Properties of Cysteine-Added Soy Protein–Wheat Gluten Films

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ABSTRACT

Soy and wheat proteins as film ingredients are advantageous due to relative abundance, biodegradability, and their renewable nature. Research objectives were to evaluate effectiveness of gluten and cysteine addition in improving properties of soy protein-based films. Thickness, mechanical, and barrier properties were evaluated. Gluten addition and pH 3.0 lowered water vapor permeability and thickness. Mechanical properties were enhanced at pH 7.0 with cysteine addition. Cysteine increased tensile strength of some treatments due to increase in disulfide bond formation. Best compromise between barrier and mechanical properties was observed for the cysteine-added soy:gluten (4:1) film at pH 7.0. These films could find application as primary packaging for low moisture foods.

Key Words: wheat gluten, SPI, film, cysteine

INTRODUCTION

Some proteins that have been studied as potential film-forming agents include collagen and gelatin, corn zein, casein, whey protein, wheat gluten, Soy protein isolate (SPI), rice bran proteins, serum albumin, peanut and cottonseed proteins, and egg white protein (Gennadios et al., 1993a; Gnanasambandam et al., 1997, Cuq et al., 1998). Incorporating various polysaccharides and lipids with soy protein has also been investigated (Gennadios et al., 1993c; Wu and Bates, 1972). Soy protein films are flexible, smooth, transparent, and clear compared to other films from plant proteins. Corn-zein forms films with good water vapor permeability properties, but zein coatings and films may have objectionable opaqueness and yellowness and have to be decolorized (Guilbert, 1986).

Combining secondary components such as wheat gluten and cysteine with SPI may cause physical and chemical interactions that may improve film properties. An important edible film property is water vapor permeability. Wheat gluten as a secondary component could improve water barrier properties of SPI films due to the large number of non-polar amino acids which contribute to hydrophobic interactions, the few ionizable amino acids, and covalent disulfide bonds which contribute to gluten insolubility (Wall and Beckwith, 1969, Reiners et al, 1973). Besides its effect on barrier properties of soy protein films, gluten could contribute cohesive and elastic properties (Wall et al., 1968).

Soy protein film formation is a result of polymerization of heat denatured proteins with disulfide and hydrophobic bonds being the main forces maintaining the film network (Fukushima and Van Buren, 1970). Wheat gluten has a cysteine content of 130 mg/g of N (Simmonds, 1981). The cysteine groups can undergo polymerization via sulfhydryl-disulfide interchange reactions during heating to form a continuous covalent network upon cooling (Lindsay, 1985). The addition of cysteine may thus be advantageous due to disulfide rearrangement. Research objectives were to evaluate how wheat gluten and cysteine addition would modify the physical and mechanical properties of soy protein films.

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MATERIALS & METHODS

Commercial SPI (Ardex D) was obtained from Archer Daniels Midland Co. Decatur, IL. Spray-dried wheat gluten was obtained from Midwest Grain Products, Inc, (Atchison, KS). The macro-nutrient composition of the wheat gluten and SPI was as follows: wheat gluten had a protein content of 75% dry basis, with maximum moisture and lipid contents of 7% and 2% respectively. The SPI had a protein content of 90% dry basis; moisture and lipid contents were 6.5 % and 1% respectively. Cysteine was purchased from Sigma Chemical CO, St. Louis, MO. Acetic acid, sulfuric acid and ammonium hydroxide, glycerol, cupric sulfate, 5,5= dithiobis (2-nitrobenzoic acid)/ DTNB, sodium sulfite, EDTA, and urea were purchased from Fisher Scientific, Fair Lawn, NJ. Tris base was purchased from Bio-Rad, Hercules, CA. All chemicals were reagent grade.

Soy protein-wheat gluten film preparation

SPI (7.5g) was weighed for the control film. This was dispersed in 120 mL water and plasticizer (2.5g glycerol) was added. For the composite soy protein-gluten films, part of the SPI was substituted with wheat gluten, resulting in various SPI:wheat gluten ratios (soy protein: gluten 1:0, 4:1, 3:1 and 2:1). To solubilize the gluten, soy protein-gluten mixtures were dispersed in 72 mL of 95% ethanol and 48 mL water. Glycerol (2.5g) was added to each solution. To determine the effects of cysteine, 2 solutions at each soy protein:gluten ratio were prepared and cysteine (1% w/w) was added to one of the solutions. To determine the effects of pH, 3 solutions at each soy protein: gluten and cysteine ratios were made, and pH was adjusted to 3.0, 7.0, or 9.0 with acetic acid or 6N ammonium hydroxide. The solutions were heated to 80°C while stirring on a magnetic stirrer/ hot plate. Solutions were then cooled to ambient and 15 mL solutions were poured into polystyrene weighing boats (Fisher Scientific) and air dried (24–48h). Films formed were 10 cm in diameter. 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 12N sulfuric acid solution (Gnanasambandam et al, 1997).

