Mechanical Properties and Water-Vapor Permeability of Soy-Protein Films Affected by Calcium Salts and Glucono-δ-lactone

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Edible films were prepared from solutions of soy protein with calcium salts and glucono-δ-lactone (GDL). Calcium salts cross-linking interactions with soy-protein isolate (SPI) could result in the formation of films with rigid three-dimensional structure. GDL contributed to the formation of a homogeneous film structure due to increased protein−solvent attraction. Tensile strength (TS) of calcium sulfate treated SPI film (8.6 MPa) was higher than the TS of calcium chloride treated SPI films (6.4 MPa) and the control SPI film (5.5 MPa). Puncture strength (PS) of calcium sulfate treated SPI film (9.8 MPa) was higher than the PS of calcium chloride treated SPI films (8.5 MPa) and the control SPI film (5.9 MPa). SPI film formulated with GDL had larger elongation at break (39.4%) than that of SPI control film (18.2%). Calcium salts and GDL-treated SPI films had lower water-vapor permeability than the SPI control film.

INTRODUCTION

The increased interest in proteins as packaging and coating agents has prompted the development of edible films to protect against negative quality changes in food products. Protein can be tailored to produce edible films, and they act as barriers to control transfer of moisture, oxygen, and oxidizing or reducing agents; undesirable changes of pigments and appearance; and loss of volatile flavors and aromas (*1*). Moisture migration is one of the most serious problems in food preservation, and it can cause adverse effects on the texture, nutritive value, salability, and wholesomeness of food products. The application of edible coatings and film depends on their capacity to act as an adjunct for improving overall food quality, extending shelf life, and possibly improving economic efficiency of packaging materials.

Soy-protein films have been reported to be an ineffective moisture barrier, as expected for hydrophilic materials (*2, 3, 4*). Moisture barrier properties of soy-protein-based films may be enhanced by calcium cross-linking of soy protein and by adjusting to the isoelectric point to optimize protein−protein interactions (*5*). Calcium chloride, a divalent salt, is incorporated in coatings to improve the texture and color of food products (). Adding calcium salts to soy-protein coatings may control darkening of fruits and vegetables.

Proteinaceous materials have the ability to form films which have many potential applications. An important aspect of these films is the renewable nature of the raw materials. Soy-protein films have been studied for food and nonfood applications (*3, 4, 6, 7*). Commercialization of soy-protein films requires improvement of their mechanical and water barrier properties. The objectives of this study were to produce soy-protein films with calcium salts or glucono-δ-lactone (GDL), and to investigate their mechanical properties including the tensile strength (TS), puncture strength (PS), elongation to break (ETB), and water-vapor permeability (WVP).

MATERIALS AND METHODS

Materials. Soy-protein isolate (SPI, ARDEX D) was obtained from Archer Daniels Midland (Decatur, IL). Calcium sulfate ($CaSO_4 \cdot 1/2H_2O$), calcium chloride ($CaCl_2$), and GDL were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical reagent grade and were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma (St. Louis, MO).

Solubility of Calcium Salts. Prior to adding calcium salts to the film-forming solution, the salts were evaluated to measure their solubility (*8*). Each calcium salt at 0.015% (wt/vol in 100 mL of water at pH 9.0) was stirred at 90 °C for 20 min. These solutions were then poured into preweighed filter paper and dried in an oven (105−110 °C) for 4 h. After the filter paper was cooled, the lost weight was recorded as percent calcium solubility.

Preparation of Films. Film-forming solutions of SPI (5 g/100 mL water) were prepared. Glycerin was added into film-forming solutions (2 g/100 mL) to ensure film plasticization. Film-forming solutions were adjusted to pH 9.0 with 1.0 N NaOH. After calcium salts and GDL (0.1/ 0.2/ 0.3%, wt/wt of SPI) were added, the film-forming solutions were stirred thoroughly for 15 min. These mixtures were then maintained at 90 °C for 20 min in a water bath to prevent protein irreversible coagulation. Film-forming solutions (15 mL) were poured into 8.6 cm^2 polystyrene weighing boats (Fisher Scientific) and air-dried at room temperature (22−25 °C). After

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Table 1. Hunter Color Values (*L*, *a*, and *b*) and Total Color Difference (ΔE) of SPI Films with Calcium Salts and GDL

