

Characteristics of Soy Protein Isolate Films Plasticized by Mixtures of Crystalline and Aqueous Sorbitol or Glycerin

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솔비톨 혼합물과 글리세린 가소제에 의한 분리 대두단백질 필름의 특성연구

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Abstract

The effects of sorbitol mixtures as plasticizers on moisture sorption property (MSP), water vapor permeability (WVP), color, tensile strength (TS), elongation at break (E), and total soluble matter (TSM) of soy protein isolate (SPI) films were investigated. Two different types of sorbitols, aqueous and crystalline, were added to film-forming solutions in various ratios of crystalline to aqueous (0:1, 0.25:0.75, 0.5:0.5, 0.75:0.25, or 1:0, based on weight). In addition, the characteristics of the SPI films plasticized by sorbitol mixtures and glycerin were compared with moisture sorption rate against time. Sorbitol-plasticized films had higher in TS, but lower in WVP and E than the glycerin-plasticized films. However, the properties of SPI films did not differ appreciably by the type of sorbitol added to film-forming solutions. To explain the high solubility and low WVP of sorbitol-plasticized films, cumulative amounts of moisture content gained during adsorption and lost during desorption of films were compared between sorbitol and glycerin-plasticized films. The results suggest that use of sorbitol as a plasticizer for preparing SPI films improves moisture barrier properties of the films. However, the high solubility of sorbitol-plasticized films needs to be reduced for improving the functionality of SPI films in potential packaging applications.

Key words : soy protein, protein films, edible films, sorbitol, glycerin, tensile properties, water vapor permeability

INTRODUCTION

Research interest in edible films and coatings for use in food packaging and preservation has increased, although commercial applications remain limited. Edible films are produced from renewable resources and can enhance the organoleptical and textural properties of foods. Proteins which have been investigated for film development include collagen, gelatin, whey protein, casein, egg albumen, soy protein, wheat gluten, corn zein, rice bran protein, peanut protein, and

cottonseed protein. However, due to the inherent hydrophilicity of proteins and the substantial amounts of added hygroscopic plasticizers, protein films perform poorly as moisture barriers (1).

A plasticizer is defined as "a substantially nonvolatile, high boiling, non-separating substance which, when added to another material, changes the physical and mechanical properties of that material"(2). Generally, protein film researchers have been plasticizing edible films with glycerin, crystalline sorbitol, or polyethylene glycol. In certain aspects, sorbitol has given films with better physical properties, particularly lower water vapor permeability, than glycerin (3, 4). Glycerin as a plasticizer increases film flexibility, but

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also results in films of high water vapor permeability. Modifying the amount of added glycerin and the pH of the film-forming solutions has decreased water vapor permeability of protein films, but not to a desirable level (5, 6). Because plasticization increases diffusion through the film, it is possible that replacing glycerin with a higher molecular weight solute, such as sucrose or a polyol, may help retard diffusion, given that the rheological properties are acceptable.

Polyols are polyhydroxy alcohols or sugar alcohols used in many diverse fields including foods, pharmaceuticals, and cosmetics. Manufacturers of soft gelatin capsules (soft gels) use mixtures of sorbitol and sorbitol anhydrides to plasticize gelatin shells (7). Such mixtures do not re-crystallize ("bloom") over time as is the case with crystalline sorbitol (8, 9). Knowledge of the phase equilibria in systems containing biological products, such as polyols, is important for modeling and designing industrial processes (10).

The objective of this study was to prepare sorbitol-plasticized SPI films in combination of aqueous and crystalline sorbitol in various ratios ranged from 0 to 1, and to characterize the modified SPI films by measurement of tensile strength (TS), elongation at break (E), water vapor permeability (WVP), moisture sorption property against time (MSP), and total soluble matter (TSM). In addition, cumulative amounts of moisture content of both sorbitol- and glycerin-plasticized films during adsorption and desorption were compared. It may help understanding the higher solubility and lower WVP of sorbitol-plasticized films than those of glycerin-plasticized films

