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Preparation of a Silk Fibroin Film Containing Catechin and Its Application

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Abstract Silk fibroin (SF) film containing catechin was prepared and the antimicrobial activity as well as physical property of the film was examined. Tensile strength of the SF film decreased with increasing concentration of catechin, and water vapor permeability of the film decreased. The film's antimicrobial activity against *Escherichia coli* O157:H7 increased with increasing catechin concentration. Sausage samples were inoculated with *E. coli* O157:H7 and *Listeria monocytogenes*, and the sausage packaged with the SF film containing catechin had a decrease in the populations of *E. coli* O157:H7 and *L. monocytogenes* by 0.83 and 0.85 log CFU/g after 12 days of storage, respectively, compared to the control. In addition, the sausage had a better quality than the control regarding lipid oxidation. Our results indicate that sausages can be packed with the SF film containing catechin to extend shelf life.

Keywords: silk fibroin film, catechin, sausage, antimicrobial activity

Introduction

Appropriate food packaging increases the shelf life of food products and prevents quality deterioration during storage. Edible film to inhibit the growth of pathogenic bacteria in food products during storage is an active research interest, and new edible packaging films have been studied for environmental concern (1). In particular, antimicrobial edible films have been used to extend the shelf life of foods, and antimicrobials used in the films are grapefruit seed extract, chito-oligosaccharides, lysozyme, nisin, chitosan, and catechin (2,3). Among them, catechin is appealing since it is also known to be efficient antioxidants (4,5).

Contamination of ready-to-eat (RTE) meat products by foodborne pathogens is of a great concern to consumers. Sausage is a widely consumed RTE meat product, but there is a hazard of food poisoning caused by pathogenic bacterial contamination during manufacturing of sausage (6). In addition, lipid oxidation deteriorates food quality, and decreases the shelf life of meat products (7). Therefore, development of edible films containing antioxidants and antibacterial materials in RTE meat products may be useful.

As one of the sources for edible films, silk fibroin (SF) is a good candidate. SF has been considered as a natural biopolymer that has unique properties such as non-toxicity, biodegradability, biocompatibility, and good water vapor permeability (8).

Therefore, the objectives of this study were to prepare the SF films containing catechin, and then to apply as a sausage package having antimicrobial activity.

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Materials and Methods

Materials Silk fibroin (SF) was obtained from World Way Co. (Yeongi, Korea). Glycerol, polyethylene glycol, and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of film forming solution SF was dissolved in calcium chloride/distilled water/ethanol (1:8:2 mol) solution at 90°C for 1 hr and dialyzed against distilled water for 3 day (9). After dialysis, SF solution was strained through cheese cloth. SF solution was then treated with plasticizer. As plasticizer, 3% glycerol and polyethylene glycol (75:25, w/w) were added, respectively. Different amounts (0, 50, 100, and 150 mg/100 mL of film solution) of catechin were immediately incorporated into each film forming solution and stirred well enough to be dissolved. 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 (24×30 cm). Uniform film thickness was maintained by casting the same amount (80 mL) of film forming solution on each plate. Plates were dried at 25°C for 24 hr. Dried films were peeled intact from the casting surface. Specimens were cut for water vapor permeability (2×2 cm) and tensile strength (2.54×10 cm).

Measurement of tensile strength (TS) and elongation The film's TS and elongation at break (E) were determined using an Instron Universal Testing Machine (Model 4484; Instron Co., Canton, MA, USA) according to the method described in the previous study (10). The film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 day. An initial grip distance of 5 cm and cross-head speed of 50 cm/min were used. The TS was calculated by dividing the maximum load by the initial cross-sectional area of a specimen, and the elongation was

expressed as a percentage of the change in the initial gauge length of a specimen at the point of sample failure. Five replicates of each film were tested.

Measurement of water vapor permeability (WVP) The WVP of the SF films was determined according to the method described in the previous study (10) using a polymethylacrylate cup (11). The cup was filled to 1 cm with distilled water and covered with a film specimen. The film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 day. The weight loss of the cups with time was measured. A linear regression analysis was performed to calculate the slope. The WVP (ng m/m^2 sec·Pa) values were then calculated using the following formula:

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

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

Culture preparation *Escherichia coli* O157:H7 (NCTC 12079) and *Listeria monocytogenes* (KCTC 3710) cultures were grown at 37°C for 24 hr in 50 mL Corning tubes that contained 25 mL of Luria-Bertani (LB, Difco Co., Detroit, MI, USA) broth and brain heart infusion (BHI, Difco) broth, respectively.

