Influence of Sorghum Wax, Glycerin, and Sorbitol on Physical Properties of Soy Protein Isolate Films

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ABSTRACT: Sorghum wax, sorbitol, glycerin, and soy protein isolate (SPI) composite films were prepared. Effects of sorghum wax, sorbitol, and glycerin concentrations on various films were evaluated using response surface methodology. All independent variables significantly ($P < 0.05$) affected film water vapor permeability (WVP), tensile strength (TS), elongation at break (E), total color difference, and total soluble matter (TSM). Increasing the sorghum wax concentration decreased WVP and E. As sorbitol content increased in the composite films, WVP and TS increased. Sorbitol had a critical point of 2–5 g/5 g SPI for low values of TSM. The addition of sorbitol contributed more to the properties of the film than did glycerin.

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KEY WORDS: Edible films, RSM design, sorghum wax, soy protein isolate, water vapor permeability.

Hydrophilic edible films, including protein films, provide barriers to oxygen and carbon dioxide and are friendly to the environment because of their degradibility and renewability. However, they have higher water vapor permeability and weaker mechanical properties than synthetic films. Poor performance as a water vapor barrier is one of the main limitations of protein films. This is attributed to the hydrophilic nature of proteins and to the considerable amount of hydrophilic plasticizers typically incorporated into protein films (1).

One way of improving moisture barrier properties of protein films is to include additives that are hydrophobic in nature. Accordingly, lipidic materials such as neutral lipids, FA, or waxes have been incorporated into protein films. 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 emulsified 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 (2).

Cast protein–lipid films have been reported to have lower water vapor permeability (WVP) values than control protein films produced using caseinates (3), whey protein (4), wheat gluten (5), or zein (6). It is well known to film scientists that protein–lipid composite films are difficult to prepare, requiring 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. Unrefined wax, which naturally contains some extraneous polar and hydrophilic materials, may be more miscible in an aqueous film-forming solution than refined wax. Wax recovered from extraction with ethanol contains some hydrophilic materials (7). Therefore, wax of unrefined origin may serve as a useful additive to improve the water vapor barrier properties of protein films. It likely would be less expensive than refined wax, and its extraneous material would aid film formation.

Glycerin, as a plasticizer, increases not only film flexibility but also WVP. Polyols, such as sorbitol, plasticize effectively due to their ability to reduce internal hydrogen bonding while increasing intermolecular spacing. Sorbitol was reportedly very compatible with glycerin in soy protein isolate (SPI) films or wheat gluten films. Sorbitol was more effective at smaller added amounts than glycerin as a plasticizer in casein films of equal mechanical properties such as tensile strength (TS) and elastic modulus (8).

The objective of this study was to gain a better understanding of plasticizer influences on properties of SPI film under different unrefined grain sorghum wax concentrations. The effects and interactions of various levels of sorghum wax, glycerin and sorbitol on WVP, TS, elongation at break (E), total color difference (ΔE), and total soluble matter (TSM) of films were studied using response surface methodology.

MATERIALS AND METHODS

Statistical design. A response surface experiment was designed to identify the relationships between three independent variables—content of sorghum wax (%, w/w protein, X_1), sorbitol (g/5 g protein, X_2), and glycerin (g/5 g protein, X_3)—and measured film properties. The specific experimental design adopted was a central composite response surface design (9). The independent variables were coded as $-\alpha$, –1, 0, 1 and α. The actual values of the independent variables were chosen based on preliminary studies, and the corresponding coded values of independent variables are given in Table 1. The complete design consisted of 19 experimental points that included five center points. Treatments from one to eight were tested in duplicate. Films were prepared in random order. Responses or film property values under observa-

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TABLE 1
Response Surface Level Combinations of Independent Variables in the Experimental Design and Responses of Dependent Variables

