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Interactions of Kraft Lignin and Wheat Gluten during Biomaterial Processing: Evidence for the Role of Phenolic Groups

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The chemical interactions between Kraft lignin and wheat gluten under processing conditions were investigated by determining the extent of the protein network formation. To clarify the role of different chemical functions found in lignin, the effect of Kraft lignin was compared with that of an esterified lignin, in which hydroxyl groups had been suppressed by esterification, and with a series of simple aromatics and phenolic structures with different functionalities (conjugated double bonds, hydroxyl, carboxylic acid, and aldehyde). The protein solubility was determined by using the Kjeldahl method. The role of the hydroxyl function was assessed by the significantly lower effect of esterified lignin. The importance of the phenolic radical scavenging structure is evidenced by the effect of guaiacol, which results in a behavior similar to that of the Kraft lignin. In addition, the significant effect of conjugated double bonds on gluten reactivity, through nucleophilic addition, was demonstrated.

KEYWORDS: Polyphenol; protein; mixing; wheat gluten; Kraft lignin; esterification; cross-linking

INTRODUCTION

Plastic waste is now regarded as a worldwide environmental problem. Biodegradable materials from renewable agricultural resources such as carbohydrates, starches, and proteins have attracted much attention as possible replacements. This will allow for sustainable development and reduce environmental concerns. Among these resources, wheat gluten appears to be one raw material of interest because of its low cost and availability in large quantities. Wheat gluten is a byproduct of the wheat starch industry (*1*). It is a mixture of (monomeric) gliadin and (polymeric) glutenin (about 65:45, w/w, respectively). In native gluten, the cysteine groups of the gliadin polypeptides are all involved in intrachain covalent disulfide bonds. Due to its monomeric structure, gliadin is mainly responsible for the viscous behavior of gluten. Glutenin, which provides the elastic properties of gluten, consists of a polymeric assembly of different subunits connected by disulfide bonds. Gluten-based materials are amorphous and can be prepared using common thermoplastic processing methods such as extrusion or thermomolding (*2*−*4*). The addition of a plasticizer overcomes the final product brittleness and provides better processability. Small polar molecules such as water, sorbitol, and fatty acids can be used for that purpose, but glycerol is the most commonly used additive (*5*−*7*).

During processing, the wheat gluten structure can be greatly modified by aggregating proteins through interchain disulfide bonds (*8*). This type of covalent cross-link requires that cysteine plays a key role in the formation of the structure of the product. A loss of solubility in sodium dodecyl sulfate (SDS) buffer is usually used as evidence and measure of the extent of cross-linking (*2*, *9*). The first step of the cross-linking reaction is the rupture of existing intramolecular disulfide bonds, so that the final material structure is highly dependent on mechanical and thermal inputs, both controllable during processing. Both radical and nucleophilic reaction pathways are involved in gluten aggregation (*10*). It is thus possible to control the extent of gluten reactivity by the addition of some compounds able to interact with some of those pathways. A recent study conducted by our group has demonstrated that Kraft lignin can impair gluten cross-linking during mixing and thermal treatment, leading to an increase of the soluble protein content (*11*). Two mechanisms were hypothesized. The first one is complexing through interaction forces (such as hydrogen bonding, dipole−dipole interactions, or other interaction forces) between Kraft lignin and wheat gluten during processing and which, by modifying the three-dimensional structure of the protein, might have modified its reactivity. The second is a direct chemical interaction of some of the functional chemical groups found in Kraft lignin with the gluten cross-linking mechanism, which mainly implies involvement of thiol radicals and thiolate anions.

Kraft lignin is the byproduct of the alkaline (Kraft) pulping process. Its structure, resulting from the alkaline modification of the native lignin of wood, is rather complex. Native lignin is basically formed by the combination of three monomers, namely,

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p-coumaryl, coniferyl, and sinapyl alcohols, resulting in a three-dimensional polymeric structure (*12*). Its characterization implies that it is partially degraded; thus, the real molecular weight of lignin in plants cannot be determined. The chemical structure of Kraft lignin depends both on the properties of the native lignin (wood species, agricultural conditions of growth) and on the intensity of the Kraft process, which causes the cleavage of native lignin aryl−ether linkages (*13*−*15*).

Kraft lignin is thus basically a polyphenolic compound, with a high thermal decomposition temperature (*13*), containing mainly aromatic groups (*16*) with different functions ranging from hydroxyl (phenolic or alkyl), conjugated double bond, methoxyl, sulfonate, etc. Such phenolic structures are known for their radical scavenging properties: the conjugation of the aromatic nucleus in their structure induces the resonance stabilization of the phenoxy radical (*17*−*19*). The radical scavenging properties of various phenolic structures have been characterized, showing a significant dependence on the functions presents on the side chains of the aromatic ring (*20*). As a result, the antioxidant properties of Kraft lignin have been investigated for the prevention of polypropylene degradation (*21*) or as an additive for cosmetic products (*22*, *23*). Kraft lignin oxidation is likely to occur in the presence of radicals formed during gluten processing and can give rise to the formation of various kinds of aldehydes (*24*). This complex structure makes it difficult to analyze its interactions with the wheat gluten protein blend, which itself is very complex. However, understanding these interactions is a main goal for the production of low-cost materials made from natural polymers.

The objectives of this work are to investigate wheat gluten cross-linking in the presence of lignin with an emphasis on the potential chemical interactions. Two routes were followed: the first was to compare the effect of Kraft lignin with an esterified lignin, in which we have suppressed hydroxyl groups to clarify their influence on the reactivity. The second was to evaluate independently the effect of different commercially available simple aromatic structures to check precisely the effect of their different functions on gluten reactivity. Among all of the chemical functions that can be found in Kraft lignin (or in its degradation products obtained by oxidation), we have focused our research on four kinds of functions that are more likely to interact with the gluten cross-linking through nucleophilic reactions or with radicals. We have evaluated the effect of hydroxyl and conjugated double bonds (in which the nucleophilic reactivity has been reinforced by the addition of carboxylic or aldehyde groups), all of them located on an aromatic structure. The effects of those compounds on protein plasticization and cross-linking were separately investigated.

