DOI:10.1021/jf901183z

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Modification of the Wheat Gluten Network by Kraft Lignin Addition

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The effect of Kraft lignin (KL) on wheat gluten (WG) network formation during biomaterial processing was investigated. Gluten plasticized with glycerol was blended with a variable content of KL and processed into material by mixing and hot molding. The effect of KL on WG cross-linking was assessed by size-exclusion chromatography coupled with specific detection of KL by fluorescence. Whereas processing of WG usually results in cross-linking and solubility loss, KL addition promoted an increase of gluten protein solubility in sodium dodecyl sulfate buffers. The feature demonstrates that KL functional groups hinder WG aggregation. A radical scavenger activity of KL toward the thiyl radicals produced during gluten mixing is proposed. Mixing also promotes the association of KL with WG as evidenced by the coelution of KL and WG in size exclusion high-performance liquid chromatography. Finally, gluten aggregation and cross-linking can be obtained by immersion of the materials in a dioxane−water solution, thereby demonstrating the occurrence of stabilized radicals on WG material mixed with KL.

KEYWORDS: Protein; polyphenol; interaction; processing

INTRODUCTION

The increase in petroleum prices and emergence of environmental concerns have led to a growing interest in the design of innovative biodegradable materials based on agropolymers, among which polysaccharides and proteins have been mainly studied (*1*−*3*). However, the concurrence between food and nonfood uses should in the future favor the use of byproducts, which are not valorized in alimentation or are difficult to valorize in large quantities. Wheat gluten (WG), which is a coproduct of starch in the starch industry (*4*), responsible for celiac disease of gluten-intolerant persons, and Kraft lignin (KL), which is a byproduct of the paper industry (*5*), are thus interesting challengers for the building of new materials.

WG is mainly composed of two types of proteins, namely, gliadin and glutenin, which are responsible for its unique viscoelastic properties. During thermal and thermomechanical treatment, gluten protein aggregates through disulfide bonding, a phenomenon that is well-documented. (*6*−*8*). However, the underlying mechanism of protein reactivity during processing is still under investigation. Shear stress during mixing or extrusion is postulated to be the starting point of free radical formation (thyils), leading to gluten cross-linking (*7*, *8*). A recent study (*9*) has proposed that the gluten aggregation mechanism during mixing is a combination of successive radical and nucleophilic mechanisms. **Figure 1** helps to understand the gluten cross-linking during processing, which can be influenced by additives able to interact with radicals formed in the first step or able to modify the pH, which impacts equilibrium 3.

A previous study (*10*) has shown that the KL addition strongly modified the mechanical and rheological properties of WG-based materials, especially by decreasing the rubbery modulus by a few orders. As WG mechanical properties are highly correlated with the extent of protein aggregation (*7*, *11*, *12*), it could be hypothesized that this effect reflects an interaction between the KL and the aggregation pathway, resulting in a reduced cross-linking. Indeed, lignin, a polyphenolic compound, has been used in materials for its antioxidant and free radical scavenging properties, which allow free radicals capture (*13*). Moreover, because of its available reactive hydroxyl groups, lignin can chemically react by esterification and etherification (*5*).

From a more general point of view, polyphenolic structures are well-known to interact with proteins, leading to the formation of protein−polyphenol complexes. For example, plant and dietary polyphenols such as tannin can precipitate salivary proteins via hydrophobic forces and hydrogen bonding (*14*−*16*). Zahedifar et al. (*17*) have shown that an industrial lignin (hydrolytic lignin) can precipitate protein and protect protein from degradation in the rumen.

Few studies have reported on the alkaline lignin reactivity in agropolymer blends. Lepifre et al. (*18*) have shown that covalent cross-linking between starch and soda lignins is likely to occur under electron beam irradiation. Soy protein/KL or lignosulfonate blends were realized by Huang et al. (*19*, *20*). The resulting modification of the mechanical properties of the blend conducted the authors to hypothesize the formation of a cross-linked network involving both lignosulfonate and soy proteins.

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 Published on Web 08/21/2009

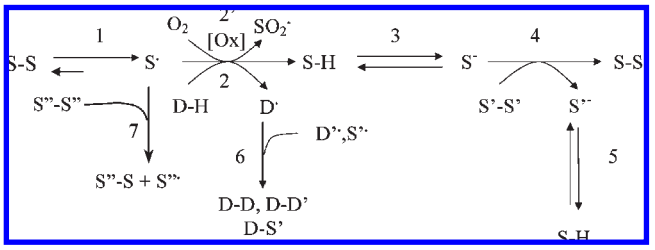

Figure 1. General mechanism of gluten aggregation (*9*).