	Independent variable				Dependent variable				
Design point	Sorghum wax (% g/g)	Sorbitol (g/5 g SPI)	Glycerin (g/5 g SPI)	MC[a] (%)	WVP[b]	TS[c] (MPa)	E[d] (%)	ΔE[e]	TSM[f] (%)
	X_1	X_2	X_3		Y_1	Y_2	Y_3	Y_4	Y_5
1[g]	–1 (6.08)	–1 (2.03)	–1 (1.01)	19.25	6.18	4.58	89.37	18.33	91.56
2[g]	–1 (6.08)	–1 (2.03)	1 (3.98)	41.92	14.80	1.07	139.23	18.41	52.57
3[g]	–1 (6.08)	1 (7.97)	–1 (1.01)	19.59	11.0	0.79	109.25	18.51	100.68
4[g]	–1 (6.08)	1 (7.97)	1 (3.98)	32.06	34.81	0.33	118.68	16.49	72.26
5[g]	1 (23.9)	–1 (2.03)	–1 (1.01)	19.24	2.23	7.60	32.25	19.64	62.98
6[g]	1 (23.9)	–1 (2.03)	1 (3.98)	39.60	6.11	2.03	48.04	23.39	50.23
7[g]	1 (23.9)	1 (7.97)	–1 (1.01)	17.13	8.14	1.17	70.91	22.09	88.43
8[g]	1 (23.9)	1 (7.97)	1 (3.98)	28.61	13.67	0.44	74.85	22.51	67.99
9	–α (0.00)	0 (5.00)	0 (2.50)	25.87	18.54	0.9	217.00	22.54	61.06
10	α (30.0)	0 (5.00)	0 (2.50)	23.95	7.79	1.74	69.81	23.66	46.42
11	0 (15.0)	–α (0.00)	0 (2.50)	29.44	5.35	9.42	20.56	23.88	25.91
12	0 (15.0)	α (10.00)	0 (2.50)	26.59	13.89	0.65	36.75	18.08	82.73
13	0 (15.0)	0 (5.00)	–α (0.00)	11.83	4.24	4.15	47.80	17.03	98.13
14	0 (15.0)	0 (5.00)	α (5.00)	40.61	17.27	0.56	106.25	22.33	58.87
15[h]	0 (15.0)	0 (5.00)	0 (2.50)		13.33	1.19	118.60	26.22	54.59
16[h]	0 (15.0)	0 (5.00)	0 (2.50)		10.96	1.29	122.12	25.30	57.21
17[h]	0 (15.0)	0 (5.00)	0 (2.50)	23.82[i]	11.12	1.23	103.73	24.97	53.87
18[h]	0 (15.0)	0 (5.00)	0 (2.50)		8.81	1.41	104.57	25.58	56.67
19[h]	0 (15.0)	0 (5.00)	0 (2.50)		8.02	1.31	84.21	25.86	60.73

[a] Moisture content, mean of three replications.
[b] Water vapor permeability (WVP) expressed $g \cdot m/m^2 \cdot h \cdot Pa$, mean of three replications.
[c] Tensile strength (TS), mean of five replications.
[d] Elongation at break (E), mean of five replications.
[e] Total color difference (ΔE), mean of ten replications.
[f] Total soluble matter (TSM), mean of three replications.
[g] Mean of two replications from 1–8 design points.
[h] Central points.
[i] Mean of five central points.

tions were WVP, TS, E, TSM, and ΔE. Data were analyzed to fit the following third-order equation for each response variable:

$$\begin{aligned} Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 \\ + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 \\ + b_{33}X_3^2 + b_{111}X_1^3 + b_{222}X_2^3 + b_{333}X_3^3 \end{aligned} \qquad [1]$$

where the b_n values were regression coefficients and X_1, X_2, and X_3 were the coded independent variables. All statistical calculations were performed using the SAS/STAT™ statistical package (10).

Extraction of wax. Approximately 800 g of whole grain sorghum (red sorghum: Golden Harvest H512 harvested in Lancaster County, Nebraska, in 1996) was mixed with approximately 800 mL of ethanol (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 (Coors 60246) fitted with a Whatman No. 2 filter paper (Whatman International Ltd., England) and overlaid with a coffee filter to remove impurities. The filtrate was collected and stored at –18°C for at least 8 h to precipitate the wax. Filtrates containing wax precipitates were filtered using a 10-cm Büchner funnel (Coors 60243) fitted with Whatman No. 42 filter paper. It is important to note that not all of the ethanol passed through the filter paper. Some remained with the wax, creating a wax/ethanol paste (unrefined wax) containing 2–3% wax. Wax/ethanol paste was stored at –18°C in cap-sealed bottles until it was used for film preparation.

Film preparation. Film-forming solutions were prepared by mixing 100 mL of distilled water and 5 g of SPI (Supro 620; Protein Technologies International, St. Louis, MO). Glycerin (USP grade; Mallinckrodt, Paris, KY), sorbitol (crystalline sorbitol, SORBOGEM™; SPI Polyols, Inc., New Castle, DE) and wax were added at levels consistent with the response surface design, on a dry basis. Sodium hydroxide (2 N) was used to adjust the solution pH to 10.00 ± 0.01. Then, solutions were held for 15 min in a 75°C water bath and strained through cheesecloth (Cheesecloth Wipes™; VWR Scientific Products, Chicago, IL) to remove any bubbles and lumps (only minuscule amounts were present). The film-forming solutions were cast on flat, level, Teflon®-coated glass plates (21 × 35 cm). Films were peeled from the plates after drying at room temperature for 20 h. Dried films were conditioned at 50% RH and 25°C for 48 h.

