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Impact of Protein Size Distribution on Gluten Thermal Reactivity and Functional Properties

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Wheat gluten structure was modified in different ways: Disulfide bonds were reduced by sulfitolysis, or protein chains were enzymatically hydrolyzed at three different degrees of proteolysis. A kinetic study of the thermal reactivity of the modified glutens showed that gluten aggregation kinetic was slowed in consequence to the shift of gluten size distribution toward smaller proteins. In contrary to sulfitolysis, proteolysis also affected the gluten reactivity potential because of the formation of numerous nonreactive species. Moreover, the thermally induced browning reaction was greatly enhanced by proteolysis, which increased the amount of free amine residues, substrates of the Maillard reaction. On the contrary, a whitening effect was observed for reduced gluten with bisulfite. Proteolysis was also found to decrease plasticized gluten viscosity, to increase gluten-based materials water solubility, and to enhance gluten adhesiveness properties but to reduce its mechanical performance. Sulfitolysis was considered as a possible way of extending gluten processability by extrusion or injection molding, whereas proteolysis was found to confer enhanced gluten stickiness that suggests new potential end uses of gluten in the pressure sensitive adhesives domain.

KEYWORDS: Wheat gluten; sulfitolysis; proteolysis; thermal reactivity; pressure sensitive adhesive

INTRODUCTION

Wheat gluten, a byproduct of the wheat starch industry, is available in large amounts and at relatively low cost (about 1 Euro/kg). Wheat gluten proteins consist mostly of monomers (gliadin) and polymers (glutenin) in roughly equal weight fractions. Glutenin consists of discrete polypeptides (subunits) linked together by interchain disulfide bonds to form high molecular weight polymers (*1*, *2*). The unique viscoelastic properties of gluten are at the origin of the breadmaking potential of wheat flour (*3*). Therefore, the first end use of wheat gluten is presently the improvement of flour strength, for breadmaking. The continual increase in gluten production together with its remarkable thermoplastic behavior allow one to consider its potential as a renewable source for the production of biodegradable biomaterials (*4*).

Thermoplastic properties of gluten proteins enable the production of materials in dry conditions, using plasticizers and temperature (*5*). However, gluten thermoplastic processability is sometimes very limited due to its high viscosity and its important thermal reactivity (*6*). Actually, changes in gluten rheological properties are observed following gluten heat treatment, with a large increase in elasticity and viscosity (*7*). Heat treatment of gluten induces an aggregation reaction that led to an increase in covalent cross-linking of gluten proteins by disulfide bonds (*8*, *9*). This cross-linking is useful for the stabilization of the macromolecular gluten network after shaping. Nevertheless, gluten thermal reactivity, whose kinetics is enhanced by high temperature and shear (*10*, *11*), limits extrusion of gluten to a very narrow window of operating conditions: If gluten cross-linking occurs too early during extrusion, gluten material will not have sufficient molecular mobility to support the high strains in the die without breaking (*6*). The control of gluten viscosity and thermal reactivity is therefore required so that gluten can be processed by extrusion or injection molding, which would represent a real challenge for the production of gluten-based materials.

As disulfide bonds play a key role in gluten aggregation mechanism, their sulfitolysis will modify gluten thermal reactivity. According to Cecil and McPhee (*12*), the active species during sulfitolysis is SO_3^{2-}, whereas HSO_3^- does not react ($SO_3^{2-} + H^+ \leftrightarrow HSO_3^-$, $pK_a = 6.8$). The less nucleophilic sulfur involved in a disulfide bond between two peptidic chains (RSSR′) is then substituted by the sulfite anion, according to the following equation (*13*):

$$RSSR' + SO_3^{2-} \leftrightarrow RSSO_3^- + R'S^- \qquad \text{(eq. 1)}$$

Released thiol and S-sulfonate groups from sulfitolysis can become available for the formation of new disulfide linkages (*14*). Gluten thermal reactivity can therefore be preserved, but its kinetics can be modified.

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Table 1. Size Distribution Profile of the Different Glutens Used, Analyzed by SE-HPLC

gluten type	proteins (% of total proteins)		
	>95 kDa	20−95 kDa	<20 kDa
native gluten	43	44	13
sodium bisulfite treated gluten	30	59	11
low proteolyzed gluten	21	57	22
medium proteolyzed gluten	11	47	42
high proteolyzed gluten	11	33	56

A good way of modifying gluten viscosity is the enzymatic proteolysis. Gluten proteolysis, leading to a decrease in protein molecular size, may also be a way of modifying gluten thermal reactivity since high molecular size gluten proteins are the first species involved in gluten aggregation (*15*). In addition, proteolyzed gluten is known to have various additional properties such as increased water solubility as well as foaming, emulsifying, and adhesive properties (*16*−*19*). Adhesive applications were already considered for gluten (*20*). The performance of pressure sensitive adhesives (PSAs) is known to depend on their viscoelastic behavior (*21*), and it is well-established that the balance between gluten viscous and elastic properties is related to the gliadin/glutenin ratio and to the size distribution of the glutenin polymers (*22*, *23*). Because proteolysis will change this distribution, it would thus modify gluten adhesive properties. By controlling the enzymatic reaction conditions, it is possible to obtain gluten with different proteolysis degrees, which enables us to adjust the properties (*17*, *24*).