Table 1. Composition Ratio of WG-Based Materials Containing Different KL Contents

composition (% wt)	sample 0% KL	10% KL	20% KL	30% KL	40% KL	50% KL	60% KL	70% KL
WG	70	60	50	40	30	20	10	0
KL	0	10	20	30	40	50	60	70
glycerol	30	30	30	30	30	30	30	30
WG/KL weight ratio	no KL	6	2.5	1.33	0.75	0.4	0.17	no WG

The objective of this study was to investigate the effect of KL on WG protein cross-linking during biomaterials processing. First, the WG protein aggregation was evaluated. A combination of size exclusion high-performance liquid chromatography (SE-HPLC) with both ultraviolet (UV) and fluorescence detectors gave us evidence of a KL association with WG. Unprocessed KL/WG blends were prepared and compared to processed blends to demonstrate the effect of thermomechanical treatment on the KL/WG reactivity. In addition, the effect of solvent on the protein aggregation of KL/WG blends was measured.

EXPERIMENTAL PROCEDURES

Materials. Commercial vital WG was obtained from Amylum Group (Aalst, Belgium). Its protein content was 77% (dry matter) according to the manufacturer. Its moisture content, determined by weighting after heating at 104 °C for 24 h, was 10.6% (wet basis). The density was 1.31 g/cm^3 (*21*).

KL was provided by Westvaco (Charleston, SC). Its M_w and M_n were estimated by gel permeation chromatography to be, respectively, 4080 and 850 g/mol (*13*).

Anhydrous glycerol was purchased from Fluka Chemie (Buchs, Switzerland) in p.a. quality. Chemicals for biochemical analysis of the samples were obtained from Sigma or Merck in p.a. quality.

Sample Preparation. Materials containing a mixture of WG:KL:glycerol in a weight ratio ranging from 70:0:30 to 0:70:30 were prepared by mixing and compression molding. In all of those materials, the plasticizer content was constant at 30 wt % as shown in **Table 1**.

Mixing Process. Fifty grams of WG, KL, and glycerol was mixed in a two blade counter-rotating batch mixer turning at 100 rpm, at a 3:2 differential speed (Plasti-corder W50, Brabender, Duisburg, Germany). The torque and product temperature were continuously recorded during mixing. The emperature of the mixing chamber was regulated at 30 °C using a regulation temperature unit (Julabo F34, Seelbach, Germany) that provides water circulation in the double jacket of the mixer.

Compression Molding. Twenty-five grams of the mixed blend was deposed in a squared mold (9 cm × 9 cm) and thermomolded at 80 and 100 °C for 10 min in a heated press (PLM 10 T, Techmo, Nazelles, France). A spacer was used to obtain materials with the same thickness of 2.3 mm.

SE-HPLC. Changes in the molecular size distribution of the WG proteins were characterized by SE-HPLC. Exhaustive presentation of the method is given in Redl et al. (*22*). Briefly, samples were ground in the presence of liquid nitrogen using a laboratory ball mill (Prolabo, France) and then blended with soluble wheat starch (1/5 g/g), which allows the glycerol absorption. The obtained powder (160 mg) was 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 (first extract). The pellet was suspended in 5 mL of SDS-phosphate buffer containing 20 mM dithioerythritol (DTE). 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 were disrupted by those chemicals, whose efficiencies were further increased thanks to ultrasonic waves. As a result, these treatments brought insoluble protein from the pellet into the 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 to alkylate the 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 (second extract).

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 for UV measurement. The apparent molecular weight of proteins was estimated by calibrating the column with protein standards (*22*).

For the fluorescence measurement, the excitation wavelength was 310 nm, and KL was recorded on an emission wavelength of 375 nm. The eluted molecules were detected by fluorescence about 10 s after the UV measurement. Therefore, the elution times were corrected for the comparison between the fluorescence and the UV absorbance chromatograms.

When the KL extraction preceded SE-HPLC analysis, the ground materials were three times successively immersed under agitation in a 1,4-dioxane:water (9:1) solution and centrifuged (1 h, 20000*g*, 20 °C). The remaining pellets were dried over P_2O_5. Then, dried samples were extracted and characterized by SE-HPLC as indicated above.

Free Lignin Quantification. Materials (prepared by mixing and hot molding at 80 °C for 15 min) were immersed in a 1,4-dioxane:water (9:1) solution at room temperature for 1 week. The quantity of KL in the solution was then determined with a spectrophotometer (Utrospec2000, Pharmacia Biotech) at 280 nm, assuming that its extinction coefficient is not modified by processing and that the extinction coefficients of the various KL fractions (F) are similar. The unextractible content was calculated as the difference between the initial KL mass in the sample and the extracted one.

