The Comparison of Total Phenolics, Total Antioxidant, and Anti-Tyrosinase Activities of Korean Sargassum Species

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Received 4 November 2020; Revised 21 December 2020; Accepted 6 January 2021; Published 18 January 2021

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Sargassum species, a group of marine brown algae consumed in Asian countries, have shown various health benefits, such as improving the conditions of cardiovascular disease, osteoarthritis, and hypopigmentation. Also, these benefits are associated with their phenolic content and strong antioxidant capacities. However, the antioxidant capacities of different Sargassum species had not been thoroughly explored and compared. Thus, this study aimed to compare the total phenolic contents, total flavonoid contents, total antioxidant capacities, and anti-tyrosine activity of eleven Sargassum species harvested off the Korean coast. The results revealed that the total phenolic content (from 20.57 to 88.97 mg gallic acid equivalent/g dry weight (dw)), flavonoid content (from 22.08 to 82.33 mg quercetin equivalent/g dw), anti-tyrosinase activity (from 13.30 to 126.30 mg kojic acid equivalent/dw), and antioxidant capacities of the 11 Sargassum species had wide ranges. Among them, S. miyabei Yendo and S. hemiphyllum showed the highest total antioxidant capacities while S. miyabei Yendo exhibiting the highest total phenolic and flavonoid contents. The highest anti-tyrosinase activity was seen in S. fillicinum and S. yendoi. Sargahydroqunoic acid and sargachromanol, two alga-derived meroterpenoid compounds with strong antioxidant activity, were detected and quantified in S. miyabei Yendo and S. serratifolium. Our findings guarantee further investigation of the health benefits of Sargassum species and maximize the commercial usage of these species.

1. Introduction

Seaweed farming cultivates and harvests seaweed for a food source and non-food usage. According to the World Fisheries and Aquaculture, global production of farmed seaweeds increased from 13.5 million tons in 1995 to over 30 million tons in 2016. Sargassum, family Sargassaceae, order Fucales Kylin, class Cyclophorophyceae, is one of the eight genera that provide the majority (96.8%) of the world seaweed aquaculture production in 2018 [1]. With 361 taxonomically accepted species, Sargassum is considered one of the richest genera among the large brown seaweed [2].

Sargassum species inhabit shallow water and coral reefs throughout the world. Asian countries have a long history of consuming Sargassum species. The nutritional assessment showed that Sargassum contains a diversity of bioactive compounds, including vitamin C, vitamin E, β-carotene and dietary fiber, phenolic compounds, and polysaccharides. The known health benefits of Sargassum species include reducing the risk of chronic diseases including obesity, types of cancers, type 2 diabetes, and neurodegeneration diseases [3].

Endogenous reactive oxygen species (ROS) have critical physiological functions, such as signal transmission and defense against external substances. However, excessive
generation of ROS can cause oxidative stress which contributes to the development of various chronic diseases, such as type 2 diabetes and cardiovascular disease [4]. Along with antioxidant enzymes, dietary antioxidant intake is essential for the physiological redox balance [5]. Compared to the widely studied fruits and vegetables-derived antioxidants, algae are less acknowledged as a potent antioxidant source. A growing body of evidence shows that algae can synthesize a great variety of primary and secondary metabolites with strong antioxidant capacity, including carotenoids, phenolic compounds, and vitamins [6].

Tyrosinase is a multi-copper enzyme that functions as a rate-limiting enzyme in the synthesis of melanin, critical for undesired browning of fruits and vegetables and skin pigmentation [7]. Screening and development of effective tyrosinase inhibitors are of great importance for their applications in the cosmetic and food industries.

To the best of our knowledge, the nutritional facts of different Sargassum species had not been thoroughly compared. Large quantities of different species of Sargassum grow along the shores of South Korea. Here, we compared their nutrition density. By measuring the total phenolic content, total flavonoid content, antioxidant capacities, and anti-tyrosinase activities, we want to provide information for their use in human and animal nutrition. By measuring the total phenolic content, total flavonoid content, antioxidant capacities, and anti-tyrosinase activity of 11 species of Sargassum inhabiting in Korean coast, this study tried to present a nutrition spectrum of Korean Sargassum species and provide information for optimizing Sargassum culture based on their nutrition density.

2. Materials and Methods

2.1. Sample Preparation. Eleven standardized Sargassum extract powders (50 g of dried Sargassum sample was extracted with 10 volumes of 70% ethanol) were officially obtained from MBRIS. For analysis, 10 mg of Sargassum extract powders were dissolved with 1 mL of 80% methanol.

