Wheat Gluten Fractions as Wood Adhesives—Glutenins Versus Gliadins

Petra Nordqvist,[1] Dalia Thedjil,[1] Sara Khosravi,[1,2] Mark Lawther,[3] Eva Malmström,[1] Farideh Khabbaz[4]

[1]*Department of Fibre and Polymer Technology, Royal Institute of Technology, SE-100 44 Stockholm, Sweden*
[2]*AkzoNobel, Casco Adhesives AB, SE-850 13 Sundsvall, Sweden*
[3]*TLA development I/S, DK-4000 Roskilde, Denmark*
[4]*AkzoNobel, Casco Adhesives AB, SE-100 61 Stockholm, Sweden*

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ABSTRACT: Plant proteins, such as wheat gluten, constitute attractive raw materials for sustainable wood adhesives. In this study, alkaline water dispersions of the protein classes of wheat gluten, glutenin, and gliadin were used as adhesives to bond together wood substrates of beech. The aim of the study is to measure the tensile shear strength of the wood substrates to compare the adhesive performance of glutenin and gliadin and to investigate the influence of application method and penetration of the dispersions into the wood material. A sodium hydroxide solution (0.1*M*) was used as dispersing and denaturing agent. Dispersions with different protein concentrations and viscosities were used, employing wheat gluten dispersions as references. Two different application methods, a press temperature of 110°C and a press time of 15 min, were employed. The tensile shear strength and water resistance of the wood substrates were compared, using a slightly modified version of the European Standard EN 204. The bond lines of the substrates were examined by optical microscopy to study the penetration and bond-line thickness. The results reveal that the adhesive properties of gliadin are inferior to that of both glutenin and wheat gluten, especially in terms of water resistance. However, the tensile shear strength and the water resistance of gliadin are significantly improved when over-penetration of the protein into the wood material is avoided, rendering the adhesive performance of gliadin equal to that of glutenin and wheat gluten. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 1530–1538, 2012

Key words: adhesives; mechanical properties; proteins; renewable resources; biopolymers

INTRODUCTION

The strive toward a sustainable society has initiated a significant amount of research on materials based on renewable resources as replacement for fossil-based materials. This is evident in the field of adhesives where a major fraction of the presently used systems are based on the nonrenewable and limited fossil source. Today's wood adhesives range from formaldehyde-based resins to latex-based systems, all being fossil based. A sustainable alternative to these adhesives, which has been proposed during the last years, is adhesives based on proteins, e.g., wheat gluten or soy bean proteins. In this study, wheat gluten has been investigated. Besides being derived from a renewable resource, wheat gluten is also an attractive raw material for adhesive applications because of its thermoplasticity and good film-forming ability. Some research has been performed regarding its ability to bond wood.[1–4]

Wheat gluten is an industrial by-product from wheat starch processing, and the annual worldwide production is ~ 400,000 metric tons.[5] It possesses unique viscoelastic properties and is mainly used in the bakery industry, although the field of food applications varies worldwide.[6–8] Its nonfood uses are also diverse, including usage in pet food and cosmetics.[6,9] Approximately 80% of wheat gluten consists of wheat storage protein, while polysaccharides, lipids, and minerals constitute the rest.[5,10] The wheat storage protein can be divided into two broad protein classes: gliadins and glutenins, because of the solubility of gliadins in aqueous ethanol (60–70%). Wheat gluten contains approximately equal amounts of gliadins and glutenins, and both protein fractions are rich in the amino acids proline and glutamine. They contain a high amount of hydrophobic amino acids, although the amount is slightly lower for the glutenins. The gliadins consist of distinct polypeptide chains, while the glutenins are comprised of polypeptide chains linked together by interchain disulfide bonds. Most of the gliadins contain six or eight cysteine residues

Correspondence to: F. Khabbaz (farideh.khabbaz@akzonobel.com).

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forming intrachain disulfide bonds.[10–12] Furthermore, while the molar mass of gliadins ranges from 30,000 to 60,000 g/mol, the molar mass of glutenins extend from around 500,000 g/mol to above 10^7 g/mol.

In a previous study, the adhesive properties of wheat gluten were compared and found to be inferior to those of soy protein isolate.[3] However, extensive research has shown that the gliadin and glutenin fractions of wheat gluten possess very different properties and render different results in both food and nonfood applications.[5,6,9,13–15] Inspired by these findings, it was hypothesized that the aforementioned differences also may result in different adhesive performance, possibly rendering one of the fractions superior to that of the others or to wheat gluten. The aim of this study is to compare the tensile shear strength of wood substrates bonded with glutenin and gliadin, respectively, and to investigate the influence of application method and degree of penetration of the proteins into the wood material.

