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COMPOSITION OF TWO SEAWEED-OLIGOSACCHARIDE-LYSATES DERIVED FROM AGARASE AS-II AND THEIR FERROUS-CHELATING ANTIOXIDANT POWER

Fu-Jin Wang*, Sheen-Kye Kang**, Chorng-Liang Pan**, and Shao-Chi Wu***

Key words: β-agarase, antioxidative activity, neoagarohexaose, neoagarotetraose.

ABSTRACT

Seaweed-oligosaccharide-lysates (SwOSLys) Gra-C108, Mon-C108, Gra-AS-II, or Mon-AS-II are seaweed polysaccharide extracts (SwPSExts) Gra or Mon, which are digested by either *Aeromonas* (*A.*) *salmonicida* MAEF108 crude agarases or Agarase AS-II that was produced from marine bacteria *A. salmonicida* MAEF108. High-performance liquid chromatography revealed that there were neoagarohexaose and neoagarotetraose in both Gra-C108 and Gra-AS-II and that neoagarooligosaccharide was absent in both Mon-C108 and Mon-AS-II. The polyphenol content of SwOSLys was higher than that of SwPSExts, though without significant difference ($p < 0.05$) on lysates, as digested by crude MAEF108-agarases or Agarase AS-II. Comparison of the chelating effects of Gra-C108 and Gra-AS-II on Fe^{2+} shows that not only do the polyphenolic compound but also the neoagarooligosaccharide show antioxidative properties.

I. INTRODUCTION

Oxidative stress has been reported to be a result of an imbalance that occurs when a survival mechanism is unable to adequately deal with a reactive oxygen species (ROS) present in the cells [2]. The interest in ROS in biology and medicine stems from their strong relationship with phenomena, such as Alzheimer's disease, arthritis, cancer, cardiovascular diseases, diabetes, tissue damage caused by ischemia-reperfusion, and Parkinson's disease [12].

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It has been observed that the production of ROS in algae is stimulated by various environmental stresses, such as high light levels, heavy metals, high salt concentrations, UV irradiation, and other factors. Algae generally have higher antioxidative activity due to their higher content of various nonenzymatic antioxidant components, such as reduced glutathione (GSH), ascorbic acid, α-tocopherol, β-carotenoids, flavonoids, hydroquinones, phycocyanin, proline, mannitol, myoinositol, phenolics, and/or polyamines [8]. As a result, algae have become good candidates as a source of natural antioxidants [17].

In recent years, our laboratory has focused on the preparation of four seaweed polysaccharide extracts (SwPSExts) from *Gelidium* sp., *Gracilaria* sp., *Monostroma* (*Mon.*) *nitidum*, and *Porphyra dentate,* or their seaweed-oligosaccharide-lysates (SwOSLys) source, as digested by the two agarases produced from *A. salmonicida* MAEF108 or *Pseudomonas* (*P.*) *vavicularis* MA103, as well as their antioxidative properties, which were evaluated through several antioxidative tests [15, 16]. Our laboratory purification of Agarase AS-II, as produced from *A. salmonicida* MAEF108, SwPSExts from *Gracilaria* sp., or *Mon. nitidum* has recently been further digested by Agarase AS-II. Thus, the current study focuses on the composition of SwOSLys, as derived from *Gracilaria* sp., or *Mon. nitidum,* and evaluates their antioxidative capacity by ferrous-chelating antioxidant power.

II. MATERIALS AND METHODS

1. Agarase-Producing Strain

An agarase-producing strain, *A. salmonicida* MAEF108, with agar softening, was isolated from the seawater off the coast of Keelung, Taiwan [15].

2. Preparation of Agarase AS-II from *A. salmonicida* MAEF108

1) Crude Agarases Preparation

A modified method was derived from preliminary experiments of Wu *et al.* [16]. *A. salmonicida* MAEF108 produced the highest agarase activities in the following mediums

and incubation conditions. A 2% activated culture of *A. salmonicida* MAEF108 was inoculated into 100 mL MMB-MAEF108 of a medium composed of 0.40 g agar (Difco, MI, USA), 3.74 g marine broth 2216 (Difco), 0.60 g fructose (Sigma Chemical Co., MO, USA), 1.20 g NaCl (Panreac, Barcelona, Spain), 50 mg $(NH_4)_2HPO_4$ (Panreac), and 12 mg $MnCl_2$ (Panreac) with its pH level adjusted to 6.0, which was suitable for a 250-mL flask, at 20°C and a required 240 rpm rotation. After a 12-h incubation period, the culture suspension was centrifuged, at 4°C with 30,700 × g, for 15 min. The crude enzyme supernatant thus collected was named MAEF108-agarases.

2) Concentration via Ultrafiltration

All procedures were carried out below 4°C. Crude MAEF108-agarases were concentrated using a disposable modular tangential flow system, the Vivaflow50, with a 30-kDa molecular weight cut-off (MWCO) membrane (Vivaflow 50, Vivascience, Hannover, Germany), which was confined with a peristaltic pump (Masterflex® 7553-10, Masterflex, IL, USA) to promote the concentration of the crude MAEF108-agarases solution, and with a flow rate maintained at 200 mL/min. When the ratio of the volume of concentrate, as compared with the original, reached 1:20, the agarases concentrate was dialyzed and subjected to the same concentration devices described above, against 10 X volume, with 20 mM Tris-HCl (Panreac) buffer (pH 6.2), which included 1.0 M NaCl and 2 mM $CaCl_2$ (Panreac). Finally, the agarases concentrate were filtrated using a 0.2-μm membrane (Millex-GS, Millipore, MA, USA) for sterilization [16].

