Effects of Extraction Methods on *In Vitro* Biological Capacities and Rheological Properties of Polysaccharides from Red Pepper Stems

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ABSTRACT: The purposes of this study were to produce polysaccharides from red pepper stems using different extraction methods and evaluate their chemical composition, *in vitro* biological capacities, and rheological properties. Two polysaccharides were extracted from red pepper stems using an autoclave and alkali treatments, and the extracts were named PAU and PAL, respectively. The contents of total phenolics and flavonoids were significantly higher in PAU than those in PAL. PAU exhibited greater scavenging activities on 2,2-diphenyl-1-picrylhydrazyl radicals, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radicals, superoxide radicals, and nitrite compared to PAL, suggesting that PAU served as better antioxidants. Similarly, *in vitro* inhibitory abilities against carbohydrate hydrolyzing enzymes of PAU were higher than those of PAL. Steady shear rheological analysis demonstrated that PAU showed higher psuedoplastic shear-thinning behavior compared to PAL. Based on the results from dynamic shear rheological properties, it was found that both samples had predominantly viscous behavior rather than elastic behavior.

Keywords: red pepper stem, polysaccharide, antioxidant ability, antidiabetic ability, rheological property

INTRODUCTION

Globally, agricultural areas produce agricultural biomass such as oil seeds, cereals, and other crops, resulting in the accumulation of agricultural waste every year. Most of these wastes are discarded and only a little amount of them are used as ingredients for animal feed or energy production. Agricultural crop residues, including plant stem and cereal straw, are known as rich sources of dietary fibers, which are composed of polysaccharides, oligosaccharides, lignin, and other plant related materials (1, 2). Therefore, it is important to study the structural, physicochemical, and functional characteristics of dietary fibers derived from agricultural crop residues in order to use them as functional ingredients in nutraceuticals or food products.

Polysaccharides, which are the main dietary fiber constituents, have been extensively investigated (3-5). It was revealed that polysaccharides obtained from plants had various therapeutic properties, such as antioxidant, antidiabetic, anticancer, and antimicrobial activities (6,7). Besides, polysaccharides can be used as fat replacers, gelling agents, emulsifiers, thickeners, and stabilizers in the food industry (8,9).

Among the agricultural crops, which are usually consumed in Korea, red peppers are one of the most commonly cultivated vegetable crops. Accordingly, a large number of agricultural crop residues, mainly stems, are produced, but these are left unused and discarded, resulting in considerable waste for producers (10). Supposing that the agricultural crop residues are an under-utilized source of dietary fibers, which have specific physicochemical properties and health benefits, studies on characterization of polysaccharides extracted from red pepper stems are important to evaluate their application in value-added food products.

Until now, many researchers have studied efficient extraction methods of polysaccharides from various fiber-rich sources and evaluated the structural, physicochemical, and biological characteristics of extracted polysaccharides (11,12). Commonly used extraction methods of polysaccharides are hot-water extraction, alkali or acid extraction, enzyme extraction, and microwave or ultrasonic assisted extraction. It is reported that extracted pol-

ysaccharides have different characteristics depending on the extraction methods (13). To the best of our knowledge, there are only a few studies regarding polysaccharides extracted from red pepper stems using different extraction methods (14). In the present study, two different polysaccharides were extracted from red pepper stems using an autoclave (hot water) and alkali extraction methods and their chemical composition, *in vitro* biological properties (antioxidant activities and α-glucosidase and α-amylase inhibitory activities), and rheological properties were investigated.

MATERIALS AND METHODS

Materials

For polysaccharide extraction by different methods, pepper stems were obtained from a local farm (Sangju, Korea) and rinsed off with tap water. The rinsed pepper stems were dried in a dry oven (DMC-122SP, Daeil Engineering Co., Gyeonggi, Korea) at 50°C, ground to a 80 mesh, and stored in a refrigerator at 4°C. D-glucose, D-galacturonic acid, Folin-Ciocalteu reagent, gallic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Griess reagent, α-glucosidase from *Saccharomyces cerevisiae*, *p*-nitrophenyl-α-D-glucopyranoside, α-amylase from porcine pancreas, and 3,5-dinitrosalicylic acid (DNS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents used in this study were of analytical grade.

