acids. The specificity of acid hydrolysis and extent of hydrolysis are functions of the acid applied, temperature, pressure, acid concentration, and presence of non-protein materials. Hydrochloric, sulfuric, and nitric acids have all been used as acid hydrolysis agents that result in very small peptide chains (27). Acid hydrolysis is not random, but instead has varying specificity that can be used to form peptides with specific end groups. Hydrolysis with some acids does decompose the amino acid tryptophan completely and converts glutamine and asparagine into glutamic acid and aspartic acid, respectively (28). Complete degradation of some amino acids, reversion of others, and an excessive level of hydrolysis in some cases makes acid hydrolysis an unsuitable preparation technique for soy protein to be used in adhesive formulations (26).

Alkaline solutions can be used to both denature and hydrolyze soy protein. Hydrolysis under alkaline conditions requires >5% alkaline conditions and elevated pressures to reach desired temperatures. Alkaline hydrolysis results in denaturation, hydrolysis of peptide bonds, and destruction of both amino acids and carbohydrates (29). Alkaline hydrolysis is a completely random process, with no control over variation or quality from batch to batch. In addition, the strong alkaline condition causes the breakdown of amino acids and creation of others. As one example, serine can be decomposed under strong alkaline conditions to form glycine or alanine (28).

A third method of protein hydrolysis is through the use of enzymes to improve functional properties (12,30,31). Two types of hydrolysis have been tested: limited proteolysis and complete enzymatic hydrolysis. Results from all tests indicated enzymatic soy hydrolysates had improved solubilities and emulsification properties as well as decreased viscosities. The primary advantage of enzymatic hydrolysis is its high specificity and yield of protein fractions. Small amounts of enzymes are required and processing conditions are safer than those for either acid or alkaline hydrolysis. Kim, *et al* (31) hydrolyzed soy protein isolates with trypsin, rennet, chymotrypsin, and alcalase to obtain information on the effects of enzyme proteolysis on molecular and functional properties. Trypsin most effectively decreased the molecular size while retaining functional properties. Studies using enzymatic hydrolysis of soy protein for wood adhesive applications are limited (32, 33). Soy protein isolates were hydrolyzed with trypsin before being used in wood systems to test adhesive strength and water-resistance. Enzymatic hydrolysates of soy protein isolate had increased water-resistance and adhesive strength compared to alkaline hydrolysate.

## Soy Protein as a Wood Adhesive

### *Alkaline Dispersions*

Wood is a polar material, and to be a good adhesive to polar materials, an adhesive must contain a large proportion of polar groups. Soy protein contains many polar groups, but they are unavailable due to the heavily coiled structure of soy protein (6). Structural changes are necessary to uncoil protein molecules and break molecular bonds (13). Any alkaline solution can disperse soy protein at least to some extent. To gain enough bonding strength for wood applications, soy proteins must be dispersed in a strong alkali solution of at least a few percent concentration (20). The strong alkaline conditions can unfold the coiled protein and break internal bonds. As a consequence of the molecular disruption, many soy protein polar groups are exposed and available to interact with wood molecules.

Hettiarachchy, *et al* (32) observed that modifying soy protein isolates at pH 10 and 50°C enhanced adhesive strength and water-resistance relative to unmodified soy protein isolates. Kalapathy (33) modified soy protein isolates with NaCl, $Na_2SO_4$, and $Na_2SO_3$. All three salts reduced the viscosity of wood adhesives with no deleterious effect to adhesive strength or water-resistance. Sun and Bian (19) reported similar findings comparing alkali- and urea-modified soy proteins to unmodified protein. In all cases, the availability of more polar and hydrophobic groups due to protein unfolding and hydrolysis increased the adhesive strength of soy proteins. Additionally, Hettiarachchy (32) and Kalapathy (33) both showed enzymatic modification of soy protein enhanced the adhesive strength compared to unmodified controls. In cold pressed wood systems, soy protein modified with trypsin was superior to unmodified soy protein, but hot pressed wood applications with trypsin modified soy protein were inferior to unmodified control samples (33). These results indicate depending on the modification technique and type of utilization, soy protein adhesives may be susceptible to continued hydrolysis or degradation during use.

### *Crosslinking Soy Protein with Phenolic Resin*

Water-resistance is among the most important properties affecting the durability of wood adhesives. In alkaline dispersions of soy flour, carbohydrate fractions contribute to dry strength, but retain water sensitivity (34). Soy protein adhesives were prominently used in the plywood industry until the 1960's for interior grade plywood (13). Around this time, the plywood industry began making indoor-outdoor plywood, which due to its dual-purpose, required greater water-resistance than soy adhesives could provide (34). Synthetic, petroleum-based resins, such as phenol-formaldehyde (PF), urea-formaldehyde (UF), and methylene diphenyl diisocyanate (MDI), can be used as cross-linking agents, or copolymers, to react with soy protein in adhesive systems and thereby improve adhesive properties compared to soy protein alone (35, 36).

Extensive studies on plywood adhesives formulated with soy protein and phenol-formaldehyde were performed by Lambuth (13). Soy proteins could be combined with phenolic resins at various levels, but compared to other protein sources such as blood, soy protein performed comparably in water-resistance only at low levels. At high levels, the soy protein formulations were also less reactive. Carbohydrate fractions do not readily interact with high pH PF resins (37). Based on the results of Conner, *et al* (37), formulations containing 70% alkaline hydrolyzed soy protein and 30% PF resin approached the quality of plywood bonded with commercial PF resin (38). Following that work, Kuo and Stokke (39) developed another resin utilizing soy flour hydrolysate instead of dispersed soy flour, and Lo (36) formulated a plywood adhesive with dispersed soy flour and MDI resin.

Soy/MDI adhesive resin was not suitable for plywood applications however, because of the low viscosity, unreacted MDI penetrated plywood sheets too quickly.

### *Alternative Resin Systems*

In most cases, viscosity is the primary physical characteristic measured for soy protein used as adhesives. The viscosity of soy protein dispersions does not increase linearly with increasing soy protein, but rather increases drastically when soy protein concentration gets above 30 wt%. (5). For some wood adhesives, however, foaming properties are more critical. Foam extrusion of adhesives is a method used in some plywood applications (40). Foaming is another prominent functional characteristic for soy protein, and therefore may be a viable option for extruded plywood adhesives (41,42). Oil is a defoaming agent, so residual oil in soy protein products is a hindrance in foam extrusion adhesives (7). Foam volume and stability are both essential functional characteristics for foam adhesives. Hojilla-Evangelista (43) demonstrated soy flours with the least amount of protein denaturation performed best in functional tests for characterizing foam adhesives. Surface hydrophobicity increases as soy proteins are denatured, and has been shown to increase foam volume (44,45). Surface hydrophobicity does not correlate to foam stability however, and therefore does not have an appreciable meaning for the use of soy protein in foam adhesives (43,46).