3) Ion-Exchange

The ion-exchange buffer (20 mM Tris-HCl, 2 mM $CaCl_2$, pH 7.2) was first washed in a 400 mm × 26 mm DE-52 ion exchange column, containing 100 mL ion-exchange DE-52 swollen gel (Whatman, Kent, UK), overnight at 4°C. The concentrated crude MAEF108-agarases were then applied to the DE-52 column. Each of the 150 fractions collected 6 mL of eluent using the fraction collector (Retriever™ 500, Isco Inc, NE, USA). The crude MAEF108-agarases were then eluted with a linear gradient of 0 to 1 M NaCl, with 4 mL of eluent collected per fraction. After the gradient, 1 M of NaCl was employed to elute and absorb all proteins [7]. Fractions with agarase activities were collected, and then concentrated using a disposable modular tangential flow system, Vivaflow 50, with a 10 kDa MWCO membrane (Vivaflow 50, Vivascience) and a peristaltic pump, as described above.

3. Preparation of Seaweed-Oligosaccharide-Lysates (SwOSLys)

1) Seaweed Polysaccharide Extracts (SwPSExts)

Deionized water was added to *Gracilaria* sp. or *Mon. nitidum* raw seaweed powder (w/w = 0.01), and then extracted at 121°C for 4 hours. After cooling to 37°C, Cellulase R-10 (enzyme/substrate = 3%, w/w) followed by Macerozyme R-10 (enzyme/substrate = 3%, w/w), both purchased from Yalkult Honsha Co. Ltd. (Tokyo, Japan), were added for a 2-h digestion at 37°C. Enzymes were inactivated for 10 min at 100°C. Both Cellulase R-10 and Macerozyme R-10 served to decompose cellulose and hemicelluloses of seaweed tissue to single cells. Then, the *Gracilaria* sp. or *Mon. nitidum* polysaccharide supernatant was obtained by centrifugation (2,000 × g for 30 min), using a high-speed refrigerated centrifuge (Himac CR 21, Hitachi, Tokyo, Japan). The supernatant was then freeze-dried and named Gra or Mon, as detailed below, and stored at -20°C for further use [4, 13].

2) Preparation of Seaweed-Oligosaccharide-Lysates (SwOSLys)

The 500-agarase-activity (AU) crude MAEF108-agarases were added to 1% Gra or Mon solutions, and digested at an optimal temperature (40°C) for 120 min, and referred to as Gra-C108 or Mon-C108. In addition, the 500-AU purified Agarase AS-II was added to 1% Gra or Mon solutions, and digested at an optimal temperature (45°C) for 120 min, and referred to as Gra-AS-II or Mon-AS-II. These lysates were freeze-dried and stored at -20°C for further use [16].

4. Quantification of Polyphenol

Forty microliters of samples were added to 3.15 mL deionized water and 0.2 mL Folin-Ciocalteu's reagent for 6 min. Then, 0.6 mL of 20% $NaHCO_3$ was added and reacted at room temperature for another 120 min, and absorbance was then measured at 765 nm. Polyphenol content was expressed as gallic acid equivalent (GAE) μg/mg [1].

5. Ferrous-Chelating Antioxidant Power

The chelating effect on ferrous ions was determined according to a previous method [16]. The SwPSExts, or their agarase lysates, were mixed with methanol and 400 μM $FeCl_2$ (Merck), followed by the addition of 2 mM ferrozine (Sigma). After 10 min, the absorbance of the mixture was determined as 562 nm. Trolox was used as a positive control [9]. The chelating effect (%) was calculated using the following equation:

$$\text{Chelating effect (\%)} = [1 - (A_{526} \text{ of sample} \div A_{526} \text{ of blank}) \times 100]$$

6. Gel Permeation Chromatography (GPC)

Concentrated Gra or Mon and their agarase lysates were applied to GPC columns (180 mm × 15 mm, Pharmacia) containing Sephacryl® S-200 gels (Pharmacia); or GPC columns (1,000 mm × 26 mm, Pharmacia) containing Sephadex™ G-25 gels (Pharmacia). Samples were eluted by degassed deionized water at ambient temperature, and fractions were then collected with the total sugar content measured by the phenol-sulphuric-acid method using glucose as the standard at A_{480} [4].

