

Physical Characteristics of a Composite Film of Soy Protein Isolate and Propyleneglycol Alginate

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ABSTRACT

Different levels (5, 10, 15, 17.5 or 20% w/w of solid) of propyleneglycol alginate (PGA) were incorporated into soy protein isolate (SPI) films to form biodegradable composite films with modified physical properties. Color of the SPI films was affected ($P < 0.05$) by the incorporation of PGA. Tensile strength increased ($P < 0.05$) with addition of PGA up to 17.5%, while the percentage elongation at break decreased with incorporation of PGA of higher levels. Water vapor permeability and water solubility also decreased by adding PGA up to 10%, but further addition of PGA increased values for these properties. Results suggest that the site of reaction with PGA on the protein chain may become saturated with PGA at the 10% level.

Key Words: biodegradable film, soy protein isolate, propyleneglycol alginate, composite film

INTRODUCTION

INTEREST IN PRODUCTION AND UTILIZATION of edible, degradable, and compostable films and coatings prepared from various biopolymers such as polysaccharides, proteins, lipids, or combinations of these components remains great (Kester and Fennema, 1986; Gennadios and Weller, 1990; Gontard and Guilbert, 1994; Guilbert et al., 1996; Krochta and Mulder-Johnston, 1997). Such films used in food systems may function as selective barriers to movement of gases, vapors, or solutes (Gennadios and Weller, 1990), and thus increase quality, stability, and shelf life of foods.

A variety of proteins, such as corn zein (Park et al., 1994), soy protein isolate (Brandenburg et al., 1993; Gennadios et al., 1993a), wheat gluten (Gennadios et al., 1993a, b; Gontard et al., 1994), milk proteins (Avena-Bustillos and Krochta, 1993; Maynes and Krochta, 1994; McHugh and Krochta, 1994a; McHugh et al., 1994) and egg albumen (Gennadios et al., 1996a) have been studied due to their film-forming ability, renewable nature, and abundance. However, few protein films are commercially used. A main limitation of protein films is limited effectiveness as a water vapor barrier, which can be attributed to the hydrophilic nature of protein film-forming substances (Krochta, 1992; McHugh et al., 1993). Accordingly, with a focus on increasing utiliza-

tion of biopolymer films, various modifications of barrier properties or improvement of physical strength may be possible.

One way to modify physical properties of films is by inducing intermolecular and intramolecular chemical bonding through physical or chemical means. Several reported studies have been directed toward development and modification of soy protein isolate films. These studies have included treatments with alkali (Brandenburg et al., 1993), alkylation with sodium alginate or with propyleneglycol alginate (Shih, 1994), enzymatic treatment with horseradish peroxidase (Stuchell and Krochta, 1994), acylation with acetic and succinic anhydrides (Ghorpade et al., 1995), treatment with formaldehyde (Ghorpade et al., 1995), short wave UV treatment (Rubin et al., 1968) and inducing cross-linking by heating/dehydration (Gennadios et al., 1996b; Yannas and Tobolsky, 1967).

Incorporation of materials such as neutral lipids, fatty acids or waxes into carbohydrate or protein films, has also been studied to improve moisture barrier properties of such films. For this purpose, lipid bilayer films (Kamper and Fennema, 1984a, b; Greener and Fennema, 1989a, b; Gontard et al., 1995) and emulsion films (McHugh and Krochta, 1994b; Shellhammer and Krochta, 1997) have been tested. Another approach to improve physical properties of biopolymer films has been to prepare composite films through combined use of compatible polysaccharide and protein materials (Shih, 1994, 1996; Jane et al., 1993; Lim and Jane, 1993). Film properties should be improved not only by strength of one material compensating for the weakness of another, but also by any synergistic effect between components.

Multicomponent film with polysaccharide and protein materials has been studied the least. Shih (1994) demonstrated the potential use of propyleneglycol alginate (PGA) in improving water stability of multicomponent films by forming covalent complexes between PGA and soy protein. But the effect of PGA on properties of soy protein film has not been comprehensively tested.

The main objective of our study was to investigate changes in properties of soy protein films resulting from the addition of PGA. The effect of different levels of PGA on color, tensile strength (TS), elongation at break (E), water vapor permeability (WVP) and water solubility (WS) of soy protein film was determined.