2.2. Total Phenolic Content. Total phenolic amount was measured by colorimetric assay with Folin & Ciocalteu’s reagent. Ten µL of appropriately diluted samples was mixed with 130 µL of distilled water in a 96-well plate. Subsequently, 10 µL of Folin & Ciocalteu’s was added. After 6 min reaction, 100 µL of 7% Na₂CO₃ solution was added. The absorbance of the mixture at 750 nm was measured after 90 min incubation in a microplate reader (ALLSHENG, Hangzhou, China). Total phenolic content in Sargassum species was expressed as mg gallic acid equivalents (GAE)/g dry weight.

2.3. Total Flavonoid Content. Total flavonoid contents were measure by the slightly modified Zhishen, Mengcheng method [8]. In a 96-well plate, 25 µL of samples was mixed with 160 µL of distilled water. 7.5 µL of 5% sodium nitrite (NaNO₂) was added to this mixture. Five minutes later, 10% aluminum chloride (AlCl₃) was added. Another 6 minutes later, 50 µL of 1 M sodium hydroxide (NaOH) was pipetted into designated wells. After mixing, the absorbance was measured against a blank at 510 nm with UV-spectrometer. Five milligrams of quercetin was dissolved in 80% methanol and then diluted to 25, 50, 100, and 150 µg/ml as standards. Total flavonoid contents were expressed by mg quercetin equivalent (QE)/g dry weight.

2.4. ABTS Radical Scavenging Assay. Antioxidant activity was measured using a group of assays. ABTS radical scavenging assay was slightly modified from the van den Berg method [9]. In brief, 1.0 mM of 2,2‘-azobis(2-amidinopropane) dihydrochloride (AAPH) was mixed with 2.5 mM 2,2‘-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminonium salt (ABTS) in 100 mM of phosphate-buffered solution (PBS). The mixture was heated in a water bath at 75°C for 3 min to generate ABTS radical. After the ABTS radical was produced, the radical solution was filtered through 0.45 µm PVDF filter. The ABTS solution was diluted with PBS to make the absorbance at 734 nm between 0.650 ± 0.020. Four microliters of diluted Sargassum species samples was pipetted to 196 µL of the ABTS radical solution. The mixture was incubated at 37°C for 10 min. The decrease in absorbance at 734 nm was measured after 10 min. The antioxidant capacity of Sargassum species was expressed as mg vitamin C equivalent (VCE)/g dry weight.

2.5. DPPH Radical Scavenging Assay. The DPPH assay was carried out according to Blois, M. S. method with a slight modification [10]. Briefly, 7.89 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved into 80% (v/v) aqueous methanol to make the concentration to 100 µM. Subsequently, the absorbance of DPPH radical solution was adjusted as 0.720 ± 0.020 with 80% methanol at 517 nm. Five µL of diluted Sargassum species sample was mixed with 295 µL of DPPH solution. The decreased absorbance of the sample and radical solvent mixture between sample extract and the radical solution was measured at 510 nm after reaction for 30 min in a dark room at room temperature. 80% aqueous methanol was used for the reference instead of samples, and vitamin C was used to make a standard curve. The DPPH radical scavenging capacity of Sargassum species was expressed as mg vitamin C/g dry weight.

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay. Ferric reducing power of Sargassum species was measured using slightly modified Benzie and Strain’s method [11]. FRAP reagent was prepared by mixing 10 volumes of acetate buffer (300 mM, pH 3.6) with 1 volume of 10 mM TPTZ (2,4,6-tril[2-pyridyl]-s-triazine) solution, 1 volume of 20 mM of FeCl₃ solution, and 1.2 volume of distilled water. The
FRAP reagent was kept at 37°C until use. Samples were mixed with 250 μL of FRAP reagent and incubated for 4 min at 37°C. The absorbance was measured at 593 nm. Ferrous sulphate (FeSO₄) was used to make a standard curve (0.1 mM to 1.0 mM). The reducing power was expressed as mM FeSO₄ equivalent/g dw.

2.7. Superoxide Radical Scavenging Assay. Superoxide radical scavenging activity was measured by the modified method of Zhang [12]. For measuring superoxide radical scavenging ability, 300 μM nitroblue tetrazolium (NBT), 60 μM of phenazine methosulphate (PMS), and 468 μM of NADH were dissolved in 16 μM Tris–HCl buffer (pH 8.0). The sample was mixed with the same volume of NBT and NADH. Afterward, the same volume of PMS was injected for a reaction. In the reaction mixture that contained NADH and NBT, adding PMS initiates NADH oxidation to generate superoxide radicals to reduce NBT. After 5 min incubation at room temperature, the absorbance was measured at 560 nm. Inhibition rate (%) of superoxide radical scavenging assay was expressed as (A blank–A sample)/A blank × 100.

