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Effect of Plasticizer and Cross-Linking Agent on the Physical Properties of Protein Films

- Research Note -

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Abstract

To improve the physical properties of protein films, various plasticizers and cross-linking agents were used in the preparation of the films. For zein film, 3% polypropylene glycol with 3% glycerol was the best plasticizer, while 2.5% glycerol was the most suitable for soy protein isolate (SPI) film in terms of tensile strength (TS), % elongation, and water vapor permeability (WVP). Formaldehyde, glutaraldehyde, glyoxal, and cinnamaldehyde as cross-linking agents of protein films were used to further improve the physical properties of the films. All aldehydes used as cross-linking agent in this study improved TS of zein and SPI films. In particular, cinnamaldehyde was the best cross-linking agent due to its safety in foods. These results suggest that appropriate use of plasticizer and cross-linking agent like cinnamaldehyde should improve the physical properties of protein films for use in food packaging.

Key words: protein films, plasticizers, cross-linking agents, physical properties

INTRODUCTION

Recently, studies on the use of natural biopolymers for packaging films have been greatly increasing (1). Proteins, lipids, and polysaccharides have been used for materials of natural biopolymers (2-6). In particular, protein-based biodegradable films offer environmental compatibility as well as improvement in the quality and shelf life of foods (7-9). Protein films can also improve the physical properties of foods and minimize loss of volatile flavors and aromas (10). However, protein films form inferior water vapor barriers, compared with synthetic polymer films (8), since protein molecules are hydrophilic in nature and tend to absorb water (11). Therefore, to improve the physical properties of protein films, treatment with cross-linking agents, plasticizers, or ionizing radiation have been tried (9,12,13).

Because biodegradable films are generally brittle, they require the addition of plasticizers (14). Plasticizers reduce the intermolecular interactions and increase the mobility of polypeptide chains, resulting in improvement in the flexibility and the extensibility of the film (14). Cross-linking agents have also been used to improve the tensile strength and water vapor barrier properties of protein films (12,15,16). Aldehydes such as glutaraldehyde, glyoxal, formaldehyde, and cinnamaldehyde have been used as cross-linking agents for biodegradable films, to improve their mechanical and water vapor permeability.

Therefore, the objectives of this study were to elucidate the effect of plasticizers and cross-linking agents on the physical properties of protein films and to improve the physical properties of the films.

MATERIALS AND METHODS

Materials

Corn zein was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and soy protein isolate (SPI) was obtained from Dupont Protein Technologies (SUPRO 500E IP, St. Louis, MO, USA). Glycerol, polypropylene glycol (PPG), formaldehyde (37%), glutaraldehyde (Grade I, 50%), and glyoxal (40%) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Cinnamaldehyde (98%) was purchased from Fluka Co. (St. Louis, MO, USA).

Preparation of film forming solution according to plasticizer

Film forming solutions were prepared by dissolving

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Table 1. Types of zein and SPI films and added with plasticizers

Film type	Protein	Glycerol (%)	PPG (%)
ZA		6	-
ZB	Zein	3	3
ZC		1.5	4.5
SA	SPI	2.5	-
SB		5	-

zein (10 g) in 100 mL of 95% ethanol or soy protein isolate (5 g) in 100 mL of distilled water. As plasticizers, glycerol and/or PPG were added at various concentrations. The pH value of the SPI solution was adjusted to pH 10 with 1 N NaOH solution. Film forming solutions were then heated in a water bath at 70°C for 20 min. Table 1 shows the types of protein film according to the plasticizer.

Preparation of film forming solution according to cross-linking agent

As a plasticizer, glycerol (2.5%, w/v) was added to the SPI film solution. For zein film solution, both glycerol (3%, w/v) and PPG (3%, w/v) were added. A cross-linking agent (0.01% solution) consisting of formaldehyde, glutaraldehyde, glyoxal, or cinnmaldehyde was then added. Film forming solutions were heated in a water bath at 70°C for 20 min. Table 1 shows the types of zein and SPI films according to different concentrations of glycerol and PPG as a plasticizer. Plasticizers for zein films were glycerol and PPG, whereas only glycerol was added for SPI film.