The purpose of our work was to first investigate in detail the influence of sulfitolysis and proteolysis on gluten thermal reactivity in order to determine how it can be modified to better satisfy requirements of thermoplastic processes. Second, we concentrate more specifically on the impact of proteolysis on gluten viscosity and adhesive properties. Other important sample characteristics (such as color and water solubility) that could limit the use of gluten-based materials were finally investigated.

MATERIALS AND METHODS

Materials. Four different modified glutens were prepared at Amylum Group (Aalst, Belgium). The modified glutens were prepared on pilot scale as described by Linares et al. (*25*). Briefly, wet gluten (30% dm) was digested by protease (Corolase, AB Enzymes) at 60 °C and pH 6 (= pH as is). For the high proteolyzed sample, the enzyme concentration was 2 g/kg (dmb) and the reaction time was 2 h; for the medium and low proteolyzed samples, the enzyme concentration was 1 g/kg (dmb) and the reaction time was 1 h and 30 min, respectively. Alternatively, wet gluten was reacted with sodium bisulfite (Aldrich) at 30 °C and 2 g/kg (dmb) for 30 min. All samples were spray-dried (Niro, production minor) immediately after preparation at an inlet temperature of 200 °C and an outlet temperature of 95 °C. The proteolysis degree remained below 2% in all cases, and the hydrolysis extent was assessed from the protein size distribution [determined by size exclusion high-performation liquid chromatography (SE-HPLC), see beyond] as high, medium, and low according to their proportion in low molecular weight protein fragments (**Table 1**). Protein contents were 77.7, 78.8, and 73.9% (wet basis) for gluten with low, medium, and high proteolysis levels, respectively. Corresponding water contents were 3.7, 4.3, and 4.7% (wet basis).

Anhydrous glycerol used as a plasticizer was purchased from Fluka (Buchs, Switzerland). Chemicals for biochemical analyses of the samples were obtained from Prolabo, BDH, or Sigma-Aldrich in p.a. quality.

Gluten-Based Materials Preparation. Each different gluten (vital and modified) was mixed for 15 min with 30% (weight/total weight) glycerol as plasticizer in a two blade counter-rotating batch mixer turning at a 3:2 differential speed (Plasti-corder W50, Brabender, Duisburg, Germany). Torque and product temperature were continuously recorded during the mixing process. The temperature of the mixing chamber was regulated at 20 °C using a regulation temperature unit (Julabo F34, Seelbach, Germany) that provides water circulation in the double jacket of the mixer. The mixing speed was 30 rpm. The mixer chamber (volume of 50 cm3) was filled with 45 g of total mass. All mixing conditions were fixed to avoid a sample heating above 55 °C, to limit the gluten aggregation reaction.

To obtain plates and perform different thermal treatments, the mixed blends were pressed between two Teflon sheets to approximately 2.3 mm thickness (thanks to spacers) in a heated press (PLM 10 T, Techmo, Nazelles, France) at different temperatures and for different times. To analyze the aggregation reaction kinetic of the different glutens, the following treatment parameters were performed at 80 °C for 4, 30, and 70 h; at 100 °C for 10 and 80 min and 3, 6.5, and 13.5 h; at 120 °C for 5, 10, 15, 30, and 60 min.

Gluten Proteins Size Distribution. Changes in the molecular size distribution of the gluten proteins were characterized by SE-HPLC. Exhaustive presentation of the method is given in Redl et al. (*26*). Briefly, samples were ground in the presence of liquid nitrogen using a laboratory ball mill (Prolabo, France) and then blended with soluble wheat starch (Sigma-Aldrich, 1/5 g/g). The obtained powders were stirred for 80 min at 60 °C in the presence of 20 mL of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% sodium dodecyl sulfate (SDS). The SDS soluble protein extract was recovered by centrifugation (30 min at 39000*g* and 20 °C), and 20 μL was submitted to SE-HPLC fractionation (SDS soluble fraction). The pellet was suspended in 5 mL of SDS−phosphate buffer containing 20 mM dithioerythritol. After it was shaken for 60 min at 60 °C, the extract was sonicated (Vibra Cell 20 kHz, Bioblock scientific) for 3 min at 30% power setting. Disulfide and weak bonds are disrupted by those chemicals, whose efficiencies are further increased thanks to ultrasonic waves. As a result, these treatments bring insoluble protein from the pellet into solution. After centrifugation (30 min, 39000*g*, 20 °C), a part of the supernatant was then mixed volume to volume with SDS−phosphate buffer containing 40 mM iodoacetamide in order to alkylate thiol groups. The reaction was carried out for 1 h in darkness, at room temperature. Twenty microliters of this solution was submitted to SE-HPLC fractionation (SDS insoluble fraction).

