Biomacromolecules **2006,** *7,* 2837−2844

Wheat Gluten−Thiolated Poly(vinyl alcohol) Blends with Improved Mechanical Properties

Rebecca M. Dicharry, Peng Ye, Gobinda Saha, Eleanor Waxman,
Alexandru D. Asandei, and Richard S. Parnas*

University of Connecticut, Institute of Materials Science, 97 North Eagleville Road U-3136, Storrs, Connecticut, 06269-3136

Received May 4, 2006; Revised Manuscript Received August 3, 2006

A multifunctional macromolecular thiol (TPVA) obtained by esterification of poly(vinyl alcohol) (PVA) with 3-mercaptopropionic acid was characterized by a combination of NMR, IR, transmission electron microscopy (TEM), and differential scanning calorimetry (DSC), and was used as a wheat gluten (WG) reactive modifier. The effect of TPVA molecular weight (M_w = 2000, 9500, 50 000, and 205 000) and blend composition (5, 20, and 40% w/w TPVA/WG) on the mechanical properties of compression-molded bars indicates that TPVA/WG blends increase the fracture strength by up to 76%, the elongation by 80%, and the modulus by 25% above WG. In contrast, typical WG additives such as glycerol and sorbitol improve flexibility but decrease modulus and strength. Preliminary investigations of suspension rheology, water uptake, molecular weight distribution and electron microscopy of TPVA/WG and PVA/WG blends illustrate the different protein interactions with PVA and TPVA. Further work is underway to determine whether TPVA and WG form protein conjugates or microphase-separated morphologies.

Introduction

Commodity materials made from naturally occurring biopolymers can be both a cost-effective and environmentally sound alternative to traditional petroleum-based polymers. Wheat gluten (WG), when molded into plastic, has high stiffness in the range of epoxy ($E \sim 1$ GPa[1] and 3.5 GPa[2]) and reasonable strength (20−35 MPa[1] and 50 MPa[2]) in comparison with other bioplastics.[3] However, WG is quite brittle, leading to this and previous investigations into additives to improve its ductility.

In particular, protein-based materials are particularly attractive because of their low cost and wide variety of functional groups associated with the amino acid residues. Structural modifications via physical (temperature or pressure treatment), enzymatic (hydrolysis, attachment of amines and deamidation), or chemical (cross-link) methods[4] are often used to tailor protein properties for different applications. Among them, chemical modification of the amino acid functional groups is a very powerful and versatile tool to improve protein properties, such as water resistance and mechanical performance.

Since molded WG is brittle, hydrophilic plasticizers such as water, glycerol, and sorbitol are used to screen noncovalent interactions. Plasticizers decrease the glass transition temperature and increase the flexibility of WG. Water is the most effective plasticizer, and its effects on WG are well-known.[5−17] While glycerol is an effective plasticizer, it migrates to the surface during storage, and thus WG films lose flexibility within a few days.[4,18] Sorbitol is less effective but is retained during storage.[19] Diethanolamine and triethanolamine can also plasticize WG,[20] and the plasticizing effect of a series of saturated fatty acids was approximated[21,22] to be intermediate between water and glycerol.

Plasticized WG has high flexibility, but very low modulus and strength. Thus, to increase strength, proteins can be cross-linked by several methods. Chemical and radiation treatments were applied to both the film-forming solution (pretreatment) and on the film (posttreatment).[23−28] Formaldehyde is a well-known cross-linking agent for many types of proteins derived from pea,[24] cottonseed,[25] corn zein,[29] and wheat,[45−47] and it generally decreases the elongation and increases the tensile strength. However, because of its toxicity, its use is limited. Ultraviolet and γ radiation can cross-link proteins, but the efficiency of the radiation in the cross-linking reaction depends on the protein source; soy protein is rich in tyrosine and phenylalanine and is thus sensitive to UV radiation,[23] which is not the case for pea[24] and gluten films.[30]

In addition to the use of plasticizers, various polymers were also blended with WG. This includes aliphatic polyesters,[31] poly(hydroxy ester ether),[32] maleic anhydride-modified polycaprolactone,[33] poly(ethylene-*co*-vinyl acetate)/poly(vinyl chloride),[34] and cassava starch.[35] The blends were mixed using a screw extruder. Mechanical properties were investigated on bars formed by injection molding of the extrudate pellets. In most cases, the mechanical properties were satisfactory, but the elongation was reduced compared with that of the pure polymer additive.

To circumvent these problems, other methods of improving the mechanical properties of WG are investigated herein. Since the disulfide bond plays a central role in the wheat protein network, chemical modification causes significant changes in the overall properties of wheat protein. This is evidenced by our recent work of modifying wheat protein with a low molecular weight (M_w = 1247) three-arm, thiol-terminated poly(ethylene oxide).[1] However, custom-designed additives are very expensive, and the cost is a concern when making a competitive alternative to commodity plastics.

In this work, we present a new polythiol additive based on thiolated poly(vinyl alcohol) (TPVA) via its esterification with 3-mercaptopropionic acid. The mechanical properties of TPVA/WG and PVA/WG blends were studied. Evidence of the chemical interaction of the TPVA with WG was revealed by

---

* Corresponding author. E-mail: rparnas@ims.uconn.edu.

10.1021/bm060432n CCC: $33.50

rheology and size exclusion high-performance liquid chromatography (SE-HPLC). Several of the property enhancements reported here are similar to those afforded by previous additives,[1] but some, such as the water absorption, are quite different. The discussion of the similarities and differences is begun here, and is further explored in a subsequent paper devoted to the microstructure of these blends.

Experimental Section

Materials. American vital WG was obtained from Arrowhead Mills, Hereford, TX. PVA (M_w = 9500, 50 000, and 205 000), 3-mercaptopropionic acid, and dithiothreitol (DTT) were from Sigma-Aldrich. PVA (M_w = 2000) was obtained from Acros Organics. All were used without further preparation. Chemlease PMR mold release was obtained from Chem Trend, Howell, MI. While mercaptopropionic acid has the characteristic smell associated with the vast majority of sulfur compounds, its toxicity (according to the MSDS) is not significantly higher than that of propionic acid. In addition, once it is esterified with PVA, the vapor pressure decreases considerably, as expected for a polymer, and no characteristic smell remains.

Techniques. *Nuclear Magnetic Resonance Spectroscopy.* ^{1}H NMR (500 MHz) spectra were recorded on a Bruker DRX-500 at 24 °C in D_2O (Aldrich; 1% v/v trimethylsilane as the internal standard).

Fourier Transform Infrared (FT-IR) Spectroscopy. FT-IR spectra were taken using a Nicolet Magna-IR 560 with 32 scans. The powders were ground into a dry KBr disk. The spectra were analyzed using Omnic software, version 7.2a, from Thermo Electron Corporation.

Differential Scanning Calorimetry (DSC). Modulated DSC (MDSC) was performed using a DSC-Q100 from TA instruments. The modulation was ± 0.5 °C/min, with a heating rate of 2 °C/min from 20 to 140 °C, using 10 mg samples. The second heating is reported.