Traditional soy-protein based resins, were prepared via a low-temperature process at 70°C and 8-12% sodium hydroxide. The downfall of such alkaline dispersions and more recent crosslinking technologies with PF resin include biological instability, low solids, short pot life, limited to poor water-resistance, and longer press times compared to resole resins (47). No literature states a specific level, but high levels of denaturation are also seen as a limiting factor in soy protein adhesives (23). Crosslinking with phenol can be used to overcome many of these problems depending on the intended application of the soy-phenol copolymer. Compared to a control PF, this type of resin was shown to have a similar pot life (4 weeks), comparable press times and curing kinetics, and comparable board properties according to ASTM D1037 (48,47).

Liu and Li (49) see the key to commercial development of soy protein adhesives is regaining at least in part the strength properties that are forfeited compared to PF or UF. Instead of denaturing or breaking down soy protein, Liu and Li took the approach of addition reactions to soy protein. Mussels support themselves in water by excreting proteins, and the active ingredients in mussel feet has been identified as 3,4-dihydroxyphenyl-alanine (DOPA) (50, 51,52). Soy protein and DOPA have been shown to be complementary in adhesive properties. DOPA was grafted to soy protein isolates (SPI) via amide linkages, which imparted phenolic-group functional properties to SPI. Adhesive strength and water-resistance increased with increasing DOPA synthesized with SPI (49).

## Summary

Soy protein is an abundant, renewable polymer with a history of use in the wood adhesives industry. The functional properties of soy protein allow for a variety of potential uses and modifications to fit into a variety of adhesive systems. For use in adhesive systems, soy flour almost certainly requires modification to increase functional properties such as adhesive strength and water-resistance properties. Protein hydrolysis and cross linking soy with other adhesives are the most common techniques for current soy protein adhesive research. A limited amount of research has been carried out on enzymatic modifications for soy flour used in adhesives, and the array of enzymes possible for use in preparing adhesive systems has great potential for making a functional soy flour for use in wood adhesives.

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# CHAPTER 2

## Water Extraction and Polymerization Time Characterization of Soy-flour Hydrolysate and Phenol-formaldehyde Resin Blends

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### Abstract

The time required for gelling (polymerization) of soy hydrolysates blended with phenol formaldehyde (PF) wood adhesive resins and the percentage of cured resin blends extracted after boiling was evaluated. Soy hydrolysate-PF resin blends were formulated at 5, 10, 20, 40, and 60% PF solids replacement for two different PF resins. Soy flour hydrolyzed using an alkaline chemical process was compared against three enzymatic hydrolysates prepared using three different enzyme combinations to investigate the effects of different enzyme treatments on hydrolysate properties. The time required for the polymerization of the hydrolysate-resin blends was not significantly different for all blends at low levels, but the differences between the blends increased steadily with increased hydrolysate replacement. Likewise, the amount of cured resin extracted in water increased with greater hydrolysate replacement levels. The actual amount extracted, however, indicated there was an interaction between the hydrolysate and the resin that prevented the hydrolysate from being extracted from the cured hydrolysate-resin blend.

Key words: soy flour, soy hydrolysates, soy adhesives, protein adhesives, enzyme, enzyme processing, biorenewable technologies, industrial utilization

## Introduction

Before the widespread use of petroleum-based resins, soy protein-based glues were an important adhesive in plywood production. Initial constraints on the soymeal included the material be defatted with negligible loss in solubility (1). These were the prescribed factors needed for the use of plywood adhesives of that time. Initial soybean flour adhesives were essentially soy flour dispersed in alkali solutions with very few added chemical modifiers to improve the adhesive properties of the protein. Increased pH decreased the protein activity, but uncoiling led to greater dispersion of flour and thereby increased the protein adhesion potential. The soy protein adhesives were highly viscous materials only suitable for roll-coat applications of plywood. Another drawback was these resins were more prone to wet wood failure than the petroleum-based resins that were later developed (2). The wood products industry has not used soybean-protein-based glues since the 1960's due to the availability of better performing adhesives such as phenol-formaldehyde and urea-formaldehyde. In recent years research efforts have once again focused on renewable agricultural products for wood adhesives (3). Renewed interest exists because of rising and fluctuating costs of petroleum-based reactants for adhesives and the effort to find more environmentally friendly “green products” that will decrease the potential of harmful chemicals in synthetic adhesives; particularly those that release volatile organic compounds into the environment.

Most research efforts have focused on soy protein isolates because of the functional characteristics of the protein (4,5). The major downside to using protein isolates is their higher cost relative to petroleum-based ingredients. For many years, soy protein isolate (SPI) prices have been approximately one dollar per pound. Phenol is the most expensive ingredient in phenol-formaldehyde resins, and by comparison, typically the price of phenol has been less than$0.50/lb. Any ingredient for adhesives made from soy protein isolates has simply been cost prohibitive. Alternative technologies utilize soy flours, with many commercial flour products available currently for $0.15-0.20/lb. Kuo, et al. (6) showed soy flour could be modified by treatment with an aqueous solution of sodium bisulfite and be used in a blend of 70% soy/30% PF combination for wood composite applications. The adhesive resin in this system had adequate mechanical properties and dimensional stability. Additional work with soy flour has shown that alkaline treatments similar to those used with SPI could be used for soy flours to make a product for cross-linking with PF resins.

One issue associated with soybean proteins in adhesives is they are highly viscous if untreated. Additionally, although soybean proteins contain many polar groups that function as the primary binding sites for adhering the wood and other cellulosic fibers, they are heavily coiled leaving the polar groups unavailable to bond with polar wood fibers (2). To offset both of these problems, soy components are often hydrolyzed under alkaline conditions to reduce viscosity and expose more functional groups on the proteins available for reaction. The reaction is simply carried out by the high temperature and presence of the strong alkaline salt. High concentrations of alkaline salts are used to form the maximum adhesive strength, but the resulting procedure is energy intensive, uses high concentrations of harsh chemicals, and results in a dark colored product capable of staining glue-lines or creating dark colored composite board compared to adhesives without hydrolyzed soy (5). Furthermore, alkaline hydrolysis is an uncontrolled process and the only variation to hydrolysates produced is the extent of hydrolysis, achieved by altering the time and/or temperature of the procedure.

One alternative to the alkaline process is to use enzymes to hydrolyze the flour. Enzymatic treatments of soy flour have the potential to reduce the variation among hydrolysis treatments, creating the potential to tailor soy flour components to individual adhesives and specific uses. Chief among the concerns for using any soy component in adhesives are the binding strength and water susceptibility of proteins compared to synthetic resins.

Although soy hydrolysates made from enzymes can be similar to alkaline produced hydrolysates, the enzymatically-produced hydrolysates have the potential to be very different in their performance and chemical nature. Enzymes can be more specific in their action, resulting in hydrolysates that can react differently in an adhesive formulation. Furthermore, the carbohydrates present in the flour have to be considered in the choice of enzymes because they are not affected by protease enzymes whereas they are affected in the alkaline process. In order to investigate whether there are any differences in the performance of the enzymatically produced hydrolysate compared to the alkaline hydrolysate, a study of the performance of the adhesives is needed. The present study evaluated the performance of alkaline and enzymatically-produced hydrolysates using two factors related to the performance of phenol-formaldehyde adhesives; the time required for the adhesive resin to polymerize and the water susceptibility of petroleum adhesive-soy hydrolysate blends.