Infrared Spectroscopy. Infrared spectra of the material were acquired on a Nicolet 6700 FT-IR spectrometer with a PbSe detector. For each spectrum, 64 scans were collected at a resolution of 4 cm^{-1}.

pH. Suspensions were obtained by dispersing 3.5 g of powder (WG and/or KL) and 1.5 g of plasticizer (30%) in 10 mL of water under magnetic stirring at 200 rpm for 15 min. The pH was measured with a pH meter (WTW inoLab, level 1).

RESULTS

Protein Aggregation. The changes in protein aggregation in the presence of KL were investigated by SE-HPLC. Because of strong intermolecular interactions (hydrogen bonds, hydrophobic interactions, etc.), WG proteins are water-insoluble. The addition of strong anionic surfactant like SDS allows the suppression of the intermolecular interactions between proteins and brings them into solution (SDS-soluble fraction). The remaining SDS-insoluble protein fraction can be further extracted in the same SDS buffer, after the cleavage of the disulfide bridges, which are responsible for the WG interchain cross-linking. **Figure 2a** (SDS-soluble) and **b** (SDS-insoluble) present the elution profiles of native WG and KL powders as received. The WG profile is usually divided in six fractions, from the larger molecule (short elution time) to the smallest one (long elution time). KL, which is a smaller molecule, is observed at higher elution times, after 16 min. The large peak observed between 18 and 21 min can be

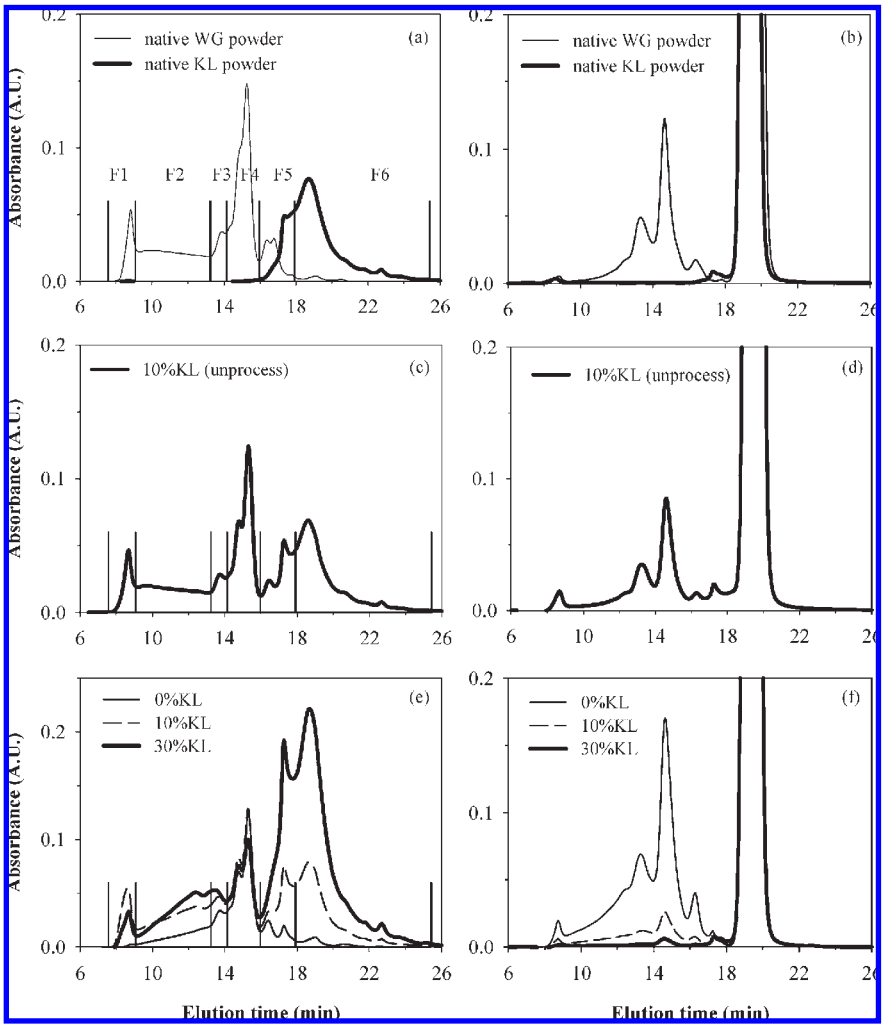

Figure 2. UV-size exclusion distribution profiles of SDS-soluble (**a**, **c**, and **e**) and SDS-insoluble (**b**, **d**, and **f**) protein fraction of various materials.

attributed to DTE (**Figure 2b**,**d**,**f**). KL powder shows a very little content of SDS-insoluble material.