Wheat gluten was therefore separated into glutenin and gliadin. Water dispersions of alkali-denatured glutenin and gliadin, respectively, were used as wood adhesives to bond together wood substrates of beech. Dispersions with different protein concentrations and viscosities were used, and corresponding dispersions of wheat gluten were used as references. Two different application methods were employed before hot pressing. The tensile shear strength and water resistance of the wood specimens were compared to determine which fraction of wheat gluten is the most promising for wood adhesive applications. To study how the tensile shear strength correlates with penetration and bond-line thickness, bond-line cross sections were examined by optical microscopy.

EXPERIMENTAL

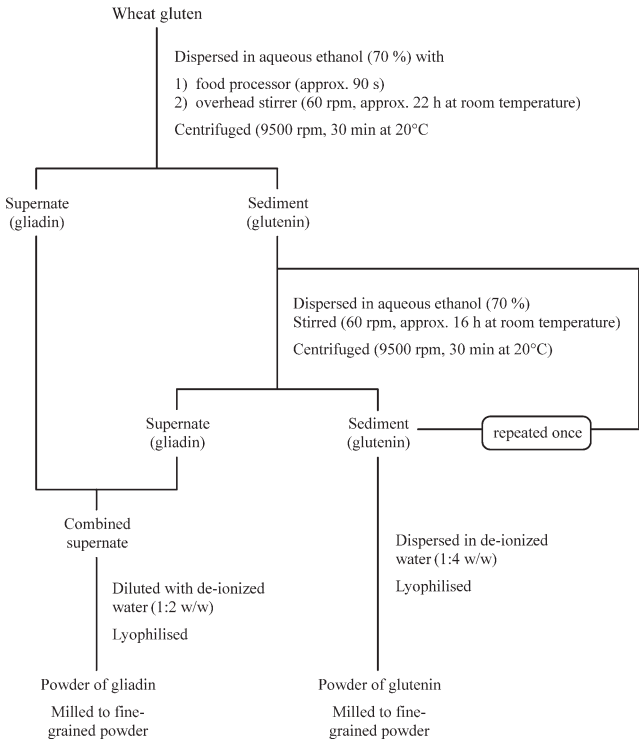
Materials

Wheat gluten Reppe Vital (kindly supplied by Lantmännen Reppe AB, Sweden), containing ~ 85% protein, was employed in this study.

Beech wood pieces were purchased from Konrad Bruckeder (Rosenheim, Germany).

Separation of wheat gluten into glutenin and gliadin

Wheat gluten was separated into glutenin and gliadin using aqueous ethanol (70%) according to Osborne's solubility test.[16] Thus, wheat gluten (350 g) was dispersed in aqueous ethanol (70%; 1225 mL) in a food processor (Type HR 2375/CN, Philips, Holland) for ~ 90 s (Scheme 1). Thereafter, the mixture was transferred to a beaker and stirred with an overhead stirrer at 60 rpm and room temperature for ~ 22 h. The mixture was centrifuged (9500 rpm for 30 min at 20°C), and the supernate and sediment, containing gliadin and glutenin, respectively, were separated. The sediment was dispersed in an additional amount of aqueous ethanol (70%; 1225 mL) and stirred at 60 rpm and room temperature for ~ 16 h. The mixture was centrifuged (9500 rpm for 30 min at 20°C), and the supernate and the sediment were separated. This sediment, emanating from the second extraction step, was treated in equivalent manner as the treatment of the sediment from the first extraction procedure.



Scheme 1 Flow diagram for the separation of wheat gluten into glutenin and gliadin.

The gliadin-containing supernatant fractions were combined, and the sediment fraction containing glutenin was collected. Prior to lyophilization, the gliadin fraction was diluted with deionized water (1 : 2 w/w), while the glutenin fraction was dispersed in deionized water (1 : 4 w/w). The resulting dry powders were milled (Type A 10, Janke and Kunkel GmbH, Germany) to homogenize to fine-grained powders.

Amino acid composition

The analysis of the amino acid compositions was performed by Eurofins Food and Agro Sweden AB according to methods SS-EN ISO 13903 : 2005 and 13904 : 2005.

TABLE I
Conditions Used for Preparing Dispersions of Glutenin (GU), Gliadin (GA), and Wheat Gluten (WG). The Dispersions Were Prepared at Room Temperature with 0.1*M* NaOH as Dispersing Agent. The Stirring Rate Was 300 rpm. Viscosity of the Dispersions

Dispersion	Protein	Conc.[a] (%, w/w)	Theoretical solids content (%, w/w)	Stirring time after addition (min)	Viscosity[b] (mPa)	Spindle, speed[c] (rpm)
14-GU	glutenin	14	13	65	36,000	LV4, 6
14-GA	gliadin	14	13	60	29	LV1, 100
30-GA	gliadin	30	28	50	48,000	LV4, 6
14-WG	wheat gluten	14	13	70	46	LV1, 100
24-WG	wheat gluten	24	22	40	48,000	LV4, 6

[a] Concentration of protein powder in the dispersions.
[b] Viscosity was measured the day after the dispersions were prepared. The viscosity values were recorded 10 s after starting the measurement.
[c] Different spindles and speeds had to be used, because of the large difference in viscosity between the dispersions.