Extraction of polysaccharides

Autoclave treatment: The powder of dried red pepper stems (10.0 g) was extracted with distilled water (10%, w/v) by autoclaving for 2 h at 121°C. The extract was filtered using filter paper (8 μm pore size), and the filtrate was concentrated in a rotary evaporator (N-1000S, EYELA, Tokyo, Japan) under reduced pressure at 40°C. The polysaccharide was precipitated by the addition of 80% ethanol (v/v) and incubated overnight at 4°C. The precipitated polysaccharide was then collected by filtration and dried in a dry oven at 35°C for later use. This polysaccharide was designated PAU.

Alkali treatment: The powder of dried red pepper stems (10.0 g) was extracted with 100 mL of potassium hydroxide solution (10%, w/v) by autoclaving for 2 h at 121°C. The extract was filtered using filter paper (8 μm pore size), and the filtrate was neutralized by 1 N acetic acid to pH 7.0. The polysaccharide was precipitated by the addition of 80% ethanol (v/v) and incubated overnight at 4°C. The precipitated polysaccharide was then collected by filtration and dried in a dry oven at 35°C for later use. This polysaccharide was designated PAL.

Chemical composition analysis

Moisture, crude ash, crude lipid, and crude protein contents in PAU and PAL were analyzed by standard AOAC methods (15). Carbohydrate content was determined by the phenol-sulphuric acid method, and D-glucose was used as a standard (16). Uronic acid content was colorimetrically measured according to the *m*-hydroxydiphenyl-sulphuric acid method, with D-galacturonic acid as a standard (17).

Determination of total phenolic contents

Total phenolic contents of PAU and PAL were determined according to the Folin-Ciocalteu colorimetric method (18) with slight modifications. The sample solution (1 mL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was mixed with 1 mL of Folin-Ciocalteu reagent (1 N). The mixture was vortexed and allowed to stand at 25°C for 5 min. Next, the mixture was mixed with 1 mL of sodium bicarbonate (10%) and then incubated at 30°C for 1 h. Finally, the absorbance of the reaction mixture was measured at 700 nm, using a UV-visible spectrophotometer. Gallic acid was used as a standard, and the total phenolic contents in both samples were expressed as mg gallic acid equivalents (GAE) per 100 g of dry mass.

Determination of total flavonoid contents

Total flavonoid contents of PAU and PAL were determined according to the aluminium chloride colorimetric method (18) with slight modifications, using catechin as a standard. The sample solution (1 mL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was mixed with 200 μL of sodium nitrite solution (5%), followed by vigorous vortexing, and allowed to stand at room temperature for 5 min. Next, the mixture was mixed with 300 μL of aluminium chloride solution (10%) and allowed to stand at room temperature for 5 min. Finally, 1.0 mL of sodium hydroxide solution (1 N) was added into the mixture, and the absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. The total flavonoid contents in both samples were expressed as mg catechin equivalents (CE) per 100 g of dry mass.

Analysis of *in vitro* antioxidant properties

DPPH radical scavenging activity: The DPPH radical scavenging activities of PAU and PAL were measured according to a modified version of the method by Shimada et al. (19). The sample solution (200 μL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was added to 1.2 mL of DPPH solution in ethanol (0.1 mM). The mixture was vortexed and then incubated in the dark for 30 min at room temperature. Finally, the absorbance of the reaction mixture was monitored spec-

trophotometrically at 517 nm. L-ascorbic acid was used as a reference antioxidant. The antioxidant capacity was expressed as percentage inhibition of DPPH radical relative to the control and was determined, based on the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without the tested sample) and A_{sample} is the absorbance in the presence of the tested sample. The sample concentration providing 50% of DPPH radical scavenging activity, IC_{50} value, was obtained by interpolation of linear regression analysis.

