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Film-Forming Properties of Proteinaceous Fibrous Material Produced from Soybean Fermented by *Bacillus natto*

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Abstract The effectiveness of a proteinaceous fibrous material formed during commercial fermentation of soy protein (PFSP) and cysteine addition were evaluated in order to improve on the properties of soy protein-based films. Nine types of films were prepared at pH 7, 9, and 11, with heat treatments at 70°C and 90°C for 30 min, by casting 5% (w/w) PFSP aqueous solution, containing 2.25% (w/w) glycerol, on to polystyrene plates. The tensile strength (TS) of films ranged from 3.88 to 6.87 MPa. The highest puncture strength (PS) was observed with pH 7.0 films prepared from PFSP solution heated at 70°C ($P<0.05$). Alkaline pH and temperature caused a decrease in both the TS and PS of the films. The thickness of films ranged from 58 to 74 μm. Water vapor permeabilities of the films decreased with increasing pH and temperature. To produce films from PFSP, pH value of 7.0 to 9.0 and heat treatment of 70°C to 90°C were needed. A soluble nature of PFSP films in water might be useful for preparation of hot water-soluble pouches. Cysteine addition could be necessary to produce films with increased TS and enhanced barrier properties. The combination treatment that provided the best combination of barrier and mechanical properties was the PFSP film prepared at pH 7.0 with addition of 1% cysteine. The films were good oxygen barriers.

Key words: Fibrous material, *Bacillus natto*, edible film, cysteine, food packaging material

Edible films offer alternative packaging without serious environmental pollution because of their biodegradable nature. Edible films are not meant to totally replace synthetic packaging films, but they do have the potential to reduce packaging and to limit moisture, aroma, and lipid migration between food components where synthetic packaging cannot function. An important aspect of these films is the renewable nature of the raw materials used for their production. Edible coatings and films can prevent quality changes in foods by acting as barriers to control transfer of moisture, oxygen, carbon dioxide, lipid oxidation, and loss of volatile flavors and aromas. They can also carry food ingredients and nutritious additives. Edible films and coatings used on pharmaceutical products, confections, fruits, vegetables, and some meat products are typically derived from lipids, proteins, carbohydrates, or composites of the three [3, 8]. Proteins that have been studied as potential film-forming agents include collagen, gelatin, corn zein, casein, whey protein, wheat gluten, soy protein isolate, rice bran proteins, serum albumin, peanut and cottonseed proteins, and egg white protein [7, 11, 20, 22]. Soy protein is one of the widely used proteins, because of its abundance and bioactivities [24]. Incorporation of various polysaccharides and lipids to soy protein has also been investigated [6, 23]. Recently, an enhancement of food storability has been studied utilizing fermented soy protein film [9, 10].

A fibrous material from soybean is usually observed on the surface of soybean paste during fermentation. This material appears first as small strands that eventually develop into long fibrous structures [13]. Preliminary observations indicated the material to be primarily protein. Proteinaceous fibrous material formed during commercial fermentation of soy protein (PFSP) may be an enzyme derived from natto, a food of boiled soybeans fermented with *Bacillus natto*. Some study has shown that a fibrous material from soybean supports the body in breaking up and dissolving unhealthy coagulated blood and maintains fibrinolytic activity. A fibrous material from soybean appears to be promising in support of areas such as cardiovascular disease, stroke, angina, venous stasis, thrombosis, emboli, atherosclerosis, fibromyalgia/chronic fatigue, claudication, retinal pathology, hemorrhoid, varicose veins, soft tissue

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rheumatisms, muscle spasm, poor healing, chronic inflammation and pain, peripheral vascular disease, hypertension, tissue oxygen deprivation, infertility, and gynecology conditions (e.g., endometriosis and uterine fibroids).

Advantages of soy protein films include their flexibility, smoothness, transparence, and clearness, compared with other films from plant proteins. Formation of soy protein film is the result of polymerization of heat-denatured proteins with disulfide and hydrophobic bonds, which are the main force to maintain the film network [5]. The cysteine groups can undergo polymerization via sulfhydryl-disulfide interchange reactions during heating to form a continuous covalent network upon cooling [9]. The addition of cysteine may thus be advantageous, owing to disulfide rearrangement.

The objectives of this research were to determine the effects of pH and heat treatment on the tensile strength (TS), puncture strength (PS), water vapor permeability (WVP), protein solubility in water, and color of PFSP films, and to evaluate how cysteine addition would modify the physical and mechanical properties of PFSP films.