WG and KL powders were blended and extracted in SDS buffer using the same mass basis as in the extracts shown in **Figure 2a**,**b**. **Figure 2c** (SDS-soluble) and **d** (SDS-insoluble) show the elution profiles of these unprocessed blend samples. The profiles equal those of the added profiles obtained from the separated extracts of KL and WG (**Figure 2a**,**b**), which demonstrates that the WG and KL extinction coefficients are not modified by simple blending. In addition, from a simple comparison between **Figure 2a**,**c** and **b**,**d**, SDS buffer seems able to largely prevent WG and KL interaction through weak bonds. **Figure 2e** shows the elution profiles of the SDS-soluble fraction of processed materials with 0−30% KL content. The KL peak position is unmodified when compared to **Figure 2a**,**c**, suggesting that a large quantity of KL remains free and is not modified either by thermomolding or mixing with WG. However, an increase of F1−F4 areas with increasing KL content can be noticed. **Figure 2f** shows a clear decrease of the insoluble fraction when the KL content increases.

The quantity of soluble and insoluble protein in each material was calculated from the chromatograms after subtraction of the KL contribution, which was estimated from the profiles of the original powder (**Figure 2a**,**b**, bold line) and the known material composition. The remaining area is thus only corresponding to WG and is expressed as a percentage of the initial WG content of each material. In that case, we assume that the extinction coefficients of both WG and KL were not modified by mixing and eventually processing them together. **Table 2** summarizes the resulting percentage of protein content in SDS-soluble and SDS-insoluble fractions, for materials molded at 80 and 100 °C. As expected, in the absence of KL, the SDS-soluble fraction decreases with the molding temperature (*11*, *12*, *23*). Indeed, the extent of WG aggregation is highly sensitive to the applied thermal treatment.

The simple addition of KL in the protein−surfactant solution (unprocessed blends) results in an incomplete protein recovery, even after DTE addition. This could be attributed to the well-known protein binding and precipitating properties of polyphenol compounds (*14*).

Contrary to the unprocessed blend, two phenomena are observed for processed blends with KL addition. For those materials, KL was blended with WG during its processing with plasticizer and is thus susceptible to interact with the complex radical and nucleophilic mechanisms, which normally result in those conditions, in the gluten aggregation.

The first phenomenon is a strong increase in the SDS-soluble protein content, with estimated content higher than the total real protein content of the sample (thus higher than 100% in **Table 2**). For example, at 30% KL and whatever the molding temperature, the calculated protein content is twice higher than the real protein quantity present in the material. This evolution can only be explained by the modification of the absorbance of the soluble compounds during their processing. As the absorbance increase is

Table 2. Protein Content (%) in SDS-Soluble and SDS-Insoluble Fractions of Native WG, Unprocessed Blends, and Materials Molded at 80 and 100 °C

	protein area/total theory protein area (%)		
sample	soluble (F1−F6)	insoluble (F1−F5)	total
native WG	77	23	100
	unprocessed blends		
10% KL	68	21	89
30% KL	57	29	86
	material molded at 80 °C		
0% KL	56	44	100
10% KL	110	9	119
30% KL	180	0	180
	material molded at 100 °C		
0% KL	41	54	95
10% KL	98	23	120
30% KL	179	0	179

only observed on processed WG/KL blends, these new structures are formed only under processing conditions, if the two compounds are present together.

The second phenomenon is a strong decrease in the SDS-insoluble fraction whatever the molding temperature. This fraction can even be smaller than that of native WG. Thus, the KL addition not only suppresses the protein cross-linking that normally occurs during mixing but also induces a significant WG protein "depolymerization", even at low KL content.