Antimicrobial activity of the films against *E. coli* O157:H7 Antimicrobial activity of the SF film was determined using the method of Ko *et al.* (12) with a modification. *E. coli* O157:H7 was cultured at 37°C in LB broth up to 10^9 CFU/mL. Fifteen μL of the bacterial suspension was placed on the film discs (0.02 g). The film discs were then incubated at room temperature for 1 hr. After the incubation, the discs were placed in 0.98 mL of 0.1% peptone water and homogenized for 3 min. The solution was then diluted with 0.1% peptone water and poured on Chromogenic *E. coli*/Coliform medium (Oxoid, Basingstoke, UK) plates. All the plates were incubated at 37°C for 24 hr. Each microbial count was the mean of 3 determinations, and the microbial count was expressed as a log colony forming unit (CFU).

Inoculation on sausages The sausages were cut into 5 g slices which were individually dipped in the mixed culture of *E. coli* and *L. monocytogenes* for 10 min. The sausage samples were then allowed to dry for 30 min under a clean bench.

Packaging of sausages with the SF film The inoculated sausages were tightly packed with the SF film that contained catechin (100 mg/mL). The SF film itself was very adaptable regarding tightness of packing. The sausage that was not packed with a SF film was used as the control. All the sausage samples were covered with a sterile polypropylene film and stored at 4°C for 12 day.

Microbiological analysis Five g of the inoculated sausage samples was placed in 95 mL of 0.1% peptone water. The samples were then homogenized in a sterile stomacher bag using a stomacher (MIX 2; AES Laboratoire, France) for 3 min, filtered through a sterile cheese cloth, and diluted with peptone water to determine microbial count. To determine their microbial counts, serial dilutions were performed in triplicate on each selective agar plate. *E. coli* counts were determined by plating the appropriately diluted samples onto Chromogenic *E. coli*/Coliform medium (Oxoid) plates. *L. monocytogenes* counts were determined by plating the appropriately diluted samples onto *Listeria* selective agar (Oxoid) plates. The plates for the *E. coli* and *L. monocytogenes* counts were each incubated at 37°C for 36 hr. Each microbial count was the mean of 3 determinations, and the microbial count was expressed as a log CFU/g.

Measurement of lipid oxidation The degree of lipid oxidation of the sausages was determined using the method of Ahn *et al.* (13). Five g of the sausage samples were homogenized in 15 mL distilled water using a blender for 1 min. One mL sample solution was transferred into a disposable test tube, and a 2 mL 2-thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution was added. The mixture was then boiled in a water bath for 15 min. The sample was cooled at room temperature for 10 min and centrifuged for 15 min at 2,000×g. The absorbance of the resulting supernatant solution was determined at 531 nm. The thiobarbituric acid-reactive substances (TBARS) value was expressed as mg malondialdehyde/kg sample (MDA/kg).

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

Results and Discussion

Physical properties of the films Film thickness of the SF film was determined, and there were slight differences among treatments (Table 1). TS of the SF film decreased with the increase of catechin concentration (Table 1). The SF film had the TS of 14.92 MPa for the control, whereas the film having catechin of 150 mg/100 mL had 11.01 MPa. The decrease in TS of the SF film can be explained by inhibition of protein-protein association in the SF film network (14). In general, increase of TS accompanies decrease of % elongation of the film (10). Percent elongation of the SF film increased with increasing catechin concentration (Table 1). Cha *et al.* (15) reported that edible films such as carrageenan films containing antimicrobial materials had TS values in the range of 6-14 MPa. Our results represented that the SF film had a similar TS value as other edible films.

WVP is one of the most important functional properties of protein films. WVP for the SF film decreased with the increase of catechin concentration (Table 1). The WVP of the SF film without catechin was 4.79 ng m/m^2 sec·Pa, whereas that of the film with 150 mg catechin was 3.35 ng m/m^2 sec·Pa. This difference can be explained by the network formation between amino acids such as alanine and tyrosine of the silk fibroin and catechin (16). Our WVPs are comparable with the WVP of the gelatin film, where it decreased with the increase of antioxidant

Table 1. Effect of catechin concentration on the thickness, tensile strength, % elongation, and water vapor permeability (WVP) of the silk fibroin film

