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Incorporating Grapefruit Seed Extract into *Gelidium corneum*−Whey Protein Isolate Blend Packaging Film Increases the Shelf Life of Fish Paste

□ Research Note □

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Abstract

The *Gelidium corneum* (GC)-whey protein isolate (WPI) blend film containing grapefruit seed extract (GSE) was prepared by incorporating different amounts (0, 0.02, 0.04, 0.08, 0.1%) of GSE into the film. The film's tensile strength (TS) and water vapor permeability (WVP) were improved by the addition of GSE. The film containing 0.1% GSE had a TS of 3.27 MPa, whereas the control had 2.64 MPa. WVP of the film was also significantly decreased by the addition of GSE. Addition of 0.1% GSE decreased the populations of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium by 1.0, 1.6, and 0.6 log CFU/g, respectively, compared to the control. Fish paste was packed with the GC-WPI blend film containing GSE, and microbial change in the fish paste inoculated with *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium during storage was examined. Populations of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were decreased by 0.60, 0.48, and 0.85 log CFU/g, after 7 day of storage, respectively. These results suggest packaging fish paste in the GC-WPI blend film containing GSE can extend the shelf life.

Key words: blend film, fish paste, *Gelidium corneum*, whey protein isolate

INTRODUCTION

Gelidium corneum, a type of red algae, has high amounts of dietary fiber and diverse physiological functions (1). In particular, extract of *Gelidium corneum* as a byproduct of red algae pulp production contains lots of agar, which is a suitable raw material for manufacturing edible films (2).

Fish paste made from fish meat, sodium chloride, and other various materials by cooking is often consumed as a ready-to-eat food in Korea (3). However, the fish paste is easily contaminated during manufacturing and storage. Typically, the shelf life of vacuum packed fried fish paste during low temperature storage is about 10 days (4). Therefore, to increase the shelf life of the fish paste, gamma irradiation treatment (5) and use of chitosan hydrolysates (6) or alginic acid hydrolysates (7) have been tried.

To extend shelf life of food, edible films have been used as packaging materials (8-10). Edible films include starch or protein based films, and they may contain antimicrobials such as lysozyme (8), chitosan (9), and rosemary oil (10). Among the antimicrobials, grapefruit seed extract (GSE) includes ascorbic acid, ascorbyl palmitate, and tocopherol that make cell wall and membranes of pathogenic bacteria weak, resulting in inhibition of the bacteria (11). GSE has been known to have antimicrobial activity (12,13). In addition, GSE has been applied to minimally processed vegetables (14) and sausages (15) to decrease pathogenic bacteria.

Therefore, the objectives of this study were to make a GC-WPI blend film containing GSE and to study it preservative properties in fish paste.

MATERIALS AND METHODS

Materials

The *Gelidium corneum* (GC) used in this study was harvested from Jeju Island, Korea. Whey protein isolate (WPI) was purchased from Davisco Foods International Inc. (Bipro, Le Sueur, MN USA). Grapefruit seed extract (GSE) was obtained from ABC Techno Inc. (Desfan-100, Tokyo, Japan). Glycerol was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Fish paste was purchased from a local retail market.

Preparation of edible film

The GC was washed to remove foreign substances and bleached using 5% chlorine dioxide at 60°C for 90 min, followed by treatment with 8.5 g/h ozone gas. The bleached and dried samples were cut, ground, and prepared using a 200-mesh sieve. To make the film, 0.75% GC powder was dissolved in distilled water and mixed

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with 5% WPI (w/v) and 5% glycerol. The film-forming solutions were then heated in a water bath at 90°C for 30 min. Different amounts (0, 0.02, 0.04, 0.08, 0.1%) of GSE were incorporated into each film-forming solution. The film-forming solutions were strained through a cheese cloth and cast on flat, teflon-coated glass plates (24 cm × 30 cm). A uniform film thickness was maintained by casting the same amount (80 mL) of film-forming solution on each plate. The plates were then dried at 25°C for 24 hr. The dried films were peeled intact from the casting surface. Specimens were cut to measure water vapor permeability (2 cm × 2 cm), tensile strength (2.54 cm × 10 cm).

Determination of film thickness

The film specimens were conditioned in an environmental chamber at 25°C and a 50% relative humidity (RH) for 2 days. The film thickness was measured using a micrometer (Model No. 2046-08, Mitutoyo, Tokyo, Japan) at five random positions, and the mean value was determined.

