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Wheat Gluten Blends with a Macromolecular Cross-Linker for Improved Mechanical Properties and Reduced Water Absorption

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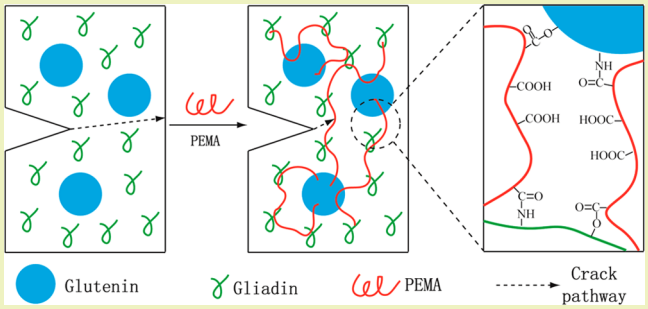
Supporting Information

ABSTRACT: Wheat gluten (WG) is reactively blended with a macromolecular cross-linker, polyethylene-*alt*- maleic anhydride (PEMA), to simultaneously improve strength, stiffness, strain, and reduce water absorption for the first time. FTIR illustrates the cross-linking reaction. An increase in T_g measured by DSC and a decrease in protein extractability measured by SE-HPLC demonstrates an increase in cross-linking as PEMA content increases. The modified WG is thermo-molded into solid bars and tested for flexural properties. The flexural testing results indicate that the maximum strain and stress of the modified WG can be improved by as much as 95% and 120%, respectively. Addition of PEMA to WG lowers the water absorption by as much as a factor of 4 at the same time as improving the mechanical properties. The results are consistent with a single phase, intermolecular, cross-linked morphology. The improvements attained make these blends approach the properties of polystyrene and aerospace grade epoxies.



KEYWORDS: *Wheat gluten, Polyethylene-alt-maleic anhydride, Intermolecular cross-linking, Mechanical properties, Water absorption*

INTRODUCTION

Wheat gluten (WG) is the protein extracted from wheat flour and affects up to 1% of the population with allergy symptoms such as celiac disease.[1] That and other factors lead to a large quantity of WG produced as a byproduct each year. As environmental concerns caused by conventional plastics increase, biodegradable plastics are sought as a substitute for synthetic plastics. WG has received attention as a potential biodegradable plastic due to its mechanical properties, low cost, and complete biodegradability.[2] It has been made into composites, foam, fibers, membranes, drug delivery agents, and other products.[3−9] Nevertheless, broad application of WG is restricted by two main drawbacks, brittleness and high water absorption. Brittleness could induce premature failure during service. High water absorption makes WG-based materials susceptible to failure by absorbing water and then softening.[3,10]

WG is a heterogeneous biopolymer composed of monomeric gliadin ($\alpha/\beta,\gamma,\omega$ gliadins, MW 28 ∼ 55 KDa) and polymeric glutenin (MW 100 kDa ∼ 10 MDa).[11] Disulfide bonds between cystine residues play an important role in defining the protein conformation.[12] Gliadin, accounting for about 60−75 wt % of gluten protein, has three to four intramolecular disulfide bonds to stabilize the folded protein conformation. Glutenin, accounting for roughly 25−40 wt % of gluten protein, has both intramolecular and intermolecular disulfide bonds, in which the intramolecular disulfide bonds keep the monomeric protein units in certain conformations, and the intermolecular disulfide bonds link monomeric proteins to form polymeric protein.[11] These two protein components interact through weak secondary bonding, leaving a notable lack of chain entanglements and network structure in the native material, which is thought to contribute to the brittle mechanical properties and high water absorption. Many other proteins also lead to brittle materials with high water absorption.[13]

Attempts to improve protein-based materials have been done in both rubbery and glassy states. Rubbery WG materials are generated by the addition of plasticizers such as glycerol, sorbitol, and other low molecular weight compounds[14,15] to yield low stiffness and highly ductile materials suitable for films. Cross-linking rubbery WG materials is often performed to improve stiffness and strength. Small molecule cross-linkers such as aldehydes[16−18] and diisocyanates[19] have been used due to high reactivity and easy processing. Hernandez-Munoz et al. and Balaguer et al. used formaldehyde and cinnamaldehyde to cross-link WG in the rubbery state where glycerol was included,

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and their three works reported a decrease in strain and an increase in modulus and strength.[16−18]

Glassy WG materials are processed without plasticizers in order to keep its high modulus, strength, and glass transition temperature.[2,10,20−24] In order to enhance its properties, different techniques such as denaturing, blending with synthetic polymers, and cross-linking have been tried. Denaturing can be accomplished by breaking disulfide bonds and disrupting hydrogen bonding.[2,23−26] Woerderman et al.[2] pioneered the use of thiol-functionalized molecules to improve WG properties by both denaturing and cross-linking WG via thiol−disulfide interchange reactions and suggested that the increased cross-linking degree contributed to the improvement. Jansens et al.[23,24] used monothiol, trithiol, and polythiol additives and achieved similar results in all cases and concluded that both the cross-linking and mixture morphologies are important factors for improving mechanical properties. Blends of WG with poly(vinyl alcohol)[27] and other polymers[28,29] have yielded a variety of results in which the blend often has relatively poor mechanical properties due to large-scale phase separation from the protein.