The SE-HPLC apparatus was a Waters model (Alliance) controlled by Millenium software (Waters). A TSK G4000-SWXL (Tosoh Biosep) size exclusion analytical column (7.8 mm × 300 mm) was used with a TSK SWXL (Tosoh Biosep) guard column (6 mm × 40 mm). The columns were eluted at ambient temperature with 0.1 M sodium phosphate buffer (pH 6.9) containing 0.1% SDS. The flow rate was 0.7 mL/min, and proteins were recorded at 214 nm. The apparent molecular weight of proteins was estimated by calibrating the column with protein standards (*27*).

Rheological Measurements. Low deformation rheological analyses were done on an Advanced Rheometric Expansion System (ARES, Rheometric Scientific, Piscataway, NJ) with a parallel plan geometry. Circular disks of 25 mm of diameter were cut into sample plates obtained after a thermal treatment at 100 °C for 10 min. The upper plan has a diameter of 25 mm, and the lower one is thermoregulated with a Peltier system and oscillates at a controlled frequency and strain. First, samples were submitted to a strain sweep test at 25 °C, at a frequency of 1 rad/s, from 0.01 to 1% of strain, to determine the linear domain of strain. Second, a time sweep test was carried out at 80 °C, at a frequency of 1 rad/s, at a strain corresponding to the linear domain, for 1 h, to check that the sample rheology does not evolve during such a time of analysis. Finally, dynamic frequency sweep tests were performed at 20, 40, 60, and 80 °C, at a strain corresponding to the linear domain, from 100 to 0.01 rad/s. Each measurement was done with a new disk sample.

High deformation rheological analyses were done on a Rheo TAXT2 rheometer (Champlan, France). Dumbbell-shaped specimens of 75 mm overall length and 4 mm width for the elongating part (5A type, standard ISO 527-2, 1993) were cut into sample plates obtained after a thermal treatment at 100 °C for 10 min. They were preconditioned at 20 °C

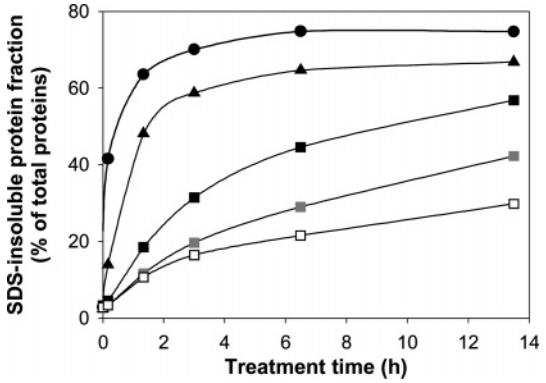

Figure 1. Increase of the SDS insoluble protein fraction during heating at 100 °C. Native gluten (circles), reduced gluten with sodium bisulfite (triangles), and slightly (solid squares), moderately (gray squares), or highly (open squares) proteolyzed glutens.

and 50% relative humidity over a saturated salt solution of $Mg(NO_3)_2$. The sample thickness was measured with a caliper. The initial grip separation was 50 mm, and the elongation speed was 1 mm/s. Stress values (MPa) were calculated by dividing the measured force values (N) by the initial cross-sectional area of the specimen (mm^2). Strain values were expressed in a percentage of the initial length of the elongating part of the specimen ($L_0 = 20$ mm). The tensile strength at break is defined as the maximum tensile stress sustained by the test specimen just before it breaks. Maximum elongation is defined as the strain value at tensile strength at break. Measurements were done on four replicates.

Gluten-Based Materials Color. The color of the different samples was determined using a Minolta Chroma Meter (CR 300, Minolta Chroma Co., Osaka, Japan). A CIE Lab color scale was used to measure the degrees of lightness (L^*), redness ($+a^*$), and yellowness ($+b^*$) of the samples. The instrument was standardized using a Minolta calibration plate with D_{65} illuminant ($L^* = 97.70$, $a^* = -0.10$, and $b^* = 1.89$).