MATERIALS AND METHODS

Materials

PFSP was collected from the surfaces of soybean paste using a perforated stainless steel spoon and stored at 4°C for further processing. Commercial SPI was obtained from Archer Daniels Midland Co. (Decatur, IL, U.S.A.). The protein content in the SPI was 90% of dry weight, and moisture and lipid contents were 6.5% and 1%, respectively. Cysteine was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetic acid, sulfuric acid, ammonium hydroxide, glycerol, cupric sulfate, 5,5' dithio-bis(2-nitrobenzoic acid)/DTNB, sodium sulfite, EDTA, and urea were purchased from Fisher Scientific (Pittsburg, PA, U.S.A.). Tris base was purchased from Bio-Rad (Hercules, CA, U.S.A.). All chemicals were of reagent grade.

PFSP Film Preparation

Film-forming solutions were prepared by dispersing PFSP (5%, w/w) and glycerol (2.25%, w/w) in deionized water. The solution was mixed using a mechanical homogenizer (Virtishear Tempest, The VirTis Co., Gardiner, NY, U.S.A.) at setting 6 for 30 min. To determine the effect of pH, PFSP film solutions were adjusted to pH 7.0, 9.0, or 11.0 using either 0.1 or 1.0 N NaOH. Subsequently, the solutions were heated at 70°C or 90°C in a water bath for 30 min. To determine the effect of cysteine, PFSP film solutions were prepared and cysteine (1%, w/w) was added to one of the solutions. SPI (7.5 g) was weighed for the control film, dispersed in 120 ml of water, and plasticizer (2.5 g glycerol) was added. After degassing under vacuum for 20 min, 12 ml of the solution was poured on the polystyrene plates (casting surface area 75 cm^2). Cast solutions were allowed to dry overnight at room temperature. Upon drying, films were peeled from the polystyrene weighing boats and were placed in a 4-shelf desiccator (50 cm×35 cm×30 cm) until further evaluation. Desiccator conditions were 23°C and 55% RH, and humidity was maintained using 12 N sulfuric acid solution [18].

Film Thickness

Film thickness was measured with an electronic digital micrometer with a sensitivity of 1.25 μm (Perkin's Elmer, Model 2804-10, Mitutoyo, Japan). Filmstrips were placed within the micrometer and the gap was reduced until first contact was noted. Measurements were taken at 6 locations, and the mean thickness was used to calculate barrier and mechanical properties.

Mechanical Properties

Mechanical properties were measured with a texture analyzer (TA.XT2, Texture Technologies, Corp., New York, U.S.A.). Sample preparation and handling for texture analyses were carried out according to the standard methods D 882-91 (ASTM, 1991). Filmstrips, measuring 100 mm×25 mm, were mounted onto the texture analyzer. The filmstrips were pulled 7.5 mm apart at a speed of 2 mm/s in tension mode. Tensile strength (TS) in MPa was calculated by dividing the peak load developed during the test by the film cross-sectional area. Puncture strength (PS) was measured as follows: circular film samples with 6.5-cm diameter were mounted onto a cup and secured between a metal rim and rubber gasket by 6 screws placed symmetrically around the circumference. With a cylindrical probe (3-mm diameter) at compression mode (TA.XT2), the films were punctured, and the force at the point of rupture was recorded in N and expressed as PS. Percentage puncture deformation was calculated by multiplying the deformation at the moment of rupture by 100%.

Water Vapor Permeability

Circular film samples of about 12-cm diameter were placed over the open mouth of aluminum cups (area 33 cm^2) and secured between a metal rim and rubber gasket. Water vapor permeability (WVP) was measured in a chamber at 22°C, conditioned at 50% relative humidity (RH). Air velocity was approximately 163 m/min over the surface of the cups to remove the permeating water vapor. Distilled deionized water was placed in the cups with an air gap of 1.4 cm above the water surface. The cup assembly was weighed every 30 min for a minimum of 10 h. Weight loss vs. time was ploted. Linear regression-derived slopes were used to estimate water vapor transmission rate, and WVP measurements expressed in g/m·s·Pa were performed as described by McHugh *et al.* [14, 15].