## Experimental Procedures

*Materials.* Defatted soybean flour, HoneySoy90, from Cenex Harvest States (Mankato, MN) was hydrolyzed under a variety of conditions. Chemical hydrolysis was performed under extreme alkaline conditions (8% sodium hydroxide at 140°C for 2-h). Additionally three enzymatic treatments were prepared using a single protease treatment (Protease), a carbohydrase enzyme preparation with a broad spectrum of activities (Carbohydrase), and the combination of protease and carbohydrases (ProCarb). Conditions for the various enzyme treatments were based on optimal temperatures for enzyme activity and adequate time for maximum enzymatic reaction. Two types of phenol-formaldehyde resins (PF) were used in the experimental procedure as well. A commercial oriented strand board face resin (PFC) was tested in all hydrolysate-resin blends, as was as a PF resin synthesized in the lab (PFL). Specifications for the lab-made resin included molar ratios of 2.4 formaldehyde:phenol and 0.1:1 sodium hydroxide:phenol.

*Test Procedures.* Soy hydrolysate-resin blends were prepared at 5, 10, 20, 40, and 60% solids replacement of PF resin. Hydrolysate-resin blends were adjusted to pH

11 with 50 wt% sodium hydroxide and then mixed for 30 minutes in a 40°C water bath. Two separate tests were performed, each using the same soy flour hydrolysates-resin blends.

*Polymerization Test*. The polymerization test was used to determine the effects of the hydrolysates on the time required to reach the gel point for resin blends relative to PF resins alone. The time required for polymerization of resins is a general tool used in resin synthesis to gauge the relative energy needed to cure a thermoset resin (7). The polymerization test was carried out on a steel plate 2 cm thick, maintained at an internal temperature of 200°C. 1 g samples were placed on the hot plate and mixed thoroughly. Mixing was continued until polymer strands were formed at which point the time was recorded.

*Water Extraction Test.* The water extraction test was performed to determine the effect of the hydrolysate on the water-resistance of the resin. Samples were cured in a furnace at 150°C for 15 min. The fully cured samples were broken into smaller pieces (>#7 mesh), and a 1 g sample was boiled in 10 ml of distilled water for 1 h. After boiling, the liquid was decanted and centrifuged for 10 min at 5 x g. A 5 g sample of the supernatant was dried at 110°C for 4 h, and the percentage of soy hydrolysate-resin blend extracted from the original cured sample was calculated by difference.

*Statistical Analysis.* Three batches of enzyme hydrolysates were prepared by Genencor Inc, Int. (Rochester, NY) and three alkaline hydrolysates were prepared, all in 1 kg batches. Polymerization and water extraction testing followed a randomized complete block design. Polymerization tests for each adhesive blend were replicated 10 times and water extraction was replicated in quadruplicate. Differences were deemed significant at a level of $p < 0.05$. All data was subjected to Analysis of Variance (ANOVA) using the general linear model (GLM) procedure by the Statistical Analysis Software Program version 9.1 (SAS Institute, Inc., Cary, NC).

## Results and Discussion

All four soy flour hydrolysates were in the range of 37-39% solids on wet basis. Both PFL and PFC had solids contents between 49-50%. Table 1 shows the polymerization times for all resin blends tested. With increased replacement of the hydrolysates, the time required for polymerization increased for both PF resins. One of the major properties affecting the time required for polymerization is the solids content. Since the hydrolysates were lower solids content than PF resin, as replacement level increased, the solids content decreased. Additionally, as the replacement level of hydrolysate increased, so too did the time for polymerization. In addition to more water present increasing the polymerization time, the conformation and reactivity of soy with PF may have slowed the polymerization process as well. He and Riedl (8) found the presence of wood decreases the polymerization process of chemical resins because it leads to a series of localized polymerization instead of bulk polymerization. Any soy in the system not reacting, or reacting differently than the polymerization of pure PF, would likely cause the same phenomena. This was true for all hydrolysates, but significant differences did exist among the combinations of hydrolysates and resins.

At the low replacement levels (5 and 10%) the hydrolysates acted similarly in the resins. At the low replacement levels only two hydrolysate blends were significantly different from the pure resin. The first was 5% alkaline hydrolysate in the PFC and the other was alkaline hydrolysate at 5% in PFL. The 5% alkaline hydrolysate in PFC actually significantly decreased the polymerization time, but 5% carbohydrase in PFL increased the polymerization time by 9% compared to the pure PFL. At 10% replacement all four hydrolysates were similar and all significantly longer than the resin alone. The similarities continued at the 20% replacement levels, but significant differences in the reaction with PF resins were observed beginning at 40% replacement. As shown in Figures 1 and 2 and Table 1, the alkaline hydrolysate had a polymerization time nearly 50% greater than the other enzymatic replacements at 60% replacement. Another interesting result is that neither Protease nor ProCarb polymerized with PFC at 60% replacement. There were no significant differences in the appearances or viscosities of Protease or ProCarb from Alkaline or Carbohydrase, but instead of forming polymer strands, both hydrolysates prepared with protease enzymes formed hard aggregates as the blend was heated on the hotplate.

The second part of the experiment was designed to characterize the water susceptibility of the cured resin blends. The raw extraction values for all treatments are reported in Table 2. The two resins with no added hydrolysate behaved very differently. After the 1 h extraction, 6.05% of the PFC was extracted from the cured mass, whereas only 0.13% of the PFL was extracted under the same conditions. The difference in extraction values for resin alone was a clear indication of formulation differences which when blended with additional ingredients, like soy flour hydrolysates, could react differently.

The extraction values in Table 2 show the increasing nature of the material removed from the cured resin during the extraction procedure. Extraction values for PFL and PFC were also dramatically different from one another as reported in Table 2. Due to the extraction differences in the two PF resins, it was more difficult to discern the effect of soy hydrolysates on resin blends and the effect of enzymatic treatment versus chemical modification by just considering the extraction values. In an effort to understand the effect of the curing treatment on the hydrolysates without PF resin, each of the pure hydrolysates were exposed to the same heat treatment used for curing the resin blends. The same water extraction was performed on the hydrolysates without any added PF. It was assumed that if no interaction took place between the hydrolysate and resin then the same percentage of the hydrolysate in each resin blend would be extracted as well. This value was referred to as the *expected value* and was compared to the actual extraction value by taking the ratio of the two values. Therefore, values in Table 3 are the percentages of the resin blend extracted compared to the amount of hydrolysate expected to be extracted if no reaction or interaction between the hydrolysate and resin occurred.

The results in Table 3 show that all values for the PFL were positive, but for PFC, all 5% replacement values and 10% replacements with carbohydrase treatments were negative. In these instances less material was extracted from the resin blends than PFC alone. In blends with PFL there were no significant differences among type of hydrolysate below 20% replacement. Differences between the two PF resin systems were more evident from the information in Table 3. A steady increase in the ratio of extracted material to the expected value was observed for PFL with increasing hydrolysate, but the same trend was not present for blends with PFC. The protease treated values changed from 40-60% replacement, but blends with the other two hydrolysates were similar between the two replacement levels. In blends with PFL, there were significant differences among all hydrolysates at 40 and 60% replacement only the carbohydrase treated soy flour was different from any of the others. The opposite was the case for blends with PFC. The carbohydrase blend at 40% was different from the other three hydrolysates at 40%, but at 60% there was no significant difference among any of the four soy-flour hydrolysates.