Preparation of dispersions of glutenin, gliadin, and wheat gluten

Table I summarizes the dispersions of glutenin (GU), gliadin (GA), and wheat gluten (WG) that were used in this study. A sodium hydroxide solution (0.1*M*; pH 13) was used as dispersing and denaturing agent. The protein was added while stirring at room temperature. Slightly different stirring times had to be used to obtain homogeneous dispersions.

Viscosity measurements of the protein dispersions

The viscosity was measured the day after the dispersions were prepared, and the data acquisition was started 10 s after starting the measurement. A Brookfield Viscometer (Model DV-II+ Pro, VWR International AB, Stockholm, Sweden) and computer program Rheocalc V2.5 (Brookfield Engineering Labs) were used for the measurements (Spindle LV1 and LV4; speed 100 rpm and 6 rpm).

Preparation of wood substrates and evaluation thereof

Application methods

Two different application methods were used to be able to compare the adhesive properties of the dispersions despite their large differences in viscosity.

Application method 1. The 1-day old dispersions were used to bond together two panels of beech, with dimension 5 × 135 × 400 mm (thickness × width × length). On one side of each panel, 180 g/m^2 of dispersion was applied. A press temperature of 110°C, a press time of 15 min, and a pressure of 0.7 MPa were used.

Application method 2. The 1-day old dispersions were used for bonding. The same type of beech panels was used as in application method 1 (AM1).

The dispersion was applied on one side of each panel (180 g/m^2), and the dispersion layer was allowed to dry in a conditioned room [(20 ± 2)°C and (65 ± 5) % relative humidity] for 24 h. Panels with one, two, and three layers of dispersion, respectively, were produced and allowed to dry in the conditioned room after each applied layer of dispersion. The treated surfaces of the panels were rewetted with water (~ 170 g/m^2) prior to bonding. However, to a set of the panels with one dry layer of dispersion, 180 g/m^2 of dispersion was applied instead of water prior to bonding. Identical press conditions as for AM1 were employed.

The nomenclature of the wood substrates is presented in Table II. Please note that lowercase letters refer to the wood substrates, while capital letters refer to the dispersions.

Tensile shear strength measurements

The bonded panels were conditioned and evaluated according to slightly modified versions of the European Standards EN 204 and EN 205.[17,18] They were bonded together with what according to the standards is classified as a thin bond line (adhesive layer 0.1-mm thick). The panels were cut into test pieces, which were treated according to the conditioning sequences shown in Table III. A summary of the minimum values of adhesive strength that must be reached for the classification of thermoplastic adhesives into the durability classes D1 to D3 is also displayed in Table III.

The length of the test pieces was 100 mm instead of 150 mm, which is the standard (EN 205). Ten test pieces were tested for each conditioning sequence and application method. An Alwetron tensile testing machine (model TCT 50, Lorentzen and Wettre, Sweden) was used for all such measurements.

TABLE II
Nomenclature of Wood Substrates Bonded with Dispersions of Glutenin (GU), Gliadin (GA), and Wheat Gluten (WG). The Substrates Were Produced Either Using Application Methods 1 or 2

Nomenclature of wood substrate[a]	Dispersion	Application method	Applied layers of dispersion
14-gu-1	14-GU	1	1
30-ga-1	30-GA	1	1
24-wg-1	24-WG	1	1
14-gu-2(1)	14-GU	2	1
14-gu-2(2)	14-GU	2	2
14-gu-2(3)	14-GU	2	3
14-gu-2(1+1)	14-GU	2	2
14-ga-2(1)	14-GA	2	1
14-ga-2(2)	14-GA	2	2
14-ga-2(3)	14-GA	2	3
14-ga-2(1+1)	14-GA	2	2
14-wg-2(1)	14-WG	2	1
14-wg-2(2)	14-WG	2	2
14-wg-2(3)	14-WG	2	3
14-wg-2(1+1)	14-WG	2	2

[a] The two-digit number and the subsequent two-letter abbreviation refer to the dispersion used for bonding, the digit following refers to the application method. The digit shown in brackets refers to the number of layers of the dispersion that was applied to the wood substrate. The combination (1+1) means that one dry layer of the dispersion was rewetted with an additional layer of the dispersion directly prior to bonding. The wood substrates with one to three layers of dispersion (application method 2) were rewetted with deionized water prior to bonding. Please note that lowercase letters refer to the wood substrates, while capital letters refer to the dispersions.

Optical microscopy

A Leica DMRM optical microscope (Leica Microsystems AB, Stockholm, Sweden), equipped with a fluorescence filter (Leica filter cube H3) and a CCD camera (Leica DFC 280), was used for examining the bonded joints of the wood substrates. Prior to the analysis, the cross sections of the wood substrates were prepared either with razor-blade cutting by hand or with UV-laser irradiation (UV-laser ablation).[19,20] The ablated substrates were prepared using a UV excimer laser (Luminox). A wavelength of 248 nm, an irradiation energy of 333 mJ, and a repetition frequency of 4 Hz were employed. All the wood substrates were stained with 0.01% aqueous Safranine-O (Basic Red 2, ICN Biomedicals) prior to the optical microscopy analysis.