ABTS radical scavenging activity: The ABTS radical scavenging activities of PAU and PAL were performed according to the method described by Re et al. (20) with slight modifications. The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 2.5 mL of ABTS stock solution (7 mM) with 0.5 mL of potassium persulfate (15 mM), and then, the mixture was left in the dark at room temperature for $12 \sim 16$ h before use. At the moment of use, the $ABTS^{+\cdot}$ solution was freshly diluted with ethanol to get the absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30°C. Each sample solution (0.2 mL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was added to a 0.8 mL of $ABTS^{+\cdot}$ solution. The decrease in absorbance was measured at 734 nm after the reaction at 25°C for 15 min. L-ascorbic acid was used as a standard antioxidant. The $ABTS^{+\cdot}$ scavenging effect was expressed as shown in the following formula:

$$ABTS^{+\cdot} \text{ scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control ($ABTS^{+\cdot}$ solution without the tested sample) and A_{sample} is the absorbance in the presence of the tested sample. The IC_{50} value was obtained by interpolation of linear regression analysis.

Superoxide radical scavenging activity: The superoxide radical scavenging activities of PAU and PAL were assessed according to the method of Marklund and Marklund (21) with slight modifications. Each sample solution (200 μL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was mixed with 3 mL of Tris-HCl buffer (50 mM, pH 8.5) and 0.2 mL of pyrogallol (7.2 mM). The reaction was kept for 10 min at room temperature and 0.1 mL of HCl (1 N) was added to the above mixture to terminate the reaction. The absorbance was taken at 420 nm, and L-ascorbic acid was used as a positive control. The scavenging ability of superoxide radical was ex-pressed according to:

$$\text{Superoxide radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control (a mixture of distilled water and 1 mM of $NaNO_2$) and A_{sample} is the absorbance in the presence of the tested sample. The IC_{50} value was obtained by interpolation of linear regression analysis.

Nitrite scavenging activity: The nitrite scavenging activities of polysaccharides extracted by PAU and PAL were performed according to a method using Griess reagent (22), with slight modifications. Each sample solution (1 mL) in distilled water at concentrations of 0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL was mixed with 1 mL of $NaNO_2$ (1 mM) and then 0.1 N HCl was added dropwise to the mixture until the pH was 1.2. The pH-adjusted mixture was diluted with distilled water to make the final volume up to 10 mL and incubated at 37°C for 1 h. After incubation, 5 mL of 2% acetic acid and 0.4 mL of Griess reagent were added to the reaction mixture. The reaction mixture was incubated at room temperature for 15 min and then the absorbance was measured at 520 nm. L-ascorbic acid was used as a standard antioxidant. The result was calculated by the following equation:

$$\text{Nitrite scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control (a mixture of distilled water and 1 mM of $NaNO_2$) and A_{sample} is the absorbance in the presence of the tested sample. The IC_{50} value was obtained by interpolation of linear regression analysis.

Analysis of *in vitro* α-glucosidase and α-amylase inhibitory activities

α-Glucosidase inhibitory activity: The α-glucosidase inhibitory activities of PAU and PAL were measured according to a modified version of the method by Ranilla et al. (23). Each sample solution (500 μL) in distilled water at concentrations of 0, 100, 200, 300, 400, and 500 μg/mL was mixed with 1.0 mL of phosphate buffer (0.1 M, pH 6.9) containing α-glucosidase solution (1.0 U/mL). After incubation at 25°C for 10 min, 500 μL of *p*-nitrophenyl-α-D-glucopyranoside solution (5 mM) in phosphate buffer (0.1 M, pH 6.9) was added to the mixture at timed intervals, and then the mixture was incubated at 25°C for 5 min. Before and after the incubation period, the absorbance of the reaction mixture was measured spectrophotometrically at 405 nm and compared to a blank, which contained 500 μL of buffer solution in place of sample

solution. The percentage of α-glucosidase inhibition was calculated by:

$$\% \text{ Inhibition} = \frac{\Delta Ao_{405} - \Delta Ae_{405}}{\Delta Ao_{405}} \times 100$$

where ΔAo_{405} is the absorbance of control without sample solution, and ΔAe_{405} is the absorbance in the presence of sample solution.