MATERIALS AND METHODS

Materials

SUPRO[®] 620, a soy protein isolate (SPI), was obtained from Protein Technologies International Company (St. Louis, MO, USA) and stored at refrigerator prior to use. Glycerin (U.S.P. food grade) was purchased from Mallinckrodt (Paris, KY, USA), and aqueous sorbitol (SORBO[®]) and crystalline sorbitol (SORBOGEM[™]) were purchased from SPI Polyols, Inc. (New Castle, DE, USA). Other chemicals were purchased from J.T. Baker Chemical (Phillipsburg, NJ, USA)

Film preparation

SPI films were prepared essentially according to the procedure described by Rhim *et al.* (26). Film-forming

solutions were prepared by dissolving 5 g of SPI in 100 mL of distilled water and 2.5 g of plasticizer. Glycerin, aqueous sorbitol, and crystalline sorbitol were added to film-forming solution as plasticizers. Aqueous sorbitol (70% w/w) and crystalline sorbitol were used separately and added in combination of aqueous and crystalline in the ratios of 0.25:0.75, 0.5:0.5, or 0.75:0.25 (w/w). Sodium hydroxide (1 N) was used to adjust the pH of the film-forming solutions to 10.00 ± 0.01 . After pH adjustment, the solutions were held for 15 min in a water bath at 75°C and strained through cheesecloth (Cheesecloth wipes, VWR Scientific Products, Chicago, IL, USA) to remove any bubbles and lumps. Film-forming solutions (90 mL) were cast on flat, level Teflon[®]-coated glass plates (21 cm x 35 cm). Films were peeled from the plates after drying at ambient temperature for about 20 h. Dried films were conditioned at 50% RH and 25°C for 24 h prior to testing.

Thickness

Film thickness was measured to the nearest 2.54 mm (0.1 mil) with a hand-held micrometer (B.C. Ames Co., Waltham, MA, USA). Five thickness measurements were taken on each water vapor permeability 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.

Color

Color values of the 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 to white), a = -80 to 100 (greenness to redness), and b = -80 to 70 (blueness to yellowness). Standard values for the white calibration plate were L = 96.89, a = -0.07, and b = 1.98. Color changes were determined by comparing total color differences among films. Total color difference (E) was calculated as:

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

Tensile strength and percentage elongation at break

TS and E were determined with an Instron Universal Testing Machine (Model 5566, Instron Corp., Canton, MA, USA) following the guidelines of ASTM Standard Method

D 882-91 (11). Initial grip separation was set at 50 mm and cross-head speed was set at 500 mm/min. TS was expressed in MPa and calculated by dividing the maximum load (N) by the initial cross-sectional area (m²) of the specimen. E was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (50 mm) and was expressed as a percentage. TS and E measurements for each type of film were replicated five times.

Total soluble matter

TSM was calculated as the percentage of film soluble matter to initial dry matter during immersion in distilled water for 24 h. Film pieces (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, USA) and stored at 25°C for 24 h. After discarding the water remaining in the beakers after 24 h, residual film pieces were rinsed gently with distilled water. The pieces were then dried in an air-circulating oven (105°C) for 24 h. The weight of solubilized matter was calculated by subtracting the weight of unsolubilized matter from the weight of initial dry matter and expressed as a percentage of the initial dry matter content using the following relationship:

$$TSM(\%) = [(S_o - S) / S_o] \times 100$$

where S_o = initial dry matter calculated by multiplying the initial sample weight by solid content and S = unsolubilized dry matter. TSM measurement for each specimen was replicated three times.

Water vapor permeability

Five film specimens were tested for each type of film. WVP (gm/m²hPa) was calculated as: WVP = (WVTRL)/p where WVTR was measured water vapor transmission rate (g/m²h) through a film specimen, L was mean film specimen thickness (m), and p 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 (12) as described by Gennadios, Weller & Gooding (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 moving the air with velocity of 196.3 m/min over the surface of film specimens to remove the permeating water vapor. The weights of the cups were recorded six times at one-hour intervals. Linear

regression was used to estimate the slope of this line in units of mass (g) per unit time (h).

