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Soy Protein Structures and Wood Adhesive Performance Properties

Charles R. Frihart

USDA Forest Products Laboratory, Madison, WI, USA

Abstract

Formaldehyde emissions from urea-formaldehyde bonded wood products have led to consumer health concerns and to companies looking for no-added formaldehyde adhesives. Most of soy flour/meal is not consumed by humans and has a reasonable cost leading to research on soy wood adhesives. In the literature there is considerable valuable information about soy modification, but there are also important weaknesses in the understanding of fundamental protein structure. Nature developed non-structural proteins to be compact dispersions (rice-like) for dealing with very crowded cell environments making the common speculation that the hydrophobic proteins can become completely uncoiled (spaghetti-like) in water unreasonable. Changes in the internal protein structure (denaturation) can influence the soy colloidal properties and improve the wood adhesive performance by further hydrophobic/hydrophilic aggregation of proteins, but these changes do not eliminate the ability of the protein to absorb water and lower wet strength for the bonded wood products. Crosslinking of the proteins in soy products by adding other chemicals has been shown to play an important role in improving the wet strength of soy-bonded wood products at commercially viable levels. Thus, the model of tying together the dispersed particles, like sticky rice, has been accepted by food researchers and fits with the results in the adhesive literature.

Keywords: Soy, protein, wood, adhesive, agglomeration, water-resistance, folding, dispersion

Email: charles.frihart@usda.gov; cfrihart4@gmail.com

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18.1 Background

The very low-cost urea-formaldehyde adhesives have worked well for decades in producing interior wood panels (plywood, particleboard, and fiberboard) that meet the needs of producers and consumers, but these adhesives release formaldehyde by depolymerization under elevated heat and humidity conditions, providing an opportunity for protein adhesives to displace the urea-formaldehyde adhesives [1]. Of the available proteins, soy leads the list because the flour is available in large volumes at reasonable cost for a high protein content agricultural product [2, 3]. With proteins being approximately 45 % of the flour by weight, the remainder contains a variety of carbohydrates and other compounds that contribute to dry bond strength but lower wet bond strength. The protein content can be enhanced by aqueous ethanol extraction to yield soy concentrate (approximately 75% protein) or a two-step purification that terminates with precipitation of the protein to yield soy protein isolate (SPI) (>90% protein) [4, 5]. Although the flour is inexpensive enough for use as a wood adhesive, the high cost of the purified proteins due to the processing steps and the low yield make them too expensive for use in wood bonding. While examining the properties of purified components often helps to understand the chemistry and properties of mixtures [6], the commercial soy protein isolates (CSPI) have been so highly modified for performance in food applications that they do not provide a model for the native proteins in the flour [5]. The research on native soy protein isolate has mainly been limited to two groups: Sun's group at Kansas State University and the wood adhesive group at the Forest Products Laboratory. For understanding soy protein adhesives, an appreciation of the fundamentals of protein behavior using prior research in food science and general biochemistry is valuable [6] as are other general protein publications [7–9]. The food research is important since commercial soy products were developed to meet the desired properties for the food applications, and most of the general literature on protein structure is also related to this application.

18.2 Protein Structure

Each native protein has a well-defined folded structure due to its sequence of amino acids [6–9]. There can be some natural variation of the amino acid sequences if it does not alter the overall protein folding [10–14]. This sequence of amino acids is known as the primary structure and it controls

the secondary structure of helices and sheets, and the overall folding of the tertiary structure [7, 12–16]. The α-helix and β-sheets are controlled by certain sequences of amino acids [9, 16, 17]; the helices are stabilized by hydrogen bonds within a segment, and the sheets use hydrogen bonds between different segments of the same protein chain. The helices and sheets provide stabilization to the overall tertiary structure and are not disturbed until enough energy is applied to unfold these elements, which is referred to as the denaturation temperature [18]. In addition to the sheets and helices, after the hydrophobic collapse the tertiary protein structure is stabilized by hydrogen, polar, hydrophobic, disulfide, and acid-base bonding between individual pairs of atoms within the chain and can be altered by the pH, ionic strength, and chaotropic agents in the external aqueous environment. Because the proteins are mainly hydrophobic due to many of amino acids' side chains being aliphatic or aromatic groups and the backbone amide groups being shielded [7], they minimize their water interactions by folding into a globular shape [9–18]. The entropy of developing this tertiary structure is overcome by minimizing the interactions of hydrophobic residues with water environment and developing internal hydrogen bonding in addition to acid-base and disulfide bonds. The development of this native structure is described as an energy well [10, 12, 13, 19]. However, the sequence of amino acids does not let all the hydrophobic groups fold to the interior, leaving some of them on the surface [14, 17].