RESULTS AND DISCUSSION

The wood adhesive performance of glutenin and gliadin was evaluated and compared, and the influence of application method and substrate penetration was investigated.

Ratio between glutenin and gliadin, and the amino acid composition of the fractions

The yield of glutenin and gliadin from the separation of wheat gluten was 54% and 46% (w/w), respectively. This result is dependent on the separation procedure, but environmental conditions during growth also affect the ratio of glutenin and gliadin, and the protein composition. However, the result agrees with previous findings.[5,10,21]

The results from the determination of the amino acid composition of the glutenin and gliadin fractions are summarized in Table IV. The analysis does not distinguish between glutamic acid and glutamine, but both glutenin and gliadin contain mainly glutamine.[5,10] It is found that both fractions contain high amounts of glutamic acid/glutamine and proline, although the amounts are highest for the gliadin fraction (Table IV). The amino acid compositions of wheat gluten and its fractions are on par with results reported by Rombouts et al.[22] Moreover, the amount of hydrophobic amino acids in glutenin is slightly higher than in gliadin according to Guilbert.[5] This is supported by the results presented in Table IV.

The amount of raw protein [N*6.25 (%)] of the glutenin and gliadin fractions as well as wheat gluten were calculated to be 75%, 91%, and 79% (w/w), respectively.

Preparation of protein dispersions, viscosity measurements, and the choice of application techniques

Wheat gluten is water dispersible at a pH both below and above its isoelectric point (pH $\sim$ 7.3).[23] In this study, alkaline conditions were chosen for the comparison of the adhesive properties of wheat gluten and its two fractions of protein: glutenin and gliadin. The proteins were dispersed and denatured in 0.1*M* NaOH (aq).

In a previous study, the adhesive properties of wheat gluten and soy protein isolate were

TABLE III
Conditioning Sequences and Minimum Values of Adhesive Strength for Thin Bond Lines

Conditioning sequences: duration and condition	Adhesive strength (MPa)	Durability classes
7 days[a] in standard atmosphere[b]	$\geq$10	D1, D2, and D3
7 days[a] in standard atmosphere,[b] 3 h in water at (20±5)°C, and 7 days[a] in standard atmosphere[b]	$\geq$8	D2
7 days[a] in standard atmosphere[b] and 4 days[a] in water at (20±5)°C	$\geq$2	D3

[a] 1 day = 24 h.
[b] (20±2)°C and (65±5)% relative humidity.

TABLE IV
The Amino Acid Composition of Glutenin, Gliadin, and Wheat Gluten

Amino acid		Glutenin amount (mol %)	Gliadin amount (mol %)	Wheat gluten amount (mol %)
Alanine	Ala	4.6	3.0	3.7
Arginine	Arg	3.0	1.9	2.5
Aspartic acid/Asparagine[a]	Asx	3.8	2.4	3.1
Cystin	$(Cys)_2$	1.2	1.4	1.3
Glutamic acid/Glutamine[b]	Glx	30	37	34
Glycine	Gly	8.3	3.3	5.8
Histidine	His	1.8	1.6	1.7
Isoleucine	Ile	3.2	3.9	3.7
Leucine	Leu	6.8	7.0	6.9
Lysine	Lys	2.3	0.6	1.5
Methionine	Met	1.4	1.3	1.4
Phenylalanine	Phe	3.1	4.4	3.7
Proline	Pro	12	17	14
Serine	Ser	7.2	6.1	6.5
Threonine	Thr	3.3	2.2	2.7
Tryptophan	Trp	0.8	0.5	0.6
Tyrosine	Tyr	2.8	1.9	2.3
Valine	Val	4.3	4.2	4.4
Raw protein [N*6.25 (%, w/w)]		75	91	79

[a] The value corresponds to the total amount of aspartic acid and asparagine.
[b] The value corresponds to the total amount of glutamic acid and glutamine.

investigated.[3] As in this study, the proteins were dispersed in 0.1*M* NaOH. The wood panels were bonded at different press temperatures (90, 110, and 130°C) and press times (5, 15, and 25 min). The study showed improved bonding results for a wheat gluten dispersion (24%) when a higher press temperature than 90°C or a longer press time than 5 min was employed. On the basis of these previous results, a press temperature of 110°C and a press time of 15 min were selected for this study.

