

## Characterization of Polysaccharides Obtained from Purslane (*Portulaca oleracea* L.) Using Different Solvents and Enzymes

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**Abstract** Physicochemical properties, such as yield and molecular weight distribution of polysaccharide fractions, of polysaccharides in the enzymatic hydrolysates of purslane were investigated and characterized. A higher amount of micro nutrients, such as potassium (9,413 mg/100 g), phosphorus acid (539 mg/100 g), leucine, alanine, lysine, valine, glycine, and isoleucine, was present in whole purslane. The yield of water soluble polysaccharides (WSP) was 0.29, 7.01, and 7.94% when extracted using room temperature water (RTW), hot-water (HW), and hot temperature/high pressure-water (HTPW), respectively, indicating that HW or HTPW extraction may be effective to obtain WSP from purslane. The average ratio of L-arabinose:D-galactose in the WSP was 37:49, 34:37, and 27:29, when extracted using RTW, HW, and HTPW, respectively. These results indicate that water was a suitable extraction solvent for preparation of the arabinogalactan component of whole purslane. A higher yield and total carbohydrate content was obtained by using Viscozyme L instead of Pectinex 5XL during extraction of the WSP, which indicates that enzymatic treatment of purslane may be an effective method to control the Mw of polysaccharides. Finally, it was confirmed that Viscozyme L is a suitable enzyme for the hydrolysis and separation of polysaccharides obtained from purslane.

**Key words:** purslane (*portulaca oleracea* L.), physicochemical property, polysaccharide, isolation, arabinogalactan

### Introduction

Purslane (*Portulaca oleracea* L.) is a summer annual vegetable that is grown worldwide. This plant is widely used as a vegetable because of its mild flavor, palatability, and mucilaginous quality. The entire plant is edible raw, cooked, or in the pickled state, and can be used to make a salad, either alone or in combination with other vegetables (1).

Purslane has a long history of use for human food, animal feed, and medicinal purposes. Purslane has been used as a folk or traditional medicine for thousands of years in many countries throughout the world, and is known to have been used by the ancient Greeks, Persians, and Indians. In Korea, this plant is utilized as a traditional remedy to prevent vomiting, bleeding, hepatitis, and gastric mucosal diseases (2, 3). Additionally, it is used in Middle Eastern countries for treatment of small tumors and inflammation, urinary disorders, liver obstructions, and ulcers of the mouth and stomach (4, 5).

The aqueous extracts of purslane contain dopa, dopamine, catecholamines, potassium, organic acids, amino acids, monoterpene glucoside, and portuloside (6-8). It has been reported that the aqueous extracts of purslane possess muscle-relaxant properties and can decrease muscle spasticities (9, 10). Purslane has a high percentage of  $\alpha$ -linoleic acid and is a richer source of fatty acid than any other green leafy vegetable (11-13).

Whole plant extracts of purslane in ethanol are known to be inhibitory to *Bacillus subtilis* and those extracted in chloroform, ethanol, and hexane are known to be inhibitory to *Rhizobium leguminosarum* (14). Testing of hepatoprotective, diuretic, and anti-inflammatory activities of water extracts of purslane showed that they were 59.4% in s-GPT and 55.8% in s-GOT when compared with the use of sylimarin against CCl<sub>4</sub> intoxication and 43.7% diuretic activity when compared with furosemide in mice (15). When the antimicrobial activities of methanol extracts of purslane were tested against food spoilage or foodborne disease microorganisms, high antimicrobial activities against *Pseudomonas citreonigrum*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were observed (16).

The mucilage of purslane can be divided into acidic and neutral fractions. The acidic fraction consists of galacturonic acid residues joined by  $\alpha$ -(1→4)-linkages; 60% of which are present as calcium salt, whereas no esterified galacturonic acid residues are present. The neutral fraction is composed of 41% arabinose and 43% galactose residues, as well as traces of rhamnose residues (17). Polysaccharide complexes extracted from purslane have been fractionated into neutral arabinogalactan and polydispersed pectin-like polysaccharides using anion exchange chromatography (18). Additionally, the interaction of gum extracted from purslane using casein has been evaluated to determine the competitive adsorption, emulsion stability, and emulsification properties in O/W emulsions to determine if it can be used as a hydrocolloid or nonionic emulsifier in the food industry (19-21). Moreover, arabinogalactan (AG) is known to be an excellent source of

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dietary fiber that is able to increase short-chain fatty acid production (primary butyrate) via vigorous fermentation by intestinal microflora (22). Additionally, AG may also be effective when combined with cancer therapies due to its ability to stimulate NK cell cytotoxicity, to stimulate the immune system, and to block metastasis of tumor cells into the liver (23). Furthermore, its unique physical characteristics allow arabinogalactan obtained from purslane to be used in various food, beverage, and nutraceutical applications. Therefore, it is worthwhile to conduct additional research to facilitate recovery of arabinogalactan from purslane through methods such as extraction, enzymatic hydrolysis, and separation.