The individual chains can lower their energy further by associating with other protein globules to form a stable association classified as the quaternary structure. In the case of soy proteins, these are mainly the glycinin and conglycinin, which are, respectively, the 11S and 7S by size separation [20–22] (S is a Svedberg unit measured as sedimentation coefficient). These aggregates stay together under the basic and acidic conditions used to purify the SPI. X-rays can be used to generate the 3-D image of the 11S protein crystals with their well-ordered structure of 2-stacked hexagons, with each having six proteins (3 acidic and 3-basic) [20–24]. The 7S is not simple since it is made of 3 different protein aggregates, consisting of individual proteins which are often glycosylated [20, 22–24].

Although the helices and sheets are important structural elements of the protein configuration, these elements are physically connected to the rest of the protein chain by polar bonds and lipophilic attractions. About 50% of the amino acids in a globular protein are in a regular secondary structure (helices and sheets) with the remainder in defined structures such as turns and loops, and other connectors [25, 26]; the turns are often aided by proline amino acids due to their cyclic structure. In the wood adhesive literature, the tendency is to consider that the proteins are completely

unfolded to become a free-floating spaghetti-like polymer without explaining how the hydrophobic proteins can be stabilized in this extended form surrounded by water molecules [27–32]. When the temperature is very high or other extreme conditions, they can become uncoiled, but upon cooling return to the colloidal state [33, 34].

If the proteins find it difficult to completely unfold, how do the small protein globules (rice-like particles) bridge the millimeter distances between wood surfaces. First, as pointed out previously, the individual proteins aggregate with other proteins to form quaternary structures [17, 25, 34–38]. The driving force for these structures, in some cases, is interactions of polar groups, but in general polar surface interactions can be easily disrupted by water. On the other hand, the folding of the proteins results in some hydrophobic patches on the globular surface as illustrated in Figure 18.1 for more water-resistant hydrophobic attractions that result in the main protein aggregates of glycinin and conglycinin. Even these protein aggregates are not large enough to bridge the mm gap between the wood surfaces, since the individual crystals are about 10 nm in diameter [24]. At room temperature, the soy protein colloids are like sticky rice due to the hydrophobic surface interactions with high affinity for each other until enough shear is provided. Additionally, they are likely to form larger aggregates in more concentrated dispersions. The associations of proteins have been attributed to both hydrophilic and hydrophobic interactions between the surfaces of different proteins [25, 39–41]. It seems natural that the hydrophobic associations are likely to be less water sensitive because these interaction sites are kept away from water by the large bulk of the proteins. As illustrated in Figure 18.1, the protein surfaces are not smooth so that

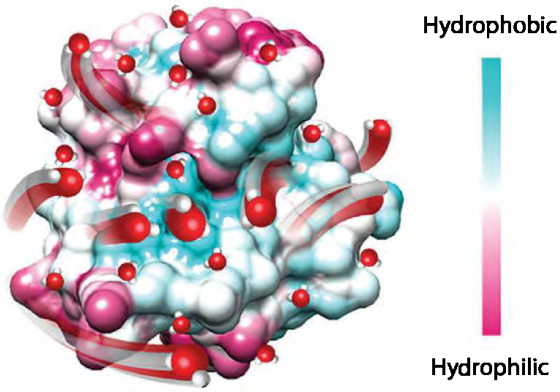


Figure 18.1 Protein surface showing polar and non-polar domains along with irregular surface and water molecules (red balls) with blue representing hydrophobic areas and purple representing hydrophilic domains (https://han.chem.ucsb.edu/research/role-hydration-water-molecular-recognition).

surfaces with the right topologies can form a tighter fit in between protein globules. An important property is that the protein globules develop higher order associations to form chains of beads [21]. This issue is explored more fully in another paper on soy protein adhesives [37].