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 24 h. Dried films were peeled intact from the casting surface. Specimens were cut for measuring water vapor permeability (2 cm ×2 cm), and tensile strength (2.54 cm×10 cm).

Determination of film thickness

Film specimens were conditioned in an environmental chamber at 25°C and 50% relative humidity (RH) for 2 days. 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 strengths (TS) and elongation at break (E) were determined with an Instron Universal Testing Machine (Model 4484, Instron Corp., Canton, MA, USA) according to the ASTM Standard Method D882-91 (17). Film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. Initial grip distance of 5 cm and crosshead 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 protein films was determined according to the modified ASTM E 96-95 method (18) at 25°C and 50% RH using polymethylacrylate cups (19,20). The cup was filled to 1 cm with distilled water and covered with a film specimen. Film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. Weight loss of cups over time was measured. A linear regression analysis was performed to calculate a slope. WVP (ng m/m^{2}s Pa) values were then calculated from:

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

where water vapor transmission rate (WVTR, g/m^{2}s) 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.

Statistical analysis

Analysis of variance and Duncan's multiple range tests with significance at $p<0.05$ were performed to analyze the results statistically using a SAS program (1999, SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Thickness of the films prepared in this study were 160 μm for zein film and 81 μm for SPI film, respectively. Film thickness values were not significantly different among treatments because the same amount of film forming solution was used.

Table 2 and Fig. 1 show the effect of different plasticizers on the physical properties of zein and SPI films. The TS value of zein film increased with increasing PPG content. TS value of Film ZC (1.5% glycerol, 4.5% PPG) was 6.3 MPa, compared to 3.3 MPa of Film ZA (6% glycerol only). However, the TS value of zein film containing PPG only was very low (Data not shown) and the film could not be made. Film ZB (3% glycerol, 3% PPG) had a 40 fold increase in % elongation, compared to that of Film ZA. Because a major disadvantage of zein films is their low % elongation, use of an appropriate plasticizer like glycerol and/or PPG is required. Film ZA had the lowest WVP (Fig. 1), but its % elon-

Table 2. Effect of plasticizers on physical properties of zein and SPI films

Film type	Tensile strength (MPa)	Elongation (%)
ZA	3.3±1.6[1)a2)]	6.8±0.3[a]
ZB	3.7±0.0[a]	276.9±7.0[b]
ZC	6.3±0.2[b]	157.8±14.9[c]
SA	3.2±0.1[a]	173.5±29.9[c]
SB	0.9±0.1[c]	187.0±10.8[c]

[1)]Mean of five replicates±standard deviation.
[2)]Any figures in the same column with the same letter are not significantly ($p>0.05$) different by Duncan's multiple range test.

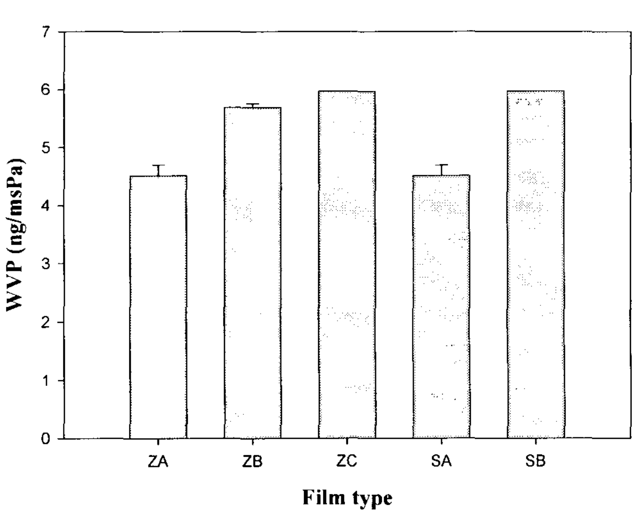

Fig. 1. Effect of plasticizers on water vapor permeability of protein films.
ZA: zein film containing 6% glycerol, ZB: zein film containing 3% glycerol and 3% PPG, ZC: zein film containing 1.5% glycerol and 4.5% PPG, SA: SPI film containing 2.5% glycerol, SB: SPI film containing 5% glycerol.

gation was not suitable. Therefore, considering WVP as well as TS and % elongation, the best plasticizer was the combination of 3% glycerol and 3% PPG. Our results are in good agreement with Park and Manjeet's report (19) that zein film containing a mixture of polyethylene glycol and other plasticizer had higher elongation than zein film containing glycerol only.