Under thermomechanical treatment, gluten aggregation results from a complex mechanism, involving sulfhydryl/disulfide interchange reactions and implying both radical and nucleophilic pathways. It has been proposed that in a first step, shear and/or temperature results in the formation of thiyl radicals in place of some of the initially present disulfide bonds. The presence of hydrogen donors results in their transformation in thiol groups, in equilibrium (depending on the pH) with thiolate anions, which should react with intramolecular disulfide bonds, giving rise to the formation of the intermolecular disulfide bonds (called aggregation phenomenon). As protein cross-linking is strongly sensitive to pH, the KL/WG blends pH was checked. The obtained data give a pH value of 6.22 for a material without KL, 6.38 for a sample with 30% KL, and 6.44 for a material only composed of KL and glycerol. The pH is not significantly modified by KL addition, demonstrating that WG depolymerization has to be attributed to a specific interaction with KL during processing. Indeed, even if a plasticization of WG by KL should explain a part of this depolymerization, the amplitude of the observed phenomenon is of such an order that it has to be explained by a chemical interaction between the KL and the gluten aggregation chemical pathway.

It was shown previously that the addition of additives able to interact with WG through both radical and nucleophilic routes can result in WG depolymerization during processing (*9*). Lignin is a complex polyphenol, which presents different functional groups and is well-known for its antioxidant or free radical scavenging properties. Accordingly, it can be supposed that lignin polyphenol groups could impair the gluten cross-linking occurring during mixing and thermomolding, due to their specific chemical structure.

The observed depolymerization should partially explain the low rubbery modulus of WG/KL materials observed in our previous study (*10*). Ullsten et al. have previously shown that the enlargement of gluten extrusion window can be obtained by the incorporation of salicylic acid in plasticized WG, which decreased the degree of aggregation due to its free radical scavenging effect (*24*). Our results support that WG depolymerization during processing can be due to the radical scavenging effect of KL. Therefore, KL should be a promising additive to facilitate the gluten-based materials processing.

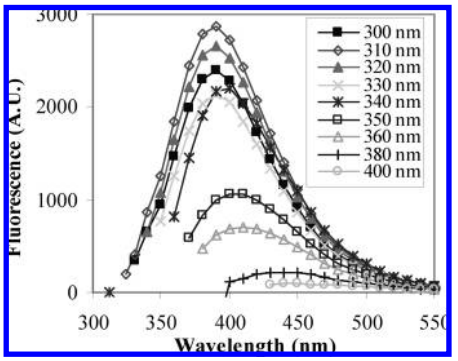

Figure 3. Emission spectra of KL dissolved in SDS solution for various excitation wavelengths ranging from 300 to 400 nm.

KL Association with Gluten. Lignin fluorescence is a well-known behavior. It has been proposed (*25*) that it originates in a nonradiative energy transfer from lignin chromophores (excited between 240 and 320 nm), to some phenylcoumarone structures present in lignin (emitting at about 360 nm). As SDS was demonstrated (although on some others fluorescent compound) to modify the fluorescence spectrum (*26*) (*27*), the fluorescence of KL was measured in a SDS solution, with the same surfactant concentration as used for protein solubility determination. A fluorescence emission spectrum maximum at 375 nm for an excitation at 310 nm was identified (**Figure 3**). These excitation and emission wavelengths are close to the one reported in the literature for spruce lignin (*25*) and KL (*28*), which exhibit, respectively, a maximum emission at 360 nm and at above 400 nm for excitation wavelengths ranging from 240 to 320 and from 300 to 400 nm. The small difference with our data can originate in structural differences or on the effect of SDS.

As WG does not exhibit any significant fluorescence at the studied excitation−emission wavelengths, fluorescence detection appears to be a good way to check precisely the presence of KL. The KL passage at the exit of the SE-HPLC column was thus specifically followed using a fluorescence detector. **Figure 4** shows the elution profiles of the SDS-soluble fraction of a KL powder, KL/WG unprocessed blends with 10 and 30% KL, and two materials thermomolded at 80 °C and containing 10 and 30% KL.

The KL powder chromatogram shows that the free KL molecules are eluted after 16 min, without any elution at earlier times. In contrast, a fluorescence signal was recorded at small elution times in KL/WG unprocessed blend, which demonstrates that some KL molecules coelute with the largest WG proteins, indicating the association of both compounds. This association was previously not detectable from the UV analysis. There is evidence that some nonspecific binding of KL on WG protein can occur upon simple blending, despite the destructuring effect of the SDS buffer. For the processed blends, profiles similar with the ones observed in UV were obtained, but the fluorescence signals were stronger than those of unprocessed blends. Blend processing undoubtedly promotes KL association with WG. However, as it is not experimentally possible to ensure that this association does not modify (reduce or increase) the fluorescence of the KL