18.3 Wood Adhesive Bond Strength

The largest adhesives market is in bonding wood for making panel products, such as particleboard and plywood, and structural products, like wood I-joists and glulam, and interior products, including cabinets and furniture. In some applications, the products not only need to meet dry strength, but in many applications, there are also wet strength requirements to account for the fact that wood expands and shrinks with variation in water exposure, causing internal strain on the bondline and adding to any externally applied force [78].

Proteins are natural adhesives, with some having good wet strength such as in the case of attaching mussels to different surfaces [39, 40]. These adhesives use a specific amino acid dihydroxyphenylalanine (L-dopa) that would be hard to obtain and expensive to use commercially for bonding wood. In contrast, plant proteins are widely available, especially soy proteins [2, 37, 42]. In the literature, several different tests have been used to measure the strength of wood bonds under dry conditions and sometimes after water exposure. The Forest Products Laboratory has been using the ABES (Automated Bond Evaluation System) test as described in ASTM D-7998 to evaluate protein-wood bond strength [43, 44] due to its use of thin, smooth veneers, rapid attainment of the set bondline temperature, low sensitivity to dispersion viscosity, and less time consuming to run, additionally giving a similar failure mode as other standard shear wood bond tests for interior plywood products [45, 46], but it is not a replacement for established product performance tests. The protein and other chemicals are dispersed in water at predetermined concentrations, spread on the wood and then bonded in a hot press before testing dry and wet after water soaking. However, because the wet tests are more difficult for adhesives to pass than the dry tests, with the wet tests often leading to bondline failure rather than the wood failure with the dry tests, the reported data has concentrated on the wet strengths [5, 45–47].

Soy flours come in several degrees of denaturation with those from Cargill being the native 90 PDI (protein dispersibility index), slightly denatured 70 PDI, and highly denatured 20 PDI, based on dry heat exposure conditions. PDI measures the percent of the protein that is replaced by

non-settling soluble in water after vigorous dispersion in water, followed by centrifugation as described in the American Oil Chemists Society's Protein Dispersibility Index (PDI) Ba 10b-09 test. As illustrated in Figure 18.2, there is not much difference in bond strength with varying degree of denaturation although the denatured proteins showed slightly improved wet strengths [48]. These flours have good dry strengths but do not come close to lower limit of 3 MPa for suitable wet strength; however, improved performance is observed at higher bonding temperatures [46], but bonding at 120°C is the upper limit for 2-5 minutes used for commercial applications. Additionally, the bondline temperatures are lower than 120°C due to greater insulation effect for thicker wood veneers, use of greater number of plies, heat required for evaporating the water in the adhesive

Thus, the soy flour is not suitable by itself under typical commercial bonding conditions, but some improvement was observed if the flour was processed to raise the protein content from about 45% to greater than 90% by removing the soluble and insoluble carbohydrates [5, 47]. However, an improvement was observed, especially in wet strength, again if the bonding temperature was increased (Figure 18.2) [46]. Two soy protein isolates SPIs were tested; the laboratory one isolated under milder conditions and a commercial one that had been jet cooked [5]. The higher temperature in improving the wet bond strength is a protein effect as shown in Figure 18.3 for both the native and jet-cooked soy protein isolates compared to Figure 18.2 for the flours; the effect is present also with added PAE (polyamidoamine-epichlorohydrin) crosslinker [46]. The explanations for this effect

