Characterization of red ginseng residue protein films incorporated with hibiscus extract

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Abstract An edible film was prepared from red ginseng residue protein (RGRP) and incorporated with hibiscus extract (HE). RGRP was extracted from red ginseng residue, which is an inexpensive by-product of the red ginseng processing industry. Different concentrations of HE were added to an RGRP film-forming solution as a natural antioxidant. The prepared RGRP films without HE had a tensile strength of 16.9 MPa and an elongation at break of 25.1%. The antioxidant activity of the RGRP film increased with increasing concentration of HE. In addition, the RGRP film with 1% HE exhibited the lowest value of water vapor permeability ($1.88 \times 10^{-9} \text{ g} \cdot \text{m} / \text{m}^2 \cdot \text{s} \cdot \text{Pa}$), which indicates that the film has high water barrier property. The results present the production of edible films from discarded red ginseng residue, and the antioxidant activity of RGRP films as a packaging material can prevent lipid oxidation and quality loss of food products.

Keywords: antioxidant activity, hibiscus extract, protein film, red ginseng residue

Introduction

Recently, research interests in biopolymers for food packaging have increased owing to environmental problems caused by non-renewable synthetic plastic waste (1). Therefore, biopolymers obtained from biomass or food processing by-products are being used to produce edible films (2). Edible films prepared from biopolymers, e.g., proteins and polysaccharides, are biodegradable and can be derived from renewable waste resources. However, they have some limitations, such as a high production cost as well as poor thermal and physical properties (3). Thus, production of edible films using cheaper sources with improved physical and thermal properties is needed to compete with synthetic plastic.

For thousands of years, ginseng (*Panax ginseng* C.A. Mey.) has been among the most widely recognized common herbal medicines in Korea and has been used throughout Asia to treat various diseases (4). Red ginseng residue (RGR) is a by-product of red ginseng extraction and has 15% protein content (5). RGR production has been increasing recently because of the popularity of healthy foods and drinks containing red ginseng. Annually, 1,000 tons of RGR is produced in the ginseng processing industry in Korea; however, owing to its high moisture content, RGR is either discarded as waste or used in animal feed (6). Therefore, RGR can be considered to be an inexpensive by-product and utilized as a potential source of film base material for edible film preparation.

Hibiscus (*Hibiscus sabdariffa* L.), a tropical annual shrub, mainly

grows in Thailand, Mexico, Egypt, and China (7). Hibiscus has been traditionally used in foods, such as soft drinks, and beverages, and as a flavoring agent. Dried flowers of hibiscus are rich in minerals, organic acids, anthocyanins, and phenolic compounds (8). Previous studies have suggested that hibiscus has antioxidant and antimicrobial properties (8–10). However, it has never been used in edible films as an antioxidant or antimicrobial agent.

This study aimed to prepare edible films using protein extracted from inexpensive by-products obtained during red ginseng processing incorporated with hibiscus extract (HE) as an antioxidant agent.

Materials and Methods

Materials RGR was obtained from Oseong Ginseng Co. (Jeungpyung, Korea). Dried hibiscus was purchased from Areumdre Co. (Ulsan, Korea).

Extraction of RGR protein (RGRP) The method described by Lee *et al.* (11) was used to extract RGRP. RGR was dried at 70°C for 8 h and ground using a blender (WB-1; Osaka Chemical Co., Osaka, Japan). RGR powder (100 g) was mixed with distilled water (1,900 mL) for 30 min using a magnetic stirrer and homogenized using a sonicator (Model-GE 750; Sonics & Materials, Newtown, CT, USA) for 10 min. After stirring at 70°C for 2 h, the solution was centrifuged at 10,000×g for 25 min, and the supernatant was precipitated with 70%

ammonium sulfate at 4°C for 1 h. After precipitation, the solution was centrifuged at 10,000× for 25 min, and the pellet was obtained, dialyzed for 9 h using a dialysis membrane (Spectra/Por RC tubing, MWCO 3.5 kDa, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA), and freeze-dried.

Extraction of HE Hibiscus extraction was performed according to the method described by Yang *et al.* (10). To make HE, dried hibiscus was ground in a blender (Osaka Chemical Co.). Hibiscus powder (30 g) was mixed with 30% ethanol (300 mL) and stirred at 75°C for 1 h, and the solution was filtered using a cheesecloth. After filtration, the filtrate was concentrated on a rotary evaporator (VV2011; Heidilph, Schwabach, Germany) at 55°C to remove the solvent and then freeze-dried. The extraction yield of HE was approximately 48% (w/w).