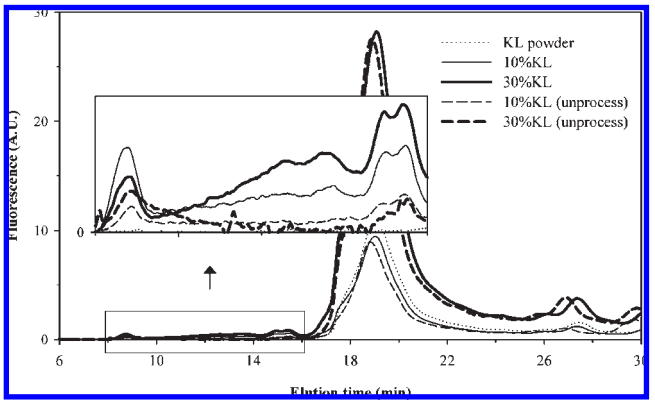

Figure 4. Fluorescence-size exclusion distribution profiles of the SDS-soluble fraction of KL powder (dotted line), gluten-based material containing 10 (solid line) and 30% (bold line) KL, molded at 80 °C, and unprocessed KL/WG blends containing 10 (dash line) and 30% (bold dash line) KL.

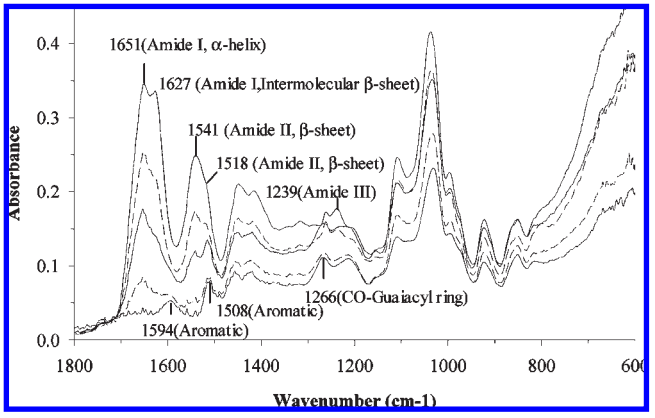

Figure 5. Infrared spectra of material containing 0, 10, 30, 50, and 70% KL content (top to bottom) molded at 80 °C.

molecule, it was not possible to precisely quantify the amount of association.

The significantly higher fluorescence signal for the processed blend (than for the unprocessed one) indicates that during processing, a specific interaction occurred between both polymers, resulting in a stronger association. Two kinds of association between KL and WG can be envisaged: an association through interactions forces or a covalent linkage through the "grafting" of KL on WG. These hypotheses will now be discuss, keeping in mind two main observations. First, the over-recovery of protein (from UV detection, **Table 2**), which can be a result of a change in the UV absorbance, which is not observed for unprocessed blends. Second, the WG depolymerization, which demonstrates an interaction of KL with the cross-linking mechanism of WG. To demonstrate the existence of a new chemical bond, infrared analysis was conducted on materials with various KL contents (**Figure 5**), but it was not possible to establish any evidence of this hypothetical bond.

The properties of polyphenol to interact and precipitate protein in solution has been extensively studied in the literature, for example, to study haze formation in beverages (*29*) or the astringency feeling in the mouth (*30*). Those studies, which attribute this behavior to a noncovalent bonding, have clearly demonstrated that the proline amino acid plays a key role in this association (*31*). WG proteins, which are usually called "prolamine", have a high content in proline, which arise for more than

Table 3. KL Extractability from Materials with Various KL Contents Plasticized with 30% Glycerol

	g/100 g		
gluten	KL	soluble lignin	insoluble lignin
0	70	71.09	−1.09
10	60	57.87	2.13
20	50	46.95	3.05
30	40	35.85	4.15
40	30	24.91	5.09
50	20	13.72	6.28
60	10	5.97	4.03

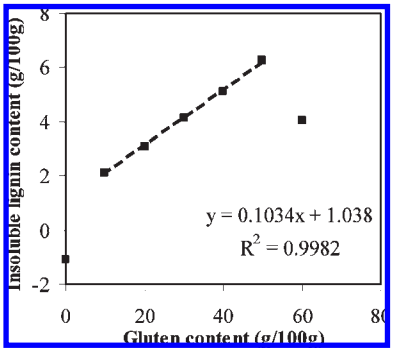

Figure 6. Insoluble KL content (in g of lignin per 100 g of WG-KL-glycerol materials) as a function of the WG content.

