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Physical Properties of Silk Fibroin Films Treated with Various Plasticizers

– Research Note –

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Abstract

Silk fibroin (SF) films were prepared using various plasticizers and their physical properties were determined. Polyethylene glycol (PEG)-SF and polypropylene glycol (PPG)-SF films had tensile strengths (TS) of 23.71 MPa and 24.51 MPa, respectively, whereas the glycerol (G)-SF film had the lowest TS of 14.24 MPa. G-SF film had the highest % elongation, compared to PPG-SF and PEG-SF films. Water vapor permeability (WVP) of SF films varied with addition of plasticizers, and PEG-SF film had the lowest WVP. There was no significant difference in Hunter L value among treatments, but PEG-SF film had higher Hunter a and b values. These results suggest that SF film could be applied to food packaging and that the addition of plasticizers should improve the physical properties of SF film.

Key words: silk fibroin, protein film, plasticizer, physical property

INTRODUCTION

There are concerns regarding the environmental impacts of the disposal of non-biodegradable packaging materials. Therefore, there have been many studies of biodegradable films made from proteins, lipids, and polysaccharides for manufacturing packaging materials of natural biopolymers that are biodegradable (1). In particular, protein-based films can offer environmental compatibility as well as improvement in the quality and self life of foods (2-5). Protein-based films can also improve the physical properties of foods and minimize loss of volatile flavors and aromas (5).

Silk fibroin (SF) is a natural biopolymer believed to possess unique properties such as nontoxicity, biodegradability, biocompatibility, and good water vapor permeability (6,7). These unique properties of silk fibroin have been applied to enzyme immobilization, oxygen permeable membranes, and used as a matrix for mammalian cell culture (8). However, SF has never been used as a food packaging material. Silk fiber from silkworm *Bombyx mori* consists of two components, silk fibroin and sericin. Silk fiber is known to be one of the strongest and toughest materials because of the dominance of well orientated β-sheet structures of polypeptide chains (9,10). However, the prepared SF film, in which the random coil conformation is predominant, is brittle, and needs to be treated with plasticizers.

Plasticizers are used to overcome the brittleness of protein films (11). Brittleness is mainly due to extensive intermolecular interactions. Plasticizers can reduce the intermolecular interactions, soften the rigidity of the film structure, and increases the mobility of polypeptide chains, resulting in improvement in the flexibility and the extensibility of the films. On the other hand, addition of plasticizers usually increases gas and water vapor permeability (12,13).

In this study, SF film was prepared and three types of plasticizer: glycerol (G), polyethylene glycol (PEG), and polyprophylene glycol (PPG) were used to improve the physical properties of the film. The objectives of this study were to prepare SF film as food packaging material and to examine the effect of plasticizers on the physical properties of the SF films.

MATERIALS AND METHODS

Materials

Silk fibroin was obtained from World Way Co. (YeonKi, Korea). Glycerol, polypropylene glycol, and polyethylene glycol were purchased from Sigma-Aldrich Chemical Co. (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

Preparation of film-forming solution

Silk fibroin was dissolved in a calcium chloride/distilled water/ethanol (1:8:2 mole) solution at 90°C for 1 h and dialyzed against distilled water for 3 days (14).

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After dialysis, the silk fibroin solution was strained through cheese cloth and then treated with plasticizers. Plasticizers were either 3% glycerol, polyethylene glycol, or polypropylene glycol, respectively. Film-forming solutions were conditioned in a water bath at 70°C for 20 min.

Film casting and drying

Film-forming solutions were strained through cheese cloth and cast on flat, Teflon-coated glass plates (24 cm × 30 cm). Uniform film thickness was maintained by casting the same amount of film-forming solution on each plate. Plates were dried at 25°C for 48 h. Dried films were peeled intact from the casting surface. Specimens were cut for water vapor permeability (2 cm × 2 cm), tensile strength (2.54 cm × 10 cm), and color (7 cm × 7 cm) measurements. Film specimens were conditioned in an environmental chamber at 25°C and 50% relative humidity (RH) for 2 days.