Oxygen Permeability

Oxygen permeability was measured as described by Sabato *et al.* [19]. Film samples were masked with precut aluminum foil (2.0 cm^2 uncovered area) for mechanical support and a more uniform thickness. These were clamped in the testing cells, and a leak test was done to ensure that the oxygen detected by the sensor was oxygen transmitted through the sample and not that admitted through leaks. For the first 10 min, O_2 was purged across the feed side of the film to remove any trace impurities in the line. Permeability measurements were made using the conditions previously described: at 35°C, 55% RH, and 103 kPa. To determine the flux of gas through the film, a Baratron transducer (MKS Instruments Inc., Andover, MA, U.S.A.) was used to measure the increase of downstream pressure with time. The testing time for each cell in the test cycle (dwell time) was about 12 h. Oxygen permeabilities were calculated as described by Sabato *et al.* [19].

Disulfide Bond Concentrations in Film Solutions

Sodium sulfite, DTNB, cupric sulfate, Tris base, EDTA, and urea were used to synthesize 2-nitro-5-thiosulfobenzoate (NTSB), according to the method of Sabato *et al.* [19]. The film-forming solutions were diluted 25-fold and mixed with the NTSB assay solution (1:1, v/v). Absorbance was measured at 412 nm for 20 min against a blank (Varian spectrophotometer, Varian Instrument Division, Palo Alto, CA, U.S.A.). For converting the absorbance to disulfide bond concentration, an A_{412} value of 13,600 $M^{-1}cm^{-1}$ was used.

Protein Solubility

Small pieces of films (25–30 mg) were placed in 5 ml of deionized water. Film samples were conditioned at ambient temperature and 50% RH for 48–52 h prior to the solubility test. The film suspension was incubated at room temperature (22–25°C) for 24 h by gentle shaking. Solubility was determined by measuring the protein content of the supernatant using the micro Kjeldahl method [1], and percent solubility was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{Protein in supernatant} \times 100}{\text{Total protein in sample}}$$

Color Values

Color values of prepared films were measured with a Gardner Colorimeter (ColorGard System/05, Pacific Scientific, Silverspring, MD, U.S.A.). Film specimens were placed on the surface of a white standard plate (Calibration Plate White-1415), and Hunter L, a, and b color values were measured. The ranges of the three color coordinates were L (0 black to 100 white), a (−greenness to +redness), and b (−blueness to +yellowness) [4]. Total color difference (ΔE) was calculated:

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

Standard values refer to the white calibration plate (L= 92.93, a=−0.83, b=−0.89).

Statistical Analysis

Each replication included an individual preparation of film from the film-forming solutions. Thickness, % puncture deformation, and tensile and puncture strengths were means of six measurements. Disulfide content and barrier properties were means of three measurements. These replications were performed in a completely randomized design. A minimum of three observations for each property was collected. Data were analyzed using the general linear model (GLM) procedure of the SAS package (version 6.03, 1995) to determine differences between treatment means. Pairwise comparison of 15 treatment means shown to be significantly different by GLM was performed using the Least 6 Significant Difference (LSD) procedure, with significance defined at $P<0.05$.

RESULTS AND DISCUSSION

Film Thickness

Film thickness is important, because it is used to calculate mechanical and barrier properties. The mean film thicknesses for various types of films are presented in Table 1. Film thicknesses ranged from 58 to 74 μm. Control films had a less even surface and transparent appearance than the other film samples. The lack of film smoothness of control films might have resulted in greater thickness readings. The thickness of control films was close to the upper limit owing to a bumpy surface, although this did not create a problem for the thickness measurement. Therefore, property evaluation on the control films was performed with confidence. There were no significant differences ($P>0.05$) among the PFSP film thicknesses. However, the film thickness of 58 μm for the PFSP-90 film at pH 11.0 was significantly ($P<0.05$) thinner than the other films, except for the PFSP-70 film at pH 11.0 (61 μm). As shown in Table 2, cysteine addition had variable effects on film thickness, which remained unchanged by most treatments, while increasing or decreasing by other combinations. The high pH and temperature probably cleaved the protein portion of PFSP into smaller units, thus making it more soluble. In addition, high temperature denatured the protein molecule and enhanced protein-protein interaction, resulting in a transparent and thinner film with a more uniform structure. The intrinsic difference in the proteins may have resulted in the variability of thickness.

