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RESEARCH ARTICLE

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Ovalbumin has unusually good wood adhesive strength and water resistance

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Abstract

The interest in non-formaldehyde adhesives has reinvigorated studies using proteins from plant and animal sources, with an emphasis on plant-based sources, especially the oilseed plants such as soybean. Although animal-based sources have received much less attention, and ovalbumin from egg whites has not been studied at all, it provides a surprising low viscosity and high wet strength as a wood adhesive. Of the animal and plant proteins used in the past, egg protein has been used for centuries as a binder in tempera, for book illumination, and for gilding, but not as a water-resistant wood adhesive. The isolated ovalbumin is the best, but dried egg whites and whole egg have good adhesive strength. The parameters for ovalbumin wood bonding are examined with thin veneers using ASTM D 7998 and plywood tests using D 906. Sodium metabisulfite, sodium periodate, urea, and polyamidoamine-epichlorohydrin (PAE) improve the wet bond strength, but the surfactants CTAB and SDS at low concentrations do not. Although the high protein content of the ovalbumin is important for good strength, the low viscosity of the protein adhesive provides further insight into protein–protein interactions.

KEYWORDS

modifiers, ovalbumin, proteins, wood adhesives

1 | INTRODUCTION

In the past, many proteins from different sources were used as adhesives, including plant (soybean) and animal (blood, several collagen sources, and casein from milk).[1–5] However, synthetic adhesives have mostly replaced these bio-based materials by having better water resistance, ease of use, and lower cost. Recently, the use of biomass for adhesives and other applications has received increasing interest.[1,3,6–9] Using natural materials can be advantageous because they are polymeric materials with many different functional groups available for adhesion to a variety of surfaces and/or for further modification.[9] However, problems with biomass materials often result from the presence of undesired components and variability in composition, in contrast to synthetic materials that are made from monomers allowing the construction of designed polymer structures.[10]

The most common oilseed, soy, has been used extensively in wood adhesives because of its consistent composition and availability in large quantities at a low cost.[11–15] However, the flour contains about 45% protein with about an equal amount of carbohydrate. The protein can be separated from the carbohydrate to provide commercially available soy protein isolates, but the cost goes up by a factor of

Linda F. Lorenz and Charles R. Frihart contributed equally to this study.

about 10. In addition, like many proteins and carbohydrates, soy dispersions are viscous unless the solids content is very low.[16] Soy protein,[14,17] like many plant proteins,[1,18] is a complex mixture of associated proteins made up of different protein subunits. Purified soy proteins are commercially available, but they are still a mixture and are usually altered so that they are not good models for the native soy proteins to try to understand adhesive strength.[19–21] The native soy proteins can be prepared in the laboratory and are lower in viscosity and in strength to most of the commercial soy proteins.

In contrast to soy protein, ovalbumin from egg whites is quite pure and readily available, so it was included as a model protein for studying protein wood adhesive properties and because it has not been studied as a wood adhesive. The unusually low viscosity and very good wet bond strength of ovalbumin as an adhesive with wood has been demonstrated in our prior research.[22] The effect of bonding parameters and additives on the ovalbumin bond strength with wood has been more extensively examined in this subsequent research and compared to that of soy proteins. Our attempts to find a synergy of improved strengths or lower viscosity by mixing soy with ovalbumin to obtain good adhesives were not very successful.

2 | RESULTS

The first step of adhesive testing used the ABES equipment for making small specimens according to ASTM D 7998 because the bonding variables of temperature and time can accurately be tested, and both dry tensile strength and wet strength after water soaking can be determined.[23] The water soaking is important because many adhesives with acceptable dry strength do not retain sufficient strength after the water soak. The other advantage of the ABES with smooth maple face veneers is that it measures the cohesive strength of the adhesive with little influence of veneer quality on the wood bond. It does not replace the standard adhesive tests for product evaluation but allows a better understanding of the performance of adhesives with very different viscosities.