Adhesion refers to the interaction between the surfaces of adhesive and substrate, and both mechanical and chemical aspects influence adhesion. There are several theories of adhesion.[24–26] However, in this case, adhesive strength is believed to be mainly due to mechanical interlocking and secondary chemical interactions between adhesive and substrate. For a proper bond to form, the adhesive need to come in close contact with the wood substrate. The adhesive must be able to wet the surface of the wood material, flow over it, and penetrate into the wood. There is usually an interest in maximizing the dry content of the adhesive, partly to reduce the amount of water left in the adhesive joint and wood material after pressing. However, if the viscosity is too high, the ability of the adhesive to properly wet, flow over, and penetrate into the wood substrate will decrease. On the other hand, if the viscosity of the dispersion is too low, the dispersion will either drain off or over-penetrate the substrate, leaving the bond line too thin.[24] In this study, the concentration of glutenin, gliadin, and wheat gluten in the dispersions could not be increased to more than 14, 30, and 24% (w/w), respectively, before a too high viscosity was obtained. However, despite the high water content, it was possible to use the dispersions as adhesives. Nevertheless, to facilitate the comparison of adhesive properties, it is desirable to be able to use dispersions with similar viscosity and the same concentration of protein regardless of protein type. However, the viscosity of the glutenin dispersion differs significantly from the dispersions of gliadin and wheat gluten, although similar protein concentrations were employed (Table I). Because of this difference, different spindles and speeds had to be employed during the viscosity measurement, which must be considered when comparing the results. Furthermore, the viscosities of the 14% dispersions of gliadin and wheat gluten were too low for conventional application methods.

To accommodate the differences in protein concentrations and viscosities of the dispersions, two different application techniques were employed. In the first case (AM1), dispersions with similar viscosities but different concentrations of glutenin, gliadin, and wheat gluten were used. These dispersions (14-GU, 30-GA, and 24-WG) were applied to the wood panel immediately prior to pressing. Equal amounts of the dispersions were applied to the panels rendering bond lines with different amount of solid content.

In the second case [application method 2 (AM2)], equal amounts of the dispersions 14-GU, 14-GA, and 14-WG, respectively, were applied to the panels resulting in bond lines with equal amounts of solid content. These dispersions contain the same concentration of protein, but the viscosities of 14-GA and

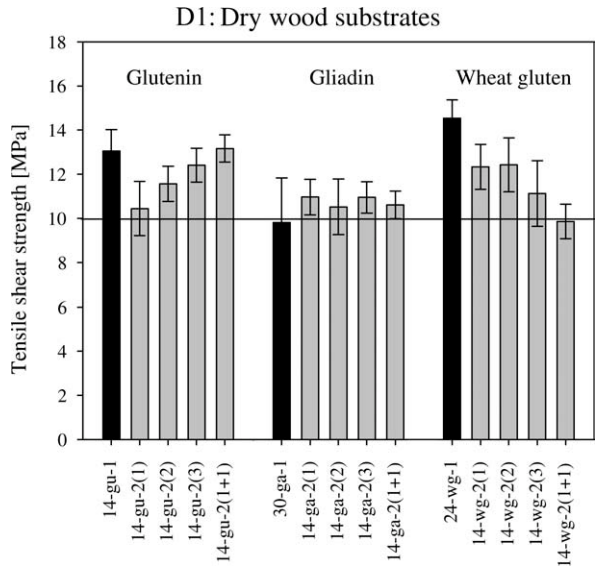


Figure 1 Tensile shear strength measurements of dry wood substrates, application method 1 (black bars) and application method 2 (gray bars). The horizontal black line at 10 MPa indicates the limit for passing the test according to the European Standard EN 204.

14-WG are extremely low. However, employing AM2 prevents the dispersions draining off or over-penetrating the wood material. One, two, or three layers of dispersion were applied for comparison of different amounts of solid contents.

In conclusion, when AM1 is used, the viscosities of the dispersions are similar, but the amount of protein in the adhesive joint differs. This difference in protein amount has been eliminated in AM2. The protein concentration of the dispersions is the same, but since their viscosities differ, the proteins might behave differently during drying and rewetting, which could influence the adhesion properties. However, using results from both application techniques render it possible to confidently compare the adhesive performances of glutenin, gliadin, and wheat gluten. The techniques complement each other and enable a broader understanding on how the proteins interact with the wood material.

Comparison of results from tensile shear strength measurements

The tensile shear strength of the wood substrates bonded with glutenin, gliadin, and wheat gluten were measured to compare the adhesive properties of the proteins. The wood substrates were conditioned and evaluated according to slightly modified versions of the European Standards EN 204 and EN 205 using tensile shear testing (Table III).[17,18] All dry wood substrates, except 30-ga-1 (AM1) and 14-wg-2(1 + 1) (AM2), passed the test according to standards (>10 MPa; Fig. 1). Furthermore, although the substrates 30-ga-1 and 14-wg-2(1 + 1) failed the test, their average tensile shear strength values are just below 10 MPa. In conclusion, the results for the dry wood substrates are similar, regardless of type of dispersion, dry content, and number of dispersion layers.