Transmission Electron Microscopy (TEM). TEM was carried out with a Philips 300 electron microscope using an operating voltage of 80 kV. Thin sections (~70 nm) were microtomed from compression-molded TPVA and PVA specimens at room temperature with a diamond knife using an LKB ultra microtome. PVA specimens were cut dry. TPVA specimens were cut, and the sections were floated on water before being collected on Cu grids. No external staining was used, since staining in RuO_4 vapor did not improve contrast.

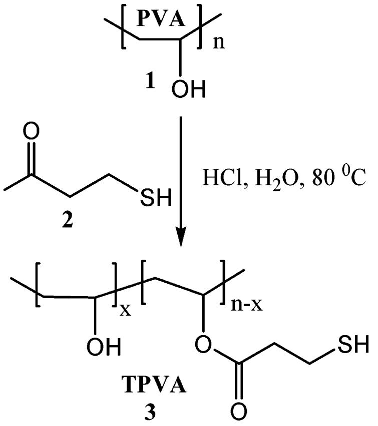
Synthesis of TPVA. TPVA was synthesized by the esterification of PVA with 3-mercaptopropionic acid in the presence of hydrochloric acid. PVA (5 g, 113.6 mmol repeat units) was dissolved in 20 mL of water at 80 °C, and a mixture of 3-mercaptopropionic acid (6 g, 56.82 mmol) and HCl (1 mL 7N, 7 mmol) was added dropwise over 20 min. After 8 h at 80 °C, the mixture was slowly poured into methanol (for PVA, M_w = 9500, 50 000, and 205 000) or acetone (for PVA, M_w = 2000), and the white precipitate was collected, washed repeatedly by methanol or acetone, and dried under vacuum at room temperature.

Elemental Analysis of TPVA. A 10−20 mg (±1 μg) portion of sample was weighed and combusted in a Schoniger Oxygen flask. The gaseous products were captured in a peroxide-absorbing solution, which was made 80% ethanolic before titration with a Ba^{2+} solution to a Thorin indicator endpoint.

Modification of Wheat Protein. Varying amounts of TPVA and PVA (5, 10, 20, 30, or 40% (w/w) with respect to gluten) were dissolved in 0.05 M acetic acid at room temperature; WG was dispersed at a 1:10 (w/v) ratio, and the mixture was stirred overnight. The mixture was subsequently freeze-dried and ground into powder with a mortar and pestle. As a control, WG was mixed with acetic acid in a 1:10 (w/v) ratio, and processed without any additive.

Preparation of Molded Specimens. Compression molding was performed at 150 °C for 10 min at 88 964 N (20 000 pounds) of force in a multicavity mold. The WG/polymer powder was molded into brown homogeneous bars with dimensions of 4 × 0.5 × 0.2 cm. The mold was coated by PMR mold release agent before molding. Although the molding temperature of 150 °C is quite high relative to previous reports,[36] no apparent degradation occurred within the short molding period. Comparison to properties obtained at 110−160 °C indicated that 150 °C was the best molding temperature.[37]

Scheme 1. TPVA Synthesis by the Esterification of PVA with 3-Mercaptopropionic Acid.



Mechanical Properties Experiments. After molding, the specimens were kept in a desiccator for 3 days, after which time a three-point bending test was performed according to the standard test methods for flexural properties, ASTM D790-02. The tests were conducted on a computer-interfaced Instron 1011 with a 500 or 50 N load cell, according to the actual load needed. The rate of crosshead motion was 1 mm/min, the data acquisition rate was 10 points per second, and three to five replicates were made of each test. Stress−strain curves were constructed from the data, and the modulus was determined by fitting a straight line to the initial part of the curves. The water content of the specimens, measured after the mechanical tests, was kept to 2.5−3 wt %.

Water Absorption Experiment. The original weight of molded specimens of WG, TPVA, PVA/WG (5, 20, and 40%), and TPVA/WG (5, 20, and 40%) was recorded. Specimens were then immersed in deionized water. At certain times, the specimens were taken out, surface dried with a tissue, and weighed. The weight was recorded as a function of time. Three replicates were measured for each material at each time point.

Rheology Study of WG in Acetic Acid. The viscosity of PVA, TPVA, and their blends with WG in acetic acid was determined using a Physica UDS 200 (Anton Paar GmbH) at room temperature using a bob and cup fixture. A steady shear rate of 500 s^{-1} was used to monitor the viscosity changes during mixing.

SE-HPLC Study. Samples (1.0 mg/mL) were extracted (1 h at room temperature) with 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) sodium dodecyl sulfate (SDS) and centrifuged (10 min, 10,000*g*). For molded specimens, a file was used to create a powder from the bar, and this powder was extracted in the buffer. Supernatants were filtered (0.45 μm) and loaded (50 μL) on a Phenomenex BioSep-SEC-S4000 (300 × 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 Kontron HPLC 332 detector (Kontron Instruments, Ltd., Buckinghamshire, U.K.) at 210 nm. Proteins were classified into four groups: (1) unextractable polymeric proteins, (2) extractable polymeric proteins, (3) oligomeric proteins and high molecular weight glutenin subunits (HMW-GS), and (4) low molecular weight glutenin subunits (LMW-GS) and gliadins.[1]

Results and Discussion

Synthesis and Characterization of TPVA. The thiolated TPVA derivative was synthesized by the esterification of PVA (**1**) with 3-mercaptopropionic acid (**2**) in the presence of hydrochloric acid[38] (Scheme 1). The reaction was carried out