There were differences in water susceptibility among the types of PF resin and hydrolysate. The most significant result however, was that in no blend combination was the actual amount extracted equal to the expected value. This indicated that to some extent there was reaction or interaction between the soy flour hydrolysate and PF resin. The increase in water extraction values and the ratio of actual to expected may indicate there is a limit to the level of replacement which can be used. With increased hydrolysate there was less PF for the hydrolysate to form interactions with, and thereby increased the water susceptibility of the resin blend. The idea of less PF available for interactions with soy hydrolysate causing increased extraction is similar to the conclusions reported in the literature that PF polymerization in the bulk liquid was altered by the presence of wood flour in particleboard systems (8). Based on the differences between resins however, results indicate PF resin could be formulated to react differently with various types of soy-flour hydrolysates. Additionally, it may also be possible to alter the enzymatic reactions, either by type of enzymes used or extent of their reaction, to form a hydrolysate that will interact with petroleum resins differently.

## Acknowledgements

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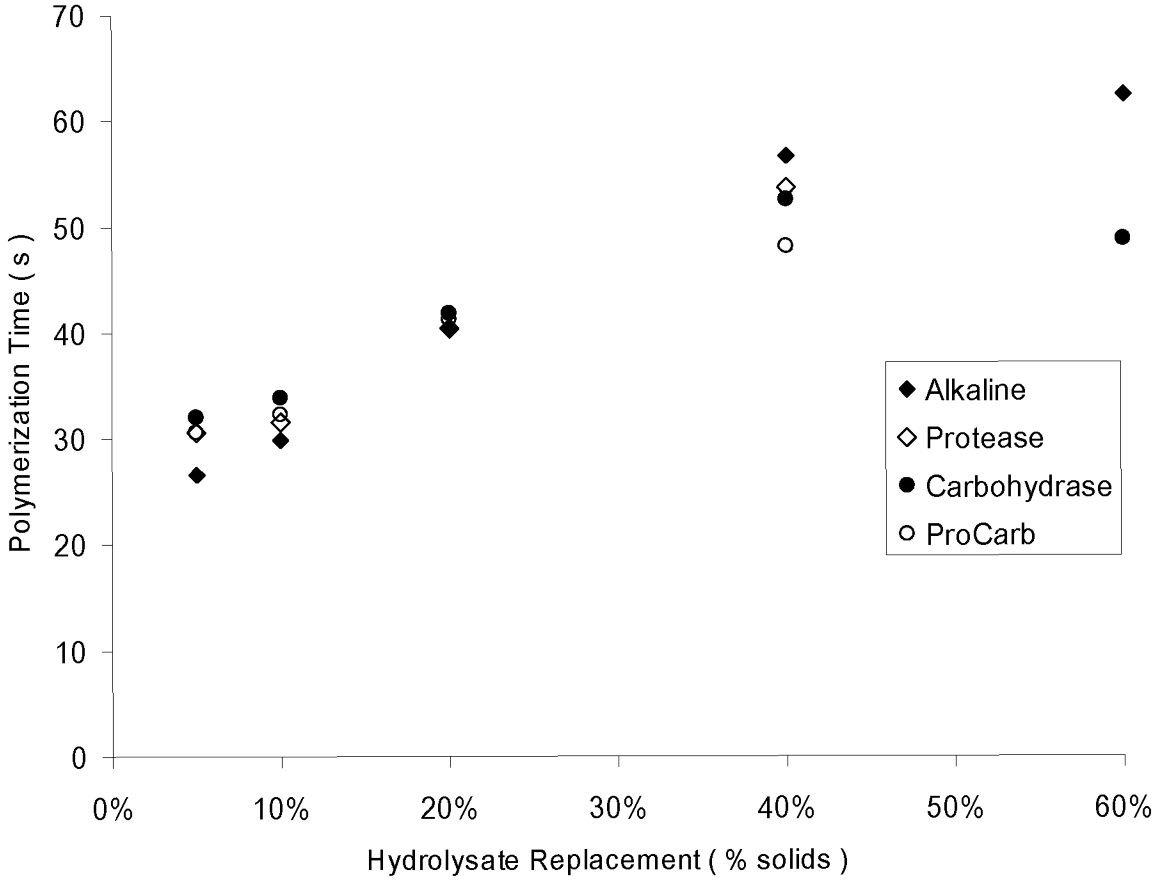

