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Effect of Hydrolysis and Denaturation of Wheat Gluten on Adhesive Bond Strength of Wood Joints

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ABSTRACT: In this study the adhesive bond strength of different wheat gluten modifications and the relationship between molecular weight and adhesive strength was examined. Guanidine hydrochloride and sodium hydroxide were used as denaturation and dispersing agent. Additionally wheat proteins were hydrolyzed by alkaline conditions and enzymes. Effects of different treatments were observed by viscosity measurements and gel electrophoresis. Wood lap joints were prepared with modified proteins and tensile shear strength was tested under dry and wet conditions. *In situ* hardening of different formulations was analyzed by means of DMA with two-layered specimens in a three-point bending test set-up. Higher solubility had no positive effect on dry bonding strength and wet bonding strength was even reduced. Depending on the degree of hydrolysis, significant improvement of adhesive bond strength was observed. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 2429–2434, 2013

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INTRODUCTION

Wheat gluten can be characterized as a "cohesive, visco-elastic proteinaceous material, which is gained as a by-product during the isolation of starch from wheat flour".[1,2] The storage proteins of wheat grain mainly consist of two protein-fractions, gliadins (prolamine), and glutelins (glutenin). Gliadin comprises individual polypeptide chains, which are mainly associated via hydrogen bonds and hydrophobic interactions. Glutenin contains high- as well as low-molecular-weight subunits covalently linked via disulfide bonds.[2,3] Among others hydrogen bonding between the repeat regions of the high-molecular-weight glutenin subunit is mainly responsible for the elasticity of gluten. On the other hand gliadin with its globular structure contributes to gluten viscosity.[4]

Highest amount of wheat gluten is consumed by the food industry. Several processes were used to improve solubility. Mainly hydrolysis and denaturation is applied to proteins in order to reduce molecular weight and to improve solubility in water based-mediums.[5,6] However, low water solubility of gluten limits its use for numerous applications in food production, aside as additive in breads and noodles.[5]

Compared to other proteins wheat gluten is the cheapest protein source.[1] Because of the limited application of wheat gluten in the food production this bio-based polymer proves also high potential for material application, e.g., environmental friendly wood adhesives.[7] New standards and limits for formaldehyde emissions from wood-based panels in the European Union and other countries promoted the development and enhancements of bio-based adhesives.[8] Therefore in the last years several efforts have been made to establish proteins as raw material for wood bonding.[6,9–11] Although the awareness of reducing the negative impact of common resins in interior rooms is growing, formaldehyde-based resins are still dominating the market for wood products. Beside economic aspects the main limitation of natural adhesives is the low resistance against humidity. Therefore, one of most important challenges is to make adhesive bonds from biological resources more durable against moisture.[9]

The research was primarily concentrated on soy,[7,12,13] and also in a lower intensity on wheat proteins.[10,14,15] For improving applicability and water resistance different modifications were used, e.g., hydrolysis or denaturation by adding GdnHCl.[10,14,16,17] Although several works describing the adhesive properties of gluten used as a glue for wood joints were published,[7,12,13] the relation between molecular structure and adhesive performance is scarcely investigated. The research was mainly focused on the development of adhesive systems with sufficient bonding strength. However, a systematic investigation

to identify parameters influencing bonding characteristics is still missing.

Several methods and techniques are known for the characterization of reactivity and cross-linking behavior of adhesives, e.g., NMR, infrared spectroscopy, viscosity measurements and differential scanning calometry. The main limitation of most methods is that the interaction of adhesive and the wooden substrate is not taken into consideration. Therefore, results and conclusions cannot be transferred to real bonding conditions. There are only few methods observing *in situ* curing of resins on a wooden surface. One of them is DMA, measuring the storage modulus of the glue between two wooden veneers in three-point bending modus. Because of this configuration reactivity and response of mechanical properties to temperature can be examined.[18–21]

The purpose of this study was to investigate adhesives prepared from wheat gluten as an environmentally friendly product for the wood industry. To enlarge adhesive properties WG was modified by denaturation agents, alkaline and enzymatic hydrolysis. The research was focused on the characterization of molecular structures, which are responsible for adhesive strength under dry and wetted conditions.