Mechanical Properties

Mechanical properties of protein films provide an indication of expected film integrity under stress conditions that would occur during processing, handling, and storage. The TS values of films are given in Table 1. The highest TS

Table 1. Selected physical properties of cast films from a soybean fermentation-derived proteinaceous fibrous material[1].

Treatments		Thickness (mm)	Relative humidity (%)	Water vapor permeability (g·mm/kPa·h·m^2)	Tensile strength (MPa)	Puncture strength (N)	Protein solubility in water (%)
Con-70	pH 7.0	93±1.6^a	66±1.1^a	6.56±1.53^b	5.08±0.46^b	6.65±0.13^d	6.8±1.4^d
	pH 9.0	90±2.6^{ab}	73±0.9^b	5.31±1.88^{bc}	4.50±0.28^c	5.69±0.27^d	9.8±2.5^c
	pH 11.0	93±7.4^a	76±1.8^b	10.51±1.26^a	4.22±0.41^c	4.67±0.21^d	17.5±1.2^b
Con-90	pH 7.0	87±2.9^a	70±0.8^a	8.29±1.75^a	6.67±0.21^a	6.21±0.14^a	6.3±2.7^d
	pH 9.0	91±4.6^a	72±1.2^a	9.47±0.92^a	6.37±0.27^a	6.34±0.25^a	9.7±1.9^c
	pH 11.0	83±2.5^a	71±1.3^a	10.82±1.33^a	5.22±0.24^b	5.27±0.41^a	12.6±2.2^c
PFSP-70	pH 7.0	74±2.5^a	72±1.4^b	3.78±0.93^c	6.87±0.43^a	9.29±0.39^a	7.3±0.5^d
	pH 9.0	70±3.6^{ab}	75±1.1^b	4.18±0.79^c	6.56±0.52^a	8.93±0.48^a	16.6±0.7^b
	pH 11.0	61±4.2^{bc}	72±1.3^b	4.72±0.55^c	4.48±0.32^c	8.09±0.41^b	29.9±3.2^a
PFSP-90	PH 7.0	72±9.8^a	71±1.6^b	4.29±1.21^c	5.27±0.88^b	7.36±0.16^c	8.2±0.8^c
	pH 9.0	69±3.7^{ab}	77±1.5^b	5.72±0.49^{bc}	4.64±0.30^c	7.06±0.18^c	17.6±1.4^b
	pH 11.0	58±5.5^c	76±1.4^b	6.75±0.89^b	3.88±0.68^c	7.36±0.37^c	34.9±3.9^a

[1]Means of three replicates ± standard deviation. Any two means in the same column followed by the same letter are not different (P>0.05).
Con-70, prepared from SPI solution heated at 70°C for 30 min; Con-90, prepared from SPI solution heated at 90°C for 30 min; PFSP-70, prepared from PFBP solution heated at 70°C for 30 min; PFSP-90, prepared from PFBP solution heated at 90°C for 30 min.

was 6.87 MPa for the PFSP-70 film at pH 7.0. The TS values of pH 7.0 films were significantly (P<0.05) higher than those of pH 9.0 or pH 11.0 films. There were no significant (P>0.05) differences between pH 9.0 and pH 11.0 films. Again, alkaline pH and low temperature caused a decrease of TS, probably due to decomposition of PFSP. Gennadios *et al.* [6] reported that the TS of soy protein isolate films was significantly reduced under high alkaline condition. The highest PS was observed with pH 7.0 films prepared from PFBP solution heated at 90°C (P<0.05) (Table 1). The Con-70 film had the lowest PS value at pH 11.0. In general, alkaline pH and low temperature caused a decrease of PS. At pH 9.0, repulsive forces might have been developed between negatively charged soy protein and protein chains of fibrous materials, probably resulting in lower TS or no improvement of TS above neutral pH for the composite films. Similar to TS, the PS of films was the highest for pH 7.0 films. A more favorable molecular orientation at pH 7.0 than pH 9.0 or pH 11.0 might have contributed to the higher TS and PS at pH 7.0. At pH 9.0, the protein denaturation might have resulted in repulsive charges, which hindered film formation. Intermolecular protein repulsive forces with highly negative charges at alkaline pH and highly positive charges at extreme acidic pH would inhibit film formation [6].

