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Effect of Kraft Lignin on Protein Aggregation, Functional, and Rheological Properties of Fish Protein-Based Material

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ABSTRACT: The main drawbacks of protein-based bioplastics are a high sensitivity to water, insufficient mechanical properties, and a narrow window of processing conditions. The objective of this work was to study the effect of Kraft lignin (KL) on protein aggregation, functional, and rheological properties of fish protein (FP)-based bioplastic. FP powder was blended with 30% glycerol and 0–70% KL. Then, blends were thermoformed by compression molding. KL addition increased protein solubility in sodium dodecyl sulfate buffer, indicating a decrease of protein molecular weight. An introduction of KL in protein blend increased mechanical properties and decreased water absorption of materials. KL addition resulted in a decrease in storage modulus in rubbery state of protein blends. It also resulted in a decrease in viscosity of protein blends at processing temperature, as determined by capillary rheometry. Therefore, KL is an alternative additive to enlarge the protein thermal processing window and improve functional properties of FP-based materials.
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INTRODUCTION

Most of plastics are petroleum-based and non-biodegradable, they are responsible of a pollution which leads to serious environmental problem. Biodegradable plastics obtained from natural resources can be an environmentally friendly alternative. A growing demand is observed for various applications such as short-lived applications for agriculture like plant pot and mulching films to cover soil, and also for food and non-food packaging.[1] Those products should be degraded in a relatively short period of time. Resultantly, the development of bioplastics from agricultural resources such as carbohydrates and proteins to substitute synthetic polymer has become an important challenge.[2,3] However, such materials usually not possess sufficient mechanical properties, and therefore, natural polymer blending is an active field of investigation to produce materials with better properties such as flowability, mechanical performance, and water resistance.[4]

For this purpose, the use of industrial byproduct presents many advantages, including in general a low cost and the absence of competition with food uses. Thailand, which is one of the largest surimi producers in Southeast Asia, generates for this production about 2.8 million liters of wastewater, which protein content is about 4.8–5.4%.[5] Protein extracted from wastewater and also protein wastes from industry are an interesting source for producing bioplastics, for example for edible packaging applications.[6] These materials can be obtained by thermoforming, which allows to tune their mechanical properties as a function of processing conditions[7] and plasticizer content.[8] The existence of covalent crosslinks has been hypothesized in those materials,[9] similarly to what exist for others proteins-based materials, such as for wheat gluten (WG).[10] In this later case, it have been evidence that covalent crosslinks formed during thermal processing are the results of a combine radical and nucleophilic reaction which generates disulfide intermolecular bonds between thiol groups.[11] In such WG-based materials, properties can be improved by the addition of Kraft lignin (KL), a byproduct of the alkaline pulping process.[12]

KL is a polyphenolic thermoplastic compound that is known for its radical scavenging properties.[13] KL addition significantly modifies the WG crosslinking extent,[14] which can be attributed to the presence of phenolic structures and reduces water sensitivity of WG-based material due to the nature of KL that is relatively hydrophobic.[15]

The objective of this work was to study the effect of KL on protein aggregation, functional, and rheological properties of fish protein (FP)-based materials. To evidence any change in the protein network formed during processing due to the addition of KL, protein aggregation and molecular weight were determined by protein solubility measurements in sodium dodecyl sulfate (SDS) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Assuming that radical routes might be implied in the KL-FP interactions, the presence of free radicals in the product by electron spin resonance (ESR) was investigated. Finally, in view of an industrial production of new biodegradable materials, material processability and functional properties were characterized. To do so, mechanical properties (at large and small deformation), capillary rheology, and water absorption properties of the materials were determined.

EXPERIMENT

Materials

Threadfin bream (*Nemipterus* sp.) were purchased from Prachauthid 61 market. (Bangkok, Thailand). KL was obtained from Raja engineering (Bangkok, Thailand). Anhydrous glycerol was purchased from Roongsub Chemical. (New South Wales, Australia) in analytical grade. Chemical reagents were obtained from Ajax Finechem, Merck and Carlo Erba in analytical grade.

Preparation of FP Powder

Threadfin bream (*Nemipterus* sp.) were gutted and headed. After that, fish mince were washed, chopped, and dried in hot air oven at 50°C for 5 h and 40°C for 24 h. Finally, the dried FP was ground below 425 μm. The composition of FP powder was determined as described by Association of Official Analytical Chemist (AOAC, 1996). Protein, fat, ash, and moisture contents were 84.9 ± 3, 4.3 ± 0.8, 2.8 ± 0.2 and 6.9 ± 0.2%, respectively.