Catechin concentration (mg/100 mL)	Thickness (mm)	Tensile strength (MPa)	Elongation (%)	WVP
0	0.089±0.02$^{a1)}$	14.88±1.75^{a}	96.94±12.21^{b}	4.79±0.06^{a}
50	0.076±0.00^{ab}	14.10±2.12^{a}	104.97±30.13^{b}	4.34±0.12^{ab}
100	0.065±0.01^{b}	13.85±1.10^{ab}	110.85±20.91^{ab}	3.49±0.45^{b}
150	0.061±0.01^{b}	10.66±2.21^{b}	165.23±36.54^{a}	3.35±0.36^{b}

$^{1)a-b}$Any means in the same column followed by different letters are significantly different ($p<0.05$).

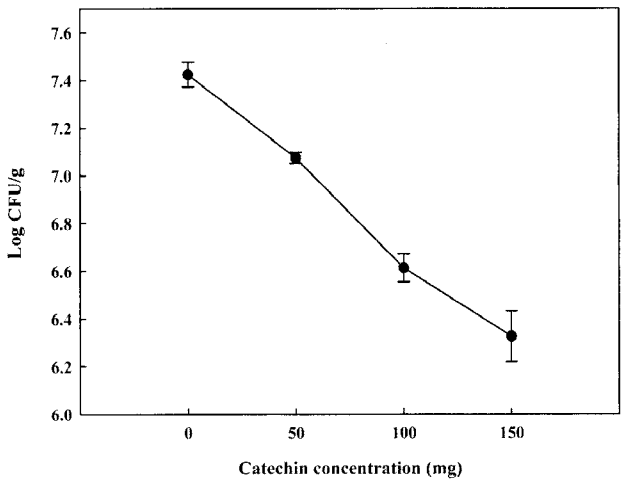

Fig. 1. Effect of catechin concentration on the antimicrobial activity of the silk fibroin film against *E. coli* O157:H7.

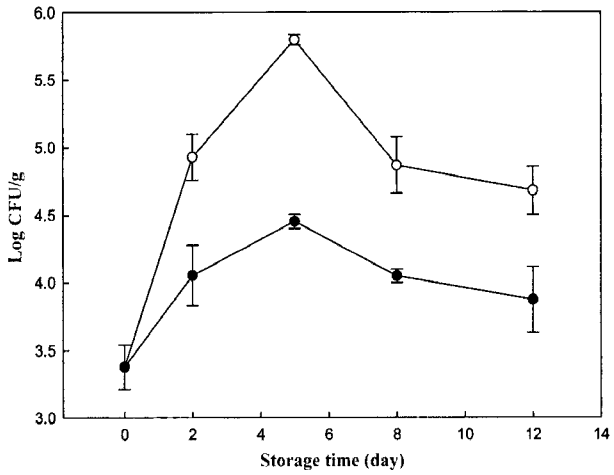

Fig. 2. Antimicrobial activity of the silk fibroin film against *E. coli* O157:H7 inoculated in sausage during storage at 4°C for 12 day. ○, Control; ●, SF films containing catechin.

materials (17). In addition, the SF film containing catechin had a lower WVP, compared to the hydrolyzed sago starch-alginate film having WVP as 4.0 ng m/ m^2 sec·Pa (18).

Antimicrobial activity of the SF film Incorporation of catechin into the SF film increased the antimicrobial activity with increasing catechin concentration (Fig. 1). Catechin has antimicrobial activity as well as anti-oxidative property (19). Sung (20) also reported that the populations of *S. typhimurium* were reduced by 0.9 log CFU/g within 24 hr by addition of 1% green tea extract. Based on the physical properties and the antimicrobial activity results of the SF film as well as cost of catechin, we determined the optimal catechin concentration of the film as a sausage package as 100 mg/100 mL.

Figure 2 represents the microbial growth and quality change of the sausages packaged with the SF film during storage. The initial inoculum level of *E. coli* O157:H7 and *L. monocytogenes* in the sausage samples were 3.2 and 3.5 log CFU/g, respectively. The populations of *E. coli* O157:H7 and *L. monocytogenes* in the sausages packaged with the SF film containing catechin were reduced significantly, compared to the control. During storage, the populations of the *E. coli* O157:H7 in the sausages increased until day 5 of storage, and then decreased. These results are in good agreement with those of Nissen *et al.* (21), where *E. coli* O157:H7 in beef increased until day 7 of storage, and then decreased during storage. This can be attributed to the difference in growth condition and availability of nutrients for the growth of the bacteria during storage. The populations of the bacteria in the sausages packed with the SF film were lower than the control during the entire storage period. In particular, there was 1.4 log CFU/g difference among the samples on day 5. After day 12, the sausage packaged with the SF film containing catechin had 0.81 log cycle reduction, compared to the control, maintaining the inhibition of microbial growth even after 12 day of storage.