	L	a	b	ΔE
SPI (control)	53.11 ± 1.34 [b]	-3.94 ± 0.11 [b]	6.75 ± 0.12 [b]	41.18 ± 1.34 [b]
CaSO$_4$ 0.3%	46.35 ± 1.17 [b]	-4.23 ± 1.24 [b]	7.23 ± 0.11 [b]	48.92 ± 0.36 [b]
CaCl$_2$ 0.3%	46.29 ± 1.61 [b]	-4.51 ± 0.04 [c]	6.92 ± 0.21 [b]	48.94 ± 0.54 [b]
GDL 0.3%	42.77 ± 1.47 [b]	-5.62 ± 1.04 [a]	7.64 ± 0.28 [a]	52.61 ± 1.18 [a]

[a-c] Means within each column with same italic superscripts are not significantly different ($n = 3$, $P < 0.05$).

the films were dry, they were peeled from the polystyrene plates and tested for film properties. Control SPI film (5% SPI) was prepared under the same conditions without adding calcium salts and GDL.

Film Thickness. Film thickness was measured using a micrometer with a sensitivity of 1.00 mm (model 2804-10, Mitutoyo, Japan). Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thicknesses (μm) of films were determined from the average of measurements at five locations.

Color. Color of films was measured using a Gardner Colormeter (ColorGard System/05, Pacific Scientific, Silverspring, MD). 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) (*9*). Total color difference (ΔE) was calculated:

$$\Delta E = [(L_{\text{film}} - L_{\text{standard}})^2 + (a_{\text{film}} - a_{\text{standard}})^2 + (b_{\text{film}} - b_{\text{standard}})^2]^{0.5} \quad (1)$$

Standard values refer to the white calibration plate ($L = 94.47$, $a = -0.81$, $b = -0.86$).

Film Surface and Cross-Sectional Morphology. The surface morphology and cross-sectional images of films were examined by scanning electron microscopy (SEM, JEOL, JSM-5800). Film samples were dried in vacuo for 12 h and cooled in a desiccator to prevent absorption of moisture. For evaluation of cross-sectional images, samples were deep-frozen in liquid nitrogen and fractured to reduce damage to the films. Each specimen was fixed to stub with silver conducting paint and was coated with gold with a Gold Sputter Coater (SPI sullier, SPI-MODULE). The surface and cross-section of specimen were observed at 10 kV of electron velocity.

Mechanical Properties. Mechanical properties of films were measured with a texture analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY). Sample handling for texture analyses was carried out according to ASTM standard method D 882-91 (*10*). Film samples were conditioned at ambient temperature and 50% relative humidity for at least 48 h prior to textural analysis (*10*). Film strips measuring 50 × 25 mm were mounted onto the texture analyzer. Initial grip distance was 40 mm and cross-head speed was set at 5 mm/s in a 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) of films was measured by mounting circular film samples of 16-mm diameter on a specially designed cup (12-mm diameter) and securing them with a gasket. Using a 3-mm probe at 5 mm/s constant speed in a compression mode (TA.XT2), the films were punctured and the force required (in newtons) was recorded and expressed as puncture strength. Elongation to break (ETB) is expressed as a dimensionless measure of a film's ability to stretch. The percent change in length experienced by a material due to pulling stress before breakage, and percent elongation at break (% ETB) was calculated as

$$\% \text{ ETB} = (D_0 - D_1)/D_0 \times 100 \quad (2)$$

where D_0 is initial distance and D_1 is distance at break.

Water-Vapor Permeability. ASTM E96-90 (*11*) was used to measure water-vapor permeability (WVP) with aluminum metal cups. The wells in the WVP metal cups (area, 33 cm²) 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 mouths of metal cups, and each was secured between a metal rim and rubber gasket by six screws placed symmetrically around the cup's circumference. The container was weighed to the nearest 0.001 g at 30 min intervals for 10 h to determine by weight the amount of moisture migration across the film in that time. Weighing every 30 min enabled us to obtain enough points for plotting weight loss vs time. The WVP was measured at 23 °C and 55% relative humidity, and linear regression-derived slopes were used to estimate water-vapor transmission rate; WVP measurements were performed as described by McHugh et al. (*5*). The WVP was expressed in g/m·s·Pa and was calculated using the following equations.

$$\text{WVTR} = [P \times D \times \text{Ln} \,[(P - P_2)/(P - P_1)]]/R \times T \times \Delta Z \quad (3)$$

where P = total pressure; D = diffusivity of water through air at 25 °C; R = gas law constant; T = absolute temperature (298K); ΔZ = mean stagnant air gap height; P = corrected vapor partial pressure at solution surface; P_2 = corrected water-vapor partial pressure at film inner surface in cup.

$$\text{WVP} = [\text{WVTR}/(P_2 - P_3)] \times \text{Thickness} \quad (4)$$

where P_3 = water-vapor partial pressure at film outer surface in desiccator.

