Preparation and Characterization of Soy Protein Isolate Films Modified with Sorghum Wax

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ABSTRACT: The effect of increasing the concentration of sorghum wax paste on the characteristics of soy protein isolate (SPI) films was investigated. Water vapor permeability (WVP), tensile strength (TS), elongation at break (E), and total soluble matter (TSM) of cast SPI films were determined. Sorghum wax paste extracted with ethanol was added to film-forming solutions of SPI at 5, 10, 15, or 20% w/w of protein. As the concentration of wax paste increased, mean WVP, E, and TSM values of SPI-sorghum-wax-paste composite films decreased and were lower than those of control SPI films. Mean TS values were lower than the control upon addition of 5 and 10% wax paste; however, TS values increased at 15 and 20% wax concentrations. Although no differences in components of sorghum wax were observed between paste extracted with ethanol and wax extracted with hexane, paste extracted with ethanol was miscible with the film-forming solution. SPI-sorghum wax paste films had better water barrier and physical properties compared to control films.

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KEY WORDS: Edible films, ethanol extraction, sorghum wax, soy protein isolate, water vapor permeability.

Proteins from several plant sources have been studied as film-formers. Films prepared from soy protein have poor moisture-barrier properties due to the hydrophilic nature of protein isolate (SPI) and the substantial amounts of hydrophilic plasticizer used in film preparation (1). Nevertheless, there is considerable interest in edible and degradable soy protein films because they are produced from a renewable resource.

Several studies have concentrated on improving the mechanical and barrier properties of soy protein films through physical, chemical, or enzymatic treatments, or addition of hydrophobic compounds. They included treatments to change pH (2); alkylation with sodium alginate or propylene glycol alginate (3); cross-linking with aldehydes (4,5) and UV irradiation (6,7); a heat-curing treatment (8); and enzymatic cross-linking (9,10). Although such treatments improved mechanical film properties, water resistance was only incrementally increased.

Generally, protein films provide limited resistance to moisture transmission because of their inherent hydrophilicity. In contrast, hydrophobic lipids, such as neutral lipids of glycerides, long-chain FA, waxes, resins, oils, and surfactants exhibit good moisture-barrier properties. Composite protein–lipid films, in a laminated or an emulsion form, may be prepared so as to combine the good structural and oxygen-barrier properties of protein films with the good moisture-barrier characteristics of lipids (11). Addition of lipid was reported to lower water vapor permeabilities (WVP) for protein films from caseinates (12), whey protein (13), wheat gluten (14), and zein (15).

Wax, a lipid with long carbon chains in its structure, is a good water barrier because of its high hydrophobicity. The surface waxes on the plant and seeds of grain sorghum play important roles in preserving the water balance by reducing evaporation from their surfaces (16).

Various methods for extracting plant and seed waxes using relatively nonpolar solvents have been reported. Because surface waxes are largely in a hydrophobically associated state, they may be extracted with solvents such as hexane (17,18), benzene (19,20), chloroform (19,20), light petroleum ether (21), or acetone (18). Sorghum waxes extracted using nonpolar solvents are not miscible in aqueous film-forming solutions.

Protein–lipid composite films are difficult to prepare and require additional processing such as heating and homogenizing. Lipid materials are not miscible in aqueous film-forming solutions. Most experiments have been performed with refined lipid materials. Wax, containing polar and hydrophilic materials, may be more miscible in an aqueous film-forming solution than refined wax. Wax recovered by extracting with ethanol likely contains some hydrophilic materials. Therefore, it may serve as a useful additive to improve the water vapor barrier properties of protein films.

Our objectives were to produce a lipid–SPI film using a crude ethanol-extracted sorghum wax and to measure film WVP, total soluble matter (TSM), tensile strength (TS), elongation (E), and its components.