α-Amylase inhibitory activity: The α-amylase inhibitory activities of PAU and PAL were measured according to the method described by Ranilla et al. (23) with slight modifications. Each sample solution (500 μL) in distilled water at concentrations of 0, 100, 200, 300, 400, and 500 μg/mL was mixed with 500 μL of sodium phosphate buffer (0.02 M, pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/mL), followed by incubation at 25°C for 10 min. After the pre-incubation, 500 μL of starch solution (1%) in sodium phosphate buffer (0.02 M, pH 6.9 with 0.006 M sodium chloride) was added to the mixture. After re-incubation at 25°C for 10 min, the reaction was stopped with 1.0 mL of DNS color reagent. Next, the mixture was incubated in boiling water bath for 5 min and cooled to room temperature. After diluting the mixture with 10 mL of distilled water, the absorbance of the reaction mixture was measured spectrophotometrically at 540 nm. The percentage of α-amylase inhibition was calculated by:

$$\% \text{ Inhibition} = \frac{\Delta Ao_{540} - \Delta Ae_{540}}{\Delta Ao_{540}} \times 100$$

where ΔAo_{540} is the absorbance of control without sample solution, and ΔAe_{540} is the absorbance in the presence of sample solution.

Rheological measurements

Preparation of polysaccharide dispersions: Polysaccharide dispersions (20% w/v) were prepared by mixing PAU and PAL with distilled water. The dispersions were stirred for 1 h at room temperature and then heated at 95°C in a water bath for 30 min with mild agitation in order to avoid the entry of air into the dispersions. The hot sample dispersions were immediately transferred to a rheometer plate to measure the rheological properties.

Steady shear rheological measurement: The steady and dynamic shear rheological properties of the polysaccharide dispersions were obtained using a strain controlled rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) with a parallel plate system (5 cm diameter) at a gap of 0.5 mm. Each sample dispersion was transferred to the rheometer plate at 30°C.

Steady shear data were obtained over the shear rate in the range of $0.1 \sim 1000 \text{ s}^{-1}$. To describe the steady shear

rheological properties of the samples, the data were fitted to the well-known power law [Eq. (1)] and Casson [Eq. (2)] models, which are as follows:

$$\sigma = K\dot{\gamma}^n \tag{1}$$

$$\sigma^{0.5} = K_{oc} + K_c\dot{\gamma}^{0.5} \tag{2}$$

where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index (Pa·s^n), n is the flow behavior index (dimensionless), and $(K_c)^2$ is the Casson plastic viscosity (η_c). Casson yield stress (σ_{oc}) according to the Casson model [Eq. (2)] was determined as the square of the intercept (K_{oc}) that was obtained from the linear regression of the square roots of the $\dot{\gamma}$ versus σ data. Using the magnitudes of K and n, apparent viscosity ($\eta_{a,100}$) at 100 s^{-1} was calculated. All steady shear rheological measurements were performed in triplicate.

Dynamic shear rheological measurement: Dynamic shear rheological properties [storage modulus (G′), loss modulus (G″), complex viscosity (η^*), and tan δ (G″/G′)] of polysaccharide dispersions were determined on a strain controlled rheometer. Dynamic rheological data were obtained from frequency sweeps over the range of $0.63 \sim 63$ rad/s at 2% strain. The 2% strain was in the linear viscoelastic region. Frequency sweep tests were also conducted at 30°C. Rheometer Data Analysis software (Rheoplus version 3.62) was used to obtain the experimental data and to calculate the G′, G″, η^*, tan δ. All dynamic shear rheological measurements were performed in triplicate.

Statistical analysis
All statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was done using the general linear models procedure in order to determine significant differences amongst the samples. Means were compared using Fisher's least significant difference procedure. All experiments were replicated at least three times for each treatment and their means±standard deviation were reported. Values of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Chemical composition analysis
Moisture, crude ash, crude lipid, crude protein, carbohydrates, uronic acids, total phenolics, and total flavonoids contents of PAU and PAL are summarized in Table 1. The predominant component of both PAU and PAL was carbohydrates (93.90% and 92.77%, respectively). Additionally, in both samples, the crude ash and lipid were

not detected and only a trace amount of proteins existed. These results indicated a high purity of the extracted polysaccharides.