2.1 | Ovalbumin and egg results

In trying to understand the adhesive strength results relative to the viscosity of soy adhesives,[11,16,21] we decided to study ovalbumin from egg whites, which is a readily available protein that can be obtained as a purified protein.[24–27] Table 1 points out the large differences in strength between commercial soy flour (about 45% protein) and commercial and laboratory soy protein isolates (CSPI and LSPI, about 95% protein), especially when the bond is tested wet. The ovalbumin was better in wet strength than the best commercial soy product (CSPI) but had 1/430 of its viscosity at twice the concentration. While this result was very exciting, it indicated that ovalbumin was probably not a good model for studying soy proteins due to the great differences in viscosity. One limitation of soy adhesives is that the solids content cannot be increased without becoming too thick for normal application processes. Soy adhesives can have a much higher viscosity because they are shear thinning and become less viscous with stirring, in comparison to phenolics which are Newtonian. For bonding wood, the viscosity of soy adhesives can be as high as 50 Pa s, whereas the viscosity of phenolics is about 0.5 Pa s.

Because ovalbumin was a good adhesive compared to CSPI (Table 1), but less viscous,[22] we decided that its properties as a wood adhesive needed to be studied further. First, various dried egg products (whole, white, and yolks) were tested to find out which provided the most dry and wet strength. In Table 2, the data shows not surprisingly, that the white portion with the highest protein content has the best wet strength. Additionally, the more purified Grade III ovalbumin did not provide a great improvement over the crude Grade II, so further work used Grade II.

LSPI had low viscosity and low wet bond strength, while CSPI was just the opposite with high viscosity and high wet bond strength.[21] The commercial SPI that we used was jet cooked for greater functionality, which greatly increased its viscosity. There are commercial SPIs with low viscosity, but these also have low strength. Thus, even though the ovalbumin is not a good model for soy, it is an interesting adhesive by itself.

At 30% solids, ovalbumin was low in viscosity which could be a problem in that less viscous adhesives often result in poor veneer bonding due to over-penetration of the wood resulting in a starved bondline. Thus, the bond strength of ovalbumin from 20% to 50% solids at pH 6 and 7 was determined **(**Figure 1**)**. Wet strength improved at higher concentrations (from 1.8 MPa at 20% solids to 4.9 MPa at 50% solids) with higher strengths at pH 7 compared to pH 6. At pHs higher than 7 the strengths did not increase. Higher dry strengths were also observed for the higher concentrations, but as the solids increased the mixtures became difficult to mix completely. The higher strengths are probably due to more protein in the bondline because the viscosity of the 40% ovalbumin is 28 times the viscosity of the 30% ovalbumin, but there is not an equal increase in the strength of the 40% compared to the 30% ovalbumin, as shown in Table 1.

Because soy adhesives increased greatly in wet strength with higher bonding temperatures,[28] it was of

TABLE 1 Properties of soy and ovalbumin adhesives with the viscosity in Pa s and ABES lap shear strength in MPa.

		Bond strength (MPa)	
Adhesive	Viscosity (Pa s)	Dry	Wet
Soy flour, 30% solids	6.4	5.8 ± 0.1	0.3 ± 0.1
CSPI, 15% solids	108.0	9.4 ± 0.5	2.8 ± 0.3
LSPI, 15% solids	5.7	5.2 ± 0.8	1.2 ± 0.2
Ovalbumin II, 30% solids	0.25	7.1 ± 0.4	3.4 ± 0.1
Ovalbumin II, 40% solids	7.2	7.8 ± 0.9	4.6 ± 0.2

TABLE 2 Properties of egg proteins with the ABES lap shear strength in MPa.

			Bond strength (MPa)	
Adhesive	Protein (%)	Fat (%)	Dry	Wet
Ovalbumin, Grade II, 30% solids	62–88	NA	7.1 ± 0.4	3.4 ± 0.1
Ovalbumin, Grade III, 30% solids	>90	NA	6.5 ± 0.2	3.6 ± 0.3
Egg whites, 35% solids	>80	0	5.2 ± 1.9	3.7 ± 0.7
Egg yolks, 35% solids	>30	>56	2.2 ± 0.2	0.4 ± 0.2
Whole egg, 35% solids	>45	>40	3.5 ± 0.2	1.5 ± 0.2

Note: The values for ovalbumin represent the ovalbumin content, not the protein content. NA, not available.