Sorption isotherm measurements

Isotherms were determined according to the procedure described by Lang *et al.* (14) and Kanade & Pai (15). The proximity equilibration cell (PEC) method of Lang *et al.* (14) offers an increased surface area of the saturated salt solution per unit vapor volume as well as a shorter mean free path for the water vapor. This decreases the equilibration time from more than 30 days to about 7 days. For adsorption measurement, film samples (20 - 30 mm²) were dried in a vacuum desiccator at 40°C over potassium hydroxide (KOH) solution with a water activity (a_w) of 0.082±0.014 for 1 wk. For desorption measurements, film pieces were hydrated in closed desiccators at 25°C over distilled water for 3 days. These dried and hydrated films were placed in a proximity equilibration cell (PEC) containing saturated salt solutions for 24 h at 25°C. The dried and hydrated samples were equilibrated with saturated salt solutions of known a_w at 25°C for 7 days. The salts used and the corresponding a_w values of their saturated solutions are shown in Table 1. Dried and hydrated film pieces were immediately weighed and dried for 24 h in an air-circulating oven at 105°C to determine their moisture content. Equilibrated moisture contents were calculated from weight gain or loss. Measurements were made in triplicate.

Table 1. Equilibrium water activity (a_w) at 25°C of saturated salt solutions used for film sample equilibration (Bell & Labuza (20))

Salt	a _w
Potassium hydroxide	0.082±0.070
Lithium chloride	0.113±0.003
Magnesium chloride	0.328±0.002
Magnesium nitrate	0.529±0.002
Cobalt chloride	0.649±0.035
Sodium chloride	0.753±0.001
Potassium chloride	0.843±0.003
Potassium sulfate	0.973±0.005

For water vapor sorption, film samples were dried in vacuum desiccators at 40°C over KOH solution (a_w=0.082±0.014) for 1 wk or hydrated in desiccators containing saturated potassium sulfate (K₂SO₄) solution (a_w=0.973 ±0.005) for 3 days. These dried and hydrated films were placed in PEC’s (equilibrated at 25°C for 48 h) containing

a saturated K_2SO_4 solution ($a_w=0.973\pm0.005$) or KOH solution ($a_w=0.082\pm0.014$). The dried and hydrated samples were equilibrated in the PEC at 25°C for 48 h. Weight gain or loss was recorded at specific intervals. Measurements were made in triplicate.

Statistical analysis

The experimental design was a completely randomized design (CRD). ANOVA tables were generated for TS, E (%), WVP, and total color difference (ΔE) using the General Linear Models (GLM) procedure in the SAS statistical software package (SAS Institute Inc., Cary, NC, USA). Significantly ($P<0.05$) different means were separated with Duncan's multiple range test.

RESULTS AND DISCUSSION

Color

Color of protein films can be of importance for consumer acceptance in packaging application. Total color difference (ΔE) values of SPI films plasticized with sorbitol in various ratios of crystalline to aqueous and plasticized with glycerin were compared (Table 2). For sorbitol-plasticized films, there was no significant difference in ΔE values by sorbitol types and ratio. However, all sorbitol-plasticized film had lower ΔE values than a glycerin-plasticized film. The lower ΔE values in sorbitol-plasticized film was attributed to higher positive b (yellowness) and negative a (greenness) values than a glycerin-plasticized film. A similar observation on total color difference in protein based films plasticized with sorbitol and glycerin was reported for egg albumen films (4)

Total soluble matter

All SPI films plasticized with sorbitol or glycerin were completely soluble in water. The protein network is not likely to solubilize or disperse in water. High interaction density and the presence of intermolecular covalent bonds are responsible for insolubility of protein-based films (24). Hydrophillic plasticizers enhance solubility in water for films based on wheat gluten or whey protein (27, 29). Stuchell & Krochta (27) pointed out increases in the content of protein solubilized in water when the hydrophillic plasticizer content increased for whey protein-based films. A decrease in the polymer network interaction density due to plasticizer presence was thus associated with this increase in solubility properties. Kim & Ustunol (28) had shown that solubility

of whey protein films was dependent on plasticizer type. For example, Sorbitol-plasticized film had higher solubility in water than glycerin-plasticized film, even though glycerin was more hygroscopic and higher EMC at all a_w ranges than sorbitol. In our present study, complete solubility of SPI film plasticized with both sorbitol and glycerin may be due to high plasticizer content (0.5g plasticizer/g SpI).

