Degradation Behavior of Soy Protein–Wheat Gluten Films in Simulated Soil Conditions

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Films containing soy protein and wheat gluten were exposed to simulated farmland soil mix over a period of 30 days and monitored for degradation. The simulated farmland soil mix (topsoil/sand/Sunshine compost/vermiculite, 59:6:25:10, wt %) was mixed and stored at ambient humidity (48–55%) and temperature (20-24 °C); the soil mix was constantly maintained at 15% moisture by weight. Research focused on evaluating the effectiveness of gluten and cysteine additions on biodegradable behavior in the simulated farmland soil conditions. The four types of films, soy protein (S:G 1:0); soy protein with cysteine addition (S:G 1:0 + CYS); soy protein—wheat gluten (S:G 4:1); and soy protein—wheat gluten with cysteine addition (S:G 4:1 + CYS), were prepared at pH 7.0 for degradation studies. Soy protein—gluten film rapidly degraded with 50% weight loss in about 10 days and with up to 95% weight loss in 30 days. Tensile strength and elongation of all soy protein—gluten films significantly decreased in 3 days. However, cysteine addition delayed the degradation rate of soy protein—gluten films. Soy protein—wheat gluten film disintegrated after 20 days in the simulated farmland soil environment. These results suggest that wheat gluten and cysteine addition to soy protein-based films could delay degradation rates due to their high disulfide contents.

Keywords: Cysteine; film degradation; protein film; soy protein; wheat gluten

INTRODUCTION

Currently, nondegradable petroleum-based synthetic polymers are used for food preservation and storage. Protein films find strong competition from nonedible synthetic films but have an environmental advantage in that they are renewable and biodegradable. Substituting some of this synthetic packaging with edible packaging could help reduce solid waste. Over 5 billion tons of packing-related solid wastes are discarded every year, and 30% of the wastes are plastics (Fleming, 1992; Rowatt, 1993). Plastic biodegradation is a slow process, and the rate is affected by the nature of the material and its form. It takes a several hundred years to degrade petroleum-based synthetic plastics, which have caused serious solid waste contamination in the world. Hence, a demand exists for natural biodegradable films from renewable sources as an alternative to synthetic polymers.

During the past two decades, the use of increasingly large amounts of synthetic, nonbiodegradable packaging and wrapping materials has created disposal and environmental problems. This study focuses on the possibility of replacing synthetic polymers with biodegradable polymers derived from renewable agricultural sources. The development of biodegradable packaging films from agricultural polymers would not only ease environmental problems but would also provide new uses for surplus agricultural products.

The following methods have been used to assess the biodegradation of plastics: changes of mechanical prop-

erties in soil (Goheen and Wool, 1991); biological oxygen demand (BOD) changes or CO_2 evolution in slugs (ASTM D5209-92, 1992a; ASTM D5271-92, 1992b); molecular changes by enzymatic or microbial treatment (Lee et al., 1991; Cacciari et al., 1993; Tokiwa and Suzuki, 1981); mechanical property changes or CO_2 evolution in soil (ASTM D5338-92, 1992c; Gilmore et al., 1992; Johnson et al., 1993); gas evolution by anaerobic digestion (ASTM D5210-91, 1991b); and mechanical property changes in several compositing soil conditions (Breslin, 1993; McCarthy et al., 1992).

Recently, considerable research has been reported on the preparation and the evaluation of biopolymer films derived from renewable resources to replace petroleumbased packaging materials (Park and Chinnan, 1990; Aydt et al., 1991; Gennadios et al., 1993; Gennadios and Weller, 1994). Soy protein films without other secondary components besides proteins do not have good mechanical and barrier properties (Wu and Bates, 1973; Gennadios et al., 1993). High water vapor permeability of soy protein films is a major limiting factor. Cysteine and gluten addition can be used to produce films with increased tensile strength (TS) and enhanced moisture and oxygen barrier properties (Were et al., 1999).

Soy protein—wheat gluten films could find applications as viable packaging materials and primary wrappers for low-moisture foods. In addition, these films could benefit soy protein and wheat producers by providing a market for the readily available soy protein isolate and wheat gluten. To commercialize these films as viable packaging materials, the soy protein—wheat gluten films must still retain all of the physical properties expected by the consumer, and when placed in the appropriate environment, they must degrade more rapidly than conventional disposable plastics. In this study, the burial test was used as a confirmatory test

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method in the simulated real farmland environment and indicates the degree of biodegradation. The objectives of this study were to prepare protein films using soy protein—wheat gluten and to investigate the effectiveness of gluten and cysteine additions in biodegradable behavior in the simulated farmland soil conditions.