Table 1. Mechanical Properties of Compression-Molded WG Blends

#	additive	additive M_w	additive thiol content[a]	additive amount[b]	strength (MPa)	modulus (GPa)	elongation (%)
1	none − 1			0	51 ± 2.4	3.5 ± 0.08	1.5 ± 0.08
2	none − 2			0	34.5 ± 5.6	2.33± 0.14	1.51 ± 0.27
3	PVA	2000	0	20	43.8 ± 4.86	2.98± 0.51	1.53 ± 0.29
4	TPVA	2000	5.5	20	46.3 ± 9.6	2.92± 0.21	1.70 ± 0.30
5	PVA	9500	0	5	48 ± 5	3.4 ± 0.2	1.4 ± 0.1
6	PVA	9500	0	20	45 ± 5	3.8 ± 0.1	1.4 ± 0.1
7	PVA	9500	0	40	39 ± 5.9	3.93± 0.16	1.47 ± 0.16
8	TPVA	9500	6.96	5	62 ± 4	3.4 ± 0.3	2.1 ± 0.1
9	TPVA	9500	6.96	20	76 ± 4	3.1 ± 0.2	2.25 ± 0.1
10	TPVA	9500	6.96	40	68.2 ± 4.3	2.9 ± 0.2	1.95 ± 0.07
11	PVA	50 000	0	5	45 ± 3.5	3.8 ± 0.2	1.2 ± 0.1
12	PVA	50 000	0	10	44 ± 3	3.5 ± 0.2	1.2 ± 0.1
13	PVA	50 000	0	20	43 ± 2	3.8 ± 0.1	1.1 ± 0.1
14	PVA	50 000	0	30	47 ± 3.2	4 ± 0.2	1.2 ± 0.1
15	PVA	50 000	0	40	45 ± 3.1	3.6 ± 0.2	1.4 ± 0.13
16	TPVA	50 000	4.94	5	74 ± 3.3	4 ± 0.1	2.1 ± 0.1
17	TPVA	50 000	4.94	10	79 ± 3.2	4 ± 0.1	2.3 ± 0.1
18	TPVA	50 000	4.94	20	89 ± 3	4 ± 0.2	2.7 ± 0.1
19	TPVA	50 000	4.94	30	75 ± 4	4.3 ± 0.1	2 ± 0.1
20	TPVA	50 000	4.94	40	78 ± 2	4.2 ± 0.1	2.1 ± 0.1
21	PVA	205 000	0	5	61.5 ± 3	4 ± 0.2	1.68 ± 0.5
22	PVA	205 000	0	20	52 ± 3	3.9 ± 0.2	1.4 ± 0.1
23	PVA	205 000	0	40	55 ± 5	3.4 ± 0.3	1.69 ± 0.17
24	TPVA	205 000	3.73	5	70.7 ± 3	4 ± 0.1	1.95 ± 0.09
25	TPVA	205 000	3.73	20	73 ± 4	3.8 ± 0.1	2.1 ± 0.1
26	TPVA	205 000	3.73	40	64.5 ± 4.8	3.6 ± 0.2	2.19 ± 0.23

[a] From elemental analysis of the additive. [b] g/g WG.

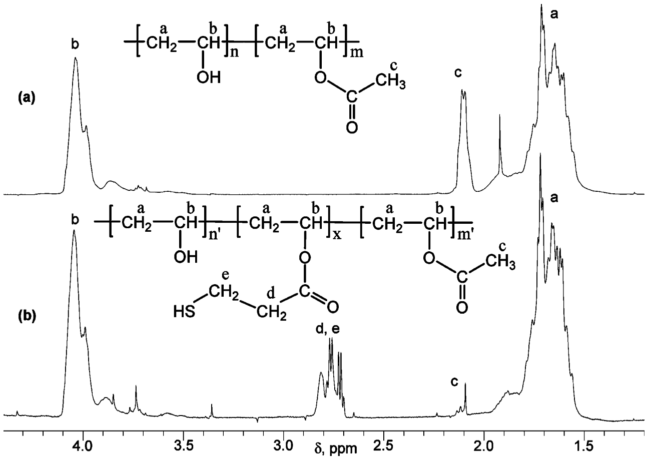


Figure 1. 500 MHz H^1 NMR spectra of (a) PVA and (b) TPVA in D_2O.

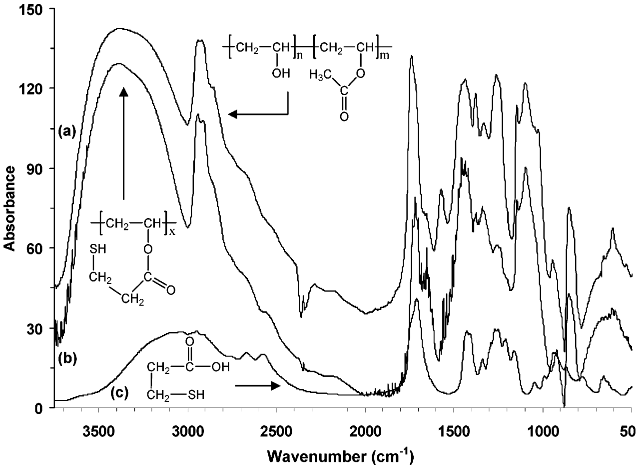
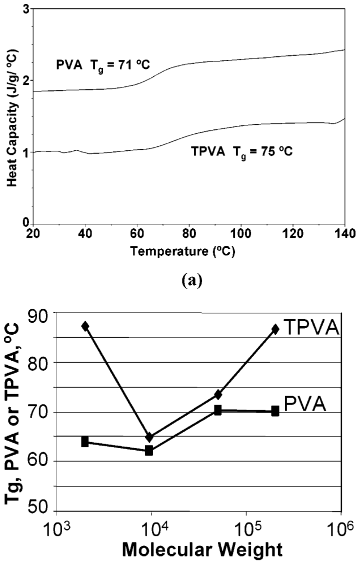


Figure 2. FT-IR spectra of (a) PVA, (b) TPVA, and (c) 3-mercaptopropionic acid.

with 1 mol of 3-mercaptopropionic acid for every two moles of hydroxyl group in PVA. Four different molecular weights of PVA were used to prepare the TPVA (**3**). Since the molecular weight has an influence on the solubility and reactivity of PVA, slightly different sulfur contents were produced for each molecular weight. The products were soluble in water at room temperature. However, in contrast to PVA specimens, compression-molded TPVA samples were water insoluble.

Figure 1 presents a comparison of the ^{1}H NMR spectra of TPVA and the precursor PVA (M_w = 50 000). The broad multiplet at $\delta \sim 1.4-1.9$ ppm corresponds to the main chain $-CH_2-$ resonance in both polymers. The resonance at $\delta \sim 2.1$ ppm corresponds to the methyl of an acetate group, $-O-CO-CH_3$. Commercial PVA is obtained by the hydrolysis of poly(vinyl acetate) and, consequently, is in fact poly(vinyl alcohol-*co*-vinyl acetate) containing about 10% acetate groups. The main chain $-CH-$ is observed in both polymers at $\delta \sim 3.8-4.1$ ppm. The decreased intensity of the acetate groups in TPVA to below 1% indicates their hydrolysis during the acid-catalyzed thiolation. In addition, the distinctive feature of TPVA is the presence of the multiplet at $\delta \sim 2.7-2.8$ ppm, which corresponds to the 3-mercaptopropionic ester fragment, $-O-CO-CH_2-CH_2-SH$, and allows the estimation of a percent of thiolation at about 5.2% for the sample in Figure 1. The percent of thiolation was also determined via elemental analysis (Table 1) and found to agree satisfactorily with the NMR values.

Figure 2 presents the FT-IR spectrum of PVA and of the corresponding TPVA (M_w = 50 000). The spectrum of the 3-mercaptopropionic acid, also included for comparison, con-



(a)

(b)

Figure 3. MDSC of PVA and TPVA: (a) MDSC data for MW 50 000 PVA and TPVA, and (b) T_g values plotted against molecular weight for PVA and TPVA.

tains characteristic absorption associated with the stretching mode of the $-$SH group at 2500$-$2700 cm^{-1}.[39] Although the degree of thiolation is low, the same peaks are also observed in the TPVA sample but are absent in PVA. For both polymers, the broad absorption at 3000$-$3800 cm^{-1} corresponds to the OH stretching vibrations, while the sharper absorbance at 2900$-$3000 cm^{-1} is associated with the C$-$H stretching mode. In addition, the C=O stretching absorption of an ester group at 1700 cm^{-1} is observed in both polymers as a result of the acetate functionality in PVA and the mercaptopropionic ester group in TPVA, respectively.

