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RESEARCH NOTE

Physical Properties and Antimicrobial Activities of a Persimmon Peel/Red Algae Composite Film Containing Grapefruit Seed Extract

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Abstract As a by-product of dried persimmon production, persimmon peel (PP) can be utilized as an edible film base material. In this study, PP/red algae (RA) composite films were prepared, and their physical properties were determined. Among the PP composite films, the 4% PP/1% RA composite film had the best physical properties (7.31 MPa, 8.16%, and 4.99 ng m/m^{2}s Pa for tensile strength, elongation at break, and water vapor permeability, respectively). The optical property of the PP composite film was desirable because the color of the film had an intrinsic persimmon color. In addition, the 4% PP/1% RA composite film containing 1% grapefruit seed extract was prepared as an antimicrobial film. These results suggest that this PP composite film can be applied to food packaging.

Keywords: composite film, edible film, persimmon peel, red algae

Introduction

Edible film and coating can affect food surface by incorporating functional compounds such as antimicrobials and antioxidants (1). In particular, an edible film containing antimicrobials could enhance microbial safety and extend the shelf-life of foods (2). However, the physical properties of edible films are inferior to those of plastic films, and more importantly, the cost is higher than plastic films (3,4). Therefore, various agricultural raw materials have been studied as an inexpensive edible film source using under-utilized food processing by-products (3-5).

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Persimmon peel (PP) is generated during the processing of dried persimmon products, but this product is primarily discarded. PP is a known good source of dietary fiber and antioxidant. Therefore, PP could be used as an edible film source if it had a film-forming ability. To enhance the mechanical properties of films, red algae (RA) such as *Gelidium corneum* have been used as a base material of composite films to improve the mechanical properties of protein films (6). Essential oils and plant extracts such as lemongrass, rosemary, and grapefruit seed extract (GSE) are used as antimicrobials in foods (1). In particular, edible films containing GSE can be applied to food packaging to extend the shelf-life of various foods (6-8). Therefore, the objectives of this study were to prepare a composite film using PP as an inexpensive by-product of dried persimmon and to construct an antimicrobial film containing GSE.

Materials and Methods

Materials PP was obtained as a by-product in the manufacturing of dried persimmon from a persimmon farm (Sangju, Korea), and *G. corneum* as RA was harvested on Jeju Island, Korea. GSE derived from the seeds of grapefruit was obtained from ABC Techno Inc. (Tokyo, Japan). Sucrose and cinnamaldehyde as a plasticizer and a cross-linking agent, respectively, were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Preparation of the PP film-forming solution PP was washed to remove foreign substances, ground using a blender (WB-1; Sanplatec, Osaka, Japan), and screened through an 80 mesh sieve. To minimize the particle size of the PP powder, 5% (w/v) PP solution in distilled water was autoclaved at 121°C for 15 min and homogenized using a homogenizer (Ultra-Turrax T25; IKA, Staufan, Germany) at 20,000 rpm for 20 min. The homogenized solution was filtered using cheese cloth and treated with a high pressure homogenizer (M-110Y; Microfludics, Newton, MA, USA) with 1 passage at 13,000 psi. The film-forming solution was then degassed, and 1.5% (w/v) sucrose and 0.01% (v/v) cinnamaldehyde were added, based on preliminary experiments using different concentrations of plasticizer and cross-linking agent (data not shown). To prepare the PP/RA composite films, the PP (3, 4, and 5 g) solution and filtered RA (2, 1, and 0 g) solution were used for the film-forming solution (total 5 g of PP and RA per 100 mL) after heating at 90°C for 30 min, based on the preliminary experiments. In addition, various quantities (0.7, 1.0, 1.2, or 1.5%) of GSE were added to the film-forming solution to construct an antimicrobial film.

Film casting and physical property measurement Eighty millilitres of PP/RA film solution was poured on flat, Teflon-coated glass plates (24×30 cm). The PP/RA film was dried at ambient temperature for 24 h, and the dried film was peeled away from the plate and conditioned at 25°C and 50% relative humidity (RH). The thickness of each film was measured using a micrometre (model No. 2109S-10; Mitutoyo, Tokyo, Japan), and the tensile strength (TS) and elongation at break (E) of the films were determined using an Instron machine (M250-2.5CT; Testometric Company Ltd., Rochdale, UK) according to the ASTM D638M method. The water vapor permeability (WVP) of the PP/RA films was determined according to a modified ASTM E 96-80 method at 25°C and 50% RH using a polymethylacrylate cup (8).

