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RESEARCH NOTE

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Development of Antimicrobial Edible Film Incorporated with Green Tea Extract

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Abstract This study investigated the antimicrobial activity of soy protein isolate (SPI) film containing green tea extract (GTE, 1-4%, w/w) on dental caries-inducing bacterium (*Streptococcus mutans*), food pathogenic (*Staphylococcus aureus, Escherichia coli O157, Salmonella typhimurium*), and spoilage (*Pseudomonas aeruginosa*) bacteria. The physical and mechanical properties of the SPI film containing GTE were also studied. The SPI film containing GTE (4%, w/w) exhibited good antimicrobial activity against *S. mutans* and *S. aureus*. The antimicrobial activity of the SPI film containing GTE increased against *S. mutans* as the concentration of GTE increased up to 4%(w/w). SPI films containing GTE showed lower tensile strength and elongation, and higher total soluble matter than those of control SPI film. Therefore, GTE can be used as one of antimicrobial agents for anti-dental caries and food packaging films.

Keywords: green tea extract, antimicrobial activity, *Streptococcus mutans, Staphylococcus aureus*, edible film

Introduction

Functional food packaging systems using various biopolymers, including edible films and coatings, have gathered great attention and the beneficial effects obtained by using edible film and coating have been reported in many publications (1-3).

The possibility of edible films or edible coatings to carry some food additives such as antioxidants, antimicrobials, colorants, flavors, fortified nutrients, and spices have also been studied. In addition, the mass transfer studies such as moisture, oxygen, and organic compounds permeability on edible films and coatings have been investigated (4-6). However, most of these studies have focused on the development or modification of packaging film for new active packaging, instead of edible film such as anti-dental caries film.

Antimicrobial film became part of a large category of new food packaging concept known as 'active packaging materials'. Active packaging materials can release antimicrobial compounds into foodstuffs slowly, and, therefore, can be used to prevent or slow down bacterial growth during storage of foods. Antimicrobial agents used in food application include organic acids, bacteriocins, enzymes, alcohols, and fatty acids. In addition, spice extracts have been introduced because of their ability to control meat spoilage (7-9).

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Naturally derived compounds and other natural products may have applications in controlling pathogens in foods. Recently, separation of bioactive materials from natural resources, which have been safely consumed, has been actively studied. Among these resources, green tea has the longest history of safe consumption and, together with coffee and cocoa, is one of the three most popular beverages in the world. It is consumed in approximately 160 countries everyday (10, 11). Green tea is composed of approximately 30% of polyphenols (dry basis), such as flavanols, flavandiols, flavonoids, and phenol acids (12). Polyphenols have been well known for their excellent biological activities including inhibition of tooth decay, allergy, and oxidation, reduction of blood pressure, and prevention of gout (11-15).

The objective of this study was to develop antimicrobial edible film using green tea extract (GTE). GTE was incorporated into soy protein isolate (SPI) film-forming solution in order to apply GTE for antimicrobial films against tooth decay, food pathogens, and food spoilage bacteria. Physical and mechanical property changes of the SPI film caused by the addition of GTE were also investigated.

Materials and Methods

Preparation of films Film-forming solutions were prepared by mixing 100 mL of distilled water with 7.5 g of SPI (Kwangil Co. Ltd., Seoul, Korea) and 3.75 g of glycerin (USP grade, Sigma Chemical Co, St. Louis, MO, USA). After adjustment of the solutions with sodium

hydroxide (1 N) to pH 10.0±0.01, they were held for 30 min in a water bath at 90°C and cooled to 23±2°C. After adding 1, 2, 3, or 4%(w/w) of GTE (powder type, Hanmi Flavor Chemical Co. Chunan, Chungnam, Korea), the solutions were strained through cheesecloth (Cheesecloth Wipes™; VWR Scientific Products, West Chester, PA, USA) and degassed under vacuum to remove any bubbles and lumps. The film-forming solutions (90 mL) were then cast on flat, level Teflon®-coated glass plates (21 cm × 35 cm). The films were peeled from the plates after drying at ambient temperature for about 20 hr and the dried films were conditioned at 50% RH and 25°C for 48 hr.

Tensile strength and percentage elongation at break Tensile strength (TS) and elongation at break (E) were determined with a Texture analyzer (TA-XT2; Stable Micro System, Surrey, UK) following the guidelines of ASTM Standard Method D 882-91 (16). Initial grip separation and cross-head speed were set at 50 mm and 500 mm/min, respectively. TS was expressed in MPa and calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen. E was calculated as the ratio of the final length of the point of sample rupture to the initial length of a specimen (50 mm), and recorded as a percentage. TS and E tests for each type of film were replicated five times.