Effect of Cysteine Addition on Mechanical Properties of Film

PFSP films at pH 7.0, 9.0, and 11.0 were used to determine the effect of cysteine on mechanical properties. The TS of the PFSP films with cysteine was not different between pH 7.0 and 9.0, but decreased at pH 11.0. The highest TS for the PFSP-70 film was observed at pH 7.0 with added cysteine, and this was the film that exhibited the highest disulfide content (Table 3), and highest % increase in disulfide content as a result of cysteine addition. The TS of the PFSP-70 film at pH 7.0 had the highest value of 6.87 MPa. The TS of the PFSP-70 film at pH 9.0 and 11.0 were 6.32 and 5.51, respectively. The highest TS was observed at pH 7.0 for most treatments, and these results

Table 2. Thickness (μm) of PFSP films in the presence of cysteine.

Sample	pH 7.0		pH 9.0		pH 11.0	
	-cys	+cys	-cys	+cys	-cys	+cys
Con-70	93±1.6^a	121±4.2^b	90±2.6^{ab}	132±4.2^d	93±7.4^a	123±4.2^e
Con-90	87±2.9^c	106±4.2^b	91±4.6^e	102±4.2^e	83±2.5^d	119±4.2^b
PFSP-70	74±2.5^c	94±4.2^b	70±3.6^b	90±4.2^d	61±4.2^a	83±4.2^d
PFSP-90	72±9.8^b	97±4.2^b	69±3.7^a	85±4.2^c	58±5.5^c	71±4.2^b

Con-70, prepared from SPI solution heated at 70°C for 30 min; Con-90, prepared from SPI solution heated at 90°C for 30 min; PFSP-70, prepared from PFSP solution heated at 70°C for 30 min; PFSP-90, prepared from PFSP solution heated at 90°C for 30 min.
cys, Cysteine.
Thickness was a mean of 6 values±standard error.
Means followed by the same superscript are not different (P>0.05).

Table 3. Tensile strength of PFSP films in the presence of cysteine.

Sample	pH 7.0		pH 9.0		pH 11.0	
	-cys	+cys	-cys	+cys	-cys	+cys
Con-70	4.85±0.3[b]	4.85±0.3[b]	2.88±0.4[a]	5.16±0.4[b]	2.95±0.2[a]	2.99±0.7[a]
Con-90	5.68±0.5[c]	6.37±0.7[c]	3.67±0.5[a]	4.14±0.5[a]	5.22±0.5[c]	3.39±0.4[a]
PFSP-70	5.04±0.3[b]	6.87±0.2[c]	3.68±0.2[a]	6.31±0.3[b]	3.16±0.6[a]	5.51±0.5[c]
PFSP-90	3.70±0.6[a]	6.72±0.2[c]	5.87±0.4[c]	3.92±0.6[a]	4.85±0.4[b]	5.38±0.3[c]

Con-70, prepared from SPI solution heated at 70°C for 30 min; Con-90, prepared from SPI solution heated at 90°C for 30 min; PFSP-70, prepared from PFSP solution heated at 70°C for 30 min; PFSP-90, prepared from PFSP solution heated at 90°C for 30 min.
cys, Cysteine.
Means followed by the same superscript are not different (P>0.05).

confirmed are in good agreement with the conclusion that pH>8.0 did not improve soy protein film properties [3]. PS data shown in Table 4 also followed the same trend as TS, with cysteine addition resulting in increased PS. Sulfhydryl-disulfide interchange, disulfide-disulfide interchange, and thiol-disulfide interchange have been reported to affect film formation [16, 21]. Indeed, cysteine addition increased the disulfide content of film-forming solutions (Table 5). Therefore, addition of cysteine may have caused rearrangement of some disulfide bonds, resulting in increased TS for most films.

Water Vapor Permeability

WVP is an important functional film property that determines utility in food systems. The WVP values of films measured were compared with calculated actual RH conditions at the underside of the films during testing (Table 1). The WVP values of films decreased with increasing pH and temperature, although there was no significant (P>0.05) difference among WVP values. Con-70 and 90 showed the highest WVP values at pH 11.0, which was significantly (P<0.05) different from that of other films. In addition to changes in carbohydrate portion, heating the film-forming solution denatures proteins, thus allowing increased interaction and higher packing, and reduces mobility of polymer chains, causing a decrease of WVP [21]. Therefore, the lower WVP of films from alkaline heated solutions was attributed to alkaline pH conditions and high temperature, resulting in a denser and smoother film structure. Similarly, Gennadios *et al.* [6] reported that the WVP of soy protein isolate films decreased with increasing pH of film-forming solutions. The WVP values were higher for films casted from unheated solution than those from solution heated at 85°C [21]. The increase of WVP with increasing pH may have been due to unfolding of soy proteins, which exposes more hydrophilic residues to the surface, thus increasing the affinity of water vapor molecules to the hydrophilic residues. High WVP of PFSP films at pH 7.0 may also have been due to less intermolecular protein crosslinking. In general, all protein-based films are known to have a high WVP, and this is attributed to the hydrophilic nature of the protein polymers and polar nature of plasticizer (glycerol) used [15]. Since a major function of films is to prevent water migration in foods, the high WVP of protein-based films would limit the use of these films to low or intermediate moisture foods.