**FIGURE 1.** Polymerization trends for hydrolysates with PFC

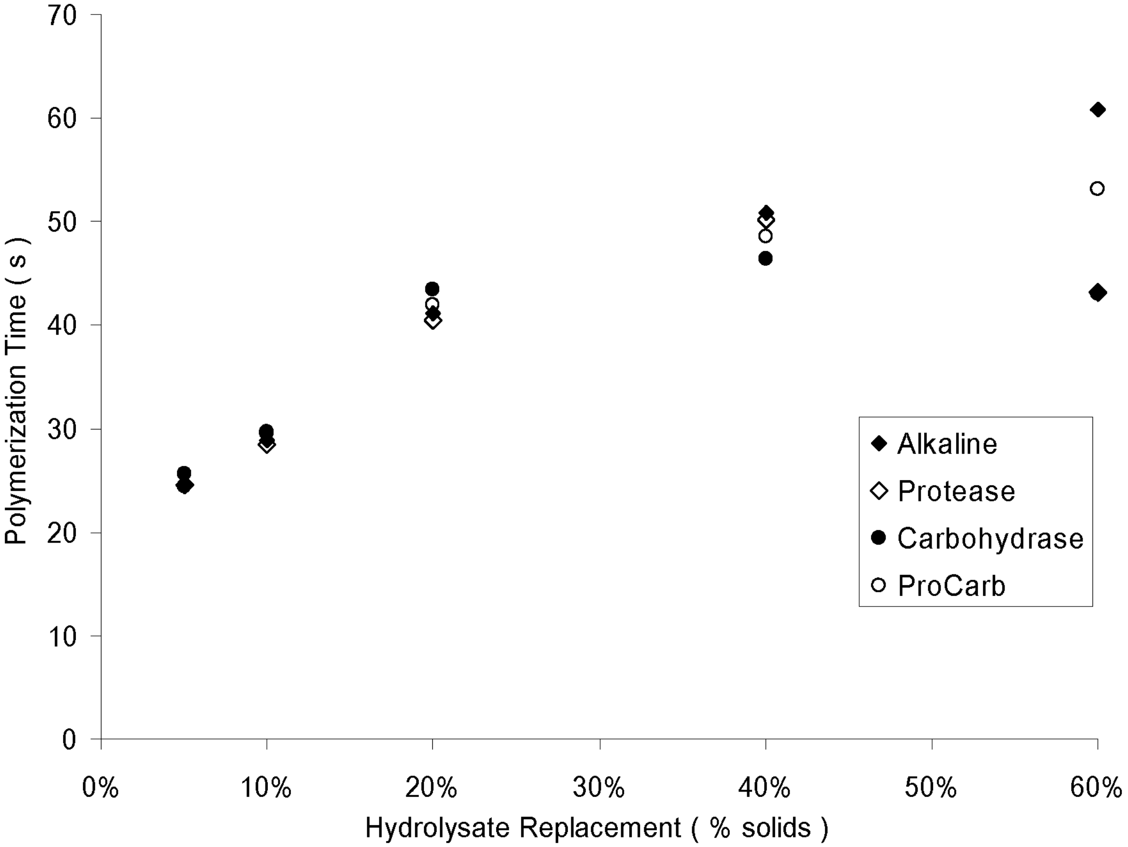

**FIGURE 2.** Polymerization trends for hydrolysates with PFL

**TABLE 1**

**Polymerization times for soy hydrolysate-PF resin blends**

| Hydrolysate ( % ) | Resin | Alkaline | | Protease | | Carbohydrase | | ProCarb | |
|---|---|---|---|---|---|---|---|---|---|
| | | Time (s) | Increase (%) * | Time (s) | Increase (%) | Time (s) | Increase (%) | Time (s) | Increase (%) |
| 5 | PFL | 24.5 a | 1 | 24.6 b | 5 | 25.7 b | 9 | 24.4 b | 4 |
| | PFC | 26.6 z | -14 | 30.6 y | -1 | 32.0 z | 3 | 30.6 y | -1 |
| 10 | PFL | 28.8 a | 22 | 28.5 a | 21 | 29.7 a | 26 | 29.6 a | 26 |
| | PFC | 29.9 z | -3 | 31.6 z,y | -2 | 33.9 y | 9 | 32.3 z, y | 4 |
| 20 | PFL | 41.2 a | 75 | 40.4 a | 72 | 43.4 a | 85 | 41.9 a | 78 |
| | PFC | 40.4 z | 30 | 40.4 z | 30 | 41.9 z | 35 | 41.3 z | 33 |
| 40 | PFL | 50.8 a | 116 | 50.1 a | 113 | 46.3 b | 97 | 48.6 a,b | 107 |
| | PFC | 56.7 z | 83 | 53.7 z | 73 | 52.5 z, y | 69 | 48.1 y | 55 |
| 60 | PFL | 60.8 a | 159 | 43.2 b | 84 | 43.0 b | 83 | 53.1 c | 126 |
| | PFC | 62.4 z | 101 | Did not gel | N / A | 48.8 y | 57 | Did not gel | N / A |

* Compared to each individual resin tested without hydrolysate added

Pure Resin Values: PFL 23.5 s
PFC 31.0 s

Statistical differences shown by letters within each replacement level and type of resin, $p < 0.05$

**TABLE 2**

**Percent of cured resin blend extracted in boiling water**

| Hydrolysate ( % ) | Resin | Alkaline | Protease | Carbohydrase | Protease & Carbohydrase |
|---|---|---|---|---|---|
| 5 | PFL | 0.17 | 0.17 | 0.15 | 0.17 |
| | PFC | 5.61 | 5.74 | 5.51 | 5.75 |
| 10 | PFL | 0.30 | 0.28 | 0.30 | 0.34 |
| | PFC | 6.46 *c* | 6.24 *c* | 5.54 *a* | 5.96 *b* |
| 20 | PFL | 1.01 *c* | 0.56 *a* | 0.92 *b,c* | 0.84 *b* |
| | PFC | 6.54 *c* | 5.86 *a* | 6.23 *b* | 6.21 *b* |
| 40 | PFL | 2.34 *a* | 3.37 *c* | 3.27 *c* | 2.84 *b* |
| | PFC | 7.87 c | 6.94 *a* | 7.24 *b* | 6.92 *a* |
| 60 | PFL | 6.85 *c* | 4.76 *a* | 5.06 *b* | 5.19 *b* |
| | PFC | 8.93 *b* | 8.71 *b* | 7.65 *a* | 8.42 *b* |
| 100 | - - - | 18.1 *d* | 12.7 | 8.72 *a* | 16.9 |

| Pure Resin Values: | |
|---|---|
| | PFL 0.13% |
| | PFC 6.05% |

Statistical differences shown by letters within each replacement level and type of resin, $p < 0.05$

**TABLE 3**

**Ratio of extracted hydrolysate to expected hydrolysate extracted**

| Hydrolysate (%) | Resin | Alkaline | Protease | Carbohydrase | Protease & Carbohydrase |
|---|---|---|---|---|---|
| 5 | PFL | 0.04 | 0.07 | 0.04 | 0.05 |
| | PFC | -0.49 | -0.50 | -1.24 | -0.36 |
| 10 | PFL | 0.09 | 0.12 | 0.19 | 0.13 |
| | PFC | 0.22 | 0.14 | -0.59 | -0.05 |
| 20 | PFL | 0.24 *a* | 0.17 *a* | 0.45 *b* | 0.21 *a* |
| | PFC | 0.14 | -0.07 | 0.10 | 0.04 |
| 40 | PFL | 0.31 *a* | 0.64 *b* | 0.90 *c* | 0.40 *a* |
| | PFC | 0.25 | 0.17 | 0.34 | 0.13 |
| 60 | PFL | 0.62 a | 0.61 a | 0.94 b | 0.50 *a* |
| | PFC | 0.27 | 0.35 | 0.31 | 0.23 |

Statistical differences shown by letters within each replacement level and type of resin, $p < 0.05$

# CHAPTER 3

## Chararterization of Soy Flour Enzymatic Hydrolysates in Wood Adhesive Applications

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### Abstract

Medium-density fiberboards (MDF) were made using soy flour hydrolysate resin blended with phenol formaldehyde (PF). Soy hydrolysate-PF resin blends were created at 5, 10, 20, and 40% PF solids replacement and sprayed onto wood fiber at a 12% resin load. Soy flour hydrolyzed using an alkaline chemical process was compared to enzymatically produced hydrolysates prepared in a way to elucidate the effects of different enzymes on hydrolysate properties in a resin system. MDF samples were tested according to ASTM standards for both mechanical and water-resistance properties. Decreases in properties were not observed with increased hydrolysate content, but for strength characteristics such as internal bond and thickness swell after a 2-hr boil, differences between the alkaline and enzymatic treatments were observed as well as the effect of different types of enzymes used. Water-resistance and internal bond strength did not show the same trend results for the type of enzyme used however. Compared to the pure resin, hydrolysate-PF blends can perform similarly for mechanical properties, but with increasing replacement water-resistance properties decrease and are less resistant than the control samples with pure PF resin.