2.8. Tyrosinase Inhibitor Activity. To measure tyrosinase inhibitor activity, first 110 μL of 0.1 M phosphate buffer saline (pH7.0), 10 μL of the diluted sample, and 10 μL of 1,000 units/ml mushroom tyrosinase were injected in 96 well plates and incubated for 30 min at 37°C. Additionally, 20 μL of 1.5 mM tyrosine was added to the incubated mixture. After incubating 15 min at 37°C, the absorbance was measured at 490 nm using a spectrometer. Ten milligrams of kojic acid was dissolved in 0.1 M PBS and then diluted to 62.5, 125, 250, and 500 mg/L for standard. Tyrosinase inhibitor activity was expressed as mg kojic acid equivalent (KAE)/g dw. And, the tyrosinase inhibitory activity used by L-DOPA was measured in a microtube to add 170 μL of 0.1 M PBS (pH 7.0), 10 μL of properly diluted samples, and 10 μL of mushroom tyrosinase (1,000 units/ml) and mixed by using vortex. After transferring the mixture to 96 well plates, incubated for 6 min at 37°C, 10 μL of 3 mM L-DOPA was injected and incubated for 1 min at 37°C. Absorbance was measured at 490 nm. The standard for tyrosinase inhibitor activity with L-DOPA was calculated by using 31.12, 62.50, and 125 mg/L kojic acid and expressed as mg KAE/g dw.

2.9. UPLC Chromatographic Analysis. Quantification of Sargahydroquinic acid (SHQA) and sargachromanol (SCM) were conducted by Waters UPLC, Aquity-PDA device. The column used was Acquity UPLC C18 1.7 μm × 2.1 × 50 mm column (Waters, Milford, MA, USA) for UPLC. And, column's temperature was 35°C. The linear gradient of mobile phases between 0.1% formic acid in distilled water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) was used as follows: 0 min (90:10), 1 min (90:10), 3 min (50:50), 6 min (30:70), 11 min (0:100), 12 min (0:100), 15 min (50:50), 17 min (90:10), and 19 min (90:10). The flow rate was 0.400 ml/min. And, scanning range was 210–600 nm. Each compound was identified by retention time (RT). SHQA and SCM were detected at 270 nm absorbance. As a standard for quantification, purified SHQA and SCM were kindly provided by Dr. Heyung-Rak Kim's laboratory in Pukyong National University. The limit of detection and the limit of quantification were expressed 3 * Sᵦ/b and 10 * Sᵦ/b, where Sᵦ was the standard deviation of the reposed and b was the slope of the standard curve, the standard deviation of y residuals of regression line.

2.10. Statistical Analysis. All analyses were repeated three times. One-way ANOVA and Tukey’s post hoc test were performed using Graphpad 7.0 (Graphpad Software, San Diego, USA). Significant differences between the results were analyzed using a Tukey post hoc test at a significance level of P < 0.05.

3. Results

3.1. Total Phenolic and Total Flavonoid Contents. Phenolic compounds have a specific structure, hydroxy-substituted benzene ring, which can make them have strong antioxidant capacity. As shown in Table 1, the total phenolic content ranged from 20.57 to 88.97 mg gallic acid equivalent of dry weight which was about 4 times different between the highest and lowest samples. S. miyabei Yendo (88.97 ± 4.34) and S. hemiphyllum (83.22 ± 1.72) exhibited the highest phenolic content, while S. confusum (20.57 ± 1.00), S. coreanum (23.82 ± 0.88), S. macrocarpum (26.32 ± 1.72), and S. micracanthum (28.62 ± 1.00) had the lowest.

Total flavonoid contents of Sargassum species are also listed in Table 1. The total flavonoid content ranged from 22.08 to 82.33 mg quercetin equivalent of dry weight which was 3.7 times different between the highest and lowest samples. Same as phenolic content, the 2 species that showed the greatest amount of flavonoid were S. miyabei Yendo (82.33 ± 3.23) and S. hemiphyllum (72.58 ± 4.91), and S. coreanum (22.08 ± 0.65), S. macrocarpum (22.08 ± 1.32), S. confusum (23.58 ± 3.23), and S. micracanthum (23.95 ± 2.50) had the lowest flavonoid content.