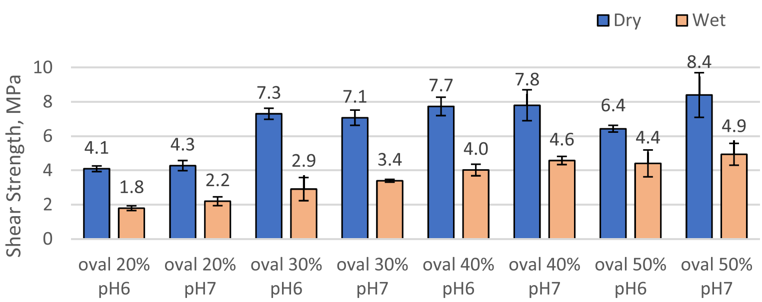

FIGURE 1 ABES dry and wet strength of ovalbumin at pH 6 or 7 and 20% to 50% solids bonded at 120°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

interest to see if ovalbumin had a similar result. The wet bond strength increased from 100 to 150°C, but then decreased at 160°C **(**Figure 2**)**, so the optimum bonding temperature was around 150°C when bonded for 2 min in the ABES. These results are very different from the soy wet strengths that continue to increase significantly even at 180°C, the highest temperature tested.[28] The development of good wet bond strength at all temperatures indicates that the ovalbumin cures readily.

2.2 | Modified ovalbumin

Literature on soy adhesives has claimed improved wet bond strengths with added denaturants,[29,30] but we have not observed these effects.[31] This difference can be due to a difference in viscosity because most studies have used plywood tests which are more sensitive to viscosity for obtaining good adhesion than the ABES test. Figure 3 shows the dry and wet shear strength of ovalbumin (20% solids) with urea (chaotropic agent), or two surfactants, cetyl trimethylammonium bromide (CTAB) or sodium dodecyl sulfate (SDS) added. Urea increased the ovalbumin strength and CTAB decreased them. SDS increased the strengths with ovalbumin at 8% addition in contrast to LSPI where it decreased the strengths. This is another example of the difference between ovalbumin and soy protein. An explanation is that when heated, ovalbumin undergoes a conformational change from its soluble structure into an insoluble all-β-sheet structure with exposed hydrophobic regions[32] which can react with the SDS to increase the bond strength.

2.3 | Reacted ovalbumin

In contrast to adding chemicals that can denature proteins and change their properties, such as chaotropic or surfactant agents, adding reactants increased the wet strength of soy proteins.[29,30] The reactants can be divided

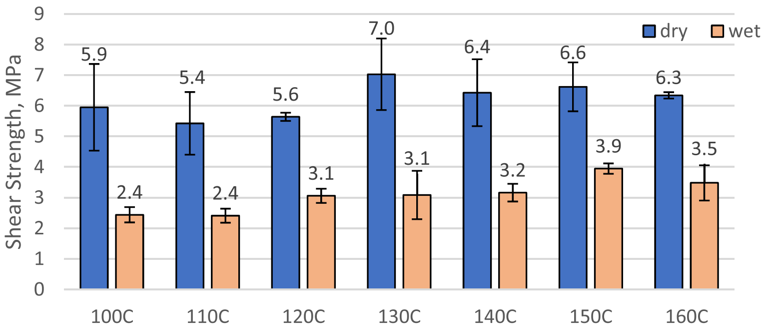

FIGURE 2 Dry and wet ABES shear strengths of ovalbumin at 30% solids bonded at temperatures from 100 to 150°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

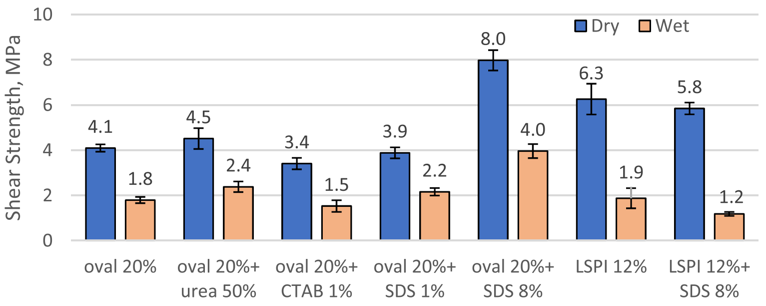

FIGURE 3 ABES dry and wet shear strength of ovalbumin (20%) plus urea, CTAB, or SDS or LSPI (12%) plus SDS bonded at 120°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