In this study, the physicochemical properties of domestic purslane were evaluated. The specific objectives of this study were (1) to investigate the effects of different solvents on the extraction characteristics of polysaccharides obtained from purslane and (2) to study the effects of enzymatic hydrolysis on the yield and molecular weight of polysaccharide fractions of purslane.

## Materials and Methods

**Materials** Purslane (*Portulaca oleracea* L.), collected at Pangyo, Gyeonggi, Korea (July, 2005), was freeze dried, ground in a hammer mill, and then frozen. Commercial pectinases (Pectinex 5XL and Viscozyme L) were purchased from Novo Co. (Krogshoejvej, Bagsvaerd,

Denmark).

**Proximate analysis** The moisture, crude protein, crude fat, and ash content of purslane were determined using the AOAC procedure (24). The Kjeldahl method was used to analyze the crude protein, and the Soxhlet method was used to analyze the crude fat. The ash content was determined by ashing in an electric furnace at 550°C.

**Micro nutrients** Micro nutrients were determined using a portion of the digested purslane sample by dry ashing and measuring the inductively coupled plasma atomic emission spectrometry (model JY 38 Plus; Horiba Jobin-Yvon, rue du Canal, Longjumeau, France).

**Fatty acid composition** Fatty acid composition was analyzed using a gas chromatograph (Varian 3800; Arian Inc., Walnut Creek, CA, USA) with a Supelcowax 10 fused-silica capillary column (i.d. 0.25 mm × 30 m, Supelco, Bellefonte, PA, USA).

**Amino acids** Amino acids were analyzed following the hydrolysis of dried samples using a HPLC (PU-980; Jasco, Tokyo, Japan) equipped with an 851-AS auto sampler, a 150 mm internal diameter Waters pico-tag column and a UV-975 UV/VIS detector (Waters Co., Milford, MA, USA)