MATERIALS AND METHODS

Wax extraction. Approximately 800 g of whole grain sorghum (red sorghum: golden Harvest H512, harvested in Lancaster County, NE, in 1996) was mixed with approximately 800 mL of ethanol (absolute, 99.5%) in a 2-L round-bottomed flask, heated to the boiling point of ethanol, and refluxed for 30 min. Vacuum filtration was performed using a 2-L side-armed Erlenmeyer flask attached to a 16-cm Büchner funnel fitted with a Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, England) and overlaid with a coffee filter to remove impurities. The filtrate was collected and stored at

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−18°C for at least 8 h to precipitate the wax. Filtrates containing wax precipitates were filtered using a 10-cm Büchner funnel fitted with Whatman No. 42 filter paper. Not all of the ethanol passed through the filter paper. Some ethanol remained with the wax, creating a wax–ethanol paste of approximately 2–3% solids content (dry basis) upon drying in an oven at 104°C for 10 h. Paste was removed from the filter paper and stored at −18°C under a tight seal (to prevent oxidation) until further use. In the case of hexane-extracted wax for TLC analysis, the process was the same as for the ethanol extraction but the wax was a white, solid flaky material after drying at ambient temperature. Wax remaining on the filter paper was collected and stored at −18°C until further use.

Film preparation. Film-forming solutions were prepared by mixing 100 mL of distilled water, 5 g of SPI (Supro 620, Protein Technologies International, St. Louis, MO) and 2.5 g of glycerin (USP grade; Mallinckrodt, Paris, KY). Wax was added at levels of 0, 5, 10, 15 or 20 g wax/100 g SPI on a dry basis and mixed by magnetic stirrer. Sodium hydroxide (2 N) was used to adjust the pH to 10.00 ± 0.01. After pH adjustment, the solutions were held for 15 min in a 75°C water bath and then strained through cheesecloth to remove any bubbles and lumps (only minuscule amounts were present). The film-forming solutions (90 mL) were cast on flat, level, Teflon®-coated glass plates (21 × 35 cm). Films were peeled from the plates after drying at room temperature for about 20 h. Dried films were conditioned at 50% RH and 25°C for 24 h. Each treatment was performed in triplicate.

Thickness. Film thickness was measured to the nearest 2.54 µm (0.1 mil) with a micrometer. Five thickness measurements were taken on each WVP sample, one at the center and four around the perimeter, and the mean was used for calculating WVP. For calculating tensile strength, five thickness measurements were taken along the length of each sample and the mean was used.

Color. Color values of films were measured using a portable colorimeter (CR-300 Minolta Chroma Meter; Minolta Camera Co., Osaka, Japan). Film samples were placed on a white plate, and the HunterLab color scale was used to measure color: L = 0 to 100 (black and white), a = –80 to 100 (greenness and redness), and b = –80 to 70 (blueness and yellowness). Standard values for the white calibration plate were L = 96.86, a = –0.07, and b = 1.98. The change of color was evaluated by comparing total color differences between films. Total color difference (ΔE) was calculated as:

$$\Delta E = \begin{bmatrix} \left(\mathrm{L}_{\text{standard}} - \mathrm{L}_{\text{sample}}\right)^2 + \left(\mathrm{a}_{\text{standard}} - \mathrm{a}_{\text{sample}}\right)^2 \\ + \left(\mathrm{b}_{\text{standard}} - \mathrm{b}_{\text{sample}}\right)^2 \end{bmatrix}^{0.5} \quad [1]$$

Color tests for each type of film were replicated five times.

TS and E at break. TS and E were both measured with an Instron Universal Testing Machine (Model 5566; Instron Corp., Canton, MA) following the guidelines of ASTM Standard Method D 882-91 (22). Initial grip separation was set at 50 mm, and cross-head speed was set at 500 mm/min. TS was expressed in MPa and calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the sample. E was calculated as the ratio of the final length of the point of sample rupture to the initial length of a sample (50 mm), as a percentage. TS and E tests for each type of film were replicated five times.

TSM. TSM was expressed as the percentage of film dry matter dissolved during immersion in distilled water for 24 h. Film pieces (20 × 20 mm) were placed in 50-mL beakers containing 30 mL of distilled water. Beakers were covered with Parafilm 'M' wrap and stored undisturbed at 25°C for 24 h. Undissolved dry matter was determined by removing the film pieces from the beakers, gently rinsing them with distilled water, and drying them in an air-circulating oven (105°C) for 24 h. The weight of dissolved dry matter was calculated by subtracting the weight of insoluble solid matter from the initial weight of solid matter (5). TSM tests for each type of film were replicated three times.