Film thickness

Film thickness was measured with an electronic digital micrometer (Cole-Parmer Instrument Co, Vernon Hills, IL). Film strips were placed within the micrometer and the gap 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). Sample preparation and handling for texture analyses were carried out according to standard methods D 882-91 (ASTM, 1991). Film strips measuring 100 mm × 25 mm were mounted onto the texture analyzer. The film strips 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 was measured as follows: circular film samples 6.5 cm diam 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 diam) at compression mode (TA.XT2), the films were punctured and the force at the point of rupture was recorded in N and expressed as puncture strength. Percentage puncture deformation was calculated by multiplying the deformation at the moment of rupture by 100%.

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 Kella and Kinsella (1985). The film forming solutions at pH 7.0 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). For converting the absorbance to disulfide bond concentration, an A_{412} value of 13,600 $M^{-1}cm^{-1}$ was used.

Apparent water vapor permeability (WVP_{app})

"Apparent" implicates the WVP_{app} results apply only to the specific thickness and water gradient studied. The wells in the WVP_{app} metal cups were filled with distilled water resulting in an air gap of 2.5 cm from the film specimen. Film samples were placed over the open tops of metal cups (area=33 cm^2), and secured between a metal rim and rubber gasket by 6 screws placed symmetrically around the circumference. The cups were weighed at 30-min intervals for 10h to determine by weight the amount of moisture migration across the film. The WVP_{app} was measured at 23°C, and 55% RH, but there was lack of air circulation in the desiccators. Weight loss vs time plots were made. Linear regression-derived slopes were used to estimate water vapor transmission rate and WVP_{app} measurements expressed in g/m.s.Pa were performed as described by McHugh et al. (1993).

Oxygen permeability

Oxygen permeability of pH 7.0 films was measured as described by Gnanasambandam et al. (1997), and O'Brien et al. (1986). 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 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 previous conditions 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) was used to measure increases in downstream pressure with time, for a known downstream volume through a fixed film surface area. The testing time for each cell in the test cycle (dwell time) was ~12h. Oxygen permeabilities were calculated as described by O'Brien et al. (1986).

Statistical analysis

Thickness, % puncture deformation, tensile and puncture strength were means of 6 measurements. Disulfide content, and barrier properties were means of 3 measurements. Statistics on a completely randomized design were determined using the general linear model procedures of SAS Institute, Inc. (1994) to determine the influence of variables on film properties. The sources in the model were soy protein:gluten ratio, pH, presence and absence of cysteine, and all their interactions. Significance of difference was defined at $P \leq 0.05$.

RESULTS & DISCUSSION

Film thickness

Film thickness is important because it is used to calculate mechanical and barrier properties. As shown (Table 1), cysteine addition had variable effects on film thickness which remained unchanged for most treatments, while increasing or decreasing for other combinations.

In general the soy protein control film (S:G 1:0) at pH 7 and 9.0 exhibited no difference in thickness with films made with a higher soy protein concentration (S:G 4:1, 3:1, and 2:1). The films with a higher soy protein content had a lower thickness compared to those with a higher gluten concentration. When soy protein was in a lower concentration, thickness ranged from 119 mm for S:G 1:3 film with added cysteine at pH 9.0 to 213 mm for the S:G 1:2 film at pH 7.0. When soy protein concentration was higher than that of the wheat gluten, thickness was lower and ranged from 102 mm for the S:G 1:0 film with added cysteine at pH 3.0 to 145 mm for the S:G 2:1 film with added cysteine at pH 7.0. The intrinsic difference in the proteins may have resulted in the thickness variability (Gennadios et al., 1993b).

