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Thermoformed Wheat Gluten Biopolymers

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The quantity of available wheat gluten exceeds the current food use markets. Thermoforming is an alternative technical means for transforming wheat gluten. Thermoforming was applied here to wheat gluten under chemically reductive conditions to form pliable, translucent sheets. A wide variety of conditions, i.e., temperature, reducing agents, plasticizers and additives were tested to obtain a range of elastic properties in the thermoformed sheets. These properties were compared to those of commercially available polymers, such as polypropylene. Elasticity of the gluten formulations were indexed by Young's modulus and were in the range measured for commercial products when tested in the 30−70% relative humidity range. Removal of the gliadin subfraction of gluten yielded polymers with higher Young's modulus since this component acts as a polymer-chain terminator. At relative humidity less than 30% all whole gluten-based sheets were brittle, while above 70% they were highly elastic.

KEYWORDS: Wheat gluten; wheat gliadin; wheat glutenin; protein; cross-linking; elasticity; thermoforming; bio-based products

INTRODUCTION

One-half of wheat produced in the U. S. is surplus to domestic needs. A declining export market has raised interest in the development of new markets based on new foods and biobased products from wheat components (*1*). Research to define wheat biorefining as a source of platform glucose for chemicals and ethanol has recently been reported (*2*−*5*). A number of novel nonfood, industrial uses of refined wheat starch also have been proposed. These include the use as a packaging polymer and as a component of lightweight concrete (*6*, *7*). Wheat starch is now used in cosmetics and as a binder or glue for corrugated cardboard.

Wheat starch is produced by several water-intensive, refining methods (*8*) that also produce wheat gluten in the ratio of 1 unit of gluten per 5.5 units of starch. The refining removes water-soluble albumin protein and hemicellulose. The emergence of new, potentially high volume uses for wheat starch creates opportunities for consideration of new uses for wheat gluten protein. Wheat gluten recovery is a key factor in wheat-based biorefining (*4*). Wheat gluten is now used to fortify weak bread-making flours, to formulate breakfast cereals, to serve as a meat substitute, and to create low-carbohydrate baked-goods. Nonfood uses of wheat gluten are not common but have a history that includes the molded magneto coil case of the 1916 Model T Ford shown in **Figure 1** (*9*).

Wheat gluten is an enriched protein (70−80 wt %) complex containing water-insoluble, ethanol soluble prolamines (gliadin) and water and ethanol insoluble glutelins (glutenin) in combination with small amounts of wheat oils, starch, and insoluble hemicellulose. The amount of gliadins and glutenins in vital wheat gluten are approximately equal. The gluten complex is produced in most wheat-growing regions and is sold after drying as "vital" wheat gluten with a 2004 US import value of ∼1.32USD/kg. Its value is approximately 7× the value of the wheat from which it is derived (*4*).

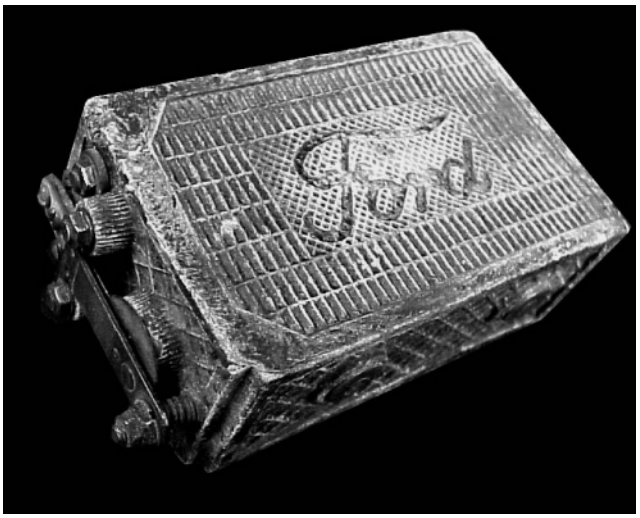

Figure 1. Magento coil from Model T Ford (∼1916). The case is reportedly formulated from wheat gluten and asbestos.