PAU had significantly higher contents of total phenolics and flavonoids compared with PAL (Table 1). According to Hromádková et al. (24) and Mandalari et al. (25),

Table 1. Chemical composition of PAU and PAL

Compounds	PAU	PAL
Moisture (%)	5.35 ± 0.75^b	7.38 ± 0.89^a
Crude ash (%)	ND[1]	ND
Crude lipid (%)	ND	ND
Crude protein (%)	0.91 ± 0.21^a	0.06 ± 0.023^b
Carbohydrates (% D-glucose equivalent)	93.90 ± 0.49^a	92.77 ± 0.28^b
Uronic acids (% GalA equivalent)	6.55 ± 0.22^b	7.65 ± 0.91^a
Total phenolics (mg GAE/100 g)	315.33 ± 3.19^a	231.11 ± 3.81^b
Total flavonoids (mg CE/100 g)	243.71 ± 7.24^a	155.83 ± 9.12^b

Each value represents the mean of triplicate experiments± standard deviation.
Mean values in the same row with different letters (a,b) are significantly different ($P<0.05$).
PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.
GAE, gallic acid equivalents; CE, catechin equivalents.
[1]Not detected.

the alkali treatment attacked the cell wall matrix and hydrolyzed some alkali-labile bonds between the polysaccharides and phenolics, therefore lowering the content of phenolics. Furthermore, it was suggested in the present study that the contents of phenolics and flavonoids, which were covalently or physically bound to PAU and PAL, could contribute to the pharmacological effects including antioxidant activity and carbohydrate hydrolyzing enzyme inhibitory activity, as shown in Fig. 1 and Fig. 2.

Analysis of *in vitro* antioxidant properties

In vitro chemical methods including DPPH, ABTS, and superoxide radicals- and nitrite-scavenging activities were used to evaluate the antioxidant properties of PAU and PAL. As shown in Fig. 1, the antioxidant activities in 5 different concentrations (2.0~10.0 mg/mL) of both PAU and PAL were analyzed. A dose-response relationship was observed in all assays, and the antioxidant abilities were raised as the concentration increased for each sample. Additionally, the IC_{50} values of PAU and PAL obtained from each assay are presented in Table 2.

In the DPPH radical scavenging assay, the radical scavenging ability ranged from 7.7% to 49.1% for PAU and 6.5% to 38.2% for PAL. The IC_{50} value (9.88 mg/mL) of PAU was significantly lower than that of PAL (13.36 mg/
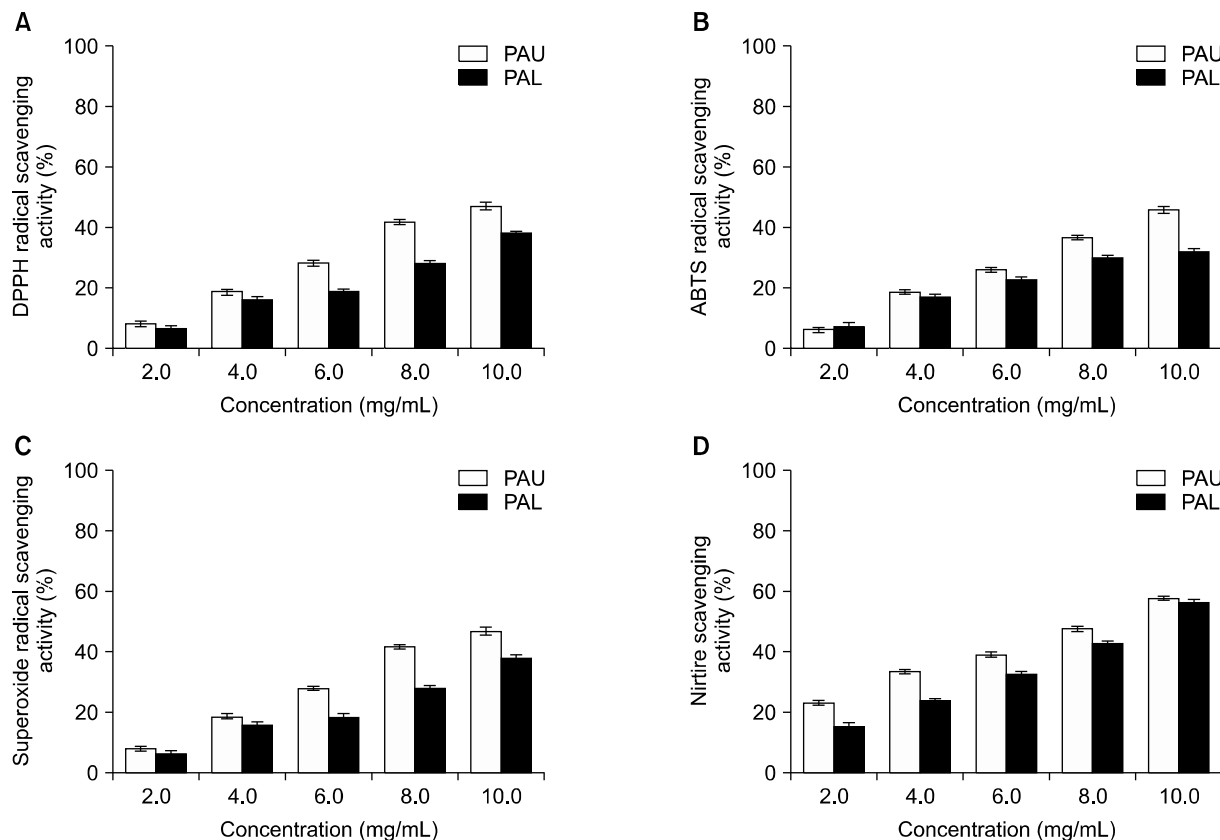