MDSC (Figure 3a) of PVA and TPVA (both M_w = 50 000), indicates that the glass transition temperature (T_g) increased slightly from 71 to 75 °C upon thiolation. Figure 3b illustrates the T_g of TPVA at several molecular weights, showing large effects due to thiolation at both low (MW 2000) and high (MW 205,000) molecular weight. Transmission electron microscopy (TEM, Figure 4), illustrates the differences in the morphologies of compression-molded samples of these two polymers. PVA was microtomed dry because of its high solubility in water and has a smooth appearance with some banding, which is probably due to compressive folding of PVA during microtoming. The compression-molded TPVA is not water soluble and, because it was floated off the sample onto water, has a highly textured appearance. Although some of the differences in appearance may be due to the differences in microtoming procedures, it is possible that TPVA may be partially microphase separated into sulfur-rich and sulfur-poor regions. The sulfur-rich regions may likely form cross-links, raising the T_g of TPVA relative to that of PVA and rendering the TPVA insoluble in water.

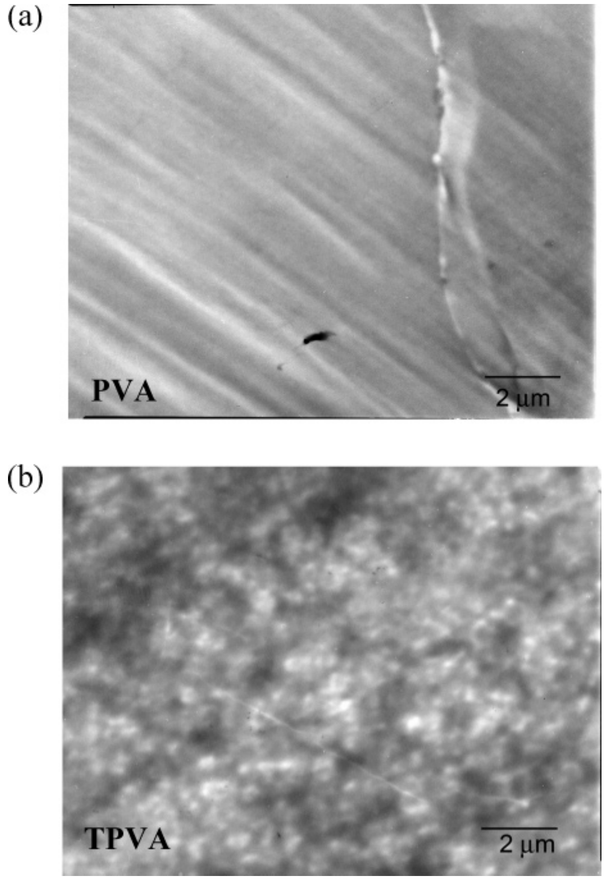
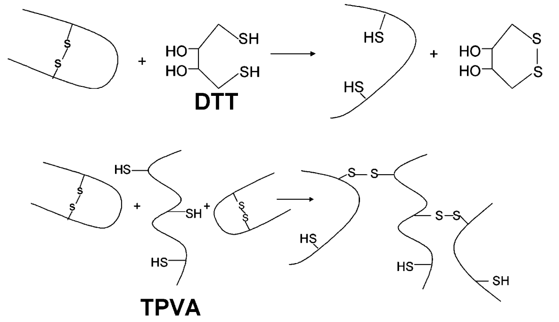


Figure 4. TEM images (×6600) of compression-molded samples of (a) dry cut PVA and (b) wet cut TPVA.

Scheme 2. Reversible Sulfhydryl/Disulfide Interchange Reaction

protein-S-S-protein + R-SH $\rightleftharpoons$ protein-S-S-R + protein-SH



Rheology of WG Blends. Previous studies show that (*2R,3S*)-3,4-dimercaptobutane-1,2-diol (dithiothreitol or DTT)[1] and similar reducing agents decrease the dough mixing time of wheat flour and its resistance to breakdown.[40,41] The effects of DTT on wheat flour rheology and other properties is widely thought to be due to the sulfhydryl/disulfide interchange reaction, illustrated in Scheme 2.[42,43] DTT is a very effective protein-reducing agent because this reaction is nearly irreversible as a result of the very stable intramolecular disulfide formed in the DTT reaction product. We hypothesize that TPVA also reduces proteins by this reaction. The three-arm thiol-terminated poly(ethylene oxide) previously blended with WG also reduced disulfide linkages in the protein, but did not appear to form the internal disulfide bond typical of DTT.[1]

The disulfide reduction reactions that reduce the dough mixing time are also expected to reduce WG suspension viscosity. Quantitative viscosity measurements may therefore provide insight into the kinetics of the interactions between thiolated additives such as TPVA and WG. TPVA (M_w = 50 000) was mixed with a suspension of 10% (w/v) WG in 0.05 M acetic acid at a level of 2% (w/v). This corresponds to an amount of thiol equivalent to that of the SH groups in WG. The corresponding control experiments were performed mixing PVA/WG as well as TPVA, PVA, and WG alone. These data were compared with WG mixed with DTT. Mixing was performed at a steady shear rate of 500 s^{-1}, and the viscosity was monitored for periods of time up to 10 h. (Figure 5).

Both PVA and TPVA display very similar, time-independent viscosity profiles. A slightly higher viscosity, which remains

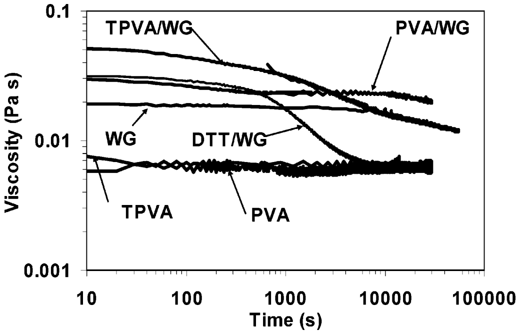


Figure 5. The time dependence of viscosity for WG and additive solutions in 0.05 M acetic acid at a 500 s^{-1} shear rate.

constant up to about 10 000 s and which is followed by a slow decrease, is observed for WG. Both PVA/WG and DTT/WG start out with comparable viscosities. However, while the PVA/WG blend behaves similarly to both PVA and WG alone and maintains a constant viscosity, a dramatic decrease in viscosity with time occurs for the DTT/WG blend after about 800 s. The TPVA/WG blend starts at higher initial viscosities, indicative of a more complex interaction of TPVA with WG and follows a viscosity decrease similar to, albeit less intense than that of the DTT/WG blend, finally converging with that of WG alone after ~10 000 s. The sharp contrast in viscosity decrease between the thiol blends and all other materials can be explained by the thiol reduction of disulfide bonds in the WG blends, leading to the formation of smaller aggregates and possible partial solubilization.

The rheological behavior illustrated in Figure 5 is due to several interactions, including particle size changes and aggregation[44] and sulfhydryl/disulfide interchange reactions. Although a full understanding of these data requires additional investigation, they are presented here without further interpretation only to demonstrate the importance of thiol-mediated interactions with wheat proteins.