Tensile strength

TS can be used to describe how the mechanical properties of films relate to their chemical structures (16). TS of SPI films plasticized with sorbitol in the ratio of crystalline to aqueous state ranged from 12.1 to 15.8 MPa, but did not significantly ($p>0.05$) differ (Table 2). Irrespective of the ratio crystalline to aqueous, sorbitol-plasticized SPI films, however, had higher TS values than glycerin-plasticized SPI film (6.0 MPa) at an equal amount of plasticizer. This is good agreement with other observations reported by other researchers for egg albumen and casein films (4, 17).

Elongation at break

Interestingly, E(%) values of SPI films plasticized by sorbitol in the various ratio of crystalline to aqueous were significantly different (Table 2). A SPI film plasticized only with aqueous sorbitol had the lowest E(%) value(36.45%), whereas a SPI film plasticized with equal amount of aqueous and crystalline sorbitol had the highest E(%) value (86.80%). This elongation behavior in SPI films as plasticized with the combination of crystalline and aqueous sorbitol could not be generalized, and understanding the behavior remains a complex subject. Using nuclear magnetic resonance techniques in starch-sorbitol films, Gaudin *et al.* (25) had reported that sorbitol in the films showed the existence of two competitive effect; one associated with a decrease in plasticizing effect and the other with enhanced the effect, depending on the amount of added sorbitol and water binding property at different relative humidities.

On the other hand, all sorbitol-plasticized SPI films, regardless of the ratio, had substantially lower E(%) values than a glycerin-plasticized film. This is consistent with the results in egg albumen(4) and casein(17) films.

Water vapor permeability

The incorporation of plasticizers modifies the molecular organization of the protein network with an increase in free volume. The network becomes less dense and as a consequence more permeable (2, 23). Permeability increase

with the addition of plasticizer can be related to hydrophilicity of plasticizer molecules. Introducing hydrophilic plasticizers, favorable to adsorption and desorption of water molecules, is known to enhance the water vapor permeability of protein-based films (3, 5, 19).

SPI films plasticized with the combination of crystalline and aqueous sorbitol showed the slight difference in WVP among film samples (Table 2), but there was no significant difference except for a SPI film plasticized with only aqueous sorbitol.

Table 2. Mean values and standard deviations of total color difference (ΔE), tensile strength (TS), percentage elongation at break (E%), and water vapor permeability of SPI films plasticized with crystalline sorbitol (C), aqueous sorbitol (S), or glycerin

Plasticizer	ΔE	TS (MPa)	E (%)	WVP ($\text{g}\cdot\text{m}/\text{m}^2\cdot\text{h}\cdot\text{Pa})\times 10^{-6}$
C:S = 0:1	13.98 \pm 2.17	14.0 \pm 0.6	36.45 \pm 18.47 ^c	3.23 \pm 0.23 ^b
C:S = 0.25:0.75	13.42 \pm 1.69	13.7 \pm 0.8	69.99 \pm 25.85 ^{ab}	3.73 \pm 0.73 ^{ab}
C:S = 0.50:0.50	13.03 \pm 0.78	12.1 \pm 0.4	86.80 \pm 19.50 ^a	3.75 \pm 0.29 ^{ab}
C:S = 0.75:0.25	13.11 \pm 0.53	14.3 \pm 1.4	52.62 \pm 16.43 ^{bc}	4.25 \pm 0.35 ^a
C:S = 1:0	12.64 \pm 1.10	15.8 \pm 1.8	67.49 \pm 18.28 ^b	3.72 \pm 0.47 ^{ab}
Glycerin	15.42 \pm 1.62	6.0 \pm 1.0	172.5 \pm 60.67	9.28 \pm 1.16

^{a-c}Any two means in the same column followed by the same small-case letter are not significantly ($P>0.05$) different by Duncan's multiple range test.