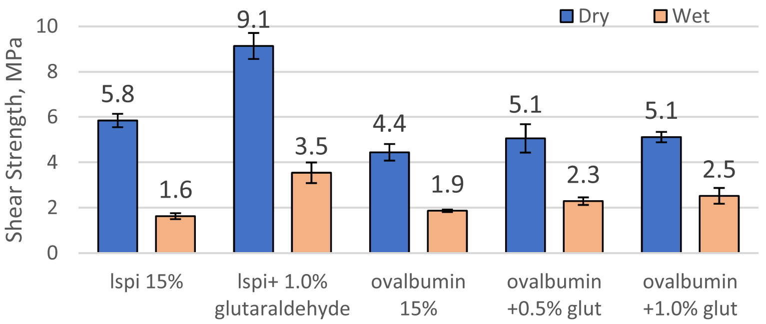

FIGURE 4 Ovalbumin (15% solids, plus glutaraldehyde (glut) compared to LSPI (15% solids) using ABES and bonded at 120°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

into two groups: those that just modify the protein to improve its cohesive strength and those that are adhesives that bond wood on their own in addition to reacting with the protein.

Aldehydes, especially formaldehyde, have long been known to react with proteins and improve their strength as adhesives,[33,34] but recent work has used glutaraldehyde to replace the disfavored formaldehyde.[7] Figure 4 shows the dry and wet shear strength of ovalbumin (15% solids) with glutaraldehyde added compared to LSPI. The wet bond strengths increased with increasing glutaraldehyde from 0.5% to 1.0% of ovalbumin solids, which is consistent with what we have seen with soy adhesives, but the effect is smaller with ovalbumin.[34]

The increased strengths of LSPI or soy flour with added glutaraldehyde could be the aldehydes linking groups like lysine together on different proteins. On the other hand, sodium metabisulfite (SMBS) breaks disulfide cross links to form sulfide groups which can decrease strength due to cleavage of the protein cross-links but adding SMBS to ovalbumin increased the strength (Figure 5). The difference in strength between LSPI and ovalbumin with addition of SMBS is perhaps because the SMBS disrupted the structure of the LSPI to reduce the wet strength but opened up the structure of the ovalbumin to increase bonding to the wood and increased the wet strength. The literature indicates that ovalbumin gelation is due to hydrophobic interactions of the proteins and not due to disulfide formation.[32]

We have discovered that periodate can improve the strength of oilseed flours, potentially by cleaving the carbohydrates into dialdehydes that can react with the lysine groups in the proteins.[34] Although this reaction was intended to improve the wet strength of flours with abundant carbohydrates to modify, it also improved the strength of purified proteins. The data in Figure 5 not only showed an increase in the ovalbumin and LSPI strength, but also the highest wet strengths for proteins

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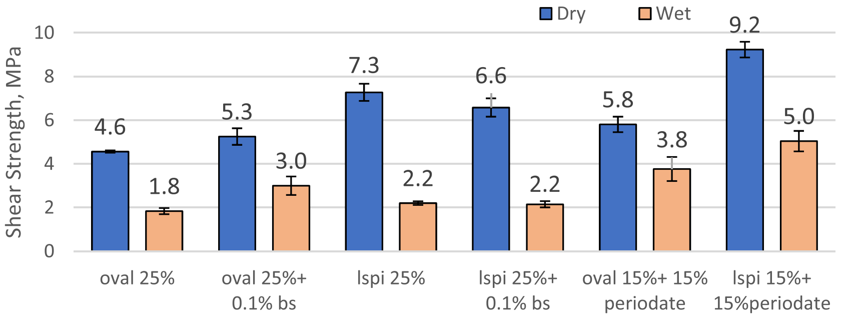

FIGURE 5 Ovalbumin (25% solids) compared to LSPI (25% solids) plus sodium metabisulfite (bs), or ovalbumin (15% solids) compared to LSPI (15% solids) plus periodate using ABES and bonded at 120°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