**Isolation of polysaccharides** Figure 1 shows the production

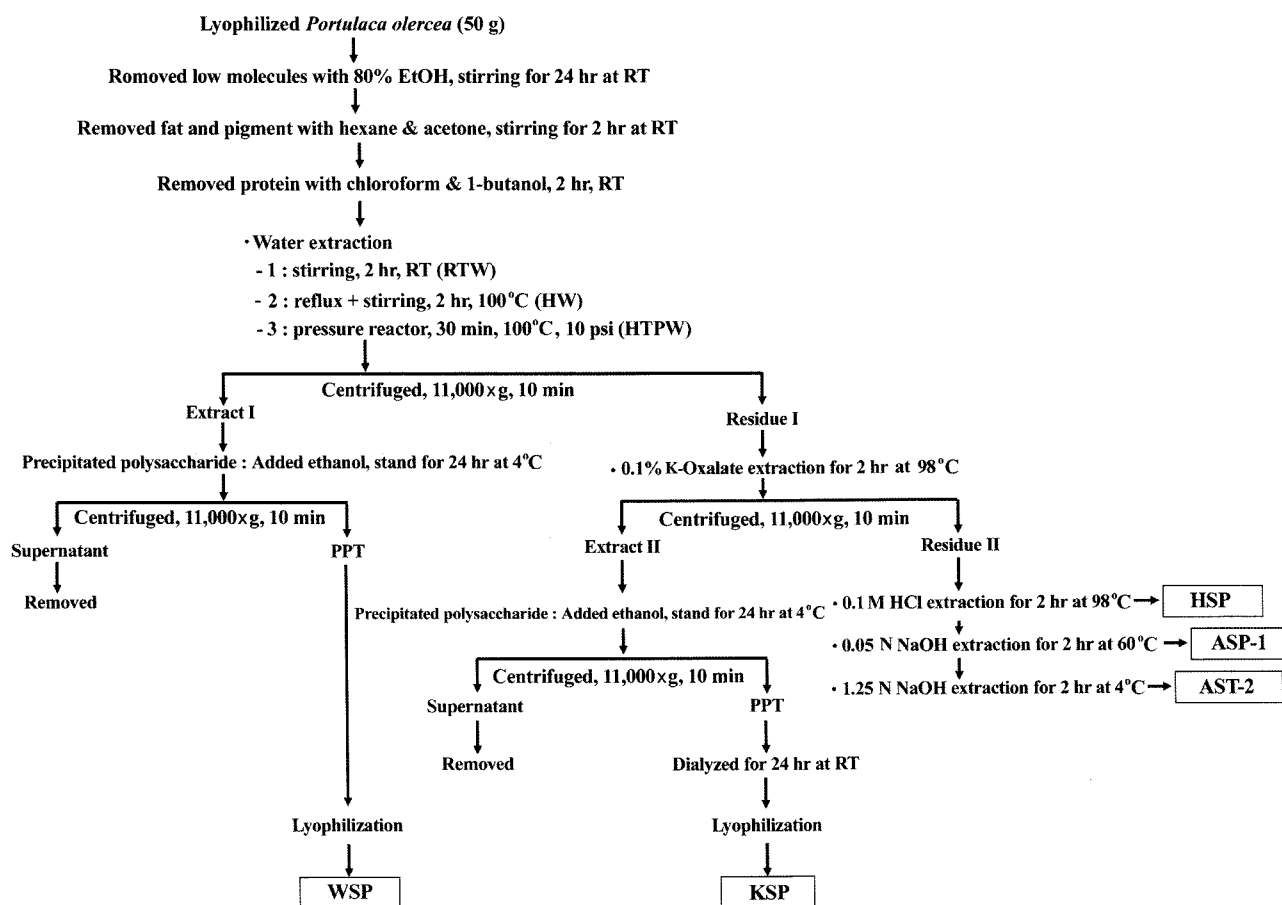


Fig. 1. Fractionation of water, alkaline, and acidic soluble polysaccharides from purslane.

processes used to prepare water soluble (WSP), potassium oxalate soluble (KSP), acid soluble (HSP), and alkali soluble (ASP) polysaccharides. The monosaccharides were extracted by placing the ground purslane in 4 volumes of 80% ethanol and stirring for 24 hr at room temperature. Next, to remove the fat and pigments, the residue pulp was extracted using *n*-hexane and acetone for 2 hr at room temperature. The residue was then dispersed in a mixture of chloroform and butanol (2:1) to precipitate the protein, and the dry residual matter was then used as the raw material. Water, acid, and alkali extractable polysaccharides were obtained after fractionation following different extraction methods, such as water, refluxing, and pressure reactor extraction. The WSP were obtained by water extraction followed by ethanol precipitation and lyophilization. The KSP were obtained by conducting potassium oxalate extraction, ethanol precipitation, and dialysis on the residue from the water extraction (Residue I). The HSP were obtained by conducting potassium oxalate and hydrochloric acid extraction on Residue I. ASPs were obtained by conducting potassium oxalate and sodium hydroxide extraction on the residue from the water extraction, following the method described for extraction of the HSP.

**Enzyme treatments** After the low weight molecules were removed from the purslane, it was dispersed into 500 mL of distilled water (1:10). Next, a mixture of Pectinex 5XL (0.25 mL) and Viscozyme (0.25 mL) was added to each reaction. Enzymatic reactions were performed at a pH of 5.0 and 50°C for 2 hr.

**Polysaccharides analysis** The total carbohydrates present in the samples were determined using the phenol-sulfuric method (25), and the composition of the sugars was determined using the Uppsala method (26). Polysaccharides (250-500 mg) were hydrolyzed with 12 N H<sub>2</sub>SO<sub>4</sub> (3 mL) at 30°C for 1 hr. After dissolution occurred, 74 mL of distilled water and 10 mL of myo-inositol were added, and the solution was then autoclaved for 1 hr. After being autoclaved, the solution was allowed to cool and then filtered using a glass funnel. The neutral sugars were quantified using a GC (Agilent 6890 Series; Hewlett Packard Chromatograph, Wilmington, DE, USA) equipped with a SP-2330 carbohydrate column (i.d. 0.25 mm × 30 m, film thickness 0.2 µm, Supelco). Methylated alditol acetates were identified by their fragment ions.

**Mw determination** To investigate the Mw distribution and fractionation of the WSP and HSP, 20 mg of each fractionated sample was dissolved in 2 mL of 0.3% sodium hydroxide solution and the sample solution was then eluted into a gel permeation chromatograph (Shodex Series, Showa Denko K.K., Tokyo, Japan) equipped with a Shodex SB-806 HQ column (i.d. 8 mm × 300 m, Showa Denko K.K.) at a flow rate of 1.0 mL/min and a temperature of 30°C. The void volume of the column was determined by application of blue dextran (2 mg/mL eluent buffer, Mw 2×10<sup>6</sup>). Solutions (100 mg/mL) containing dextrans of known Mw (78.8×10<sup>4</sup>, 40.4×10<sup>4</sup>, 21.2×10<sup>4</sup>, 11.2×10<sup>4</sup>, 4.73×10<sup>4</sup>, 2.28×10<sup>4</sup>, and 1.18×10<sup>4</sup>) were used for calibration.

**Statistical analysis** All results expressed represent the mean ± standard deviation (SD). The significant differences between group means were assessed using the Student's *t*-test (27). A *p* value of 0.05 was considered statistically significant.