Gluten-Based Materials Water Solubility. Disk samples of 20 mm diameter were immersed in 50 mL of distilled water (containing 0.1% NaN_3 to avoid microbial growth) at 25 °C for 24 h and then dried on P_2O_5 (until constant weight). Measurements were done in duplicate. The sample solubility during water immersion was determined as follows:

$$\text{solubility (\%)} = (W_d - W_{wd})/W_d \times 100 \qquad \text{(eq 2)}$$

where W_d is the initial sample dry weight and W_{wd} is the sample dry weight after water immersion. The sample water content was determined after drying on P_2O_5 until constant weight.

RESULTS AND DISCUSSION

Influence of Sulfitolysis and Proteolysis on Gluten Thermal Reactivity. Native gluten and four modified glutens, differing in their initial proteins size distribution profile, were used to make gluten-based materials by mixing with glycerol. These materials were then subjected to various thermal treatments that led to cross-linking of gluten proteins by disulfide bonding (*8*, *9*). The evolution of the SDS soluble and insoluble fractions of native and modified glutens as a function of the applied thermal treatments (80, 100, and 120 °C for different times) was followed by SE-HPLC. For all gluten types, the total chromatogram surface area of the SDS soluble fraction decreased as the treatment time and temperature increased, while the SDS insoluble fraction increased. This progressive insolubilization is due to the gluten aggregation reaction that is temperature-dependent (*15*, *28*−*30*). As an example, **Figure 1** shows the evolution of the SDS insoluble fraction of native and modified glutens during the thermal treatments at 100 °C. Results reveal differences in protein insolubilization rates according to the initial gluten structure. Gluten treated by sodium bisulfite presents a similar evolution profile to native gluten, although the initial SDS insolubilization rate was lower as well as the maximum percentage of SDS insoluble proteins reached. Concerning proteolyzed glutens, the higher the extent of proteolysis, the lower the rate of protein solubility loss. In addition, although no maximum percentage of SDS insoluble gluten proteins was reached by the time the thermal treatments were performed, this parameter seems to decrease with proteolysis degree.

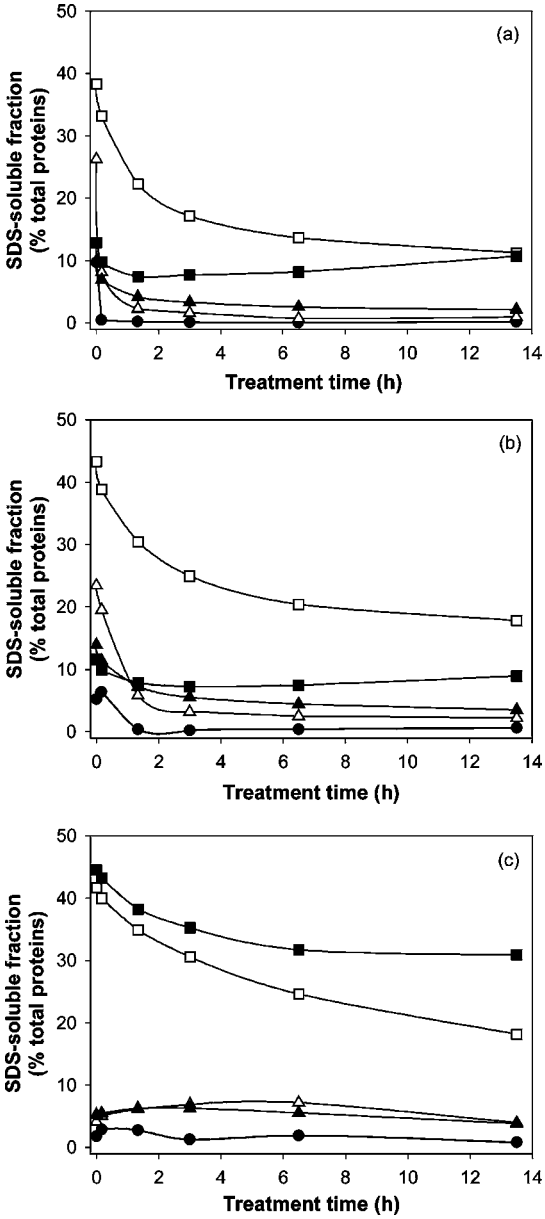

Figure 2. Changes in SDS soluble protein fractions of different molecular size separated by SE-HPLC for native gluten (**a**), reduced gluten with sodium bisulfite (**b**), and moderately proteolyzed gluten (**c**) during heating at 100 °C. Fractions consist in proteins above 680000 (F1, circles), ranging from 95000 to 680000 (F2, open triangles), from 55000 to 95000 (F3, solid triangles), from 20000 to 55000 (F4, open squares), or under 20000 (F5, solid squares).