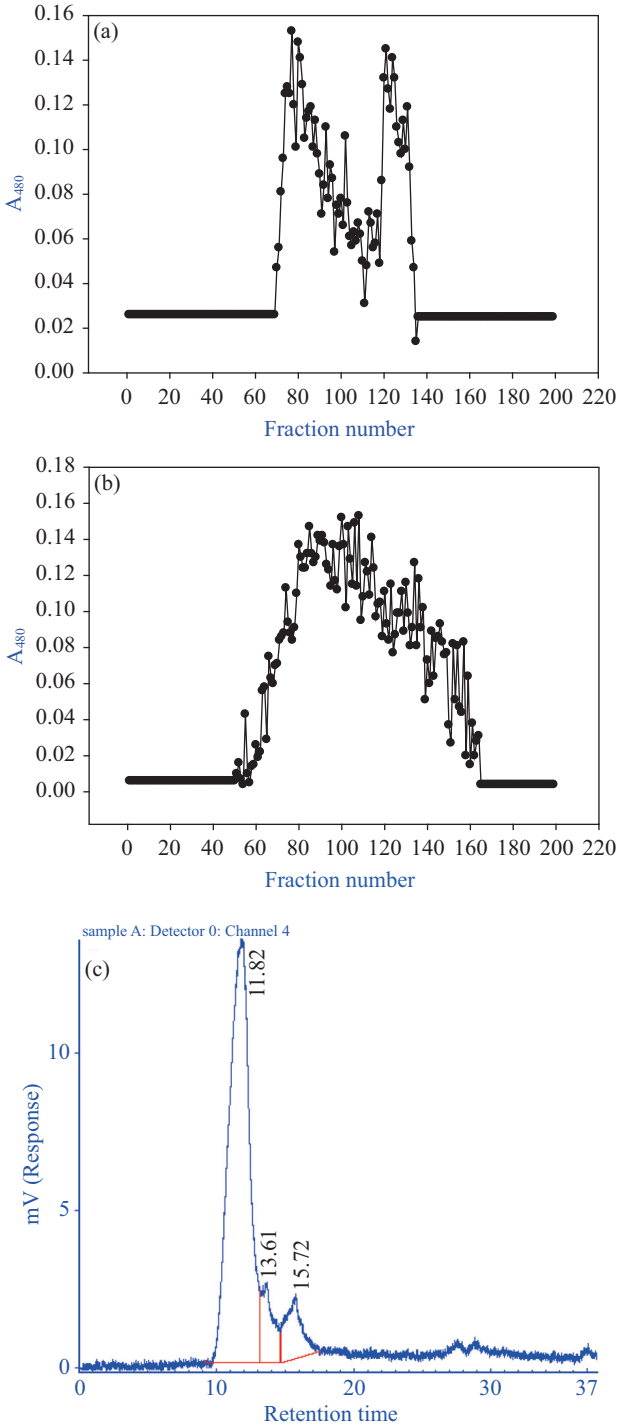

Fig. 1. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Gracilaria* sp. extract polysaccharides (Gra).
[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm x 300 mm; column temperature: 30°C; injection volume: 20 µL; flow rate: 0.4 mL/min; detector: ELSD].

7. High-Performance Liquid Chromatography (HPLC)

The SEC–ELSD system consisted of a pump PU-1580 from Jasco (Tokyo, Japan), an Asahipak GS-320 HQ multi-mode column (300 × 7.6 mm) preceded by an Asahipak GS-2G 7B guard column (50 × 7.6 mm) (Shodex, Showa Denko, Tokyo, Japan), and an ELSD ZAM 3000 (Schambeck SFD GmbH, Bad Honnef, Germany). The temperature settings for the column oven and detector nebulizer were 30°C and 80°C, respectively. Samples were injected via a CO-150 sampler with a 20-µL sample loop (Rheodyne, Cotati, CA, USA). The mobile phase for the SEC was deionized water with a flow-rate of 0.4 mL/min. For the NH_2-HPLC–ELSD system, the same pump, ELSD injector sampler and sample loop were used as above. However, an Asahipak NH_2 P-50 4E multi-mode column (250 × 4.6 mm) and an Asahipak NH_2P-50G 4A (10 × 4.6 mm) (Shodex, Showa Denko, Tokyo, Japan) were used, where the temperature settings for the column oven and detector nebulizer were 40°C and 80°C, respectively. The mobile phase was methanol:H_2O = 30:70 with a flow-rate of 0.5 mL/min. Chrom Manager 5.8 software from Analytical Based Development Center (Taichung, Taiwan) was used for online data monitoring and analysis [6].

8. Statistical Analysis

All antioxidative results of the samples are expressed as mean ± SD (n = 3). Data were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences among the groups, multiple comparisons among the means were performed, using Duncan's new multiple range tests. Statistical significance was determined by setting the aggregate type I error at 5% ($p < 0.05$) for each set of comparisons, using the Statistical Analysis System software package [10].

III. RESULTS AND DISCUSSION

1. Composition of Seaweed Polysaccharide Extracts or Their Lysates

To monitor the components of Gra samples, Gra-C108, Gra-AS-II, Mon, Mon-C108, and Mon-AS-II were applied to gel permeation chromatography, using Sephacryl® S-200 gels, as shown in Fig. 1(a) to Fig. 6(a); and using Sephadex™ G-25 gels, as shown in Fig. 1(b) to Fig. 6(b). HPLC was performed using an Asahipak GS-320 HQ size-exclusion column to monitor the components of SwPSExts, and their agarase lysate samples, as shown in Fig. 1(c) to Fig. 6(c). As can be seen, the GPC profile with Sephacryl® S-200 gels of Gra [Fig. 1(a)], Gra-C108 [Fig. 2(a)], and Gra-AS-II [Fig. 3(a)] shows major peaks of neoagarooligosaccharides for fractions 120-140, 140-180, and 120-160, respectively. Moreover, the neoagarooligosaccharide fractions of Gra, Gra-C108, and Gra-AS-II bring in absorption values of 0.15, 0.70, and 0.20 at A_{480} of 130, 160, and 140, respectively; implying neoagarooligosaccharide contents in the order of Gra-C108 > Gra-AS-II > Gra. The GPC profile with Sephacryl® S-200 gels of Mon [Fig. 4(a)] and