Total soluble matter Total soluble matter (TSM) content was expressed as the percentage of dry film matter dissolved during immersion in distilled water for 8 hr. Film pieces (10×30 mm) were placed in 50-mL beakers containing 40 mL of distilled water. The beakers were covered with Parafilm 'M' wrap (American National Can™; Chicago, IL, USA) and stored at 25°C for 8 hr. Subsequently, the water remaining in the beakers was discarded and the residual film pieces were rinsed gently with distilled water. The pieces were then dried in an air-circulating oven (105°C) for 24 hr. The weight of dissolved dry matter was calculated by subtracting the weight of insoluble solid matter from the initial weight of solid matter (1). TSM tests for each type of film were replicated five times.

$$TSM = [(\text{Initial dry wt} - \text{Final dry wt}) / \text{Initial dry wt}] \times 100$$

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 Hunter L, a, b color scale was used to measure color: L = 0 to 100 (black to white), a = -80 to 100 (greenness to redness), and b = -70 to 70 (blueness to yellowness). Standard values for the white calibration plate were L = 96.86, a = -0.07, and b = 1.98. Color tests for each type of film were replicated five times.

Indicator bacteria and cultures One dental caries induction strain and three food pathogenic bacteria, and one food decay bacterium were used in this study. *Streptococcus mutans* KCTC 3065 was used as dental caries inducing bacterium. Brain heart infusion broth (Difco, Detroit, MI, USA) was used for cultivation of *S. mutans* KCTC 3065 under anaerobic condition at 37°C for 3 days (14). *Staphylococcus aureus* KCTC 2199, *Escherichia coli* 0157 KCTC 0019, *Salmonella typhimurium* KCTC 2053, and *Pseudomonas aeruginosa* KCTC 1636 were used as food pathogens and spoilage bacteria. These strains were maintained at -70°C in a nutrient broth containing 33.3% glycerol and subcultured twice in the nutrient broth at 37°C for 1 day before use.

Antimicrobial assay Antimicrobial activity test of films was carried out using the agar diffusion method. Films were cut into a disc of 5 mm diameter using a circular knife. The cut sample film discs were placed on BHI agar plate for *S. mutans* KCTC 3065 or nutrient agar (2%w/v agar) plate for *S. aureus* KCTC 2199, *E. coli* 0157 KCTC 0019, *S. typhimurium* KCTC 2053, and *P. aeruginosa* KCTC 1636. The media were previously seeded with 0.1 mL of inoculum containing each microorganism in the range of 10^5-10^6 CFU/mL. The plates were then incubated at 37°C for 24 hr (8, 14). Diameter of clear zone (mm) surrounding the film discs was measured to evaluate the inhibitory activity. Antimicrobial activity tests for each type of film were replicated three times.

Results and Discussion

Film properties In preliminary experiments, SPI films containing over 5%(w/w) of GTE produced film with inferior quality due to immiscibility with SPI film-forming solution. Therefore, the maximum concentration of GTE in SPI film was limited by 4%(w/w). The results of TS and E of SPI film with 4% GTE are shown in Table 1. The mean values of TS and E of SPI films with 4% GTE were 0.44 ± 0.05 MPa and 91.3 ± 18.2%, respectively. By addition of GTE in the SPI solution, TS and E values decreased to 23 and 40%, respectively. This result indicates that the GTE interrupts protein-protein association in SPI film networks, implying the deterioration of physical properties of SPI film. Sai (17) reported the negative effects of GTE on protein interactions in his study.

The optical appearance of SPI film was retained in distilled water (25°C) for 8 hr, while the SPI film with 4% GTE was totally collapsed under the same condition. The TSMs of SPI film and SPI film with 4% GTE were 33.3 ± 2.4 and 39.7 ± 1.3%(w/w), respectively. These results also showed that GTE negatively affected the protein interactions between protein molecules. SPI films with GTE were more brownish than the control SPI films. Compared to control SPI film, the lightness ('L' value) of SPI film with GTE was decreased from 61.08 to 49.98, while the redness ('a' value) and the yellowness ('b' value) were increased from -1.24 to 6.64 and from 5.56 to 19.08, respectively.