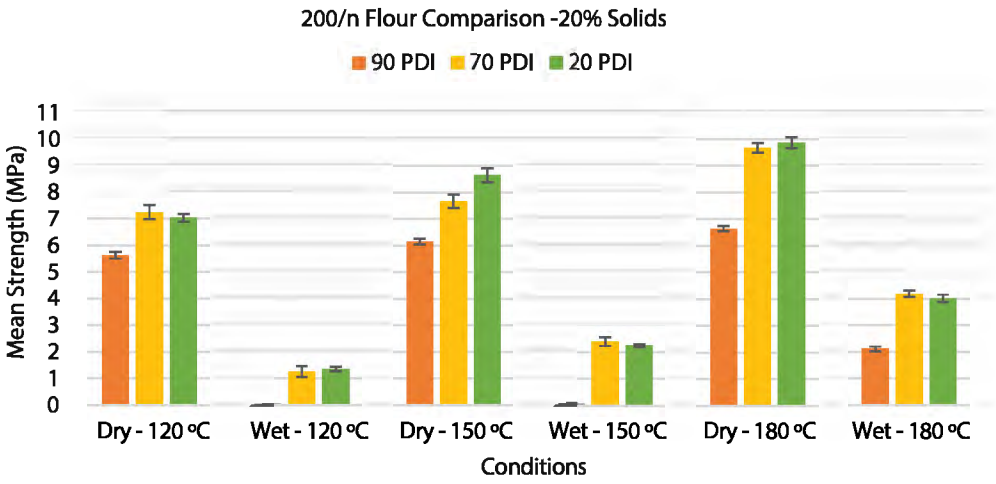


Figure 18.2 ABES bond strength before and after water soaking for soy flour of n=20, 70 and 90 PDI of 200 mesh particle size and at 20 wt% flour aqueous dispersion, using bonding temperatures of 120, 150 and 180°C [46].

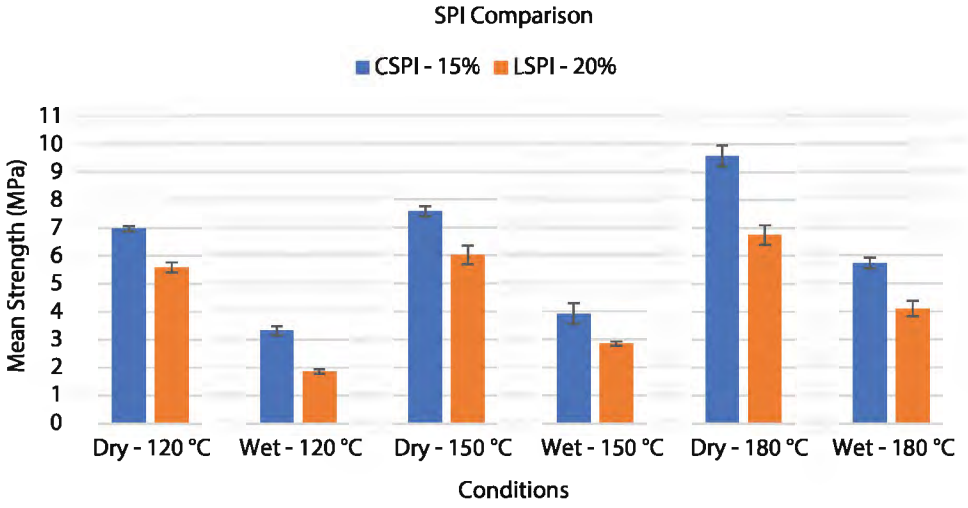


Figure 18.3 The effect of bonding temperature on dry (blue bars) and wet (red bars) strength, tested with maple veneers using ABES with both native (laboratory prepared soy protein isolate, LSPI) and commercial soy protein isolates (CSPI) [46].

can be that the proteins are rearranging either to form stronger bonds between individual protein globules, to entangle the protein chains near the surface, or to provide some protein crosslinking reaction. Since the purified proteins have a similar result with higher temperature bonding as the flour, the result is unlikely to involve carbohydrate-protein reactions: thus, Maillard crosslinking is unlikely.

Since increased heating in the bonding step was shown to increase the bond strength, as well as dry heating of the flour before bonding had an effect (denatured 20 PDI versus native 90 PDI in Figure 18.2), we wondered why did heating affect the bonding of the soy products and did this explain the difference between commercial and native SPI (soy protein isolate) [5, 47]. Figure 18.4 summarizes our findings about the difference between flours and isolates before and after two types of heating (wet autoclaving and jet cooking) [5]. The data show that while wet autoclaving improved the strength, this strength is considerably below that of the jet cooking results. Jet cooking also involves wet heating, but the heating time is shorter, the shearing of the sample is much higher, and the cooling of the sample is very rapid compared to the autoclave heating and cooling. Thus, jet cooking provides a very different soy protein structure that results in the strongest soy bond with no additives. However, this strength is unlikely to be due to entanglement of the protein chains since autoclave heating of the commercial SPI causes a significant reduction of the wet bond strength of the jet cooked SPI. Figure 18.4 also shows that either adding or