## Results and Discussion

**Proximate analysis of purslane** The results of the proximate analysis of purslane are shown in Table 1. The protein and fat contents of purslane were 11 and 2.87%, respectively. This may be due to cultivar or environmental differences, as well as developmental factors or the plant growth stage at the time of harvest. Mohamed and Hussein (28) reported that the nutrient component of purslane was different at different growth stages, however, in their study, the nutrient values were evaluated using a different method. In their study, the leaves were found to have the highest protein content at all stages of growth, however, the protein content of the roots declined significantly. Omara-Alwala *et al.* (11) found that the fat content of the entire plant was significantly lower than that of its leaves. The fatty acid content of whole purslane found in this study is presented in Table 2. The most abundant of the ω-3 polyunsaturated fatty acids (PUFA) was 18:3 ω3. This acid is known to be the precursor of ω-3, longer chain PUFA, therefore the use of purslane can be considered as an alternative to marine sources of ω-3 PUFA. Omara-Alwala *et al.* (11) also reported that eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids are present in purslane. However, the longer-chain omega-3 fatty acids, such as EPA and DHA, were not detected in this study, which is an agreement with the findings of Liu *et al.* (13) and Guil *et al.* (29).

**Micro-nutrients** The micro-nutrients found in whole purslane are shown in Table 3. A greater amount of micro-

**Table 1. Proximate analysis of purslane (% d.b.)**

Composition	Content <sup>1)</sup>
Crude protein	11.0±0.23
Crude fat	2.8±0.13
Total carbohydrate	69.5±0.37
Ash	16.7±0.73

<sup>1)</sup>Values are the mean of values in triplicate.

**Table 2. Fatty acid composition of purslane (mg/g, d.b.)**

Fatty acid	Content <sup>1)</sup>
C16:1	ND
C18:2 ω6	10.3±0.34
C18:3 ω3	18.4±0.47
C20:4	ND
C20:5 ω3	ND
C22:5 ω6	ND

<sup>1)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

**Table 3. Micro-nutrients composition of purslane**

Composition	Content (mg/100 g) <sup>1)</sup>	Tolerance limit (mg/kg)
Ca	463.84±2.52	
Fe	12.20±0.18	
Pb	0.085±0.01	< 0.3
Cd	-	< 0.1
Mn	13.01±0.24	
P	538.92±14.72	
Zn	2.84±0.24	
Cu	0.96±0.01	
K	9,412.96±358.87	
Se	-	
Ge	-	

<sup>1)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

nutrients, such as potassium (9,413 mg/100 g), phosphorus (539 mg/100 g), and calcium (464 mg/100 g), were present in whole purslane as compared to those obtained by Kesden and Will (30). The potassium and phosphorus contents of the whole purslane found in this study were significantly higher than those found by Mohamed and Hussein (28), whereas the calcium level was similar to those of Mohamed and Hussein (28). The iron, manganese, and copper content found in this study also much higher than those found by Mohamed and Hussein (28). However, in other reports, the reported calcium content of whole purslane much lower, being 79 and 103 mg/100 g dry weight (30, 31). It has been reported that the highest potassium level occurs during the first growth stage, and that this level decreases as the plants mature. Therefore, the nutritional component of purslane may be affected by cultivar or environmental factors. Horan *et al.* (32) showed that a high potassium diet showed to be correlated with lower blood pressure, therefore, purslane may reduce high blood pressure due to its high potassium content compared to that of other vegetable crops.

**Amino acids** The amino acid composition of whole purslane is shown in Table 4. Glutamic acid was the most prevalent amino acid (1,605 mg/100 g), followed by aspartic acid, leucine, alanine, lysine, valine, glycine, and isoleucine (1,184.3, 905.9, 740.9, 738.3, 637.3, 633.3, and 548.0 mg/100 g, respectively). These results are consistent with those of Miller *et al.* (33), who suggested that purslane has a good balance and concentration of essential amino acids with a sufficiently high chemical score.

**Extraction of polysaccharides** The yield, total carbohydrate, and uronic acid contents of extracted polysaccharides and non-starch polysaccharides were analyzed to investigate their chemical properties. The extraction of polysaccharides from whole purslane was accomplished using methanol-chloroform with different solvents, such as water (WSP), 1% potassium oxalate (KSP), 0.1 N HCl (HSP), 0.05 N NaOH (NSP-1), and 1.25 N NaOH (NSP-

**Table 4. Amino acid composition of purslane**

Amino acid	Content <sup>1)</sup>	
	mg/100 g	% Ratio
Aspartic acid	1,184.3±5.52	12.1
Serine	310.4±2.24	3.2
Glutamic acid	1,605.8±7.21	16.4
Glycine	633.3±5.63	6.5
Histidine	249.3±2.22	2.5
Threonine	357.8±3.34	3.6
Arginine	470.3±4.57	4.8
Alanine	740.9±6.86	7.5
Proline	514.5±5.95	5.2
Cysteine	-	-
Tyrosine	280.8±4.74	2.9
Valine	637.3±5.69	6.5
Methionine	100.3±2.42	1.0
Lysine	738.3±6.77	7.5
Isoleucine	548.0±5.58	5.6
Leucine	905.9±8.79	9.2
Phenylalanine	544.1±6.67	5.5
Total	9,821.3±5.26	100