On the other hand, all Sorbitol-plasticized SPI films had lower WVP(overall mean value of $3.74 \times 10^{-6} \text{ g}\cdot\text{m}/\text{m}^2\cdot\text{h}\cdot\text{Pa}$) than a glycerin-plasticized film ($9.28 \times 10^{-6} \text{ g}\cdot\text{m}/\text{m}^2\cdot\text{h}\cdot\text{Pa}$). This result as affected by different type of plasticizer had been also observed for other protein-based films such as whey protein (3), egg albumen (4), wheat protein (18), and casein (17) films. Cherian *et al.* (19) had reported that the WVP of glycerin-plasticized wheat gluten films was reduced when glycerin was partially substituted with sorbitol.

Moisture Sorption isotherms and sorption curves

The equilibrium moisture sorption curves of SPI films plasticized with sorbitol or glycerin during adsorption and desorption were presented in Fig 1. The sorption curves of SPI films were typical of moisture sensitive (hydrophilic) polymers. At high a_w ranges ($a_w \geq 0.65$), the EMC of both sorbitol- and glycerin- plasticized films increased greatly while at low a_w range, increase in EMC was reduced, leading to J-shaped isotherm curves. Sorption isotherms for SPI films were influenced by the type of plasticizer incorporated. Glycerin-plasticized SPI films absorbed more moisture at all a_w ranges investigated than did sorbitol films, possibly due

to the higher moisture affinity of glycerin.

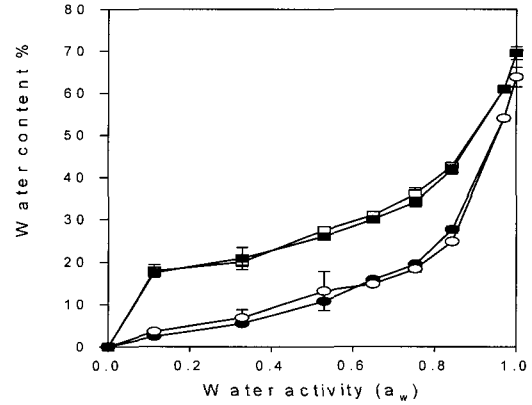


Fig. 1. Water vapor sorption isotherms at 25°C of SPI films plasticized with sorbitol (circles) or glycerin (squares). Solid circles and squares correspond to adsorption measurements; blank circles and squares correspond to desorption measurements.

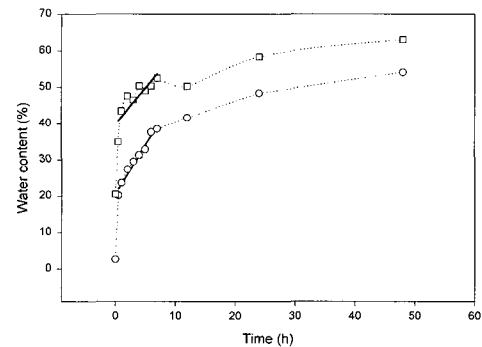


Fig. 2. Water vapor adsorption by SPI film plasticized with sorbitol (O) or glycerin (□) placed in a desiccator over a saturated potassium sulfate solution ($a_w=0.973$) at 25°C.

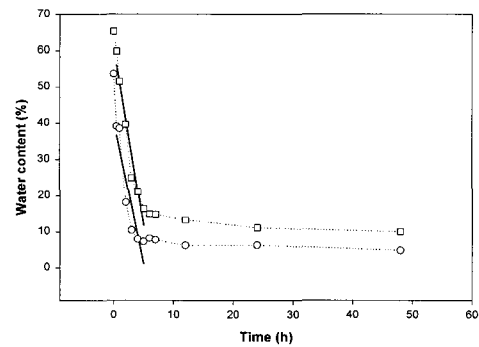


Fig. 3. Water vapor desorption by SPI film plasticized with sorbitol (O) or glycerin (□) placed in a desiccator over a potassium hydroxide solution ($a_w=0.082$) at 25°C.

Typically, a comparison of adsorption and desorption isotherms of most hydrophilic polymer systems shows some hysteresis (20). Hysteresis can result from slow swelling due to conformational changes involving structural relaxation (21, 22). However, in our study, the hysteresis between adsorption and desorption isotherms of SPI films plasticized with sorbitol or glycerin was minimal or negligible.