Oxygen Permeability

Oxygen permeability of films at pH 7.0 was tested, since pH 7.0 films exhibited enhanced mechanical properties. As shown in Table 6, O_2 permeability of pH 7.0 films with cysteine addition was higher than the control films. An inverse relation between WVP and O_2 was observed. Permeability is affected by the chemical nature of polymers, and the soy protein control film may have had more hydrogen bonding than the PFSP films.

Table 4. Puncture strength of PFSP films in the presence of cysteine.

Sample	pH 7.0		pH 9.0		pH 11.0	
	-cys	+cys	-cys	+cys	-cys	+cys
Con-70	7.73±0.3[b]	9.50±0.7[c]	5.73±0.3[a]	6.49±0.3[a]	5.08±0.4[a]	7.35±0.4[b]
Con-90	8.02±0.4[c]	9.84±0.8[c]	9.59±0.8[c]	6.17±0.5[a]	6.23±0.3[a]	5.90±0.3[a]
PFSP-70	9.21±0.7[c]	10.68±0.2[c]	6.75±0.5[a]	9.49±0.9[c]	7.16±0.4[b]	6.66±0.5[a]
PFSP-90	5.66±0.6[a]	8.13±0.1[b]	7.95±0.2[b]	7.91±0.6[b]	5.54±0.3[a]	6.54±0.2[a]

Con-70, prepared from SPI solution heated at 70°C for 30 min; Con-90, prepared from SPI solution heated at 90°C for 30 min; PFSP-70, prepared from PFSP solution heated at 70°C for 30 min; PFSP-90, prepared from PFSP solution heated at 90°C for 30 min.
cys, Cysteine.
Means followed by the same superscript are not different (P>0.05).

Table 5. Disulfide bond concentration (M) of PFSP film solutions at pH 7.0.

Sample	S-S content (M)		% increase of S-S
	-cys	+cys	
Control	5.74×10^{-5}	1.32×10^{-4}	▲ 56.6
PFSP-70	6.24×10^{-5}	1.42×10^{-4}	▲ 54.7
PFSP-90	4.64×10^{-5}	1.28×10^{-4}	▲ 63.8

PFSP-70, prepared from PFSP solution heated at 70°C for 30 min; PFSP-90, prepared from PFSP solution heated at 90°C for 30 min.
cys, Cysteine.
The S-S content values are an average of three measurements of the film-forming solutions diluted 25-fold.

Protein Solubility

The solubility of film protein was determined to evaluate their integrity in an aqueous environment (Table 1). High solubility may be desired for some applications such as water-soluble coatings and films. The solubility of film protein increased with increasing pH and temperature. The pH 11.0 film prepared from PFSP solution heated at 90°C gave the highest protein solubility (34.9%) ($P<0.05$), whereas the lowest solubility was observed with Con-90 films at pH 7.0 (6.8%). The PFSP-90 at pH 11.0 and PFSP-70 at pH 11.0 film pieces immersed in water were broken apart at the end of 24 h incubation. The combination of high alkaline pH and temperature treatment probably caused cleavage of the protein portion of PFSP into smaller units [2]. Heat treatment denatures proteins, possibly releasing small peptides, which remain bound in the unheated proteins [21]. In addition, degradation of proteins could be possible to a smaller extent, because of high alkaline pH and temperature. Consequently, a highly soluble film structure was observed. Similarly, an increase of protein solubility was observed with soy protein isolate films heated at 85°C [21]. However, Roy *et al.* [18] reported that, after heating wheat gluten film-forming solutions at 55, 65, 75, 85, or 95°C for 10 min, the solubility of films in water decreased with increasing temperature. The high soluble nature of the PFSP-90-pH 11 film in water might be useful for preparation of hot water-soluble pouches.