On the other hand, the results from tensile shear strength measurements of water-soaked wood specimens distinctly show that there is a difference in tensile shear strength and water resistance between the three dispersions, Figures 2 and 3. All the 3-h-water-soaked substrates passed the test according to standards (>8 MPa), except for those bonded with the dispersion 30-GA (AM1) (Fig. 2), while all specimens soaked in water for 4 days failed the test (<2 MPa) (Fig. 3). Almost all wood specimens bonded with the dispersion 30-GA (AM1) fell apart during water soaking, while those bonded with the dispersion 14-GA and AM2 showed similar tensile shear strength as those specimens bonded with 14-GU, 14-WG, and 24-WG (Figs. 2 and 3). Thus, the choice of application method has a large impact on the tensile shear strength of the wood specimens bonded with the gliadin dispersions. The bond strength, especially the water resistance of the bond, is markedly improved when AM2 is used. Also noteworthy is that the tensile shear strengths of the glutenin and wheat gluten dispersions are not affected by the application method.

AM2 also gives rise to similar results from each conditioning sequence, irrespectively of type of dispersion and amount of protein applied to the wood

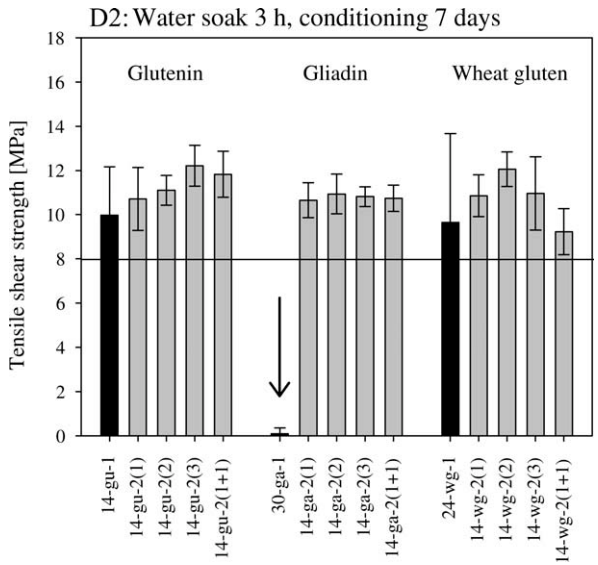


Figure 2 Tensile shear strength measurements of wood substrates soaked in water for 3 h and conditioned for 7 days, application method 1 (black bars) and application method 2 (gray bars). The horizontal black line at 8 MPa indicates the limit for passing the test according to the European Standard EN 204. Please note that to draw attention to the low value of 30-ga-1, the bar is marked with an arrow.

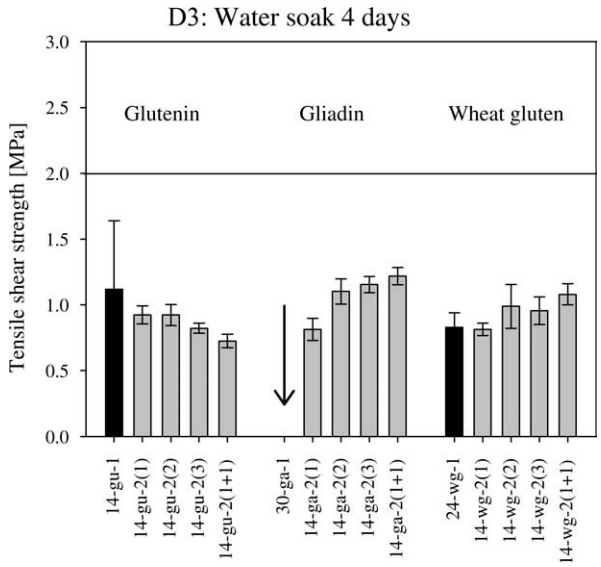


Figure 3 Tensile shear strength measurements of wood substrates soaked in water for 4 days, application method 1 (black bars) and application method 2 (gray bars). The horizontal black line at 2 MPa indicates the limit for passing the test according to the European Standard EN 204. Please note that the measured value of 30-ga-1 is zero, and the missing bar is indicated with an arrow.

panel (25 to 76 g/m^2). Minor differences in tensile shear strength are found to be within the experimental error of the method. It seems as if two, or even one layer, of protein dispersion will result in maximum tensile shear strength. Additional amount of protein is redundant and does not increase the strength. Furthermore, the tensile shear strength was not significantly improved when one dry layer of dispersion was rewetted with an additional layer of dispersion just prior to bonding, compared with rewetting two dry layers of dispersion with water.