Table 1. Tensile strength (TS) and elongation at break (E) values of soy protein isolate (SPI, 7.5%, w/w) film and SPI film containing green tea extract (GTE, 4%, w/w)

	TS (MPa)	E (%)
SPI + GTE	0.44 ± 0.05	91.3 ± 18.2
SPI	0.57 ± 0.04	151.6 ± 19.8

Antimicrobial activity Antimicrobial activity of SPI film incorporated with GTE (4%, w/w) against *P. aeruginosa* KCTC 1636, *E. coli* O157 KCTC 0019, *S. typhimurium* KCTC 2053, *S. aureus* KCTC 2199, and *S. mutans* KCTC 3065 is shown in Table 2. In case of control SPI film, no antimicrobial activity was observed in our study. The SPI film with GTE did not show inhibitory activity against *P. aeruginosa* KCTC 1636, *E. coli* 0157 KCTC 0019, and *S. typhimurium* KCTC 2053. Several researchers (13,15,18) reported that *P. aeruginosa*, *E. coli*, and *S. typhimurium* were highly tolerant against the antimicrobial activity of green tea. Park (18) also pointed out that GTE showed higher antimicrobial activity against Gram (+) bacteria (*S. aureus*) than Gram (-) bacteria (*E. coli* 0157, *S. typhimurium*).

The SPI film with GTE showed selective inhibitory activity against food pathogens examined in our study. The growths of *S. aureus* KCTC 2199 and *S. mutans* KCTC 3065 were inhibited (Table 2). This phenomenon was consistent with the results of previous researches (11, 13, 15, 18). Yang *et al.* (11) and Yeo *et al.* (13) reported polyphenol compounds from green tea primarily inhibit the glucosyltransferase activity and growth of *S. mutans* in their studies. Antimicrobial activity against *S. mutans* increased with the concentration of GTE in SPI film within the range of 1-4% (Table 3). Figure 1 illustrates the inhibitory zone of antimicrobial SPI film containing GTE (4%, w/w) against *S. mutans* KCTC 3065.

In conclusion, SPI film incorporated with GTE showed not only the selective antimicrobial activity against food pathogenic bacterium (*S. aureus*) as well as dental caries-inducing bacterium (*S. mutans*), but also the change of physical properties due to the immiscibility between GTE and SPI. These results suggest that the edible film containing GTE can be applied for anti-caries edible film that melts in the mouth as well as for food active packaging film. For future work, it can be suggested that the functionality and feasibility of using antimicrobial edible film should be considered according to various purposes such as edible films and other packaging film layers.

Table 2. Antimicrobial activity of soy protein isolate film containing green tea extract (4%, w/w) against pathogenic bacteria and dental caries inducing bacterium

Strains		Inhibition zone (mm)
Pseudomonas aeruginosa	Gram (-)	-[1)]
Escherichia coli O157	"	-
Salmonella typhimurium	"	-
Staphylococcus aureus	Gram (+)	6.8 ± 0.6[2)]
Streptococcus mutans	"	11.0 ± 1.3

[1)]No inhibition.
[2)]Diameter.

Table 3. Effect of concentrations of green tea extract (GTE) on antimicrobial activity of soy protein isolate film against *Streptococcus mutans* and *Staphylococcus aureus*

	Concentration of GTE (w/w)			
	1%	2%	3%	4%
Streptococcus mutans	-[1)]	9.3 mm[2)]	9.8 mm	11.0 mm
Staphylococcus aureus	-	5.6 mm	6.8 mm	6.8 mm

[1)]No inhibition.
[2)]Diameter of inhibition zone.

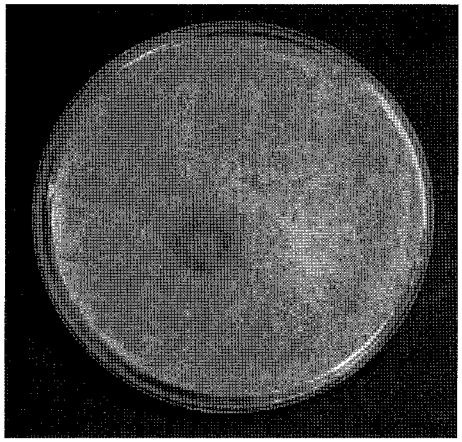

Fig. 1. Antimicrobial activity of soy protein isolate film containing green tea extract (4%, w/w) against *Streptococcus mutans*.

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