Macromolecular cross-linkers have been tried with WG in the glassy state in an effort to produce network morphologies. Cross-linking agents with multiple thiol groups were developed to break the disulfide bonds thereby opening the WG structure and then cross-linking the protein to form a network structure. While improved mechanical properties were obtained, the water absorption was either not improved significantly or became even worse.[10] In the most closely related work by Sun,[29] soy protein isolate and poly(ethylene-*co*-ethyl acrylate-*co*-maleic anhydride) were blended as solid powders in a mixer without a plasticizer. The strength and modulus decreased with addition of more copolymer, which may have been due to poor chemical interaction in the dry mixing conditions.

In the present work, a new macromolecular cross-linking agent is introduced using maleic anhydride groups in an alternating copolymer with ethylene in an attempt to form a network structure without breaking the disulfide bonds. The hypothesis is that polyethylene-*alt*-maleic anhydride (PEMA) will preferentially form intermolecular cross-links, rather than intramolecular cross-links, with the protein due to its high reactivity but low mobility within the protein structure. Experimental results illustrate the macromolecular cross-linker, PEMA, dramatically improves WG properties. While the results of cross-linking WG with PEMA can be directly compared to the previous work cross-linking WG with macromolecular polythiols,[10] comparing the effects of macromolecular cross-linkers in the glassy state with the effects of small cross-linkers in the rubbery state[16−18] is difficult and is addressed in the discussion below.

MATERIALS AND METHODS

Materials. American Vital Wheat gluten was obtained from Arrowhead Mills with 80% protein, 10% moisture, and 10% other (starch, lipid, ash, etc.). WG was vacuum-dried for 12 h before use, and the resulting moisture content was about 8 wt %. Polyethylene-*alt*-maleic anhydride (MW 100,000−500,000) and ethyl acetate were purchased from Sigma-Aldrich. DMSO was purchased from Fisher Scientific.

WG Modification. WG was dispersed in DMSO at the ratio of 1:40 (wt:vol) at 60 °C and stirred for 1 h and then subsequently cooled to room temperature. PEMA was dissolved in DMSO at the ratio of 1:100 (wt:vol). PEMA solution was added dropwise into WG dispersion to obtain mixed ratios of 5, 10, 20, and 50 wt % under rigorous stirring at room temperature. A rapidly increasing viscosity upon mixing indicates rapid association and perhaps rapid reactions between PEMA and WG. The mixture was stirred at 60 °C for 1 h to blend the mixture homogeneously. Subsequently, the blended mixture was precipitated in ethyl acetate at the ratio of 1:10 (v/v). The white precipitate was collected carefully and washed 5 times with ethyl acetate and dried under vacuum overnight before thermo-molding. The moisture contents of WG/5%PEMA, WG/10%PEMA, WG/20% PEMA, and WG/50%PEMA are 12.83, 11.24, 13.29, and 15.75 wt %, respectively.

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were taken using a Nicolet Magna-IR 560 with 32 scans at 4 cm^{-1}. The powders were ground into a dry KBr disk. The spectra were analyzed with Omnic software from the Thermo Electron Corporation.

Thermal Gravimetric Analysis (TGA). TGA was performed on a TGA 2950 instrument. Triple replicate samples before molding of WG, PEMA, WG/5%PEMA, WG/10%PEMA, WG/20%PEMA, and WG/50%PEMA were loaded and heated to 700 °C at the rate of 10 °C/min.

Differential Scanning Calorimetry (DSC). WG, PEMA, WG/5% PEMA, WG/10%PEMA, WG/20%PEMA, and WG/50%PEMA samples before molding were analyzed using modulated DSC (DSC-Q100 from TA Instruments). Modulation amplitude was 0.5 °C every 60 s. Ten milligram samples were loaded and sealed in aluminum pans. Samples were heated at 10 °C/min from 0 to 230 °C, held at 230 °C for 2 min, cooled at 5 °C/min back to 0 °C, held at 0 °C for 5 min, and heated to 230 °C at 5 °C/min. The second heating is reported below. Three replicates were performed for each sample. Glass transition temperature (T_g) values were obtained by analyzing the data using the Universal Analysis software from TA Instruments.