Optical microscopy analysis. Correlation with the tensile shear strength measurements

Optical microscopy was used to study penetration and bond-line thickness to further clarify why different tensile shear strengths were obtained for glutenin, gliadin, and wheat gluten [Fig. 4(a–l)]. The dispersions 14-GU, 30-GA, and 24-WG had similar viscosities and were applied using AM1 [Fig. 4(a,e,i)]. The specimens bonded with 14-GU and 24-WG are relatively similar [cf. Fig. 4(a,i)], but the penetration appears to be slightly more extensive when 24-WG is used. The difference in the amount of protein applied to the wood panels most likely accounts for the small difference in joint thickness. However, regardless of these minor differences, these specimens reveal similar tensile shear strengths (Figs. 1–3). Interestingly, the bond line obtained using 30-GA is completely different [cf. Fig. 4(e) with (a,i)]. The penetration of 30-GA is significant [Fig. 4(e)], and the dispersion has penetrated into and partly filled some of the larger wood cells, in some

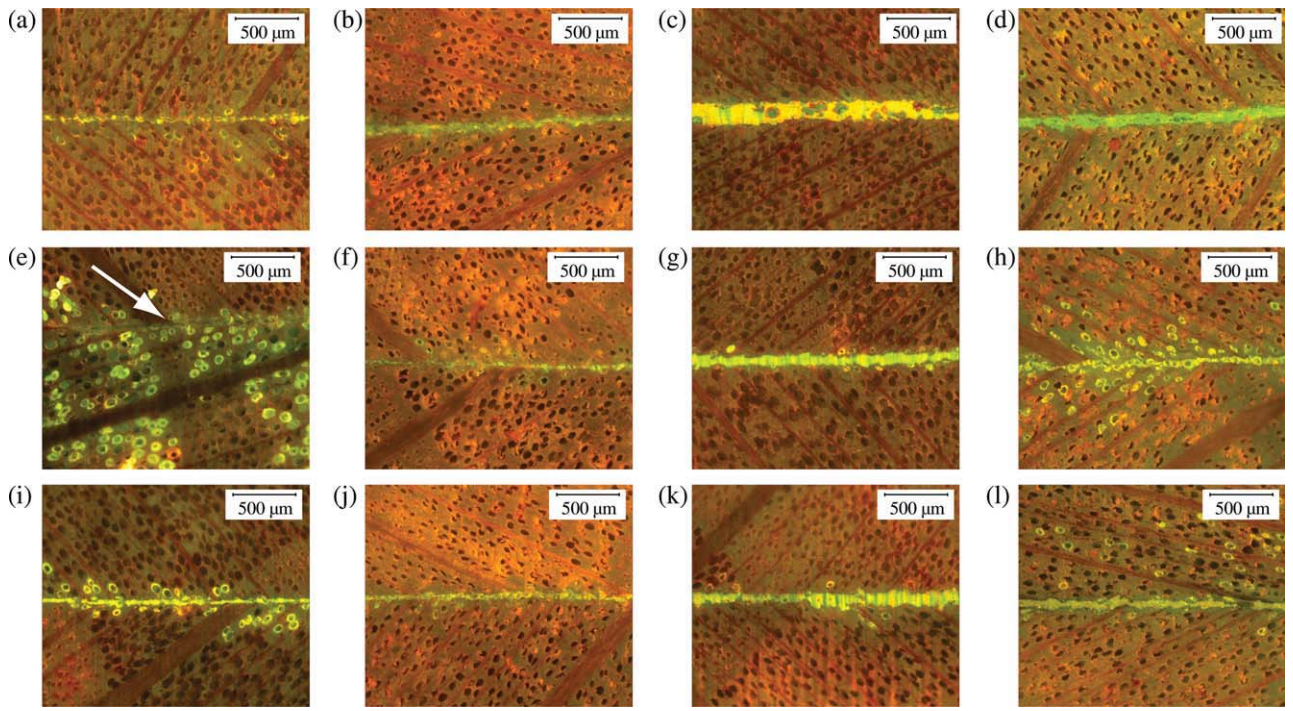


Figure 4 Optical microscopy images of wood substrates bonded with different dispersions and application methods. Method 1: Figures a, e, and i; Method 2: Figures b–d, f–h, and j-l. (a) 14-gu-1, (b) 14-gu-2(1), (c) 14-gu-2(2), (d) 14-gu-2(1 + 1), (e) 30-ga-1, (f) 14-ga-2(1), (g) 14-ga-2(2), (h) 14-ga-2(1 + 1), (i) 24-wg-1, (j) 14-wg-2(1), (k) 14-wg-2(2), and (l) 14-wg-2(1 + 1). The horizontal bond line of 30-ga-1 (e) is indicated with an arrow.

cases even as far as ~ 1000 μm from the joint. Nevertheless, the extensive penetration leaves the bond line very thin, and the tensile shear strength is low (wood substrates 30-ga-1, Figs. 2 and 3), although a larger amount of protein was applied to the wood panels with the gliadin dispersion than with the other dispersions. The small amount of adhesive left in the bond line is insufficient to properly join the wood surfaces. Penetration of the adhesive into the wood substrate is important to establish a strong and good bond between the adhesive and the wood material.[24] However, a good balance between the amount of adhesive in the joint and in the wood material is crucial.