The moisture adsorption/desorption curve of SPI films plasticized with sorbitol or glycerin at given relative humidities as a function of time were presented in Fig. 2 and Fig. 3. Moisture adsorption for both sorbitol- and glycerin-SPI films at a_w of 0.97 was more rapid in the initial stages of moisture adsorption and a lesser amount of moisture was adsorbed as adsorption time increased. Then, the moisture content of SPI film reached a plateau, indicating that the SPI film became equilibrated with storage relative humidity. Moisture desorption behavior for the films at a_w of 0.082 showed similar trends to moisture absorption.

The slope of the adsorption curve in the initial stages for sorbitol-plasticized film was 2.65 ($R^2=0.97$), while the slope for glycerin-plasticized SPI film was 1.68 ($R^2=0.71$). It may indicate that films plasticized with sorbitol equilibrated faster than glycerin-plasticized film at given relative humidity. It is interesting to note that even though glycerin molecules have more hydroxyl group to which water molecules can be adsorbed and thus are more hydrophilic than sorbitol molecules, sorbitol-plasticized film adsorbed moisture faster in the initial stages. The results could be associated with higher incorporation ability of sorbitol to protein network than that of glycerin. Our present studies on sorption isotherms and initial sorption rates in SPI films may partly explain the lower WVP and higher solubility of SPI films plasticized with sorbitol, compared to SPI films plasticized with glycerin.

요 약

가소제로서 솔비톨 혼합물의 첨가가 분리대두단백 필름의 수분흡습특성, 수분투과도, 색, 인장강도 및 신장률, 그리고 용해 특성에 미치는 영향을 조사하였다. 솔비톨 혼합물은 액상형과 결정형의 상태로 다양한 비율로 혼합하여 첨가하였고, 가소제로 글리세린을 첨가한 대조구 필름의 물리적 특성과 비교하였다. 솔비톨이 첨가된 필름은 대조구 필름에 비해 높은 인장강도를 갖는 반면 낮은 수분투과도 및 신장률을 보였다. 한편 솔비톨 첨가 형태에 따른 영향은 필름 신장률에는 유의적 차이는 보였으나 기타 수분투과도, 색, 인장강도 등의 필름특성에는 커다란 차이는 보이지

않았다. 솔비톨 첨가된 필름은 높은 수분차단 효과 및 필름 강도를 갖는 대신 높은 용해성을 보여, 상업적 활용성을 증진시키기 위해서는 이를 극복하는 연구가 필요 할 것으로 사료된다.

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REFERENCES

- Gennadios, A., McHugh, T.H., Weller, C.L., and Krochta J.M. (1994) Edible films based on proteins. In J.M. Krochta, E.A. Baldwin, & M. Nisperos-Carriedo, Edible coatings and Coatings and Films to Improve Food Quality. Lancaster, PA: Technomic Publishing, (1st edition), p.201-277
- Banker, G.S. (1966) Film coating theory and practice. J. Pharm. Sci., 55, 81-89
- McHugh, T.H., and Krochta, J.M. (1994) Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. J. Agri. Food Chem., 42, 841-845
- Gennadios, A., Weller, C.L., Hanna, M.A, and Froning, G.W. (1996) Mechanical and barrier properties of egg albumen films. J. Food Sci., 61, 585-589
- Gontard, N., Guilbert, S., and Cuq J-L. (1993) Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. J. Food Sci., 58, 206-211
- Jo, K.H., Park, H.J., Jung, S.T., Rhim, J.W. and Weller C.L. (1996) Water vapor permeability and mechanical properties of soy protein films as affected by plasticizer, solvent, pH and salt. Foods and Biotech., 5, 243-248
- Reich, G. (1996) Effect of sorbitol specification on structure and properties of soft gelatin capsules. Pharmaceutical Industry, 58, 941-946
- Anker, M. Stading, M., and Hermansson, A.M. (2001) Aging of whey protein films and the effect on mechanical and barrier properties. J. Agri. Food Chem., 49, 989-995
- Gennadios, A. (2001) Soft gelatin capsules. In A.