At the same soy protein-gluten and cysteine concentration, some films at pH 3.0 had lower thickness than those at pH 7.0 and 9.0. This could be because pH 7.0 and 9.0 were close to the isoelectric point of wheat gluten. The main protein fractions in wheat gluten are gliadin and glutenin. The isoelectric point of gliadin and glutenin have been estimated at 8.1 and 7.1 respectively, while that of wheat gluten is 7.5 (Wu and DimLer, 1963). The pH values of 7.0 and 9.0 were too close to the isoelectric point of wheat gluten proteins, resulting in poor wheat gluten dispersion in the film forming solutions. Films cast from solutions of pH 7.0 or 9.0 were "grittier" due to undissolved protein particles. The lack of film smoothness may have resulted in greater thickness readings. In contrast pH 3.0 was far from the isoelectric point of the gluten proteins, resulting in good gluten dispersion, smooth films, and lower thickness readings. These results confirmed earlier findings where viscosity differences due to pH of the film forming solutions, and the isoelectric point of gluten proteins, affected film thickness (Gennadios et al., 1993c).

Effect of cysteine addition on film mechanical properties

Soy protein: gluten ratios of 1:0, 4:1, 3:1, and 2:1 at pH 3.0, 7.0, and 9.0 were used to determine the effects of cysteine on mechanical properties, as preliminary studies indicated that films with greater gluten content had lower tensile and puncture strength. Cysteine addition increased TS of 6 treatments (S:G 1:0 at pH 7.0 and 9.0, S:G 4:1 film at pH 3.0, 7.0, and 9.0, and S:G 2:1 film at pH 3.0), lowered TS of 2 (soy protein control film at pH 3.0, and S:G 3:1 film at pH 9.0) and had no effect on the other 4 (S:G 3:1 film at pH 3.0 ,7.0, S:G 2:1 at pH 7.0 and 9.0). Sulfhydryl-disulfide interchange, disulfide-disulfide interchange, and thiol-disulfide interchange have been reported to affect film formation (Kasarda et al., 1971). Addition of cysteine may have caused rearrangement of some disulfide bonds that resulted in increased TS for most films. Cysteine addition increased disulfide content of film forming solutions (Table 2). This increase in disulfide content may have increased TS. This was similar to findings reported where sodium sulfite increased TS (Gennadios et al., 1993c). Highest TS was observed for the pH 7.0 S:G 4:1 film with added cysteine, and this was the film that exhibited the highest disulfide content (Table 2), and highest % increase in disulfide content as a result of cysteine addition.

Puncture strength (PS) data (Table 3), followed the same trend as TS, with cysteine addition resulting in increased puncture strength in 4 trials, reduced puncture strength in 2 others, and had no effect for the other treatments. With one exception, cysteine addition had no effect (Table 4) on % puncture deformation.

Effects of soy protein-wheat gluten concentration on film mechanical properties

Films without cysteine. At pH 3.0, soy protein control films (S:G 1:0) had the highest TS of 5.87 MPa, and TS was not different ($P>0.05$) at the other soy protein: gluten ratios with values ranging from 2.88–3.68 MPa. At pH 7.0, all the composite soy protein- wheat

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Table 1—Thickness (μm) of soy protein: wheat gluten films

Soy protein: gluten ratio	pH 3.0 -cys	pH 3.0 +cys	pH 7.0 -cys	pH 7.0 +cys	pH 9.0 -cys	pH 9.0 +cys
S:G 1:4	132±4.2[c]	121±4.2[b]	203±4.2[e]	182±4.2[d]	184±4.2[d]	193±4.2[e]
S:G 1:3	133±4.2[c]	126±4.2[b]	200±4.2[e]	202±4.2[e]	164±3.9[d]	119±4.2[b]
S:G 1:2	130±4.2[c]	124±4.2[b]	213±4.2[e]	190±4.2[d]	175±4.2[d]	163±4.2[d]
S:G 2:1	123±4.2[b]	117±4.2[b]	103±4.2[a]	145±4.2[c]	144±4.2[c]	141±4.2[c]
S:G 3:1	113±4.2[b]	111±4.2[b]	141±4.2[c]	141±4.2[c]	139±4.2[c]	132±4.2[c]
S:G 4:1	131±4.2[c]	124±4.2[b]	136±4.2[c]	144±4.2[c]	139±3.9[c]	134±4.2[c]
S:G 1:0	117±4.2[b]	102±5.9[a]	146±3.9[c]	144±3.9[c]	135±3.9[c]	142±3.9[c]

[a-e]Means followed by the same superscript are not different (P>0.05).
[f]Thickness was a mean of 6 values ± standard error. S:G is soy protein:wheat gluten in the soy protein-wheat gluten mixture; cys is cysteine.