Preparation of an RGRP film incorporated with HE On the basis of preliminary experiments using various plasticizers (data not shown), sorbitol was used as a plasticizer. RGRP (1 g) was dissolved in distilled water (20 mL), and the solution was sonicated. The solution was then heated at 70°C for 30 min in a water bath and cooled. Sorbitol (40%, w/w, on a dried RGRP basis) and HE (0.25, 0.5, 0.75, and 1.0%, w/v, on film solution basis) were added and stirred for 30 min so that they were completely dissolved. The mixture was then filtered using a cheesecloth, and the filtrate (12 mL) was poured into a petri dish and dried at 25°C for 16 h to fabricate RGRP films.

Physical properties of RGRP films The tensile strength (TS) and elongation at break (E) of the RGRP films were determined in five replications for each experiment according to the ASTM method D638M (12).

Optical properties of RGRP films The color of the RGRP films was measured with a colorimeter (CR-400; Minolta, Tokyo, Japan). The Hunter values of each film were measured five times. Analysis of the films were conducted by placing them on a standard white plate. The Hunter values of the standard plate were as follows; $L=96.75$, $a=0.14$, and $b=2.1$. The opacity of the RGRP films (2.5×1 cm) was determined by measuring the absorbance at 600 nm with a spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan).

Water vapor permeability (WVP), moisture content, and water solubility of RGRP films The WVP of the RGRP films (2×2 cm) was determined in triplicate according to the ASTM E96-80 method (13). The method described by Basiak *et al.* (14) was used to measure the moisture content and water solubility of the films. The test was performed in triplicate.

Antioxidant activity of RGRP films ABTS and DPPH radical scavenging activity assays were performed to measure the antioxidant activity of the RGRP films. Prior to the experiment, the film-extract solution was

obtained by mixing the film sample (0.5 g) and distilled water (5 mL). The method described by Yang *et al.* (1) was used to measure the ABTS radical scavenging activity, wherein 100 µL of the film-extract solution was mixed with 1.9 mL of the ABTS solution and incubated in a dark room for 10 min. After incubation, it was centrifuged at 1,600×*g* and 20°C for 3 min, and the absorbance of the filtrate was measured at 734 nm. DPPH radical scavenging activity was determined according to the method described by Lee *et al.* (2). The film-extract solution (100 µL) was mixed with a 0.1 mM DPPH solution (3.9 mL), and the mixture was kept in a dark room for 1 h. After centrifugation, the absorbance of the filtrate was measured at 517 nm. The results of the ABTS and DPPH assays were expressed as the percentage of the radical scavenging activity of the film-extract solution. The test was repeated five times.

Thermal properties of RGRP films Thermogravimetric analysis (TGA) was performed with a Mettler Toledo thermogravimetric analyzer (Mettler Toledo, Columbus, OH, USA). A small sample of the film (4.0±0.1 mg) was analyzed under the following condition: heating at a rate of 10°C/min from 25 to 600°C under nitrogen atmosphere (flow rate, 50 mL/min). Differential scanning calorimetry (DSC) measurements were performed with a Mettler Toledo DSC 1. A small sample of the film (7.5±0.35 mg) was placed into a hermetic pan and sealed. An empty pan was used as a reference cell (control). Analysis was performed under the following condition: heating at a rate of 10°C/min from 0 to 350°C.

Statistical analysis Statistical analysis of the experimental data with five replications, except for the tests measuring WVP, moisture content, and water solubility ($n=3$), was performed using SAS (SAS Institute, Inc., Cary, NC, USA). Statistical significance at $p<0.05$ was evaluated using analysis of variance and Duncan's multiple range tests.