The viscosity of plain WG and PVA/WG suspensions remains constant for a relatively long time, as no reduction of the WG occurs in these systems. Figure 5 also sheds some light on the kinetics of the reducing reaction. It appears that DTT reacts with WG protein much faster than TPVA, as seen from the decrease in the DTT/WG blend viscosity relative to the levels of TPVA. This may be due to the much higher molecular weight of the TPVA ($M_w = 50\,000$) compared to that of the DTT reducing agent ($M_w = 154$).

Mechanical Properties. TPVA and PVA of various molecular weights were used to modify WG and form compression-molded bars. Specimens from both WG and TPVA/WG blends have a homogeneous look and brown color. The bars molded out of PVA/WG blends are slightly darker and have obvious heterogeneities in the form of light-colored spots.

The mechanical properties of protein/polymer blends of WG and varying amounts of TPVA or PVA polymers ($M_w = 50\,000$) were determined by three-point bending tests, and are summarized in Table 1 and Figure 6. The PVA/WG blends have similar or lower stress and strain to failure versus WG. By contrast, the TPVA/WG blends provide larger strain at break and flexure strength and a slightly higher modulus than pure WG and PVA/WG. For example, the 20% TPVA/WG blend has a breaking strain of 2.7% compared to 1.1% in the corresponding PVA/WG blend, and a flexure strength of 89 MPa compared to 43 MPa in the corresponding PVA/WG blend. The mechanical properties first increase with increasing TPVA content, and then begin to decrease. Optimum mechanical properties are observed in the range of 20−30% (w/w) TPVA. This additive concentration roughly corresponds to an equivalent amount of thiol in the additive and in the WG. This stoichiometry was determined by amino acid analysis of the WG, which yielded 0.301 mmol cysteine residues/g of WG. Cysteine is the only thiol-containing amino acid of naturally occurring proteins.

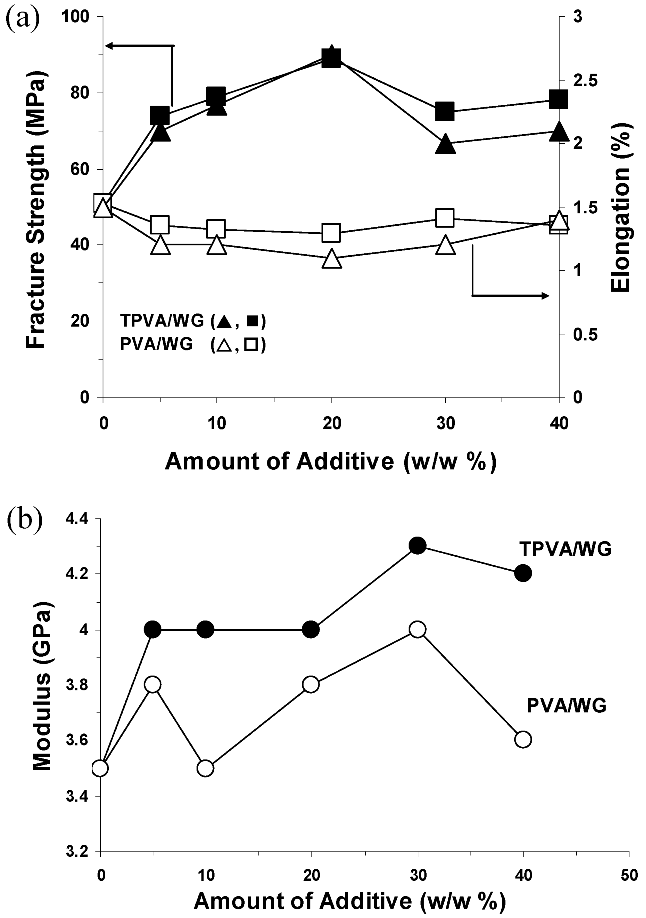


Figure 6. The dependence of (a) fracture strength (■, □), elongation (▲, △), and (b) modulus (●,○) of TPVA/WG and PVA/WG blends on additive ($M_w = 50\,000$) content.

Figure 6 also indicates that TPVA does not plasticize WG, as the elongation to break increases without the penalty of decreased modulus. This superior performance is in line with our previous results,[1] and it contrasts strongly with the behavior of WG plasticizers, which increase ductility at the expense of modulus,[14−16,45−48] or WG cross-linkers, which increase modulus at the expense of ductility.[49−52]

It is also interesting to note that, for this thiolation level (5.2 mol %), an additive content of 27% w/w would contain an amount of thiol equivalent to that of the WG. However, the 20% w/w blend generally had better properties than the 30% w/w blend, illustrating the complex interactions in this system. It is not surprising that the properties should be quite different at additive levels higher than the thiol-equivalent amount, as the multiple −SH functionalities along the TPVA chain allow for multiple disulfide−sulfhydryl exchange reactions, which alter the distribution of inter- and intramolecular disulfide linkages in WG as well as within TPVA. This is also apparent in the water absorption experiments (*vide infra*) in that the molded TPVA is not water soluble as a result of disulfide cross-links.

Effect of TPVA Molecular Weight. PVA samples with $M_w = 9500$, 50 000 and 205 000 were used for PVA/WG and

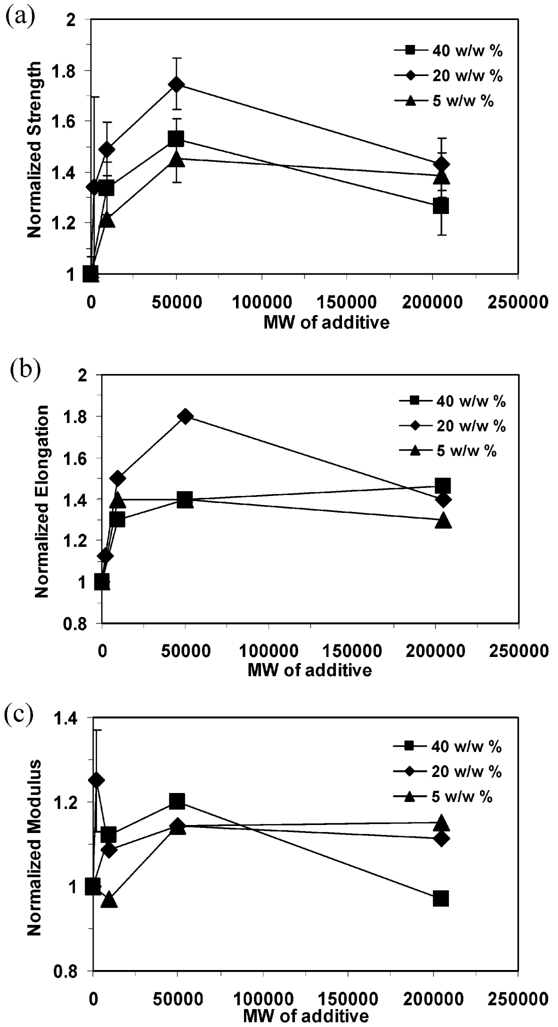


Figure 7. The dependence of normalized (a) strength, (b) elongation, and (c) modulus on the additive molecular weight and TPVA/WG blend composition: 5% w/w (▲), 20% w/w (◆) and 40% w/w (■). Values were normalized by dividing by the value for the control unmodified WG sample.

