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Variation in Fucoidan Contents and Monosaccharide Compositions of Korean *Undaria pinnatifida* (Harvey) Suringar (Phaeophyta)

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Three different forms of *Undaria pinnatifida*, the southern form (*U. pinnatifida* f. *typica*), the northern form (*U. pinnatifida* f. *distans*), and Samcheok form (recently cultivated strain), were examined for the contents and compositions of fucoidans. Fucoidans were extracted from the dried edible portions of three forms of *U. pinnatifida* in low pH condition, mainly by ethanol precipitation and $CaCl_2$ treatment. It was shown that Samcheok form contains 1.8 and 3.5 times more fucoidans than the northern and the southern forms, respectively. The monosaccharide compositions of individual fucoidans were also varied. The fucoidans from the southern and the northern forms were shown to be composed of mainly fucose and galactose with the molar percentage ratios of 83.5%:16.5% and 87.4%:12.6%, respectively, indicating that these are F-type fucoidans. The fucoidan from Samcheok form, however, consisted of fucose (62.7%), galactose (32.9%), and small amount of glucose (4.4%). The results of this study showed that both amount and monosaccharide compositions of fucoidans are variable depending on *U. pinnatifida* forms.

Key Words: fucoidan, monosaccharide composition, *Undaria pinnatifida*

INTRODUCTION

Undaria pinnatifida (Harvey) Suringar is an annual brown alga that is composed of haptera, stipes, blades and sporophylls. The sporophyte generally grows to 1.5-2 m, sometimes up to 3 m, within a year and shows heteromorphic alternation of generation. The microscopic gametophyte is dioecious. *U. pinnatifida* produces various polysaccharides including fucoidan.

Fucoidan is a group of marine sulfated polysaccharides containing large proportions of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, mannose and glucuronic acid (Fig. 1). These polysaccharides are known to display various biological activities such as anti-inflammatory (Angstwurm *et al.* 1995; Maruyama *et al.* 2005), antiviral (Preeprame *et al.* 2001; Lee *et al.* 2004), and antioxidant activity (Ruperez *et al.* 2002). Fucoidans also prevent *Helicobacter pylori* infection and reduce the risk of associated gastric cancer (Shibata *et al.* 2003). For these reasons, production and applications of fucoidans as therapeutic agents have been increasingly important topics of intensive researches.

Undaria pinnatifida is cultivated widely in Korea, China and Japan where it is a commercially important food (Guiry and Blunden 1991). In Korea, *U. pinnatifida* cultivation and marketing in 2005 recorded about 286,611 metric tons (wet) and 44,000,000 US$ (44,326,784,000 won). There are two different forms of *U. pinnatifida* that are commercially cultivated in Korea, i.e. *U. pinnatifida* f. *typica* (southern form) and *U. pinnatifida* f. *distans* (northern form) (Okamura 1936, Kang 1968). Compared to the southern form, the northern form has a longer stipe with sporophylls arising from the lower region with a deeply divided blade (Lee and Sohn 1993; Ohno and Matsuoka 1993). A new form of *U. pinnatifida* originated from Samcheok on the east coast of Korea is recently cultivated in Korea (Samcheok form).

In this study, we isolated and compared the amount of fucoidans from three forms (southern, northern, and Samcheok forms) of *U. pinnatifida* that are cultivated in Korea, and also compared the monosaccharide compositions in the isolated fucoidans.

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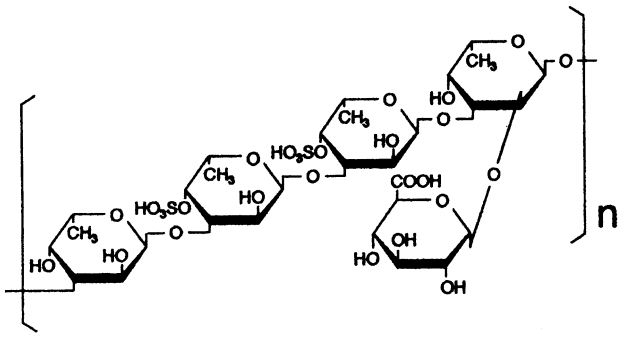

Fig. 1. A typical structure of fucoidan derived from *Cladosiphon okamuranus* (Adapted from Sakai *et al*. 2003).