To get a better understanding of the differences in protein aggregation kinetics, evolution of the five SDS soluble protein fractions of decreasing molecular size separated by SE-HPLC was analyzed. **Figure 2** gives the results for native, sulfitolyzed,

and one proteolyzed gluten during thermal treatment at 100 °C. When native gluten is subjected to the thermal treatment, we observed that changes in SDS soluble fractions depend on their respective molecular size (**Figure 2a**): Glutenin reacts faster than gliadin, and the last eluting fraction remains roughly constant, around 10% of total proteins. This result is in agreement with those reported by Domenek et al (*15*) who ranked gluten protein in classes of different reactivity according to their molecular size.

In the case of gluten reduced by sodium sulfite, the same tendency is observed, but all dropping rates are slowed as compared to native gluten (**Figure 2b**). It is possible that at first, disulfide cross-linking between sulfitolyzed polypeptide chains lead to the production of SDS soluble polymers. A small increase in the fraction of highest molecular size (F1) can effectively be observed (**Figure 2b**). Aggregation of native gluten is thought to result from the conversion of intramolecular disulfide bonds from different polypeptide chains into interchain ones. The mechanism involves sulfhydryl/disulfide exchanges. Oxidation of S-sulfonated groups into disulfide would proceed according to another type of mechanism (see eq 1). It is possible that this different reaction pathway accounts for the slowest insolubilization rate of sulfitolyzed gluten.

Concerning proteolyzed glutens, the analysis of the different SDS soluble fractions during thermal treatment showed a completely different evolution (**Figure 2c**). Fractions of higher molecular sizes (F1−F3) first increased before decreasing by order of molecular size. The fraction of smaller size F4 decreased continuously, while fraction F5 (smallest size) first decreased and then reached a plateau around 17, 33, or 40% of total proteins for low, medium, or high proteolyzed glutens, respectively. Wheat gluten proteolysis led to a pronounced decrease in protein mean molecular size, glutenin polymer (F1 and F2) being almost absent while fraction F5 highly increased (**Table 1**). From the transitory evolution of the different fractions, it seems that large protein species are first created from smaller ones before being able to form SDS insoluble aggregates. The gluten aggregation reaction appears thus to proceed more gradually, a critical molecular size having to be reached before solubility loss could occur.

As proteolyzed glutens are almost lacking of high molecular size proteins, the overall aggregation kinetic is consequently slowed. In addition, most of the small proteolyzed fragments, included in fraction F5, were nonreactive since after an initial dropping phase this fraction leveled off at a high protein proportion as compared to native or sulfitolyzed glutens (**Figure 2**). In gluten proteins, most of the cysteine residues are located at the C-terminal part of the polypeptide chains. Considering the uneven distribution of cysteine, it is likely that many hydrolytic peptides will lack of it. Deprived of cysteine residue, those peptides, like the ω-gliadins (*30*), have no chance to react during thermal treatment. This feature means that maximum protein solubility loss will be lower for proteolyzed gluten, depending on proteolysis extent.

Gluten sulfitolysis and proteolysis were then found to both modify gluten thermal reactivity but in different ways. In the case of sulfitolyzed gluten, the aggregation kinetic is only slowed. On the contrary, proteolysis both slows down gluten aggregation kinetic and limits the reactivity potential through the formation of nonreactive protein species. Sulfitolysis may consequently be the way to slow the too fast reactivity of gluten during extrusion or injection-molding processes without altering its aggregation potential, thereby enlarging the possible operating conditions of such processes for gluten transformation.

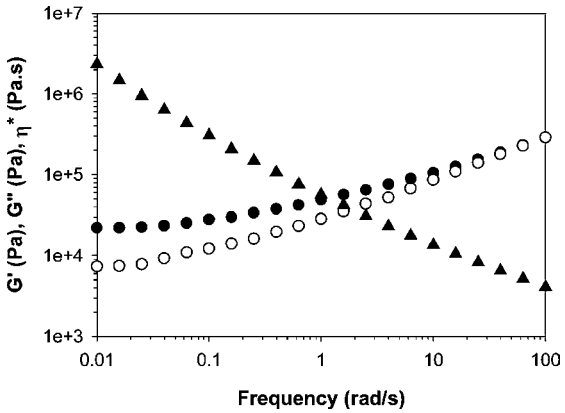

Figure 3. Mechanical spectra obtained at 80 °C for the medium proteolyzed gluten plasticized with 30% glycerol. G′, solid circles; G″, open circles; and η^*, solid triangles.