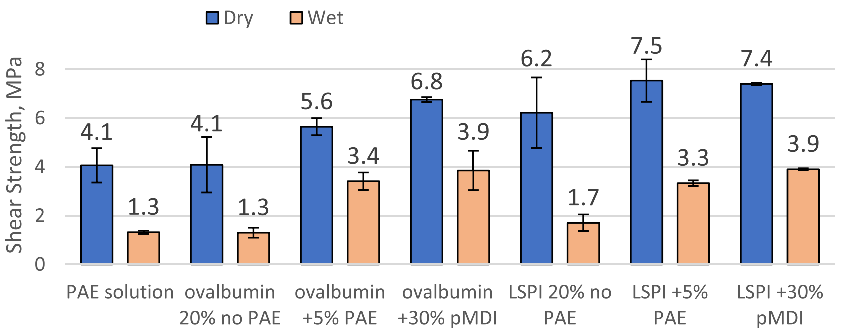

FIGURE 6 Dry and wet ABES shear strength properties for ovalbumin (20%) and (LSPI 20%) with PAE (5%) and pMDI (30%) bonded at 120°C for 2 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

that we have tested using the ABES method and bonding at 120°C. This could be the glycosides attached to ovalbumin and LSPI glycoproteins reacting with periodate and/or the α-amino acids in the protein reacting with the periodate.[35]

In addition to the additives that can modify the proteins but are not adhesives themselves, co-adhesives can be used with the proteins. A common co-adhesive that is used with soy for interior adhesives is polyamidoamine-epichlorohydrin (PAE), which is combined with soy flour for plywood adhesives.[29,30] In Figure 6, it is clear that the PAE at 5% of ovalbumin or LSPI solids greatly improves the dry and wet bond strength. Another co-adhesive, polymethylenediphenyl diisocyanate (pMDI) at 30% of solids, also increased the dry and wet strength, but more pMDI (30%) had to be used to obtain as much wet strength as PAE at 5%.

2.4 | Plywood

Although the ABES testing is very informative, it does not replace the standard product testing. The main market for protein adhesives has been interior plywood assembly; thus, it was important to test the ovalbumin adhesive with the standard ASTM D 906 plywood test.[36] By themselves, soy flour or LSPI will not pass this test, but they do well when combined with PAE.[37] Plywood can be made with CSPI at 15% solids, but higher solids are more difficult to use due to a high viscosity. The viscosity was 108 Pa s for 15% CSPI solids and too high to determine for 20% CSPI solids compared to ovalbumin at 7.2 Pa s for 40% and 38.0 Pa s for 45% solids. Because the ovalbumin is low in viscosity, an adhesive of 45% solids

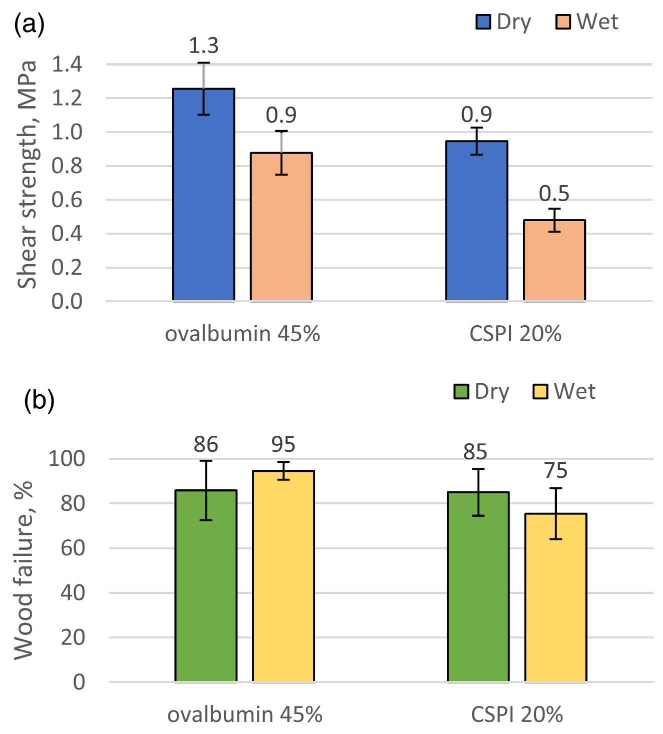

FIGURE 7 (a) Dry and wet shear strength and (b) wood failure of 3-ply poplar plywood bonded with ovalbumin (45% solids) compared to CSPI (20% solids) using ASTM D 906 and bonded at 150°C for 5 min. The vertical lines within the bars are one standard deviation. [Color figure can be viewed at wileyonlinelibrary.com] could be used for bonding the three-ply samples. The veneers used in standard plywood tests are much rougher than those used in the ABES testing and require higher viscosities to provide adequate holdout on the surface. As shown in Figure 7a,b, the CSPI at 20% solids had good bond strength and good wood failure in both the dry and wet shear tests. The ovalbumin showed high strength dry and wet and good wood failure at 45% (Figure 7a,b). High wet wood failure has not normally been found with protein adhesives, making the ovalbumin results very unusual.