MATERIALS AND METHODS

Seaweed materials

Three different forms of *Undaria pinnatifida* were collected from an aquaculture area in Gijang near Busan, on the southeastern cost of Korea, on January 20 2005: southern form (*U. pinnatifida* f. *typica*), northern form (*U. pinnatifida* f. *distans*), and Samcheok form. The former two species have been domestically cultivated, and the latter had been naturally grown in Samcheok and was cultivated from 2004 winter for the first time.

Extraction of fucoidans

Extraction of fucoidan was performed as described by Kim *et al*. (2004). Edible portions of wet *Undaria* were taken out, freeze-dried and then cut into appropriate sizes (~ 3 × 3 cm). One hundred g of the sample were suspended in 1 L of 0.1 N HCl and allowed to stand for 24 h at room temperature. After filtration through Scotch Bright pad, the filtrates obtained were neutralized with NaOH and re-filtered to remove insoluble debris. The retentate (about 20 g) was, on the other hand, subjected to re-extraction for 2 h at 70°C with 5 vol. of 0.2 N HCl. The filtrates were pooled together and concentrated at 60°C to 1 L by vacuum evaporation. To this, 3 volume of 95% ethanol was added under the gentle stirring and allowed to stand for 2 h at ambient temperature. After centrifugation at 8,000 x g for 15 min at 4°C, the precipitates were completely dissolved in appropriate volume of distilled water (dH_2O) by adjusting the pH to 2 with HCl. To this, $CaCl_2$ was added to the final concentration of 2 M and the mixture was centrifuged as above. The supernatant obtained was treated with 3 volume of 95% and centrifuged. The precipitates were dissolved in dH_2O and repeatedly flash-evaporated (N-1000, Eyela Co., Japan) to remove residual ethanol, re-dissolved in dH_2O (pH 2), dialyzed at 4°C (MWCO 14,000 Da) for 48 h and then freeze-dried.

Acid hydrolysis of fucoidans for monosaccharide composition analysis

To determine the monosaccharide compositions of fucoidans obtained from three different samples, 50 mg each of fucoidans was dissolved in 1 ml of dH_2O, and equal volume of 4 M trifluoroacetic acid (TFA) was added and allowed to stand for 4 h at 100°C under gentle stirring. After the reaction, the mixture was filtered through 0.45 μm syringe filter and vacuum dried using Speed-Vac (Module spin 40, Biotron, Korea). The dried material was re-dissolved in 0.1 ml of dH_2O and dried. This was repeated three times to completely remove any residual TFA.

Monosaccharide analysis by HPAEC-PAD

The monosaccharide analysis of TFA-hydrolyzed fucoidans was performed by HPAEC (High Performance Anion-Exchange Chromatography) using BioLC (DX 500 Chromatography System, Dionex Co., USA) equipped with pulsed amperometric detector (ED 50, Dionex Co., USA). The dried sample was dissolved in 0.1 ml of dH_2O and filtered through microspin filters (0.45 μm, PGC Scientifics, Frederick, MD). After the filtration, 20 μL of the sample were injected and fractionated on a CarboPac PA-1 column (4 × 250 mm, Dionex Co., USA) which was pre-equilibrated with 100 mM NaOH and eluted in an isocratic mode at a rate of 1 ml/min. The fucoidan purchased from Sigma (USA) was acid hydrolyzed in the same way as above and used as a reference. L-fucose, D-glucose, and D-galactose (Sigma Co. St Luis, USA) were used as standard monosaccharides. All the reagents used for fucoidan extraction and carbohydrate analysis were of ACS grade.