Statistical Analysis. Each replication included individual preparation of film from film-forming solution. Three replications were performed in a completely randomized design. A minimum of three observations were collected unless specified otherwise. Data were analyzed using the general linear model procedures of SAS (*12*) to determine differences between treatment means. Pairwise comparison of all treatment means was performed using the least significant difference (LSD) procedure with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

Film Thickness. Film thickness is used to calculate mechanical and moisture barrier properties. Calcium chloride addition had variable changes (98−128 μm) on film thickness which remained unchanged for calcium sulfate addition (112−124 μm). Films cast from solutions containing 0.3% calcium chloride (wt/wt of SPI) were "grittier" because of coagulated protein particles. The lack of film smoothness containing 0.3% calcium chloride (wt/wt of SPI) may have resulted in greater thickness (128 μm) readings. The films with a higher GDL concentration had a lower thickness (84−98 μm) when compared to that of those with a higher calcium sulfate concentration.

Film Color. Color is an important property of edible films because it could affect consumer acceptance in potential edible food applications. Hunt *L*, *a*, and *b* color values and total color differences (ΔE) for films are shown in Table 1. SPI films with calcium sulfate at pH 10.0 showed the higher negative *a* (greenness) and positive *b* (yellowness) values. SPI with GDL at pH 9.0
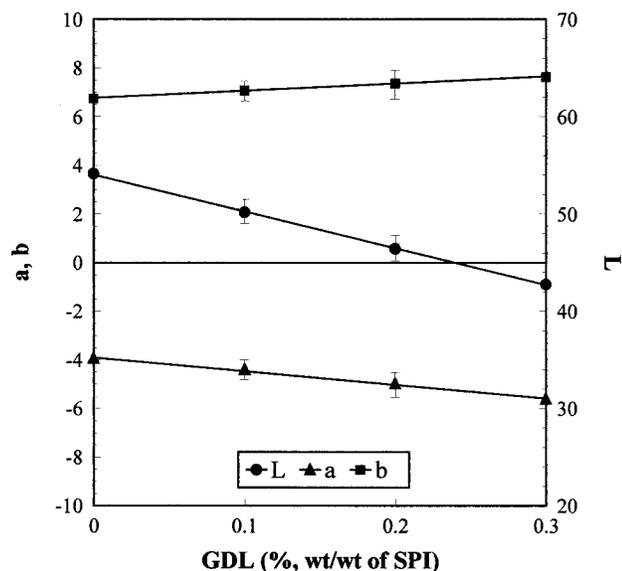
Figure 1. Effect of GDL on the Hunter *L*, *a*, and *b* color values of SPI films ($n = 3$, $P < 0.05$).

Table 2. Effect of Calcium Salts Concentration on Mechanical Properties of SPI Films

calcium salts (wt/wt of SPI)	tensile strength (Mpa)	puncture strength (MPa)	elongation to break (%)
SPI (control)	5.5 ± 0.8 [b]	5.9 ± 0.7 [d]	18.2 ± 1.6 [d]
CaSO$_4$			
0.1%	6.8 ± 1.1 [ab]	8.2 ± 0.5 [b]	20.7 ± 0.9 [cd]
0.2%	8.3 ± 1.3 [a]	9.6 ± 0.6 [a]	26.7 ± 1.2 [a]
0.3%	8.6 ± 1.5 [a]	9.8 ± 0.7 [a]	28.5 ± 1.2 [a]
CaCl$_2$			
0.1%	5.3 ± 0.7 [b]	7.9 ± 0.3 [b]	23.6 ± 0.7 [b]
0.2%	6.4 ± 0.9 [ab]	8.5 ± 0.7 [ab]	24.2 ± 0.9 [ab]
0.3%	5.0 ± 0.8 [b]	7.2 ± 0.5 [c]	21.9 ± 0.8 [c]

[a-d]Means within each column with same italic superscripts are not significantly different ($n = 3$, $P < 0.05$).

were generally clearer (highest *L* value) and more uniform. Effect of GDL on Hunter color values of SPI films at 0.1, 0.2, and 0.3% (wt/wt of SPI) induced substantial changes in film color properties. Figure 1 shows that increasing GDL concentration resulted in linear ($R^2 = 0.99$) decrease of L, a linear ($R^2 = 0.99$) increase of negative *a* (greenness), and a linear ($R^2 = 0.98$) increase of positive *b* (yellowness).