Characterization of FP and KL Powder

Glass transition temperatures of FP and KL powder are 167.2 ± 1.0 and 151.7 ± 0.7°C, respectively, determined by the second heating curve of differential scanning calorimetry (Mettler Toledo DSC 822^e, Zurich, Switzerland) scan with 10°C/min of heating rate.[16] Thermal decomposition (T_d) of FP and KL are 192 and 165°C, respectively, tested by thermal analysis instrument (Mettler Toledo TGA/SDTA 851^e, Zurich, Switzerland) with 10°C/min of heating rate.[17]

Preparation of FP/KL Materials

Proteins-based materials, and for the particular case of this study, FP-based materials, are known to exhibit a strong sensitivity to the plasticizer content. Therefore, in this study, materials FP: KL: glycerol composition is ranging from 70 : 0 : 30 to 0 : 70 : 30 (weight ratio), thus with a constant glycerol content of 30 wt %.

Mixing Process. FP/KL and glycerol (50 g) were mixed in an internal mixer (Plasti-corder W50, Brabender, Duisburg, Germany) at 80°C, 100 rpm for 15 min. Torque and product temperature were continuously recorded during mixing.

During mixing, torque and temperature evolutions were followed for each sample as an indicator of the mixing state. For all samples, torque increased rapidly to a maximum value, then decreased and stabilized, while temperature continually increased before to stabilize, as described in literature.[18] Maximum and stabilized torques decreased when the KL content increased from 0 to 70%, respectively, from 39 to 10 Nm and 27 to 5 Nm. Temperature evolution presented the same trend: the addition of KL decreased the stabilized temperature from 98 to 81°C for 0 and 70% KL, respectively. For 15 min of mixing time, torque and temperature of all samples were stabilized, which is indicative of the completeness of the mixing.

Compression Molding Process. Ten or twenty-five grams of the blends were placed in a square mould (9 × 9 cm^2) and thermoformed at 100°C for 15 min in a Hydraulic Press Machine (20 T., SMC TOYO METAL Co., Thailand). A load of 1 ton was directly applied to the sample in a mould. The thickness of material was approximately about 1 or 2 mm depending on initial weight of blends.

Characterization of FP/KL Polymer Materials

Protein Solubility. Measuring the protein solubility in SDS is a convenient way to characterize the extent of crosslink in such materials.[14] The protein powder (26.67 mg) was stirred for 80 min at 60°C in the presence of with 20 mL of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% SDS. The SDS-soluble protein extract was recovered by centrifugation (50 min at 15,000 g and 20°C), and 1000 μL was used to determine protein content. 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, and the extract was sonicated for 3 min. These treatments brought insoluble protein from the pellet into the solution. After centrifugation (50 min, 15,000 g, 20°C), a part of the supernatant and precipitate was used to determine protein content using the Kjeldahl method (Kjeltex System-Texator, Sweden).

SDS-PAGE. Proteins were characterized by SDS-PAGE. SDS-soluble and insoluble proteins of samples with 0–60% KL were solubilized in an SDS-solution containing 10 mL of 10% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.5 M Tris-HCl (pH 6.8), and Trace bromophenol blue 0.0003 g. The amount of protein loaded onto the polyacrylamide gel was 30 μL. A total of 100 μL of each protein were mixed with 400 μL of SDS buffer then, used for gel electrophoresis. Stacking gel and separating gel were made of 4% (w/v) and 15% (w/v) polyacrylamide, respectively. The amount of protein loaded onto the polyacrylamide gel was 15 mg. The Precision Plus Protein^{TM} standard (10–250 kDa) was also injected at the loading of 20 μL in the left lanes of the gel as a marker and the electrophoresis was carried out at 200 V for about 60 min. After electrophoresis, gels were stained with silver.

ESR Spectroscopy. The radicals formed on material after treatments were identified by ESR spectroscopy.[19] First, samples were collected immediately after mixing and molding. Then,

they were put into liquid nitrogen and stored frozen before analysis. In testing, 8 mg of samples were weighed and placed into ESR glass containers. Samples were packed to a height of 4 mm that was sufficient to completely fill the ESR cavity.

Tests were conducted using a JEOL (JES-RE2X) at liquid-nitrogen temperature (77 K) to eliminate nonresonant effects of water on free radical signals and to detect thiyl and disulfide radicals that often cannot be detected at room temperature.[20] ESR spectra (first derivatives) were recorded at 77 K, 1 mW microwave power, and 0.5 mT modulation amplitude and 9.4 GHz microwave frequency. The g values were determined by standardization with α, α'-diphenyl-β-picryl hydrazyl. The g value is calculated from the relationship $hv = g\beta B$, where h is Plank's constant (6.63×10^{-34} J s), v is the microwave frequency (9.4 GHz, measured by a frequency counter), β is the Bohr magneton (9.27×10^{-24}A m^2), and B is the magnetic field (G). Spectra were recorded during runs.