Table 2. Complex Viscosity Obtained for the Different Proteolyzed Glutens at 1 rad/s and 80 °C

gluten type	η^* (Pa s) at 1 rad/s
low proteolyzed gluten	1.18×10^5
medium proteolyzed gluten	4.71×10^4
high proteolyzed gluten	2.35×10^4

Influence of Proteolysis on Gluten Viscosity and Adhesive Behavior. The rheological behavior of proteolyzed glutens was found to remain roughly stable during the time sweep tests at 80 °C, meaning that no reactivity will have to be considered during the analyses. The mechanical spectrum at 80 °C of the medium proteolyzed gluten is given in **Figure 3**. As the strain frequency decreases, G' and G'' moduli decrease first linearly in a log/log scale before reaching the viscoelastic plateau. The complex viscosity (η^*) continuously increases when the strain frequency decreases, which is characteristic of a shear thinning material. Complex viscosity values obtained for the different proteolyzed glutens at 1 rad/s are given in **Table 2**. This value was lowered by 80% for the highest proteolyzed gluten as compared with that of the lowest proteolyzed gluten, confirming the ability of proteolysis to decrease gluten viscosity.

Concerning adhesive properties, the performance of PSAs is known to depend on their viscoelastic behavior (*21*) and can be characterized by the Dahlquist criterion (*31*) or by the viscoelastic windows concept of Chang (*32*). Dahlquist defined the requirement for a good PSA to have a storage modulus lower than 3.3×10^5 Pa (*31*). Moreover, as adhesive bonding is a low rate process while debonding is a high rate process, a frequency sweep test enables us to characterize viscoelastic properties during both phenomena. To integrate this feature, Chang proposed a concept of viscoelastic windows, obtained by plotting four coordinates (G'' at 0.01 rad/s and G' at 100 rad/s, G'' at 100 rad/s and G' at 100 rad/s, G'' at 100 rad/s and G' at 0.01 rad/s, and G'' at 0.01 rad/s and G' at 0.01 rad/s) on a log−log plot of G' as a function of G'' (*32*). According to Chang, general purpose PSAs have moduli at the two frequencies between 10^4 and 10^5 Pa.

Consequently, to characterize the adhesive behavior of the different proteolyzed glutens, moduli values at 100 and 0.01 rad/s were used to obtain the viscoelastic window of Chang. The windows obtained at 80 °C for the proteolyzed glutens are shown in **Figure 4**. Below this temperature, none of them satisfied Dhalquist criterion; the moduli values were too high. When the proteolysis degree increases, moduli are lower and

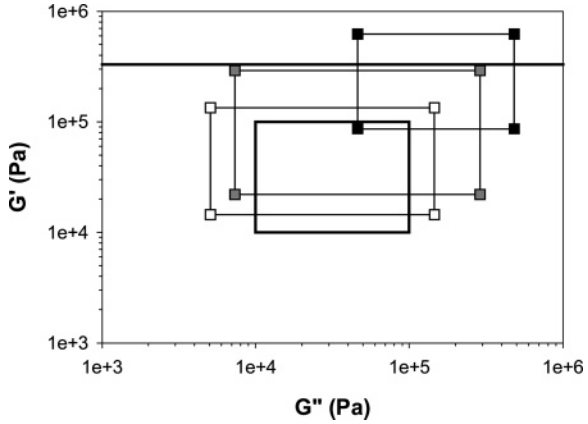

Figure 4. Viscoelastic windows obtained at 80 °C for slightly (solid squares), moderately (gray squares), or highly (open squares) proteolyzed glutens plasticized with 30% glycerol. The bold line represents Dahlquist criterion, and the bold window represents the Chang viscoelastic window for general purpose PSAs.

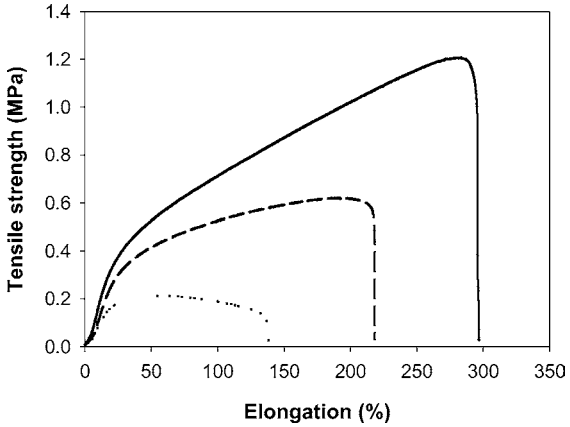

Figure 5. Tensile stress–strain curves until break for materials plasticized with 30% glycerol and heated for 10 min at 100 °C. Native gluten (solid line) and slightly (dash line) and moderately (dotted line) proteolyzed glutens.

better satisfied both Dahlquist and Chang predictions for general purpose PSAs. From these theoretical results, an adhesive behavior can be expected at 80 °C from the materials made with the medium and high proteolysis degrees.