MATERIALS & METHODS

Materials

Supro 620, a soy protein isolate (SPI) was obtained from Protein Technologies International (St. Louis, MO) and stored at 4°C prior to use. Glycerin and sodium hydroxide were purchased from J.T. Baker (Phillipsburg, NJ). PGA (Kelcoloid LVF) was obtained from the Kelco Company (Chicago, IL).

Preparation of soy films

A control (0% PGA) film-forming solution was prepared at room temperature by slowly dissolving 5g of SPI in a constantly stirred mixture of 100mL of distilled water and 2.5g of glycerin as a plasticizer. Five additional film solutions for composite film-making were prepared by using various concentrations of PGA (5, 10, 15, 17.5 or 20% as g PGA/g solid). Predetermined amounts of PGA were added into mixtures of distilled water and plasticizer, and the mixture was heated to about 80°C on a hot plate with stirring until PGA was completely dissolved. SPI was then added to the mixtures and dissolved. pH was adjusted to 10±0.1 with 1N sodium hydroxide. Each solution was heated for 20 min in a constant temperature water bath at 70°C, then strained through 8-layered cheese cloth (grade 40, Fisher Scientific), and poured onto a leveled Teflon-coated glass plate (21 cm × 35 cm). Film thickness was controlled by casting the same amounts (80 mL) of film-forming solution. Films were allowed to dry at ambient conditions for about 20h. They were peeled from the plate and samples for proper-

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ty testing were cut. Tensile testing samples were cut into 2.54 cm × 10 cm rectangular strips. Testing samples were squares of 7 cm × 7 cm (WVP), 7 cm × 7 cm (color) and 2 cm × 2 cm (WS) in size.

Thickness

Film thickness was measured to ±2.54 μm (0.1 mil) with a hand-held micrometer (B.C. Ames Co., Waltham, MA). Five thickness measurements were taken on each tensile testing sample along the length of the strip with the mean used in TS calculations. Similarly, 5 measurements were taken on each WVP sample, 1 at the center and 4 around the perimeter and the mean values were used in WVP calculations.

Conditioning

All films for TS and WVP were conditioned for 2 days in an environmental chamber (Model RC-5492, PGC Parameter Generation & Control, Inc., Black Mountain, NC) set at 50% RH and 25°C before testing (ASTM Standard Method D 618-61, ASTM, 1995a). Films for the other properties also were stored at the same conditions prior to testing.

Color

Color values of films were measured with a CR-300 Minolta Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan). This instrument was a color analyzer with an 8-mm dia measuring area. Films were placed on a white standard plate (calibration plate CR-A43) and the Hunter-Lab color scale was used to measure color: L = 0 (black) to L = 100 (white); a = -80 (greenness) to a = 100 (redness); b = -80 (blueness) to b = 70 (yellowness). Total color difference (ΔE), yellowness index (YI), and whiteness index (WI) were calculated as (Francis and Clydesdale, 1975; Bolin and Huxsoll, 1991):

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad (1)$$

$$YI = 142.86 \text{ b/L} \quad (2)$$

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (3)$$

where $\Delta L = L_{\text{standard}} - L_{\text{sample}}$; $\Delta a = a_{\text{standard}} - a_{\text{sample}}$; $\Delta b = b_{\text{standard}} - b_{\text{sample}}$. Standard values for the white plate were: L = 96.86, a = -0.02, and b = 1.99, respectively. Five measurements were taken on each film, 1 at the center and 4 around the perimeter. Color measurements were taken in triplicate for each type of film.

Tensile strength and percentage elongation at break

TS and E were evaluated with a Model 4201 Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA). Initial grip separation was set at 50 mm and cross-head speed was set at 500 mm/min. TS was calculated by dividing the maximum load

by the cross-sectional area of the sample. E was calculated as the percentage of change by dividing film elongation at the moment of rupture by initial gauge length (50 mm). TS and E measurements for each type of film were replicated 3× with individually prepared films as the replicated experimental units and each replicate being the mean of 7 tested sampling units from the same film.

Water vapor permeability

WVP (g·m/m²·s·Pa) was calculated as:

$$WVP = (WVTR \cdot L) / \Delta p \quad (4)$$

where WVTR was measured water vapor transmission rate (g/m²·s) through a film, L was mean film thickness (m) and Δp was partial water vapor pressure difference (Pa) across the two sides of the film.