Mechanical Properties. Tensile tests were performed on a Texture Analyzer (Stable Micro System, TA-XT. plus, Surrey, UK). Samples were cut into dumb-bell-shaped specimens and preconditioned at 25°C and 53% relative humidity over a saturated salt solution of $Mg(NO_3)_2$. 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 percentage of the initial length of the elongating part of the specimen. Young's modulus was determined as the slope of the linear regression of the stress–strain curve.

Water Absorption. The samples (20 mm in diameter) were dried in hot air oven at 50°C for 48 h. (W_i). Then, they were immersed in 50 mL distilled water containing 0.05% NaN_3 (to avoid the microbial growth) at 25°C. The swollen samples were wiped and weighed (W_w) after 1 week. Then, they were dried in hot air oven at 50°C for 48 h (W_f). Water absorption was calculated by the following equation.

$$\text{Water absorption}(\%) = 100(W_w - W_f)/(W_i) \quad (1)$$

Viscoelastic Properties. Viscoelastic properties were characterized with a dynamic mechanical thermal analyzer (NETZSCH DMA 242, Germany) equipped with a cryogenic system fed with liquid nitrogen. Rectangular samples ($15 \times 3 \times 1$ mm^3) were preconditioned at 25°C and 0% relative humidity over P_2O_5. A tensile test was performed with a temperature ramp from −100 to +200°C at a heating rate of 3°C/min. A variable sinusoidal mechanical stress was applied to produce a sinusoidal strain amplitude of 0.05%. During analysis, storage modulus (E'), loss modulus (E'') and tan δ (E''/E') were recorded and plotted against temperature. T_g was identified as the temperature of the tan δ maximum.

Rheological Analysis. After mixing, the melt flow behavior of FP/KL blends was determined by capillary rheometry (capillary rheometer; RHEO-TESTER 2000, Germany) with a 2-mm capillary die and a length-to-diameter ratio of 15. Measurements were carried out at 140°C under a shear rate ranging from 10 to 1000 s^{-1}. Apparent viscosity was plotted against shear rate.

Table I. Protein Content (%) of SDS-Soluble, SDS-Insoluble Fraction and Total Protein Recovery of FP Powder and FP/KL Materials Molded at 100°C

Sample	SDS-soluble protein	SDS-insoluble protein	Total protein recovery (%)
FP power	64 ± 8	24 ± 4	88 ± 5
0% KL	18 ± 2	8 ± 3	27 ± 2
20% KL	21 ± 1	11 ± 2	32 ± 4
40% KL	23 ± 4	12 ± 3	34 ± 3
60% KL	28 ± 5	9 ± 3	37 ± 8

The Rabinowitsch correction was applied to account for the influence of shear thinning in the calculation of the shear rate and corresponding viscosity, and the Bagley correction, corresponding to the adjustment for excess pressure drop at the die entrance, was applied by using three capillaries with the same radius but different length/radius ratios. A simple mathematical expression describing the relationship between viscosity and shear rate is

$$\eta = K\dot{\gamma}^{n-1} \quad (2)$$

where the consistency (K) corresponds to the viscosity value for a shear rate ($\dot{\gamma}$) of 1 s^{-1} and the power-law index (n) characterizes the deviation from the Newtonian behavior, for which $n = 1$.[21]

Fourier Transform Infrared Analysis. Dry samples were crushed with KBr and pressed into pellets (KBr pellet technique). Then, samples were tested by a Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum, Singapore). For each spectrum, 64 scans were collected at the resolution of 4 cm^{-1} in the transmittance mode.[22]

RESULTS AND DISCUSSION

Protein Aggregation

Protein Solubility in SDS. Changes in proteins solubility before and after processing are often regarded as a good indicator of crosslink formation during processing.[23] Solubility is measured in SDS solutions, a surfactant which helps breaking intermolecular hydrogen bonding and therefore, favors protein solubilization. In this test, we quantify the extent of protein that are directly soluble in SDS (called SDS-soluble proteins), and the extent of proteins that become soluble in SDS only after the action of the reducing agent DTE (called SDS-insoluble proteins). DTE is responsible for the cleavage of disulfide bonds usually involved in protein crosslinking, and therefore, the SDS-insoluble protein represents the proteins that where crosslinks through disulfide covalent bond in the material. Processing of FP protein results in a strong protein crosslinking, which is evidence here: after mixing and compression molding process, SDS soluble fraction of FP decreases dramatically from 64 (native powder) to 18% (processed material), as shown in Table I. The combined action of DTE and sonication only brings about 8% of the total protein amount in solution, showing that disulfide bonds only represent a limited fraction of the covalent crosslinks that stabilize the structure. KL addition slightly inhibits