Adhesive performance depends not only on the bond strength between the adhesive and the adherent but also on the cohesive strength of the adhesive itself. Therefore, tensile tests were performed on the materials obtained with the proteolyzed glutens (**Figure 5**). As the proteolysis degree increases, the strength at break values decreases. The test cannot be achieved for the highest proteolyzed gluten because the sample flows when tightened in the grips. Consequently, even if the most proteolyzed glutens seem to display a good adhesiveness, they may not have sufficient strength to perform a solid bond. The strength of materials made from gluten could be enhanced by an appropriate thermal treatment, leading to disulfide bonding and thus to an increase of the network cohesion (*33*). However, we showed that the thermal reactivity of proteolyzed gluten was greatly reduced by proteolysis. It will therefore be difficult to modify the mechanical properties of proteolyzed gluten by this way. Consequently, a compromise has to be found between material adhesiveness and cohesiveness. Specific tests for adhesive properties will enable us to define the suitable proteolysis degree of gluten that will lead to possible applications as PSA.

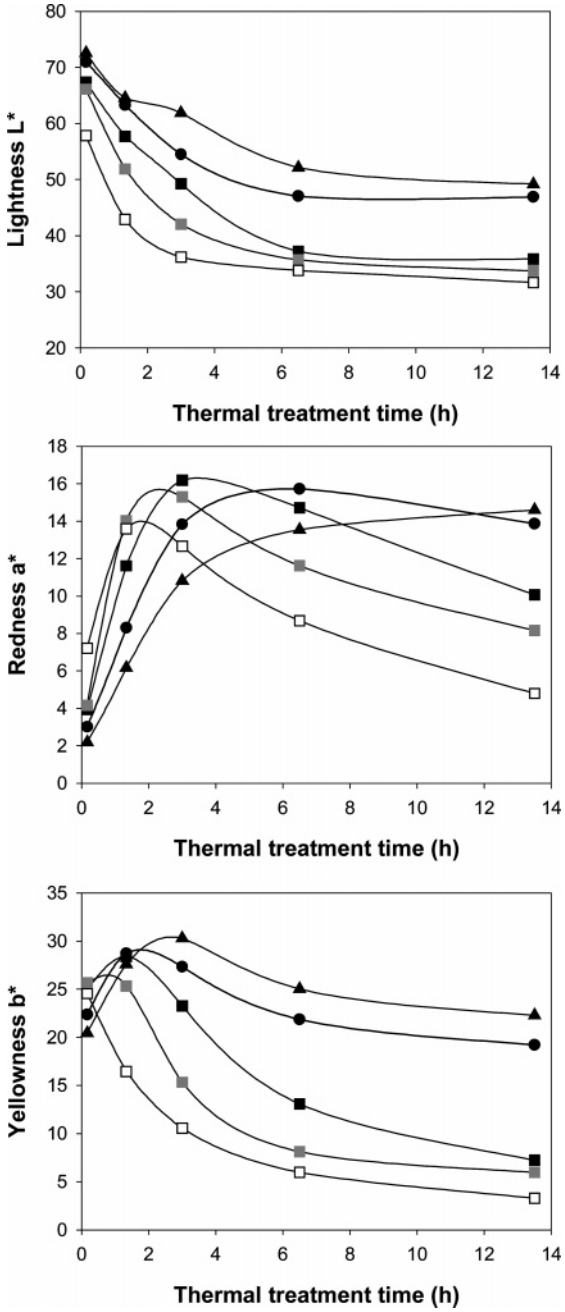

Figure 6. Evolution of color parameters for samples plasticized with 30% glycerol and heated at 100 °C. Native gluten (circles), reduced gluten with sodium bisulfite (triangles), and slightly (solid squares), moderately (gray squares), or highly (open squares) proteolyzed glutens.

Influence of Gluten Structure on Materials Browning during Thermal Treatments. With increasing thermal treatment time, the color of native gluten-based materials became darker (lower L^*) in a monotonic way, while redness (a^*) and yellowness (b^*) increase to a maximum before decreasing (**Figure 6**). The effects were more intense, and the changes were more rapid when treatment temperature increases (not shown). These changes in coloration were already observed on gluten films (*33*−*35*). The browning is likely to be due to the accumulation of Maillard products, resulting from complex reactions between aldehyde groups of reducing carbohydrates with free amino groups of amino acids.