WVTR was determined gravimetrically using a modified Method E 96-95 (ASTM, 1995b). Film specimens were mounted on poly(methyl methacrylate) cups filled with distilled water up to 1 cm from the film underside. The cups were placed in an environmental chamber set at 25°C and 50% RH. A fan was operated within the chamber creating an air velocity of 198 m/min over the surface of the cups to remove permeating water vapors. Weights of cups were recorded every hour for a period of 8 h. Steady state was reached after about 1h. Slopes of the steady state (linear) portion of weight loss versus times curves were determined by linear regression to estimate WVTR. Coefficient of determination (R²) for all reported data was 0.99 or greater. In calculating WVP, the effect of resistance of the stagnant air layer between the film undersides and the surface of water in cups was corrected (McHugh et al., 1993; Gennadios et al., 1994). For each type of film, WVP measurements were replicated 3×.

Water solubility

WS was calculated as the percentage of soluble matter to initial dry matter in each film sample (Gontard et al., 1992). Three randomly selected samples from each type of film were first dried at 105°C for 24h to determine initial dry matter. After drying, films were immersed in 30 mL of distilled water in a 50 mL beaker. Beakers were covered with Parafilm "M" wrap (American National Can, Greenwich, CT) and stored in an environmental chamber at 25°C for 24h with occasional gentle stirring. Undissolved dry matter was determined by removing the film pieces from the beakers, gently rinsing them with distilled water, and then oven drying (25°C, 24h). The weight of solubilized matter was calculated by subtracting the weight of unsolubilized dry matter from the weight of initial dry matter and expressed as a percentage of the initial dry matter content. Film samples were weighed to the nearest 0.0001g before and after drying. Dry matter and WS were determined in triplicate for each type film.

Statistical analysis

Measurements of each property were triplicated for color, TS, E, WVP, and WS with individually prepared and cast films as replicated experimental units. Statistics on a completely randomized design were determined using the General Linear Models procedure in the SAS program (SAS Institute, Inc., 1988). Mean property values were separated (P<0.05) with Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS & DISCUSSION

Color

Soy protein films produced without PGA were smooth and transparent with a greenish, yellow color having mean color values of L 92.9, a -3.27, and b 15.4 (Table 1). When PGA was incorporated, films were smooth and transparent up to the incorporation of 15% PGA. With PGA above 15%, the films became thick and gritty, as evidenced in film thickness (Table 1). This may be attributed to weak interaction between SPI and PGA, and disruption of SPI film homogeneity by bulky PGA molecules. Incorporation of PGA increased film lightness as evidenced by greater (P<0.05) L-values up to 10% PGA. Conversely, yellowness (b-values) decreased with incorporation of PGA up to 10% then increased. Greenness (a-values) decreased continuously as PGA level increased. The interaction between SPI and PGA is caused by cross-linking between the protein and PGA (Shih, 1994, 1996). Cross-linking of protein films by aldehyde treatment (Habeeb and Hiramoto, 1968) or UV curing (Weadock et al., 1984) has caused films to be yellower and darker. The SPI-PGA film we developed, however, was less yellow and lighter up to 10% PGA incorporation, but the film became more yellow and darker when the PGA level was > 10%.

Color changes due to incorporation of PGA can be more fully described using other color functions (Francis and Clydesdale, 1975; Bolin and Huxsoll, 1991), such as E which indicates the degree of total color difference from the standard color plate, YI indicates degree of yellowness, and WI indicates degree of whiteness. The addition of PGA up to 10% resulted in an increase (P<0.05) in whiteness (Fig. 1) with further additions of PGA decreasing whiteness. In contrast, yellowness decreased as PGA increased up to 10% then increased with more PGA. E showed the same pattern as YI, indicating color difference of PGA-incorporated SPI film was mainly due to changes in yellowness. Incorporation of PGA at 10% seemed to be a saturation point in the SPI-PGA reaction. This confirmed our previous result on SPI-DAS (dialdehyde starch) films (Rhim et al., 1998), in which similar saturation was found at DAS level of 10%.

Tensile strength and elongation

TS of soy protein isolate films containing

Table 1—Film thickness and Hunter color values (L, a and b) of soy protein isolate (SPI) films with various levels of incorporated propyleneglycol alginate (PGA)

PGA (%)	Thickness (μm)	L (black to white)	a (green to red)	b (blue to yellow)
0	63.3±2.1 ^d	92.90±0.25 ^e	-3.27±0.09 ^d	15.35±0.46 ^b
5	66.4±1.2 ^{cd}	93.05±0.08 ^{bc}	-2.23±0.07 ^d	13.00±0.25 ^c
10	64.2±0.3 ^d	93.43±0.04 ^a	-1.61±0.01 ^c	11.69±0.03 ^d
15	69.1±0.5 ^c	93.26±0.02 ^{ab}	-1.56±0.01 ^c	12.08±0.11 ^d
17.5	77.2±0.5 ^b	93.01±0.06 ^c	-1.36±0.03 ^d	13.12±0.11 ^c
20	93.5±4.8 ^a	92.27±0.13 ^d	-1.24±0.01 ^a	16.29±0.22 ^a