SE-HPLC Study. Samples (1.0 mg/mL) before molding were extracted for 1 h at room temperature with a 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) sodium dodecyl sulfate (SDS)[2,10] and centrifuged (10 min, 10,000*g*). Supernatants were filtered (0.45 um) and loaded (50 uL) on a Phenomenex BioSep-SEC-S4000 (300 mm × 7.8 mm) column (Phenomenex, Torrance, CA). The proteins were eluted at room temperature with 50.0% (v/v) acetonitrile containing 0.05% (v/v) trifluoroacetic acid (flow rate, 0.5 mL/min). The detection was performed with a Milton Roy SpectroMonitor 3100 detector at 210 nm. WG proteins were classified into three groups: (1) unextractable polymeric proteins, (2) glutenin, and (3) gliadins ($\alpha/\beta,\gamma,\omega$ gliadins).[22]

Mechanical Property Test. WG, PEMA, hydrolyzed PEMA, and blended WG/PEMA (650 mg) were compression molded at 150 °C for 10 min at a pressure of 4.45×10^7 N/m^2, corresponding to an applied force of 8.9×10^4 N (20,000 lbf) in a stainless steel mold to form 10 samples of 4 cm × 0.5 cm × 0.2 cm, which were subsequently kept in a desiccator for 3 days before testing. All samples were subjected to three-point bending tests performed according to the ASTM D790-02 standard on a computer interfaced Instron-1011 with a 500 N load cell. The rate of crosshead motion was 1 mm/min with a data acquisition rate of 10 points per second. Five replicates were performed for each material.

Water Absorption Test. The original weight of molded WG, WG/5%PEMA, WG/10%PEMA, WG/20%PEMA, and WG/50% PEMA samples were recorded. Specimens were subsequently immersed in DI water. At certain times, specimens were taken out, surface dried, and weighed. The sample mass reached a steady value within 48 h. Three replicates were measured for each blend.

RESULTS

FTIR Analysis. The complexity of WG renders FTIR characterization of the reactions with a PEMA copolymer difficult to interpret. This difficulty arises because, first, only a small fraction of the amino acids in WG, such as lysine, serine, threonine, arginine, and free thiol groups,[30] can react with the anhydride in the copolymer,[31] and second, the IR absorption spectra of the reacting amino acids overlap with other amino acid IR absorption bands. Nonetheless, anhydride functional

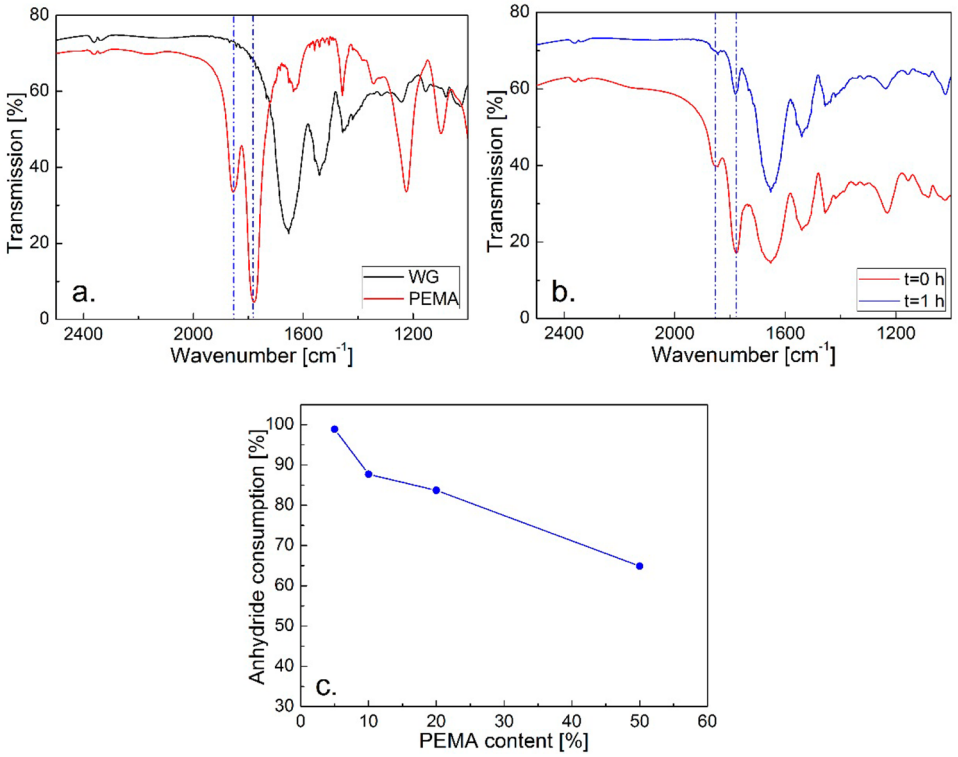

Figure 1. (a) IR spectra of pure WG and PEMA. (b) IR spectra of WG/10%PEMA before and after reaction. Spectra of other blends are included in the Supporting Information. (c) Quantitative analysis results of anhydride consumption at different PEMA fraction.