WVP. Five samples were tested for each type of film. WVP ($g \cdot m/m^2 \cdot h \cdot Pa$) was calculated as:

$$\mathrm{WVP} = (\mathrm{WVTR} \cdot L)/\Delta p \quad [2]$$

where WVTR was measured as water vapor transmission rate ($g/m^2 \cdot h$) through a film sample, L was mean film thickness (m), and Δp was partial water vapor pressure difference (Pa) between the two sides of the film sample. WVTR was determined gravimetrically using a modification of ASTM Method E 96-95 (23). Film samples were mounted on polymethylmethacrylate cups filled with 16 mL of distilled water up to 1.03 cm from the film underside. Cups were placed in an environmental chamber set at 25°C and 50% RH. A fan within the chamber moved the air at a velocity of 196 m/min over the surface of the films to remove the permeating water vapor. The weights of the cups were recorded six times at 1-h intervals. Linear regression was used to estimate the slope of this line in g/h. WVP was calculated by using a corrective equation (24).

TLC of wax. Residual solvent was removed from the wax by using vacuum. About 50 mg of either wax (extracted with hexane or ethanol) was dissolved in 5 mL of chloroform while being heated. Then 10 µL of the wax solution was spotted on a silica-gel TLC plate (20 × 20 cm, 250 µm) (Aldrich Chemical Co., Milwaukee, WI). The TLC plate was developed with hexane/diethyl ether/acetic acid (85:15:2). Developed spots were visualized by dipping the plate in 10% cupric sulfate solution dissolved in 8% phosphoric acid for 5 s, drying for 5 min, and heating in an oven at 150°C until the spots charred.

Statistical analyses. ANOVA tables were generated for TS, percent E, WVP, and TSM as a function of level of added wax using the General Linear Model (GLM) procedure, a package program of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Significantly ($P < 0.05$) different treatment means were separated by using Duncan's multiple range test.

RESULTS AND DISCUSSION

The pasty wax extracted by ethanol was miscible in film-forming solutions and mixed easily by stirring, but not the

TABLE 1
Color Values and Total Color Difference (ΔE) of Sorghum Wax–Soy Protein Isolate (SPI) Composite Films[a]

Sorghum wax concentration (% w/w of protein)	L	a	b	ΔE
0	92.51 ± 0.77^{a}	-3.82 ± 0.16^{e}	19.19 ± 1.01^{a}	18.16 ± 1.08^{a}
5	90.72 ± 0.46^{b}	-2.78 ± 0.13^{d}	19.61 ± 1.00^{a}	18.87 ± 1.06^{a}
10	$89.55 \pm 0.73^{c,d}$	-0.88 ± 0.05^{c}	17.02 ± 1.00^{b}	16.74 ± 1.21^{b}
15	89.97 ± 0.40^{c}	-0.42 ± 0.04^{b}	14.90 ± 0.54^{c}	14.65 ± 0.66^{c}
20	89.14 ± 0.69^{d}	0.003 ± 0.15^{a}	16.54 ± 0.80^{b}	16.49 ± 1.03^{b}

[a] Any two means in the same column followed by the same lowercase roman letter are not significantly ($P > 0.05$) different by Duncan's multiple range test.

hexane-extracted wax. Therefore, only SPI-ethanol-extracted sorghum wax films were studied.

Color. Changes in total color difference (ΔE) of sorghum wax–SPI composite films depended on the sorghum wax paste concentration (Table 1). ΔE decreased by adding sorghum wax paste but showed the lowest ΔE value of 14.65 ± 0.66 at 15% of sorghum wax paste. Changes in b values of composite films showed the same pattern as ΔE of the films, which indicates that the apparent color change of the film is mainly caused by the change of yellowness of the film. An increment of wax paste in SPI film decreased L values of films, which decreased the transparency of the film.