MATERIALS AND METHODS

Materials. Wheat gluten was obtained from Amylum Group (Aalst, Belgium). Its moisture content was about 11%. Kraft lignin was provided by Westvaco (Charleston SC). 1,4-Dioxane, acetic anhydride, and pyridine were purchased from Sigma-Aldrich. Glycerol was purchased from Fluka, and all of the commercially available additives were purchased from Sigma-Aldrich, except for cinnamaldehyde (SAEC). The number-average molecular weight M_n of Kraft lignin was estimated by gel permeation chromatography to be 850 g/mol (*25*). Because we focus on the reactivity of aromatic units, we deducted from the lignin structure an estimated equivalent molecular weight (MW_{eq}) per aromatic unit. We used for Kraft lignin the value of 180 g mol^{-1}, assuming that its structure is basically constituted by an assembly of the three main precursors. A value of 273 g mol^{-1} was used for the esterified lignin, as explained below.

Esterification of Kraft Lignin. Fifty grams of Kraft lignin was mixed with 250 mL of dioxane, 45 g of acetic anhydride, and 36 g of pyridine in a one-neck flask and was stirred at room temperature overnight. This mixture was then dropped into ether, and the precipitate was filtered. The reagent was separated from the precipitate by mixing the precipitate with water (1 L), stirring (30 min), and filtering. To ensure the complete removal of the reagent, the above separation was carried out six times. Finally, the precipitate was dried in an oven at 40 °C until it attained a constant weight.

Preparation of Wheat Gluten Materials. A sample was prepared by mixing 35 g of native wheat gluten and 15 g of glycerol. This composition was referred to as a "reference" material. The other samples were prepared by substituting 1, 5, or 10% by weight of the 35 g of wheat gluten with Kraft lignin, esterified lignin, or additives while maintaining the glycerol content at 15 g. The products were mixed in a two-blade counter-rotating batch mixer turning at a 3:2 differential speed (Plasti-corder W50, Brabender, Duisburg, Germany). The mixing chamber included a water circulation system that controlled its inner wall temperature. This circulating water was regulated at 30 °C by using a regulation temperature unit (Julabo F34, Seelbach, Germany). Mixing speed was 100 rpm, and mixing time was kept constant at 10 min after maximum torque had been reached. This apparatus allowed for electronic measures of the torque on the main axis and of the product temperature in the chamber, which were continuously recorded during mixing. The standard deviation for this measure, determined from five repetitions of the same experiment on a wheat gluten−glycerol mixture, was estimate to be $< 1\%$.

Compounds like glycerol, guaiacol, *trans*-anethole, and cinnamaldehyde, which are liquid at ambient temperature, were added directly into the mixer. The solid additives such as vanillin, cinnamyl alcohol, ferulic acid, and coumaric acid were first dissolved in 15 g of glycerol before mixing. Wheat gluten, Kraft lignin, and esterified lignin were added directly into the mixer.

Thermal Treatment of the Wheat Gluten Materials. The thermal treatment was performed using a thermal molding press (PLM 10 T, Techmo, Nazelles, France). Five grams of the product was compressed at 100 °C for 10 min, at a pressure of 4 MPa, directly applied to the sample. We experimentally checked that this thermal treatment did not modify the lignin solubility (*11*), which was consistent with its high thermal stability (*26*).

Wheat Gluten Protein Solubility. The wheat gluten protein solubility measurement is a common way to determine the extent of the cross-linking between proteins. The soluble proteins were first extracted in a sodium dodecyl sulfate (SDS) solution. Their content was determined according to the Kjeldahl method, which gave a precise measurement of the nitrogen content (*27*). The soluble protein content was then expressed as a percentage of the protein content originally present in the sample.

Morphological Analysis. A scanning electron microscope (JEOL JSM-5800LV) was used to study the surface morphology of the Kraft lignin and esterified lignin. The samples were first mounted on the brass stub with double-sticky tape. The samples were then coated with a thin evaporated layer of gold to improve conductivity and prevent electron charging on the surface. The scanning electron microscope was operated at 10 kV.

Fourier Transform Infrared Analysis. Fourier transform infrared spectroscopy (FTIR) was performed (Bruker Equinox 55) to characterize the chemical structure of Kraft lignin and esterified lignin. The samples were dried at 100 °C for 6 h in an oven prior to the test. The dried powder samples were mixed with KBr and pressed into the disk form by hydraulic compression. The samples were scanned at a frequency range of 4000−400 cm^{-1} with 128 consecutive scans in a 4 cm^{-1} resolution.

RESULTS AND DISCUSSION

Esterification on Kraft Lignin. Phenolic and aliphatic hydroxyl groups are found in the Kraft lignin structure (*28*, *29*), which derive from the natural lignin structure (**Figure 1**). Those functions are usually known for their plasticizing action (through hydrogen bonding) on the wheat gluten protein. Moreover, phenol is well-known for its radical scavenging properties, which affect the wheat gluten cross-linking that implies the formation and transfer of radical species. To show the effect, or absence of effect, of those structures, we conducted the esterification of the hydroxyl groups, which have been extensively documented (*30*−*34*). After esterification, the color of lignin changed from dark brown to light brown. The powder surface evolved from a smooth one on the