Table 2—Disulfide bond concentration (M) of pH 7.0 soy protein-gluten film solutions

Treatment	S-S content (M)[b]	% inc with cysteine addn
S:G 2:1	6.24×10^{-5}	
S:G 2:1+cys	1.42×10^{-4}	54.7
S:G 3:1	4.64×10^{-5}	
S:G 3:1+cys	1.28×10^{-4}	63.8
S:G 4:1	5.22×10^{-5}	
S:G 4:1 +cys	1.45×10^{-4}	63.9
S:G 1:0	5.74×10^{-3}	
S:G 1:0 +cys	1.32×10^{-4}	56.6

[a]S:G is SPI:wheat gluten ratio in the film forming solutions; cys is cysteine.
[b]The S-S content values are an average of three measurements of the film forming suolutions diluted 25 fold.

Table 3—Puncture strength of soy protein-gluten films

Soy protein: gluten ratio	pH 3.0 -cys	pH 3.0 +cys	pH 7.0 -cys	pH 7.0 +cys	pH 9.0 -cys	pH 9.0 +cys
S:G 2:1	5.08±0.38[a]	7.35±0.38[b]	7.73±0.38[b]	9.50±0.38[c]	5.73±0.38[a]	6.49±0.38[a]
S:G 3:1	6.23±0.38[a]	5.90±0.38[a]	10.02±0.3[c]	9.84±0.38[c]	9.59±0.38[c]	6.17±0.38[a]
S:G 4:1	7.16±0.38[a]	6.66±0.38[a]	9.21±0.38[c]	10.68±0.38[c]	6.75±0.38[a]	9.49±0.38[c]
S:G 1:0	5.54±0.38[a]	6.54±0.38[a]	5.66±0.38[a]	8.13±0.38[b]	7.95±0.38[b]	7.91±0.38[b]

[a-c]Any two means followed by the same superscript are not different (P>0.05).
[d]S:G is soy protein:wheat gluten ratio; cys is cysteine.

gluten films exhibited higher TS and puncture strength than soy protein control films. At pH 9.0, TS and PS was highest for the S:G 3:1 film with values of 5.22 MPa and 9.59 N respectively.

At the different soy protein: gluten ratios, S:G 1:0 control films had the highest puncture deformation at pH 7.0 and 9.0 with values of 28.36 and 20.99% respectively whereas values of the composite soy protein-gluten films ranged from 3.22–9.02% at pH 7.0, and 5.73–14.05% at pH 9.0. At pH 3.0, gluten addition increased puncture deformation of S:G 3:1 film without cysteine (26.86%) from 6.35% for the S:G 1:0 film, but no difference was detected at the other soy protein: gluten ratios.

Films with added cysteine. At pH 3.0, S:G 2:1 and 4:1 films had similar TS, but TS decreased for the S:G 3:1 film. At pH 7.0, increasing the SPI concentration increased TS ($P<0.05$). Increased TS from 4.85 to 6.87 MPa when soy protein concentration was increased from S:G 2:1 to S:G 4:1 may be because pH 7.0 was near the isoelectric point of wheat gluten. The poor dispersibility of the gluten at pH 7.0 thus resulted in increased TS when gluten content was decreased. Insolubility limits structural development (cross-linking) thus causing decreased TS when gluten was in higher concentration at pH 7.0 (Brandenburg et al., 1993). At pH 9.0, the S:G 2:1, 3:1, and 4:1 films had TS of 2.99, 3.39, and 5.51 MPa respectively. Similar to pH 7.0, increasing the SPI from S:G 2:1 to S:G 4:1 at pH 9.0 also resulted in increased TS. Highest TS and PS were observed for the S:G 4:1 film at pH 7.0. At pH 3.0, S:G 2:1 film had higher PS than the other combinations. At pH 7.0, no difference in PS was detected at the different soy protein:gluten ratios, while at pH 9.0, S:G 4:1 and 1:0 films had higher PS than films at lower soy protein ratios.