Each of the solubility classes of proteins is a complex mixture of proteins whose molecular weight distribution determines physical properties. The gliadins are mainly monomeric single chain polypeptides such as ω-gliadins (MW 70,000); whereas, the glutenins are polymeric, disulfide-linked polymeric chains or HMW-GS (MW A subunits 80,000−120,000) and LMW-GS

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(MW B subunits 40,000−55,000 and MW C subunits 30,000−40,000). The glutenin subunits have at least two terminal cysteine residues and are chain propagators, while the gliadins may have two, one, or no cysteine residues and may be propagators, terminators, or neutral (*10*). However, below 90 °C the cysteine residues of gliadins participate in only intramolecular interactions. The concentration of cysteine residues is approximately 1.8% in gliadin-1.3% in glutenin (*11*). Disulfide linkages to sulfhydryl groups in wheat protein are correlated with the strength of dough made by hydrating and developing flour (*12*).

The viscoelastic properties of hydrated dough are attributed to molecular entanglements or "transient" cross-links within the highly linear glutenin (MW of 10^5 to $\sim 10^6$) that is hypothesized to undergo reversible unfolding of the protein in the region(s) between disulfide linkages (*13*). The gluten structure is stable, but mechanical and thermal stress can disentangle, orient, and rupture the polymer network. For instance, mixing leads to polymer rupture at disulfide bonds. Heating to 80 °C in the presence of 20% or more water reduces the amount of free-sulfhydryl groups, alters glutenin conformation, and reduces hydrophobicity. Further heating reduces the number of disulfide bonds and engages the gliadins in polymer formation. Sulfhydryl-disulfide interchange reactions may act during stress as a mechanism for relief of that stress (*13*−*16*).

Three approaches have been made to induce desirable polymer properties from this platform protein concentrate: (a) evaporative casting of whole or vital wheat gluten formulations from an acetic acid or basic/ethanol extract (*17*−*19*), (b) evaporative casting of gliadin fraction formulations from aqueous ethanol (*20*), and (c) thermoforming of hydrated dough (*21*, *22*).

Chemically reductive thermoforming has been applied by us (*23*, *24*) to wool and chicken feather keratin dough formulations to increase the biopolymer elastic modulus. The method was applied at 110−130 °C and 480−1680 K Pascal, and, after cooling, produced pliable translucent sheets. $Na_2S_2O_3$ was the reducing agent. Both wool and feather contain 7−15% cysteine that forms disulfide cross-links responsible for stabilizing the native helical structure. The method employed high solid-phase concentrations and required long reaction times to obtain homogeneous, translucent sheets.

The objective of this study was to evaluate and modify elasticity properties of reactively thermoformed gluten-rich polymers. Whole gluten was used because of its availability and low cost. Glutenin subfractions of whole gluten were also evaluated even though they are not available commercially. Chemical reducing agents, cross-linking agents, and potentially reactive constituents such as keratin proteins were included in the evaluation.

MATERIALS AND METHODS

Chemicals. Vital wheat gluten was obtained from Giusto, S. San Francisco, CA.; soy protein from Protein Technologies,St. Louis, MO; silk protein from Bomdik; leather hydrolysate from Hynite Corp., Oak Creek WI.; rice hull from Wehah Fram, Richvale CA. The authors thank Dr. Walter Schmidt of USDA for the chicken feather and Mr. Trung Cao of USDA for the glutenin, which was separated from whole gluten by solubilizing the gliadin in 70% ethanol, filtration, and drying. All other chemicals were from Sigma-Aldrich, St. Louis MO.

Instruments. Gluten-based polymers were thermoformed using a heated hydraulic press (Carver Hydraulic #2518) with two 30.5 cm × 30.5 cm pressure plates. The elasticity of the thermoformed sheets were measured with a Texture Analyzer TA-XT2 (Texture Technology, Corp.).