Table 6. Oxygen permeability ($cm^3\cdot\mu m/m^2\cdot d\cdot kPa$) of PFSP films at pH 7.0 in the presence of cysteine.

Sample	-cys	+cys
Control	0.578^a	0.494^a
PFSP-70	2.1^b	2.2^b
PFSP-90	1.36^b	1.2^b

PFSP-70, prepared from PFSP solution heated at 70°C for 30 min; PFSP-90, prepared from PFSP solution heated at 90°C for 30 min.
cys, Cysteine.
Means followed by the same superscript are not different ($P>0.05$).

Table 7. Hunter color values (L, a, and b) and total color differences (ΔE) of PFSP films[1].

Treatments		L	a	b	ΔE
Con-70	pH 7.0	36.16^e	-4.06^a	6.84^a	58.91^a
	pH 9.0	42.34^d	-3.92^b	6.79^a	52.78^b
	pH 11.0	48.99^c	-4.04^a	6.62	48.16^c
Con-90	pH 7.0	40.60^e	-4.12^a	6.97^a	38.69^d
	pH 9.0	45.20^d	-3.84^b	6.82^a	39.60^d
	pH 11.0	49.40^d	-4.09^a	6.44^b	38.63^d
PFSP-70	pH 7.0	68.70^c	-4.32^a	6.89^a	40.19^d
	pH 9.0	67.80^c	-3.89^b	6.79^a	40.34^d
	pH 11.0	67.60^c	-4.16^a	6.62^b	39.64^d
PFSP-90	PH 7.0	70.30^b	-4.18^a	6.81^a	39.81^d
	pH 9.0	71.21^b	-3.96^b	6.77^a	39.69^d
	pH 11.0	73.40^a	-4.14	6.42^b	35.94^d

[1]Means of three replicates. Any two means in the same column followed by the same letter are not significantly different ($P>0.05$).
Con-70, prepared from SPI solution heated at 70°C for 30 min; Con-90, prepared from SPI solution heated at 90°C for 30 min; PFSP-70, prepared from PFBP solution heated at 70°C for 30 min; PFSP-90, prepared from PFBP solution heated at 90°C for 30 min.

Film Color

Color is an important attribute of packaging films, since it could affect consumer acceptance in potential edible or non-edible packaging applications. The Hunter L, a, and b color values and total color differences for films are shown in Table 7. Heat-treated films were generally clearer and more uniform than control films. Control films had a bumpy surface appearance owing to its solubility problem. Our present study showed that the isoelectric point of PFSP was approximately pH 4.0, and its solubility at pH 7.0 was 98%. Therefore, pH is a critical factor in film preparation from PFSP. The PFSP-90-pH 11.0 film showed the highest L value (lighter) (73.4) ($P<0.05$), whereas the Con-70 film at pH 7.0 gave the lowest L value (darker). Similar to L values, yellowness (+b values) of PFSP-90 at pH 7.0 (6.81), PFSP-90 at pH 9.0 (6.77), and PFSP-90 at pH 11.0 (6.42) films were lower than the other films. Alkaline pH and temperature caused formation of a greener color (-a values). High alkaline pH and temperature unexpectedly gave lighter and less yellow films, although such conditions would be expected to be more favorable for non-enzymatic browning. This discrepancy was probably due to increased solubility of PFSP under the experimental conditions (alkaline pH and temperature) compared with the pH 7.0 films, since particle size and distribution significantly affect color measurement.