^{a-e}Means of three replicates ± standard deviations. Any two means in the same column followed by the same letter are not significantly ($P>0.05$) different.

different levels of PGA (Fig. 2) showed that except the 20% PGA film, film TS increased ($P<0.05$) after adding PGA. The increase may be attributed to development of cross-linking between SPI and PGA (Shih, 1994, 1996). Increase in TS or puncture strength of protein films due to development of cross-linking has been reported for formaldehyde-treated SPI films (Ghorpade et al., 1995); glutaraldehyde-treated collagen films (Weadock et al., 1984); formaldehyde- and glutaraldehyde-treated zein/starch molded plastics (Jane et al., 1993); and cottonseed protein films reacted with formaldehyde, glutaraldehyde, glyoxal, and gossypol (Marquie et al., 1995). In our results, TS of SPI films increased by ≈160% with addition of PGA at a level of 17.5%, which resulted in the strongest film in the experimental ranges tested. At higher levels of added PGA (20%), TS decreased. This may indicate that the increased ratio of PGA to SPI resulted in steric hindrances preventing the hydroxyl groups on PGA from reacting with amino groups on protein chains (Nisperos-Carriedo, 1994).

Percentage elongation at break, a measure of a extensibility, for SPI-PGA films decreased from 170 to 30% as the amount of PGA increased from 0 to 20% (Fig. 3). In

general, increased TS of protein films due to cross-linking has been often accompanied by reduced film E, resulting in less elastic films. Gennadios et al. (1996b) reported increases in TS and decreases in E for SPI films cross-linked by heat curing. Weadock et al. (1984) also reported a similar effect in collagen films cross-linked by glutaraldehyde and UV curing. Different levels of PGA in SPI films showed a difference ($P<0.05$) in mean values of E of SPI-PGA films. These results support cross-linking between PGA and protein as the primary mechanism for strengthening the physical properties of composite films.

Water vapor permeability

WVP of the control SPI film was $2.46 \times 10^{-9} \pm 5.51 \times 10^{-11}$ g·m/m²·s·Pa. This value was in good agreement with reported values for SPI films of similar composition (Brandenburg et al., 1993; Ghorpade et al., 1995). Incorporation of PGA improved the WVP characteristics of the film as evidenced by decrease in WVP values (Fig. 4). WVP of SPI films decreased ($P<0.05$) to the lowest value of $1.99 \times 10^{-9} \pm 4.73 \times 10^{-11}$ g·m/m²·s·Pa with addition of 10% PGA. This decrease may also have been due to the development of cross-linking between protein and PGA. Ghorpade

et al. (1995) had reported that SPI films cross-linked by formaldehyde treatment had slightly lower WVP than untreated films. Further addition of >10% PGA increased WVP of the composite film (Fig. 4). This may have been due to partial destabilization of the protein structural matrix by bulky and hydrophilic PGA molecules widening the interstitial spaces in the protein matrix, thus allowing for an increased diffusion rate of water molecules through the films.

Moisture content and water solubility

Changes in MC of SPI films with different levels of PGA (Fig. 5) showed no clear trend, but MC generally decreased with added PGA. This could be explained by the fact that development of cross-linking between SPI and PGA reduced hydrophilic sites along protein chains decreasing protein access to water molecules. Usually, cross-linked protein films have been reported to have less water uptake capacity (Weadock et al., 1984; Jane et al., 1993). In our results, a decrease in MC may not have occurred because the PGA has many hydrophilic hydroxyl groups and the effect of cross-linking on decrease in MC was likely to be lessened. The WS of SPI films was decreased ($P<0.05$) by adding PGA up to 10%, then increased substantially with higher amounts of PGA. The initial decrease in WS could be considered as direct evidence of SPI-PGA cross-linking in the films. The property of insolubilization of proteins through cross-linking reactions has been reported (Weakley et al., 1961; Marquie et al., 1995) and has been widely applied in paper coatings and leather finishes (Lakshminarayana et al., 1985; Detlefsen, 1989). In the food industry, the increase of water resistance of edible films is an important property in applications such as food protection when water activity is high or