MATERIALS AND METHODS

Materials. Commercial soy protein isolate (SPI) ARDEX D was obtained from Archer Daniels Midland Co. (Decatur, IL). The SPI had a protein content of 87.25% dry basis and a moisture content of 6.5%. Spray-dried wheat gluten was obtained from Midwest Grain Products, Inc. (Atchison, KS). Wheat gluten had a protein content of 76.6% dry basis, with a moisture content of 7%. Cysteine was purchased from Sigma (St. Louis, MO). Glycerol was purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals were reagent grade.

Preparation of the Simulated Farmland Soil Mix. The simulated farmland soil mix (topsoil/sand/Sunshine compost/ vermiculite, 59:6:25:10, wt %) were obtained from Department of Plant Pathology at University of Arkansas and sifted to remove large clumps with a one-eighth-inch screen into plastic boxes ($15 \times 10 \times 6.5$ in.). Topsoil (pH 6.2, organic matter 6.4%, and total nitrogen 0.09%), the uppermost layer of ground, was taken from the farm of the University of Arkansas, Fayetteville. It was generally darker than the deeper layers, as it was richer in organic materials. The simulated farmland soil mix was kept moist with deionized water and stored at ambient humidity (48-55%) and temperature (20-24 °C). Film strips (100 \times 25 mm) were buried in the simulated farmland soil mix. The simulated soil mix was maintained at 15% moisture by weight. Moisture content of the simulated soil mix was determined by weighing approximately 5 g of moist soil mix in a labeled aluminum weighing pan and drying at 103-105 °C for 18-24 h. Moisture content of the simulated farmland soil mix was adjusted by adding appropriate amounts of deionized water to obtain the desired moisture content as follows:

$$W_{A} = S_{R}H[(WS_{F} - WS_{R})/(100 - WS_{F})]$$

where W_A = amount of water to be added, S_R = weight of initial soil, WS_F = the final moisture content (%), and WS_R = initial water content.

Preparation of Soy-Wheat Gluten Film. Four types of films, soy protein (S:G 1:0) soy protein with cysteine addition (S:G 1:0 + CYS); soy protein-wheat gluten (S:G 4:1); and soy protein-wheat gluten with cysteine addition (S:G 4:1 + CYS) were prepared for the degradation study. The method developed by Were et al. (1999) to produce soy-gluten-cysteine with improved TS and enhanced moisture barrier properties was used to prepare films for this investigation. SPI (5 g) was weighed for the control film and dispersed in 95 mL of water, and plasticizer (glycerol, 2.5 g) was added. For the composite soy protein-gluten films, part of the SPI was substituted with wheat gluten, resulting in optimized soy protein/wheat gluten ratios (S:G 4:1). To solubilize the gluten, soy protein-gluten mixtures were dispersed in 40 mL of 70% ethanol and 55 mL of water. Glycerol (2.5 g) was added to each solution. To determine the effects of cysteine, cysteine (1% w/w) was added to the film solutions. Film-forming solutions were adjusted to pH 7.0 with 0.1 N NaOH. The solutions were heated to 45 °C while stirring in a thermostated water bath. Air bubbles in film-forming solutions were eliminated by applying a vacuum until air bubbles disappeared. Film-forming solutions (15 mL) were then poured into 8.6-cm² polystyrene weighing boats (Fisher Scientific) and air-dried at room temperature (22-25 °C). Upon drying, the films were peeled from the polystyrene plates. Prior to all the tests, the films were conditioned in compliance with the ASTM Standard Method D882-90 (ASTM, 1990). Films were placed in desiccators and were equilibrated for a minimum of 48 h at 25 °C and 55% relative humidity.

Relative humidity in the desiccator was maintained with 12 $\,\rm N$ sulfuric acid.

Determination of Weight Loss. Degradation of the film was estimated as weight loss during degradation in the aerobic simulated farmland soil mix determined over 30 days. Each film strip was washed with water and cleaned gently with a moist tissue prior to evaluation and then dried in vacuo for 24 h before measured. Until they were analyzed, samples were kept in dry desiccator. The weight loss (%) of films exposed to the simulated soil mix was calculated as follows:

weight loss (%) =
$$(W_t/W_0) \times 100$$

where W_0 and W_t were the initial weight and the residual weight at a given time of the sample, respectively.

Determination of Total Soluble Matter. Total soluble matter of soy-gluten films was determined to evaluate the film integrity in an aqueous environment for comparison against the degradation behavior of the simulated soil mix. Total soluble matter was determined by the procedure of Gontard et al. (1992). The initial dry matter of film strips $(25 \times 25 \text{ mm})$ was measured by drying strips in a mechanical convection oven at 100 °C for 24 h and cooling in a desiccator before taking the weight. Film strips were placed in beakers containing sodium azide (0.02%, w/v) to prevent microbial growth and stored at room temperature for 24 h with occasional gentle stirring. Undissolved dry matter was determined by taking the film pieces out of the beakers and drying them in a mechanical convection oven (100 °C for 24 h). The soluble matter was determined by measuring the weight loss of films and the protein content of the supernatant solution using the micro Kjeldahl method (AOAC, 1990).