TS value of SPI film was affected by glycerol content as a plasticizer. TS value of Film SA was 3.2 MPa, while that of Film SB was 0.9 MPa. Similar results were observed in another study (20). Glycerol can be easily inserted among polypeptide chains and form hydrogen bonds with amino groups of protein molecules (14). Wang et al. (21) reported that glycerol was an effective plasticizer of soy protein film. There was no significant difference in terms of % elongation of Film SA and SB, but Film SA had higher TS and lower WVP. Therefore, these results suggest that the most suitable concentration of glycerol as a plasticizer should be 2.5%, which was better than 5%, to improve the physical properties of SPI film.

Based on the results of the effects of plasticizers in SPI and zein films, various cross-linking agents were used to further improve the functional properties of the films. Using Film ZB and SA as the control, formaldehyde, glutaraldehyde, glyoxal, and cinnamaldehyde were separately added as cross-linking agents of protein films, and their physical properties were determined. In general, cross-linking agents increase TS values of protein films by reacting with ε-amino group of lysine or guanidine group of arginine of protein molecules (22). In this study, TS values of protein films were increased by the addition of cross-linking agents, compared to the control (Table 3, 4). Zein film treated with formaldehyde had an increase in TS by 1.2 MPa, and TS of SPI film was increased by 1.6 MPa. Other aldehydes such as glutaraldehyde, glyoxal, and cinnamaldehyde had similar values. The same trend was also observed in the studies of glutenin-rich films (23) and wheat gluten films (24). However, % elongation of protein films decreased slightly due to the formation of more rigid structures by cross-linking agents except zein film treated with formaldehyde. Regarding use of cross-linking agent, it should be also noted that safety issue of aldehyde could be a serious concern when it is applied in foods (25). There-

Table 3. Physical properties of zein films containing cross-linking agents

Treatment	Tensile strength (MPa)	Elongation (%)
Control	3.7±0.0[1)a2)]	276.9±7.0[a]
Formaldehyde	4.9±0.2[b]	281.6±6.9[a]
Glutaraldehyde	4.9±0.0[b]	252.4±10.50[ab]
Glyoxal	4.9±0.1[b]	202.4±17.8[b]
Cinnamaldehyde	5.2±0.1[c]	253.9±23.7[ab]

[1)]Mean of five replicates±standard deviation.
[2)]Any figures in the same column with the same letter are not significantly ($p>0.05$) different by Duncan's multiple range test.

Table 4. Physical properties of SPI films containing cross-linking agents

Treatment	Tensile strength (MPa)	Elongation (%)
Control	3.2±0.1[1)a2)]	173.5±29.9[a]
Formaldehyde	4.8±0.2[b]	152.4±8.3[a]
Glutaraldehyde	4.4±0.2[b]	142.8±8.8[a]
Glyoxal	4.1±0.7[b]	156.9±8.7[a]
Cinnamaldehyde	4.2±0.9[b]	142.8±8.8[a]

[1)]Mean of five replicates±standard deviation.
[2)]Any figures in the same column with the same letter are not significantly ($p>0.05$) different by Duncan's multiple range test.

fore, cinnamaldehyde, which is used as a food additive in ice cream, bread, meat, and candy, can be used as a cross-linking agent for edible film due to its low toxicity. In particular, TS for zein film increased to the highest level, 5.2 MPa, when cinnamaldehyde was used. These results suggest that appropriate use of plasticizer and cross-linking agent like cinnamaldehyde should improve the physical properties of protein films for food packaging.

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