<sup>1)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

2), according to the procedure shown in Fig. 1. WSP and KSP were obtained by precipitation with ethanol and freeze-drying. The acidic and alkaline fractions (HSP, ASP-1, and ASP-2) were neutralized, exhaustively dialyzed, and then freeze-dried. The yield of WSP was found to be 0.29, 7.01, and 7.94%, when extracted by room temperature water (RTW), hot-water (HW), and hot water/high pressure-water (HTPW), respectively (Table 5). Conversely, potassium oxalate and hydrochloric acid appeared to be much more effective at solubilization of carbohydrates than water when the extraction was conducted at 25°C for 2 hr. Similarly, Wenzel *et al.* (18) also reported that the extraction yield obtained using boiling water was twice the yield obtained using water of temperatures ranging from 0 to 25°C. Additionally, the amount of total carbohydrates recovered was between 23-29% when RTW extraction was performed. Conversely, the uronic acid content of WSP extracted by RTW was much higher than that of WSP extracted by HW or HTPW. These results may indicate that HW or HTPW extraction can be an effective method to obtain WSP from purslane. However, in the hydrochloric acid and ammonium oxalate produced a greater yield of polysaccharides than sodium hydroxide when the KSP, HSP, and NSP were extracted. According to Wenzel *et al.* (18), the ethanolic precipitation of crude polysaccharides usually results in 2 insolubilized forms: a major one (83-90%), which resembles a 'cord filament' and a minor one, which resembles 'resin fines'. They also reported that the 'fines' subfraction was composed of

**Table 5. Yield and composition of polysaccharide fractions from purslane**

Extracts <sup>1)</sup>	Extraction condition <sup>2)</sup>	Polysaccharide			NSP <sup>5)</sup> (%)
		Yield (%)	TCH <sup>3)</sup> (%)	UA <sup>4)</sup> (%)	
WSP	1	0.29±0.01 <sup>6)</sup>	23.64±1.42	4.30±0.03	11.87±0.51
	2	7.01±0.24	22.74±0.02	0.57±0.01	8.12±0.45
	3	7.94±0.36	29.45±0.28	0.44±0.05	11.19±0.37
KSP	1	8.87±0.41	12.81±1.19	0.33±0.01	1.62±0.04
	2	5.04±0.35	9.12±0.02	0.21±0.02	0.69±0.02
	3	4.63±0.44	7.38±0.30	0.19±0.02	0.68±0.01
HSP	1	7.93±0.67	24.39±0.49	0.51±0.01	9.61±0.66
	2	5.28±0.49	21.79±0.23	0.45±0.07	6.18±0.54
	3	5.08±0.43	22.58±0.25	0.48±0.01	6.85±0.56
NSP-1	1	1.30±0.05	23.50±0.60	3.05±0.13	6.85±0.55
	2	1.23±0.04	22.22±0.48	3.09±0.10	5.62±0.49
	3	3.53±0.32	22.73±0.02	1.61±0.05	5.67±0.57
NSP-2	1	2.59±0.02	43.70±1.70	0.91±0.00	18.13±0.67
	2	2.70±0.17	45.37±0.48	0.94±0.05	20.19±0.82
	3	2.91±0.25	45.34±0.68	0.75±0.02	22.33±0.94

<sup>1)</sup>WSP, extraction with water; KSP, extraction with 1% potassium oxalate; HSP, extraction with 0.1 N HCl; NSP-1, extraction with 0.05 N NaOH; NSP-2, extraction with 1.25 N NaOH.

<sup>2)</sup>1, extraction at 25°C for 2 hr; 2, extraction at 100°C for 2 hr; 3, extraction at 100°C and 10 psi for 30 min.

<sup>3)</sup>Total carbohydrate.

<sup>4)</sup>Uronic acid.

<sup>5)</sup>Non-starch polysaccharide.