Thickness. Film thickness was measured to the nearest 2.54 μm (0.1 mil) with a hand-held micrometer (B.C. Ames

Co., Waltham, MA). Five thickness measurements were taken on each WVP specimen, one at the center and four around the perimeter, and the mean was used in the WVP calculation. For TS calculations, five thickness measurements were taken along the length of each specimen, and the mean was used in calculating film TS.

Color. Color values of films were measured using a portable colorimeter (CR-300 Minolta Chroma Meter; Minolta Camera Co., Osaka, Japan). Film specimens 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. ΔE was calculated as:

$$\Delta E = [(L_{\text{standard}} - L_{\text{sample}})^2 + (a_{\text{standard}} - a_{\text{sample}})^2 + (b_{\text{standard}} - b_{\text{sample}})^2]^{0.5} \qquad [2]$$

Color measurements 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 (11). Initial grip separation was set at 50 mm, and cross-head speed 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 specimen. E was calculated as the ratio of the final length of the point of sample rupture to the initial length of a specimen (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 (American National Can, Chicago, IL) and stored at 25°C for 24 h. Dissolved dry matter was determined by discarding the water from the beakers and drying the remaining film specimen 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 (12). TSM tests for each type of film were replicated three times.

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

$$\text{WVP} = (\text{WVTR} \cdot l)/\Delta p \qquad [3]$$

where WVTR was measured water vapor transmission rate ($g/m^2 \cdot h$) through a film specimen, l was mean film specimen thickness (m), and Δp was partial water vapor pressure difference (Pa) between the two sides of the film specimen. WVTR was determined gravimetrically using a modification of ASTM Method E 96-95 (13). Film specimens 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 was operated in the chamber to move the air with a velocity of 196.3 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 corrective equation (14).

RESULTS AND DISCUSSION

Statistical analysis and response surfaces. Table 2 summarizes the results of the ANOVA for each of the dependent variables with their corresponding coefficients of multiple determination (R^2). The three independent variables had effects on film properties. TS (y_2) and TSM (y_5) had significant lack of fits ($P < 0.05$) but had sufficiently high R-square values to indicate that the data were adequately explained. The TS and TSM models were considered approximate and can be used for trend analysis. 3D response surfaces (Figs. 1–3), generated by the regression equations of Table 2, show effects of sorbitol, glycerin, and sorghum wax on prescribed film properties.

WVP. Three factors influenced WVP of SPI film. The shape of the response surface (Fig. 1) was characteristic of the interaction of three variables. The lowest WVP values were observed at the lowest concentration of sorbitol. Generally speaking and as would be expected, the lowest WVP was obtained with the 30% (w/w SPI) sorghum wax. The addition of sorbitol gave lower WVP values compared to glycerin-plasticized whey protein films (15) or starch films (16), but even the absences of glycerin and sorbitol did not decrease the WVP in this study. This result could have been due to the nonwax components of the ethanol-extracted sorghum wax. It likely contained some hydrophilic materials. The outer surface of the cuticle of grain sorghum kernels is covered with epicuticular waxes that can take diverse forms. Intracuticular waxes are embedded in the cutin polymer and little information is available on their composition. Wattendorf and Holloway (17) reported that plant cuticles also contain nonlipid, hydrophilic constituents such as polysaccharides. Although previous reports were related to leaves and fruits, it is highly probable that wax extracted using ethanol contained hydrophilic constituents. Therefore, it is assumed that sorbitol or glycerin molecules have linked with hydrophilic materials of wax or try to occupy the hydrophilic sites of protein competitively.

At the low concentration of sorbitol, glycerin did not affect WVP. Otherwise, glycerin increased the WVP at the high concentration of sorbitol. Sorghum wax concentration decreased the WVP. Lipids or waxes are effective water vapor barriers. The lowest WVP value of 2.23×10^{-6} $g \cdot m/m^2 \cdot h \cdot Pa$ was obtained at the fifth design point. It was lower than the lowest value of 4.6×10^{-6}g and 3.4×10^{-6} $g \cdot m/m^2 \cdot h \cdot Pa$ reported for soy protein/FA and soy protein/lipid emulsified film, respec-

TABLE 2
Regression Coefficients and Analyses of Variance[a]