Effect of Calcium Salts on Mechanical Properties. Mechanical properties of protein films provide an indication of expected film integrity under conditions of stress that would occur during processing, handling, and storage. The strength and flexibility of film is described by its tensile strength (TS), puncture strength (PS), and elongation to break (ETB). Effects of varying concentrations of calcium salts on SPI films are shown in Table 2. The results indicated that calcium sulfate treated SPI (CaSO−SPI) films increased the TS and PS, whereas calcium chloride treated SPI (CaCl−SPI) films did not improve significantly. Because the pH of film-forming solutions containing calcium salts were above the isoelectric pH of soy proteins, the protein side chains would have a predominance of negative charges to which these cations could bind. Calcium ions may bind to negative charges on the soluble protein fraction, changing the ionic interactions among proteins. The increase in protein solubility by calcium salts in film-
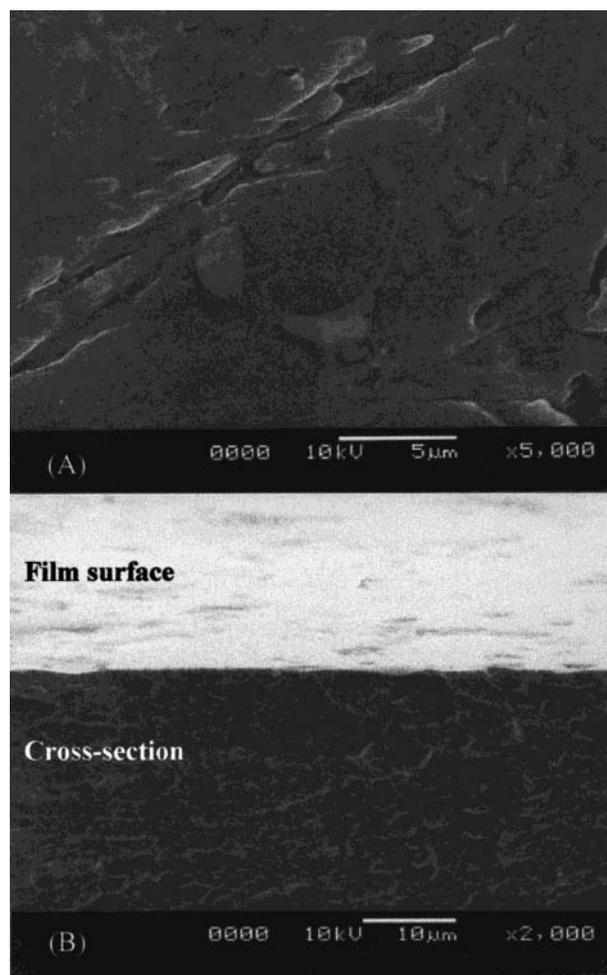
Figure 2. Typical SEM micrograph of 5% SPI film: (A) surface morphology of the SPI film; (B) cross-section of the SPI film.

forming solution is attributed to greater electronegativity of divalent calcium cations. This property would enable divalent calcium cations to bind strongly with polar groups on protein to form denser three-dimensional networks in SPI films. However, solubility of each calcium saltcould affect the mechanical properties of protein films. The solubility was about 96% for calcium chloride and about 74% for calcium sulfate. From the results, it could be considered that addition of calcium chloride to protein showed faster coagulating rate than that of calcium sulfate. Therefore, CaSO−SPI films had a homogeneous structure, but CaCl−SPI films had a heterogeneous structure. Figure 2 shows a typical SEM micrograph of SPI films. SPI films had a bumpy surface appearance and thick mass cross-sectional structure. The appearance of calcium salts treated films was generally clearer and more uniform than that of SPI films, as shown in Figure 3. However, CaCl−SPI films had more coagulated protein spots on the surface. The increase in protein hydrophobicity could be attributed to divalent calcium cations forming cross-linking between negatively charged carboxyl groups on polypeptide chains, thus preventing those charged sites from interacting with water, rendering rigid structure to films and contributing to mechanical properties. Figure 4 , cross-sections of calcium salts treated SPI films, shows that the CaSO−SPI films were denser and had more homogeneous structures than CaCl−SPI films.