EXPERIMENTAL

Materials

All reagents were of at least analytical grade from Sigma–Aldrich (Steinheim, Germany) unless otherwise specified. Neutrase 0.8 L (EC 3.4.24.28, from Bacillus amyloliquefaciens) was purchased from Novozymes (Denmark). Roti®-PAGE Gradient (4–20%) gels with dimensions of 9 × 10 cm was accquired from LACTAN (Graz, Austria).

Native wheat proteins, Amygluten 110 was purchased from Tate and Lyle (London, United Kingdom). Following main ingredients were determined: water 7.05 ± 0.07, proteins 81.51 ± 0.12, starch 8.73 ± 0.14 and fat 1.41 ± 0.04 (w/w% based on dry matters). The moisture content was determined by the mass-loss after 48 h in a drying chamber at 103°C, while the protein content was determined according to the Kjehldal method (N × 5.68). Total starch content was determined by means of amyloglucosidase and α-amylase treatment (Megazyme International Ireland, Wicklow, Ireland) according to AACC method 76–13. The fat content was calculated after soxhlet-extraction with ethyl ether and drying afterwards according to AACC method 30–25. These results were almost consonant with the data received from the producer. The amount of proteins and starch were little bit less in respect to own analysis.

Wheat Gluten Modification and Adhesive Formulations

Wheat gluten hydrolysates (WGHs) were achieved by alkaline and enzymatic treatments. All modifications were carried out with 10% WG (w/w) suspensions under constantly stirring. Neutrase 0.8 L (Novozymes) was used for enzymatic hydrolysis under neutral conditions and a temperature of 50°C according to Kong et al.[5] WG was suspended in water and the reaction was started by the adding the enzyme to give a final enzyme-to-substrate ratio of 1 : 100 (w/w). A treatment time of 60 min was applied and the reaction was interrupted by short heating to about 95°C. For alkaline hydrolysis pH was adjusted to 13 with concentrated sodium hydroxide solution. The dispersions were heated up to 50°C for 1 and 4 h, respectively. The hydrolysis was stopped by neutralization to pH of 7 with concentrated HCl. All WGHs were vacuum dried at 40°C and milled using a 0.25 mm mesh.

Viscosity Measurements of the Protein Dispersions

Dispersions with a solid content of 15% were prepared with different wheat gluten (WG) modifications and dispersing agents. A Bohlin CVO Rheometer (Malvern Instruments, United Kingdom) with a coaxial cylinder C 25 at 20°C and a speed of 10 rpm was used. Integration time was set to 15 s and for each run 10 values were recorded. All measurements were done in triplicate.

SDS-PAGE

For SDS-PAGE a gradient polyacrylamide gel (4–20%) was used to cover a broad range of molecular weight. The samples were incubated in extraction buffer (0.25*M* Tris, 1.92*M* Glycine, 1% DTT, and 0.1% SDS) over night and afterwards reduction of disulfide bonds was performed at 100°C for 5 min. All samples were centrifuged at 4000 × *g* for 10 min, and the supernatants were used to load the gels. The separating gel was run at a constant current of 20 mA for about 1.5 h. The gels were stained in Coomassie brilliant blue. Molecular weights of protein hydrolysates were estimated by using Roti®-Mark 10-150 (Roth, Germany).

Sample Preparation and Mechanical Testing

Lap joint samples for tensile shear strength tests were prepared according to EN 204[22] with a gluten suspension as an adhesive. All adhesive systems were composed of 15% solids based on dry maters. The suspensions were homogenized for 20 min by magnetic stirring. Because of this pretreatment homogeneous mixtures were generated, except for the gluten-water dispersion. This dispersion contained some lumps because of the bad solubility of gluten in polar solvents, whereby a patchy distribution of glue was achieved. As references a melamine-urea-formaldehyde resin (10H116, Dynea, Austria, Krems) and a polyvinyl acetate adhesive (Leifa PV/H D3, Henkel Central Eastern Europe GmbH, Ausrtia, Vienna) with water resistance of class D3 according to EN 204 were used. These adhesives have different attributes, which reflect the different properties and curing behaviors of WG modifications. The MUF resin is a hot setting condensation resin, which is very brittle. In contrast curing of PVAc is a physical process and the hardened glue has a high elasticity.[8,21]