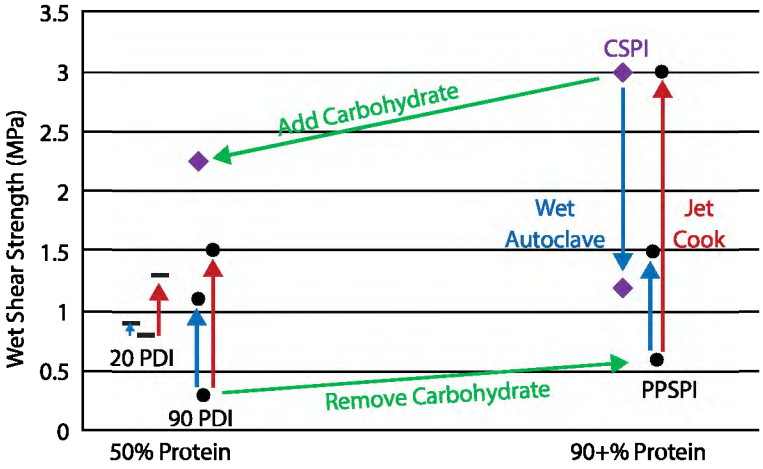


Figure 18.4 The wet strengths of bonds made with different soy formulations with emphasis on protein content from 45% for flour to 95% for SPI, and exposure to different heating conditions (autoclave and jet cooking prior to adhesive formulation). Starting materials: Black circle, 90 PDI flour; black bar, 20 PDI flour; purple diamond, CSPI (PROFAM® 974) and native isolate PPSPI made in the pilot plant. The arrows show the effect of different heating conditions and either adding carbohydrates to SPI or removal from soy flower. Source: [5].

removing the carbohydrates causes only a small change in the wet strength. Both of these heating processes increased the size of the gel particles of the uncured soy as exemplified by the higher viscosities for the dispersions [5].

18.4 Soy Dispersion Rheology

Protein rheology leads to a better understanding of the higher-order structures of the proteins [48–53]. The standard Brookfield cup-and-bob viscosity can indicate differences in the soy flours, but the shear history needs to be kept constant to be able to compare the measured values due to the agitation altering the viscosity value [48]. A better understanding of the protein structure is obtained using dynamic rheology that measures the properties starting from low shear that is ramped over time and separates the solid-like elastic data from the liquid-like flow data [51, 52]. This method shows that soy dispersions are initially thinned by both time-dependent and shear-dependent factors until a steady state condition when measured at low shear rates [53]. These changes are assumed to be caused by increased dispersion of the soy flour into smaller particles. These amplitude-sweep data were obtained using an Anton Paar MCR302

(Graz, Austria) with a CC27 cup and bob measuring system. The 90 PDI native, partially denatured 70 PDI, and the highly denatured 20 PDI flour show a similar steady state storage and loss modulus before dramatically decreasing (Figure 18.5) [53]. The globular structure of native state 90 PDI and a hypothetical extended linear (uncoiled) structure for the denatured 20 PDI soy would not be expected to have very similar curves contrary to what was observed; the uncoiled protein would not have a large initial plateau but show a steady decrease in viscosity with increasing shear. This data is consistent with the native and denatured soy containing larger associations of globular proteins held together by polar and non-polar surface interactions until enough force is applied to break them apart into component globules. The 20 and 70 PDI flours showed a more elastic response (difference between storage and loss moduli) initially compared to the 90 PDI flour as would be expected by a less compact globule after loss of the minimum volume for the native state. Additionally, both loss and storage moduli of the dispersion increased with the denaturation. One explanation is that the less compact denatured protein is taking up a larger volume, leaving less solvent available for flowing, making the entire mixture more rigid. Another interesting point is that the denaturing of the flour increases the amount of shearing needed to break up the initial rigid gel structure. The unusual second shear-thinning step indicates that while the first step breaks apart the gel structure to create a flowing liquid, the second