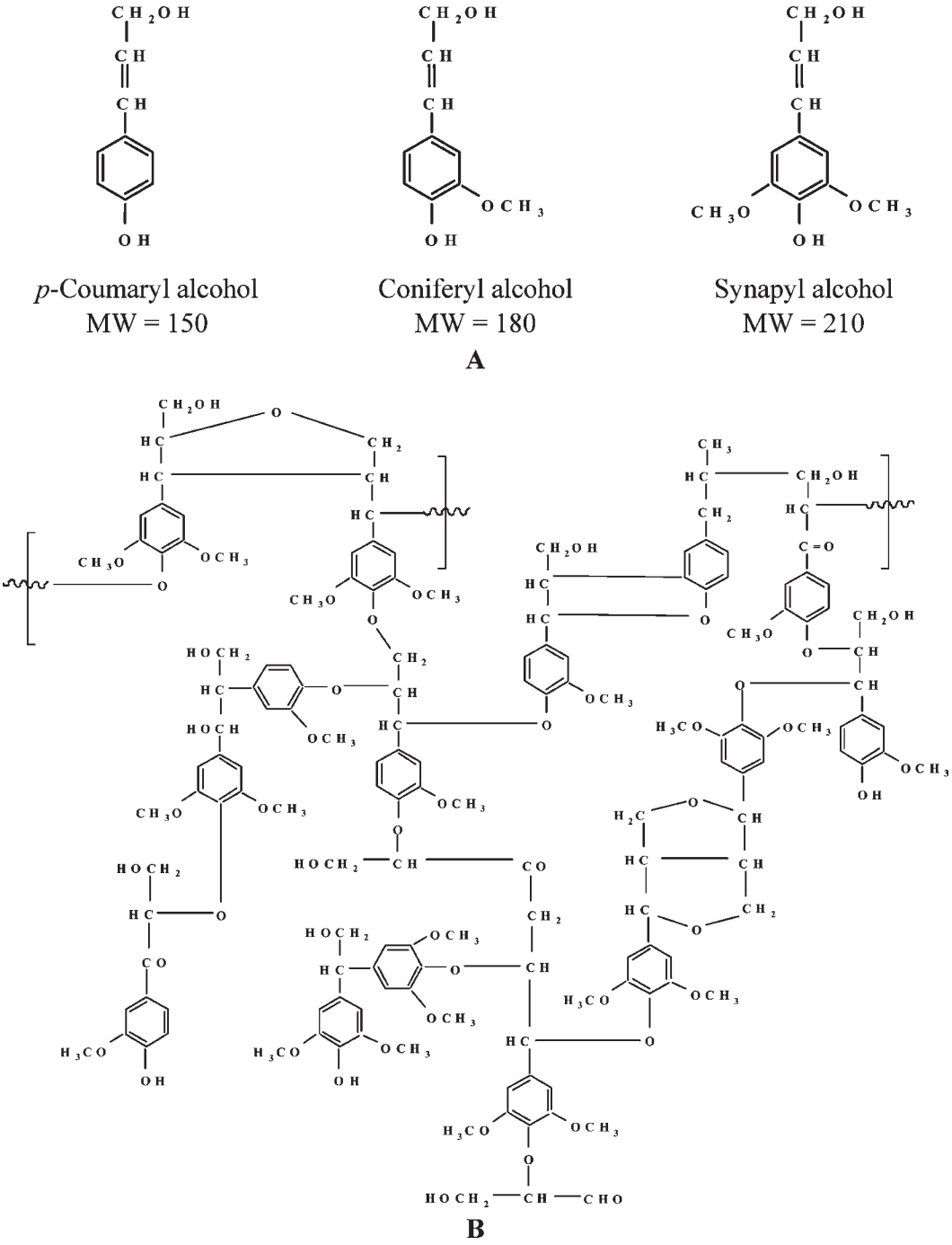

Figure 1. Representation of the main primary monolignols of lignin (**A**) and of a lignin polymer structure (**B**).

Kraft lignin to a rough one on the modified sample (**Figure 2**). The FTIR analysis conducted on esterified lignin and Kraft lignin showed a significant decrease in the hydroxyl peak (at 3407 cm^{-1}) after the esterification and the appearance of two new bands at 1743 and 1762 cm^{-1}, which corresponded to the C═O stretching in the ester group (**Figure 3**). It has been shown that in the presence of imidazole, a basic catalyst, this reaction is complete (*35*). We therefore chose to use pyridine, another basic compound (*36*). The OH groups still present in the structure are thus likely to be mainly ones associated with acidic functions initially present in the product. Lignin modification has been extensively studied, either for the characterization of its structure or for the modification of its properties, to test its use for various specific purposes. It has been shown that the chemical modification usually results in a modification to its solubility (*35*). However, we did not observe the formation of any residual solid during the evaporation of the ether phase after modification. From this we concluded that the lignin modification that occurred was not associated with a fractionation. The molecular weight of the esterified lignin was estimated by assuming that each aromatic group has two OH functions (one aromatic and one aliphatic carried by a side chain) that were completely modified. This calculation aimed to give a rough estimation of the molar content of aromatics groups in the mixture, to compare it with the effect of more simple additives. In this study, the additives and lignin molar concentrations are varied by 1 order of magnitude (from 1 to 10 wt %), which is much more important than the uncertainty on the molar concentration that might result from this calculation (< 30%). This approximation is thus sufficient to allow for a comparison with the cysteine content of the protein or with the other additives used.

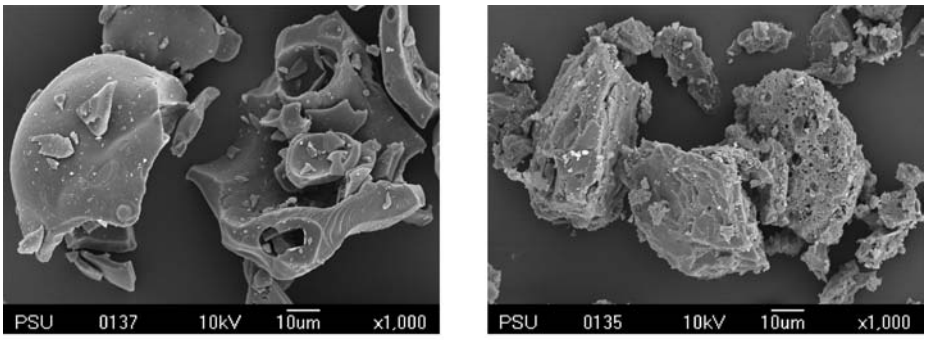

Figure 2. SEM micrographs of Kraft lignin (**A**) and esterified lignin (**B**).