TPVA/WG blends containing 5, 20, and 40% (w/w) additive. TPVA and PVA with $M_w = 2{,}000$ were blended with WG at 20% (w/w). The mechanical properties of PVA/WG and TPVA/WG blends are summarized in Table 1. The properties of the TPVA/WG blends are also shown in Figure 7.

Statistical analysis (t-test) of the mechanical property data indicates that the differences were significant (95% confidence level) between the measured strength, elongation, and modulus of the TPVA/WG blends and the unblended WG, except in the cases of $M_w = 2000$ TPVA blended at 20% (wt/wt) and $M_w = 205\,000$ TPVA blended at 40% (w/w). The mechanical properties of TPVA/WG blends were also significantly different from those of PVA/WG blends with 95% confidence, except for the same two exceptions noted above. Overall, the best results were achieved with intermediate molecular weight (50 000) TPVA at a 20% (wt/wt) composition (Figure 7).

There exist many possible reasons why the MW 50 000 TPVA gives the highest strength and strain to failure. One possible explanation stems from the MDSC results summarized in Figure 3b. The elevated T_g of both MW 2000 TPVA and MW 205 000 TPVA, relative to PVA, indicates that the interactions of TPVA with itself are strongest at the lowest and highest molecular weights used in this study. Perhaps the intermediate sized TPVA of MW 50 000 interacted with WG more strongly than the lower and higher molecular weight TPVA because it interacted less strongly with itself.

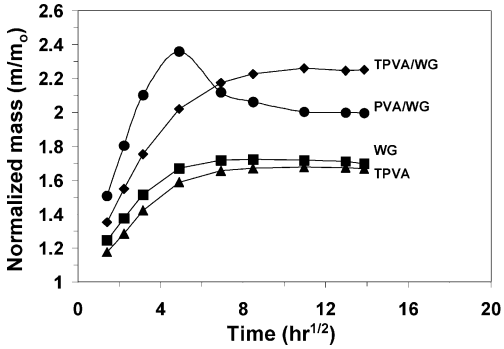


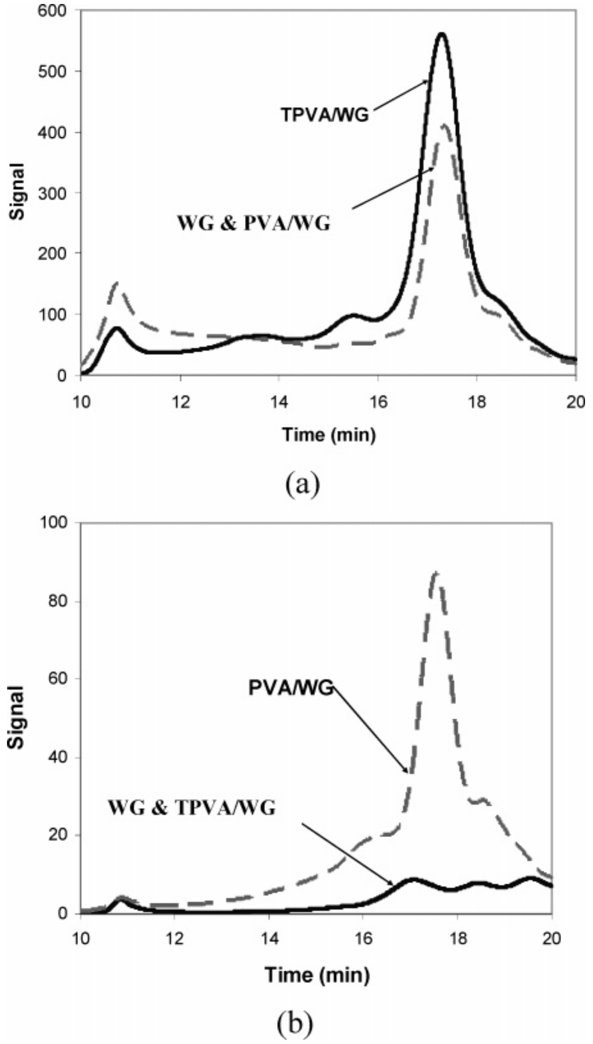
Figure 8. Water absorption properties of PVA/WG 20% w/w (●), TPVA/WG 20% w/w (◆), WG (■), and TPVA (▲). The normalized mass of the sample plotted on the ordinate is the sample mass, *m*, after absorbing water for a period of time divided by the initial sample mass, m_0, before immersion.

Water Absorption Experiments. The water absorption data of the compression-molded, blended material is presented in Figure 8. The experiments were stopped after 8 days because of mold growth. Since molded PVA is water soluble, PVA water absorption was not measured. During the first 2 days of the experiment, WG absorbed 70% of its original weight in water and reached a plateau of normalized mass of 1.7. While TPVA absorbed roughly the same amount of water as WG and at a similar rate, in contrast to PVA, it did not dissolve, most likely because of the formation of intra/intermolecular disulfide cross-links.

The PVA/WG blend absorbed much more water than did WG. Thus, after 1 day, the weight at least doubled. Then, it began to decrease with time because of the dissolution of PVA and its slow diffusion out of the specimen. In contrast, the water absorption of the TPVA/WG blend increased continuously with time and leveled off after 100 h. Interestingly, the TPVA/WG blend absorbed more water than either component. This behavior is significantly different than our previously reported blends with lower molecular weight thiols.[1] Although the water uptake mechanism may be quite complex in these materials, the initial slopes of the normalized mass in Figure 8 illustrate some interesting comparisons between the water transport kinetics. For example, the rate of water uptake in the TPVA/WG blend is much larger than in either individual component. This nonideal behavior may be indicative of complex blend morphology, with interfacial zones that are more hydrophilic than either component.

SE-HPLC Analysis of Extractable Gluten Proteins. SE-HPLC (Figure 9) was used to investigate the effect of the molding process on the molecular weight distribution of the extractable protein fractions of WG and PVA/WG and TPVA/WG blends. WG alone had almost identical profiles with PVA/WG before molding and with TPVA/WG after molding.

Figure 9a,b presents the molecular weight profiles before and after molding from WG blends using PVA and TPVA with $M_w = 50\,000$, which were extracted as previously described[1] using SDS buffer. Longer times on the *x*-axis in Figure 9 correspond to lower molecular weight species. As shown before, gliadins and LMW-GS (M_w up to ~70 000) emerge from the column at 16−19 min, oligomeric proteins and medium molecular weight glutenin subunits (MMW-GS, $M_w \sim 65\,000{-}90\,000$) emerge at 12−16 min, while extractable polymeric proteins (the highest molecular weight species, HMW-GS, $M_w > 90\,000$) elute at about 10 min.[33]



(a)

(b)

Figure 9. SE-HPLC chromatographs of WG, PVA/WG, and TPVA/WG (20% w/w) blends (a) before and (b) after molding.