Determination of film thickness

Film thickness was measured with a micrometer (Mitutoyo, Model No. 2046-08, Tokyo, Japan) at five random positions and the mean value was used.

Measurement of tensile strength and elongation

Film tensile strength (TS) and elongation at break (E) were determined with an Instron Universal Testing Machine (Model 4484, Instron Co., Canton, MA, USA) according to the ASTM Standard Method D882-91 (15). An initial grip distance of 5 cm and cross-head speed of 50 cm/min were used. TS was calculated by dividing the maximum load by initial cross-sectional area of a specimen, and elongation was expressed as a percentage of change of initial gauge length of a specimen at the point of sample failure. Five replicates of each film were tested.

Measurement of water vapor permeability

Water vapor permeability (WVP) of SF-films was determined according to the modified ASTM E 96-95 method (16) at 25°C and 50% RH using 20 mL polymethylacrylate cup (17,18). The cup was filled to 1 cm with distilled water and covered with a film specimen. Weight loss of cups with time was measured. A linear regression analysis was performed to calculate a slope. WVP (ng m/m^2s Pa) values were then calculated from:

$$WVP=(WVTR \times L)/\Delta p$$

where water vapor transmission rate (WVTR, g/m^2s) was calculated by dividing the slope by the open area of the cup. L is mean thickness (m), and Δp is corrected partial vapor pressure difference (Pa) across the film specimen.

Color measurements

Color values of the silk protein films were measured using a colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan). Film specimens (7 cm × 7 cm) were placed on a white standard plate and the Hunter L, a, and b color was used to measure color: L=0 (black) to L=100 (white); a=-80 (greenness) to a =100 (redness); and b=-80 (blueness) to b=70 (yellowness). Five measurements were taken at different locations on each specimen.

Statistical analysis

Analysis of variance and Duncan's multiple range tests were performed to analyze the results statistically using a SAS program (SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

For preparation of SF film, a suitable solvent is needed for dissolving the silk fibroin. Dissolving silk fibroin requires breakage of the hydrogen bond network and swelling of the compact fibrous structure of the individual fibroin molecules. An ideal solvent is one capable of penetrating into the fiber and dissolving it. The most common solvent system for silk fibroin, $CaCl_2/H_2O/$EtOH mixture (19) was used in this study. SF film formed without plasticizer was very brittle and easily broken when it was peeled off the plate. Therefore, we could not determine the physical properties of the film. Hydrophilic plasticizers such as glycerol (G), polyethylene glycol (PEG), and sorbitol are usually used to improve the mechanical properties of protein films (19-21). Plasticizer's size, shape, and composition influence its ability to disrupt hydrogen bonding of the protein chain (19). In this study, we used 3 different plasticizers: G, PEG, and PPG.

Film thickness of each film was measured using a micrometer. Film thickness values (Table 1) were not significantly different among treatments since the same amount of film-forming solution was used.

Table 1 shows the effect of different plasticizers on the physical properties of SF films. G-SF film (silk fibroin film treated with glycerol) had the lowest tensile strength (TS) value, 14.24 MPa. There were no significant differences between PPG-SF (silk fibroin film treated with polyethylene glycol) and PEG-SF film (silk fibroin film treated with polypropylene glycol) with regard to TS value, but PEG-SF film had a better elongation at break (% elongation). In general, increase of TS was accompanied by decrease of % elongation. Due to that reason, G-SF films had high % elongation values, 73 times of PPG-SF film elongation and 25 times of

Table 1. Physical properties of silk fibroin films

Type of film	Type of plasticizer	Thickness (mM)	Tensile strength (MPa)	Elongation (%)
G-SF	Glycerol	0.083±0.004[1)]	14.24±1.085[b2)]	184.44±8.027[a]
PEG-SF	Polyethylene glycol	0.080±0.005	23.71±0.971[a]	7.23±0.472[b]
PPG-SF	Polypropylene glycol	0.077±0.004	24.51±4.071[a]	2.51±0.579[b]

[1)]Means of five replications±standard deviations.
[2)]Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test.