3.2. Antioxidant Capacity. An array of assays was adopted to determine the antioxidant capacity of various species of Sargassum. As shown in Table 2, in the same with the total phenolic and flavonoid contents, antioxidant capacities showed a wide range. The strongest antioxidant activity was 8.8, 8.4, 2.2, and 36.8 times higher than the lowest activity, based on ABTS, DPPH, superoxide radical scavenging, and FRAP assays, respectively.

S. hemiphyllum and S. miyabei Yendo showed the highest ABTS and superoxide scavenging activities, whereas S. miyabei Yendo exhibited higher DPPH and FRAP activities than S. hemiphyllum.

The four species, S. coreanum, S. macrocarpum, S. confusum, and S. micracanthum, containing the lowest phenolic and flavonoid contents also showed the lowest ABTS, DPPH, and FRAP activities. The range of superoxide radical scavenging activity was less wide compared to the other three activities. There were no significant differences among 10 out of
ability in 11 species of Sargassum species. Total antioxidant capacity of Sargassum species.

### Table 1: Total phenolic and total flavonoid contents of Sargassum species.

| Species       | Total phenolic content (mg GAE/g dw) | Total flavonoid content (mg QE/g dw) |
|---------------|------------------------------------|-------------------------------------|
| S. confusum   | 20.57 ± 1.00d                       | 23.58 ± 3.23d                       |
| S. coreanum   | 23.82 ± 0.88d                       | 22.08 ± 0.65d                       |
| S. filicinum  | 56.21 ± 1.00b                       | 65.38 ± 7.77b                       |
| S. hemiphyllum| 83.22 ± 1.72a                       | 72.58 ± 4.91ab                      |
| S. horneri    | 36.09 ± 1.00c                       | 36.58 ± 2.63c                       |
| S. macrocarpum| 26.32 ± 1.72d                       | 22.08 ± 1.32d                       |
| S. micranthum | 28.62 ± 1.00d                       | 23.95 ± 2.50d                       |
| S. miyabei Yendo | 88.97 ± 4.34a | 82.33 ± 3.24a |
| S. serratifolium| 55.63 ± 6.22b | 46.83 ± 2.48a |
| S. silicuasrum| 40.11 ± 1.72c                       | 32.95 ± 2.02cd                      |
| S. yendoi     | 42.41 ± 1.00c                       | 40.70 ± 4.20c                       |

Table 1: Total phenolic and total flavonoid contents of Sargassum species.

Table 2: Antioxidant capacity of Sargassum species.

| Species       | ABTS (mg VCE/g dw) | DPPH (mg VCE/g dw) | Superoxide radical (%) | FRAP (mM Fe2+ equivalent/g dw) |
|---------------|--------------------|--------------------|------------------------|-------------------------------|
| S. confusum   | 25.65 ± 1.22d      | 38.56 ± 4.82d      | 36.71 ± 0.77b          | 0.026 ± 0.003d               |
| S. coreanum   | 32.59 ± 0.46d      | 39.96 ± 3.38d      | 23.11 ± 2.30bc         | 0.092 ± 0.009d               |
| S. filicinum  | 140.05 ± 4.69b     | 78.21 ± 0.61c      | 40.39 ± 1.80b          | 0.158 ± 0.011cd              |
| S. hemiphyllum| 205.65 ± 7.17a     | 137.86 ± 5.40b     | 51.94 ± 3.07a          | 0.645 ± 0.065b               |
| S. horneri    | 74.19 ± 3.70c      | 61.02 ± 1.61c      | 30.98 ± 1.11bc         | 0.135 ± 0.020d               |
| S. macrocarpum| 32.85 ± 1.22d      | 27.68 ± 0.61d      | 32.21 ± 3.54bc         | 0.068 ± 0.006d               |
| S. micranthum | 23.25 ± 2.43d      | 23.12 ± 2.43d      | 34.76 ± 2.26b          | 0.038 ± 0.020d               |
| S. miyabei Yendo | 186.19 ± 23.15a  | 193.65 ± 23.19a    | 43.66 ± 3.38ab         | 0.956 ± 0.094a               |
| S. serratifolium| 99.52 ± 2.88c     | 75.40 ± 1.82c      | 34.76 ± 3.38b          | 0.286 ± 0.020c               |
| S. silicuasrum| 76.32 ± 2.12c      | 66.28 ± 4.86c      | 40.39 ± 0.71ab         | 0.195 ± 0.018b               |
| S. yendoi     | 89.39 ± 4.41c      | 59.61 ± 2.79d      | 38.04 ± 5.07b          | 0.236 ± 0.043c               |

Table 2: Antioxidant capacity of Sargassum species.