For the blends, a possible difficulty in interpreting the SE-HPLC data could arise if the signal due to wheat protein and dissolved polymer cannot be differentiated. However, control UV absorption experiments reveal that, at 210 nm (the SE-HPLC detector wavelength), the UV absorption of WG is about 140 times stronger than that of PVA per unit mass. While the UV absorption of TPVA was not determined, the low degree of thiolation (~5%) will not strongly affect the absorption results since the ester and the thiol groups are not very strong UV chromophores. Thus, the data discussed below refers almost exclusively to wheat protein.

Before molding, both blends contain a large fraction of extractable low molecular weight protein subunits, especially for TPVA/WG. The reduced extraction of high molecular weight protein and increased extraction of low molecular weight protein from the TPVA blend indicates some reduction in the protein disulfide bonds and thus conversion of some of the polymeric gluten to lower molecular weight subunits. This change in molecular weight distribution and increase in extracted material versus WG or PVA/WG is consistent with rheology data, indicating disulfide bond reduction. The total protein extracted from the TPVA/WG sample increased by 39% over that extracted from the WG sample, as measured by the area under the chromatogram from 10 to 20 min.

After molding, as expected because of the temperature-induced cross-linking reactions,[53] the fraction of extractable proteins decreases significantly for both blends, as evidenced by the decrease in the signal from samples made from the same weight of material. This is due to the formation of strong molecular associations and the incorporation of LMW-GS into larger aggregates. However, the peak of the low molecular weight proteins from TPVA/WG or WG alone is drastically reduced. This indicates a larger post-cure cross-linking density in WG and TPVA/WG blends versus PVA/WG blends.

The data in Figure 9b does not indicate any detectable difference in extractable protein or molecular weight distribution between WG and TPVA/WG after molding. Therefore, the improvement in mechanical properties of the TPVA/WG blend over WG is probably not due to increased cross-linking due to TPVA. While a strong interaction of TPVA with WG is apparent from Figure 9a and from the rheology (Figure 5), it does not appear to increase the cross-linking of WG above the intrinsic cross-linking caused by the compression molding process.

Conclusions

Esterification of commercially available PVA with 3-mercaptopropionic acid provides easy access to multifunctional macromolecular thiols, as confirmed by a combination of NMR, FT-IR, TEM, and DSC investigations. The resulting TPVA was used to reactively modify WG blends. SE-HPLC and viscosity measurements of WG as well as WG/PVA and WG/TPVA suspensions illustrate the ability of TPVA to reduce WG disulfide bonds via the disulfide/sulfhydryl interchange reaction.

The effect of additive molecular weight and blend composition on the mechanical properties of compression-molded bars was studied with three-point bending tests for both TPVA/WG and PVA/WG blends and compared with the WG baseline. In contrast to conventional WG additives, which typically improve flexibility but decrease modulus, the TPVA/WG blends greatly increase both flexure strength and strain to failure while also slightly increasing modulus over pure WG and PVA/WG blends. An optimum was observed for $M_w = 50\,000$ in a 20% (w/w) TPVA/WG blend, which improved the fracture strength by 76% and elongation by 80% while increasing modulus by roughly 25% above that of WG. Similar improvements in mechanical properties were reported previously using a three-arm, thiol-terminated poly(ethylene oxide) ($M_w = 1247$) to modify WG.[33]

In contrast with the previous results using the trithiol additive, the water absorption of TPVA/WG blends increased well above the water uptake by either WG or TPVA alone. This nonideal behavior is not well understood, but probably indicates that the TPVA/WG blend is cross-linked at a level less than or equal to that of WG and may contain complex morphology, including interactions of TPVA with the polysaccharide domains of WG. Accordingly, SE-HPLC chromatograms of SDS-extractable proteins from compression-molded bars of WG and TPVA/WG blends are virtually identical, supporting this assertion. Previously, the trithiol/WG blend absorbed only half the water of pure WG, and SE-HPLC indicated that the trithiol promoted cross-linking in the WG blend upon compression molding. However, previous experiments were carried out with Amylum WG (Aalst, Belgium), while current data refer to Arrowhead Mills WG (Hereford, TX).

Improvements in WG blend properties have been achieved with two thiolated additives, but apparently by different mechanisms. It is possible that TPVA forms a microphase-separated blend, with the thiol groups compatibilizing the polymer with the protein. A detailed characterization of the morphology of PVA/WG and TPVA/WG blends is currently in progress.

Acknowledgment. This project is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, Grant No. 2005-35504-16058.

References and Notes

(1) Woerdeman, D. L.; Veraverbeke, W. S.; Parnas, R. S.; Johnson, D.; Delcour, J. A.; Verpoest, I.; Plummer, C. J. G. *Biomacromolecules* **2004**, *5*, 1262–1269.

(2) The smaller values were obtained from tensile data collected at a strain rate of 0.1 min^{-1} (erroneously reported as 10 s^{-1} in ref 1), while the larger values were obtained using a three-point bend test (ASTM standard D790-02). We believe that neither set of values is more correct than the other. Rather, the discrepancy in the mechanical values is a reflection of polymeric time-dependent behavior.

(3) Mohanty, A. K.; Liu, W.; Tummala, P.; Drzal, L. T.; Misra, M.; Narayan, R. Soy protein based plastics, blends, and composites. In *Natural Fibers, Biopolymers and their Biocomposites*; Mohanty, A. K., Misra, M., Drzal, L. T., Eds.; CRC Press Books: Boca Raton, FL, 2005.

(4) De Graaf, L. A.; Kolster, P.; Vereijken, J. M. In *Plant Proteins from European Crops*; Gueguen, J., Popineau, Y., Eds.; Springer-Verlag: Berlin, 1998; pp 335–339.

(5) Kalichevsky, M. T.; Jaroszkiewicz, E. M.; Blanshard, J. M. V. *Int. J. Biol. Macromol.* **1992**, *14*, 257–266.

(6) Gontard, N.; Guilbert, S.; Cuq, J. L. *J. Food Sci.* **1993**, *58*, 206–211.

(7) Gennadios, A.; Weller, C. L. *Trans. ASAE* **1994**, *37*, 535–539.

(8) Gennadios, A.; Brandenburg, A. H.; Park, J. W.; Weller, C. L.; Testin, R. F. *Ind. Crops Prod.* **1994**, *2*, 189–195.

(9) Noel, T. R.; Parker, R.; Ring, S. G.; Tatham, A. S. *Int. J. Biol. Macromol.* **1995**, *17*, 81–85.

(10) Gontard, N.; Ring, S. *J. Agric. Food Chem.* **1996**, *44*, 3474–3478.

(11) Gontard, N.; Thibault, R.; Cuq, B.; Guilbert, S. *J. Agric. Food Chem.* **1996**, *44*, 1064–1069.

(12) Pouplin, M.; Redl, A.; Gontard, N. *J. Agric. Food Chem.* **1999**, *47*, 538–543.