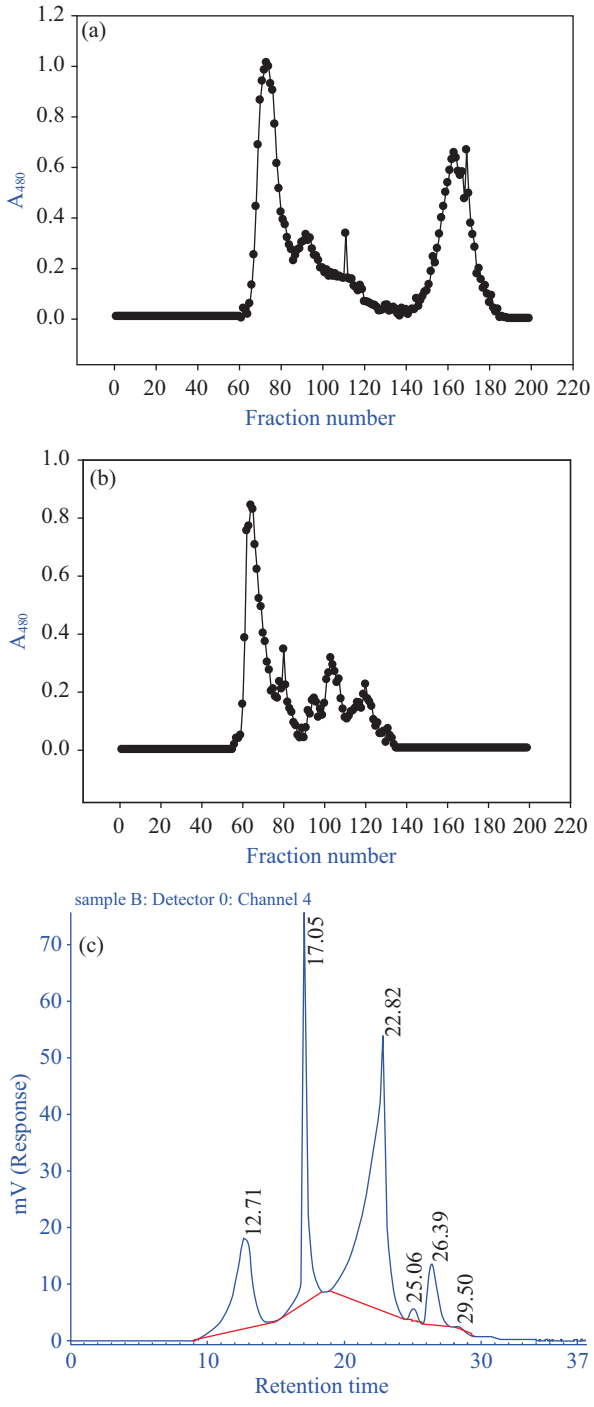

Fig. 2. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Gracilaria* sp. extracted polysaccharides digested by *A. salmonicida* MAEF108 crude agarases (Gra-C108).
[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm x 300 mm; column temperature: 30°C; injection volume: 20 µL; flow rate: 0.4 mL/min; detector: ELSD].