Results and Discussion

Preparation of RGRP films Preliminary studies were conducted to optimize the extraction condition of RGRP from RGR. Under optimum conditions, the maximum yield of RGRP from RGR was approximately 9.30%. In this study, different plasticizers were used to prepare the extracted RGRP film with appropriate physical properties. Lee *et al.* (3) reported that plasticizers provide flexibility to the films by reducing the interactions between polypeptide chains. To select a suitable plasticizer, fructose, glycerol, and sorbitol were used. The concentration of each plasticizer was 40% of RGRP (based on dry weight). Among the plasticizers used, the mechanical property of the film with fructose was not determined because it was too fragile. The film plasticized with glycerol was too sticky. On the other hand, the film with sorbitol had a TS value of 16.9 MPa and an E value of 25.1% (data not shown). The reason for this difference is mainly the volume

introduced by sugar alcohol molecules and the hydrophilic nature of the plasticizer (1). Yang *et al.* (1) also observed a similar trend in distiller dried grains with soluble protein films, where sorbitol was used as a plasticizer. To further optimize the concentration of the plasticizer, different concentrations, such as 30, 40, 50, and 60%, of sorbitol were added to RGRP. As the concentration of sorbitol increased, the TS value of the film decreased and the E value of the film increased. The RGRP film containing 30% sorbitol had the lowest value of E (10.1%), and the film containing 60% sorbitol had the lowest value of TS (5.69 MPa) (data not shown). Therefore, 40% sorbitol was used as the appropriate plasticizer concentration for RGRP films. Mohammad *et al.* (15) reported that the mechanical properties of the films are affected by molecular interactions, such as hydrogen bonding between the plasticizer and polymer in the film matrix.

Physical properties of RGRP films incorporated with HE To provide an antioxidant property to the RGRP film, HE was added as a natural antioxidant to the film-forming solution. RGRP films containing different concentrations of HE were prepared. The TS value of the RGRP film incorporated with 1.0% HE decreased from 16.94 to 3.73 MPa in comparison with the RGRP film without HE (Table 1). However, there was no significant difference among the films containing 0.25–1.0% HE. In contrast, the E value of the RGRP film containing 1.0% HE increased from 25.1 to 87.1%. Shin *et al.* (16) also reported that the TS value of red algae film decreased and the E value increased with the increasing amount of grapefruit seed extract. This difference is mainly due to the addition of phenolic compounds to the films, which affects polymer–polymer interactions in the film matrix (17). In the case of the RGRP film, hydrogen and covalent bonds between phenolic compounds in HE and polypeptides in the RGRP interfered with protein–protein interactions, resulting in the weakening of the film network.

WVP of the films is an important factor and the film with low WVP can be a good barrier between the environment and packaged food (18). The WVP of the RGRP films incorporated with 0 to 0.75% HE showed no significant difference. However, the WVP ($1.88 \times 10^{-9} \text{ g} \cdot \text{m} / \text{m}^2 \cdot \text{s} \cdot \text{Pa}$) of the RGRP film containing 1.0% HE decreased as compared to that of the RGRP film without HE ($2.22 \times 10^{-9} \text{ g} \cdot \text{m} / \text{m}^2 \cdot \text{s} \cdot \text{Pa}$). This might be due to the complex film matrix formed at the higher concentration of HE and the more complex route of water passage (19).

Lee *et al.* (11) reported that the addition of thyme essential oil (TEO) decreased the WVP of skate skin gelatin film owing to the increased hydrophobicity of the film network by the addition of TEO. Bahram *et al.* (20) also reported the decreased WVP of a whey protein concentrate film with cinnamon essential oil owing to increased hydrophobic compounds in the film. In this study, addition of 1.0% HE decreased the WVP of RGRP films compared with the films without HE, indicating that the addition of HE provides a high water barrier property to the RGRP film. This can be beneficial for the long-term storage of foods by preventing sogginess and microbial spoilage.

Water solubility of RGRP films Water solubility is an essential property of edible films. A high solubility of edible films is suitable for food products during cooking, whereas a low solubility is appropriate for the storage of foods. The moisture content of the films increased from 7.48 to 11.37% with the addition of 1.0% HE (Table 1). The RGRP film containing HE had a high water retention capacity in comparison to the RGRP film without HE. Similar to our study, Nouri and Nafchi (21) reported an increased moisture content of a sago starch film containing betel leaves extract owing to the hygroscopic characteristics of the film as well as the high content of phenolic compounds in the betel leave extract. In contrast to the moisture content, no significant difference was observed in the water solubility of the films by the addition of HE up to 0.75%, but the water solubility decreased with the addition of 1% HE. Nie *et al.* (22) also reported that the water solubility of a myofibrillar protein-based film decreased owing to aggregation of polymer molecules by phenolic compounds of grape seed and green tea. From the results obtained in this study, it is apparent that the addition of HE increased water resistance of the RGRP films.