Highest TS values of wheat gluten films have been reported to be produced between pH 12-13 (Gennadios et al., 1993b). The highest pH we tested was 9.0. Wheat gluten at high concentrations did not contribute positively to the TS of the films because the films may not have been produced at the optimal pH for wheat gluten usage. Producing films was not practical at the optimal pH for maximum TS of wheat gluten for edible packaging because of the high alkalinity. Gluten addition decreased puncture deformation at pH 9.0, and had no effect at the pH 3.0, and 7.0, with the exception of S:G 4:1 film at pH 7.0, which had a lower puncture deformation than the S:G 3:1 and S:G 2:1 film.

Effect of pH on film mechanical properties

Films without cysteine. At the same soy protein: wheat gluten ratio and cysteine concentration, TS of most films was highest at pH 7.0 (Table 4). For the S:G 2:1 film, TS increased from 2.88 MPa at pH 3.0 to 4.85 MPa at pH 7.0, but was not different at pH 9.0 (2.95 MPa). For the S:G 4:1 film, TS was 3.69, 5.04, and 3.16 at pH 3.0, 7.0, and 9.0 respectively. Increasing the pH to 7.0 resulted in higher TS. Increasing the pH from 7.0 to 9.0 had variable effects; lower TS for the S:G 2:1, and S:G 4:1 film, higher for the S:G 1:0 film, while remaining unchanged for the S:G 3:1 film. At pH 9.0, repulsive forces may have developed between the negatively charged soy protein and wheat gluten chains that may have resulted in lower TS or no improvement in TS above neutral pH for the composite films.

Similar to TS, puncture strength of films (Table 5) was highest for pH 7.0 films. A more favorable molecular orientation at pH 7.0 than pH 3.0 or pH 9.0 may have contributed to higher TS and PS at pH 7.0. At pH 9.0, the protein denaturation may 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 (Gennadios et al., 1993b).

Films with added cysteine. Tensile strength of the S:G 2:1 film with cysteine was not different between pH 3.0 and 7.0, but decreased at pH 9.0. Tensile strength of S:G 3:1 film was highest at pH 7.0, with values of 4.14, 6.37, and 3.39 MPa at pH 3.0, 7.0 and 9.0 respectively. Tensile strengths of S:G 4:1 film were 4.78, 6.87, and 5.51 at pH 3.0, 7.0 and 9.0 respectively. Highest TS was observed at pH 7.0 for most treatments and these results confirmed reports that concluded that pH >8.0 did not improve soy protein film properties (Brandenburg et al., 1993). Since SPI was used in a higher concentration than gluten, this may be the reason why the highest TS was observed at pH 7.0 for most films.

Apparent water vapor permeability (WVP_{app})

As shown (Fig.1), cysteine addition had no effect on WVP_{app} of the films. Films with added gluten had lower WVP_{app} compared to soy protein control films. The presence of the large number of non polar amino acids and few readily available ionizable amino acids in films containing gluten may have contributed to lower WVP_{app} in films containing gluten compared to soy protein control films. Soy protein may also be more hydrophilic than wheat gluten.

The hydrophilic nature of the protein polymers contributes to thickness affecting WVP_{app}. At the same pH and cysteine level, some films with added gluten were thicker than soy protein control films. This may have contributed to films with less SPI having a lower

WVP$_{app}$. The thicker the film (more gluten concentration), the more resistance to mass transfer across it. Permeability measurements assume a plot of permeation versus thickness to yield a value of 1.00. Salame and Steingiser (1977) indicated that the slope value ranged between 0.8–1.2 so a film with double the thickness would exhibit a permeability that was 80–120 % higher. Film thickness has an effect on WVP$_{app}$ of hydrophilic films (McHugh et al., 1993). Water vapor permeability has also been shown to be directly proportional to the degree of polymerization. Films with added gluten may have had increased polymerization (Pascat, 1986).

At the same soy protein: gluten, and cysteine concentration, the WVP$_{app}$ of the composite soy protein-gluten films was lower at pH 3.0 and 7.0 compared to pH 9.0. The increase in WVP$_{app,}$ with increasing pH may have been due to unfolding of soy proteins that exposed more hydrophilic residues to the surface causing an increased affinity of water vapor molecules with the hydrophilic residues. High WVP$_{app}$ of composite soy-gluten films at pH 9.0 may also have been due to less intermolecular protein crosslinking. Lower WVP$_{app}$ for the soy protein-gluten films at pH 3.0 compared to pH 7.0 and 9.0 was in contrast to earlier reports. Acidic pH had resulted in higher WVP$_{app}$ for gluten films, however, the method of film preparation differed, and gluten was the only protein polymer in the previous study, whereas films in our current study were a mixture of soy protein and wheat gluten (Herald et al., 1995). Repulsive forces among negatively charged soy protein and wheat gluten chains may have developed, resulting in higher WVP$_{app}$ at pH 9.0.

