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Chemical Modification of Soy Flour Protein and Its Properties *

Yuzhi Xu[1,2], Chunpeng Wang[1],Fuxiang Chu[1], Charles R Frihart[2],
Linda F Lorenz [2]and Nicole M Stark[2]

[1]Institute of Chemical Industry of Forest Products,Chinese Academy of Forestry,Nanjing 210042, China

[2]Forest Products Laboratory,USDA Forest Service, Madison,WI 53726-2398, USA

[1] xuyzz@hotmail.com,[2] {cfrihart, llorenz& nstark}@ fs.fed.us

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Abstract. This work is to examine ways to chemically modify soy proteins flours and analyze the results and determine the adhesive performance. Reaction with acetic anhydride converts amine and hydroxyl groups to amides and esters, respectively that are less polar and can make the adhesive more water resistant.The succinic anhydride reacts with these same groups but the products have terminal carboxylic acid groups that can react with the polyamidoamine-epichlorohydrin (PAE) resin that is used to cross-link the soy adhesives for improving bond strength. The attenuated total reflectance infrared spectroscopy (ATR-IR) is used to examine changes in the soy flour in going from unmodified to acetylated and succinylated state.

Introduction

In the past few years, concern about formaldehyde emissions, cost issues, and interest in biobased materials has renewed interest in soy adhesives [1-6]. Although Synthetic adhesives have generally replaced biobased adhesives over the past seventy years because of their durability, low cost, and ease of use. Soy proteins provide good adhesion to wood and other materials; however, these adhesives have poor water resistance without chemical modification or cross-linking. And so many different uses of chemical modification include determination of relative reactivities of side chain groups, the identification and quantitation of individual amino acid residues required for biological activity, the development of affinity and mechanism-based reagents for pharmaceutical uses, cross-linking reagents, special techniques for bioprosthesis, blocking reagents for peptide synthesis, reagents for specific cleavages of peptide bonds, bioconjugation, and special adjunctive modifications for analytical purposes, such as mass spectrometry[7]. The cross-linker polyamidoamineepichlorohydrin (PAE) resin used as wet strength resins for paper, innovatively has been employed in providing acceptable water resistance for interior wood products bonded with soy adhesives [3,4]. And the technology for soy adhesives using PAE cross-linking has provided adhesives that are used in plywood and engineered wood flooring [5], as well as particleboard and medium density fiberboard [8], which has been actuated by lower formaldehyde emission standards set by the California Air Resources Board (ATCM 2009).The Variety of procedures for protein modification were developed and used for many years prior to any interest in, or significant understanding of the chemical basis of those processes. To have a better understanding how alteration of protein structure by chemical modification effected its performance as a wood adhesive, and acetylation of soy flour at different temperature and the succinylated were made in this study. The changes in the soy flour in going from unmodified to acetylated and succinylated state were examined using modified ninhydrine method, titration of isolation point and ATR-IR.

Method

1. Take the soy flour (lot#090809-E) 30g and acetic anhydride 50g, and then stir the mixture at room temperature,40°C and 60°C, respectively, and then filtered with toluene.
2 To compare the effect of acylation with different carbon-chain on the modification, take soy flour 20g and succinic anhydride 10g, and add chloroform 70g to make the reaction mixture. Stirred for 3 hours at room temperature, but succinic anhydride solid was still available after 3 hours, and so stayed overnight, filtered, and tested with UV.

Testing

1. Modified ninhydrine method according to the literature [9]: Ninhydrine solution(1 ml) was added to three 1%(wt%) aqueous sample solutions, respectively, and was heated at 100°C (in a boiling water bath) for 5 min and cooled to 25°C.And the absorbance was determined at 580 nm against a water blank. The absorbance indicated the number of free amino groups available for reaction with ninhydrine reagent, and the difference in absorbancies between unmodified and acylated soy flours reflected the extent of acylation.
2. Assay of isoelectronic point: Add HCl solution (1N) enough to make the sample aqueous solution (1% wt) from original PH value to ~ 2.00 and then titrate with NaOH solution (1N) at ambient temperature.
3. ATR-IR characterization: The samples solution (30%,wt) are drawn down on the glass slide at 8μm thick and then put in fume hood until water evaporated completely before ATR-IR characterization using Scientific Thermo NICOLET iZ10.
4. Bond strength testing: It was conducted according to the method [10] listed in the literature. The Automated Bonding Evaluation System (ABES) Model 311c manufactured by Adhesive Evaluation Systems Incorporated (Corvallis, OR) was used to make small bonded samples to test the adhesives. Maple veneers were cut into 114.3 (grain direction) by 19.1 mm strips, with all wood conditioned at 22°C and 50 % relative humidity. The bonded specimens were assembled by applying adhesive to one side of the 5 mm overlap. This overlap was maintained by clipping the two wood pieces together with a binder clip while being placed in the ABES unit. Each specimen was pressed by the unit at 200KPa and at 120°C for 120s. Each specimen was removed and allowed to cool and recondition overnight. Of the fourteen specimens using each adhesive, half were pulled until bond fracture at ambient conditions and half were tested after a four hour water soak..