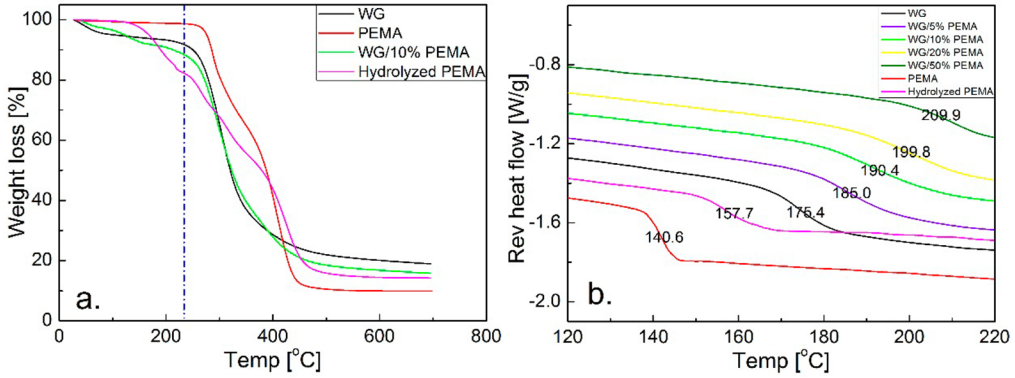

Figure 2. (a) TGA spectra of WG, WG/10%PEMA, PEMA, and hydrolyzed PEMA. WG/5%PEMA, WG/20%PEMA, and WG/50%PEMA are shown in the Supporting Information. (b) DSC spectra of WG, PEMA, hydrolyzed PEMA, WG/5%PEMA, WG/10%PEMA, WG/20%PEMA, and WG/50%PEMA, with the T_g values noted on each curve. The standard deviation of T_g is approximately 1% of the values shown based on DSC scans of three samples.

groups in a PEMA copolymer show a unique and sharp doublet peak at 1850 and 1780 cm^{-1},[32] which is absent in WG (Figure 1a). Therefore, the interaction between WG and PEMA can be characterized by comparing intensity changes of the doublet peak before and after reaction, as shown in Figure 1b. Quantitative analysis of the anhydride consumption (Figure 1c) was performed using the absorbance spectra, and detailed procedures are provided in the Supporting Information.

Thermal Analysis. In Figure 2a, TGA shows that when temperature is higher than 230 °C, WG and modified WG start decomposing. Only the curve for WG blended with 10% PEMA is included in Figure 2a for clarity, and the curves for other blends are similar and presented in the Supporting Information. Hence, all the processing and analysis in the present work is performed at temperatures below 230 °C. Moreover, when heated to 230 °C, WG samples undergo about 8 wt % loss, which is due to moisture in the protein structure.[33−35] The blend loses more mass than the WG at temperatures below 230 °C, even though the PEMA loses less mass than WG at low temperatures. Note, however, that hydrolyzed PEMA loses almost 20% of its mass by 230 °C, and the blend follows a mass loss curve between the WG and the hydrolyzed PEMA. The role of hydrolyzed PEMA is illustrated in the Discussion section.

DSC analysis in Figure 2b shows that WG has a single and broad T_g near 175 °C, which is thought to be due to the broad molecular weight distribution and complex composition.[10,35] PEMA, also with a broad molecular weight distribution, displays a narrower T_g at 140.6 °C. The hydrolyzed PEMA has a T_g near 158 °C, an increase due to hydrogen bonding.

After blending at weight ratios of 95/5, 90/10, 80/20, and 50/50, the blends show increasing T_g values of 185, 190.4, 199.8, and 209.9 °C, respectively. Measured values of WG properties and the properties of WG blends can be sensitive to moisture content, so the drying procedure used here is identical to that used previously[3,10,35] to make the most accurate comparisons. The residual moisture in the DSC samples was largely driven off during the first heating, so the reported T_g values represent well-dried WG and WG blends with PEMA before molding. This is significantly different than Jansens et al.,[20] where their measurements were performed in hermetically sealed DSC pans such that their T_g values represent the material with the contained moisture.

Furthermore, one broad T_g is observed, and the breadth of the transition appears to increase with larger fractions of PEMA in the blend. No transitions are observed in the regions where PEMA, hydrolyzed PEMA, and WG display transitions, and this single T_g indicates that WG/PEMA blends form one phase.

The appearance of a single T_g in Figure 2b, above the T_g values of the blend constituents, is in marked contrast to the blends of WG and thiolated PVA reported previously,[10,35] where two T_g values were observed between the T_g values of the constituents. The values of T_g obtained in the earlier work were indicative of a partially compatibilized and microphase separated blend with a very low degree of cross-linking. The T_g values obtained in the current work are indicative of a well-compatibilized blend with extensive cross-linking.