TS. The TS value of the control film (0% wax added) was 7.56 MPa ± 0.13 MPa (Table 2). TS were significantly affected ($P < 0.05$) by increasing wax concentration. TS decreased to 6.22 ± 0.54 and 6.44 ± 0.90 MPa at 5 and 10% wax, respectively, and it started to increase as wax concentration was increased further. The reason that TS increased as amounts of wax concentration increased may have been due to the increase of miscibility of protein with the wax paste that contains a considerable amount of ethanol and possibly water. Ethanol might extract more polar materials from sorghum, although in small amounts.

Generally, avoidance of cracks during application of wax films to foods and during subsequent handling of foods has been deemed difficult, if not impossible. In this study, sorghum wax films were flexible and adequate for handling and wrapping.

E at break. All films containing ethanol-extracted sorghum wax had significantly ($P < 0.05$) lower E values than control SPI films (Table 2), and all decreased as the concentration of sorghum wax increased. The mean value of E decreased from 134.6 ± 28.5% for the control to 42.76 ± 13.8% for 20% sorghum wax.

Elongation or extensibility of protein films has been reported to decrease as a result of the addition of various types of lipids. Acetylated MG decreased E of whey protein and caseinate films from 22.74 and 20.84 to 10.78 and 13.58%, respectively (25). Lipid-rich egg yolk solids decreased E at break of egg albumin film to 20%, whereas the mean E value of the control was 77% (26). Lauric, myristic, and palmitic acids reduced SPI film extensibility to a ratio of 0.71–0.18 to control (1).

Reduced extensibility and TS of film from proteins with added lipids were assumed to result from unstable structural integrity (1). However, SPI films containing sorghum wax paste had high TS and low E; thus, SPI–sorghum wax film had better structural integrity than films developed in previous research (1,3,5,8).

TSM. Similar to WVP, TSM offers an indication of a film's hydrophilicity (1). Incorporation of ethanol-extracted sorghum

TABLE 2
Water Vapor Permeability (WVP), Tensile Strength (TS), Elongation at Break (E), and Total Soluble Matter (TSM) of Soy Protein–Sorghum Wax Composite Films[a]

Sorghum wax concentration (% w/w of protein)	WVP ($\times 10^{-6}$ g·m/m^2·h·Pa)	TS (MPa)	E (%)	TSM[b] (%)
0	9.40 ± 1.77^{a}	7.56 ± 0.13^{b}	134.6 ± 28.5^{a}	34.35 ± 3.19^{a} (34.35 ± 3.19^{a})
5	6.73 ± 0.97^{b}	6.22 ± 0.54^{c}	105.6 ± 22.3^{b}	$30.65 \pm 0.91^{a,b}$ $(28.33 \pm 0.93^{a,b})$
10	$5.51 \pm 0.06^{b,c}$	6.44 ± 0.90^{c}	79.34 ± 13.5^{c}	$30.25 \pm 0.72^{a,b}$ $(25.59 \pm 0.71^{a,b})$
15	$4.72 \pm 0.31^{c,d}$	7.23 ± 0.91^{b}	67.09 ± 7.78^{c}	26.71 ± 3.29^{b} (19.38 ± 3.62^{b})
20	3.75 ± 0.28^{d}	8.53 ± 0.79^{a}	42.76 ± 13.8^{d}	27.96 ± 5.06^{b} (18.28 ± 6.69^{b})

[a] Any two means in the same column followed by the same lowercase roman letter are not significantly ($P > 0.05$) different by Duncan's multiple range test. For other abbreviation see Table 1.
[b] TSM values in parentheses were calculated using only SPI and glycerin weight as basis.

wax significantly reduced ($P < 0.05$) TSM of SPI films. It decreased to 27.96 ± 5.06% at a wax concentration of 20%, from a control value of 34.35 ± 3.19%. When TSM was calculated using only the amount of soluble matter from SPI and glycerin, TSM values were from 18.28 to 28.33% for the portion of SPI and glycerin in dry matter of films. Greater TSM reductions were reported for increasing levels of emulsified FA or composite SPI films (1,11). Rhim *et al.* (11) reported that, similar to WVP, water solubility is affected more by the chemical nature of films than by the structural integrity of films.