3 | DISCUSSION

Ovalbumin protein from egg whites is an interesting wood adhesive, which is not totally surprising since it was used for a long time as a binder in tempera, for book illumination, and for gilding. Its reported use as an adhesive has been limited,[22,38] because it has mainly been used in food products. Our original idea that ovalbumin could be a good model for studying soy protein adhesives is very dubious at this point because it has high strength at low viscosity, and the small increase in bond strength with high temperature bonding distinguishes ovalbumin from soy and other oilseed proteins.[34] The low associative aspect of the low viscosity of the ovalbumin adhesives compared to the soy adhesives with their high associative nature are related to their protein characteristics.

Egg proteins, especially ovalbumin, have been well studied for their properties and structure.[1,26,27,32,38–40] Egg whites are mainly albumin (about 75%), but also contain a variety of other proteins. The ovalbumin is a single protein of 45 kDa, pI = 5.1 compared to soy, which is mainly 11 S glycinin aggregate (360 kDa, pI = 6.4) made up of acidic (37 kDa, pI = 4.6–5.4) and basic (20 kDa, pI = 8–8.5) subunits and 7 S aggregate (360 kDa, pI = 4.5) made up of α (58 kDa, pI = 5.2), α' (57 kDa, pI = 4.9), and β (42 kDa, pI = 3.5) subunits. In addition, ovalbumin is a phosphoglycosyl protein containing one N-glycosylation site, while neither fraction of soy is phosphorylated and mainly the 7 S subunits are glycosylated. The ovalbumin proteins are single chains with little interaction with each other. In contrast, most of the soy proteins are aggregated globules that most likely have many of the reactive amino acids inaccessible to reaction with denaturing chemicals.

While both the whole egg and white portion of the egg have wet strength, the egg white portion has the highest protein content and highest strength. The clarity of the egg white portion indicates that the proteins are dispersed in very small particles or are soluble. The low molecular weight of the protein and the literature data indicate that the protein is not aggregated under neutral conditions.[26,27,39] This factor explains the low viscosity of the ovalbumin compared to the highly aggregated proteins of the soy products in Table 1.

Hu reported "The native ovalbumin shows a mixture of α-helix and β-sheet, while both the heat and alkali treatments are able to transform the native protein into a predominance of β-sheet secondary structure." The results reported here substantiate that the altered protein forms bearing increased β-sheet structures are prone to hydrophobic aggregation.[32] This denaturation and aggregation could be an explanation for the high strength of the bonded ovalbumin.

An additional difference in ovalbumin is that it contains four non-exposed sulfide groups. The addition of SMBS was to prevent the thiol groups from joining ovalbumin molecules together by limiting sulfide to disulfide formation during adhesive bond formation. However, the data in Figure 5 show that the SMBS actually improved the bond strength rather than decreased it; thus, aggregation by disulfide bond formation does not seem to be a good model for explaining the ovalbumin wet bond strength. The presence of phosphate groups in the ovalbumin might be an explanation because casein is a good wood adhesive that also contains phosphate groups.[41] Efforts to show that phosphorylation of soy proteins would improve soy flour strength were somewhat successful,[42] but it is not clear that it was specifically the phosphate groups on the protein or denaturation from the reaction conditions that improved the strength. Although the reaction with SMBS and SDS was different between ovalbumin and soy protein, the limited effectiveness in adding denaturants (urea and CTAB) to ovalbumin was similar to that with soy protein.

The reactants (glutaraldehyde, PAE, pMDI, and periodate) that increased wet strength of soy protein also increased wet strength of ovalbumin. From the literature, the glutaraldehyde is expected to cross-link the lysine groups on different protein chains,[1,34] while the PAE can react with the carboxylic acid groups on the proteins and carbohydrates,[43] and pMDI can react with almost any functional group. Periodate reacts with both carbohydrates[31] and proteins.[34]

4 | CONCLUSIONS

Although the egg white ovalbumin is quite different from soy proteins, compositionally and structurally, it can also be used as a wood adhesive and is better in wood bonding than soy proteins, producing very good bonds when tested dry or wet. The real advantage of ovalbumin is the low viscosity compared to soy and other protein adhesives, and the higher solids at low viscosity results in easier application of the adhesive.