SE-HPLC Analysis. WG and modified WG were extracted by sodium dodecyl sulfate solutions as previously described.[2,10] Figure 3 presents the size exclusion chromatography elution

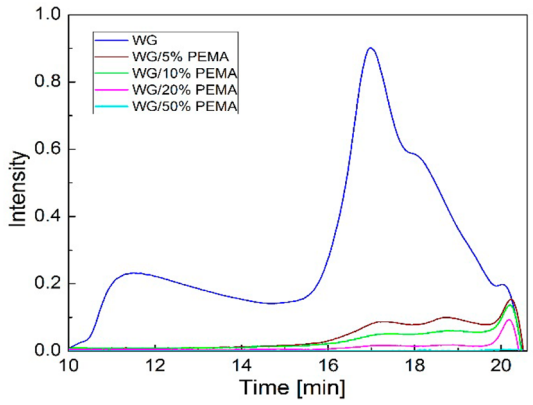

Figure 3. SE-HPLC curves of WG, WG/5%PEMA, WG/10%PEMA, WG/20%PEMA, and WG/50%PEMA extracted from the blend by SDS buffer.

profiles of WG and modified WG, longer times on the *x*-axis corresponding to lower molecular weight components. WG's broad size distribution is further confirmed with broad peaks lasting from 10 to 21 min. The gliadins (MW 28 KDa to 55 KDa) emerge from the column at 16−19 min, and glutenin (MW > 100 kDa) elutes at 10−16 min.[2,10] Resolution of the polymeric proteins was not possible on the column used due to a high MW limit of roughly 500 kDa.

After blending with PEMA, WG becomes much less extractable, indicating the formation of a cross-linked structure. In Figure 3, the peak intensity drops to nearly zero from 10 to 16 min for all the blends, illustrating that even 5% addition of PEMA cross-links the glutenin fractions. From 16 to 20 min, the intensity of the gliadin peak decreases dramatically upon addition of PEMA, indicating increased incorporation of the gliadins into the cross-linked structure as the PEMA addition increased. At 50% additive, the gliadin peak intensity decreases to near zero indicating complete cross-linking by PEMA.

The quantity of extractable protein decreases to a much greater extent when blended with PEMA compared to previous blends of WG with thiolated PVA.[10] This is consistent with the DSC results above that indicate a higher degree of cross-linking by PEMA than by thiolated PVA.

Flexural Test. Flexural testing results for WG, PEMA, hydrolyzed PEMA, and blends of WG/PEMA are summarized in Table 1 and Figure 4. Compared to WG, the modified WG specimens exhibit improved mechanical properties, initially following an upward trend as the PEMA additive increases. At 20% PEMA additive, modified WG obtains the highest mechanical properties with strength and strain increases of 120% and 95%, respectively, and a 20% increase in modulus relative to WG.

Table 1. Mechanical Properties of WG, PEMA, and WG/PEMA Blends[a]

	stress (MPa)	strain (%)	modulus (GPa)
WG	42.7 ± 2.2	0.97 ± 0.09	4.40 ± 0.10
PEMA	78.2 ± 3.9	1.73 ± 0.16	4.73 ± 0.15
hydrolyzed PEMA	34.8 ± 3.1	0.57 ± 0.03	5.80 ± 0.14
WG/5%PEMA	61.6 ± 6.4	1.37 ± 0.06	4.60 ± 0.60
WG/10%PEMA	75.4 ± 2.3	1.66 ± 0.12	4.90 ± 0.12
WG/20%PEMA	92.9 ± 3.3	1.89 ± 0.12	5.24 ± 0.16
WG/50%PEMA	87.0 ± 2.8	1.89 ± 0.10	5.15 ± 0.21
polystyrene[b,36]	76	3.3	2.7

[a]Average values and standard deviations calculated from at least five tests. [b]Polystyrene is used as the reference.

Water Absorption. Upon immersion in DI water, specimens start absorbing water and reach saturation by 48 h. The water absorption ratio (W_a) is calculated as water absorbed over the initial weight. The W_a during the first 9 h is displayed in Figure 5a, and steady state W_a is summarized in Figure 5b. Compared to WG, the modified WG specimens have a lower W_a. At 50% additive, W_a of modified WG decreases to 27% from 115% for WG.

DISCUSSION

It appears that for the first time, a blend of WG with a synthetic polymer has produced a material with increased strength, stiffness, strain to failure, and T_g, while simultaneously reducing water absorption. WG amino acid analysis shows that primary amine and hydroxyl are the functional groups in WG able to react with the anhydride in PEMA.[30] The reactivity of these groups with PEMA is also expected to depend upon the protein conformation in solution due to steric hindrance and diffusion rate considerations. In DMSO, gliadins are soluble and partially denatured because the DMSO weakens their intramolecular hydrogen bonds. The few intramolecular disulfide bonds in the gliadins restrict the protein conformations,[11,12,37] increasing steric hindrance, especially for the high MW PEMA. Polymeric glutenin is not soluble in DMSO as a result of the large MW and large number of intra- and intermolecular disulfide bonds.[37] Nevertheless, due to similar polarity of glutenin and DMSO, polymeric glutenin can be suspended evenly in DMSO, with reactive amine and hydroxyl on the protein surface available for further reaction.