Coefficient	Water vapor permability, (WVP) (Y_1)	Tensile strength, (TS) (Y_2)	Elongation at break (E) (Y_3)	Total color difference (ΔE) (Y_4)	Total soluble matter (TSM) (Y_5)
b_0	11.12*	10.85***	57.76	11.29***	127.9***
Linear					
b_1	–0.6525*	0.2622**	–9.560***	–0.3163	–3.194***
b_2	–3.4336*	–3.544***	31.54***	–0.02904	8.1327
b_3	0.1461	–2.015***	42.45**	9.054***	–41.91***
Quadratic					
b_{11}	0.01511**	–0.002356	0.1755**	0.04248*	0.02341
b_{22}	0.6981**	0.4519***	–3.009***	0.3134	–2.223*
b_{33}	0.1571*	0.08056	–4.293	–2.704**	4.802***
Two-factor cross					
b_{12}	0.05389	–0.01651**	0.3121	0.01558	0.2130
b_{13}	–0.002182	–0.02211	–0.3746	0.05764**	0.6140**
b_{23}	1.123***	0.2237***	–1.483	–0.1537**	0.9524
Cubic					
b_{111}	—	—	—	–0.001269**	—
b_{222}	–0.0469**	0.2237***	—	–0.03534**	0.16338**
b_{333}	—	—	—	0.2172**	—
b_{123}	–0.04308**	—	—	—	–0.0581
Lack of fit	0.2373	0.0415	0.8445	0.1756	0.0020
R-square	0.9602	0.9580	0.8103	0.9436	0.9281
F	32.93	36.54	8.07	14.35	17.62
Probability of *F*	<0.0001	<0.0001	0.0001	<0.0001	<0.0001

[a] Model for analysis of property values (Y) used X_1 = sorghum wax concentration (%, w/w soy protein), X_2 = g of glycerin in 5 g of soy protein, and X_3 = g of sorbitol in 5 g of soy protein, and $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{111}X_1^3 + b_{222}X_2^3 + b_{333}X_3^3$.
*Significant at 10% level, **significant at 5% level, ***significant at 1% level.

tively (2). However, it was higher than whey protein–lipid emulsion films and wax films (4).

TS. Increasing the sorbitol and glycerin contents decreased film TS. Decreases in TS with increases in plasticizer concentration have been reported for whey protein isolate films plasticized with sorbitol and glycerol together (15); for egg albumen films plasticized with sorbitol, glycerol, or polyethylene glycol (18); and for films made from gelatin, soluble starch, and poly-

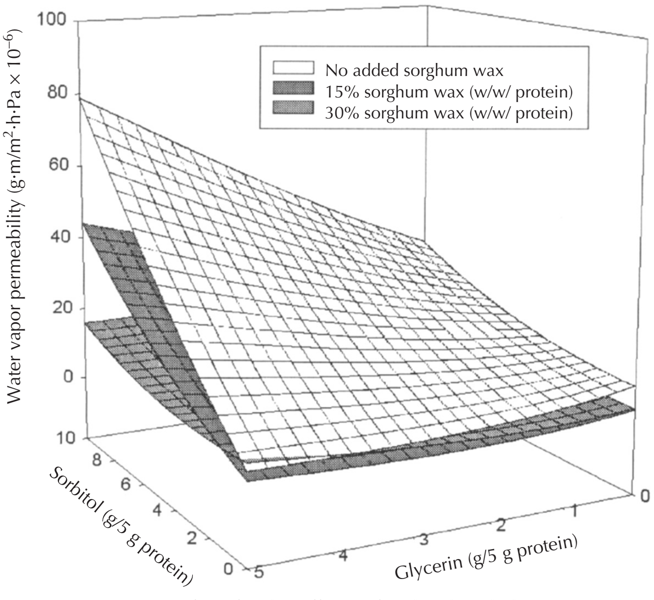

FIG. 1. Response surface for the effects of sorbitol and glycerin concentrations on the water vapor permeability of sorghum wax–soy protein isolate composite films.

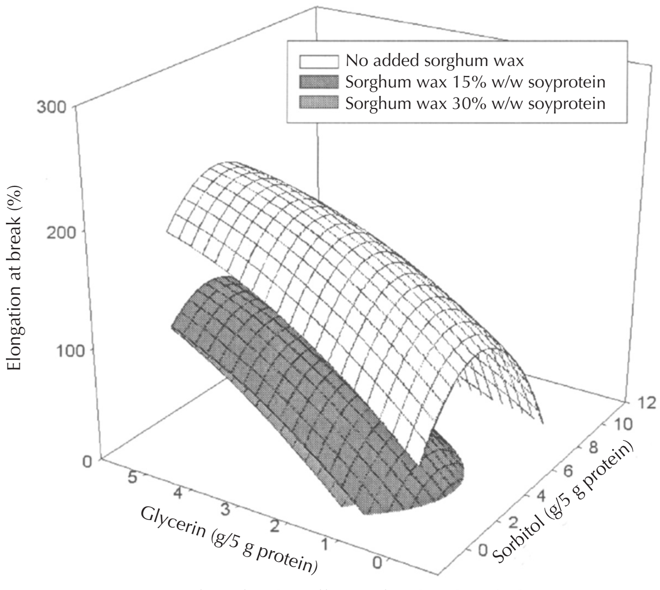

FIG. 2. Response surface for the effects of sorbitol and glycerin concentrations on the elongation at break (%) of sorghum wax–soy protein isolate composite films.