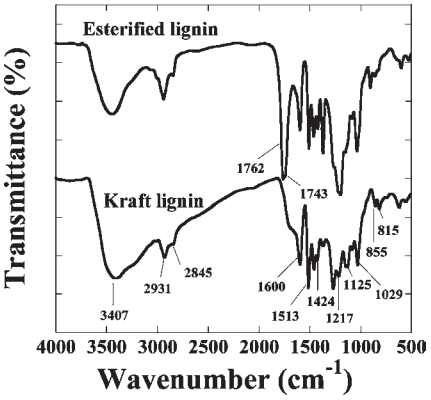

Figure 3. FTIR spectrum of Kraft lignin and esterified lignin.

Materials Processing. *Effect of Glycerol Content*. Glycerol is a well-known plasticizer of wheat gluten. The evolution of the sample during processing, from a wheat gluten/glycerol mixture to a homogeneous sample, was assessed by the torque and internal temperature changes. **Figure 4A** presents the torque and temperature evolution of wheat gluten processed with various amounts of glycerol: the reference sample contained 30 wt % glycerol, whereas the other samples contained additional amounts of glycerol. The amount of this additional glycerol was the same, on a weight basis, as used for the different additives in the subsequent experiments. The initial torque rapidly increased to a maximum value, then gradually decreased, and stabilized when a homogeneous mixture was obtained. The lag time before the torque increase is mainly a function of the wettability of the wheat gluten powder by the plasticizer and is thus linked to the dynamics of the system during mixing. The torque enhancement reflected an increase in the viscosity (*6*, *37*), so that the plasticizing efficiency is assessed by the torque level at the plateau. Despite the use of a recirculating water bath, the mixing chamber temperature increased, which is commonly attributed to an important viscous dissipation of the mechanical energy transmitted to the medium, which cannot be compensated by a sufficiently fast calorie extraction. The temperature increased and stabilized after the torque reached its maximum value. **Figure 4A** shows that this evolution is highly sensitive to the glycerol content, which acted as a plasticizer. In protein-based materials, a plasticizer is a small molecule able to interact through hydrogen bonding with the protein, thus reducing the direct protein−protein interaction (*6*, *38*). As its content increases in the sample, the torque increase is delayed, while the maximum torque, stabilization torque, and stabilization temperature reached during processing decreased, and this reflected the lower viscosity of the mixture.

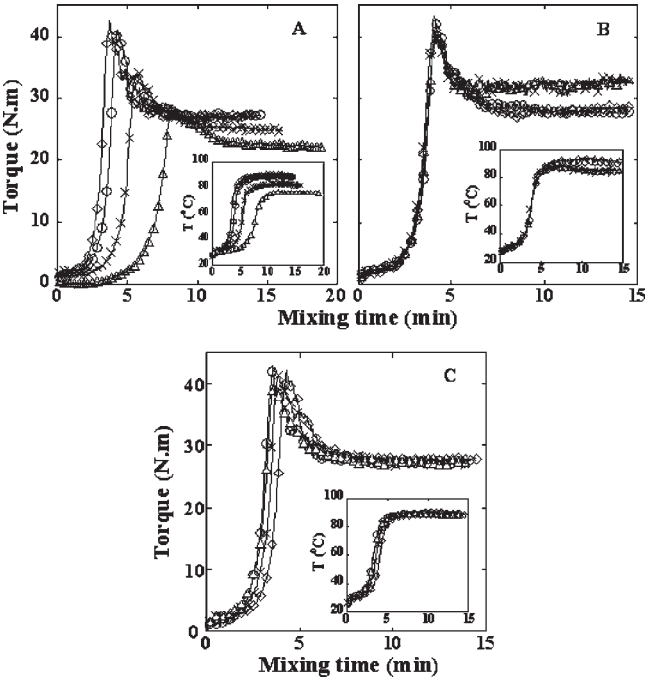

Figure 4. Effect of glycerol (**A**), Kraft lignin (**B**), and esterified lignin (**C**) contents on the torque and temperature evolution of wheat gluten: wheat gluten reference (◇), 1% (○), 5% (×), and 10% (△).

Effect of Kraft Lignin and Esterified Lignin. Kraft lignin addition resulted in an increase of the stabilization torque, whereas the stabilization temperature decreased (**Figure 4B**). This effect is uncommon, as, usually, added compounds result in an increase (or a decrease) in both parameters, as shown previously with glycerol. At a macroscopic level, either Kraft or esterified lignin might form agglomerates in the medium. In that case, lignin acts as a filler, which explains the torque increase, previously observed with fibers in similar conditions (*37*). At a molecular level, a number of interactions between protein and Kraft lignin can be envisaged (*11*): $\pi-\pi$ interaction between aromatic structures, hydrophobic interactions, hydrogen bonding, and covalent linkages. Among these, hydrogen bonding is supposed to have a strong influence and is primarily responsible for protein plasticization, which usually results in a viscosity decrease (*6*). The presence of hydroxyl groups in the Kraft lignin structure might favor interactions between wheat gluten and Kraft lignin, giving rise to a strong reinforcing effect. The influence of the hydroxyl groups was evidenced by the fact that their esterification resulted in a different evolution during processing: when esterified lignin

Table 1. Maximum Torque and Stabilization Temperature of the Samples

	maximum torque (N·m)			stabilization temperature (°C)		
additive	1 wt %	5 wt %	10 wt %	1 wt %	5 wt %	10 wt %
glycerol	41	34	28	89	83	76
Kraft lignin	42	40	41	90	84	86
esterified lignin	43	41	40	90	89	88
guaiacol	42	35	29	89	82	75
vanillin	36	31	25	81	77	72
trans-anethole	41	37	32	88	80	61
ferulic acid	35	31	27	79	73	70
coumaric acid	38	32	29	85	76	72
cinnamaldehyde	42	38	36	92	84	74
cinnamyl alcohol	40	34	26	90	82	71