PEG-SF films' value. Cuq et al. (22) reported that at the same mass basis in film formulation, lower molecular weight (Mw) plasticizers produced films that were more plastic than higher Mw plasticizers. Therefore, G-SF film treated with glycerol of lower Mw had the highest % elongation value, 184%. Lee et al. (23) reported that protein films such as zein and SPI films had TS values in the range of 3~4 MPa. Our results clearly showed that SF-films prepared in this study had higher TS values than other protein films. SF films had a higher TS, compared to synthetic polymer films such as commercially used LDPE (low density polyethylene) having TS as 9~15 MPa. TS of PEG-SF and PPG-SF films had 1.5 times of LDPE TS value.

WVPs of SF films treated with different plasticizers are shown in Fig. 1. WVPs of G-SF and PPG-SF films were 3.92 and 4.12 ng m/m^2s Pa, respectively, while PEG-SF film had the lowest value, 1.32 ng m/m^2s Pa. Banker (13) reported that addition of plasticizers generally increased gas and water vapor permeability of the film. Because high WVP of films causes decay of foods during storage, protein films need to decrease WVP. Protein films usually form inferior water vapor barriers, compared to synthetic polymer films, since protein molecules are hydrophilic in nature and tend to absorb

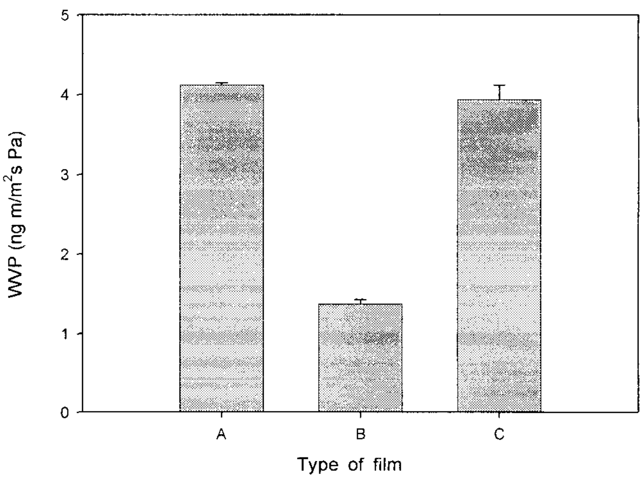

Fig. 1. Water vapor permeability of silk fibroin films. A, silk fibroin film containing glycerol (G-SF film); B, silk fibroin film containing polyethylene glycol (PEG-SF film); C, silk fibroin film containing polypropylene glycol (PPG-SF film).

Table 2. Hunter L, a, and b values of silk fibroin films

Type of film	L	a	b
G-SF	96.10±0.39[1)]	-0.51±0.02[b2)]	3.77±0.11[ab]
PEG-SF	96.01±0.35	-0.70±0.04[c]	4.07±0.33[a]
PPG-SF	95.70±0.06	-0.38±0.02[a]	3.38±0.16[b]

[1)]Means of five replications±standard deviations.
[2)]Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test.

water. Therefore, few protein films are used commercially. However, SF film had a lower WVP, compared with zein film having WVP of 5.30 ng m/m^2s Pa (24). Compared with other hydrophilic films such as SPI, wheat gluten, and methylcellulose (MC), SF-film has a very low WVP due to its insoluble properties, and can provide a good barrier to water vapor during storage of foods. Therefore, in terms of WVP, SF film is an excellent biomaterial and PEG-SF film is the best candidate, considering WVP, TS, and % elongation of the film.

Color of protein film might be an important property because it could affect consumer acceptance in food packaging. Table 2 shows Hunter L, a, and b color values of SF films. There were no significant differences in Hunter L value of SF films depending on plasticizer type, but PEG-SF film had a little higher a and b values. However, overall, treatment with various plasticizers did not affect significantly the color of SF film.

In conclusion, these results suggest that SF film could be applied as food packaging and addition of plasticizers should improve the physical properties of SF film. Among plasticizers used in this study, PEG was the most suitable for SF films with regard to WVP, TS, and % elongation.

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