<sup>6)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

**Table 6. Sugar composition of polysaccharide fractions obtained from purslane using different extraction methods**

Extracts <sup>1)</sup>	Extraction condition <sup>2)</sup>	Sugar composition (%)					
		Rha	Ara	Xyl	Man	Gal	Glu
WSP	1	9.42±0.18 <sup>3)</sup>	37.09±2.26	0.26±0.36	-	48.83±1.36	4.41±0.74
	2	8.43±0.01	34.28±0.04	0.48±0.01	0.62±0.10	36.97±0.05	19.22±0.09
	3	6.45±0.21	27.04±0.29	0.57±0.02	0.89±0.08	28.75±0.16	36.29±0.72
KSP	1	9.41±0.01	33.00±0.30	1.72±0.01	1.43±0.11	32.85±0.19	21.60±0.71
	2	9.18±0.07	16.89±0.71	2.63±0.01	0.88±0.09	34.58±0.40	35.83±0.67
	3	8.58±0.14	18.40±0.04	2.25±0.14	0.88±0.10	30.55±0.95	39.34±0.64
HSP	1	6.48±0.98	12.59±0.01	0.42±0.04	0.61±0.08	29.78±4.81	50.12±8.05
	2	10.38±0.11	12.50±0.42	0.67±0.01	0.60±0.01	30.98±0.03	44.86±0.32
	3	9.34±0.27	9.79±0.11	0.72±0.03	0.63±0.08	26.19±0.21	53.33±0.31
NSP-1	1	15.10±0.10	37.78±0.33	1.05±0.02	0.51±0.11	35.17±0.19	10.39±0.15
	2	22.39±0.24	18.72±0.23	5.72±0.02	0.88±0.13	40.28±0.37	12.01±0.25
	3	21.67±0.55	18.38±0.68	3.37±0.01	0.77±0.01	41.31±0.04	14.51±0.18
NSP-2	1	2.67±0.14	6.06±0.21	48.47±0.14	2.75±0.14	10.45±0.28	29.60±1.41
	2	3.01±0.41	7.12±1.34	46.04±0.20	2.99±0.13	10.21±0.30	30.63±0.30
	3	2.58±0.01	11.39±0.07	37.18±0.14	3.96±0.14	9.90±0.78	34.98±2.33

<sup>1)</sup>WSP, extraction with water; KSP, extraction with 1% potassium oxalate; HSP, extraction with 0.1 N HCl; NSP-1, extraction with 0.05 N NaOH; NSP-2, extraction with 1.25 N NaOH.

<sup>2)</sup>1, extraction at 25°C for 2 hr; 2, extraction at 100°C for 2 hr; 3, extraction at 100°C and 10 psi for 30min.

<sup>3)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

acidic sugars, O-acetyl substituents, and protein, therefore they might be suggested that it was enriched in the arabinogalactan component.

**Sugar composition of polysaccharides** The sugar composition of polysaccharide fractions solubilized using various extractants is shown in Table 6. Increasing the

extraction temperature and pressure increased the glucose content of the WSP from 4.4 (RTW) to 19.2% (HW) and 36.3% (HTPW). Additionally, considerable amounts of galactose, arabinose, rhamnose, and glucose were found in the WSP, KSP, and HSP, which indicates that these fractions contain a mixture of starch and pectin. These results may also be due to the gelatinization of starch during extraction, which is known to be the primary solubilizing mechanism of plant polysaccharides. The HSP showed a significant increase in glucose content via the acid hydrolysis of starch. Additionally, the concentration of sodium hydroxide had a strong effect on the glucose content of the NSP. The average distributions of L-arabinose:D-galactose of the WSP were 37:49, 34:37, and 27:29 when extracted with RTW, HW, and HTPW, respectively. It has been reported that water extraction is a suitable process for the preparation of the arabinogalactan component of whole purslane. Amin and El-Deeb (17) also reported that the acidic fraction consisted of galacturonic acid residues joined by  $\alpha$ -(1  $\rightarrow$  4)-linkages, and the neutral fraction was composed of 41% arabinose and 43% galactose residues in the polysaccharides of purslane. The structure of ionic (charged at pH>2.0) arabinogalactan in the complex is not well defined, which makes it somewhat similar in its internal composition and structure to gum arabic (22).

**Enzymatic treatments and Mw distributions** In order to investigate the effect of enzymes on the extractability of polysaccharides, purslane was treated with pectinases prior to preparation of the WSP and HSP after removal of the

**Table 7. Effect of enzyme treatment on the extraction yield of purslane<sup>1)</sup> (%)**

Enzyme treatment <sup>2)</sup>	WSP		HSP	
	Yield (%)	TCH <sup>3)</sup> (%)	Yield (%)	TCH (%)
Control	7.01±0.62 <sup>6)</sup>	22.74±0.02	5.28±0.44	21.79±0.02
P	11.10±0.71	17.73±0.66	8.87±0.67	19.14±1.03
V	13.12±0.76	29.62±1.53	6.76±0.54	21.61±0.05
PV	9.44±0.68	20.33±0.73	7.24±0.73	21.61±0.16

<sup>1)</sup>Values represent the mean of values obtained from experiments conducted in triplicate.

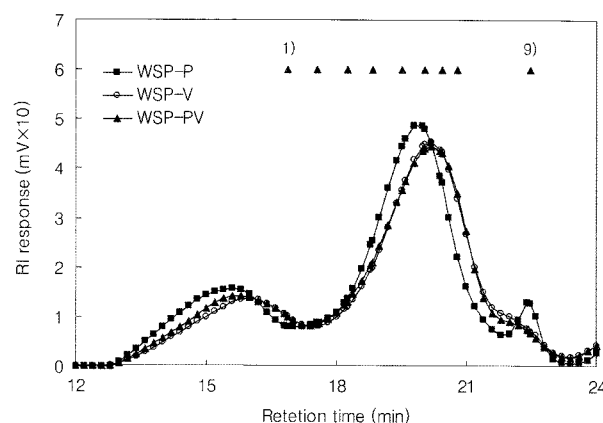
<sup>2)</sup>Control, no enzyme treatment; P, Pectinex 5XL; V, Viscozyme L.; PV, Pectinex 5XL+Viscozyme L.