- Gennadios, Protein based Film and Coatings. Boca Raton, FL: CRC Press, In press.
10. Ninni, L., Camargo, M.S. and Meirelles, A.J.A. (2000) Water activity in polyol systems. *J. Chem. Eng. Data*, 45, 654-660
 11. ASTM. (1995) Standard test methods for tensile properties of thin plastic sheeting. In *Annual book of ASTM standards* (Vol. 8.01). West Conshohochen, PA: American Society for Testing and Materials, p. 182-190
 12. ASTM. (1995). Standard test methods for water vapor transmission of materials. In *Annual book of ASTM standards* (Vol. 4.06). West Conshohochen, PA: American Society for Testing and Materials, p. 697-704
 13. Gennadios, A., Weller, C.L. and Gooding, C. H. (1994) Measurement errors in water vapor permeability of highly permeable, hydrophilic edible films. *J. Food Eng.*, 21, 395-409
 14. Lang, K.W., McCune, T.D. and Steinberg, M.P. (1981) A proximity equilibration cell for rapid determination of sorption isotherms. *J. Food Sci.*, 46, 936-938
 15. Kanade, P.B. and Pai, J.S. (1988) Moisture sorption method for hygroscopic samples using a modified proximity equilibration cell. *J. Food Sci.*, 53, 1218-1219
 16. Ninnemann, K.W. (1968) Measurement of physical properties of flexible films. In O.J. Sweeting, *The Science and Technology of Polymer Films*. London: Interscience, p. 546-649
 17. Chick, J. and Ustunol, Z. (1998) Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films. *J. Food Sci.*, 63, 1024-1027
 18. Pouplin, M., Redl, A. and Gontard, N. (1999) Glass transition of wheat gluten plasticized with water, glycerol, or sorbitol. *J. Agri. Food Chem.*, 47, 538-543
 19. Cherian, G., Gennadios A., Weller C.L. and Chinachoti P. (1995) Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chem.*, 72, 1-6
 20. Labuza, T.P. (1984) Moisture sorption: practical aspects of isotherm measurements and use. St. Paul, MN: American Association of Cereal Chemists.
 21. Bryan, W.P. (1987) Thermodynamics of water-biopolymer interactions: irreversible sorption by two uniform sorbent phases. *Biopolymers*, 26, 387-401
 22. Slade, L., Levine, H. and Finlay, J.W. (1989) Protein-water interactions: water as a plasticizer of gluten and other protein polymers. In R.D. Phillips, & J.W. Finlay, *Protein Quality and the Effects of Processing*. New York: Marcel Dekker, p. 9.
 23. Ashley, R.J. (1985). Permeability and plastics packaging. In *Polymer Permeability*; Comyn, J., Ed.; Elsevier Applied Science Publishers: London, pp. 269-308.
 24. Cuq, B., Gontard, N., Cuq, J-L. and Guilbert, S. (1997). Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *J. Agric. Food Chem*, 45, 622-626.
 25. Gaudin, S., Lourdin, D., Le Botlan, D., Ilari, J.L. and Colonna, P. (1999). Plasticisation and mobility in starch-sorbitol films. *Journal of Cereal Science*, 29, 273-284.
 26. Rhim, J.W., Gennadios, A., Handa, A., Weller, C.L. and Hanna, M.A. (2000). Solubility, tensile, and color properties of modified soy protein isolate films. *J. Agric. Food Chem*, 48, 4937-4941.
 27. Stuchell, Y.M. and Krochta, J.M. (1994). Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.*, 59, 1332-1337.
 28. Kim, S.J. and Ustunol, Z. (2001). Solubility and moisture sorption isotherms of whey-protein-based edible films as influenced by lipid and plasticizer incorporation. *J. Agric. Food Chem*, 49, 4388-4391.
 29. Gontard, N., Guilbert, S. and Cuq, J.L. (1992). Edible wheat gluten films; Influence of the main process variables on film properties using response surface methodology. *J. Food Sci.*, 57, 190-195, 199.