All films however had high water vapor permeability and this was attributed to the hydrophilic nature of the protein polymers and polar nature of plasticizer (glycerol) used (Kester and Fennema, 1986). Since a major function of films is to prevent water migration in foods, the high WVP$_{app}$ would limit the use of these films to low or intermediate moisture foods (such as nuts).

Oxygen permeability

Oxygen permeability of films at pH 7.0 was tested, since pH 7.0 films exhibited enhanced mechanical properties. As indicated (Table 6, Fig. 1), O_2 permeability of pH 7.0 films with gluten addition was higher than the S:G 1:0 films with the exception of S:G 3:1 film where no difference was detected, while WVP$_{app}$ of films with added gluten was lower. An inverse relation between WVP$_{app}$ and O_2 was observed. Highest O_2 permeability was detected in the film with the highest gluten content (S:G 2:1 film). Permeability was affected by the chemical nature of the polymers, and soy protein control film may have had more hydrogen bonding than the composite soy protein: wheat gluten films. Films we produced had good oxygen barrier prop-

Table 4—Tensile strength of soy protein-gluten films

Soy protein: gluten ratio	pH 3.0 -cys	pH 3.0 +cys	pH 7.0 -cys	pH 7.0 +cys	pH 9.0 -cys	pH 9.0 +cys
S:G 2:1	2.88±0.203^{a}	5.16±0.203^{b}	4.85±0.203^{b}	4.85±0.203^{b}	2.95±0.203^{a}	2.99±0.203^{a}
S:G 3:1	3.67±0.203^{a}	4.14±0.203^{a}	5.68±0.203^{c}	6.37±0.203^{c}	5.22±0.203^{c}	3.39±0.203^{a}
S:G 4:1	3.68±0.203^{a}	4.78±0.203^{b}	5.04±0.203^{b}	6.87±0.203^{c}	3.16±0.203^{a}	5.51±0.203^{c}
S:G 1:0	5.87±0.203^{c}	3.92±0.203^{a}	3.70±0.203^{a}	6.72±0.222^{c}	4.85±0.222^{b}	5.38±0.203^{c}

[a-c] Any two means followed by the same superscript are not different. (P>0.05).
[d] S:G is soy protein: wheat gluten ratio; cys is cysteine.

Table 5—% Puncture deformation of soy protein- gluten films

Soy protein: gluten ratio	pH 3.0 -cys	pH 3.0 +cys	pH 7.0 -cys	pH 7.0 +cys	pH 9.0 -cys	pH 9.0 +cys
S:G 2:1	16.87±3.89^{abcde}	9.02±4.5^{defgh}	9.02±4.5^{defgh}	9.32±3.89^{abcd}	11.62±3.89^{cdef}	10.78±4.50^{cdefg}
S:G 3:1	26.86±4.5^{ab}	16.57±3.89^{bdefg}	4.43±4.5^{fgh}	11.28±4.5^{cdef}	5.73±4.5^{efgh}	16.07±4.5^{cdefg}
S:G 4:1	12.23±3.89^{cdefgh}	6.07±3.89^{efg}	3.22±4.5^{h}	3.21±4.5^{h}	14.05±3.89^{cdefgh}	.84±4.5^{fgh}
S:G 1:0	6.35±3.18^{efgh}	19.79±3.89^{cde}	28.36±4.5^{ab}	7.46±3.18^{cdefgh}	20.99±3.89^{abc}	4 26.77±3.89^{ab}

[a-h]Any two means followed by the same superscript are not different (P>0.05).
[i]S:G is soy protein:wheat gluten ratio; cys is cysteine.
S:G is SPI: wheat gluten ratio in the film forming solutions, cys is cysteine.The S-S content values are an average of three measurements of the film forming solutions diluted 25 fold.