Key words: soy flour, soy hydrolysates, soy adhesives, protein adhesives, enzyme, enzyme processing, medium-density fiberboard, biocomposites

## Introduction

Soy flour adhesives were important in the wood products market prior to the availability of petroleum-based adhesives. Early soy adhesives were made from defatted soy flours and used in roll-coat applications for plywood manufacture. The soy flour adhesives had properties equal to other wood adhesive available and were less costly than other adhesives in use at that time (1). Soy flour adhesives grew to peak production in the 1960's of 100 million pounds annually. Throughout the growth period the basic technology remained the same as initially used by Davidson and Laucks. To improve the adhesive properties, soy flour was dispersed in a strong alkali (2). Alkaline treatment caused denaturation of the proteins and exposed more polar amino acid residues thereby increasing the adhesive capabilities of the soy flour. The major downfall of the early soy flour adhesives was their lack of water-resistance and short pot-life caused by increases in viscosity. Additional chemical compounds were added, such as calcium hydroxide or sodium silicate, to increase water holding capacity and stabilize viscosity (2). In the later stages of soy flour adhesive use, crosslinking agents became an important part of adhesive formulation. Compounds including, but not limited to, carbon disulfide, thiourea, and potassium xanthate were used to increase water-resistance and to stabilize the denatured structure from further hydrolysis (3).

Once the availability of petroleum-based adhesives became widespread the utilization of soy flour adhesives quickly decreased. Synthetic resins such as urea-formaldehyde and phenol-formaldehyde were cheaper, more water resistant, and more consistent. In recent years there have been many efforts to replace petroleum-based chemicals with materials from renewable resources, including chemicals derived from both proteins and carbohydrates from various plant sources. The resurgence of adhesives formulated with biobased materials has been fueled by increased petroleum costs and the increased demand for more environmentally friendly products. Several research groups have investigated the use of soy protein isolates in wood adhesive formulations (4-7). Steele *et al.* (8) developed a 50% soy protein isolate and 50% phenol-resorcinol-formaldehyde (PRF) cold-setting resin that was used in commercial finger-jointed lumber. Most systems with protein isolates are expensive however, since isolates generally sell for more than $1/lb. The application demonstrated by Steele, *et al.* (8) was economical however, because PRF is a costly adhesive compared to other adhesive resins.

Kuo, et al. (9) demonstrated soy flour could be modified with a sodium bisulfite treatment and then used in a 70% soy and 30% PF blend for flakeboard applications. Sodium bisulfite soy flour dispersions and alkaline hydrolyzed soy flour perform similar to, if not better, than pure PF resins in southern pine plywood (10). The natural foaming properties of soy flour can be utilized in plywood adhesives as well. Foamed plywood adhesives made with soy material actually performed better when the soy component was flour or protein concentrate compared to protein isolate (11). Expanding upon the work of Steele (8), soy flours have been tested in finger joint applications with PRF. Similar results were reported, indicating high reactivity between soy and PRF with the added benefit of a lower cost adhesive component (12)

Based on the fore mentioned work, it is widely accepted that soy flour used in adhesive formulation requires some type of modification to provide proper adhesive properties. Most research emphasizes a similar type of alkaline treatment that was used in early soy flour adhesives. Such chemical treatments unfold the proteins exposing more polar groups to aid in bonding with polar wood fiber molecules (2). The problems with alkaline modification are that the hydrolysis reactions are uncontrolled, highly caustic, require expensive reactors, and is carried out at relatively high temperatures. Enzymatic treatments of soy flours have the potential to modify flours in a similar manner as alkaline treatments, but in a safer way since enzymatic modification is done at lower temperatures and at more neutral pH's. The use of enzymes can lead to the creation of many soy flour hydrolysates with different properties. The present study evaluated the effect of enzymatically produced soy flour hydrolysates compared to an alkaline hydrolysate of soy flour when blended with PF resin for medium-density fiberboard (MDF).

## Experimental Procedures

*Materials.* Defatted soybean flour from Cenex Harvest States (Mankato, MN) was hydrolyzed under a variety of conditions. A chemical method was performed under extreme alkaline conditions (8% NaOH at 140°C for 2-h). Four enzymatic treatments were prepared using a single protease treatment (Pro), a carbohydrase medley with multiple activities on specific types of carbohydrates (Carb), and two different combinations of protease and carbohydrase; using the same conditions, but done in a different order (ProCarb and CarbPro respectively). Enzyme treatments were not limited; in an attempt to cause a similar complete hydrolysis like the alkaline treatment. Conditions for the various enzyme treatments were based on optimal temperatures for enzyme activity and adequate time for maximal enzymatic reaction. The phenol formaldehyde (PFL) used in the study was synthesized in the lab with a formaldehyde:phenol molar ratio of 2.4:1 and a molar ratio of 0.1 for sodium hydroxide:phenol.

*Test Procedures*. Soy hydrolysate-resin blends were prepared at 5, 10, 20, and 40% solids replacement of PF resin. Soy hydrolysate-resin blends were adjusted to pH 11, and then blended for 30 minutes in a 50°C water bath. Viscosity measurements were taken on PF resin, soy hydrolysates, and all resin blends using a LabLine viscometer and a #4 probe at 100 rpm. MDF was prepared by spraying - 12% resin by weight onto dried pinewood fiber. The fiber mat was pressed at 50 tons for 8 min in a Wabash hydraulic press to form 0.5 in boards with a density of 50 $lb/in^3$.

*Statistical Analysis.* Three 1 kg batches of enzymatic hydrolysates were prepared by Genencor Int. and three alkaline hydrolysates were prepared. For each sample batch two 16x16 in boards were made. They were cut into appropriate sample sizes to test the MDF according to ASTM D1037 for bending, strength, and water-resistance properties (13). For modulus of rupture and elasticity (MOR and MOE respectively), there was one sample per board, or two for each resin blend per replicate. There were four internal bond strength (IB) samples per board and one sample for boiling and soaking per board. Bending and strength tests were performed using an Ultimate Testing System. All data was subjected to Analysis of Variance (ANOVA) using the general linear model (GLM) procedure by the Statistical Analysis Software Program version 9.1 (SAS Institute, Inc., Cary, NC).

## Results and Discussion

The viscosities of the hydrolysate treatments varied greatly based on the type of treatment performed (Table 1). Alkaline treatments, ProCarb, and CarbPro produced the greatest reduction in viscosity, but these viscosities were still in the range of 580-750 cP. Protease and carbohydrase treatments reduced the viscosities of the flour dispersions as well, but the final viscosity of these treatments (1500-2000 cP) was larger than the final viscosity of combined treatments because less of the total flour was hydrolyzed by the enzyme treatment(s). Considering the large range of viscosities of pure hydrolysates, a larger range of viscosities was expected for the resin blends. Table 1 shows the viscosities of resin blends after being adjusted to pH 11. There were no significant differences among the viscosities of the hydrolysate blends, the level of replacement, or compared to the pure resin itself. With the PF resin used in this study, blend viscosities were dictated by the PF resin itself, not by the hydrolysate or replacement level.