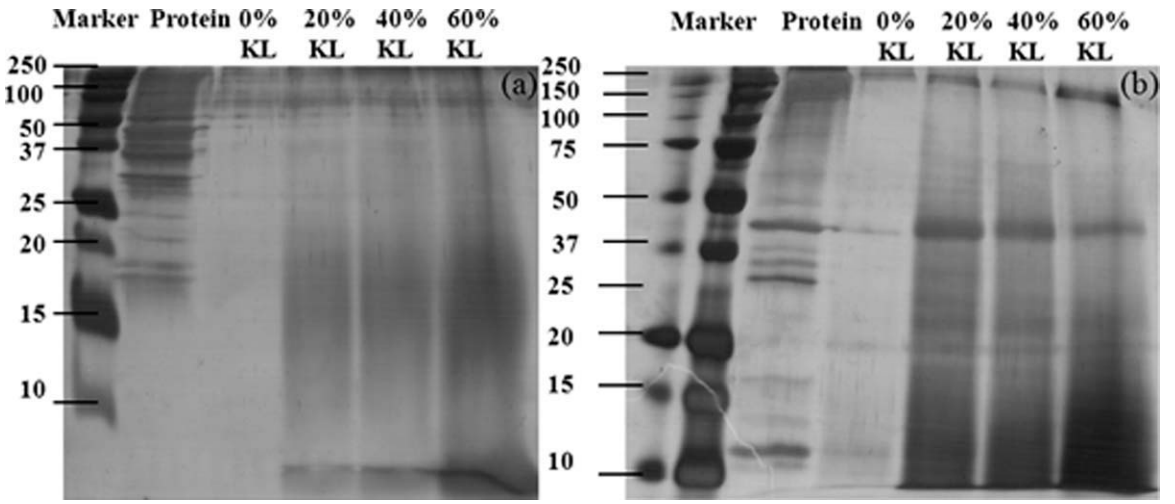

Figure 1. Protein molecular weight of SDS-soluble (a) SDS-insoluble (b) of FP/KL materials.

the mechanism responsible for the solubility loss, as assessed by an increase in SDS-soluble content and total protein recovery. The total protein recovery is less than 100% in all samples that clearly indicate the existence of very strong interactions between most proteins in those materials, making them completely insoluble in SDS.

Protein Molecular Weight. The molecular weights of the proteins solubilized in SDS before (SDS-soluble) and after (SDS-insoluble) the action of DTE were measured using 15% SDS-PAGE gel. Those results are presented on Figure 1(a, b). Molecular weights of FP powder ranging from 18 to 250 kDa are observed in both SDS-soluble and insoluble. SDS-soluble proteins exhibit a wide and discrete distribution of proteins which size is above 15 kDa, while SDS-insoluble proteins obtained after reduction also contain some smaller protein which size is comprise in between 10 and 15 kDa. After compression molding, FP-based material only presents low-intensity bands above 50 kDa in the SDS-soluble fraction and one main band between 37 and 50 kDa for the SDS-insoluble fraction. This suggests that the SDS soluble protein of high molecular weight (>50 kDa) and the SDS insoluble protein which molecular weight is comprise in between 37 and 50 kDa, are not, or at least less, involved in the non-disulfide crosslinking reaction that decreases protein solubility. Bands of materials with 20–60% KL contents present the exact same trend as materials without KL: in the studied molecular weight range (10–250 kDa), no significant change of protein molecular weight could be attributed to KL addition.

Free Radicals. To investigate whether the effect of KL on an increase of protein solubility in SDS results from free radical scavenging properties of KL, free radicals of materials were determined by ESR. Figure 2(a, b) shows ESR spectra corresponding to relatively stable free radicals of FP/KL materials after mixing at 80°C and compression molding at 100°C, respectively. FP/KL materials after mixing present ESR spectra similar to the one obtained after compression molding. The signal intensity of FP-based material (0% KL) indicates the presence of nitrogen or nitroxyl radicals at $g = 2.0057$.[24,10] Sulfur-centered radicals such as thiyl and disulfide radicals are either absent or their signal is hidden in the nitrogen or nitroxyl signal. For KL-based material (70% KL), the signal intensity indicates the presence of aromatic radicals at $g = 2.0033$.[25] The g values of nitrogen radicals of FP and aromatic radicals of KL are close, so we cannot observe any significant difference of their peaks. It is, therefore, not possible to evidence from those spectra any specific interaction between WG and KL, indeed the spectrum of plasticized KL (70% KL) already shows the presence of radicals, even in the absence of FP: this signal only appears to be smaller when KL content decreases.