RESULT AND DISCUSSION

Fucoidans were isolated from three forms of *Undaria pinnatifida*: the southern form (*U. pinnatifida* f. *typica*), the northern form (*U. pinnatifida* f. *distans*) and Samcheok form. From 100 g each of dried samples, 1.09 g, 2.08 g and 3.83 g of fucoidans were extracted with the yield of 1.0%, 2.1% and 3.8% in dry mass, respectively (Table 1). Samcheok form contained 1.8 and 3.5 times more fucoidans than the northern and the southern form, respectively. On the other hand, overall yields of

Table 1. Yields of fucoidans extracted from three forms of *Undaria pinnatifida* that were cultivated in Korea

Forms	Dry Weight (g)	Fucoidan (g)	Yield (% in mass)
southern form (*U. pinnatifida* f. *typica*)	100	1.09	1.0
northern form (*U. pinnatifida* f. *distans*)	100	2.08	2.1
Samcheok form (*U. pinnatifida*)	100	3.83	3.8

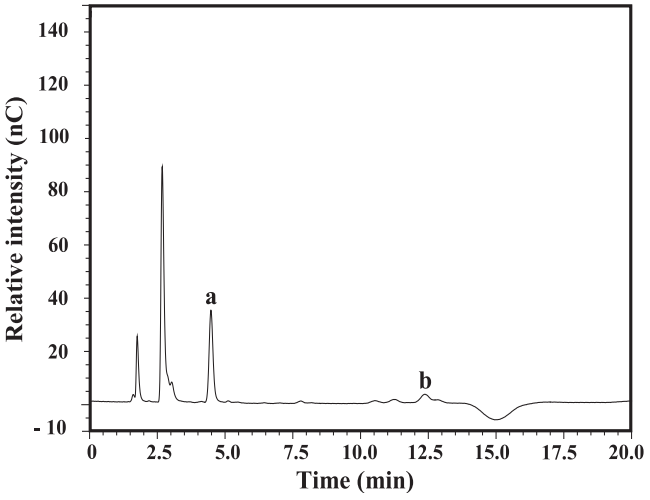

Fig. 2. HPAEC-PAD analysis for monosaccharide composition of the fucoidan extracted from southern form (*U. pinnatifida* f. *typica*). Acid hydrolysis of fucoidan was performed with 2 M triflouroacetic acid for 4 h at 100°C and the hydrolysates were analyzed by HPAEC-PAD using Bio-LC (Dionex) as described in the text. Symbol: a, L-fucose; b, D-galactose.

fucoidans extraction from these three forms in this work were somewhat lower comparing with about 4.8% yield that was previously reported from Japanese brown algae (Nishide *et al.* 1987; Nishino *et al.* 1989). They are also lower comparing with 4.8% yield from the Korean sea tangle (*Laminaria religiosa*) that was collected in Busan area (Koo *et al.* 1995). The reason for these low extraction yields was possibly due to the differences in cultivation area, times of collection, degree of maturation, etc. (Koo *et al.* 1995).

Monosaccharide composition analysis of extracted fucoidans was performed by HPAEC/PAD (BioLC) analysis after 2 M TFA hydrolysis. Each monosaccharide was quantified by comparing the peak area of sample sugar to that of standard monosaccharide of known amount. The fucoidan of the southern form was also turned out to be F-type as it contained mainly fucose