The quantity of all glues was 200 g/m^2 and spread out with a spatula on one of the wood planks. The amount of adhesive equates about 30 g/m^2 for the WG mixtures and 120 g/m^2 for reference glues based on dry matters.[11] For specimen production beech boards with a density of 700 ± 50 kg/m^3 and a thickness of 5 ± 1 mm were used. The samples for mechanical testing were produced with a pressure of 0.6 ± 0.1 MPa at 120°C for 20 min by means of a laboratory hot press (Langzauner, Austria). Afterwards specimen with a length of 150 ± 5 mm and a width of 20 ± 1 mm were gained from the prefabricated joints according to EN 204. All samples were stored at 20 ± 2°C and 65 ± 5% relative humidity for one month before testing. Samples for testing the bond strength in wet conditions were stored in a water basin at 20°C for 3 h and reconditioned afterwards (7 days) according to the durability class D2 of EN 204.

For mechanical testing a universal testing machine (Zwick/Roell) equipped with a 20 kN load cell was used. A cross head speed in the range of 0.5–0.75 mm/min was chosen to guarantee sample failure of lap joints within 60 ± 30 s. Shear strength was calculated corresponding to EN 204 by dividing the maximum load until breakage by the tested area. The generated data set was statistically analyzed by means of one way ANOVA (analysis of variance at $P < 0.05$).

Dynamic-Mechanical Analysis

Thermal-mechanical characterization of adhesive mixtures was performed with a DMA (Netzsch DMA 242 C, Germany) using a 3-point bending test set-up. Test samples were produced of two beech veneers (length × width = 60 × 10 mm, thickness = 1.2 mm). On one veneer 0.1 ± 0.02 g (=167 g/m^2) of different adhesive formulations was spread out and covered with the second veneer. Samples were first heated up from 30 to 120°C at 3°C/min heating rate. Measurements were accomplished at a strain sweep frequency of 1 Hz. The static load was 0.2 N, whereas the dynamic force applied during frequency sweep was 0.3 N.

The storage modulus (E') as well as the loss factor tan $\delta = E'/E''$, with E'' being the loss modulus were recorded. To exclude influence of wood the relative mechanical cure (rMC) was calculated by the modulus—to max modulus ratio (E) as a function of temperature. Therefore, change in mechanical properties can be assigned to polymerization and curing of the applied glue.[19–21] Data interpretation was performed with Proteus software (Netzsch, Germany). All measurements were done in triplicate.

RESULTS AND DISCUSSION

Molecular Weight Distribution

The results of the alkaline and enzymatic treatment of gluten are given in Figure 1. Native WG shows only few defined bands and moreover wider distributed fractions. The major part was located between 60 and 80 kDa, also a noticeable part at about 150 kDa and 30–40 kDa was observable. As mentioned, gluten is yielded as a by-product during starch isolation from wheat. Because of the processing of different wheat cultivars a complex mixture of storage proteins with similar, but not identical molecular weight distribution is generated. Furthermore it must be considered that for SDS-PAGE sample preparation a reduction agent was used to break up disulfide bonds between protein fragments. Therefore the real molecular weight of native protein aggregates was a multiple of the detected values.[2]

Because of the unspecific dissociation at high pH-levels a very broad distribution of protein-fragments was obtained. The alkaline hydrolysis for 4 h showed the highest impact on hydrolysis. The main quantity of peptides was distributed below 30 kDa with a very intensive area lower than 10 kDa. Only few bands were observed over 80 kDa. Alkaline hydrolysis for 1 h showed a similar distribution with a very intensive band at about 30 kDa. Compared with the longer treatment the quantity of small peptide chains is smaller and the high molecular weight fraction is slightly higher. The enzymatic treatment resulted in less decomposition. The main part is located between 60 and 80 kDa like for native WG, but the high molecular weight fraction over 100 kDa disappeared. Some defined bands between 40 and 60 kDa and an intensive area at about 30 kDa were detected. Furthermore, only few peptides below 10 kDa were observed.