Fig. 1. The scavenging activities of PAU and PAL determined by DPPH radical scavenging assay (A), ABTS radical scavenging assay (B), superoxide radical scavenging assay (C), and nitrite scavenging assay (D). Error bars represent standard deviation of three independent experiments. PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.

Table 2. DPPH radical scavenging activity, ABTS radical scavenging activity, superoxide radical scavenging activity, and nitrite scavenging activity with IC_{50} values of PAU and PAL
(unit: mg/mL)

Samples	Scavenging activities (IC_{50})			
	DPPH	ABTS	Superoxide	Nitrite
PAU	9.88 ± 0.03^b	9.77 ± 0.02^b	10.24 ± 0.09^b	8.66 ± 0.18^b
PAL	13.36 ± 0.00^a	13.32 ± 0.02^a	13.54 ± 0.02^a	9.07 ± 0.03^a

Each value represents the mean of triplicate experiments± standard deviation.
Mean values in the same column with different letters (a,b) are significantly different ($P<0.05$).
DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.
PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.

mL), indicating the stronger scavenging activity of PAU compared to PAL.

In the ABTS radical scavenging assay, PAU had 7.5~50.3% scavenging activity whereas PAL presented 6.5~38.1% scavenging activity. The IC_{50} value (9.77 mg/mL) of PAU was significantly lower than that of PAL (13.32 mg/mL). With regard to the superoxide radical scavenging assay, the scavenging activities were 46.8% and 38.3% at 10.0 mg/mL with IC_{50} values of 10.24 and 13.54 mg/mL for PAU and PAL, respectively, showing a more potent radical scavenging activity of PAU compared to PAL.

Similarly, the nitrite scavenging activity of PAU was greater than that of PAL, as shown by the IC_{50} values (8.66 mg/mL for PAU and 9.07 mg/mL for PAL). From these results, it was confirmed that PAU was a more effective antioxidant than PAL. According to previous studies (26), there is a good correlation between total polyphenol contents and *in vitro* antioxidant activity. Therefore, the greater antioxidant property of PAU could be explained by its higher total phenolic and flavonoids contents compared to PAL, as shown in Table 1.