Two types of measurements were made on the MDF boards produced in the study. The first are characterized as the dry, mechanical properties of the boards. Board density was determined from bending test specimens. Table 2 shows the mechanical properties measured by the UTS. Among all types of hydrolysates and replacement levels there were no significant differences in board density. This was important because it validated the MDF fiber/resin mats were evenly and adequately assembled. The only statistically different set of boards for density were the samples made with 20% reduction in resin. As expected, the reduced resin load resulted in a lower board density since less resin solids were added to the wood fiber. This was significant because whereas replacement with hydrolysate did not affect the board density, a reduction in resin did. The other two results obtained from bending tests were the modulus of rupture and modulus of elasticity. MOE is the numerical description of an object’s tendency to deform under an applied stress. In the case of MDF made with soy hydrolysates blended with PFL, there were few differences between sample treatments. The variation in MOE was too large to make any difference claims aside from the Pro treatment being different from ProCarb at 20% replacement. In this case the mean MOE for Pro was much higher than the means for all other treatments.

MOR is the force required to fracture or break an object of specific dimensions, and in this application gave more meaningful, interpretable results. As seen in the MOE results, a decrease in value was not seen with increasing hydrolysate replacement. Significant differences were observed between types of hydrolysate at each replacement level. No general trends, such as, the Pro treatment is different from the Carb or CarbPro at all levels can be made however for MOE results. Differences among hydrolysates were not consistent among replacement levels.

Internal bond strength is a measure of the perpendicular force required to cause failure in MDF. Differences were observed in the samples measured, and IB is the only mechanical property where differences were observed with increasing replacement amount. Both the Alkaline and Pro treatments showed decreasing IB values with increasing replacement level. Within each type of treatment, MDF samples made with resins containing carbohydrase were not different from other replacement levels. At 5% replacement there were no differences in IB of any resin blend. At replacement levels above 5% however, all treatments prepared with the carbohydrase medley were significantly higher than IB's from the alkaline treatment, and with the exception of the 20% replacement level, the carbohydrase treatment led to differences from the protease treatment as well. This result indicated that in adhesive systems using soy flour it is important to treat the carbohydrate fraction. Not only did carbohydrase reduce the viscosity to allow easy incorporation in adhesives, the carbohydrase treatment also aids in some adhesive properties of soy flour hydrolysates.

In addition to testing strength differences among hydrolysate-resin blends, control samples of pure resin were made. One set of control MDF boards were made at the same 12% resin load, and a second batch of boards with 20% less resin

(resin load equal to 9.6%) were also made for each replicate. The second set of boards with 20% less resin were made to simulate the MDF properties if hydrolysates actually have no adhesive property. If the soy hydrolysates act as a filler in the system (ie – no adhesive reaction or interaction with PF or wood fiber), the 20% replacement level properties would be equivalent to the 20% resin reduction values. In Table 3 the average values for the control samples are listed below the primary results for resin blends. Table 3 indicates similar results as previously stated. MOR and MOE were not applicable in identifying differences among hydrolysates or their usefulness in adhesive properties in this study. For the IB values however, there was a large difference between the two control samples. With 20% less resin the mean IB was one-third less than for the full resin load. For hydrolysate-resin blends at 20% replacement only Alkaline and Pro were different from the pure resin values, but Pro was also different from the 20% resin reduction.

The second type of testing performed was water-resistance tests. The first was a 24 h soak test at 24°C, and the second was a 2-h boiling test. Water absorption and thickness swell were measured before and after the test. In all cases, water absorption (measured by gain in mass) were proportional to the thickness swell. Thickness swell results for both tests are reported in Table 4 (TS-2B and TS-24S). Differences were not consistent nor show any trend in the 24 h soak test. Similarly differences were not observed between the full resin load or the 20% reduction for the 24 h test. The 2-h boil test was more intensive on the MDF samples and show a more clear difference in water-resistance among the types of hydrolysate. At 5 and 10% replacement levels, thickness swell of Alkaline and Carb treatments were lower and significantly different from the three treatments that contained proteases. This was the opposite result compared to strength testing, and indicated the hydrolysis and enzymatic denaturation of proteins in the soy flour leads to decreased water-resistance in hydrolysate-resin blends. At 20 and 40% replacement levels however, the differences were negligible. Hydrolysate replacement at low levels with protease-treated hydrolysates also led to decreased water-resistance compared to pure resin. Protease treated samples at low replacement levels were actually similar to MDF samples made with decreased amount of resin.

MDF samples made with soy hydrolysate-PF resin blends showed mixed results. For some properties, differences were not seen between the type of hydrolysate or even from the inclusion of soy hydrolysate. For tests like IB and thickness swell, simply reducing the resin load diminished strength qualities.

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**TABLE 1**

**Hydrolysate native viscosity**

| Hydrolysate | Viscosity (cP) |
|---|---|
| Alkaline | 580 |
| Carb | 1500 |
| ProCarb | 750 |
| Pro | 2000 |
| CarbPro | 700 |

**TABLE 2**

**Soy hydrolysate-PF resin blend viscosities**

| Hydrolysate | Replacement ( % ) | Viscosity ( cP ) |
|---|---|---|
| Alkaline | 5 | 120 |
| | 10 | 110 |
| | 20 | 120 |
| | 40 | 130 |
| Carb | 5 | 120 |
| | 10 | 130 |
| | 20 | 140 |
| | 40 | 140 |
| ProCarb | 5 | 120 |
| | 10 | 120 |
| | 20 | 120 |
| | 40 | 120 |
| Pro | 5 | 130 |
| | 10 | 140 |
| | 20 | 150 |
| | 40 | 160 |
| CarbPro | 5 | 120 |
| | 10 | 120 |
| | 20 | 120 |
| | 40 | 130 |
| PFL | --- | 120 |

**TABLE 3**

**Mechanical properties for soy hydrolysate-PF resin blends**

| Replacement ( % ) | Hydrolysate | Board Density ( psi ) | MOR x 1000 ( psi ) | MOE x 1000 ( psi ) | IB ( psi ) |
|---|---|---|---|---|---|
| 5 | Alkaline | 49.5 | 6.67 *a* | 569 | 134 *a* |
|  | Carb | 48.9 | 7.06 *a,b* | 621 | 140 *a* |
|  | ProCarb | 49.8 | 7.39 *b* | 671 | 137 *a* |
|  | Pro | 48.8 | 6.87 *a,b* | 638 | 144 *a* |
|  | CarbPro | 47.5 | 6.67 *a* | 587 | 129 *a* |
| 10 | Alkaline | 48.3 | 6.95 *a,b* | 598 | 122 *a* |
|  | Carb | 48.7 | 6.60 *a* | 549 | 141 *b* |
|  | ProCarb | 48.6 | 7.11 *a,b* | 645 | 150 *b* |
|  | Pro | 48.0 | 7.14 *a,b* | 672 | 127 *a* |
|  | CarbPro | 48.4 | 7.37 *b* | 666 | 145 *b* |
| 20 | Alkaline | 49.7 | 6.60 *a* | 643 | 114 *a* |
|  | Carb | 49.7 | 7.40 *b* | 667 | 141 *b* |
|  | ProCarb | 48.5 | 6.48 *a* | 595 | 139 *b* |
|  | Pro | 50.8 | 7.57 *b* | 716 | 129 *a,b* |
|  | CarbPro | 48.1 | 6.96 *a,b* | 645 | 137 *b* |
| 40 | Alkaline | 48.8 | 6.48 *a,b* | 616 | 100 *a* |
|  | Carb | 50.0 | 6.70 *b* | 610 | 139 *b* |
|  | ProCarb | 49.1 | 5.84 *a* | 565 | 135 b |
|  | Pro | 49.5 | 6.95 *b* | 665 | 109 *a* |
|  | CarbPro | 48.8 | 7.07 *b* | 661 | 129 *b* |
| PFL |  | 48.4 | 6.43 | 605 | 166 |
| 20% resin reduction |  | 46.8 | 6.36 | 610 | 99 |