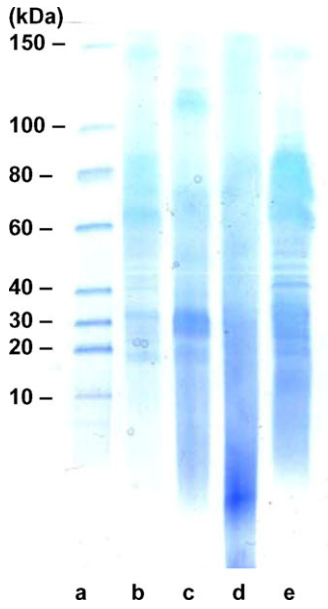

Figure 1. SDS-PAGE images of native gluten and modifications by alkaline and enzymatic hydrolysis. (a) Molecular weight marker; (b) native gluten; (c) alkaline hydrolyzed gluten (1 h); (d) alkaline hydrolyzed gluten (4 h); (e) enzymatic hydrolyzed gluten. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Viscosity of Wheat Gluten Modifications

Viscosities of different adhesive preparations are given in Table I. WG is insoluble in water, but dispersible at pH levels below and above its isoelectric point (pH about 7.3).[15] Because of the bad solubility of native gluten it was not possible to determine the viscosity of the gluten/water mixture. The untreated gluten dissolved in 0.1M NaOH and 1M GdnHCl showed the highest viscosities, 51.1 ± 2.5 mPas and 525.1 ± 8.2 mPas, respectively. During mixing for 20 min under alkaline conditions slight hydrolysis took place. Furthermore disulfide bonds can be destroyed. Thus molecular size of proteins is diminished and the viscosity is reduced. Additionally carboxyl groups were completely deprotonated and therefore solubility is improved. Also GdnHCl denatures proteins, but this process is milder compared with alkaline conditions. The high ionic strength of the GdnHCl solutions masks the electrostatic interactions. Therefore, gluten loses its native structure and the protein gets unfolded.[23] Used concentration of GdnHCl caused that the specific tertiary structure was lost.[17]

The WGHs suspensions had moreover lower viscosities. Compared to enzymatic hydrolysis higher viscosities for alkaline WGHs were obtained. The adhesive preparation consisting of enzymatic treated gluten resulted in a very thin suspension with a viscosity of only 7.7 ± 0.4 mPas. WGHs from alkaline conditions showed at least doubling in viscosity and further increasing viscosity was observed for longer treatment time. In general depending on the molecular size of WGHs different viscosities were measured. Higher degree of hydrolysis and therefore

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Table I. Adhesive Formulations of Different Wheat Protein Modifications and Viscosities of Adhesive Dispersions Formulated with the Modified Wheat Protein

Wheat gluten modification	Solvent	Concentration (%, w/w)	Viscosity[a] (mPas)
Native	Water	15	–[b]
Native	0.1 *M* NaOH	15	51.1 ± 2.5
Native	1 *M* GdnHCl	15	525.1 ± 8.2
Enzymatically hydrolyzed	0.1 *M* NaOH	15	7.7 ± 0.4
Alkaline hydrolysis (1 h)	0.1 *M* NaOH	15	14.8 ± 2.5
Alkaline hydrolysis (4 h)	0.1 *M* NaOH	15	19.0 ± 2.8

[a]Viscosity was measured with a 25 mm spindle at 20°C and 10 rpm.
[b]Due to the insolubility of gluten in water it was not possible to measure the viscosity.

smaller peptide fragments lead to higher viscosity of dispersions. The 4-h long alkaline treatment resulted in the highest viscosity among WGHs. Due to disintegration of peptide bonds more free amino and carboxyl groups were generated, which can adsorb more water molecules. Furthermore more hydrogen bondings between peptides were generated. Thus hydration radius of smaller peptides and molecular interactions were blown up, whereas viscosity increases.