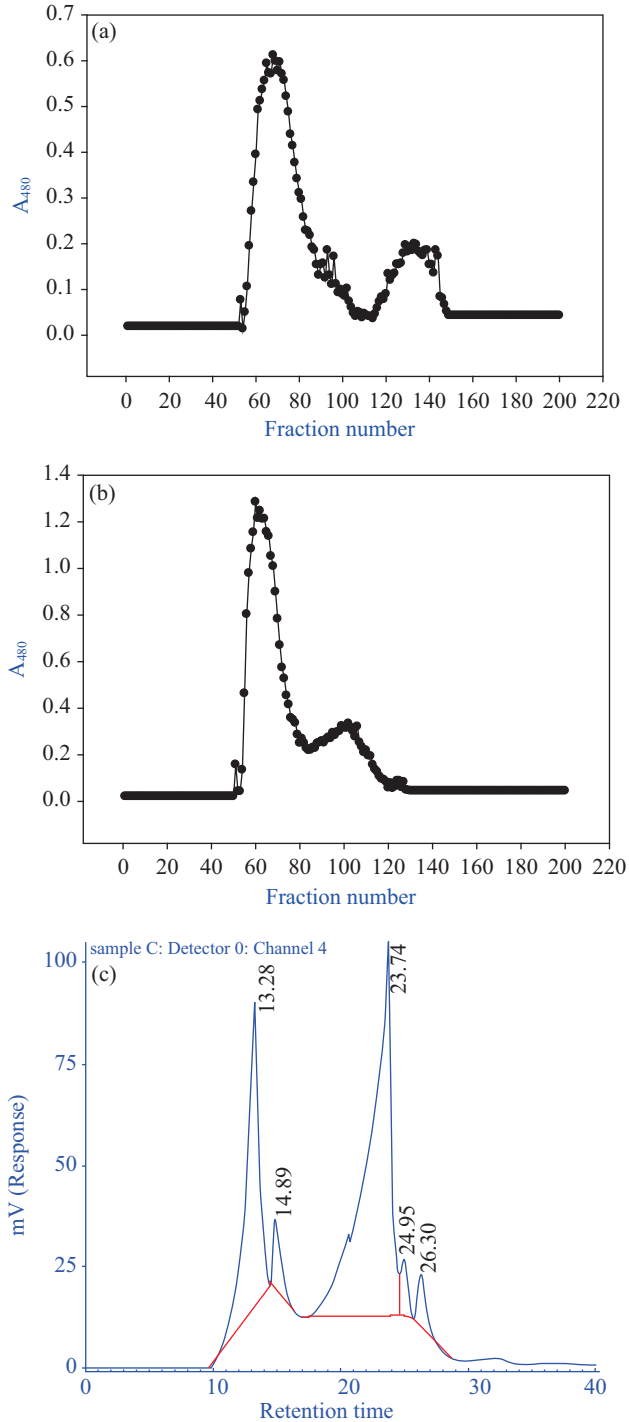

Fig. 3. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Gracilaria* sp. extracted polysaccharides digested by Agarase AS-II (Gra-AS-II).
[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm × 300 mm; column temperature: 30°C; injection volume: 20 µL; flow rate: 0.4 mL/min; detector: ELSD].

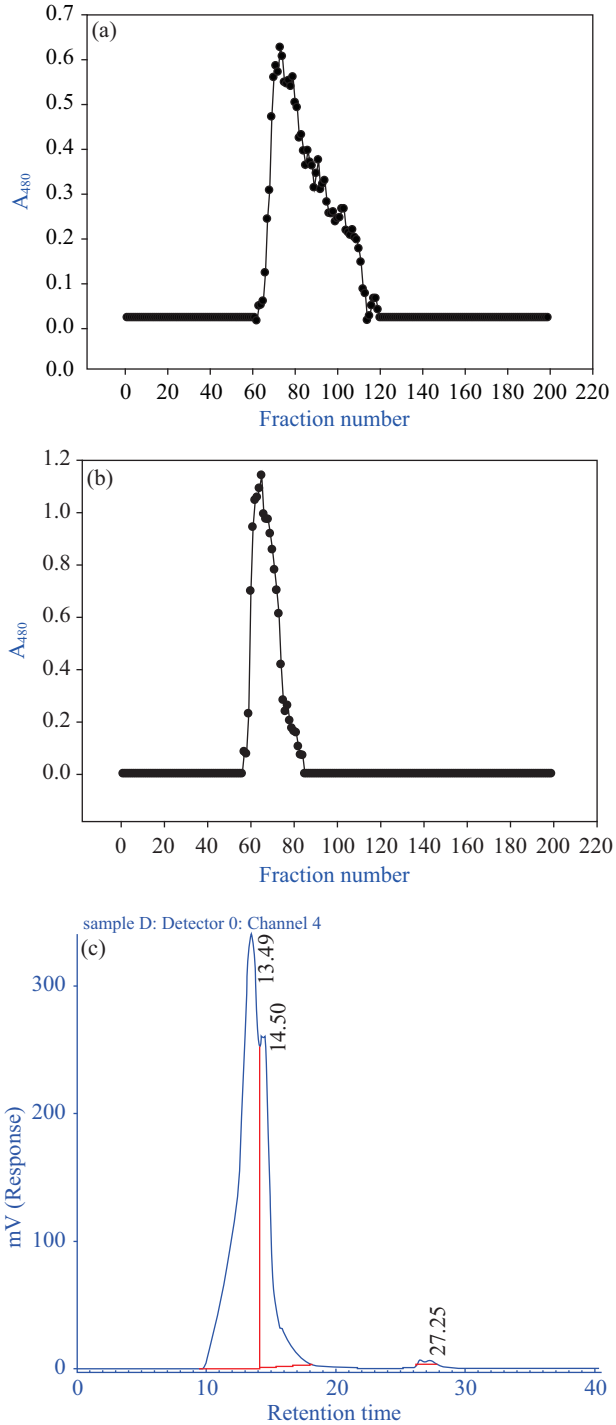

Fig. 4. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Mon. nitidum* extracted polysaccharides (Mon).

[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].

[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].

[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm × 300 mm; column temperature: 30°C; injection volume: 20 µL; flow rate: 0.4 mL/min; detector: ELSD].