Degradation of Mechanical Properties. Addition of wheat gluten and cysteine increased the TS of soy protein films due to an increase in disulfide bond formation. In this study, we attempted to elucidate the role of disulfide bonds with respect to the development of physical defects and mechanical breakdown during the degradation process. TS and elongation of films were measured with a texture analyzer (TA.XT2, Texture Technologies, Corp., Scarsdale, NY). Sample preparation and handling for texture analyses were carried out according to standard methods D 882-91 (ASTM, 1991). The films were equilibrated at ambient humidity (48-55%) before the test. Film strips measuring 100×25 mm were mounted onto the texture analyzer. The film strips were pulled 50 mm apart at a speed of 5 mm/s in tension mode. TS in MPa was calculated by dividing the peak load developed during the test by the film cross-sectional area. Elongation is expressed as a dimensionless measure of a film's ability to stretch. The percent change in length is that change experienced by a material due to pulling stress before breakage.

Statistical Analysis. Each replication included individual preparation of film from film-forming solution. Three replications were performed in a completely randomized design. A minimum of three observations was collected unless specified otherwise. Data were analyzed using the general linear model procedures of SAS (1992) to determine differences between treatment means. Pairwise comparison of all treatment means was performed using the least significant difference (LSD) procedure (P > 0.05).

RESULTS AND DISCUSSION

Physical Appearance. Film appearance is an important property of protein films since it could affect consumer acceptance of such protein films in potential edible or nonedible packaging applications. Table 1 shows Hunter *L*, *a*, and *b* color values of the soy protein–gluten films at zero time before samples were buried in the stimulated soil mix. Gluten addition resulted in lighter and more yellow films (higher *L* and +*b* values). The increased yellowness of the composite soy protein–gluten films could be due to gelatinization

Table 1. Hunter Color Values (*L*, *a*, and *b*) and Total Color Differences (ΔE) of Soy Protein–Wheat Gluten Films at Zero Time^{*a*}

soy/gluten (S:G)	L	а	b	ΔE
S:G 1:0	36.16 ^c	-4.06^{a}	6.84 ^a	58.91 ^a
S:G 1:0 + CYS	42.34^{b}	-3.92^{b}	6.79 ^a	52.78^{b}
S:G 4:1	48.99 ^a	-4.04^{a}	6.62 ^b	48.16 ^c
S:G 4:1 + CYS	49.83 ^a	-3.62°	6.42 ^c	45.32 ^c

^{*a*} Values were the mean of three values. Means within each column with same superscript are not significantly different (P > 0.05).



Figure 1. Changes in weight loss during the degradation process of soy protein–gluten film by simulated soil environment. Water content of simulated soil was 15%. Means were average of three values (P > 0.05). S:G is soy protein/gluten ratio.

of the small amount of starch contained in the wheat gluten when the solutions were heated (Gennadios and Weller, 1991). The S:G 1:0 films were clear and smooth surfaces, whereas the S:G 4:1 films were opaque with uneven surfaces and insoluble particles. This was attributed to incomplete solubilization of the wheat gluten at pH 7.0.

Each of the degraded films was checked for transparency, development of cracks and pits, and discoloration or any changes in color at various stages of degradation. The S:G 4:1 films developed an intense dark brown color with extensive pitting all over the surface for 5 days. However, they retained their shapes with rough and undulating surfaces. The S:G 1:0 films disintegrated into pieces that could not be salvaged from the wet soil after 20 days. The S:G 4:1 films with cysteine addition largely retained their shapes, surface smoothness, and color, although some minor pitting could be observed.

Weight Loss in the Soil. The definition of degradation is the conversion of large complex molecules into small fragments of less complex molecules. Weight losses of samples were obtained from the difference between the initial film weight and the sample weight after aging. Weight loss of film samples during degradation in simulated farmland soil mix is shown in Figure 1 where the mass of the sample, normalized with respect to its initial mass, is plotted versus time. Estimation of degradation by weight loss is prone to error in part because of the mass of dirt, fungi, and bacteria not removed during washing as well as the partial removal of the sample labels during degradation. Despite these potential problems, the overall trends in weight loss over time were quite constant (Figure 1). Most of the weight loss occurred in the first 5 days, and then degradation proceeded more slowly. Within 30 days, 95% of the films had been degraded.