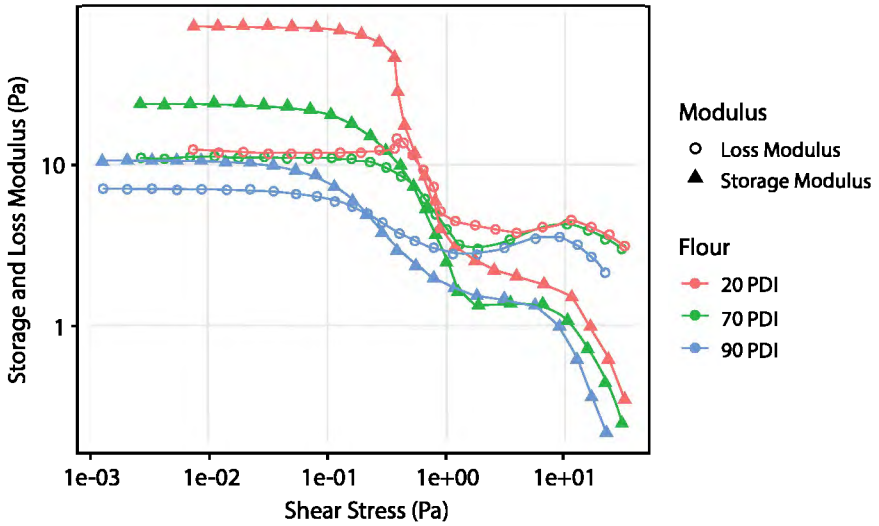


Figure 18.5 Amplitude sweep data for 20 (red), 70 (green), and 90 (blue) PDI flours at 20% solids in dynamic rheology experiments with the closed triangles showing the storage moduli and the open circles showing loss moduli. Only single replicates are shown for clarity, but the curves are quite reproducible [53].

transition mainly affects the storage aspect, but not the loss (flow) character of the dispersion, indicating a further fracture of the protein globules to give smaller particles.

One question is: whether the curve for the flours represents only the effect of the protein, or whether the other components of the flour have an effect on the rheology. Besides the protein, the other main components of the flour are the soluble and insoluble carbohydrates [5, 47, 48]. The soluble fraction consisting of low molecular-weight sugars and proteins is not likely to significantly influence the overall rheology. On the other hand, the insolubles, consisting of higher molecular-weight carbohydrates and proteins, are likely contributors to overall rheological properties of the flour because these insolubles showed significant storage and loss moduli when the insolubles were tested at the same concentration as in the flour (Figure 18.6) [53]. The native SPI made in the pilot plant (PPSPI) tested at the same concentration as in the flour looks a lot like the 90 PDI flour in the shear energy required to break the solid-like structure although the moduli are lower probably due to the overall lower solids content. This supports the assertion that the protein contributes most to the flour rheological properties.