Methods. In a typical experiment, 16 g of commercial gluten was mixed with 0.35 g of $Na_2S_2O_3$, 4 g of glycerine, used as a nonvolatile plasticizer, and 4 mL of distilled water. The mixture was first manually kneaded as a dough for 1−2 min then in a pasta making machine (Atlas, Model 150-mm Deluxe) to homogeneity. When a liquid additive was used, the amount of water was reduced to maintain the consistency or plasticity of the dough.

The kneaded dough was first placed between two sheets of unbleached parchment paper, then between two 30.5 cm × 30.5 cm stainless steel plates. This assembly was compressed on the hydraulic press heated to 110−130 °C. Pressure was applied first at 480 K Pascal for 60 s and then at 1680 K Pascal for five minutes. The resulting sheet, generally a circle with a diameter of 20−30 cm, was removed and allowed to dry at room temperature for one week. The relative humidity in the laboratory was generally around 50%, but was not controlled. The range of ambient relative humidity values was from 30 to 70% RH. Measurements in this range werfall within the experimental errors indicated in the tables. For measurements above and below the ambient range of relative humidity, the samples were equilibrated in a closed glass jar containing a humidity controlling solution for 24 h. Equilibration employed the following: (1) saturated LiBr solution in water over excess LiBr for 6% relative humidity and (2) saturated NaCl solution in water over excess NaCl for 75% RH.

Tensile tests using the Texture Analyzer, Texture Profile analysis software (force-tension mode/repeat option) were applied to strips (68 × 6.35 mm^2) that were cut from the center of equilibrated sheets. The strips were repeatedly extended and relaxed parallel to the long strip dimension. Each test used a deformation and relaxation rate of 1 mm/sec applied for five seconds. Five repeats were included. The total deformation was 5 mm or 15.7% of the 31.8 mm initial sample length exposed to elongation, ie the distance between the sample holding clamps. The applied force (Newtons) was divided by the cross-sectional area of the test strip and by the fractional deformation to determine the elastic or Young's modulus. Samples were rejected that fractured or did not recover sufficiently to allow five cycles of testing.

Table 1. Elastic Modulus for 15% Elongation of Polypropylene and Thermoformed, Chemically Reduced, and Plasticized Dough of Wheat Protein

base polymer	reduced	concentration (wt % dmb)	Young's modulus (Pascals) × 10^7 (%)
whole vital gluten	no	78.6	2.8 ± 3.5
vital gluten	yes	78.6	4.1 ± 6.2
glutenin	yes	78.6	6.5 ± 5.7
polypropylene			5.3 ± 4.5

RESULTS

Although temperatures between 90 and 140 °C could be applied successfully to produce films from wheat vital gluten, darkening discoloration without increase in strength was observed above 110 °C. At lower temperatures longer reaction times were needed, hence 110 °C was employed in all experiments discussed below.

The presence of reducing agents in the formulation reduced the consistency of the mixture facilitating ingredient mixing. A number of reducing agents were applied to test if the native disulfide linkages were effectively disrupted. Na_2SO_3, $Na_2S_2O_3$, $Na_2S_2O_4$, and a mixture of Na_2SO_3 and sulfur all produced thermoformed polymers with similar properties suggesting that even the weakest reducing agent had effectively disrupted the available bonds. The sheets without a reducing agent were not uniform and failed the test procedures.