Measurement of antimicrobial activity Agar diffusion tests were performed to determine the antimicrobial activity of the PP/RA film containing GSE against *Escherichia coli* O157:H7 and *Listeria monocytogenes* according to the method of Shin *et al.* (9). Approximately 7 log CFU/mL of *E. coli* O157:H7 (0.1 mL) and *L. monocytogenes* (0.1 mL) were plated onto Tryptic soy broth-Bacto agar (Difco, Detroit, MI, USA) and Oxford medium base agar (Difco), respectively. The PP/RA films containing GSE were cut to a 10 mm diameter and placed onto the inoculated plates. The *E. coli* O157:H7 and *L. monocytogenes* plates were stored at 4°C for 1 h to allow the GSE to diffuse and then incubated at 37°C for 24 and 48 h, respectively. The diameters of the inhibition zones were measured using a digimatic calliper (model 500-181-20; Mitutoyo Co., Kawasaki, Japan).

Measurement of optical property The color of the PP/RA composite film was determined using a colorimeter (CR-400; Minolta, Tokyo, Japan). The color values are expressed as the total colour difference (E^*), hue angle (h), and chroma (C). In addition, transmittance (%) was determined by measuring the absorbance at 660 nm using a spectrophotometer (UV-2450; Shimadzu Co., Kyoto, Japan).

Results and Discussion

To prepare a PP film, the film-forming solution containing 5% PP powder was first autoclaved and treated by homogenization and high pressure homogenization. As a result, only the film-forming solution treated by both homogenization and high pressure homogenization could form a film. High pressure homogenization has been used to improve the mechanical property of apple peel films by reducing the particle size (10).

To make the film applicable, plasticizer and crosslinking agent should be added. Polyols such as glycerol are usually used as a plasticizer for the carbohydrate-based films (11). Preliminary, various types of plasticizers such as sucrose, glycerol, sorbitol, and fructose and various amounts (0.01, 0.05, and 0.1 g) of cinnamaldehyde as a cross-linking agent were used for the preparation of the PP film. Based on the TS, E, and WVP values of the prepared films (data not shown), the PP film containing 1.5% sucrose and 0.01% cinnamaldehyde had the best physical properties and thus the condition was chosen for the preparation of the PP film.

It has been reported that incorporating RA into the film-forming solution improves the mechanical properties of the protein film (3). To enhance the mechanical properties of the PP film, 1 or 2% RA was added to the PP film-forming solution. The PP composite film containing 1% RA had a TS of 7.31 MPa, an increase of 1.26 MPa compared with the PP film (Table 1). In contrast, the PP composite film containing 2% RA had a TS of 3.32 MPa. In contrast to our results, Shin *et al*. (3) reported that the TS increased as the RA content increased in a rice bran protein/RA composite film. This difference could be attributable to the different film base material because PP is not a protein, which had a different compatibility with the RA molecules, resulting in different TS. In this study, the 4% PP/1% RA composite film was selected as the most appropriate composite film because it had the best mechanical property among the films prepared.

The color of the PP film appeals to consumers, which is along with carotenoid compounds such as β-cryptoxanthin, zeaxanthin, and lutein, which are enriched in the PP (12). Therefore, the PP film, with the intrinsic color of PP, could have an advantage as a food packaging material. The color of the PP/RA composite film was observed with regards to Hunter L^*, a^*, b^*, total colour difference, hue angle, and chroma values (Table 2). As the RA content increased, the total color difference and hue angle increased. However,

Table 1. Physical properties of persimmon peel (PP)/red algae (RA) composite films

PP (%)	RA (%)	Tensile strength (MPa)	Elongation (%)	WVP (10^{-9}g m/m^2 s Pa)
5	0	6.05±0.33[b1)]	10.80±2.00[a]	5.35±0.43[a]
4	1	7.31±0.51[a]	8.16±0.96[b]	4.99±0.24[a]
3	2	3.32±0.41[c]	2.16±0.00[c]	4.91±0.04[a]

[1)]Mean values sharing different letters within a column are significantly different by Duncan's multiple range test at $p<0.05$.