<sup>3)</sup>Total carbohydrate.

**Table 8. Mw and composition of WSP**

Samples <sup>1)</sup>	Fraction I		Fraction II	
	Mw	Composition (%)	Mw	Composition (%)
Control	62,034,119	29.01	68,668	70.93
WSP-P	2,214,487	28.01	65,099	71.99
WSP-V	1,839,860	26.10	61,965	73.90
WSP-PV	1,919,907	27.45	65,754	72.56

<sup>1)</sup>Control, no enzyme treatment; WSP-P, water soluble polysaccharide prepared using Pectinex 5XL; WSP-V, water soluble polysaccharide prepared using Viscozyme L.; WSP-PV, water soluble polysaccharide prepared using a combination of Pectinex 5XL and Viscozyme L.



**Fig. 2. Effect of enzyme treatment on the Mw of WSP.** WSP-P, water soluble polysaccharide treated with Pectinex 5XL; WSP-V, water soluble polysaccharide treated with Viscozyme L.; WSP-PV, water soluble polysaccharide treated with Pectinex 5XL+Viscozyme L.; Standard of Mw, 1)  $7.88 \times 10^5$ ; 2)  $4.04 \times 10^5$ ; 3)  $2.12 \times 10^5$ ; 4)  $1.12 \times 10^5$ ; 5)  $4.73 \times 10^4$ ; 6)  $2.28 \times 10^4$ ; 7)  $1.18 \times 10^4$ ; 8)  $5.9 \times 10^3$ ; 9) glucose.

low molecules with ethanol, as described in Fig. 1. Purslane treated with Viscozyme L exhibited a higher yield and higher total carbohydrate content than purslane treated with Pectinex 5XL in the WSP; however, there was little difference in amounts of these enzymes in the HSP, even when combined treatment using both enzymes was conducted (Table 7). The Mw distributions of the WSP fractions of purslane were determined using a Shodex SB-806 column (Table 8 and Fig. 2). The WSP treated with Viscozyme L, Pectinex 5XL, and both combined were greatly decreased in Mw and weakly tailed towards a lower Mw. The fractions were eluted as bi-modal peaks, one less intense, about Mw 2,200,000 and a second, dominant peak, with a Mw <66,000 fraction I could be easily broken down into lower molecular materials. Fraction II did not have a great change in molecular weight, however, although enzymatic hydrolysis did occur in this fraction. Overall, enzymatic treatment of purslane appears to be an effective method to control the Mw of polysaccharides. Additionally, Viscozyme L was demonstrated to be a suitable enzyme for the extraction and separation of polysaccharides from purslane.

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## References

- Lee TB. Illustrated flora of Korea. Hyangmunsa, Seoul, Korea. pp. 324-325 (1999)
- Peng PC, Haynes LJ, Magnus KE. High concentration of (-)-noradrenaline in *Portulaca oleracea* L. Nature 191: 1108 (1961)
- Chan K, Islam MW, Kamil M, Radhakrishnan MNMZ, Habibullah M, Attas A. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. sativa (Haw.) Celak. J. Ethnopharmacol. 73: 445-451 (2000)
- Miller AG, Morris M. Plants of Dhofa, the Southern Region of