The elastic properties of polymers based on gluten proteins derived from wheat were found to bracket those of polypropylene when indexed by Young's modulus for the first deformation (**Table 1**). For the same deformation, less force was required to extend whole vital gluten than polypropylene, but more force was needed for the glutenin component of the gluten. In unreduced hydrated dough prepared for baking, high molecular

Table 2. Elastic Modulus for 15% Elongation of Vital Gluten with the Addition of Minor Amounts of Protein of Animal and Plant Origin

base polymer	additive	concentration (wt % dmb)	Young's modulus (Pascals) $\times 10^7$ (%)
vital gluten			4.1 ± 6.2
	feather	4.0	5.4 ± 8.0
	leather hydrolysate	4.0	4.9 ± 2.8
	whey protein	4.0	4.5 ± 7.8
	soy protein	6.7	5.8 ± 2.4
	silk	1.0	5.9 ± 7.6
polypropylene			5.3 ± 4.5

Table 3. Elastic Modulus for 15% Elongation of Vital Gluten with Minor Amounts of Formaldehyde Cross-Link Agent

cross-linking agent	concentration (wt % dmb)	Young's modulus (Pascals) $\times 10^7$ (%)
37% formaldehyde solution	1.0	8.70 ± 3.2
formaldehyde sodium bisulfite	1.0	9.78 ± 4.5
paraformaldehyde as solid powder	1.0	9.72 ± 3.6

weight glutenin proteins participate in intermolecular S−S linkages at both termini; whereas, the lower molecular weight gliadin fraction, with usually only one terminal cysteine residue per molecule, participates less frequently and ends the cross-linking process (*10*, *13*, *14*). Therefore, the less elastic behavior of the glutenin concentrate may be due to a higher level of cross-linking and an overall higher molecular weight (*25*).

Noncereal proteins with the potential for molecular interaction with the base gluten protein by S−S cross-linking as well as through intermolecular hydrogen bonding were incorporated prior to reduction and thermoforming. Some of these proteins (keratins) are known to have higher amounts of cysteine than gluten. As disulfide links reform, complex weblike molecular structures centered on the minor protein component may be possible. The elastic moduli of these formulations were greater than that of vital gluten alone. The silk formulation produced both the highest modulus 5.9×10^7 Pascal (**Table 2**) and a specific modulus increase greater by about a factor of 4 than any other protein.

Formulations employing acids, such as hexanoic, octanoic, lauric and benzoic acids showed reduced Ym to $1.6-2.2 \times 10^7$ pascals. Saturated fatty acids have been reported to plasticize gluten films and improve water vapor permeability (*21*). An unsaturated acid, such as oleic acid, resulted in a tacky sheet that could not be separated from the press plate. However, a bivalent acid, succinic anhydride, resulted in moderately higher values ($7.1 \pm 4.5\% \times 10^7$ Pascals). A polymeric acidic carbohydrate, alginic acid, produced Ym values of $8.2 \pm 2.5\% \times 10^7$ Pascals (*26*, *27*). We have previously reported the use of acids to alter the properties of chitosan (*28*), using acids to convert the amino groups into ammonium ions.

Formulations employing formaldehyde also were effective in altering the moduli of reductively thermoformed gluten polymers at 0.75−1.5% as shown in **Table 3**. At higher percentages than those reported, the sheets were very fragile and broke under testing. Formaldehyde has been used to cross-link plasticized gluten films cast from acetic acid and ethanol (*19*). One of the first synthetic polymers, galalith, was formed by the reaction of casein with formaldehyde.

Formulations employing colloidal microcrystalline cellulose, rice hull powder, acrylic latex and natural latex are shown in **Table 4**. All of these constituents resulted in increased elastic modulus, relative to the reduced gluten, but the films obtained were not translucent and uniform. Both latex preparations diminished the Young's modulus resulting in greater elasticity.