WVP. All SPI films containing ethanol-extracted sorghum wax had significantly ($P < 0.05$) lower mean WVP values than the mean WVP of $(9.40 \pm 1.77) \times 10^{-6}$ g·m/m^2·h·Pa for the control (Table 2). A significant ($P < 0.05$) reduction in film WVP [decreased to $(3.75 \pm 0.28) \times 10^{-6}$ g·m/m^2·h·Pa] was observed as wax concentration increased to 20% (dry basis) of SPI.

Trials to decrease WVP of bilayer edible films or composite edible films by adding lipids and waxes have been reported. WVP decreases as chain length and concentration of FA increases. Although Kamper and Fennema (27) did not use sorghum wax, they reported that films coated with solid beeswax or paraffin wax were very effective barriers to the transfer of water vapor.

Gontard *et al.* (14) determined WVP for various edible bilayer films including beeswax, paraffin wax, carnauba wax, insect wax, MG, and palm oil as the lipid layer. Waxes provided excellent moisture barriers compared to MG or palm oil, and WVTR decreased significantly as concentrations of waxes increased (14).

Rhim *et al.* (11) investigated changes in selected properties of soy protein films resulting from incorporation of FA. Emulsified soy protein–FA composite films showed lower mean WVP values from 5.76×10^{-6} to 8.28×10^{-6} g·m/m^2·h·Pa, than control values of 1.26×10^{-5} g·m/m^2·h·Pa (11). SPI–sorghum wax composite films, in this study, had considerably lower WVP than the SPI–FA composite films of Rhim *et al.* (11).

TLC analysis of sorghum wax. No major differences in main components in TLC trials were observed between the sorghum waxes extracted with ethanol and those extracted with hexane (Fig. 1). Fatty aldehydes, FA, fatty alcohols, hydrocarbons, and wax esters appeared to be the dominant components in both sorghum waxes. Although compositions of wax classes were not quantified in this study, Bianchi *et al.* (19) reported that sorghum wax contained 32% aldehydes, 24% FA, 34% fatty alcohols, 1.3% hydrocarbons, and 4% wax esters, whereas Avato *et al.* (20) reported 21% aldehydes, 27% FA, 32% fatty alcohols, 7% hydrocarbons, and 13% wax esters. Despite the similarities in the major components of the ethanol- and hexane-extracted sorghum waxes, differences were noted between the cold-storage precipitates of the solvents. Precipitates of ethanol extracts formed during storage in a freezer were so pastelike that filtration was very difficult and time-consuming in the subsequent wax recovery process, but the wax extracted with hexane was readily recoverable in a dry, flaky or powdery state. This implied that ethanol might extract more polar materials and water with wax from grain sorghum. These more polar materials, although in small amounts, subsequently remained in the wax even after filtration and may have functioned as emulsifiers or plasticizers in film-making. However, further study of the minor components should be conducted to understand the function of the wax.

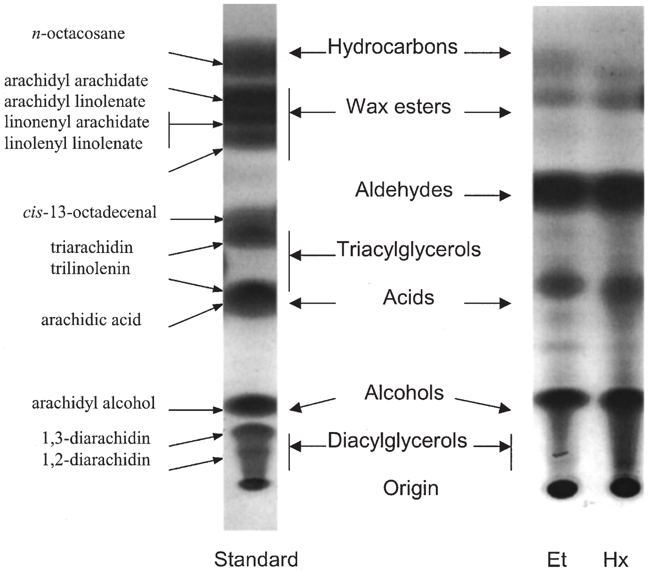

FIG. 1. TLC of waxes. Et, sorghum wax extracted with ethanol; Hx, sorghum wax extracted with hexane.

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