Measurement of tensile strength and elongation

The film's tensile strength (TS) and elongation at break were determined using the Instron Universal Testing Machine (Model 4484, Instron Co., Canton, MA, USA) according to the ASTM Standard Method D882-91 (16). The film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. 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

The water vapor permeabilities (WVP) of the edible films were determined according to a modified ASTM E 96-95 method (17) at 25°C and 50% RH using a polymethylacrylate cup (18). 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 days. 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}s 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}s) 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 (NCTC12079), *Listeria monocytogenes* (KCTC 3710) and *Salmonella typhimurium* (ATCC 14028) cultures were grown at 37°C for 24 hr in 50 mL Corning tubes containing 25 mL of Luria-Bertani (LB, Difco Co., Detroit, MI, USA) broth, brain heart infusion (BHI, Difco) broth, and tryptic soy broth (TSB, Difco), respectively.

Antimicrobial activity of the films

The antimicrobial activity of the film was determined using the method of Ku et al. (19). The *E. coli* O157:H7 (NCTC12079), *L. monocytogenes* (KCTC 3710), and *S.* Typhimurium (ATCC 14028) were incubated at 37°C in broth (LB, BHI, TSB, respectively) until $10^8 \sim 10^{10}$ 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 60 min. 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 plated in Chromogenic *E. coli* Coliform medium (Oxoid, Basingstoke, UK) plates, *Listeria* selective agar (Oxoid) plates, and *Salmonella* Chromogenic agar (Oxoid). All the plates were incubated at 37°C for 24 hr. Each microbial count was the mean of three determinations, and the microbial count was expressed as a log-colony-forming unit (CFU)/g.

Inoculation on fish paste

E. coli O157:H7 and *S.* Typhimurium were cultured in LB and TSB broth until 10^7 CFU/mL, respectively, while *L. monocytogenes* was cultured at 37°C in BHI broth until 10^8 CFU/mL. The fish paste was decontaminated by 70% ethanol for 3 min and dried in a clean bench. The decontaminated samples were then inoculated with the bacterial suspensions and left at room temperature for 15 min. The initial inoculation level of the *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were 5.04, 6.65, and 6.10 log CFU/g, respectively.

Packaging of the fish paste with the film

The inoculated fish paste was packed in direct contact with the GC-WPI film containing GSE. The sample that was packed with the GC-WPI film without GSE was used as the control. All the samples were stored at 4°C for 12 days.

Microbiological analysis

Five grams of the inoculated fish paste samples was placed in 45 mL of 0.1% peptone water. The samples

were then homogenized in a sterile stomacher bag using a Stomacher (MIX 2, AES Laboratoire, Combourg, France) for 3 min, filtered through a sterile cheese cloth, and diluted with peptone water to determine their microbial counts. To determine their microbial counts, serial dilutions were performed in triplicate on each selective agar plate. The *E. coli* O157:H7 counts were determined by plating the appropriately diluted samples onto Chromogenic *E. coli* Coliform medium (Oxoid) plates. The *L. monocytogenes* counts were determined by plating the appropriately diluted samples onto *Listeria* selective agar (Oxoid) plates. The *S.* Typhimurium counts were determined by plating the appropriately diluted samples onto *Salmonella* Chromogenic agar (Oxoid) plates. Each plate was incubated at 37°C for 36 hr. Each microbial count was the mean of three determinations, and the microbial count was expressed as a log-colony-forming unit (CFU)/g.

Sensory evaluation

Samples were analyzed for freshness, texture, flavor, color, and overall acceptability by 8 trained panelists. Sensory qualities of the samples were evaluated using a 9-point hedonic scale method. (9 point is very good, and 1 point is very poor). Analysis of variance and Duncan's multiple range test were performed to analyze the results using a SAS program.

RESULTS AND DISCUSSION

Physicochemical properties of the film

Thickness of the film decreased with increasing GSE amounts, and addition of GSE affected the mechanical properties of the film (Table 1). These results are in good agreement with those of Du et al. (20), where the thickness of apple puree edible film was decreased by increasing carvacrol concentrations. Addition of GSE to the film significantly increased TS value. The film had a TS of 2.64 MPa for the control, whereas the film containing 0.1% GSE had 3.27 MPa (Table 1). Our results are in accordance with Ko et al. (21), where TS of SPI and wheat gluten film increased to 10.43 and 3.50 MPa by the addition of nisin, compared to 8.95 and 1.95 MPa for the control. In general, increases in TS accompany decreases in % elongation (22). There was a decrease in the % elongation of the film containing GSE, compared to the control.

Water vapor permeability (WVP) is one of the most important physical properties of protein films. WVP of the film significantly was decreased by the addition of GSE (Table 1). Ku et al. (19) also reported that WVP of the GC film decreased with increasing catechin concentration. Decrease of WVP by addition of GSE can be attributed to modification of the film structure by the addition of antimicrobials, due to the cross-links formed by phenolic constituents in the GSE (23).