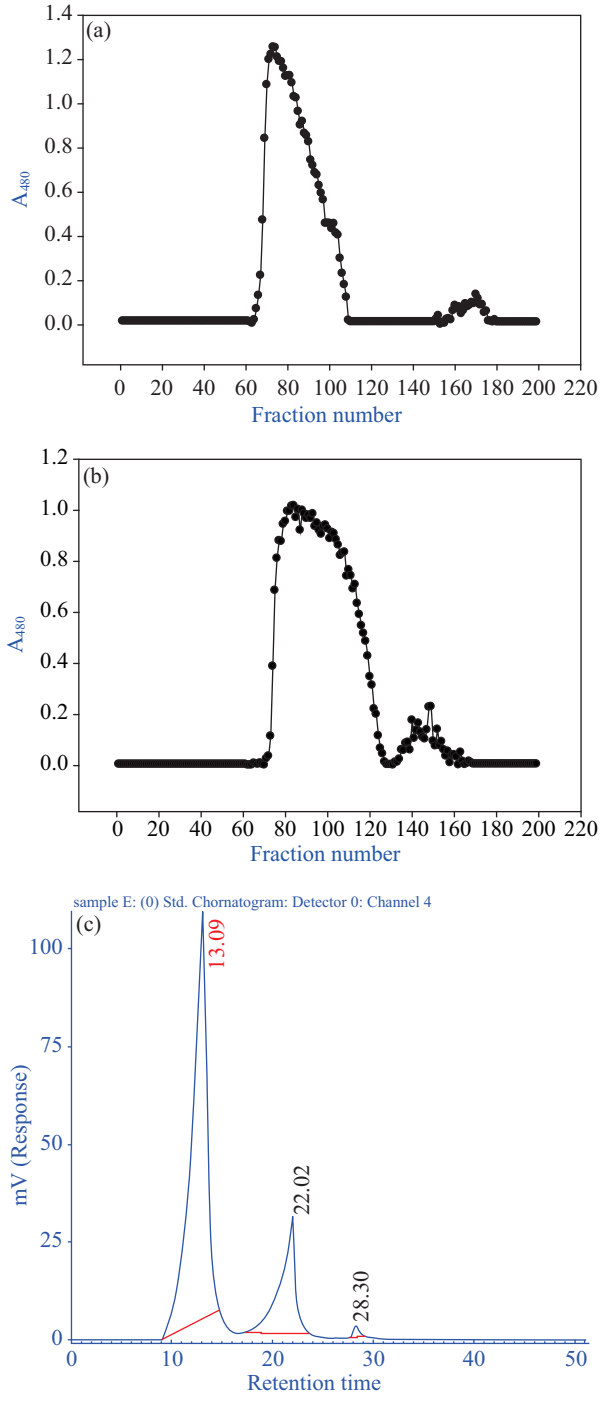

Fig. 5. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Mon. nitidum* extracted polysaccharides digested by *A. salmonicida* MAEF108 crude agarases (Mon-C108).

[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].

[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].

[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm × 300 mm; column temperature: 30°C; injection volume: 20 µL; flow rate: 0.4 mL/min; detector: ELSD].