(13) Roy, S.; Weller, C. L.; Gennadios, A.; Zeece, M. G.; Testin, R. F. *J. Food Sci.* **1999**, *64*, 57–60.

(14) Castelli, F.; Gilbert, S. M.; Caruso, S.; Maccarrone, D. E.; Fisichella, S. *Thermochim. Acta* **2000**, *346*, 153–160.

(15) Kayserilioglu, B. S.; Bakir, U.; Yilmaz, L.; Akkas, N. *J. Agric. Food Chem.* **2003**, *51*, 964–968.

(16) Zhang, X.; Burgar, I.; Do, M. D.; Lourbakos, E. *Biomacromolecules* **2005**, *6*, 1661–1671.

(17) Pommet, M.; Redl, A.; Guilbert, S.; Morel, M.-H. *J. Cereal Sci.* **2005**, *42*, 81–91.

(18) Redl, A.; Morel, M. H.; Bonicel, J.; Vergnes, B.; Guilbert, S. *Cereal Chem.* **1999**, *76*, 361–370.

(19) Cherian, G.; Gennadios, A.; Weller, C.; Chinachoti, P. *Cereal Chem.* **1995**, *72*, 1–6.

(20) Irissin-Mangata, J.; Bauduin, G.; Boutevin, B. *Polym. Bull.* **2000**, *44*, 409–416.

(21) Pommet, M.; Redl, A.; Morel, M. H.; Guilbert, S. *Spec. Publ.–R. Soc. Chem.* **2004**, *295*, 439–442.

(22) Pommet, M.; Redl, A.; Morel, M.-H.; Guilbert, S. *Polymer* **2002**, *44*, 115–122.

(23) Brault, D.; D'Aprano, G.; Lacroix, M. *J. Agric. Food Chem.* **1997**, *45*, 2964–2969.

(24) Gennadios, A.; Ghorpade, V. M.; Weller, C. L.; Hanna, M. A. *Trans. ASAE* **1996**, *39*, 575–579.

(25) Gennadios, A.; Rhim, J. W.; Handa, A.; Weller, C. L.; Hanna, M. A. *J. Food Sci.* **1998**, *63*, 225–228.

(26) Gueguen, J.; Viroben, G.; Noireaux, P.; Subirade, M. *Ind. Crops Prod.* **1998**, *7*, 149–157.

(27) Marquie, C.; Aymard, C.; Cuq, J. L.; Guilbert, S. *J. Agric. Food Chem.* **1995**, *43*, 2762–2767.

(28) Ali, Y.; Ghorpade, V. M.; Hanna, M. A. *Ind. Crops Prod.* **1997**, *6*, 177–184.

(29) Parris, N.; Coffin, D. R. *J. Agric. Food Chem.* **1997**, *45*, 1596–1599.

(30) Micard, V.; Morel, M. H.; Guilbert, S. *Spec. Publ.–R. Soc. Chem.* **2000**, *261*, 356–359.

(31) Lim, S. K.; Jung, I. K.; Lee, K. H.; Byung S. J. *Eur. Polym. J.* **1999**, *35*, 1875–1881.

(32) Wang, C.; Carriere, C. J.; Willett, J. L. *J. Polym. Sci., Part B: Polym. Phys.* **2002**, *40*, 2324–2332.

(33) John, J.; Tang, J.; Bhattacharya, M. *Polymer* **1998**, *39*, 2883–2895.

(34) Marais, S.; Nguyen, Q. T.; Langevin, D.; Metayer, M. *Macromol. Symp.* **2001**, *175*, 329–347.

(35) Hashimoto, J. M.; Nabeshima, E. H.; Cintra, H. S.; Dias, A. R. G.; Bustos, F. M.; Chang, Y. K. *J. Sci. Food Agric.* **2002**, *82*, 924–930.

(36) Gällstedt, M.; Mattozzi, A.; Johansson, E.; Hedenqvist M. S. *Biomacromolecules*, **2004**, *5*, 2020.

(37) Ye, P.; Reitz, L.; Horan, C.; Parnas, R. S. *J. Polym. Environ.* **2006**, *14*, 1–7.

(38) Kuninori, T.; Matsumoto, H. *Agric. Biol. Chem.* **1970**, *34*, 1089–1094.

(39) Silverstein, R. M.; Webster, F. X.; Kiemle, D. In *Spectrometric Identification of Organic Compounds*, 7th ed.; Wiley: New York, 2006.

(40) Bekes, F.; Gras, P. W.; Gupta, R. B. *Cereal Chem.* **1994**, *71*, 44–50.

(41) Veraverbeke, W. S.; Verbruggen, I. M.; Delcour, J. A. *J. Agric. Food Chem.* **1998**, *46*, 4830–4835.

(42) Owusu-Apenten, R. K.; Chee, C.; Hwee, O. P. *Food Chem.* **2003**, *83*, 541–545.

(43) (a) Mauritzen, C. A.; Stewart, P. *Nature* **1963**, *197*, 48–49. (b) McDermott E. E.; Pace, J. *Nature*, **1962**, *192*, 657. (c) Smithies, O. *Science* **1965**, *150*, 1595.

(44) Nielsen, L. E.; Landel, R. F. In *Mechanical Properties of Polymers and Composites*, 2nd ed.; Marcell Dekker: New York, 1994; p 378.

(45) Mangavel, C.; Barbot, J.; Bervas, E.; Linossier, L.; Feys, M.; Gueguen, J.; Popineau, Y. *J. Cereal Sci.* **2002**, *36*, 157–166.

(46) Mangavel, C.; Barbot, J.; Gueguen, J.; Popineau, Y. *J. Agric. Food Chem.* **2003**, *51*, 1447–1452.

(47) Barbot, J.; Popineau, Y.; Gueguen, J. *Biomacromolecules* **2004**, *5*, 2020–2028.

(48) Pommet, M.; Redl, A.; Guilbert, S.; Morel, M.-H. *J. Cereal Sci.* **2005**, *42*, 81–91.

(49) Hernandez-Munoz, P.; Villalobos, R.; Chiralt, A. *Food Hydrocolloids* **2004**, *18*, 403–411.

(50) Hernandez-Munoz, P.; Lopez-Rubio, A.; Lagaron, J. M.; Gavara, R. *Biomacromolecules* **2004**, *5*, 415–421.

(51) Micard, V.; Belamri, R.; Morel, M.-H.; Guilbert, S. *J. Agric. Food Chem.* **2000**, *48*, 2948–2953.

(52) Tropini, V.; Lens, J.-P.; Mulder, W. J.; Silvestre, F. *Ind. Crops Prod.* **2004**, *20*, 281–289.

(53) (a) Pommet, M.; Redl, A.; Guilbert, S.; Morel, M. J. *J. Agric. Food Chem.* **2005**, *53*, 3943–3949. (b) Fogliano, V.; Monti, S. M.; Musella, T.; Randazzo, G.; Ritieni, A. *Food Chem.* **1999**, *66*, 293–299.

BM060432N