Analysis of *in vitro* α-glucosidase and α-amylase inhibitory activities

Fig. 2 shows the dose-response curves of the inhibitory effects of PAU and PAL against α-glucosidase and α-amylase. The inhibitory activities of PAU and PAL against α-glucosidase and α-amylase were increased with an increase in the concentrations of both samples from 100 μg/mL to 500 μg/mL. PAU and PAL at the highest concentration of 500 μg/mL presented the highest α-glucosidase and α-amylase inhibitory effects with the inhibitory rate of 65.6% and 68.9% for PAU and 61.8% and 66.8% for PAL, respectively. In addition, the results obtained from each assay revealed that the inhibitory effects of PAU against α-glucosidase and α-amylase were superior at all concentrations (100~500 μg/mL) compared with those of PAL. According to Kim et al. (27), polyphenolic compounds can restrain the α-glucosidase and α-amylase activities because of their binding ability with proteins such as enzyme proteins. Therefore, the results of this study may be explained by the higher contents of total phenolic and flavonoid compounds of PAU, as indicated in Table 1. In conclusion, these outcomes indicated that PAU could potentially control the rise in post-prandial glucose by starch.

Steady and dynamic shear rheological properties

The σ versus $\dot{\gamma}$ data for PAU and PAL at 30°C were well fitted to two rheological models (power law and Casson models) with high correlation coefficient (0.99, data are not shown), as shown in Fig. 3 and Table 3. Both PAU and PAL presented a shear-thinning behavior because of the *n* values were less than 1. The pseudoplastic properties of both samples could be explained by conformational changes of polysaccharide molecules as a result of shearing. Specifically, the junction zones in the tested polysaccharides were broken in response to shearing, consequently leading to molecule disaggregation (7,28). In addition, in the present study, PAU had a lower *n* val-
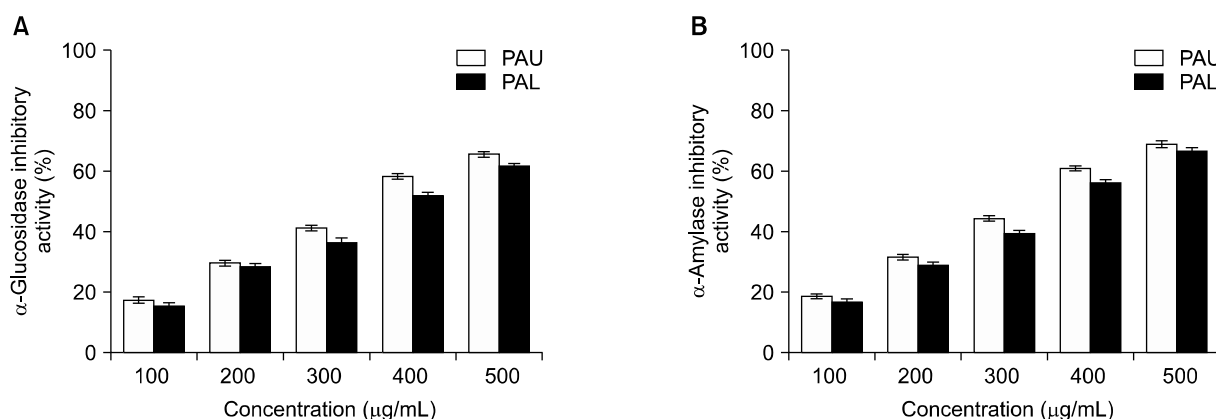

Fig. 2. α-Glucosidase inhibitory activity (A) and α-amylase inhibitory activity (B) of PAU and PAL. Error bars represent standard deviation of three independent experiments. PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.
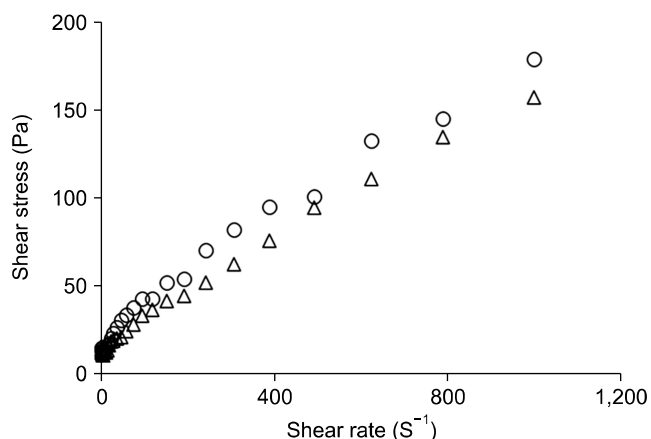
Fig. 3. Shear rate–shear stress plot for PAU (○) and PAL (△). PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.