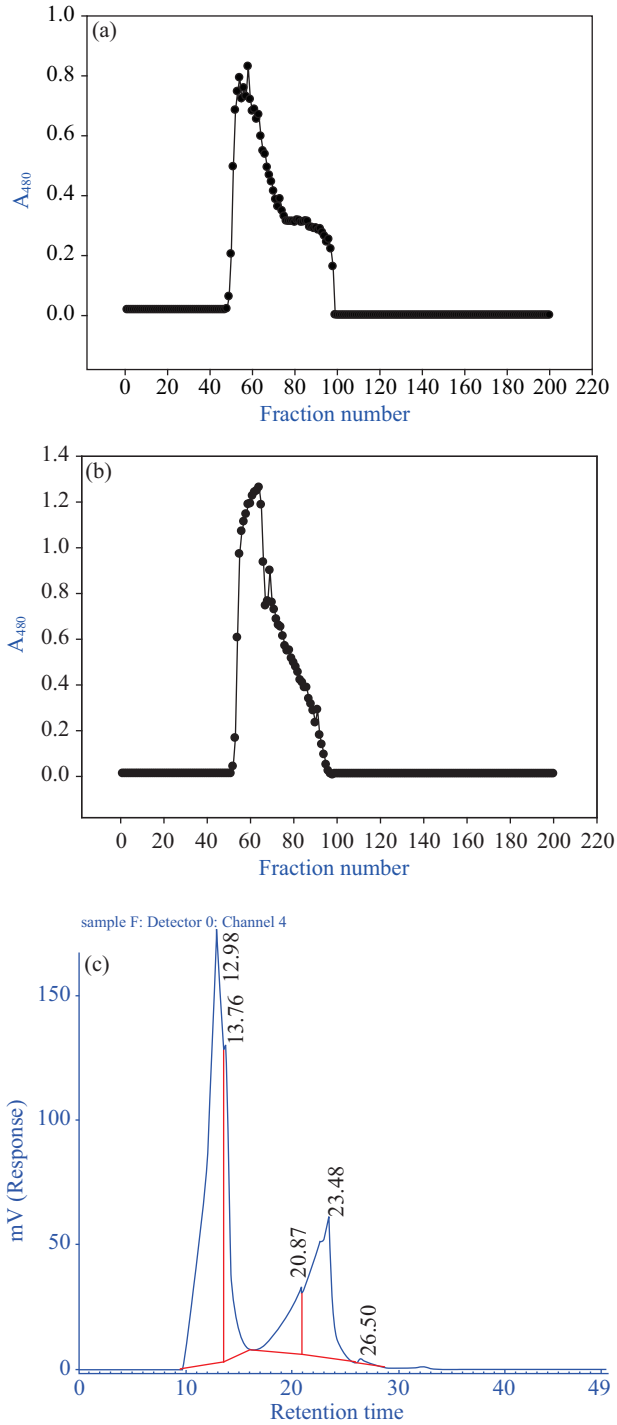

Fig. 6. (a) GPC profile with Sephacryl® S-200 gels, (b) GPC profile with Sephadex™ G-25 gels, (c) HPLC profile of *Mon. nitidum* extracted polysaccharides digested by Agarase AS-II (Mon-AS-II).
[GPC column size with Sephacryl® S-200 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[GPC column size with Sephadex™ G-25 gels: 90 cm × 2.6 cm; fraction size: 3 mL; flow rate: 0.5 mL/min; injected total sugar content: 3.65 mg/mL; injection volume: 2 mL].
[HPLC column: Asahipak GS-320 HQ, size-exclusion column, 7.5 mm × 300 mm; column temperature: 30°C; injection volume: 20 μL; flow rate: 0.4 mL/min; detector: ELSD].

Mon-C108 [Fig. 5(a)] shows major peaks of neoagarooligosaccharide for fractions 110-120 and 150-180, respectively; while the GPC profile with Sephacryl® S-200 gels of Mon-AS-II.

Fig. 6(a) shows no apparent peak of neoagarooligosaccharides. Moreover, the neoagarooligosaccharide fractions of Mon and Mon-C108 bring in absorption values of 0.05 and 0.15 at A_{480} of 115 and 165, respectively; implying neoagarooligosaccharide contents in the order of Mon-C108 > Mon > Mon-AS-II. The GPC profile with Sephadex™ G-25 gels of Gra [Fig. 1(b)] shows disorderly distribution of neoagarooligosaccharides all throughout. Gra-C108 [Fig. 2(b)] shows major peaks for fractions 60-80, 90-110, 110-130, and 130-140, while Gra-AS-II [Fig. 3(b)] shows major peaks for fractions 60-80, 80-120, and120-130. The GPC profile with Sephadex™ G-25 gels presents more distinct peak in Gra-AS-II than in Gra-C108 and Gra. The GPC profile with Sephadex™ G-25 gels of Mon [Fig. 4(b)] shows major peak for fraction 50-90. Mon-C108 [Fig. 5(b)] shows major peak for fractions 70-125 and 125-160, while Mon-AS-II [Fig. 6(b)] shows major peak for fraction 50-95.

GPC could only monitor the trends of SwPSExt digestion, whether they used crude MAEF108-agarases or Agarase AS-II. Both Sephacryl® S-200 gels and Sephadex™ G-25 gels were able to monitor different molecular weights, focused on 1,000-80,000 or 100-5,000, respectively. Before gel permeation chromatography by Sephadex™ G-25, it should be known all polysaccharides and oligosaccharides of SwPSExts or SwOSLys. After Sephacryl® S-200 gels employed for gel permeation chromatography, it could be more easy focus on fractions that contain oligosaccharides of SwOSLys to next gel permeation chromatography used Sephadex™ G-25. These two GPC gels were able to determine the carbohydrate molecular weight distribution of SwPSExts and their agarase lysates, and revealed that HPLC had higher sensitivity and could monitor the traces of oligosaccharides in agarase lysates better than GPC could. HPLC revealed that there were neoagarohexaose [Retention time (RT) = 25.06 min] and neoagarotetraose (RT = 26.32 min) in both Gra-C108 and Gra-AS-II [Fig. 2(c), and Fig. 3(c)]. The HPLC profile of Gra-C108 [Fig. 2 (c)] was more complex than that of Gra-AS-II [Fig. 3(c)]. Such results may be caused by *A. salmonicida* MAEF108 crude agarases, which contain multiple enzymes and have thus additional digestive functions. When monitored by GPC, Mon-C108 showed one major peak and one minor peak in both Sephacryl® S-200 and Sephadex™ G-25 gels [Fig. 5(a) and (b), respectively], while Mon-AS-II showed one major peak, in both Sephacryl® S-200 and Sephadex™ G-25 gels [Fig. 6(a) and(b), respectively]. *Mon. nitidum* showed no significant changes in the reduction of sugar content when its polysaccharide was digested by Agarase AS-II. It exhibit crude MAEF108-agarases that could rather digested Mon polysaccharides than utilized purified Agarase AS-II. When analyzed by HPLC, Mon also showed traces digested by Agarase AS-II, which produced rare non-reducing oligosaccharide fragments (RT = 23.48 min).

Table 1. Polyphenolic compound contents and Fe^{2+} ion chelating effects of two algal extracted polysaccharides or their agarase lysates[1].

Sample[2]	GAE/total sugar (μg/mg)[3]	Fe^{2+} Chelating effect (%)	EDTA equivalent (ppm)
Gra	$363.4 \pm 2.0^{a*}$	62.5 ± 2.5^{c}	5.3 ± 0.2^{c}
Gra-C108	586.1 ± 1.01^{c}	93.1 ± 1.2^{e}	7.5 ± 0.1^{e}
Gra-AS-II	580.4 ± 5.1^{c}	78.5 ± 0.7^{d}	6.4 ± 0.1^{d}
Mon	358.1 ± 1.8^{a}	21.3 ± 1.8^{a}	2.4 ± 0.4^{a}
Mon-C108	570.8 ± 1.0^{bc}	32.0 ± 0.1^{b}	3.1 ± 0.0^{b}
Mon-AS-II	561.8 ± 3.0^{bc}	27.3 ± 0.9^{b}	2.8 ± 0.1^{b}

[1]: Each value is the means ± standard deviation (n = 3). Data bearing different superscript letters in the same column are significantly different ($p < 0.05$).