Bonding Performance

The results of lap joint testing conditioned according to the D1 and D2 class of the European standard EN 204 are given in Figure 2. The dashed lines are indicating the minimum levels of the two classes D1 and D2. Due to bad solubility of native gluten in water, the shear strength of the mixture shows high variation. This can be assigned to the patchy distribution of the glue. For other solvents gluten solubility has been improved and less scattering of the strength values was observed.

The references PVAc and MUF passed the requirements of the D1 and D2 class according to EN 204. The MUF resin showed the best bonding capacity overall. The untreated gluten adhesives demonstrated only moderate adhesive strength of about 6–8 MPa. The alkaline solvent had a slight positive impact on the bonding strength. But all in all no significant changes in the dry tensile shear strength of WG dissolved in different solvents were detected. Compared with the gluten-water suspension wet bonding strength of WG solved in alkaline and GdnHCl solutions even decreased to a very low level below 2 MPa. Because of denaturation the native inter and intra molecular forces were weakened and cohesive strength was diminished. Therefore, the induced hydration had a higher negative impact and cohesive failure took place.

Enzymatically treated WG showed no improvement of adhesive properties. Dry and wet bonding strength were in the same range as native WG. The slight enzymatic hydrolysis altered the properties of gluten less. Indeed smaller peptides were generated, but still high molecular weight protein fragments remained. Also the very thin consistence must be considered. Starving of the glue could contribute to the bad performance, because too little glue remained in the bonding line.

Formulations of alkaline WGHs gained dry shear strength of about 7.5 and 12 MPa for short and long treatment times, respectively. The alkaline modification had significant higher tensile shear strength under wet conditions. Longer treatment time even resulted in excellent bonding performance comparable to the PVAc reference. The dry bonding strength was even a little bit higher and the wet bonding strength was in the range of PVAc glue. But the excellent bonding performance of the MUF resin was not completely accomplished. The adhesive based on a short hydrolysis time at a high pH-level carried out moisture resistance with shear strength of about 4–6 MPa. On the other hand bonding strength was not improved. However, slight alkaline hydrolysis was not sufficient to achieve requirements according to EN 204.

Tensile shear strength of the reference glues were in sound correlation with other studies.[24] Strong hydrolysis resulted in excellent bonding performance similar to synthetic resins. The good moisture resistance indicated a notable degree of cross-links. Furthermore the hydrophobic character of WG contributed to the good tensile shear strength after water storage. The solid content of the WGH glue with 15% is even very low compared to other adhesives. Usually resins contain solid amounts of at least 50% or even more.[11] Alkaline WGHs demonstrated superior bonding performance based on dry matters compared to PVAc.

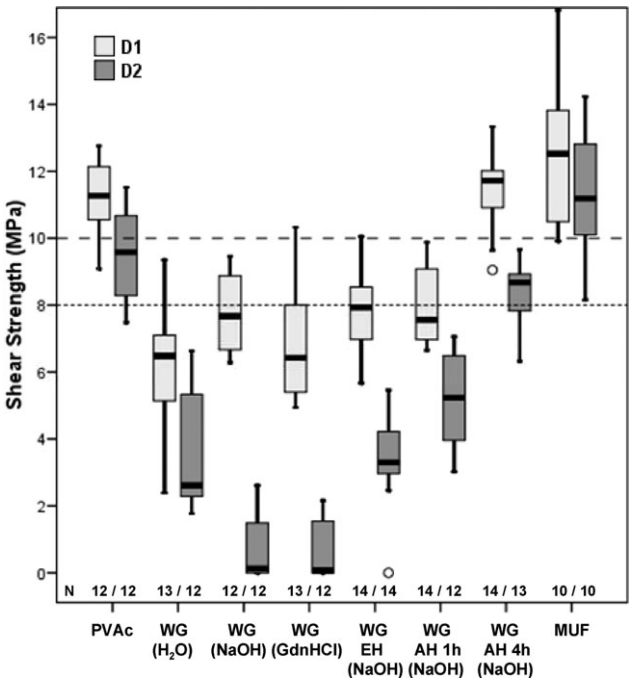

Figure 2. Shear Strength of lap joint specimen with different adhesives preparations (EH, enzymatic hydrolyzed; AH, alkaline hydrolyzed) tested according to EN 204 after dry (D1) and wet (D2) conditioning. Circles represent outliers. The used solvent is declared in brackets. Lines indicate minimum levels of required adhesive strength according to the durability classes D1 and D2 of EN 204. N shows the number of tested specimen.