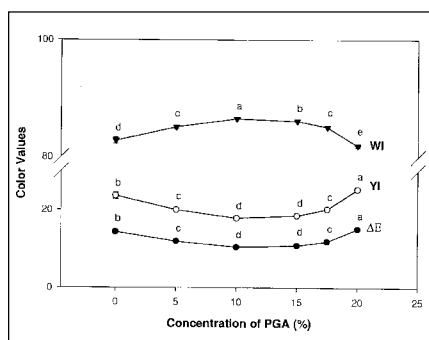


Fig. 1—Change of color values of soy protein isolate-propyleneglycol alginate composite films as related to propyleneglycol alginate content. Vertical bars represent standard deviation and are visible in cases where the error bar is larger than the symbol. Different letters show significant ($P<0.05$) differences.

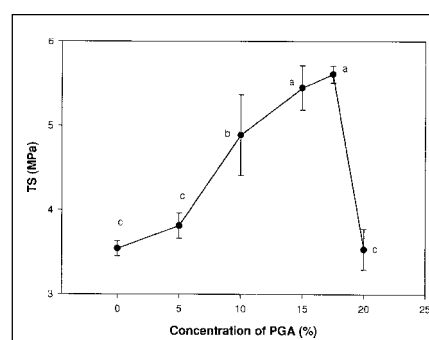


Fig. 2—Changes of tensile strength of soy protein isolate-propyleneglycol alginate composite films as related to propyleneglycol alginate content. Vertical bars represent standard deviation and are visible in cases where the error bar is larger than the symbol. Different letters show significant ($P<0.05$) differences.

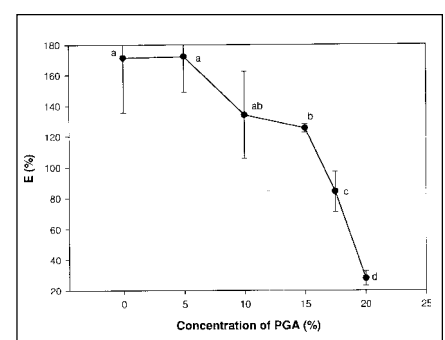


Fig. 3—Change of elongation at break of soy protein isolate-propyleneglycol alginate composite films as related to propyleneglycol alginate content. Vertical bars represent standard deviation and are visible in cases where the error bar is larger than the symbol. Different letters show significant ($P<0.05$) differences.

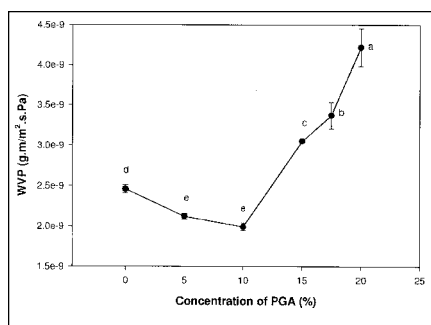


Fig. 4—Change of water vapor permeability of soy protein isolate-propyleneglycol alginate composite films as related to propyleneglycol alginate content. Vertical bars represent standard deviation and are visible in cases where the error bar is larger than the symbol. Different letters show significant (P<0.05) differences.

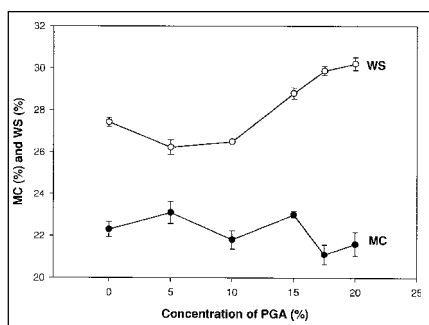


Fig. 5—Change of moisture content and water solubility of soy protein isolate-propyleneglycol alginate composite films as related to propyleneglycol alginate content. Vertical bars represent standard deviation and are visible in cases where the error bar is larger than the symbol. Different letters show significant (P<0.05) differences.

when the film must be in contact with water during processing of the food.

CONCLUSIONS

COMPOSITE FILMS COULD BE MADE BY incorporating PGA into SPI up to 20% w/w of solid. Mechanical strength, water resistance, and resistance against water vapor transfer could be increased with addition of PGA up to 10%. The improvement of such functional properties of SPI-PGA composite films may increase their use in renewable packaging applications. That PGA is edible, degradable, and nontoxic would make its use as an edible, degradable film widely applicable.

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