[2]: Gra: *Gracilaria* sp. extracted polysaccharides; Gra-C108: *Gracilaria* sp. extracted polysaccharide lysates that digested by *A. salmonicida* MAEF108 crude agarases; Gra-AS-II: *Gracilaria* sp. extracted polysaccharide lysates that digested by Agarase AS-II; Mon: *Mon. nitidum* extracted polysaccharides; Mon-C108: *Mon. nitidum* extracted polysaccharide lysates that digested by *A. salmonicida* MAEF108 crude agarases; Mon-AS-II: *Mon. nitidum* extracted polysaccharide lysates that digested by Agarase AS-II.

[3]: Polyphenolic compound contents expressed as gallic acid equivalent.

In addition, neoagarooligosaccharide was absent [Fig. 6(c)].

2. Polyphenol contents and Ferrous-Chelating Antioxidant Power of SwPSExts, and Their Agarase Lysates

Wu *et al*. [16] fractionated algal oligosaccharide lysates and found that the polyphenolic fractions showed more antioxidative abilities, thus the polyphenolic compounds in Gra, Gra-C108, Gra-AS-II, Mon, Mon-C108, and Mon-AS-II were evaluated. The results shown in Table 1 are polyphenolic compound contents expressed as gallic acid equivalent (GAE). The GAE of the SwPSExt Gra was 363.4 μg/mg, and their agarase lysates, Gra-C108 or Gra-AS-II, were increased to 586.1 or 580.4 μg GAE per mg of total sugar, respectively, without significant difference ($p < 0.05$). GAE of SwPSExt Mon was 358.1 μg/mg, while that of their agarase lysates, Mon-C108 and Mon AS-II, were 570.8 and 561.8 mg per mg total sugar, respectively, without significant difference ($p < 0.05$).

The antioxidant potential of sulfated polysaccharides of brown seaweed *Fucus vesiculosus* examined using ferric reducing antioxidant power (FRAP) assay was found to be higher than that of the agar-like sulfated galactans from red seaweed [9]. Jimenez-Escrig *et al*. [5] also indicated that algal extracts that have chelating Fe^{2+} effects may due to polyphenolic compound contents and dose dependent. Chelating effects of SwPSExts and their agarase lysates on Fe^{2+} were further evaluated. Percentages of Fe^{2+}-chelating effects, and its EDTA equivalent, are shown in Table 1. The Fe^{2+}-chelating effect on Gra was 62.5% (equivalent to 5.3 ppm EDTA); and after digestion by *A. salmonicida* MAEF108 crude agarases, the chelating effect rose to 93.1% (equivalent to 7.5 ppm EDTA). The chelating effect on Gra-AS-II was not as strong as that on Gra-C108, though it still reached 78.5% (equivalent to 6.4 ppm EDTA). Mon showed chelating effect of 21.3%; and after digestion by enzyme, Mon-C108 and Mon-AS-II showed Fe^{2+}-chelating effect of 32.0% and 27.3%, respectively. Heo *et al*. [3] reported that the total soluble polyphenol content of brown seaweeds, hydrolyzed by carbohydrases or protease, performed better antioxidative abilities than the unhydrolyzed ones. Algal oligosaccharide lysate, as derived from *Porphyra dentate,* was fractionated by Wu *et al*. [16], who reported that the polyphenolic fraction possessed antioxidative properties, when monitored via their Fe^{2+}-chelating capacities. Comparison of the Fe^{2+}-chelating effects on Gra-C108, Gra-AS-II, Mon-C108, and Mon-AS-II showed that not only polyphenolic compounds but also sulfated polysaccharides possessed antioxidative properties. Wang *et al*. [11] also separated agar oligosaccharides AOS-1, AOS-2, and AOS-3 by ethanol fractionation and anion exchange chromatography. The agar oligosaccharides exhibited antioxidative activities in several antioxidative tests, such as scavenging hydroxyl free radicals and superoxide anion radicals, and inhibiting lipid peroxidation. The fragment of the sulfate group showed stronger antioxidative activities than that without the sulfate group. Wang *et al*. [11] indicated that antioxidative activities were closely related to the molecular mass of the agar oligosaccharides and the substitute groups bound in the carbohydrate ring. Therefore, neoagarooligosaccharides in both Gra-C108 and Gra-AS-II could possess larger antioxidative capacities than Mon-C108 and Mon-AS-II, without neoagarooligosaccharides. Moreover, neoagarobiose, neoagarotetraose and neoagaorohexaose show Fe^{2+}-chelating effects of 47.0 ± 1.1, 25.1 ± 2.1, and 2.5 ± 1.5%, revealing that neoagarooligosaccharides of low molecular weight have greater chelating effect on ferrous ion than those of high molecular weight [14]. Comparing their HPLC profiles also shows higher neoagarobiose content in their SwPSExts or agarase lysates with greater chelating effect on ferrous ion.

IV. CONCLUSION

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