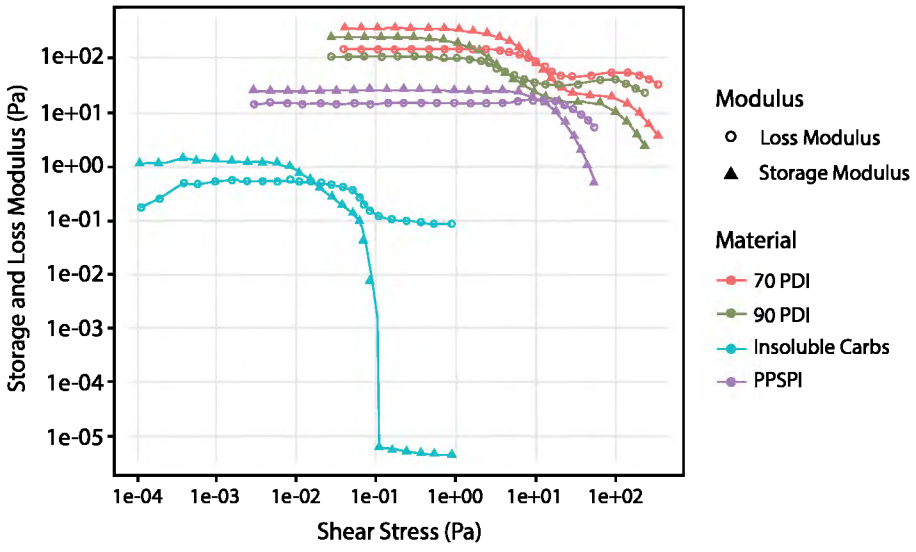


Figure 18.6 Storage and loss moduli versus applied stress for two soy flours (red for 70PDI and green for 90 PDI)) , at 30 wt. % native soy protein isolate in purple, and soy insoluble carbohydrate fraction in blue (at 15 and 7.5 wt. %, respectively, representing their proportional contribution to the soy flour). The closed triangles show the storage moduli and the open circles show loss moduli [53].

18.5 Protein Structure Revisited

Despite some wood adhesive literature claiming that the soy protein is completely unfolded in the denatured state [21, 27–32], most protein research has considered the unfolding to be a transient state between the original state and a new denatured state [7, 19, 54–63]. The arguments against soy protein staying unfolded are listed below:

- The proteins are naturally predominately hydrophobic, and many of the treatments, especially the most common thermal conditions, do not alter the portion of hydrophobic groups, but these may move more hydrophobic groups to the surface leading to greater aggregation and decreased solubility.
- For an unfolded protein to be stable, all the hydrophobic sections of the protein would need to be bound to water, resulting in the loss of the naturally strong water-water hydrogen bonds as the protein penetrates into the water domain. This would be thermodynamically very unfavorable.
- If the protein were extended, the entangled chains with the many interchain hydrogen bonds would provide a very strong bond as is exhibited by silk which does not need added crosslinkers to provide strength.
- Because of the molecular weight of 20 kDa or greater for the protein subunits [20, 42], unfolding would lead to very high viscosities for highly unfolded proteins, which are only observed at very high pHs.
- The dynamic rheology of the 20 and 70 PDI denatured flours have similar profiles to the native 90 PDI flour, which would not be expected if the denatured structures were unfolded [50, 51].
- The thermal denaturation of soy has shown that individual proteins form new combinations of aggregated subunits [62].
- The general protein literature supports the idea of the unfolded protein as just a transient state until conditions are appropriate for refolding [12–14, 19, 63–70].

Denaturation often leads to the formation of new and larger gel aggregates [25, 26, 55, 67, 69–71]. Thermal denaturation has been well studied

since it is used in food preparation and leads to gelation [36, 56, 65–69, 71]. The common model is that the intermediate cluster is a string of protein beads that becomes an interconnected gel network, as illustrated in Figure 18.7. The hydrated gel network for the cured soybean was observed by carrying out nanoindentation on a water soaked bondline made with a soybean adhesive. Despite the soy being cured under normal conditions, the indent in the bondline disappeared after being made; this is what would be expected for a gel structure where the indenter pushed water into adjacent cells and flowed back after removal of the indentation pressure. This makes more sense than the proposal of just increasing aggregate sizes [25].

Although bond strength can be formed from the linkage of proteins either by polar or hydrophobic bonds, the polar bonds are more likely to be broken by water exposure. The hydrophobic gelation hypothesis for the soy hydrocolloid is supported by the studies that show that the curing of ovalbumin from egg whites occurs by flipping the structure's surface from a polar to a non-polar configuration [68, 69, 72, 73]. In addition, from Figure 18.7, it is easy to visualize how the addition of a cross-linker can improve the strength of the gel structure by making additional or reinforcing existing connections between gel particles. This allows the data in the literature to be correct even though the papers incorrectly portray the soy protein structure.