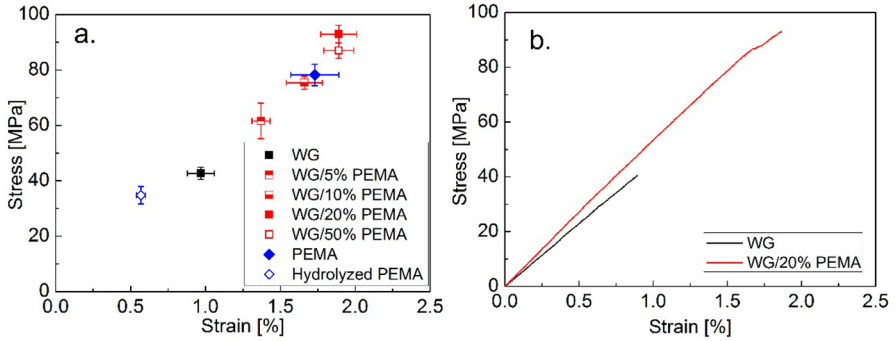

Figure 4. (a) Summary of strength and strain at failure of WG, PEMA, hydrolyzed PEMA, and WG/PEMA blends. (b) Representative stress−strain curves of WG and WG/20%PEMA. Stress−strain curves of WG/5%PEMA, WG/10%PEMA, and WG/50%PEMA are shown in the Supporting Information.

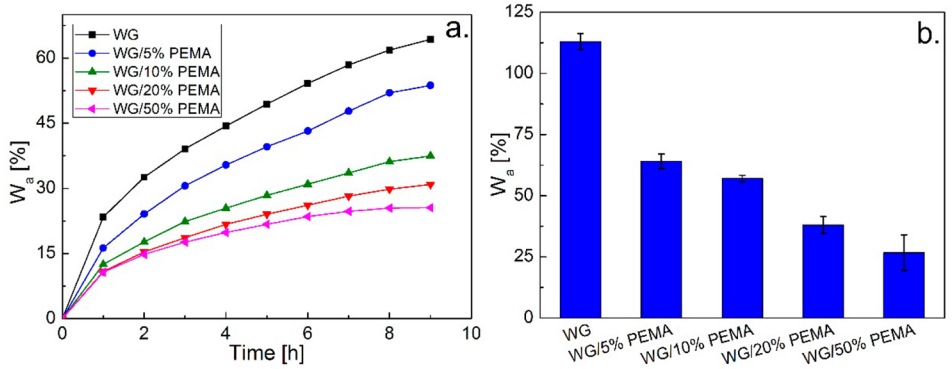

Figure 5. (a) First 9 h of water absorption ratio of WG and WG blends. (b) Stable water absorption ratio at t = 48h. (Water absorption ratio is not applicable to PEMA due to its hydrolysis and complete solubility in DI water).

A stoichiometric calculation indicates that 9.2 wt % PEMA could consume all the primary amine and hydroxyl functional groups in WG. Therefore, if only the stoichiometry of the reactions between PEMA and the protein are considered, the consumption of anhydride in the PEMA should have been 100% for the 5% PEMA addition, 91% for the 10% PEMA addition, 40% for the 20% PEMA addition, and only 10% for the 50% PEMA addition. Moreover, due to steric hindrance of the large molecules involved, lower consumption of anhydride than indicated by stoichiometry is expected. However, the quantitative IR analysis results in Figure 1c indicate that much larger fractions of the anhydride in the PEMA were consumed.

The vacuum-dried WG has approximately 8 wt % of bonded residual water (see TGA data, Figure 2a) that can hydrolyze anhydride into carboxylic acid groups.[38] A stoichiometric calculation of the hydrolysis reaction indicates that the residual water is sufficient to react with 100% of the anhydride for the blends with 5%, 10%, and 20% PEMA, and with 56% of the anhydride in the case of the 50/50 blend. Therefore, the anhydride groups in the PEMA that do not quickly react with the WG are expected to be hydrolyzed by residual water. Hence, the decreased IR doublet peak intensity assigned to the anhydride (Figure 1b) indicates PEMA reactions with both WG and residual water in WG.

Due to the hydrolysis reaction, the blends evaluated by various methods were most likely combinations of WG, PEMA, and hydrolyzed PEMA. In the cases of the 5%, 10%, and 20% PEMA blends, most of the PEMA is expected to be in the hydrolyzed form. Thus, the TGA data of the 10% blend in Figure 2a lies between the WG and the hydrolyzed PEMA, rather than between the WG and the unhydrolyzed PEMA.