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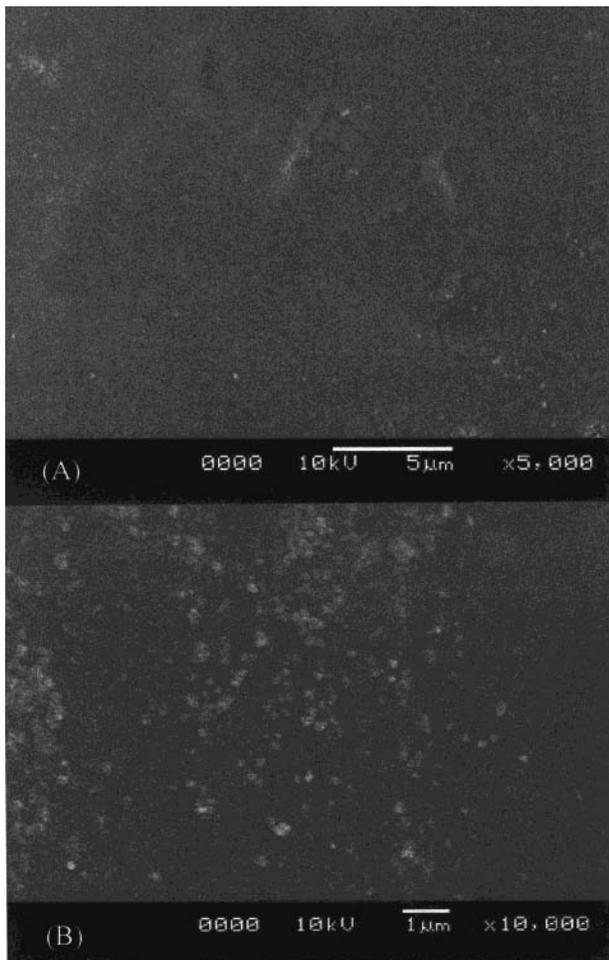
Figure 3. SEM micrographs of calcium treated SPI films: (A) surface morphology of CaSO−SPI film; (B) surface morphology of CaCl−SPI film.

Figure 4. SEM micrographs of calcium-treated SPI films: (A) cross-section of CaSO−SPI film; (B) cross-section of CaCl−SPI film.

Effect of GDL on Mechanical Properties. Effects of varying concentrations of GDL on SPI films are shown in Table 3. Tensile strengths of GDL-treated SPI (GDL−SPI) films with 0.1, 0.2, and 0.3% GDL (wt/wt of SPI) were 6.3, 7.7, and 8.3 MPa, respectively. Puncture strengths of GDL−SPI films were 7.2, 8.3, and 8.8 MPa. The results indicated that addition of GDL affected the TS, PS and ETB. It is considered that hydrogen bonds contributed to film formation in the presence of GDL, and affected mechanical properties, such as TS, PS, and ETB. Figure 5 shows SEM micrographs of the surfaces and the cross-sections of GDL−SPI films. Appearance of GDL−SPI films was more uniform and clearer than that of the SPI films. It is thought that electrostatic inter-action and disulfide bonds were involved in film forma-tion of SPI by heat (*13*), whereas hydrogen bonding as mainly responsible for formation of GDL−SPI films.

Water-Vapor Permeability. As shown in Table 4, the WVP of SPI films was affected by calcium salts and GDL. Treatment with calcium salts and GDL improved the moisture-barrier properties of SPI films. Addition of calcium sulfate improved both mechanical and water barrier properties of SPI film by reacting with protein constituents to yield insoluble proteinates and altered film consistency. Calcium bridges between protein constituents in films would have contributed to the lower WVP in films compared to that of the SPI control

Table 3. Effect of Glucono-δ-lactone on Mechanical Properties of SPI Films

GDL (wt/wt of SPI)	tensile strength (MPa)	puncture strength (MPa)	elongation to break (%)
0.1%	6.3 ± 0.4 *b*	7.2 ± 0.8 *bc*	21.1 ± 1.2 *c*
0.2%	7.7 ± 0.3 *a*	8.3 ± 0.6 *b*	27.3 ± 1.2 *b*
0.3%	8.3 ± 0.5 *a*	8.8 ± 0.7 *a*	39.4 ± 2.7 *a*