Packaging with the SF film containing catechin also decreased the microbial populations of *L. monocytogenes* (Fig. 3). During storage, the populations of the *L. monocytogenes* in the control increased on day 2 of storage, and decreased until day 12. On the contrary, the sausages packed with the SF film had a continuous decrease in the populations of the bacteria during storage. These results are in accordance with those of Ye *et al.* (22), where the populations of *L. monocytogenes* on ham steaks packaged with chitosan-coated plastic film slightly decreased during storage. The populations of *L. monocytogenes* for the control increased to 4.35 log CFU/g after day 2, while the sausages packaged with the SF film containing catechin reached 3.41 log CFU/g, resulting in a decrease by 0.94 log CFU/g. In addition, the population of the bacteria in the control was 3.36 log CFU/g after 12 day of storage, while the sausages packed with the SF film had 2.51 log CFU/g. Cagri *et al.* (23) also reported that a whey protein film with an antimicrobial *p*-aminobenzoic acid, when used as a casing for hotdogs, retarded the surface microbial growth. Our results are comparable with those reports.

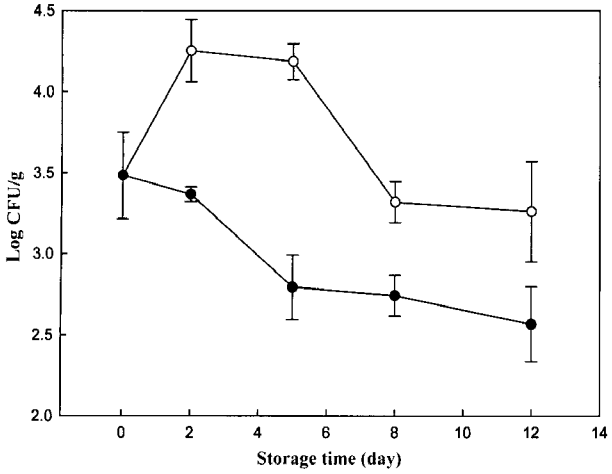

Fig. 3. Antimicrobial activity of the silk fibroin film against *L. monocytogenes* inoculated in sausage during storage at 4 for 12 day. ○, Control; ●, SF films containing catechin.

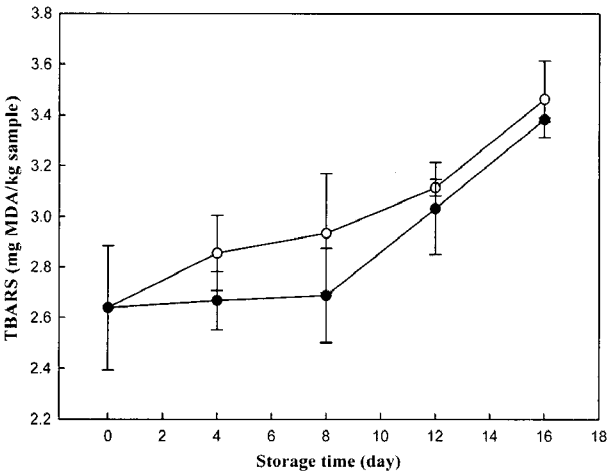

Fig. 4. Change in TBARS value of sausage during storage at 4 for 12 day. Bars represent standard error. ○, Control; ●, SF film containing catechin.

Lipid oxidation in sausages during storage TBARS value represents the degree of lipid oxidation of meat products. TBARS values of the sausage samples increased with increase of storage time (Fig. 4). The sausages packaged with the SF film containing catechin had 2.61 mg MDA/kg after day 8, in contrast to 2.92 mg MDA/kg for the control. Sheard *et al.* (24) reported that consumers could sense off odor when TBARS value was over 5.8 mg MDA/kg. Our results were lower than the criteria. In addition, after day 12, the sausages packaged with the SF film containing catechin had 2.95 mg MDA/kg, in contrast to 3.10 mg MDA/kg for the control. These results indicate that lipid oxidation of the sausage was deterred by packing with the SF film containing catechin.

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