The reaction between WG and PEMA manifests itself in the DSC and SE-HPLC results. As shown in Figure 2b, the WG/PEMA blend T_g increases progressively with addition of PEMA, with all the blend values well above the T_g values of either WG or PEMA. T_g is generally a function of cross-linking degree;[39] therefore, the increase in the blend T_g is expected from cross-linking with PEMA. Moreover, the cross-linking is also clearly illustrated by the SE-HPLC spectra in Figure 3. With addition of PEMA, the extractable glutenin is virtually eliminated, and the extractable gliadin is progressively reduced with an increasing addition of PEMA. A small amount of cross-linking is expected to nearly eliminate the extraction of uncross-linked glutenins due to their very high molecular weights. They will be trapped in the network even if uncross-linked. Uncross-linked gliadins are expected to be able to diffuse out of the cross-linked network and be extracted due to their relatively small size. However, as the cross-linking density increases, the diffusivity of gliadins is expected to decrease thereby giving a reduced extractability as the copolymer is added to the blend in larger fractions. In addition, the amount of extractable gliadin is expected to decrease as the fraction of gliadin incorporated into the cross-linked network increases. Therefore, after being cross-linked, the blend forms an unextractable network structure, resulting in a zero peak intensity from 10 to 16 min upon all PEMA ratios and a progressively decreasing gliadin peak from 16 to 20 min as the PEMA additive increases.

The steric hindrance in gliadins and the low solubility and steric hindrance of glutenins should not affect the diffusion of

small cross-linkers as much as the diffusion of macromolecular cross-linkers into the proteins. Therefore, different from small cross-linkers, PEMA is expected to react with amine and hydroxyl in the outer regions of the gliadin coils and suspended glutenin aggregates preferentially to the core regions of the proteins. Thus, the PEMA may be more effective at forming intermolecular bonds to link protein components together than forming intramolecular cross-links. A schematic illustration of this morphology model is given in Figure 6.

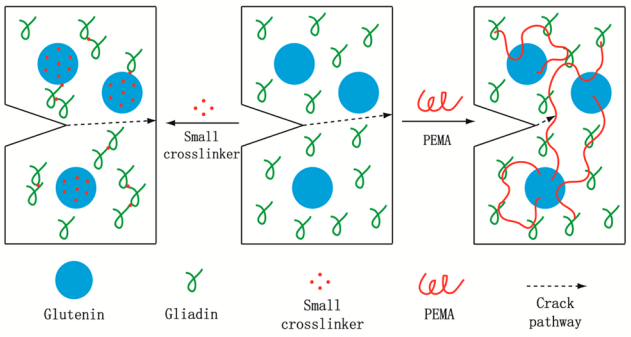

Figure 6. Morphologies expected from the reactions of small MW cross-linkers and large MW cross-linkers, PEMA, with WG. Cracks propagating through materials may encounter different levels of resistance due to different morphologies.

Despite steric hindrance and preferential reaction zones in the protein structure, phase separated regions are not shown by the DSC because only single glass transitions appear in the DSC data of Figure 2b. However, the glass transitions become broader as larger amounts of PEMA are added. This may be due to increased cross-link density,[39] or this may indicate composition fluctuations in the material but not the abrupt compositional changes associated with phase separation. However, when macromolecular polythiols were blended with WG, multiple transitions were observed in the DSC indicating phase separation.[35] Similar phase separation is also shown in the work of dry blended soybean protein with PE−PEA−MA[29] and WG blended with epoxidized soybean oil.[40] The morphology of the WG blends with macromolecular cross-linkers is therefore expected to be significantly different depending upon the processing method and chemical interactions.

Small cross-linkers generally have molecular weights of order of 100 g/mol, which is very small relative to WG components that have molecular weights ranging from 28 KDa to 10 MDa. The small cross-linkers are therefore depicted as small dots in Figure 6. Due to the big size difference, small MW cross-linkers are expected to cross-link protein functional groups in very close proximity to each other and therefore form primarily intramolecular cross-links. While that morphology may rigidify the glutenin aggregates and form gliadin aggregates, stiffening the overall material, it may not form intermolecular networks capable of dissipating the energy of a propagating crack. However, macromolecular PEMA with size of the same order as WG components is expected to form many intermolecular cross-links between glutenin aggregates and gliadins, generating a network morphology capable of blunting crack propagation.

As stated previously, a direct comparison to the work with aldehyde cross-linkers[16−18] is difficult because that work was carried out in the rubbery state created by adding plasticizers to the material. However, a plasticizer cannot reduce the disulfide bonds to denature the WG. Therefore, the original WG molecular structure should be little changed by the plasticizer. Thus, the low molecular weight aldehydes may only cross-link protein functional groups in very close proximity to each other and therefore form primarily intramolecular cross-links. The model depicted in Figure 6 is consistent with the mechanical property results for the WG cross-linked by aldehydes[16−18] and the less brittle blends formed in previous work with macromolecular polythiol cross-linkers[10] and the less brittle cross-linked proteins produced in this work, with experimental data summarized in Figure 4 and Table 1.