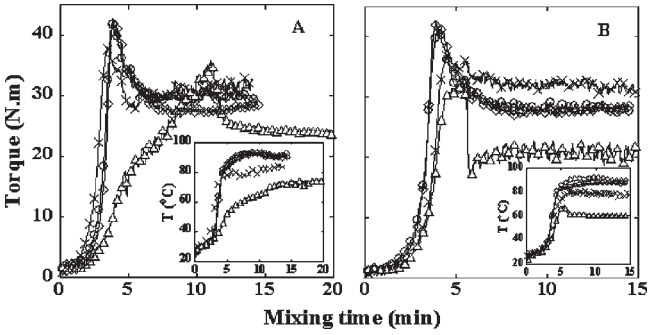

Figure 5. Effect of cinnamaldehyde (**A**) and *trans*-anethole (**B**) contents on the torque and temperature evolution of wheat gluten: wheat gluten reference (◇), 1% (○), 5% (×), and 10% (△).

was added, it did not modify in any way the processing parameters of plasticized wheat gluten, as indicated in **Figure 4C**.

Effect of Other Additives. Investigation of the effect of various functional groups was achieved by adding commercially available aromatic additives exhibiting various functions. Whereas the maximum torque reached during the processing of the reference was about 43 N·m, the one measured with additives ranged between 25 and 43 N·m (**Table 1**). Torque curves were strongly dependent on the added additive, and different behaviors were identified. Vanillin and ferulic acid produced evolutions very similar to the ones observed with increasing glycerol contents (**Figure 4A**), thereby demonstrating their plasticizing action on the wheat gluten (data not shown). Basically, guaiacol and cinnamyl alcohol gave the same evolution, except that the torque increase was not delayed. Thus, we suggest that those compounds also efficiently plasticized wheat gluten. Finally, cinnamaldehyde and *trans*-anethole both exhibited a specific behavior (**Figure 5**, panels **A** and **B**, respectively). Their torque evolution had an irregular trend, with significant oscillations (especially at 10%) that could be attributed to a wall-slip effect. Those experiments were repeated twice to check their reproducibility, which was very good. These two additives have the absence of hydroxyl groups in common, indicating that they cannot plasticize the wheat gluten proteins. From a physical point of view, they were more likely to act as a lubricant rather than as a plasticizer.

Protein Solubility after Mixing. During mixing, the mechanical energy transferred to the system resulted in a strong shear and a viscous dissipation (i.e., a temperature increase). Both phenomena have been identified as promoters of the formation of intermolecular disulfide bonds between proteins, which results in a loss of their solubility in SDS (*2*, *39*). The measure of protein solubility is a simple and direct method to quantify the extent of cross-linking. This determination can be done by a measure of the protein absorbance at 214 nm in the UV spectra. However, it has been shown (*11*) that the specific interaction between lignin and wheat gluten can change the absorbance of the protein, thus making this determination inappropriate. In this study, the protein content in the SDS solution was thus determined by quantification of the total nitrogen content, using the Kjeldahl method.

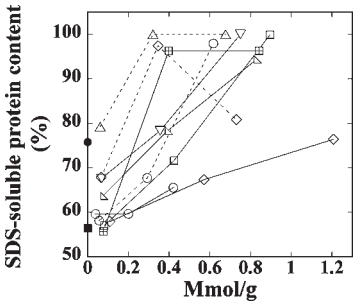

Figure 6. Effect of additives on the SDS-soluble protein content of wheat gluten after mixing (without thermal treatment): glycerol (open diamonds on solid line), Kraft lignin (open circles on dashed line), esterified lignin (open circles on solid line), guaiacol (open squares on solid line), vanillin (open diamonds on dashed line), *trans*-anethole (downward pointing open triangles on solid line), ferulic acid (upward pointing open triangles on dashed line), cinnamaldehyde (crossed square on solid line), cinnamyl alcohol (right triangles on solid line); wheat gluten reference (solid squares) and native wheat gluten (solid circles).

Guaiacol is basically a phenol, with a reactivity only slightly modified by the addition of the methoxyl group in the para-position on the aromatic ring. It has a slightly higher radical scavenging activity than a simple phenol (*40*). Thus, if this behavior is involved in the interaction with gluten, it should give rise to a more significant effect, making it easier to observe. Comparison between guaiacol and vanillin, a residue of Kraft lignin oxidation (*24*), allowed us to evaluate the effect of the presence of an aldehyde on the aromatic ring. The effect of a double bond conjugated with the aromatic ring was assessed by using ferulic acid, cinnamyl alcohol, cinnamaldehyde, and *trans*-anethole. These compounds differ by the presence of other groups such as aldehyde, hydroxyl (phenolic or aliphatic), and carboxylic acid.

The effect of the various additives on protein solubility is presented in **Figure 6**. Whereas the protein solubility of native wheat gluten (the unprocessed and unplasticized powder) is close to 76%, processing in plasticized conditions of the reference sample resulted in a decrease of the solubility of up to 56 ± 2.50%, due to protein cross-linking. The addition of 0.2 mmol/g (or more) Kraft lignin, esterified lignin, or any other additives used in this study promoted an increase in the protein extractability, in comparison with the reference. Those results obviously showed an effect of those compounds on protein cross-linking, which can originate from a specific chemical interaction, but also the possibility of protein plasticization by the additive. Indeed, protein cross-linking increased with the processing temperature. As it has been shown previously that the plasticization of the protein resulted in a lower plateau temperature when additives are added, this could cause a lower cross-linking. In these samples, the lower Brownian energy did not favor disulfide bond formation. This was shown by the fact that in **Figure 6**, glycerol addition appeared to modify the final wheat gluten solubility, whereas it was not supposed to participate in any way in the chemical mechanism involved in cross-linking. This "plasticizing" or "thermal" effect necessarily affects the protein cross-linking when additives with a plasticizing effect are added. In this study, we mainly wanted to focus on the chemical interactions between