Fourier Transform Infrared. Lignin[26] and proteins[27] Fourier transform infrared (FTIR) spectra have been already characterized; they exhibit a large number of peaks due to the structure complexity of those biomacromolecules. We used this technique to identify the presence of new bonds after the blending of those biopolymers. Spectra acquired for different concentration of KL do not show any significant difference with a simple law of mixtures, and therefore do not show the presence of a specific bond in the material (Figure 3). However, the complexity of the spectra, which shows a large number of peaks, does not allow us to definitely exclude the possibility of such bond formation.

Functional Properties

Addition of KL has a significant and positive effect on the functional properties of the materials. Up to 30 wt %, it increases Young's modulus and tensile strength by 50 and 300%, respectively, while only slightly decreasing elongation at break (Figure 4). Improving the FP materials mechanical properties clearly shows an optimum at 30 wt % KL, where Young's modulus is higher than 42 MPa, the tensile strength is close to 4.9 MPa, and the elongation at break is of 20%. This concentration is close to the point where FP and KL are present in equal quantities in the blends, which would be the case for a 35 : 35 : 30 mixture. For higher KL concentration, mechanical properties drastically drop, reaching value significantly below the ones of plasticized FP materials. It can be suggested that above this value, the KL which becomes the main component of the

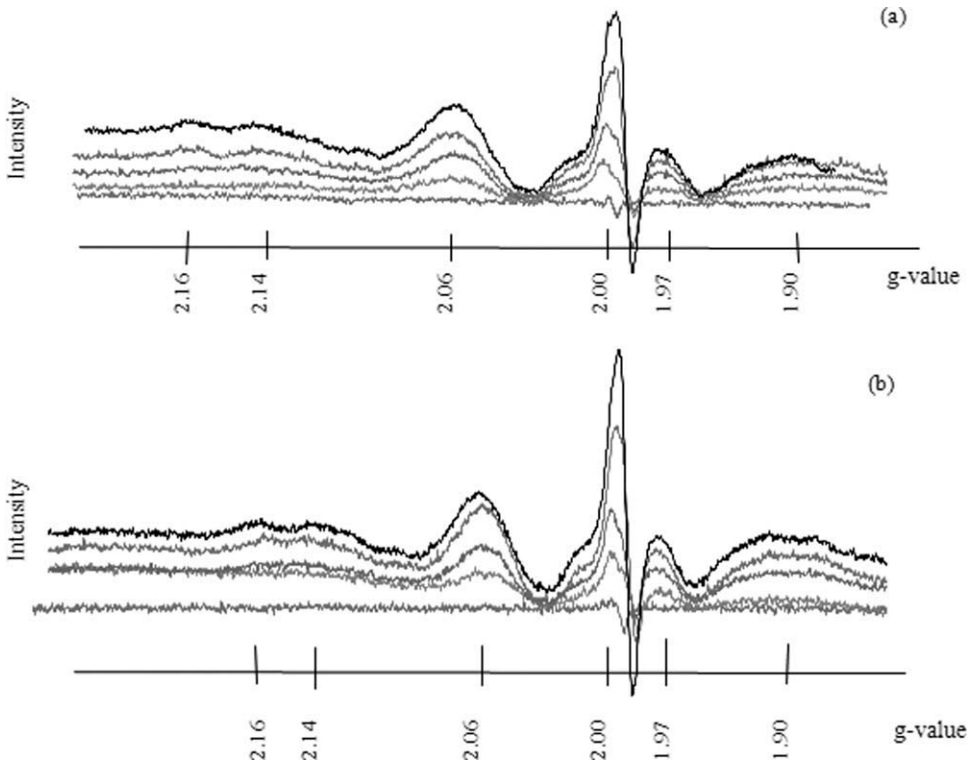

Figure 2. ESR signals of materials with 0, 20, 40, and 70% KL contents (bottom to top) after mixing at 80°C (a) and molding at 100°C (b).

blends, no longer reinforces FP but constitutes the matrix of the sample. It is clear from this study that the properties of plasticized KL materials (0 : 70 : 30) are significantly below the ones of plasticized FP materials (70 : 0 : 30), but that their blending, due to the occurrence of specific interactions, gives rise to improved properties. Those interactions can include a covalent bonding between KL and FP, which could explain the reduced polymerization observed from protein solubility measurements, and also noncovalent interactions between the polymers, which will be discussed later.