Samples made with sulfitolyzed gluten are slightly lighter (higher lightness and yellowness components, lower redness) than those made with native gluten (**Figure 6**). This feature

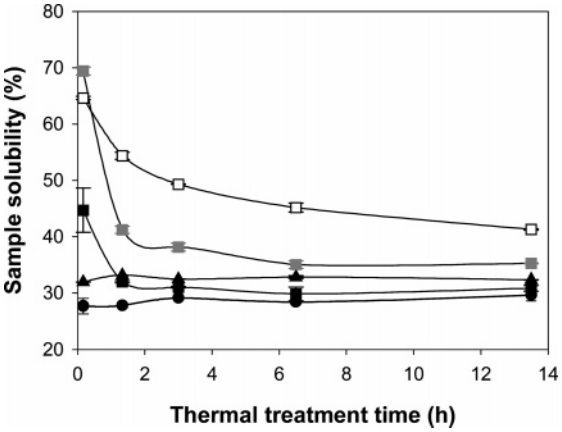

Figure 7. Change in water solubility for samples plasticized with 30% glycerol and heated at 100 °C. Native gluten (circles), reduced gluten with sodium bisulfite (triangles), and slightly (solid squares), moderately (gray squares), or highly (open squares) proteolyzed glutens.

can be explained by the whitening action of sulfite as well as its inhibiting effect on Maillard reaction (*36*). Samples from proteolyzed glutens were on the contrary much more sensible to browning: the higher their degree of proteolysis, the sooner the color evolution and the more important the differences in color coordinates with native gluten (**Figure 6**). This can be related to the proteolysis reaction that increases the content in free primary amino groups, thereby supplying additional reactive species for the Maillard reaction. This color sensibility will greatly impair the use of those materials made from proteolyzed glutens when a thermal treatment needs to be performed. For proteolyzed gluten and in order to limit Maillard reaction, it might be useful to eliminate the residual starch (8−15%) usually present in commercial wheat gluten.

Influence of Gluten Structure on Materials Water Solubility. Water immersion tests were performed on samples having undergone thermal treatments at 100 °C. Solubility results are shown in **Figure 7**. Hydrophilic plasticizers such as glycerol are known to migrate in the water during sample immersion, but native gluten remains insoluble, thus preserving sample integrity (*37*, *38*). This was confirmed by our results since, whatever the thermal treatment time, native gluten-based samples have solubility values around 29%, which roughly corresponds to their initial glycerol contents (**Figure 7**). The same behavior was observed for samples from sulfitolyzed gluten, solubility values (33%) being however slightly higher than those for native gluten. Concerning materials made from proteolyzed glutens, much higher solubility values were obtained for the shortest treatment times, and the samples were partly disintegrated. Actually, in addition to glycerol, and contrary to native gluten, proteolyzed glutens are partly soluble (*16*). Up to 70% of gluten samples with high or moderate proteolysis degree and thermally treated for 10 min at 100 °C were lost during the test (**Figure 7**). This means, assuming that all glycerol migrated into water during immersion, that around 60% of the initial proteolyzed gluten was brought into solution. The sample solubility dropped upon further heating. The feature may be related to the increase in SDS insoluble protein fraction due to cross-linking (**Figure 1**). Beyond 80 min of the thermal treatment at 100 °C, samples made from the lowest proteolyzed gluten were no more water soluble. From sample solubility values, it can be calculated that in samples treated for 13.5 h at 100 °C, 8% of the gluten with the moderate proteolysis degree is still water soluble, while this value equals 18% for gluten with the highest hydrolysis level. This behavior can be related to the limiting reactivity of proteolyzed glutens that was demonstrated before and will impair the use of such materials in application where a water resistance is required.

In conclusion, this study evaluated the potentiality of sulfitolysis and proteolysis to adjust gluten viscosity and thermal reactivity in order to extend its processability and use potential. Gluten processing by extrusion or injection molding is greatly limited by its too fast aggregation reaction leading to an early structured macromolecular network that can no more support the strain of the process.

Sulfitolysis appeared to slow gluten thermal reactivity kinetic without affecting the maximum of aggregated gluten protein that can be reached. It may thus be a way of enlarging the operating conditions window for the production of gluten-based thermoplastic materials by extrusion. In addition, resulting materials will still have an aggregation potential that can be used to improve their mechanical resistance.

Proteolysis was a good way to reduce gluten viscosity and to slow its thermal reactivity kinetic, but this was to the detriment of proteins aggregation potential, since proteolyzed fragments include nonreactive species. Proteolysis also greatly contributed to increase thermal browning and water solubility of gluten materials. Nevertheless, it appeared to be a promising way to enhance gluten stickiness. Further investigations and additional adhesive tests are still necessary to adjust the proteolysis degree and to confirm the possibility of proteolysis to extend gluten uses in the PSAs domain.

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