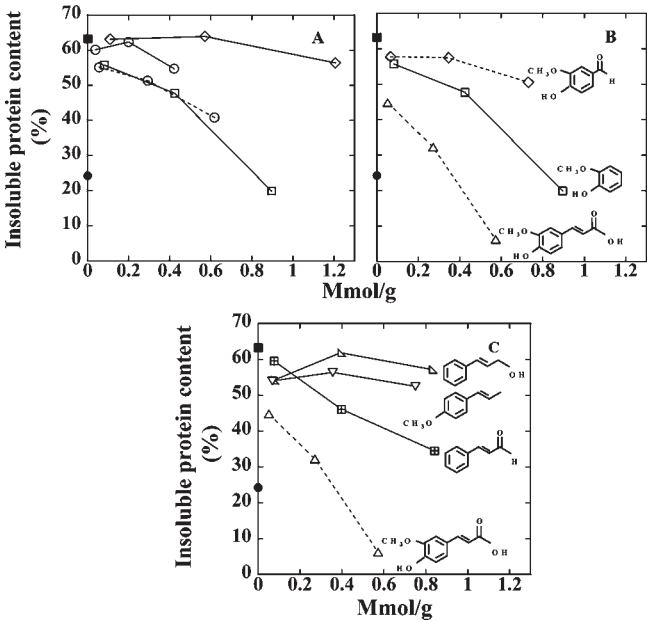

Figure 7. Effect of additives on the insoluble protein content of wheat gluten after thermal treatment; Kraft lignin, esterified lignin, guaiacol, and glycerol (**A**); phenolic compounds (**B**); and aromatic compounds containing conjugated double bond (**C**): glycerol (open diamonds on solid line), Kraft lignin (open circles on dashed line), esterified lignin (open circles on solie line), guaiacol (open squares on solid line), vanillin (open diamonds on dashed line), *trans*-anethole (downward point open triangles on solid line), ferulic acid (upward pointing open triangle on dashed line), cinnamaldehyde (crossed squares on solid line), cinnamyl alcohol (right triangles on solid line); wheat gluten reference (solid squares), and native wheat gluten (solid circles).

wheat gluten and Kraft lignin. Thus, to eliminate this effect, all of the samples were thermomolded at 100 °C for 10 min, so that their thermal history can be considered as being the same.

Protein Solubility after Mixing and Thermomolding. In **Figure 7**, we present the protein-insoluble content of the different samples after thermomolding. The protein-insoluble content (expressed in percentage and defined as 100 − the protein soluble content) is directly proportional to the extent of the protein cross-linking. It is expressed as a function of the concentration of the additives expressed in millimoles per gram of wheat gluten. This allows for a direct comparison with the wheat gluten composition in amino acids (cysteine content is assumed to be about 130 μmol/g). The "reference" wheat gluten/glycerol sample insoluble protein content is provided in each graph. A previous study (*11*) showed that Kraft lignin strongly affects protein aggregation, inhibiting the wheat gluten cross-linking and even resulting in an increase in the soluble protein content.

It can be seen in **Figure 7A** that the Kraft lignin amount (expressed in aromatic units) needed to inhibit the wheat gluten cross-linking was clearly higher than the cysteine concentration. If the inhibition of the cross-linking was due to the reaction of the added compounds with the cysteine groups (thus preventing them from forming disulfide bonds), the mechanism does not have a one-to-one stoichiometry or the reactivity of the Kraft lignin groups is weak. This is probably due to the fact that the densely interconnected structure of Kraft lignin limited its ability to interact with the protein groups. As the addition of esterified lignin had only a slight effect on protein solubility, this illustrated the effect of the phenol group on the cross-linking kinetics. This hypothesis is moreover confirmed by the fact that the addition of guaiacol resulted in an effect very similar to that of the Kraft lignin.

From the effect of the additives, it is possible to identify the influence of the various functionalities used in this study. Comparison of the effects of guaiacol, vanillin, and ferulic acid (**Figure 7B**) allows for an evaluation of the effect of an aldehyde and of a double bond (conjugated with an acid function) on the reactivity of a phenolic additive in the presence of wheat gluten. The protein cross-linking in the wheat gluten/guaiacol sample decreased as long as the guaiacol content increased in the investigated range of concentration. In that sense, this compound produced the closest behavior to the Kraft lignin. Guaiacol acts as a simple phenol, but the ortho-methoxyl substitution increase its radical scavenging efficiency (*40*). It has been suggested that the ortho-methoxyl function can form an intramolecular hydrogen bond with the phenolic hydrogen, making the hydrogen atom abstraction from the *o*-methoxyphenols easier (*41*, *42*). The evidence of a strong effect of a simple phenolic structure on wheat gluten cross-linking significantly reinforces the hypothesis that Kraft lignin can interact with wheat gluten protein by trapping the radicals formed during processing, and not only by a complexation involving interactions forces (*11*). The addition of an aldehyde group on the phenolic structure (vanillin) seems to partially inhibit its action. This observation is already consistent with some results that demonstrated that the presence of an aldehyde on the aromatic ring in vanillin reduced its radical scavenging efficiency (*40*). The addition of ferulic acid strongly reduced the wheat gluten cross-linking during processing. A similar result (not shown) was observed with coumaric acid, which has a very similar structure. As this effect is, on a molar basis, significantly more important than for guaiacol, it can be attributed to the presence of a double bond. The presence of both an aromatic structure and a carboxylic function, on both sides of this double bond, significantly favored its ability to react with nucleophilic species, such as the thiolate anion, that have been implicated in the mechanism of wheat gluten cross-linking. It could be hypothesized that in the case of ferulic acid, the presence of both a phenol group, which is well-known as a radical scavenger, and a reactive delocalized double bond contributed to inhibit the wheat gluten cross-linking that occurs during processing, even resulting in protein depolymerization in comparison to the native state.