10% of the total amino acid composition. They should thus be considered as good candidates for this kind of interaction. Moreover, it was proposed that $\pi-\pi$ interaction can occur in those systems (*31*) (*32*), which might explain the increase of the UV absorption observed in this study, as those interactions can affect the absorbance spectrum (*33*). In such a model, the KL combination with WG proteins would result in a modification of their spatial configuration, making impossible their further cross-linking through disulfide bonds. During processing, the rupture of the disulfide bond between glutenin subunits, in the first step of the mechanism described in **Figure 1**, would generate new smaller species immediately associated with KL molecules and thus not any more able to cross-link following the further step of the process. Such a behavior could explain the observed WG depolymerization.

Moreover, some others studies show evidence of the possibility for the phenolic structure to be chemically grafted on proteins (*34*). A covalent bonding is susceptible to occur, for example, via the oxidation of *ortho*-dihydroxybenzene structures and subsequent reaction with nucleophilic agents. This reactivity has been used to realize the grafting of bovine serum albumin on quercitin in oxidizing conditions (*35*). It is thus likely that in the system studied here, the presence at the same time in a highly concentrated medium of both radical species (thiyls formed on protein) and radical scavenger species (KL) should result in a specific interaction. For example, the formation of a covalent bond between the thiolate anion and some quinone structure formed during processing can be considered. The fact that we were not able to demonstrate the formation of this bond by IR could be attributed in that case to the highly complex IR spectra of the agropolymer blend, according to a large number of functions and linkages found in their structure.

Lignin Extractability. The content of lignin that can be extracted from the material was determined on materials mixed at 80 °C, in similar conditions as the one given in a previous study (*10*). A dioxane−water solution, which is a good solvent

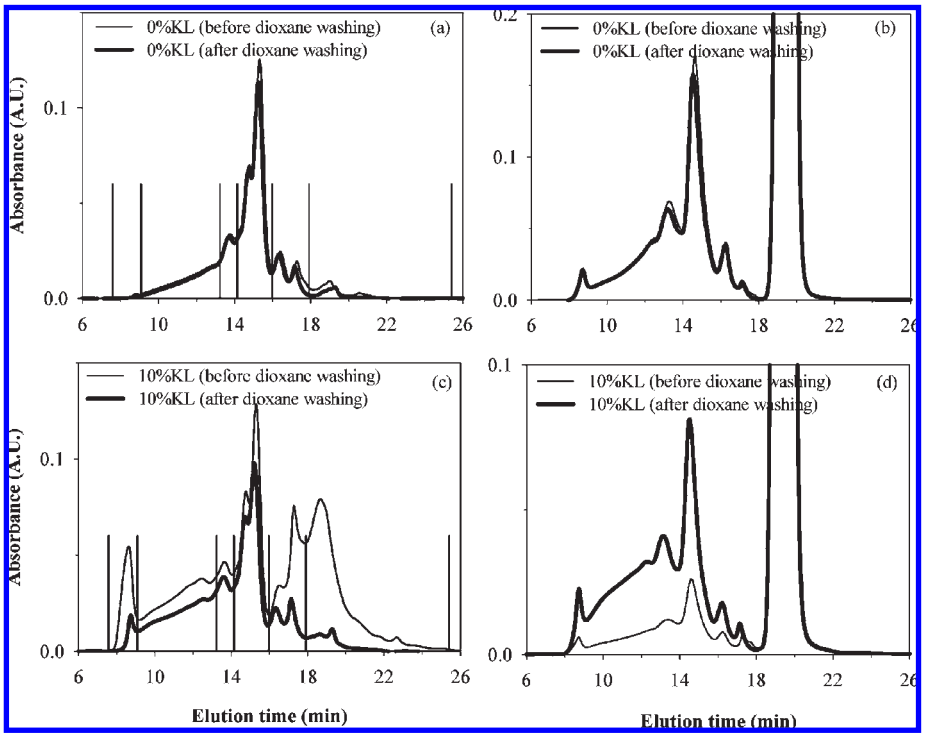

Figure 7. UV-size exclusion distribution profiles of SDS-soluble (**a** and **c**) and SDS-insoluble (**b** and **d**) protein fraction of various materials molded at 80 °C.

of lignin, was used as a medium for the KL extraction. As KL molecules are mainly based on similar phenolic structures, it is assumed that their absorbance is quite the same, whatever their size. Thus, a simple absorbance measurement should give a rough idea of their content in the medium. In **Table 3**, the extractible and unextractible KL contents are expressed as a percentage of the total material weight. In the case of a simple KL/glycerol material, thermomechanical treatment did not affect lignin extractability (measurement error is about 1%), which also confirms that the KL absorbance is not affected by the thermal treatment.