Results and Discussion

1. Assay of free amino group

Although instrumental methods such as HPLC are currently used for determining compounds containing amino group, the simple and convenient ninhydrin method still possess several advantages because of no expensive equipment required and routine analysis of large numbers of samples[11]. The different acylation temperature and side chain were used to carry out the ninhydrin reaction, and the objectives of the present research were designed to reveal the effects of reaction temperature and side-chain length on the extent of chemical modification using ninhydrin method.

The effects of reaction temperature and side chain on the colour development analyzed with UV were listed in the tableI. The temperature values of reaction were adjusted to 25°C, 40°C and 60°C. While the absorbance for 40°C and 60°C was about the same, the highest absorbance was obtained at 25°C.From the data in the table, acylation occurred in this study. Compared with acetylate group which used succinylate group to obtain comparable absorbance, one of the possible reasons for the higher intensity of colour development in succinylated soy protein sample might be its steric availability for reaction[9]. While different reactions showed different degrees of color development, the relationships between absorbance and extent of acylated modification were still observed for allmodification.

2. Titration of isoelectronic point

Every amino acid has a characteristic isoelectric point.Proteins are made of amino acids, so they also have isoelectric points, the sum of all of those of their component amino acids.The isoelectric points of changes in the soy flour proteins in going from unmodified to acetylated and succinylated state were concluded from fig.1, fig.2 and fig.3. At low pH, the amino acid is protonated at both the amine and carboxyl functions, which carries a net positive charge and can be treated as a diprotic acid. At high pH, both the carboxyl and amine groups are deprotonated, and the amino acid carries a net negative charge. At some intermediate pH, the amino acid is a zwitterions, and carries no net charge. This is called the isoelectric point of the amino acids, and is designated pHI. At this pH value, the amino acid will be stationary in an electric field. While the PHI for soy flour protein and acylated soy protein was about the same, the highest PHI was obtained at succinylated protein. From the data in the curves, acylation occurred in this study. But the isoelectric point came later in the titration curve of sample modified with succinylate group.

TABLE I UV ABSORBANCE OF SOY FLOUR PROTEIN BEFORE AND AFTER MODIFICATION IN IMPROVED NINHYDRIN METHOD

Sample	PH	Viscosity (cP)	UV
Soy flour (200-90)	6.23	51300	2.760
Acetylated soy flour protein at room temperature	4.52	236	0.540
Acetylated soy flour protein at 40°C	4.85	652	0.125
Acetylated soy flour protein at 60°C	4.20	178	0.118
Succinylated soy flour protein at room temperature	3.37	220	1.058