To demonstrate the effect of a conjugated double bond, we have checked the effect of different aromatic but nonphenolic structures that possess this structure. In **Figure 7C**, the effect of the presence of double bonds on an aromatic structure is investigated independently of the presence of a phenol and compared with the results previously shown for the addition of ferulic acid. Those compounds are likely to interact with thiol groups formed during processing, especially by radical addition on the double bond or by nucleophilic addition of a thiolate anion (*10*). Although *trans*-anethole and cinnamyl alcohol have a slight, but measurable, effect on wheat gluten cross-linking, this effect was clearly stronger when cinamaldehyde and ferulic acid were used. This observation strongly supports an interaction of the conjugated double bond through its ability to interact with thiolate anion, implied in the nucleophilic step of wheat gluten cross-linking. Indeed, aldehyde and acid functions favored the ability of the double bond to react with an anion. This observation demonstrates that the presence of double bonds, conjugated with an aromatic group, can interact with the wheat gluten cross-linking and that the most probable mechanism of this action is a nucleophilic attack on this group.

Kraft lignin and wheat gluten are both renewable resources exhibiting promising potential as new raw materials for bioplastic production. Their blending allows for the monitoring of some of the materials' key properties, especially because Kraft lignin

reduced the wheat gluten cross-linking during processing. However, due to their complex structure, the description of their interaction is not easy. In this study, suppression of the hydroxyl groups present in the lignin structure, by esterification, appeared to suppress the plasticizing effect of Kraft lignin (assessed by its effect on the torque/temperature curves recorded during mixing) and to significantly reduce its interference with wheat gluten cross-linking.

The addition of simpler compounds, based on an aromatic structure with additional functions, clarified the potential roles of the complex structures found in Kraft lignin. The analysis mainly demonstrated the effect of the phenol structures and that of the conjugated double bond. The former interacted with the wheat gluten cross-linking pathway through their ability to trap the radicals formed under shearing (and heating) on cysteine groups. The latter was more likely to interact with the thiolate anions formed during processing. Evidence of a similar behavior between guaiacol and Kraft lignin indicated that the main interaction was occurring through the aromatic hydroxyl antiradical activity. These results demonstrate that the chemical reactivity of the Kraft lignin structures can explain protein depolymerization. This constitutes a new step in understanding the polyphenol−protein interactions that are involved in the production of bioplastics from Kraft lignin and wheat gluten.