The unextractible KL quantity increases until a content of 50% of gluten in the material, as illustrated in **Figure 6**. From this graph, it can be determined that the mass of KL rendered unextractable is of about 0.1 g per gram of WG, that is, about 120 μmol/g of WG. This lignin can be ever physically blocked in a gluten network, chemically graft on the proteins, or associated through strong interaction forces with it. However, this last hypothesis is less likely as dioxane solutions are usually considered efficient for the breaking of polyphenol−protein structures formed in solution by complexation (*29*). The measured amount is close to the total estimated thiol equivalent content of WG [about 120−150 μmol SH + 2SS/g (*36*)], which may suggest that KLs interact with thiyl radicals formed during processing (*9*). To demonstrate that KL is not likely to be only physically blocked in the WG network, a SE-HPLC analysis has been conducted.

Protein Aggregation after Immersion in a Dioxane−Water Solution. After free KL extraction in the dioxane−water solution, materials (thermomolded at 100 °C) were analyzed by SE-HPLC. **Figure 7** shows the elution profiles of the SDS-soluble and SDS-insoluble fraction of the control (0%) and 10% KL material before and after dioxane−water washing.

The immersion in the dioxane−water solution does not modify the SE-HPLC protein profile of the control material (without KL, **Figure 7a**,**b**). The complete extraction of free KL is confirmed in **Figure 7c** by the suppression on the chromatogram of the KL peak (after 16 min). Thus, all of the KL present in the medium is strongly associated with WG.

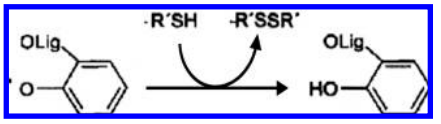

Figure 8. Transfer of a radical from a lignin phenoxy to a thiol (*39*).

Moreover, this analysis shows a surprising result: The SDS-insoluble profile intensity significantly increases after extraction, while at the same time the intensity of the SDS-soluble profile is decreasing (**Figure 7c**,**d**). Thus, immersion in the dioxane−water solution favors the protein cross-linking but only on materials containing KL. It can be observed in **Figure 7d** that even after DTE reduction, a significant content of high molecular weight proteins is observed, indicating the formation of nonreducible bonds (C−C bonds).

We previously suggest that KL impairs WG cross-linking through a radical scavenging effect that could involve different mechanisms. Phenol from lignin can be supposed to interact with the thiyl radicals produced during the material processing. Material soaking in dioxane−water solution will unbalance the situation. First, dioxane solution should be able to break the part of the noncovalent association between WG and KL, giving more mobility to the protein (*29*) (*37*). Second, because of the strong hydrogen bond acceptor character of dioxane and its action on the protonation of hydroxyl and carboxyl groups (*38*), radicals caught and stabilized by KL could be released in the medium. Indeed, the possibility in adequate circumstances of a transfer of a radical from a lignin phenoxy to a thiol (**Figure 8**) has been previously shown (*39*). Radical destabilization would promote an intense protein cross-linking through the formation of iso-peptidic bonds, as indicated by the large size of the protein extracted in the presence of DTE.

In conclusion, this study demonstrates that the introduction of KL in gluten-based material results in a strong WG protein

depolymerization. This phenomenon should be responsible for the modification of the rheological properties of KL/WG blends previously reported (*10*). The protein depolymerization may be explained by a combination of a strong noncovalent interaction, which modifies the 3D structure of the protein and thus its reactivity, and by the antioxidant or free radical scavenging properties of KL, which can capture free radicals formed during processing. The properties of KL made it a new convenient additive for the enlargement of the WG extrusion window. A strong association of KL on WG under processing conditions was evidenced. The ratio of KL associated with WG after immersion in a dioxane solution is estimated to be about 120 μmol/g, suggesting that WG equivalent cysteine groups could be involved in the bond formation. This study suggests a possible grafting of KL on WG through chemical cross-linking (covalent bond) or radical-pairing effects. Interestingly, the WG depolymerization appears reversible, and a strong repolymerization can be obtained after processing, without thermal treatment, by immersion in an adequate solvent such as a dioxane−water solution.

ABBREVIATIONS USED

DTE, dithioerythritol; F, fraction; KL, Kraft lignin; SDS, sodium dodecyl sulfate; SE-HPLC, size exclusion high-performance liquid chromatography; UV, ultraviolet; WG, wheat gluten.

ACKNOWLEDGMENT

We are very grateful to Joëlle Bonicel for the SE-HPLC analysis and to Bernard Kurek (INRA Reims) for providing KL.

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Received April 8, 2009. Revised manuscript received July 13, 2009. Accepted July 23, 2009.