The appearance of the microscopy images of the wood substrates produced with AM2 may further account for the differences in tensile shear strength obtained with glutenin, gliadin, and wheat gluten [Fig. 4(b–d,f–h,j–l)]. The degree of penetration is reduced and bond-line thickness is increased when changing from AM1 to AM2. These differences are especially evident when comparing the images of gliadin-bonded substrates [Fig. 4(e,g)]. When using AM1, the dispersions were still wet when hot-pressed, while the dry dispersion layers (AM2) were probably still dry at the border between wood and protein layer and only wet on the outer surface of the layers. Consequently, the dispersions that were dried after the application only penetrated into the wood to a minor extent, rendering the bond lines thicker. Because of this reduction in penetration, the tensile shear strengths of the wood substrates bonded with gliadin are significantly improved, being most evident for the water-soaked substrates (Figs. 2 and 3).

Furthermore, regardless of dispersion, the thickness of the bond lines increases with the number of dispersion layers, but with no significant increase in tensile shear strength [Figs. 1–3 and 4(b,c,f,g,j,k)]. Moreover, the appearance of the images of the substrates bonded with two layers of dry dispersion differs from those bonded with one dry and one wet layer of dispersion, although the amount of added protein is the same [Fig. 4(c,d,g,h,k,l)]. The bond line of 14-gu-2(2) is thicker than that of 14-gu-2(1 + 1) indicating a certain degree of penetration of the wet dispersion, although this is not obvious from the image of 14-gu-2(1 + 1) [Fig. 4(c,d)]. In addition, 14-ga-2(1 + 1) has a thinner bond line than 14-ga-2(2) because of relatively extensive penetration of the second dispersion layer [Fig. 4(g,h)]. These results indicate that the first layer of protein only partly covers the wood surface, but that it still seems to reduce penetration. The bond line of 14-wg-2(1 + 1) is also thinner than 14-wg-2(2) [cf. Fig. 4(k,l)], although the penetration is less extensive than for 14-ga-2(1 + 1). Nevertheless, it can be concluded that the reduction in bond-line thickness does not result in a significant change in tensile shear strength (Figs. 1–3).

Degree of wood penetration of protein dispersions

The optical microscopy analysis clearly reveals differences in penetration between glutenin, gliadin, and wheat gluten. Gliadin penetrates to a larger extent and more deeply into the wood material than glutenin and wheat gluten [Fig. 4(a,e,i)]. Glutenin penetrates the least. Some of these differences may be due to a higher degree of solubility of gliadin in the dispersing medium (0.1*M* NaOH), partly being due to the much lower molar mass of gliadin compared with that of glutenin. Nevertheless, it does not explain why the penetration depth is larger for gliadin than for wheat gluten. The smaller amount of gliadin present in wheat gluten would not necessarily result in reduced penetration depth. However, it has been suggested that gliadin is physically entrapped in the glutenin network.[9,10,27] This interaction between gliadin and glutenin may reduce the mobility of the gliadin molecules, resulting in a lower degree of penetration and a reduced penetration depth for wheat gluten compared with that of gliadin.

Moreover, glutenin has been shown to polymerize at elevated temperatures (50–130°C; mostly in the wet state). The crosslinking is due to formation of additional SS-bonds. These may be formed from oxidation of SH groups, but evidence for SH/SS interchange reactions has also been presented.[9,13,28–31] Gliadin, on the other hand, more or less lacking free SH groups, is not prone to polymerize. However, glutenin has been shown to react with gliadin during heating at temperatures equal or above 90°C.[28,32,33] These polymerization and crosslinking reactions ought to decrease the mobility of the protein molecules and may also partly explain the differences in penetration behavior of glutenin, gliadin, and wheat gluten. The elevated temperatures during pressing may reduce the penetration of the glutenin dispersion, while there are no or almost no crosslinking reactions involved when the gliadin dispersion is heated. However, since both gliadin and glutenin are present in wheat gluten, the mobility of the protein molecules may be reduced because of the suggested polymerization. Thus, the wheat gluten dispersion will penetrate into the wood material but not to the same extent as the gliadin dispersion. Gliadin penetrates the most; however, the results from the optical microscopy analysis indicate that its penetration is reduced when glutenin is present.

CONCLUSIONS

The performance of glutenin and gliadin as wood adhesives was evaluated, and the observed differences were related to the choice of application method and extent of penetration of the proteins into the wood material. It can be concluded that the adhesive

properties of glutenin and wheat gluten are similar, while the properties of gliadin are inferior to that of the others, especially in regard to water resistance. The tensile shear strengths obtained with glutenin and wheat gluten are similar regardless of application method, while the application method has a considerable effect on the tensile shear strength when using gliadin. This difference is mainly due to extensive penetration of gliadin dispersion into the wood material. However, the tensile shear strength and the water resistance of gliadin are significantly improved when starved bond lines are avoided through changes in application method, rendering the adhesive performance of gliadin equal to that of glutenin and wheat gluten.

Furthermore, the tensile shear strength was not appreciably influenced when the amount of the proteins applied to the wood panels was changed from 25 to 76 g/m^2, indicating that the additional amount of protein is redundant. Moreover, the tensile shear strength was not noticeably improved when the dry layer of dispersion was rewetted with wet dispersion prior to bonding, instead of rewetting with deionized water.

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