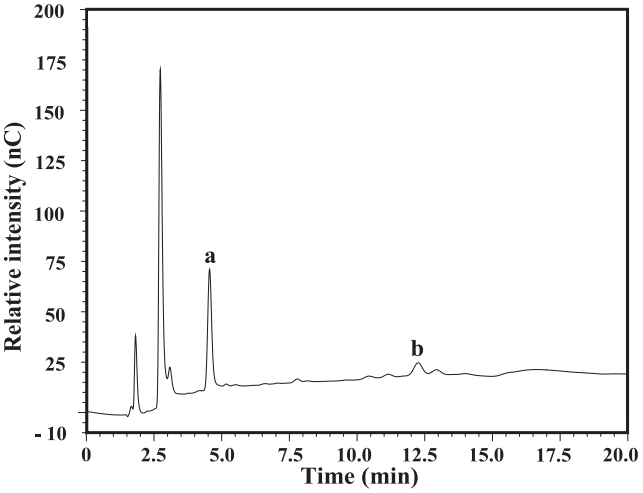

Fig. 3. HPAEC-PAD analysis for monosaccharide composition of the fucoidan extracted from northern form (*U. pinnatifida* f. *distans*). Acid hydrolysis of fucoidan and monosaccharide analysis was performed as described in the legend of Fig. 2. Symbol: a, L-fucose; b, D-galactose.

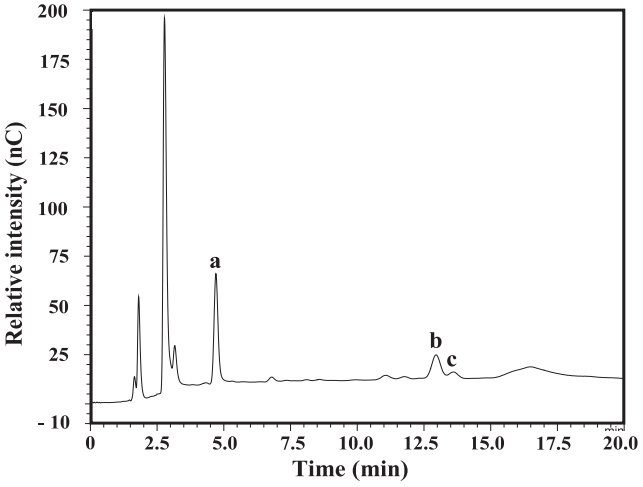

Fig. 4. HPAEC-PAD analysis for monosaccharide composition of the fucoidan extracted from Samcheok form of *U. pinnatifida*. Acid hydrolysis of fucoidan and monosaccharide analysis was performed as described in the legend of Fig. 2. Symbol: a, L-fucose; b, D-galactose; c, D-glucose.

(83.5%) and galactose (16.5%) (Fig. 2 and Table 2). The northern form was shown to be composed of mainly fucose and galactose with 87.4% and 12.6%, respectively, indicating that the fucoidan of this species is also F-type fucoidan (Fig. 3 and Table 2). The fucoidan from Samcheok form, however, consisted of fucose (62.7%), galactose (32.9%), and small amount of glucose (4.4%) (Fig. 4 and Table 2).

The results of this study showed that both amounts and monosaccharide compositions of fucoidans were variable depending on *U. pinnatifida* forms. Three forms

Table 2. Monosaccharide composition of the fucoidan extracted three forms of *Undaria pinnatifida* that were cultivated in Korea

Forms	Monomer	Molar Ratio[1)]	Relative Area (%)
southern form	L-fucose	5.0	83.5
(*U. pinnatifida* f. *typica*)	D-galactose	1.0	16.5
northern form	L-fucose	6.9	87.4
(*U. pinnatifida* f. *distans*)	D-galactose	1.0	12.6
Samcheok form	L-fucose	1.91	62.7
	D-galactose	1.0	32.9
	D-glucose	0.13	4.4

[1)] The values were obtained from the area of each peak on the HPAEC-PAD chromatogram of acid hydrolysate of the isolated fucoidan.

were cultivated in the same area and collected at the same time. Therefore, the difference of cultivation area or collection time was not the parameters affecting the fucoidan contents and monosaccharide compositions. The southern form and the northern form showed the same mitochondria 23S rDNA sequences (Muraoka and Saitoh. 2005). The mitochondria 23S rDNA of Samcheok form and other genes of those three forms of *U. pinnatifida* need to be sequenced and compared to elucidate their genetic variation. Specific biological functions of these three types of fucoidans remained to be elucidated.

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