KL is also known for its hydrophobic behavior,[28] its addition significantly reduces the sample water sensitivity, swelling is rapidly reduced from 57 to 42% with addition of only 20% KL, and then continuously decreases when KL content increases [Figure 4(d)]. In addition, the water absorption of 40–60% KL materials, in which KL is the main component of material, is close to that of 70% KL (or material without FP). This again can be attributed to the fact that in those samples, KL constitutes the main agropolymer, and therefore the matrix of the system. In this case, its high hydrophobic character (in comparison to the proteins[29]) prevents high level of water absorption by the proteins incorporated into the KL matrix.

Rheological Properties of FP/KL Blends

Viscoelastic Properties. Polymeric materials exhibit viscoelasticity, as a result their mechanical properties strongly depends on the temperature (T) and time scale at which they are characterized. The glass transition temperature (T_g) of a product is of special importance, indeed, mechanical properties will strongly depend on the difference between the T_g and the temperature of interest, ($T-T_g$). At $T = T_g$, the elastic modulus drastically decreases, this is a clear manifestation of the glass transition, alternatively, it can be identified by a peak of tan δ during a temperature ramp. It can be clearly observed both events on the samples (Figure 5); and for convenience, the last method to determine the T_g was chosen. For all FP-based materials, it can be clearly observed that two peaks of tan δ appear. The lower temperature peak can be attributed to the T_g of a glycerol rich phase,[30] whereas the higher temperature peak corresponds to the T_g of the overall plasticized materials and will be used in the following as the material T_g.

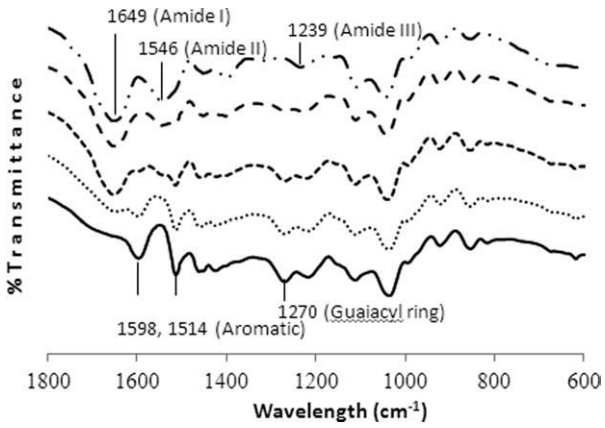

Figure 3. FTIR spectra (top to bottom) of 0, 20, 40, 60, and 70% KL contents.