Table 4. Elastic Modulus for 15% Elongation of Vital Gluten with Minor Amounts of Nonreactive Crop Residues and Latex

additives	concentration (wt % dmb)	Young's modulus (Pascals) $\times 10^7$ (%)
microcrystalline cellulose	4.8	5.4 ± 3.9
rice hull powder	4.0	7.1 ± 7.9
rice straw powder	4.0	7.8 ± 8.1
natural latex	5.0	4.1 ± 3.2
acrylic latex	5.0	2.8 ± 4.1

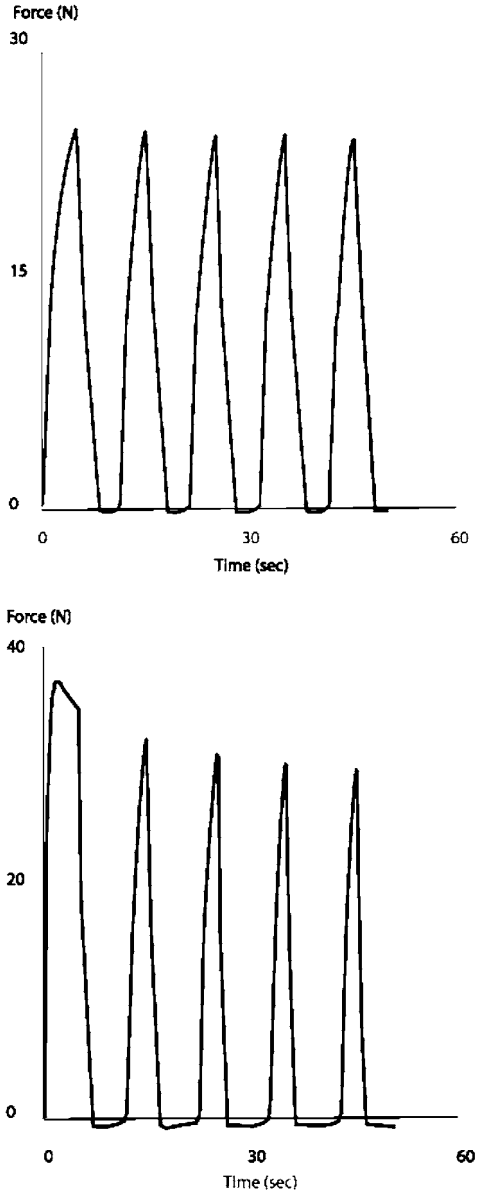

Figure 2. Repeated extension to constant 15.7% deformation for (top) polypropylene (0.41 mm thick) and (bottom) vital gluten cross-linked with formaldehyde (0.47 mm thickness).

It has been reported that gluten added to high strength polyester (10−40%) did not alter its elasticity (*29*).

While it has been reported (*21*) that the mechanical strength of gluten films was unaffected by humidity, we observed that both the weight of the sheet and its modulus varied with the relative humidity. The weight increased at high relative humidity (>70%) and the elastic modulus decreased; conversely, the weight decreased at low relative humidity (<30%) and the elastic modulus increased. Samples stored at constant humidity were stable. Cross-linking with a thiol-branched cross-linker has

been shown to reduce water absorption in compression molded wheat gluten (*30*).

Repeated elongation tests revealed differences in the elastic properties of the gluten-based formulations and a commercial polypropylene sample. Typically vital wheat gluten formulations (**Figure 2**) exhibit increasingly elastic behavior with each subsequent deformation, hence the Young's modulus determined for each extension is less than that for the previous extension. This effect is most severe between the first and second deformations where an 11% reduction occurs. The value of Ym continues to diminish for each subsequent deformation, but with a smaller 3% reduction at each step. The percentage reduction between the first and fifth deformation was 19%. The Ym for polypropylene also decreases, but with only a 4% reduction between the first and fifth extension. Nevertheless, the recoverable elasticity of the sample suggest the possible application to commercial packaging products such as six-pack beverage rings provided the elasticity can be made less dependent on relative humidity.

In conclusion, sheets formed by chemically reductive thermoforming of wheat gluten dough have elasticity comparable to commercial polymeric materials, but the Young's modulus for these materials is reduced more rapidly after repeated extension. Elastic moduli may be altered to tailor the elasticity over a narrow range of values. In the 35−70% relative humidity range the variation in Young's modulus was less than 10%. At 6 and 75% RH elasticity tesing failed. Within the 35−70% range of RH the composites show good recovery on repeated extension.

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