Fig. 4. Comparison of storage modulus (G′), loss modulus (G″), complex viscosity (η*) versus function of frequency (ω) for PAU and PAL. PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.

ue (0.46) than PAL (0.49), indicating the higher pseudoplastic (non-Newtonian) behavior for PAU.

The steady shear rheological data of both samples showed $\eta_{a,100}$, K, and σ_{oc} of PAU were significantly higher than those of PAL (Table 3). According to Tan and Gan (7), a more highly branched conformation of polysaccharides could interrupt entanglement of polysaccharide chains compared to a less branched polysaccharide. Therefore, it was speculated that PAU had a more complicated structure than PAL.

Fig. 4 provides changes in G′, G″, and η* as a function of frequency (ω) for PAU and PAL at 30°C. The magnitudes of G′ and G″ for both samples were increased with an increase in ω with a high frequency dependency. However, η* values for both samples declined with an increase in ω, presenting shear-thinning properties in accordance with the power law model. G″ values were significantly lower than G′ values at all ω values (0.63∼ 62.8 rad/s) in PAU and PAL, indicating that both samples had predominantly viscous behavior rather than the elastic behavior. In addition, G′ and G″ values of PAU were much greater than those of PAL at all values of ω (0.63∼62.8 rad/s), demonstrating the higher viscoelastic property of PAU. The greater viscoelastic properties of PAU can be associated with its tighter network struc-
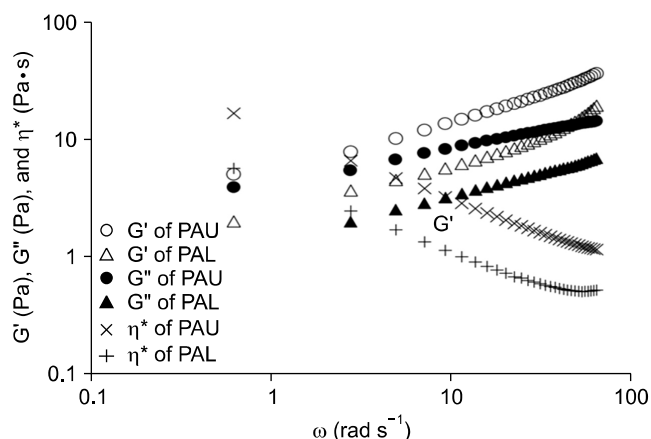
ture and greater stiffness compared to PAL. Such structure implies a more limited mobility, and subsequently the longer relaxation times, which result in higher elastic properties (29,30).

In conclusion, two polysaccharides (PAU and PAL) were extracted from red pepper stems by autoclave and alkali treatments, respectively. The *in vitro* antioxidant activities and inhibitory effects against carbohydrate hydrolyzing enzymes of PAU were significantly higher than those of PAL. According to the results obtained from steady and dynamic shear rheological analysis, when compared to PAL, PAU showed relatively stronger shear-thinning behaviors and viscoelastic properties. Based on the results, it was demonstrated in this study that alkali treatment was an effective method to obtain functional polysaccharides from red pepper stems. Moreover, it was suggested that PAU could be used as a functional ingredient or thickening agent in food industries.

ACKNOWLEDGEMENTS

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

Table 3. Steady shear rheological properties of PAU and PAL

Samples	$\eta_{a,100}$ (Pa·s)	K (Pa·s^n)	n (−)	σ_{oc} (Pa)
PAU	0.45±0.00^a	3.79±0.13^a	0.46±0.02^b	9.37±0.13^a
PAL	0.37±0.01^b	3.10±0.05^b	0.49±0.01^a	7.50±0.14^b

Each value represents the mean of triplicate experiments± standard deviation.
Mean values in the same column with different letters (a,b) significantly different (*P*<0.05).
$\eta_{a,100}$, apparent viscosity; K, consistency index; *n*, flow behavior index; σ_{oc}, Casson yield stress.
PAU, polysaccharides extracted by autoclave treatment; PAL, polysaccharides extracted by alkali treatment.

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