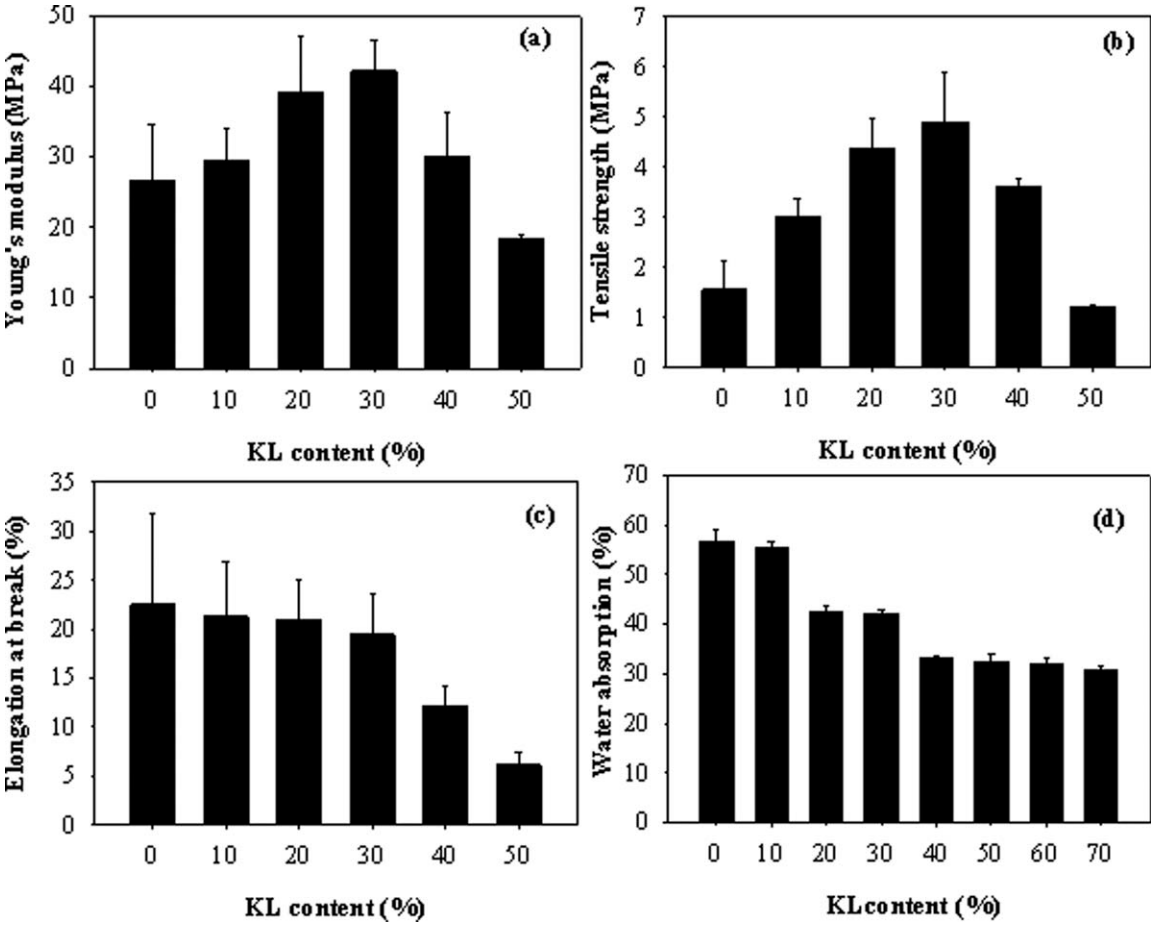

Figure 4. Mechanical properties and water absorption of FP/KL protein materials.

Table II shows the values of storage modulus E' in the glassy (30°C) and rubbery (140°C) regions, the tan δ peak height (E''/E') and the T_g determined by the temperature at maximum of tan δ peak height. The storage modulus exhibits a different behavior on KL addition in the glassy and rubbery states. In the glassy state, the modulus exhibits variation but remains in the order of 0.7–1.3 GPa until 50% KL, and then decreases. By contrast, in the rubbery state (above the T_g), the modulus continuously decreases when the KL amount increases. Viscoelastic properties of macromolecular blends are related with the size and branching of the polymer chain, therefore, this observation can be associated with the lower molecular weight of KL (MW = 2000–5000)[31,32] compared with FP (MW = 8–600 kDa). Moreover, above the T_g, KL which has a number of hydroxyl groups might be able to behave as a plasticizer of the protein network, thereby reducing its modulus.[23]

Although the T_g of plasticized FP and plasticized KL are very close to each other, the blending of the two polymers results in a small but significant increase of the blend T_g. This may be associated with a chemical bonding between FP and KL, which have been evidence on other proteins or on noncovalent interactions.[14,33,34] It is worth noting that the T_g evolution shows an optimum at the same material composition as the one at which optimum mechanical properties are observed in the previous section. Indeed, all materials where tested at the same T, while their T_g is different. Therefore, the value $T-T_g$, linked to the mechanical properties, shows an optimum when the T_g reaches its maximum value.

Capillary Rheology. To get a better representation of the rheological properties of materials during processing, the viscosity of

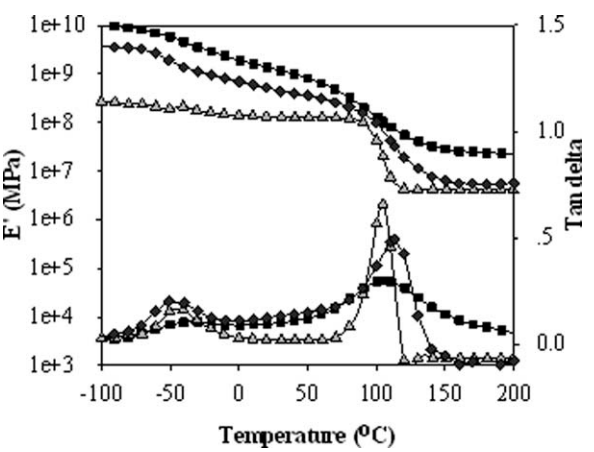

Figure 5. Storage modulus (E') and tan δ of FP/KL material with 0%KL (squares), 30%KL (diamonds), 70%KL (triangles).

Table II. Storage Modulus (E') in the Glassy State (30°C) and Rubbery (140°C) States, Glass Transition Temperature (T_g), and tan δ Peak Height of FP/KL Materials