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LITERATURE CITED

(1) Hoseney, R. C. *Principles of Cereal Science and Technology*, 2nd ed.; American Association of Cereal Chemists: St. Paul, MN, 1998.
(2) Redl, A.; Guilbert, S.; Morel, M.-H. Heat and shear mediated polymerisation of plasticized wheat gluten protein upon mixing. *J. Cereal Sci.* **2003**, *38*, 105–114.
(3) Redl, A.; Morel, M.-H.; Bonjcel, J.; Vergnes, B.; Guilbert, S. Extrusion of wheat gluten plasticized with glycerol influence of process condition on flow behavior, rheological properties, and molecular size distribution. *Cereal Chem.* **1999**, *76*, 361–370.
(4) Jerez, A.; Partal, P.; Martinez, I.; Gallegos, C.; Guerrero, A. Rheology and processing of gluten based bioplastics. *Biochem. Eng. J.* **2005**, *26*, 131–138.
(5) Pouplin, M.; Redl, A.; Gontard, N. Glass transition of wheat gluten plasticized with water, glycerol, or sorbitol. *J. Agric. Food Chem.* **1999**, *47* (2), 538–543.
(6) Pommet, M.; Redl, A.; Guilbert, S.; Morel, M.-H. Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *J. Cereal Sci.* **2005**, *42* (1), 81–91.
(7) Pommet, M.; Redl, A.; Morel, M.-H.; Domenek, S.; Guilbert, S. Thermoplastic processing of protein-based bioplastic: chemical engineering aspects of mixing, extrusion and hot molding. *Macromol. Symp.* **2003**, *197* (1), 207–218.
(8) Cuq, B.; Gontard, N.; Guilbert, S. Proteins as agricultural polymers for packaging production. *Cereal Chem.* **1998**, *75* (1), 1–9.
(9) Schofield, J. D.; Bottomley, R. C.; Timms, M. F.; Booth, M. R. The effect of heat on wheat gluten and the involvement of sulphydryl−disulphide interchange reaction. *J. Cereal Sci.* **1983**, *1*, 241–253.
(10) Auvergne, R.; Morel, M.-H.; Menut, P.; Giani, O.; Guilbert, S.; Robin, J.-J. Reactivity of wheat gluten protein during mechanical mixing: radical and nucleophilic reactions for the addition of molecules on sulfur. *Biomacromolecules* **2008**, *9*, 664–671.
(11) Kunanopparat, T.; Menut, P.; Morel, M.-H.; Guilbert, S. Modification of the wheat gluten network by Kraft lignin addition. *J. Agric. Food Chem.* **2009**, *57* (18), 8526–8533.
(12) Ralph, J.; Lundquist, K.; Brunow, G.; Lu, F.; Kim, H.; Schatz, P. F.; Marita, J. M.; Hatfield, R. D.; Ralph, S. A.; Christensen, J. H.; Boerjan, W. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. *Phytochem. Rev.* **2004**, *3*, 29–60.
(13) Tejada, A.; Pena, C.; Labidi, J.; Echeverria, J. M.; Mondragon, I. Physico-chemical characterization of lignins from different sources for use in phenol−formaldehyde resin synthesis. *Bioresour. Technol.* **2007**, *98*, 1655–1663.
(14) Chakar, F. S.; Ragauskas, A. J. Review of current and future softwood Kraft lignin process chemistry. *Ind. Crops Prod.* **2004**, *20* (2), 131–141.
(15) Pan, X.; Kadla, J. F.; Ehara, K.; Gilkes, N.; Saddler, J. N. Organosolv ethanol lignin from hybrid poplar as a radical scavenger: relationship between lignin structure, extraction conditions, and antioxidant activity. *J. Agric. Food Chem.* **2006**, *54*, 5806–5813.
(16) Balakshin, M. Y.; Capanema, E. A.; Chen, C.-L.; Gracz, H. S. Elucidation of the structures of residual and dissolved pine Kraft lignins using an HMQC NMR technique. *J. Agric. Food Chem.* **2003**, *51*, 6116–6127.
(17) Graf, E. Antioxidant potential of ferulic acid. *Free Radical Biol. Med.* **1992**, *13*, 435–448.
(18) Zang, L. Y.; Cosma, G.; Gardner, H.; Shi, X.; Castranova, V.; Vallyathan, V. Effect of antioxidant protection by *p*-coumaric acid on low-density lipoprotein cholesterol oxidation. *Am. J. Physiol. Cell Physiol.* **2000**, *279*, c954–c960.
(19) Butterfield, D. A.; Kanski, J.; Aksenova, M.; Stoyanova, A. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure−activity studies. *J. Nutr. Biochem.* **2002**, *13*, 273–281.
(20) Dizhbite, T.; Telysheva, G.; Jurkjane, V.; Viesturs, U. Characterization of the radical scavenging activity of lignins − natural antioxidants. *Bioresour. Technol.* **2004**, *95*, 309–317.
(21) Gregorova, A.; Kosikova, B.; Stasko, A. Radical scavenging capacity of lignin and its effect on processing stabilization of virgin and recycled polypropylene. *J. Appl. Polym. Sci.* **2007**, *106*, 1626–1631.
(22) Vinardell, M. P.; Ugartondo, V.; Mitjans, M. Potential applications of antioxidant lignins from different sources. *Ind. Crops Prod.* **2008**, *27*, 220–223.
(23) Vinardell, M. P.; Ugartondo, V.; Mitjans, M. Comparative antioxidant and cytotoxic effects of lignins from different sources. *Bioresour. Technol.* **2008**, *99*, 6683–6687.
(24) Villar, J. C.; Caperos, A.; GarciaOchoa, F. Oxidation of hardwood kraft-lignin to phenolic derivatives. Nitrobenzene and copper oxide as oxidants. *J. Wood Chem. Technol.* **1997**, *17* (3), 259–285.
(25) Pouteau, C.; Dole, P.; Cathala, B.; Averous, L.; Boquillon, N. Antioxidant properties of lignin in polypropylene. *Polym. Degrad. Stab.* **2003**, *81*, 9–18.
(26) Brodin, I.; Sjoholm, E.; Gellerstedt, G. The behavior of Kraft lignin during thermal treatment. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 70–77.
(27) Norm NF EN ISO 20483, BIPEA.
(28) Mansouri, N.-E. E.; Salvado, J. Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins. *Ind. Crops Prod.* **2006**, *24*, 8–16.
(29) Baptista, C.; Robert, D.; Duarte, A. P. Effect of pulping conditions on lignin structure from maritime pine Kraft pulps. *Chem. Eng. J.* **2006**, *121*, 153–158.
(30) Chen, C. L.; Lin, S. Y.; Dence, C. W. *Methods in Lignin Chemistry*; Springer: Berlin, Germany, 1992.
(31) Lewis, H. F.; Brauns, F. E.; Buchanan, M. A.; Brookbank, E. B. Lignin esters of mono- and dibasic aliphatic acids. *Ind. Eng. Chem.* **1943**, *35* (10), 1113–1117.
(32) Brauns, F. E.; Lewis, H. F.; Brookbank, E. B. Lignin ethers and esters − preparation from lead and other metallic derivatives of lignin. *Ind. Eng. Chem.* **1945**, *37*, 70–73.
(33) Mansson, P. Quantitative determination of phenolic and total hydroxyl groups in lignins. *Holzforschung* **1983**, *37*, 143–146.
(34) Glasser, W. G.; Jain, R. K. Lignin derivatives. *Holzforschung*. **1993**, *47*, 225–233.
(35) Thielemans, W.; Wool, R. P. Lignin esters for use in unsaturated thermosets: lignin modification and solubility modeling. *Biomacromolecules* **2005**, *6*, 1895–1905.

(36) Morck, R.; Kringstad, K. P. ^{13}C-NMR spectra of Kraft lignins − II. Kraft lignin acetates. *Holzforschung* **1985**, *39* (2), 109–119.
(37) Kunanopparat, T.; Menut, P.; Morel, M.-H.; Guilbert, S. Reinforcement of plasticized wheat gluten with natural fibers: from mechanical improvement to deplasticizing effect. *Composites Part A* **2008**, *39*, 777–785.
(38) Song, Y.; Zheng, Q. Improved tensile strength of glycerol-plasticized gluten bioplastic containing hydrophobic liquids. *Bioresour. Technol.* **2008**, *99*, 7665–7671.
(39) Micard, V.; Morel, M.-H.; Bonicel, J.; Guilbert, S. Thermal properties of raw and processed wheat gluten in relation with protein aggregation. *Polymer* **2001**, *42*, 477–485.
(40) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28* (1), 25–30.
(41) de Heer, M. I.; Mulder, P.; Korth, H. G.; Ingold, K. U.; Lusztyk, J. Hydrogen atom abstraction kinetics from intramolecularly hydrogen bonded ubiquinol-10 and other (poly)methoxy phenols. *J. Am. Chem. Soc.* **2000**, *122*, 2355–2360.
(42) Zhou, B.; Cheng, J.-C.; Dai, F.; Yang, L.; Liu, Z.-L. Antioxidant activity of hydroxycinnamic acid monomers in human low density lipoprotein: mechanism and structure−activity relationship. *Food Chem.* **2007**, *104*, 132–139.

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