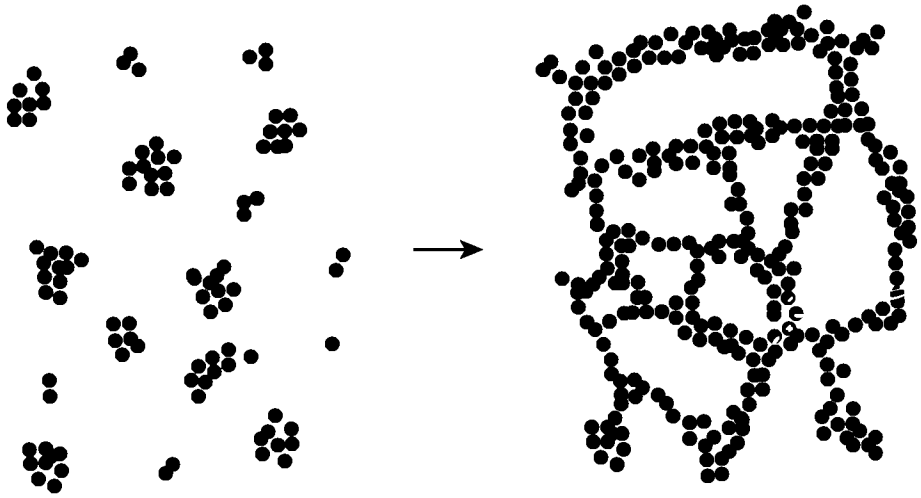


Figure 18.7 A hypothesis of soy protein curing by forming cluster of protein globules that becomes a porous gel with water filling the voids, taken from Oakenfull *et al.* [68].

18.6 Conclusion

Much of the wood adhesive literature contains incorrect information about the three-dimensional structure of denatured soy proteins, but in certain regards this does not negate the experimental data on performance properties using various crosslinking methods [37]. The unaltered fact is that the soy proteins do not form strong water-resistant wood bonds within the adhesive without some cross-linking between the proteins unless jet cooked. Some have claimed that the use of denaturing chemicals is sufficient to form strong bonds [21, 42, 74], but this has been contradicted by studies at the Forest Products Laboratory [75].

The reversible folding reaction differs from an ordinary chemical reaction in that generally no covalent bonds are made or broken when a protein unfolds and re-folds except for that some proteins are stabilized by covalently formed disulfide bonds that can be broken; the protein just re-equilibrates to different three-dimensional states in response to changed chemical and/or physical conditions that either disfavor or favor certain folded states [6, 12, 13, 18, 19, 36, 62–66]. Some unfolded state is a necessary step in going from the native structure to one of many folded denatured structures. Because proteins are predominately hydrophobic, the structures try to minimize water exposure of the hydrophobic domains by adopting globular shapes and by forming quaternary and higher-order structures to shield the surface hydrophobic domains from the water. Contrary to some of the literature, a fully extended state is unlikely to be feasible, and if it did exist, the proteins would form a highly physically crosslinked structure eliminating the need for cross-linking chemicals for water resistance.

Commercially available dry thermally denatured soy, such as 20 PDI flour, give only some improvement in wet bond strength. In contrast, the wet denatured soy isolate from heating in an autoclave or jet cooking gave improved wet strength, especially the latter [5]. It is not surprising that the presence of water makes it easier to increase gel size. Dynamic rheology showed that the native 90 PDI flour gave shear-thinning curves similar to the partially denatured 70 PDI and highly denatured 20 PDI, except that the latter ones had higher moduli, probably by occupying a greater volume [50].

The literature describes the denatured proteins as existing as a gel made using strings of beads of protein globules [68], with gelation existing as a lattice structure that can swell and weaken in the presence of water.

Crosslinking chemicals can improve wet strength by reinforcing the lattice and continue to be an active area of research [76, 77].

18.7 Summary

The importance of using soy proteins as wood adhesives has been hindered by an incorrect vision of denatured soy protein structures. Based on food and general protein literature, denaturation does not produce an uncoiled, linear structure but produces a highly aggregated structure of more loosely coiled globules. Understanding the food protein literature is important since the commercial soy products were developed for that market. In contrast, much of the soy adhesive literature incorrectly assumes complete opening of the protein structure. As an analogy, the soy adhesives in the normal pH range are rice-like rather than spaghetti-like. Thus, studies on soy adhesives should focus on dealing with the hydrocolloidal and gel properties of soy.

AI disclosure statement: The author confirms that no artificial intelligence (AI)-generated content or AI-assisted writing tools were used in the preparation of this chapter (including summaries and the abstract). All text, analysis, and conclusions are the result of the author's original work.

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