KL (%)	E' at 30°C (MPa)	E' at 140°C (MPa)	Tan δ peak height (E''/E')	T_g (°C)
0	982 ± 281	29.7 ± 4.1	0.32 ± 0.03	105.5 ± 1.9
10	764 ± 128	16.9 ± 0.0	0.34 ± 0.03	107.8 ± 2.4
20	1123 ± 195	15.2 ± 2.1	0.43 ± 0.05	113.1 ± 2.5
30	846 ± 563	10.7 ± 4.2	0.49 ± 0.01	115.1 ± 2.7
40	1506 ± 49	8.1 ± 0.2	0.59 ± 0.01	113.0 ± 0.5
50	551 ± 195	7.1 ± 1.9	0.64 ± 0.07	111.3 ± 2.9
60	244 ± 3	5.9 ± 0.6	0.59 ± 0.01	106.6 ± 0.7
70	89 ± 57	3.4 ± 1.2	0.74 ± 0.09	109.6 ± 5.0

plasticized blends with 0–70% KL was measured with a capillary rheometer. The test sample was pushed at a temperature of 140°C through a capillary die by a piston at steady shear. The shear viscosity of FP/KL blends decreases when shear rate increases (Figure 6). The shear viscosity of the blends is recorded in the shear rate range of 10–1000 s^{-1}. However, the testing region for FP blend with 0% KL could not exceed 500 s^{-1} with the same amount of sample. Generally speaking, the addition of KL results in a decrease of the shear viscosity. The relationship between shear viscosity and shear rate can be described by a power law equation [eq. (2)]. Power-law index (n) and flow behavior consistency (K) were calculated. The power-law index of FP-based material is 0.17. This value is in the range of what have been reported previously in the literature for others proteins, ranging from 0.12 to 0.78.[35,16] Power-law index and the consistency coefficient decreases when KL content increases (Table III).

Clearly, the blending with KL gives more stability under high shear and reduces the sample viscosity, both aspects being of significant interest in view of industrial processing. This again might be explained by the low molecular weight of KL (MW = 2–5 kDa)[31,32] compared to the one of FP (MW = 8–600 kDa),[36] and also by its plasticizing properties and by the depolymerization effect previously observed.[14] At high temperature (above the T_g), KL can also contribute to the FP plasticization, due to its high number of hydroxyl groups.

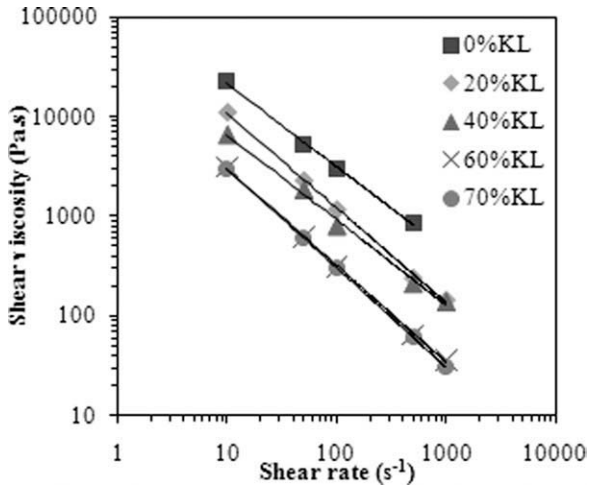

Figure 6. Viscosity of FP blends with 0–70% KL content determined by capillary rheometry at 140°C of die temperature.

Table III. Power-Law Model Parameter of FP/KL Blends

Sample	K (Pa s^n)	n
0% KL	145693 ± 21442	0.17 ± 0.01
20% KL	105266 ± 4981	0.02 ± 0.03
40% KL	47727 ± 3976	0.14 ± 0.01
60% KL	28459 ± 428	0.03 ± 0.00
70% KL	30235 ± 117	0.00 ± 0.00

CONCLUSIONS

In this study, we investigated how the addition of KL can modify and improve the properties of FP-based materials, in the view of the production of biodegradables blends from industrial byproducts. The results show that after processing, the FPs are aggregated through strong interaction, which can only partly be attributed to disulfide bonds. The addition of the KL increases the protein solubility. Beside the radical scavenging properties of KL are likely to be involved in this behavior, we are not able to evidence by ESR the specific presence of radicals due to this interaction. We demonstrate that the addition of KL significantly increases the materials Young's modulus and tensile strength, with only a slight effect on deformation at break. In terms of mechanical properties, an optimum formulation clearly exists, for which the FP/KL ratio is of 40/30, for a material plasticized with 30 wt % of glycerol. We hypothesize that above this value, KL which will become the main component of the blends will constitute the continuous phase. The water sensitivity, measured by the samples swelling in water, is considered as one of the main drawbacks of biobased materials. In the case of FP-based materials, it is reduced by more than 40% due to KL addition, which we attribute to the hydrophobic properties of KL. Finally, we evidence a strong capillary viscosity decrease resulting from the addition of KL. This can be linked to the protein reduced polymerization in presence of KL, to its plasticizing properties above the T_g, and to its lower molecular weight. In conclusion, this study shows how FP-based plastics can be improved, both in terms of functional and processing properties, by the use of KL.

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