Optical properties of RGRP films Table 2 shows the optical properties of the RGRP films containing HE. Initially, the color of the films without HE was pale yellow. Then, the color gradually became dark red with increasing amounts of HE. The RGRP films containing HE had significant decreases in lightness (L value) and increases in redness (a value) (Table 2). With the addition of 1% HE, the lightness of the RGRP film containing HE decreased from 84.66 to 39.86 in comparison to the RGRP film without HE, and the redness significantly increased from 0.41 to 48.87 owing to the increased amount of anthocyanin in the film. Wang *et al.* (23) observed color changes in

Table 1. Physical properties of red ginseng residue protein films incorporated with hibiscus extract (HE)

HE (%)	TS (MPa) ¹⁾	E (%) ²⁾	WVP ($\times 10^{-9} \text{ g} \cdot \text{m} / \text{m}^2 \cdot \text{s} \cdot \text{Pa}$) ³⁾	Water solubility (%)	Moisture content (%)
0	16.94 $\pm$ 2.62 ^{a4)}	25.10 $\pm$ 4.95 ^d	2.22 $\pm$ 0.07 ^a	91.15 $\pm$ 1.11 ^a	7.48 $\pm$ 0.99 ^c
0.25	4.54 $\pm$ 0.22 ^b	64.42 $\pm$ 4.41 ^c	2.13 $\pm$ 0.03 ^a	88.63 $\pm$ 0.85 ^a	9.32 $\pm$ 0.88 ^b
0.5	4.55 $\pm$ 0.46 ^b	74.20 $\pm$ 5.98 ^b	2.05 $\pm$ 0.04 ^a	88.29 $\pm$ 1.71 ^a	9.79 $\pm$ 0.18 ^b
0.75	4.38 $\pm$ 0.39 ^b	81.61 $\pm$ 5.72 ^a	2.10 $\pm$ 0.10 ^a	87.71 $\pm$ 2.45 ^{ba}	10.03 $\pm$ 0.29 ^b
1.0	3.73 $\pm$ 0.29 ^b	87.09 $\pm$ 3.02 ^a	1.88 $\pm$ 0.22 ^b	84.44 $\pm$ 2.41 ^b	11.37 $\pm$ 0.34 ^a

¹⁾Tensile strength ²⁾Elongation at break ³⁾Water vapor permeability

⁴⁾Values are mean $\pm$ SD. TS ($n=5$), E ($n=5$), WVP ($n=3$), Water solubility ($n=3$), Moisture content ($n=3$). Any means in the same column followed by different letters are significantly ($p<0.05$) different by Duncan's multiple range test.

Table 2. Optical properties of red ginseng residue protein films incorporated with hibiscus extract (HE)

HE (%)	Optical properties			
	<i>L</i>	<i>a</i>	<i>b</i>	Opacity ²⁾
0	84.66±0.36 ^{a1)}	0.41±0.46 ^d	19.31±0.36 ^a	5.33±0.29 ^d
0.25	64.52±0.33 ^b	30.65±1.05 ^c	10.68±0.15 ^e	7.23±0.49 ^c
0.50	51.57±0.29 ^c	43.52±0.36 ^b	11.08±0.16 ^d	8.78±0.19 ^b
0.75	43.42±1.40 ^d	48.70±0.33 ^a	13.69±0.48 ^c	8.53±0.64 ^b
1.0	39.86±0.77 ^e	48.87±0.69 ^a	14.74±0.12 ^b	9.73±0.15 ^a

¹⁾Values are mean±SD (*n*=5). Any means in the same column followed by different letters are significantly (*p*<0.05) different by Duncan's multiple range test.

²⁾Opacity unit: A/mm. A, the absorbance value at 600 nm; mm, thickness of the film (*n*=5)

Fig. 1. Antioxidant activities of the red ginseng residue protein film containing hibiscus extract. **Fig. 2.** Thermogravimetry curves of the red ginseng residue protein film containing hibiscus extract (HE).

soy protein isolate films incorporated with chestnut bur extract. Similarly, Jouki *et al.* (24) observed decreases in lightness with increasing concentrations of TEO in quince seed mucilage films owing to the phenolic content of TEO.