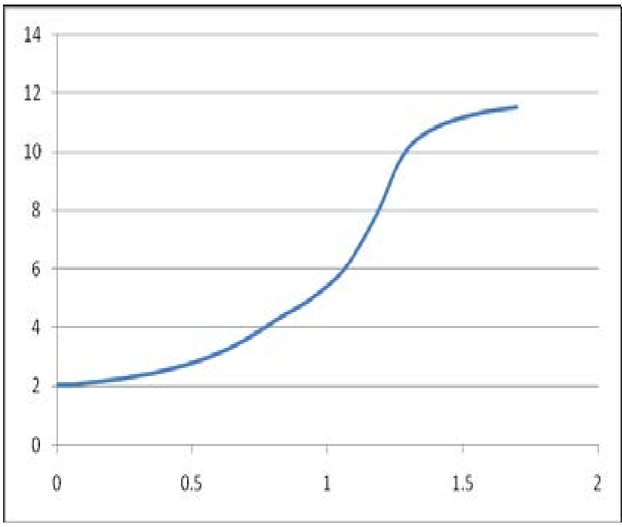

Fig.1 Isoelectric point curve of soy flour protein

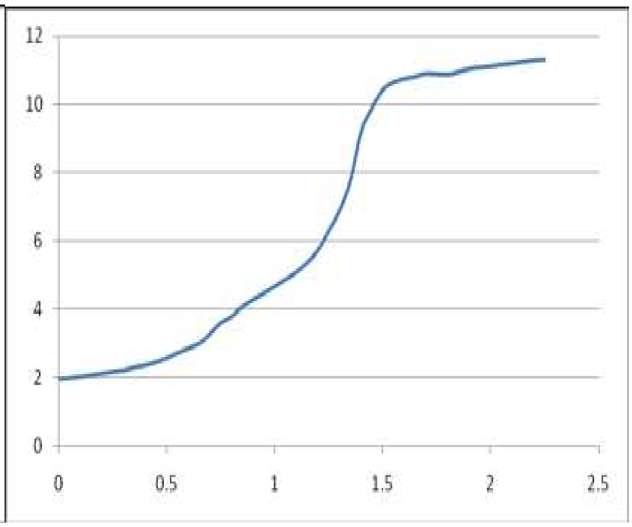

Fig.2 Isoelectric point curve of acetylated soy flour protein

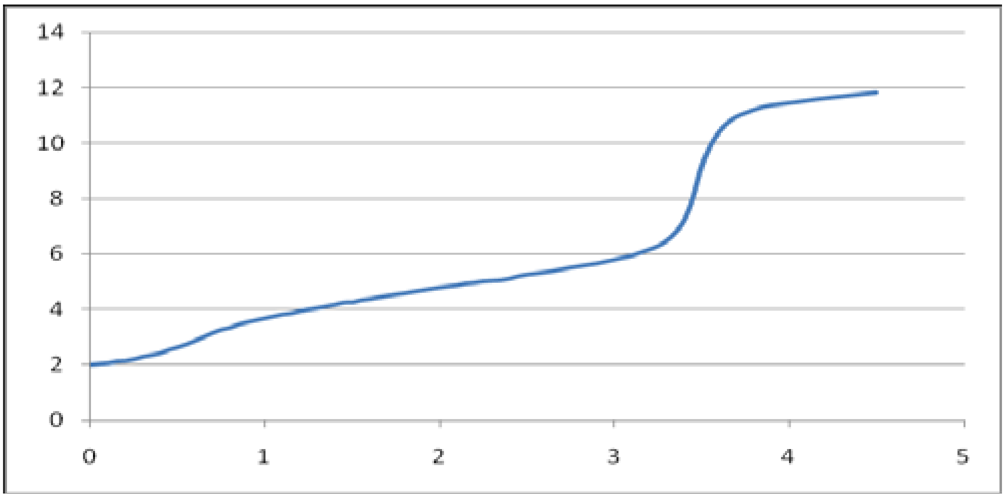

Fig.3 Isoelectric point curve of succinylated soy flour protein

3. ATR-IR

Attenuated total reflectance infrared spectroscopy (ATRIR) is an important spectroscopic tool for understanding protein structure in previous studies [12-15]. For the comparisons, the spectra were normalized using the peak at 3284cm^{-1}, which corresponded to N-H stretching of both secondary amides and secondary amines. The whole region of the spectra was to be checked for differences, but there was not any difference between soy flour with no PAE VS PAE (Fig.4). As shown in Fig.5, The peaks at 1630cm^{-1} and 1540cm^{-1} likely C=O stretching and N-H bending came from a secondary amide in the unmodified and acetylated soy flour, respectively. However, C=O stretching from a secondary amide in the succinylated soy flour shifted to around 1700cm^{-1}. And the peaks at 1400cm^{-1} and 1300cm^{-1} mostly related to CN stretching of N-C=O group and C-O stretching of O-C=O group, respectively.

4. Bond strength

The literature indicates that many other compounds can denature soy, but they fail to give us adhesive bonds with decent water resistance. Compounds, such as urea,guanidinium hydrochloride, and various surfactants, have been reported to denature the protein as a way to improve adhesion [16], but they have not seen acceptable water-resistance on their small scale veneer adhesion testing [10].