The network's effectiveness at blunting crack propagation can be well illustrated by its toughness,[41] which is the energy required to fail the sample. Toughness is calculated by integrating the stress−strain curve by the following equation

$$W = \int_0^{\varepsilon_f} \sigma d\varepsilon$$

where, W is the toughness, ε_f is the failure strain, and σ is the stress. As shown in Figure 7, the toughness increases as the

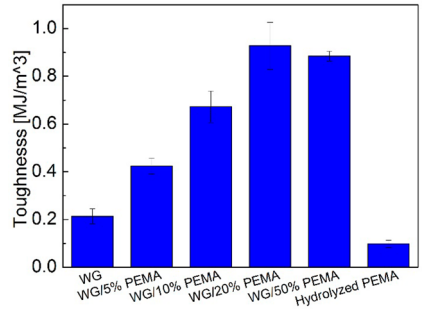

Figure 7. Toughness of WG, WG/5%PEMA, WG/10%PEMA, WG/20%PEMA, WG/50%PEMA, and hydrolyzed PEMA.

PEMA additive increases up to 20 wt % PEMA. The toughness of WG/50 wt % PEMA is statistically indistinguishable from the toughness of WG/20 wt % PEMA, and the toughness of 100% hydrolyzed PEMA is again quite low. Compared to WG, the toughness of WG/20%PEMA increased by more than a factor of 4. A much tougher material is produced by combining two brittle components, which is probably due to the intermolecular cross-linked network structure. Similar results were also observed in recently studied double network hydrogels.[42]

Besides increasing the mechanical properties, a large reduction in water absorption is observed in Figure 5, which is a significant improvement over the previous work with polythiols.[10] Polythiols strengthen WG by introducing cross-linking and chain entanglement through thiol−disulfide exchange reactions. However, the thiol−disulfide exchange also breaks the disulfide bonds in the native WG thereby opening the WG structure. WG structure denaturing by thiols is widely accepted[2,10,24,35] and can be observed by a decrease in viscosity of WG suspensions and solutions upon addition of dithiothreotol (DTT) or by macromolecular polythiols such as thiolated poly(vinyl alcohol). The blends formed are only partially miscible, as noted above, and the degree of cross-linking is very low as evidenced by DSC data and by SDS extraction data. Upon thermo-molding, the SDS extraction of the protein declines significantly, perhaps due to additional

disulfide cross-linking as well as typical protein reactions under heat.[2,10]

Breaking the disulfide bonds with thiols increases the protein hydrophilicity and WG blends with such opened structural morphology are therefore likely to be more hydrophilic. The opened structure of the protein may therefore be responsible for the increased water absorption observed in the earlier work. In contrast, PEMA does not break the disulfide bonds and furthermore, it increases the cross-linking density of the protein, which may explain the reduced W_a. Generally, W_a of a polymer is a function of cross-linking degree because increasing cross-linking reduces the ability of a polymer to swell.[39,43]

CONCLUSION

In the present work, WG modification with the macromolecular cross-linker, PEMA, was investigated. The experimental results demonstrated that PEMA is a better cross-linker for WG than small molecule cross-linkers or other macromolecular cross-linkers such as thiolated PVA. The interaction between WG and PEMA was characterized by FTIR, DSC, SE-HPLC, and flexural tests. The data indicate that PEMA effectively cross-linked the WG and that the degree of cross-linking increased as the fraction of PEMA increased. Furthermore, PEMA was largely hydrolyzed during the blending process by residual water in the WG.

The flexural test results demonstrate that blending WG with PEMA increases strain to failure rather than decreasing it as when WG is blended with small aldehyde cross-linkers.[16−18] This opposite behavior in strain to failure indicates that the blend morphology of WG/PEMA is much different than the blend morphology of WG with small molecule cross-linkers. Combining the flexural results, protein extraction results, and macromolecular and polyfunctional characteristics of PEMA indicates that the PEMA may form intermolecular cross-links connecting the protein components into a single network. The intermolecular covalent bonds between the WG components produce a tougher network structure than either the WG or the PEMA individually.

Increasing the mechanical properties as reported above has been achieved before with thiolated PVA, but simultaneously reducing water absorption is reported here for the first time. Previous work with thiolated PVA broke the disulfide linkages to make the thiol groups available as cross-linking sites. The chemistry of PEMA cross-linking does not disrupt disulfide bonds and so the PEMA did not open the WG structure. Cross-linking the protein without opening its structure and the increased cross-link density of PEMA compared to thiolated PVA are the most likely reasons that the water absorption was reduced. The improvements achieved bring WG closer to engineering applications for biodegradable plastics.

ASSOCIATED CONTENT

Supporting Information

FTIR spectra of the WG/5%PEMA, WG/20%PEMA, and WG/50%PEMA blends before and after reaction; quantitative analysis of anhydride consumption ratio procedures; TGA spectra of WG, PEMA, and hydrolyzed PEMA with WG/5% PEMA, WG/20%PEMA, and WG/50%PEMA; and representative stress−strain curves of WG/5%PEMA, WG/10%PEMA, and WG/50%PEMA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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