The opacity of the RGRP films increased from 5.33 to 9.73 (A/mm) with the addition of 1.0% HE (Table 2). Prodpran *et al.* (25) reported that the transparency of fish myofibrillar protein films decreased with the addition of phenolic acid. Moreover, Wu *et al.* (26) reported the increase in the opacity of gelatin films with the addition of green tea extract owing to the improvement in the light transmission barrier property of the film.

Antioxidant activity of RGRP films The antioxidant activity of the RGRP films incorporated with HE is shown in Fig. 1. RGRP films showed radical scavenging activity from 66.6 to 77.7% with the addition of 1.0% HE in ABTS assay. In the DPPH assay, the radical scavenging activity also increased from 37.1 to 92.0% with the addition of 1.0% HE. In general, the nature of polyphenolic compounds and concentration affect the antioxidant activities of plant extracts (1). Dietary plants have different bioactive compounds, such as vitamins (vitamin C, vitamin B₁, and vitamin B₂), minerals (sodium, calcium, and potassium), and polyphenols (anthocyanin and carotenoids), which act as antioxidants. Among them,

polyphenol is a major antioxidant agent (27). There are many phenolic compounds in HE, such as delphinidin-3-sambubioside, cyanidin-3-sambubioside, quercetin, luteolin glucoside, and chlorogenic acid (27). The phenolic compounds in HE increased the antioxidant activity of the RGRP film. Al-Hashimi (8) reported that the antioxidant activity of HE increases with the increasing concentration of HE owing to the increase in the phenolic compounds. The results of Yang *et al.* (10) are consistent with those of this study.

Thermal properties of RGRP films The TGA thermogram shows the thermal degradation behavior of the RGRP films containing HE (Fig. 2). The first stage of weight loss indicates removal of water and volatile compounds from the film (28). At the first stage, the film with HE lost more weight than the control film because of the higher moisture content in the film containing HE. Lee *et al.* (11) reported a similar trend in skate skin gelatin films containing TEO. The weight loss of the film in the second stage was due to the degradation of protein molecules, in particular the breakdown of covalent bonds of amino groups in the protein and plasticizer in the film network (28). TGA thermograms in the range between 300 and 400°C indicate that the film with HE had a higher thermal stability. These results may be attributed to the interactions between polymers affected by the phenolic compounds in HE (29).

Fig. 3. DSC curves of the red ginseng residue protein film containing hibiscus extract (HE).

Table 3. DSC of red ginseng residue protein films incorporated with hibiscus extract (HE)

Sample	T _{m1} (°C)	T _{m2} (°C)	ΔH _f (J/g)
RGRP film	230.6	297.4	482.37
RGRP film with 1.0% HE	228.9	290.7	283.19

The results of DSC are shown in Fig. 3 and Table 3. The DSC curves show melting temperature and enthalpy change. In Fig. 3, there are two endothermic peaks of each film. The control film had T_m peaks of 231 and 297°C, whereas the film with HE had peaks of 229 and 291°C. It appears that there are two different proteins in the RGRP, which have different melting points. Adebowale *et al.* (30) reported that the two endothermic peaks in the DSC thermogram indicate the presence of two different proteins in the film. The fusion enthalpy values of the control film and the film with HE were 482 and 283 J/g, respectively. Barreto and Meyer (31) reported that the fusion enthalpy of proteins increases with chain stiffness and strength of both intermolecular and intramolecular interactions. Rattaya *et al.* (32) reported a similar study in which the fusion enthalpy of fish skin gelatin films decreased from 18.2 to 9.93 J/g by the addition of seaweed extract owing to lower hydrogen bonding than the control. However, it should be noted that there is a significant variation in the fusion enthalpy values of fish skin gelatin and RGRP in this study. The reason for the variation can be explained by different amino acid compositions. Fish skin gelatin contains amino acids such as proline, glycine, and hydroxyproline (33), whereas RGRP mainly has acidic amino acids (5). From the results obtained in this study, it is apparent that phenolic compounds affect the film network, resulting in the weakening of the film matrix. Consequently, less energy is needed for the degradation of the film with HE than the control film.

In conclusion, RGRP films incorporated with HE showed antioxidant activity as well as reduced WVP and water solubility in this study. The results suggest that RGRP films incorporated with HE can be prepared at low cost and potentially be applied in food packaging to

prevent oxidation and quality loss of food products.

Disclosure The authors declare no conflict of interest.

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