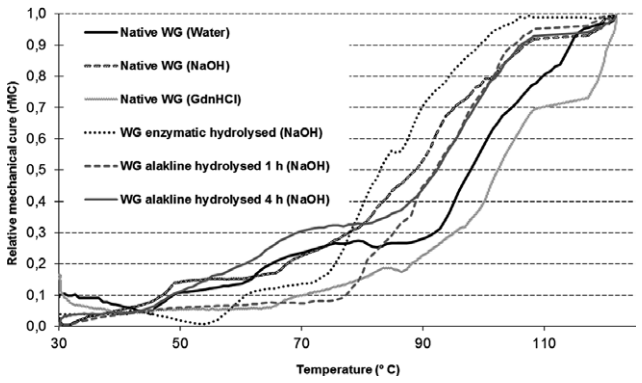

Figure 3. Relative mechanical curing progress observed by DMA in the three-point bending mode as a function of temperature. The different adhesive preparations were put between two wooden veneers and heating rate of 3°C/min was applied. Curves represent average of three measurements.

Curing Behavior

DMA experiments applied in the study allowed the observation of mechanical cure and thus of *in situ* polymerization. Curing of the different adhesives between two wooden veneer strips caused an increase of the storage modulus. The rise can be assigned to changes in viscosity and progress of cross-linking.[19,21,25] Due to the calculation of the rMC variation of wood is mainly excluded.[20] A low heating rate of 3°C/min was used to diminish thermal lags.[21]

The DMA-curves of the relative cure are presented in Figure 3. However, partially decrease of E' could be measured due to the softening of wood and viscosity thinning of the WG preparations induced by increasing temperature. In the temperature range between 50 and 60°C a first rise in E' was recorded. The main curing occurred between 80 and 100°C. At high temperature a steep increase of E' was observed with a flattening of the curve at the end of the curing process. E' remaining constant indicates that hardening of resin was accomplished.

Native Gluten showed quite weak cross-linking behavior and main rise of rMC was detected at over 90°C. But already at very low temperatures of about 30°C increased values of rMC was observed. This could be assigned to the native cohesivity of gluten. Gluten suspended in GdnHCl-solution even slowed down the progress of hardening. A two step curing was observed with beginning from over 90°C and about 115°C.

The high ionic strength repulsion of protein chains is possibly responsible for reduced cross-linking reactions. Therefore, significant higher thermal impact was necessary. WG in alkaline solution showed similar behavior like native WG, but the initial rMC rate was lower. Because of better dispersing properties above the isoelectric point also viscosity was reduced. For enzymatic modified WG highest reactivity was observed with a steep increase in rMC beginning at 75°C until curing was completed at about 100°C. Also the other WGHs showed increased reactivity compared to native WG.

Influence of WG Modifications on Adhesive Performance

In contrast to other studies the addition of one molar solution of GdnHCl did not improve bonding performance.[16] The dry shear strength remained on a poor level comparable with native WG. GdnHCl is used for food analysis as denaturation agent to enlarge solubility and thus yield of extraction.[26] Also dissolving native WG in diluted sodium hydroxide solution did not advance tensile shear strength of lap joint specimen. However, solubility of gluten seems to have no positive influence on adhesive strength to the wood surfaces. Treatments concerning to improve only solubility gained even less wet shear strength. The destruction of inter- and intramolecular bonds due to denaturation, e.g., removal of electrostatic interactions, disulfide, and hydrogen bridge linkages, weakened the polymeric network of gluten. Therefore water could penetrate easily in the bond line and disrupt the cohesive network.