Total Soluble Matter. Total soluble matter of soy protein-wheat gluten (S:G 1:0, S:G 4:1) films with or without addition of cysteine were measured. After 24 h incubation of four different films in an aqueous environment with gentle motion, films maintained their integrity, an indication that the protein polymer network remained intact and only monomers, small peptides, and nonprotein material were soluble. The total soluble matter ranged from 24.4 to 28.8% for the soy protein (S:G 1:0) films with or without cysteine addition, respectively. For the S:G 4:1 films with or without cysteine addition, lower total soluble matter (ranging from 21.1 to 23.9%) was observed, respectively. The insolubility of wheat gluten in water is attributed to the low number of ionized polar amino acids, the large number of hydrophobic interactions between nonpolar amino acids, and the presence of covalent disulfide bonds (Reiners et al., 1973). Cross-linking water-soluble soy proteins to wheat gluten could decrease the solubility of soy-gluten films. The disulfide bond is responsible for stabilizing the film network (Saio et al., 1971), thus causing a lower total soluble matter for soy-gluten films with cysteine addition.

Usually, the typical degradation pattern of protein film is as follows: in the first stage, only the molecular weight decreases caused by a random chain scission mechanism, and in the second step, mass loss and change in the rate of chain scission occur. The lag time can be explained as follows: for a film to be degraded and eroded, first water should penetrate into the matrix, and then the chains become cleaved. The lag time in erosion is thought to be the sum of time to reach equilibrium and to escape from the matrix of cleaved soy protein and wheat gluten.

Changes in Mechanical Properties. The mechanical changes occurring during burial degradation testing of protein films could be characterized by the chemical bonds in the main chains and in the side chains ruptured as evidenced by a diminution of the molecular weight and an evolution of the molecular weight gaseous products, respectively. In this study, the effect of disulfide bonds on mechanical properties was observed during the degradation process. Figure 2 shows changes in TS of soy protein-based films with wheat gluten and cysteine addition during the degradation process in the simulated soil mix. Addition of cysteine and wheat gluten was used to produce films with increased TS and enhanced barrier properties (Were et al., 1999). At zero time, the S:G 4:1 film had a significantly higher TS than the S:G 1:0 film due to disulfide bond formation. Combining secondary components such as wheat gluten and cysteine with soy protein may cause physical and chemical interactions that may improve film properties and delay film fracture. Wheat gluten as a secondary component improved water barrier properties of soy protein films due to the large number of nonpolar amino acids that contribute to hydrophobic interactions, the few ionizable amino acids, and covalent disulfide bonds



Figure 2. Changes in tensile strength during the degradation process of soy protein–gluten film by simulated soil environment. Water content of simulated soil was 15%. Means were average of three values (P > 0.05). S:G is soy protein/gluten ratio.

that contribute to gluten insolubility (Were et al., 1999; Reiners et al., 1973). The S:G 4:1 film without addition of cysteine had a TS of 8.24 MPa and corresponding values of 8.68 MPa with cysteine addition. The addition of cysteine may be advantageous due to disulfide rearrangement. Addition of cysteine may have caused rearrangement of some of the bonds that resulted in increased TS for most films. This was similar to findings reported in which sodium sulfite, another disulfide bond reducing agent, increased TS (Gennadios et al., 1993). Sulfhydryl-disulfide interchange, disulfide-disulfide interchange, and thiol-disulfide interchange are factors that have been reported to affect film formation (Kasarda et al., 1971). The TS of S:G 1:0 film deteriorated rapidly in 3 days (P > 0.05). After 7 days, all of the S:G films were totally deteriorated. However, the degradability of the S:G 4:1 films with cysteine addition during storage seems slower than the S:G 1:0 films. It was concluded that wheat gluten is high in cystine, and the cystine content favors formation of disulfide bonds that may stabilize the protein network (Mattern, 1991) during the degradation process.

Figure 3 shows the changes in elongation of soygluten films during the degradation procedure in simulated soil mix. At zero time, increases in film elongation were observed for soy protein films with the addition of wheat gluten. The high molecular weight subunits of glutenin have been proposed to be the major components responsible for the elasticity of gluten, and these subunits are held together by covalent disulfide bonds (Tatham et al., 1985). However, the elongation of all films deteriorated rapidly in the first 3 days and then slowed. From these results, we can conclude that the plasticizer (glycerol) containing three hydroxyl groups was highly hydrophilic and was preferentially removed, leaving the soy protein–gluten network (Goheen and Wool, 1991).



Figure 3. Changes in elongation during the degradation process of soy protein–gluten film by simulated soil environment. Water content of simulated soil was 15%. Means were average of three values (P > 0.05). S:G is soy protein/gluten ratio.

ABBREVIATIONS USED

S:G, soy protein—wheat gluten ratio; S:G 1:0 + CYS, soy protein film with cysteine addition; S:G 4:1, soy protein—wheat gluten ratio 4:1 film; S:G 4:1 + CYS, soy protein—wheat gluten ratio 4:1 film with cysteine addition; TS, tensile strength.

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