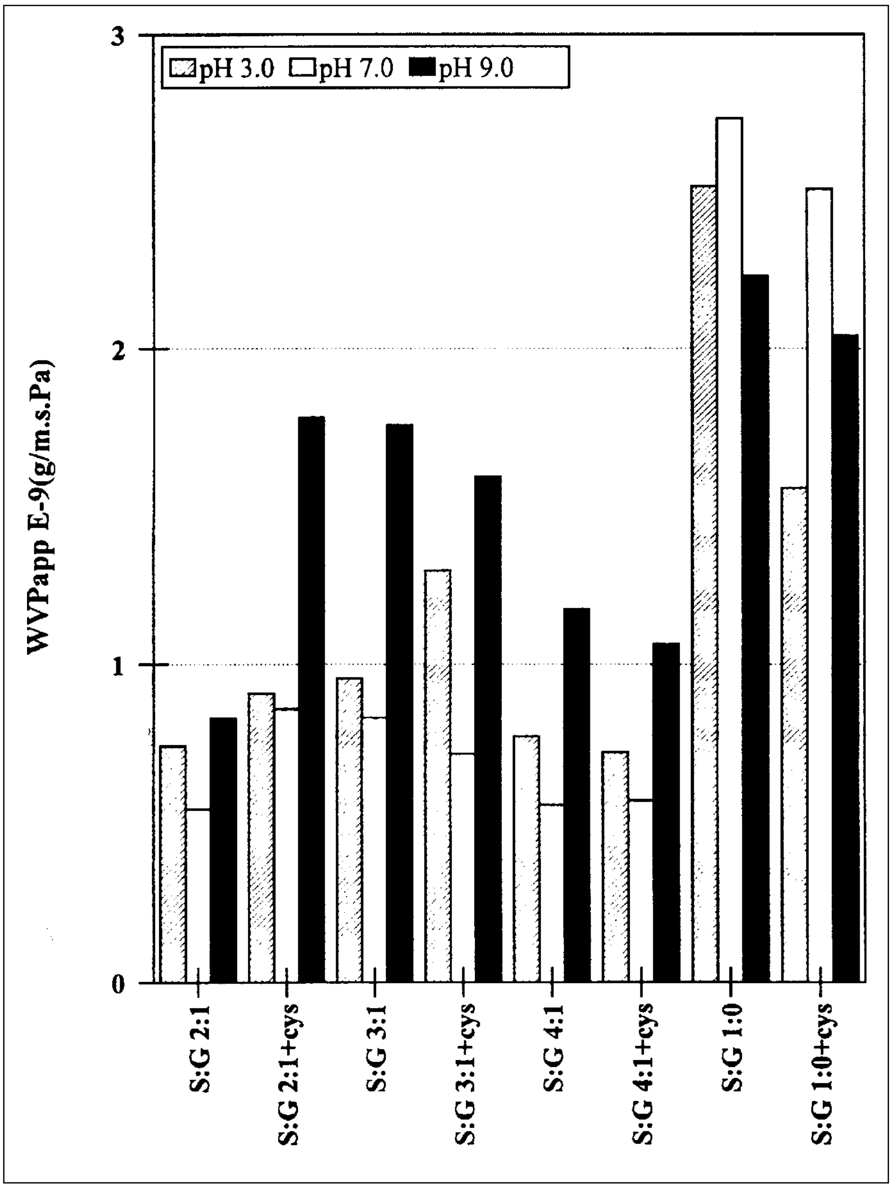

Fig. 1—Water vapor permeation of soy protein-wheat gluten films. S:G is soy protein:wheat gluten ratio in the film forming solutions; cys is cysteine.

erties, and this could be due to their relatively high water.

CONCLUSIONS

CYSTEINE AND GLUTEN ADDITION COULD BE used to produce films with increased TS and enhanced barrier properties. Addition of cysteine increased TS of 6 treatments, lowered TS of 2 treatments and no effect was detected with the other treatment combinations. Gluten addition lowered WVP_{app}. The treatment combination that provided the best combination of barrier and mechanical properties was the S:G 4:1 film at pH 7.0 with 1 % cysteine addition. The films were good oxygen barriers.

Table 6—Oxygen permeability (cm³.mm/ m².d.kPa) of pH 7.0 soy prote in-gluten films

Treatment	- cysteine	+ cysteine
S:G 2:1	2.1[b]	2.2[b]
S:G 3:1	0.525[a]	0.975[b]
S:G 4:1	1.36[b]	1.2[b]
S:G 1:0	0.578[a]	0.494[a]

[a-b]Means followed by the same superscript are not different (P>0.05.
[c]S:G is SPI:wheat gluten ratio.

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