- Oman. Traditional, Economical, and Medicinal Uses. The Office of the Advisor for Conservation of Environment, Diwan of Court, Sultanate of Oman. pp.186-192 (1988)
5. Usmanghani K, Saeed A, Alam MT. Indusyunic Medicine. University of Karachi, Karachi, Pakistan. pp. 75-81 (1997)
  6. Sakai N, Inada K, Okamoto M, Shizuri Y, Fukuyama Y. Portuloside A, a monoperene glucoside, from *Portulaca oleracea*. *Phytochemistry* 42: 1625-1628 (1996)
  7. Seo Y, Shin J, Cha HJ, Kim YA, Ahn JW, Lee BJ, Lee DS. A new monoterpene glucoside from *Portulaca oleracea*. *Bull. Korean Chem. Soc.* 24: 1475-1477 (2003)
  8. Chen J, Shi YP, Liu JY. Determination of noradrenaline and dopamine in Chinese herbal extracts from *Portulaca oleracea* L. by high-performance liquid chromatography. *J. Chromatogr. A* 1003: 127-132 (2003)
  9. Okwuasaba F, Ejike C, Parry O. Skeletal muscle relaxant properties of the aqueous extract of *Portulaca oleracea*. *J. Ethnopharmacol.* 17: 139-160 (1986)
  10. Parry O, Okwuasaba F, Ejike C. Preliminary clinical investigation into the muscle relaxation actions of an aqueous extract of *Portulaca oleracea* applied topically. *J. Ethnopharmacol.* 21: 99-106 (1987)
  11. Omara-Alwala T, Mebrahtu T, Prior DE, Ezekwe M. Omega-three fatty acids in purslane (*Portulaca oleracea*) tissues. *J. Am. Oil Chem. Soc.* 68: 198-199 (1991)
  12. Sridhar R, Lakshminarayana G. Lipid classes, fatty acids, and tocopherols of leaves of six edible plant species. *J. Agr. Food Chem.* 41: 61-63 (1993)
  13. Liu L, Howe P, Zhou YF, Xu ZQ, Hocart C, Zhang R. Fatty acids and  $\beta$ -carotene in Australian purslane (*Portulaca oleracea*) varieties. *J. Chromatogr. A* 893: 207-213 (2000)
  14. Banerjee G, Mukherjee A. Antibacterial activity of a common weed, *Portulaca oleracea* L. *Geobios -Lyon* 30: 143-144 (2003)
  15. Lim JP, Suh ES. Hepatoprotective, diuretic, and anti-inflammatory activities of the extract from *Portulaca oleracea* Linné. *Korean J. Med. Crop Sci.* 8: 189-193 (2000)
  16. Lim MK, Kim M. Antimicrobial activity of methanol extract from *soibirhym* (*Portulaca oleracea*) against food spoilage on foodborne disease microorganism and the composition of the extract. *Korean J. Soc. Food Cook. Sci.* 17: 565-570 (2001)
  17. Amin ES, El-Deeb SM. Isolation of *Portulaca oleracea* (REGLA) mucilage and identification of its structure. *Carbohydr. Res.* 56: 123-128 (1977)
  18. Wenzel GE, Fontana JD, Correa JBC. The viscous mucilage from the weed *Portulaca oleracea*, L. *Appl. Biochem. Biotech.* 24/25: 341-353 (1990)
  19. Garti N, Slavin Y, Aserin A. *Portulaca oleracea* gum and casein interactions and emulsion stability. *Food Hydrocolloid* 13: 127-138 (1999)
  20. Garti N, Aserin A, Slavin Y. Competitive adsorption in O/W emulsions stabilized by the new *Portulaca oleracea* hydrocolloid and nonionic emulsifiers. *Food Hydrocolloid* 13: 138-144 (1999)
  21. Garti N, Slavin Y, Aserin A. Surface and emulsification properties of a new gum extracted from *Portulaca oleracea* L. *Food Hydrocolloid* 13: 145-155 (1999)
  22. Grieshop CM, Flickinger EA, Fahey GC. Oral administration of arabinogalactan affects immune status and fecal microbial populations in dogs. *J. Nutr.* 132: 478-482 (2002)
  23. Causey JL, Robinson RR, Feirtag JK. Effects of larch arabinogalactan on human peripheral blood mononuclear cells. *FASEB J. Part I:* 457 (1999)
  24. AOAC. Official Method of Analysis of AOAC Intl. 16<sup>th</sup> ed. Method 991.43. Association of Official Analytical Communities, Arlington, VA, USA (1995)
  25. Southgate DAT. Determination of Food Carbohydrates. Oxford University Press, London, England. pp. 99-144 (1976)
  26. Theander O, Aman P, Westerland E, Andersson R, Pettersson D. Total dietary fiber as neutral sugar residues, uronic acid residue, and kalsol lignin (The uppsal method): Collaborative study. *J. AOAC Intl.* 78: 1030-1044 (1995)
  27. SAS Institute, Inc. SAS User's Guide. Statistical Analysis System Institute, Cary, NC, USA (1990)
  28. Mohamed A, Hussein A. Chemical composition of purslane (*Portulaca oleracea*). *Plant Food Hum. Nutr.* 45: 1-9 (1994)
  29. Guil JL, Torija ME, Giménez JJ, Rodríguez I. Identification of fatty acids in edible wild plants by gas chromatography. *J. Chromatogr. A* 719: 229-235 (1996)
  30. Kesden D, Will Jr AA. Purslane: An ubiquitous garden weed with nutritional potential. *P. Fl. St. Hortic. Soc.* 100: 195-197 (1987)
  31. Simopoulos AP. Terrestrial sources of omega-three fatty acids: Purslane. pp. 93-107. In: *Horticulture and Human Health: Contribution of Fruits and Vegetables*. Quwbedeaus B, Bliss F (eds). Englewood Cliffs, Prentice-Hall, NJ, USA (1987)
  32. Horan MJ, Blaustein MP, Dunbar JB, Grundy S, Kachodourian W, Kaplan NM, Kotchen TA, Simopoulos AP, Van Itallli TB. NIH report on research challenges in nutrition and hypertension. *Hypertension* 7: 818-820 (1985)
  33. Miller TE, Wing JS, Huete AR. The agricultural potential of selected C4 plants in arid environments. *J. Arid Environ.* 7: 275-286 (1984)