The structure of ovalbumin is very different from the soy proteins commonly tested as wood adhesives in that it is a monomeric protein rather than a protein aggregate. Also, it is phosphorylated and has free sulfide groups. Although the ovalbumin is a good adhesive by itself, the properties can be greatly improved by adding reactants such as PAE, pMDI, SMBS, periodate, and glutaraldehyde, but generally not with chaotropic agents or surfactants, except for SDS.

Research grade ovalbumin was used for this fundamental research on the wood adhesive properties of ovalbumin. The economic feasibility as a wood adhesive was not determined, but using non-food grade ovalbumin, or crude egg whites, would be less expensive than research or food grade ovalbumin.

5 | EXPERIMENTAL SECTION/ METHODS

5.1 | Materials

Cargill Inc. (Cedar Rapids, IA) provided Prolia™ 200-90 soy flour and ADM (Decatur, IL) provided commercial soy protein isolate (CSPI) (PRO-FAM 974). Laboratory soy protein isolate (LSPI) was prepared in our laboratory.[18] Ovalbumin (Grade II 62-88% and Grade III >90% purity) were obtained from Sigma Aldrich (St. Louis, MO), while the dried whole eggs, dried egg yolks, and dried egg whites were provided by Ballas Egg Products Corp. (Zanesville, OH). Columbia Forest Products (Chatham, VA) supplied the hard maple veneer for the ABES tests and poplar veneer for plywood was supplied by Besse Forest Products (Gladstone, MI). Sodium metabisulfite, sodium periodate, cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), glutaraldehyde, and urea were obtained from Sigma-Aldrich (St. Louis, MO) and used as received. Polyamidoamine-epichlorohydrin (PAE, CA1920) was obtained from Solenis (Wilmington, DE). Polymethylenediphenyl diisocyanate (pMDI) was provided by Huntsman (The Woodlands, TX).

5.2 | Adhesive preparation

Soy material (12%–30%) or egg product (15%–50%) was dispersed in water and the mixture was stirred for 5 min. When other components were added, the mixture was stirred for an additional 5 min. Amounts of additional components were calculated as a percent of protein solids.

5.3 | ABES

Adhesive was applied to 5 mm of the end of one piece of maple veneer (117 × 20 × 0.6 mm^3), which was overlapped 5 mm with another piece of veneer. Samples were hot pressed in the Automated Bonding Evaluation System (ABES) equipment (Corvallis, OR) at 0.2 MPa at 120°C for 2 min.[23] The bonded wood samples were equilibrated at 21°C and 50% relative humidity at least overnight before testing dry or wet after soaking in water for 4 h at room temperature. Three or four specimens each, dry or wet, were tested in tensile shear with the ABES and the average and one standard deviation are shown in the figures.

5.4 | Viscosity determination

Viscosities of the adhesives were measured with a Brookfield DV-E viscometer at 22°C by rapidly hand mixing the sample for 30 s, immediately inserting the #6 spindle, waiting 10 s, then turning on the motor at 20 rpm and recording a reading after 10 s.

5.5 | Plywood preparation

Poplar 3-ply plywood panels (3.2 mm/veneer ply) were bonded with ovalbumin at 45% solids or CSPI at 20% solids and tested for shear strength and wood failure.[36] The panels were bonded with ovalbumin or CSPI at 150°C for 5 min at 0.86 MPa (125 psi) after a closed assembly time of 15 min. The spread rate was 200 g m^{-2}. The panels were conditioned at 26.7°C and 30% RH for 1 week before being cut into samples. For testing shear strength, the panels were cut into 8.26 × 2.54 cm^2 samples according to ASTM D 906,[36] and half were tested dry, and half tested wet after soaking in room temperature water for 24 h. In a modification of D 906, all samples were pulled with the lathe checks open to obtain more consistent data than pulling some open and some closed.

AUTHOR CONTRIBUTIONS

Linda Lorenz: Data curation (lead); investigation (lead); methodology (lead); writing – original draft (supporting); writing – review and editing (lead). **Charles R. Frihart:** Conceptualization (lead); project administration (lead); resources (lead); supervision (lead); writing – original draft (lead).

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Mike Birkeland of Solenis, LLC raised the question about comparing ovalbumin to soy adhesives and provided useful commentary on the results.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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