*a−d*Means within each column with same italic superscripts are not significantly different ($n = 3$, $P < 0.05$).

films. Calcium bridges would maximize interactions between negatively charged molecules and might im-prove protein network and stability. GDL−SPI films showed lower values of water permeation. GDL pro-moted aggregation of the unfolded protein by increasing hydrophobicity and insolubilization. Ionic cross-linking reduced not only protein segmental mobility, but also protein solubility in water, thus reducing WVP through the protein matrix (*5*). The acidic GDL treatment could improve resistance to water-vapor diffusion in protein films. From the results, edible protein films containing calcium salts and GDL have the potential for decreased WVP. SPI films formulated with calcium salts or GDL afford potential as moisture barriers for food systems and as coating agents for food application or intermedi-ate-moisture foods.

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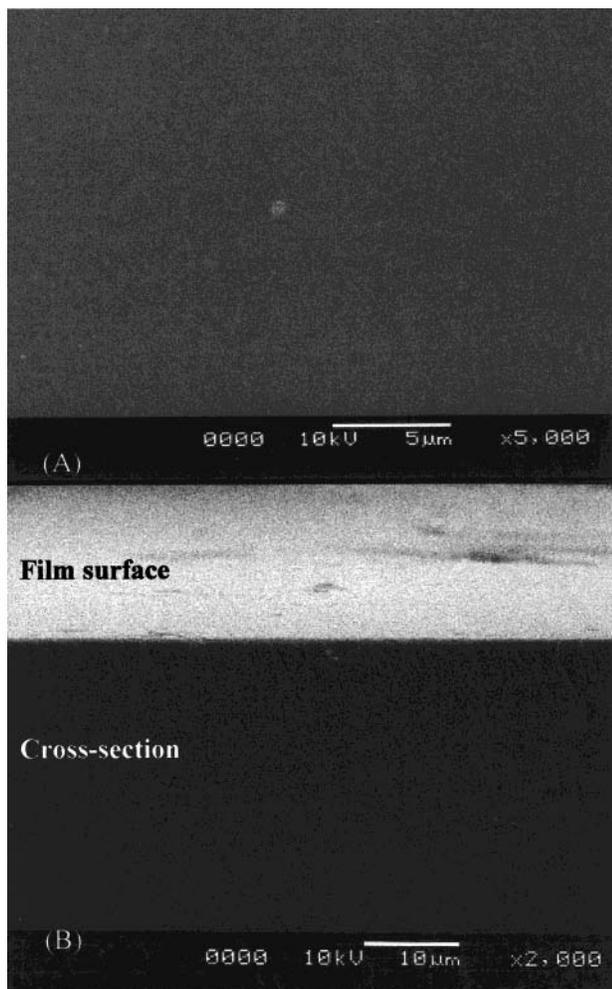
Figure 5. SEM micrograph of GDL−SPI film: (A) surface morphology of the GDL−SPI film; (B) cross-section of the GDL−SPI film.

Table 4. Water-Vapor Permeability of SPI Films with Calcium Salts or GDL

	thickness (μm)	WVP ($\times 10^{-9}$ g·m/m²·s·Pa)
SPI (control)	84 ± 8.2 [c]	2.46 ± 0.31 [a]
SPI + CaSO₄ (0.3 wt %/wt)	124 ± 5.6 [a]	1.73 ± 0.21 [b]
SPI + CaCl₂ (0.3 wt %/wt)	128 ± 6.3 [a]	2.12 ± 0.23 [a]
SPI + GDL (0.3 wt %/wt)	98 ± 4.7 [b]	1.43 ± 0.24 [c]

[a−c] Means within each column with same italic superscripts are not significantly different ($n = 3$, $P < 0.05$).

ABBREVIATIONS USED

ETB, elongation to break; CaSO−SPI, calcium sulfate treated SPI; CaCl−SPI, calcium chloride treated SPI; GDL, glucono-δ-lactone; GDL−SPI, glucono-δ-lactone treated SPI; PS, puncture strength; SEM, scanning electron microscopy; SPI, soy-protein isolate; TS, tensile strength; WVP, water-vapor permeability.

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