*Statistical differences shown by letters within each replacement level, $p < 0.05$*

**TABLE 4**

**Water resistance results for soy hydrolysate-PF resin blends**

| Replacement ( % ) | Hydrolysate | TS-2B (%) | TS-24S (%) |
|---|---|---|---|
| 5 | Alkaline | 9.8 *a* | 4.3 *a* |
| | Carb | 9.5 *a* | 4.1 *a* |
| | ProCarb | 11.9 *b* | 5.0 *a* |
| | Pro | 12.3 *b* | 9.4 *b* |
| | CarbPro | 11.7 *b* | 5.1 *a* |
| 10 | Alkaline | 10.1 *a* | 4.9 *a* |
| | Carb | 9.6 *a* | 4.1 *a* |
| | ProCarb | 12.0 *b* | 5.1 *a,b* |
| | Pro | 11.5 *b* | 4.7 *a* |
| | CarbPro | 11.7 *b* | 5.6 *a,b* |
| 20 | Alkaline | 13.1 *a* | 6.2 *b* |
| | Carb | 12.2 *a* | 4.4 a |
| | ProCarb | 12.4 *a* | 4.8 *a* |
| | Pro | 12.1 *a* | 4.8 *a* |
| | CarbPro | 13.2 *a* | 5.0 *a* |
| 40 | Alkaline | 15.4 *a* | 6.5 *b* |
| | Carb | 16.1 *a* | 5.2 *a* |
| | ProCarb | 16.2 *a* | 6.2 *b* |
| | Pro | 15.9 *a* | 5.8 *a,b* |
| | CarbPro | 15.5 *a* | 5.0 *a* |
| PFL | | 9.9 | 4.4 |
| 20% resin reduction | | 11.2 | 6.2 |

*Statistical differences shown by letters within each replacement level, $p < 0.05$*

# CHAPTER 4

# Conclusions

## Summary

The goal of these studies was to evaluate the characteristics of enzymatic soy flour hydrolysates and compare them to alkaline hydrolysate. Two different approaches were used to elucidate the characteristics. In the first study methods were used to characterize the hydrolysates when blended with two different types of PF resin. In the second study medium-density fiberboards (MDF) were made with hydrolysate-resin blends. MDF was tested according to ASTM standards as a means to evaluate the performance of resin blends containing soy hydrolysate.

Previous research studies have shown that resin systems containing as much as 70% soy could be used to adequately produce a variety of wood products, but properties of wood products made with low replacement levels of petroleum products do not exist in published data. This study looked at both low level replacements as well as higher replacements. In the characterization of resin blends significant differences were observed for the polymerization time and water extraction values for soy hydrolysate interactions with different types of PF resin. Significant differences were also observed between different hydrolysates as well. At low replacement levels polymerization time did not increase dramatically, but as the replacement amount increased, all hydrolysates did increase the time required for polymerization. Another important finding was that at 60% replacement the enzymatic treatments with protease caused the resin blends to not polymerize with one of the resins tested. The author believes this is further validation that the hydrolysis treatment has an effect on the interaction between PF resin and soy hydrolysate. Water extraction results provided similar results – resins react differently from one another, react differently with different types of hydrolysate, and show decreased water-resistance with increased replacement with soy hydrolysate.

The second study added another variable to hydrolysate characterization by adding wood fiber to the system. Testing MDF samples is in many ways an indirect test of hydrolysate compatibility with PF resins. Results in wood product systems are important as well though because even if hydrolysates and resins are reactive with one another, it must be shown they are still capable of producing strong products. Three mechanical property tests were performed on MDF samples. Modulus of elasticity and modulus of rupture were less capable of showing differences between samples. At the lowest replacement level of 5% there was no difference among the adhesive strength for any of the five hydrolysates as indicated by IB. The higher replacement levels (10, 20, and 40%) of alkaline and protease treatments had decreased IB values compared to the three treatments which utilized a carbohydrase treatment on the soy flour. Finally, water absorption tests on MDF samples showed the opposite trends relative to IB results. At low levels the thickness swell were lowest for the alkaline and carbohydrase treatment, but all three treatments with protease enzymes showed greater thickness swell after the 2-h boiling test. At high replacement levels (≥20%) there was no difference between any of the hydrolysates, but they were larger than the control resin samples.

## Potential for Future Research

As is the case at the completion of many studies, more research questions exist regarding soy flour utilization in adhesive systems as well as new ones that were created by the results of this particular work. It was shown here that enzymatic hydrolysates are compatible with PF resins. In one study three enzymatic treatments were used and in the other four. These four treatments were decided upon with the guidance of industry partners. Studies showed the type of treatment had an effect on soy flour hydrolysate interaction with PF and strength properties, both mechanical and water-resistance, of MDF produced with hydrolysate-resin blends. In all cases, the hydrolysis of soy flour was run to completion. It is of interest in the future to examine the effect that a lower degree of hydrolysis (ie – a less complete hydrolysis compared to the alkaline treatment) may have on the reactivity of soy flours with chemical adhesives. Similarly, more needs to be done on the type of enzymes used for hydrolysis. Initial enzymatic hydrolysate work was done solely with protease enzymes. Since work has expanded to include carbohydrases, the choice of proteases has not been reevaluated. The current choice of enzymes randomly cleaves proteins, but if the viscosity of soy flours is lowered by other means (carbohydrases), less reactive protease enzymes may be used to produce larger and more reactive peptide fragments for adhesive bonding.

The previous suggestions would be aided greatly if more were known about the specific reactions occurring or capable of occurring between chemical adhesives and soy flour. One of the key steps for the continued development of soy flour hydrolysates for industrial utilization is a more thorough understanding of the reactions taking place. Functional groups in the proteins will undoubtedly react differently from one another with adhesives, and even that may vary based on the type of adhesive being formulated. Likewise, thermal analysis information would be helpful in future development efforts. Polymerization results showed the incorporation of soy into PF resins increased the time required for polymerization. In industry terms this would relate to longer press times and decreased productivity. Detailed thermal studies elucidating the nature of adhesive blends would potentially aid adhesive formulations.

Limiting factors for the use of alkaline hydrolysis of soy flour have included the dark color and strong ammonia odor of the product, neither of which can be controlled. The color of enzymatic hydrolysates varied depending on the treatment, and there was no obnoxious odor given off by enzymatic hydrolysates. The general properties of enzymatic treatments may lead to other possible uses beyond adhesives. To identify more potential uses, how soy flour could be used best, and the quality of individual flour products, it would be beneficial to develop a series of functional tests and procedures that could be used for characterizing hydrolysates.

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