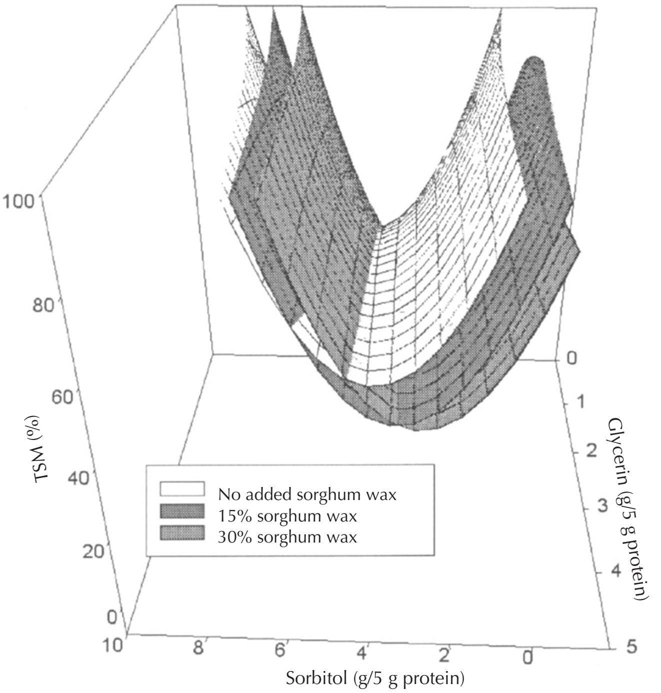

FIG. 3. Response surface for the effects of sorbitol and glycerin concentrations on the total soluble matter (TSM) of sorghum wax–soy protein isolate composite films.

ols (19). However, increases in TS were reported with increases in sorbitol concentration for lactic acid- and rennet-precipitated casein-based films (8). Sorbitol may have resulted in decreased TS strength when it was used with other plasticizers. The sorghum wax, glycerin, and sorbitol used in this study may not have linked with each other or may not have cooperated to improve film structural integrity. High TS would be expected at low levels of glycerin and sorbitol. TS of films at the fifth and eleventh design points were 7.60 and 9.42 MPa, respectively (Table 1). These values were much higher than those for films made from oils/waxes and zein (20), or soy protein and FA (2).

Elongation at break (%). Addition of sorghum wax decreased film E, but the level of the addition did not affect it. The response surface showed that glycerin increased the E value, but sorbitol had a critical concentration of approximately 2 g/5 g SPI (Fig. 2). Increases in E values with increasing concentration of plasticizers were reported for whey protein (15), egg albumen (18), and gelatin-soluble starch films (19). Generally, increasing TS accompanies decreasing elongation in films. However, the decrease in elongation of specimens of low TS may have been caused by excess amounts of sorbitol that weakened films so that they were easily torn during Instron tests.

ΔE. Response surface analysis indicated that ΔE values were of the highest value near the central points. Ranges of *L*, *a,* and *b* values near the central points were 87.8–92.4, –2.57–4.53, and 9.38–20.38, respectively. The *b* values varied drastically with film composition.

TSM. TSM decreased for sorbitol levels of 4.5, 3.5, and 2.5 g/5 g SPI at sorghum wax levels of 0, 15, and 30%, respectively. As the concentration of sorghum wax increased, the lowest value of TSM shifted to a lower value (Fig. 3). Glycerin was not effective at all in changing the TSM or else was much less effective than other factors in this study. Interestingly, TSM had its lowest values at a sorbitol level of approximately 2 g/5 g protein. This result related well to the response surface of the E value.

Implications. The proposed use of an unrefined wax in place of a refined wax in SPI films to reduce costs and improve film structural integrity appears validated. Generally, film properties of SPI films with added sorghum wax paste were similar to or better than those with no wax or refined wax. For example, WVP was lower for SPI films with sorghum wax paste than SPI films with other added refined lipid materials. The increased TS of SPI films with added sorghum wax paste over TS of SPI films with other added lipid materials may be attributed to the increased structural integrity afforded by the sorghum wax paste.

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