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REFERENCES

1. AOAC. 1990. *Official Methods of Analysis*, 15th Ed. Association of Official Analytical Chemists, Arlington, VA, U.S.A.
2. BeMiller, J. N. and R. L. Whistler. 1996. Carbohydrates, *In* O. R. Fenema (ed.), *Food Chemistry*, 3rd Ed. Marcel Dekker Inc., NY, U.S.A.
3. Brandenburg, A. H., C. L. Weller, and R. F. Testin. 1993. Edible films and coatings from soy protein. *J. Food Sci.* **58:** 1086–1089.
4. Francis, F. J. and F. M. Clydesdale. 1975. *Food Colorimetry: Theory and Applications*. AVI, Westport, CT, U.S.A.
5. Fukushima, D. and J. Van Buren. 1970. Mechanisms of protein insolubilization during the drying of soy milk. Role of disulfide and hydrophobic bonds. *Cereal Chem.* **47:** 687–696.
6. Gennadios, A., A. H. Brandenburg, C. L. Weller, and R. F. Testin. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **41:** 1835–1839.
7. Gennadios, A., T. H. McHugh, C. L. Weller, and J. M. Krochta. 1994. Edible coating and films based on proteins, pp. 201–277. *In* Krochta, J. M., E. A. Baldwin, and M. O. Nisperos-Carriedo (eds.), *Edible Coatings and Films to Improve Food Quality*. Technomic Publishing Co., Inc., Lancaster, PA, U.S.A.
8. Kester, J. J. and O. R. Fennema. 1986. Edible films and coatings: A review. *Food Technol.* **40:** 47–59.
9. Kim, H. W., E. J. Ko, S. K. Lee, S. D. Ha, K. B. Song, S. K. Park, D. H. Chung, K. S. Youn, and D. H. Bae. 2005. Physical, mechanical, and antimicrobial properties of edible film produced from defatted soybean meal fermented by *Bacillus subtilis*. *J. Microbiol. Biotechnol.* **15:** 815–822.
10. Kim, H. W., K. M. Kim, E. J. Ko, S. K. Lee, S. D. Ha, K. B. Song, S. K. Park, K. S. Kwon, and D. H. Bae. 2004. Development of antimicrobial edible film from defatted soybean meal fermented by *Bacillus subtilis*. *J. Microbiol. Biotechnol.* **14:** 1303–1309.
11. Krochta, M. J. and C. D. De Mulder-Johnston. 1997. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technol.* **51:** 61–74.
12. Kunte, L. F., A. Gennadios, S. L. Cuppett, A. Hanna, and C. L. Weller. 1997. Cast films from soy protein isolates and fractions. *Cereal Chem.* **74:** 115–118.
13. Lee, H. J., K. W. Lee, K. H. Kim, H. K. Kim, and H. J. Lee. 2004. Antitumor activity of peptide fraction from traditional Korean soy sauce. *J. Microbiol. Biotechnol.* **14:** 628–630.
14. McHugh, T. H., R. Avena-Bustillos, and J. M. Krochta. 1993. Hydrophilic edible films: Modified procedure for water vapor permeability and explanation for thickness effect. *J. Food Sci.* **58:** 889–903.
15. McHugh, T. H., J. F. Aujard, and J. M. Krochta. 1994. Plasticized whey protein edible films: Water vapor permeability properties. *J. Food Sci.* **59:** 416–419, 423.
16. Parris, N. and D. R. Coffin. 1997. Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *J. Agric. Food Chem.* **45:** 1596–1599.
17. Reed, S. M. and C. Noricote. 1983. Chemical and immunological similarities between the phloem proteins of three genera of the curcubitaceae. *Planta* **158:** 119–127.
18. Roy, S., C. L. Weller, M. G. Zeece, and R. F. Testin. 1995. Effect of heat on the physical and molecular properties of wheat gluten films. 12E-14. *1995 Annual Meeting of the Institute of Food Technologists*, Anaheim, CA, Institute of Food Technologists, Chicago, IL, U.S.A.
19. Sabato, S. F., B. Ouattara, H. Yu, G. D'Aprano, C. Le Tien, M. A. Mateescu, and M. Lacroix. 2001. Mechanical and barrier properties of cross-linked soy and whey protein based films. *J. Agric. Food Chem.* **49:** 1397–1403.
20. Salame, M. and S. Steingiser. 1977. Barrier polymers. *Polym. Plast. Technol. Eng.* **8:** 155–175.
21. Stuchell, Y. M. and M. J. Krochta. 1994. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* **59:** 1332–1337.
22. Wall, J. S., M. Friedman, L. H. Krull, J. F. Cavins, and A. C. Beckwith. 1968. Chemical modification of wheat gluten proteins and related model systems. *J. Polymer Sci.* **24:** 147–161.
23. Wu, L. C. and R. P. Bates. 1973. Soy protein-lipid films: Studies on the film formation phenomenon. *J. Food Sci.* **37:** 36–39.
24. Yang, J. I., S. H. Lee, D. H. Hahm, I. H. Kim, and S. Y. Choi. 2004. Enhancement of calcium-binding quality of proglycinin peptides by chemical phosphorylation. *J. Microbiol. Biotechnol.* **14:** 607–611.