Antimicrobial activity of the film against pathogenic bacteria

The antimicrobial activity of the film against *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium is shown in Table 2. Incorporation of GSE into the GC-WPI film increased inhibition of the bacterial growth with increasing GSE content. Addition of 0.1% GSE decreased the populations of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium by 1.0, 1.6, and 0.6 log CFU/g, respectively, compared to the control. These results suggest that the GC-WPI film containing GSE has

Table 1. Effect of GSE on the thickness, tensile strength, % elongation, and water vapor permeability of the GC-WPI blend film

GSE amount (%)	Thickness (mm)	Tensile strength (MPa)	% elongation (%)	Water vapor permeability (ng m/m^{2}sPa)
0	0.138±0.003^{a}	2.64±0.178^{b}	115.68±8.642^{a}	6.86±0.141^{a}
0.02	0.116±0.003^{b}	3.15±0.113^{a}	91.37±3.038^{b}	5.77±0.211^{b}
0.04	0.116±0.003^{b}	3.17±0.118^{a}	94.04±5.369^{b}	5.72±0.211^{b}
0.08	0.112±0.003^{bc}	3.29±0.210^{a}	90.93±2.220^{b}	5.55±0.105^{b}
0.1	0.106±0.001^{c}	3.27±0.042^{a}	98.13±8.217^{b}	5.27±0.070^{b}

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

Table 2. Effect of GSE on the inhibition of the pathogenic bacteria (Log CFU/g) in the GC-WPI blend film

Type of bacteria	GSE amount (%)				
	0	0.02	0.04	0.08	0.1
E. coli	7.15±0.201^{a}	6.87±0.228^{a}	6.84±0.088^{a}	6.24±0.337^{b}	6.15±0.213^{b}
L. monocytogenes	9.45±0.136^{a}	8.89±0.078^{b}	8.92±0.042^{b}	8.29±0.017^{c}	7.84±0.042^{c}
S. Typhimurium	8.05±0.074^{a}	7.40±0.073^{a}	7.10±0.020^{c}	7.15±0.080^{c}	7.13±0.066^{c}

$^{a-c}$Any means in the same row followed by different superscript letters are significantly different ($p<0.05$).

Table 3. Change in the populations (Log CFU/g) of the pathogenic bacteria inoculated on the fish paste during storage

Type of bacteria		Storage period (days)			
		0	2	4	7
E. coli	Control	5.05±0.111[a]	5.73±0.172[a]	4.68±0.185[a]	3.89±0.157[a]
	GSE	5.05±0.111[a]	5.20±0.265[b]	4.27±0.160[a]	3.29±0.018[b]
L. monocytogenes	Control	6.68±0.064[a]	7.44±0.167[a]	7.33±0.172[a]	6.93±0.036[a]
	GSE	6.68±0.064[a]	7.20±0.227[a]	7.12±0.072[a]	6.45±0.213[a]
S. Typhimurium	Control	6.12±0.163[a]	5.74±0.146[a]	4.64±0.148[a]	4.58±0.032[a]
	GSE	6.12±0.163[a]	5.11±0.293[b]	4.40±0.151[a]	3.73±0.067[b]

[a,b]Any means in the same column followed by different superscript letters are significantly different ($p<0.05$).

antimicrobial activity on a wide range of gram-positive and gram-negative bacteria (12).

Microbiological analysis in the fish paste during storage

Populations of pathogenic bacteria, *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium in the fish paste packed with the film during storage were determined (Table 3). The initial inoculation level of the *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium in the fish paste were 5.05, 6.68, and 6.12 log CFU/g, respectively. The populations of the *E. coli* O157:H7 in the fish paste during storage increased until day 2 of storage, but afterwards they rapidly decreased. After 7 days of storage, the film containing 0.1% GSE decreased populations of the bacteria by 0.60 log CFU/g more, compared to the control (Table 3).

In the case of *L. monocytogenes*, the populations of control bacteria increased until day 2 of storage and then decreased, whereas the sample packed with the GC-WPI film containing GSE had a gradual decrease in the populations of the bacteria during storage (Table 3). Typically, populations of *L. monocytogenes* decreased by 0.48 log CFU/g more after 7 days, compared to the control.

In contrast, populations of *S.* Typhimurium continuously decreased during storage (Table 3). After 7 day of storage, populations of *S.* Typhimurium decreased by 0.85 log CFU/g more, compared to the control. Xu et al. (14) have reported that application of GSE to fresh cut vegetables decreased the populations of *S.* Typhimurium by 2.8 log CFU/g. It has been known that the antimicrobial effect of GSE is due to hydroxy radicals (24).

In summary, our results suggest that fish paste can be packed within edible GC-WPI blend film containing GSE to inhibit the growth of pathogenic bacteria.

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