Table 2. Optical properties of the PP/RA composite film

PP (%)	RA (%)	E^*_{ab}	Hue angle	Chroma	T_{660} (%)
5	0	67.30±0.76[c1)]	71.81±0.66[c]	32.36±0.29[b]	3.79±0.33[c]
4	1	73.20±0.21[b]	77.26±0.16[b]	33.76±0.05[a]	5.29±0.12[b]
3	2	79.70±0.62[a]	82.72±0.53[a]	30.92±0.40[c]	7.16±0.19[a]

[1)]Mean values sharing different letters within a column are significantly different by Duncan's multiple range test at $p<0.05$.

Table 3. Physical properties and antimicrobial activities of the PP/RA composite films containing various amounts of GSE

GSE (%)	Tensile strength (MPa)	Elongation (%)	WVP (10^{-9}g m/m^2 s Pa)	Inhibition zone (mm)	
				E. coli O157:H7	*L. monocytogenes*
0[1)]	7.31±0.51[a2)]	8.16±0.96[d]	4.99±0.24[a]	0	0
0.7	5.17±0.23[b]	16.36±0.43[c]	5.15±0.36[a]	0	25.51±0.82[d]
1	4.35±0.18[c]	19.80±1.53[b]	5.32±0.34[a]	10.90±0.13[c]	28.52±1.59[c]
1.2	4.17±0.23[cd]	21.04±1.22[ba]	5.27±0.24[a]	12.65±0.45[b]	30.78±0.94[b]
1.5	3.85±0.14[d]	21.80±0.90[a]	5.27±0.31[a]	13.68±0.41[a]	32.64±1.49[a]

[1)]The film consists of 4% PP and 1% RA.
[2)]Mean values sharing different letters within a column are significantly different by Duncan's multiple range test at $p<0.05$.

the chroma value decreased predominantly due to the decrease in the intrinsic color of the persimmon.

The transparency of the PP/RA composite film was determined by measuring transmittance. The transmittance of the PP film without RA was 3.79% and increased with the increase in the RA content due to the transparency of the RA (Table 2). The transmittance of the PP/RA composite film increased by 1.5% compared with the PP film; however, the film had a low transmittance of 5.29%, which is enough to block light. Blocking light can be an effective means to prevent lipid oxidation during food storage (13). Therefore, the PP film can be applied to the packaging of foods containing high lipid contents.

GSE is incorporated into the film-forming solution for its antimicrobial activity. For *E. coli* O157:H7, antimicrobial activity was shown at 1% GSE and increased as the GSE content increased (Table 3). In contrast, the growth of *L. monocytogenes* was inhibited beginning at 0.7% GSE, and the inhibition zone increased as the GSE concentration increased. It has been reported that GSE can provide an antimicrobial activity to an edible film and that it is more effective against *L. monocytogenes* than *E. coli* O157:H7 (4,7). Reagor *et al*. (14) also reported that GSE had a greater antimicrobial activity on Gram-positive bacteria compared to Gram-negative bacteria. The antimicrobial activity of GSE is mainly due to the fact that GSE can disrupt bacterial membrane and release cytoplasmic content (9,15). Edible films containing GSE have been previously studied in the antimicrobial packaging of various foods (4,7,8,16).

The mechanical properties of the 4%PP/1%RA composite film containing GSE were determined (Table 3). As the GSE content increased from 0.7 to 1.5%, the TS of the film decreased from 7.31 to 3.85 MPa and the E increased from 8.16 to 21.82%. Jang *et al*. (7) also reported that the TS of a rapeseed protein/gelatin composite film decreased and the E increased after the addition of GSE, similar to our results. The reason for the decrease in TS and increase in E might be attributable to weak intermolecular interactions between PP molecules because of the phenolic acids present in GSE (17). Therefore, with the antimicrobial activity of the 4% PP/1% RA composite film containing GSE, it is suggested that 1% GSE is a suitable content for the antimicrobial PP film, along with the mechanical property of the film containing GSE.

In conclusion, as a by-product of dried persimmon production, PP can be utilized as an edible film source. The use of 1.5% sucrose and 0.01% cinnamaldehyde is suggested for the preparation of a PP film with desirable physical properties. The incorporation of 1% RA into the PP film further improved its physical properties. In addition, the addition of GSE to the composite film provided an antimicrobial activity. Therefore, these results suggest that the PP/RA composite film containing GSE can be applied to food packaging to extend the shelf-life of foods.

Disclosure The authors declare no conflict of interest.

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