With increasing degree of hydrolysis and reduced molecular weight the bonding strength was improved. Very small peptides can act as cross-linking agent. Because of decomposition more amino- and carboxyl-groups are available for cross-linking reactions. Because of the higher mobility also the reactivity was enlarged. Furthermore very small peptides or amino acids could act as plasticers.[27] Additionally compatibility between starch and protein macromolecules was increased, whereby shear strength of the system was enhanced by improving cohesive properties.[27] Compared with the enzymatic treatment alkaline conditions diminished molecular weight distribution of WG in a higher extent, especially the formation of very small peptides.

Influence of WG Modifications on Curing Behavior

In general gluten shows no cross-linking reactions by heating up to 70°C.[4,28] Gliadins appear to polymerize at temperatures over 100°C and glutelins already below 100°C.[28] Despite addition of GdnHCl modification of WG resulted in higher reactivity. For alkaline WGHs already increase of rMC was monitored at quite low temperatures, between 75 and 85°C. However, smaller peptide fragments achieved by hydrolysis significantly improved reactivity and less activation energy was needed for cross-linking reactions. Although enzymatic hydrolysis gained superior reactivity, adhesive properties were not improved. However, alkaline hydrolysis gained only moderate reactivity, but superior values of tensile shear strength were detected.

The viscosity of adhesive formulations varied in a wide range from below 10 to more than 500 mPas. Very high viscosity limits wetting and penetration of adhesives into to the wood lumina. This results in decrease of the bonding strength. In contrast, if the viscosity of the dispersion is too low the adhesive bond line is more prone for starving.[15,29,30] Compared with other synthetic resins amount of glue based on dry matters is very low. Also determined viscosities of WGHs were very low. Formaldehyde-based resins usually have dry contents above 50%[11] and viscosities ranges between 300 and 600 mPas. However, by adjusting the solid content of protein glues viscosity can easily raised[10,11,15] and requirements for industrial applications can be matched.

Although gluten is designated and sold as protein concentrate, it contains more than just protein. Usually dry gluten contains beside proteins, up to 10% water, and varying amounts of starch, lipids, and fibers. The starch and fiber become entrapped in the cohesive matrix of the protein and are difficult to remove

as the protein content increases.[1,2] Therefore further purification is not profitable. Due to the remaining amount of about 8% starch also its influence to the bonding behavior must be considered. During heating in the presence of water starch will be gelatinized and the viscosity increase dramatically.[29] In contrast to gluten increase of viscosity begins earlier. Gelatinization of native wheat starch starts from 60°C.[29] But starch organization is partially altered because of the starch isolation process and even more under alkaline conditions. Such treatments partly cause damage of the starch granules. Thus gelatinization behavior is affected, which caused lower gelatinization enthalpies and temperatures.[30]

The viscosity of WG dispersions decreased during initial heating, which could cause over-penetration of the glue. Thus the presence of starch limited starving of the bond line. This feature is also applied for synthetic resins by an addition of about 5% starch.[31] Furthermore due its polar character starch could contribute to the adhesive power.[29] These considerations were also confirmed by DMA measurements.

CONCLUSIONS

In this study basic aspects of wood to wood bonding with wheat proteins were observed. Beside molecular and structural properties of different modifications also all ingredients of the adhesive formulations were considered to explain reactivity and bonding strength. Generally it is postulated that adhesive performance of proteins is affected by dispersing properties and the ability to interact with the wooden substrate.[15,32] Because of denaturation proteins got unfolded and the solubility is enhanced. Also the native structure was altered, which affected cohesive strength of the bond line negatively after curing. The formation of smaller peptides by alkaline hydrolysis improved dry bonding strength, wet bonding strength primary after longer treatment. However, distribution of molecular weight of peptides essentially influenced bonding performance. The reactivity of different WG formulations was observed by calculating the rMC based on DMA measurements in the three point bending modus. Curves of rMC explained reaction behavior of WG modifications.

In Europe gluten has many economic benefits compared to the more expensive animal or soy-protein products. By simple alkaline hydrolysis excellent bonding strength under dry and previous moistened conditions according to the durability classes D1 and D2 of EN 204 were achieved. But further research is demanded for the use of wheat gluten glue in the wood industry.

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