The bond strength was dependent of chemical modification and cross-linking, especially the wet strength of the soy flour adhesives was improved using PAE shown in fig.6, fig.7 and fig.8. The data did not strictly support improved dry and wet adhesive strength with the use of some denaturants with soy flour, but good wet strength was observed with the use of PAE resin, although there was nearly no difference between the soy flour protein and cross-linked protein with PAE in ATR-IR spectra. The PAE resin is an effective crosslinker for soy proteins and this innovative technology [3, 4] has been the main method used to develop good water resistance for soy wood bonding applications [5, 8, and 17]. The PAE provides products that meet the no-added formaldehyde (NAF) standards [18] and green building standards [19].

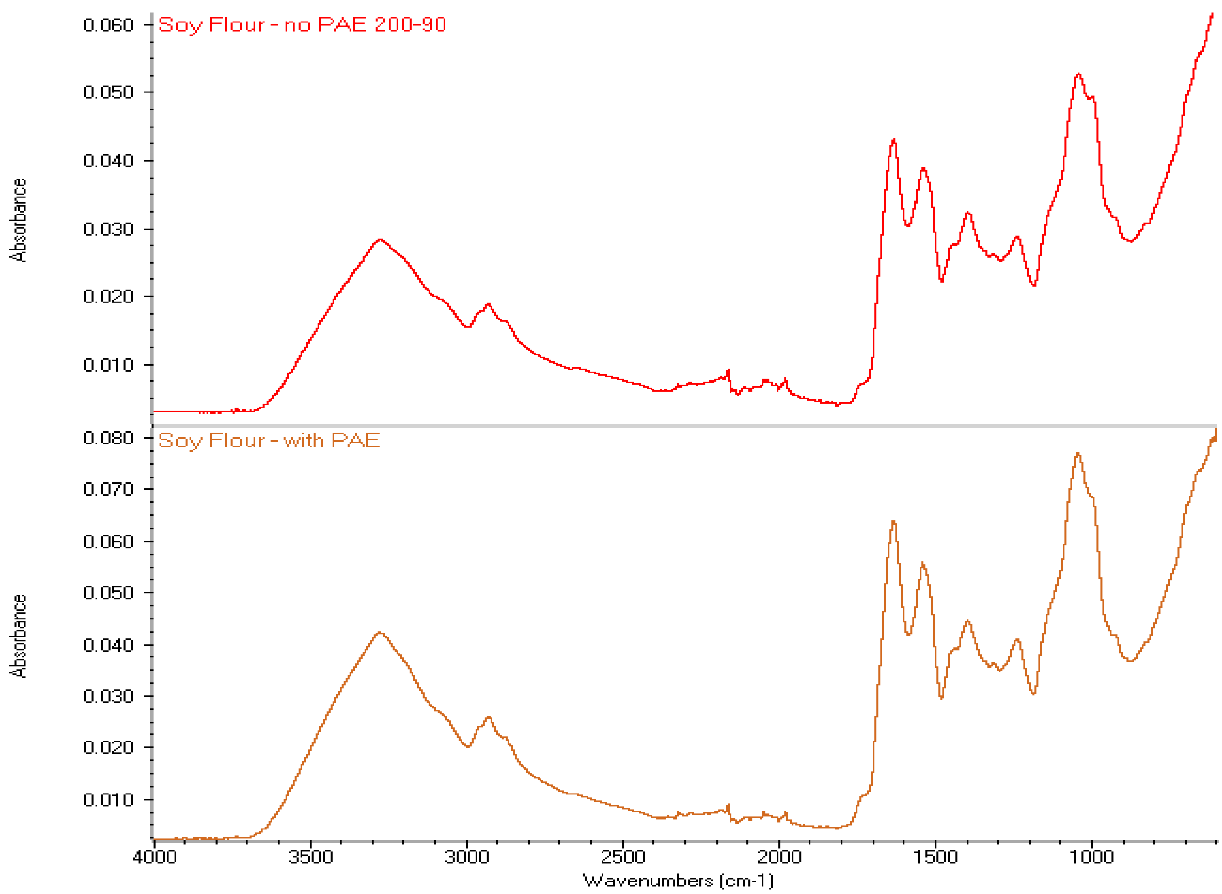

Fig. 4 ATR-IR spectra of soy flour with PAE VS no PAE

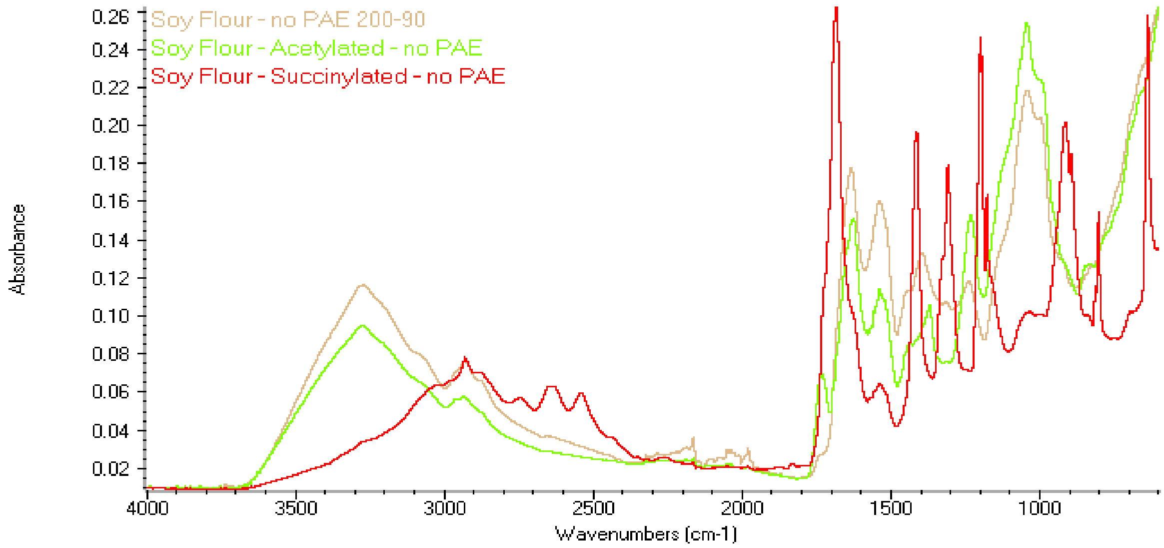

Fig.5 ATR-IR spectra of unmodified and acylated soy flour

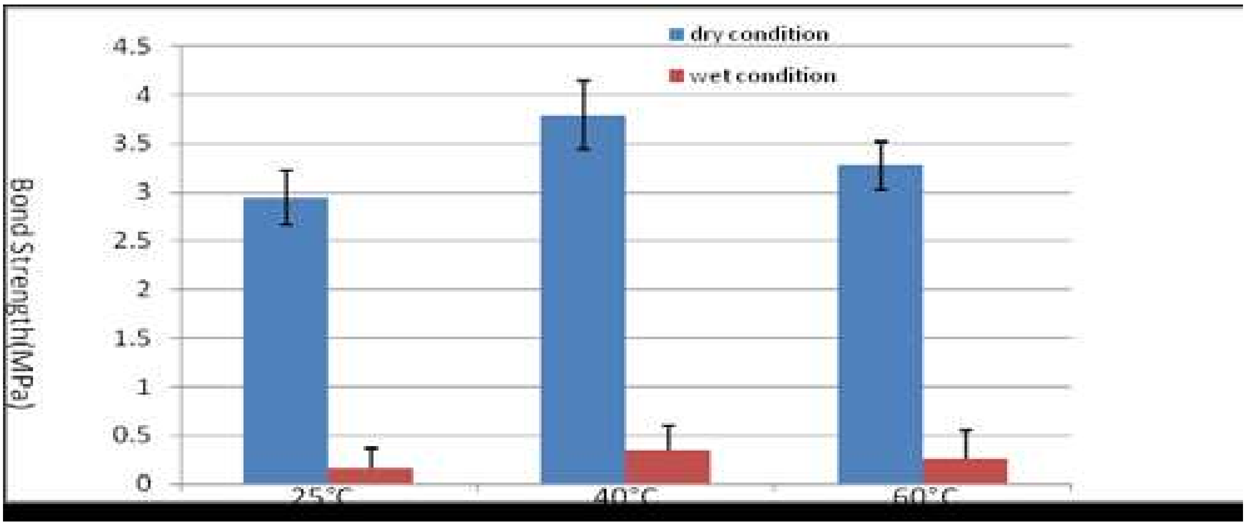

Fig.6 Bond strength of soy flour acetylated at different temperature

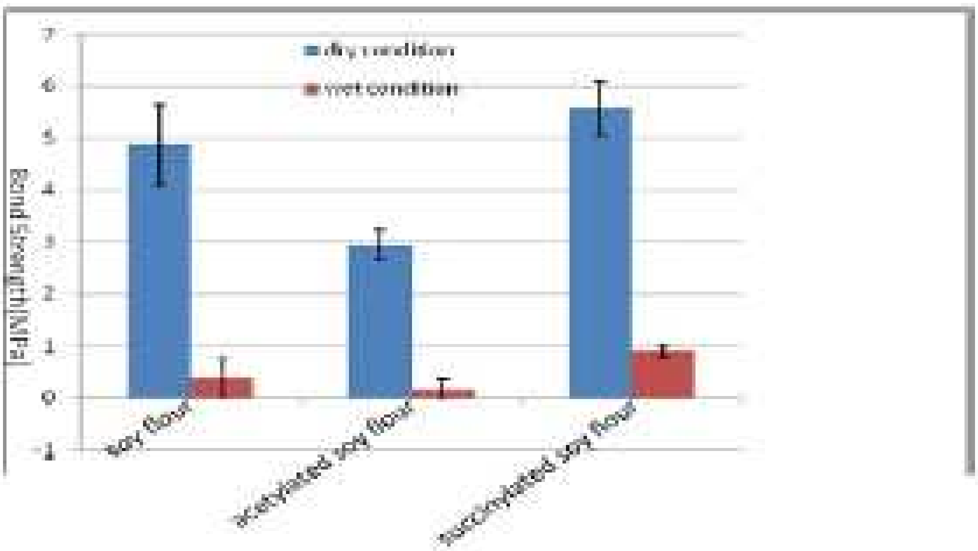

Fig.7 Bond strength of unmodified and acylated soy flour protein

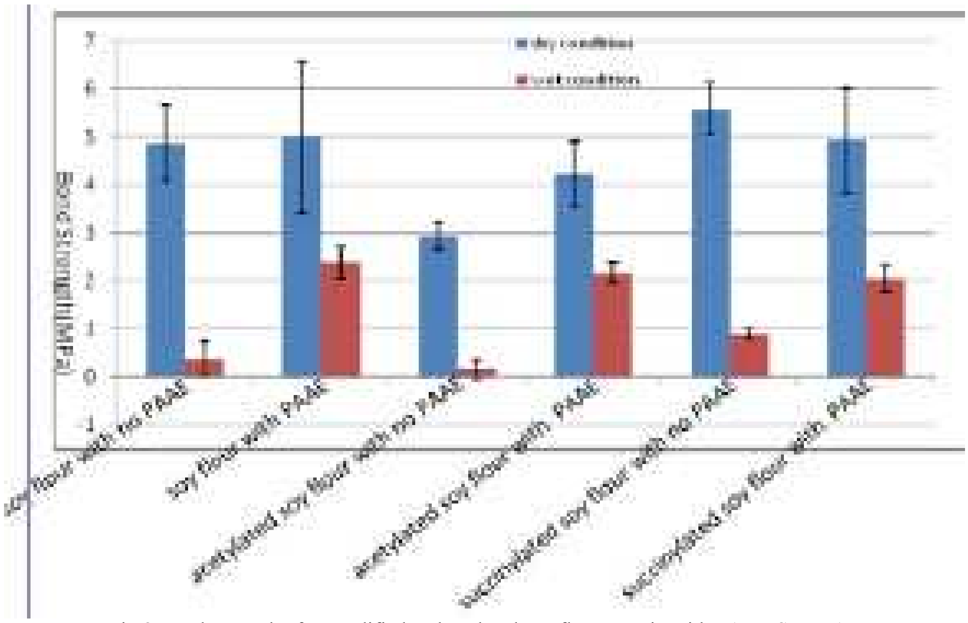

Fig.8 Bond strength of unmodified and acylated soy flour protein with PAE VS no PAE

Conclusion

The chemical changes in the soy flour in going from unmodified to acetylated and succinylated state were approved using UV and titration of isoelectronic point, especially the side chain attached to the soy flour molecular backbone was shown in ATR-IR spectra. The water resistance of Soy proteins adhesives was improved by appropriate succinylation or PAE cross-linking. In the future research, we should implement simple and environment-friendly method during the modification procedure. The reagents react with these amine and hydroxyl groups to amides and esters but the products have terminal reactive groups that can react with the PAE resin that is used to cross-link the soy adhesives for promoting the wet bond strength. And effects of additional factors on final properties of adhesives should also be considered, such as degrees of substitute, side-chain length or content of crosslinking agent.

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