Table 3: Tyrosinase inhibition activity of Sargassums.

| Species       | L-tyrosine (mg KAE/g dw) | L-DOPA (mg KAE/g dw) |
|---------------|--------------------------|----------------------|
| S. confusum   | 15.63 ± 1.76a            | 19.65 ± 3.11b        |
| S. coreanum   | 18.97 ± 4.31a            | 26.23 ± 11.13b       |
| S. filicinum  | 117.63 ± 1.61b           | 50.07 ± 1.23a        |
| S. hemiphyllum| 88.6 ± 3.61c             | 47.3 ± 1.61a         |
| S. horneri    | 43.47 ± 1.44c            | 30.32 ± 1.15b        |
| S. macrocarpum| 14.63 ± 2.36a            | 19.57 ± 0.88b        |
| S. micranthum | 13.30 ± 0.50a            | 19.98 ± 0.75b        |
| S. miyabei Yendo | 70.47 ± 2.57d         | 26.07 ± 2.92b        |
| S. serratifolium| 50.47 ± 4.16c          | 29.73 ± 7.81b        |
| S. silicuasrum| 41.97 ± 0.76c            | 24.32 ± 1.53b        |
| S. yendoi     | 126.3 ± 0.50a            | 52.65 ± 0.14n        |

Table 3: Tyrosinase inhibition activity of Sargassums.

3.3. Measurement of Tyrosinase Inhibitor Activity with L-Tyrosine and L-DOPA. Tyrosinase is an enzyme responsible for the synthesis of melanin, the pigment responsible for skin color. Presently several natural or synthetic compounds have been used to inhibit tyrosinase and have also been used by the cosmetic industry as a skin whitening and anti-wrinkling agent [13]. Tyrosinase inhibition activity assay results are shown in Table 3. It was expressed as mg kojic acid equivalent per gram of dry weight. When tyrosine was used as substrate, S. yendoi (126.30 ± 0.50) showed the highest anti-tyrosinase activity, followed by S. filicinum (117.63 ± 1.61), S. hemiphyllum (88.60 ± 3.61), and S. miyabei Yendo (70.47 ± 2.57). When L-DOPA was used as a substrate, S. yendoi (52.65 ± 0.14), S. filicinum (50.07 ± 1.23), and S. hemiphyllum (47.330 ± 1.61) showed the highest tyrosinase inhibition ability.

3.4. Correlation between Antioxidant Capacity and Anti-Tyrosinase Activity. To determine the association between total phenolic contents, total flavonoid contents, and antioxidant ability in 11 species of Sargassum, Pearson correlation analysis was conducted. In Table 4, the results showed that the total phenolic contents, total flavonoid contents, and antioxidant activity were significantly positively correlated. Through this, it was confirmed that various methods of measuring antioxidant capacity could be used to measure the antioxidant capacity of Sargassum. Besides, the total phenolic content and flavonoid content showed a significant
3.5. Quantification of SHQA and SCM. As shown in Figure 1, SCM and SHQA were measured through UPLC in 11 Sargassum species. Retention time (RT) of SHQA and SCM purified standards was detected at 5.60 and 6.68 min, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of SCM were 0.014 mg/L and 0.045 mg/L, and LOD and LOQ of SHQA were 0.28 mg/L and 0.933 mg/L. Among the 11 Sargassum species extracts, SHQA and SCM were detected in S. miyabei Yendo and S. serratifolium. In the extract of S. miyabei Yendo, SHQA was quantified as 118.44 ± 0.87 mg/g and SCM as 17.82 ± 0.10 mg/g. Also, S. serratifolium extract contained 17.75 ± 0.12 mg/g SHQA and 1.05 ± 0.01 mg/g SCM.

4. Discussion

The Republic of Korea, as a major producer of maricultured seaweed, has experience in artificial production and cultivation of Sargassum species [14, 15]. In an effort to identify the Sargassum species growing off the Korean coast and develop the nutrient intensive Sargassum species for cultivation, all the accessible Sargassum species off the Korean coast were collected from MBRIS and their polyphenol contents were analyzed.

A regular intake of phenolic compounds exhibits a potential for the prevention or reduction in the risk of degenerative disease associated with oxidative stress, such as cardiovascular disease, diabetes, and obesity [16, 17]. Among the phenolic compounds, flavonoids are the largest group that has received major attention in research. TPC and TFC from several Sargassum species had been tested in other studies. However, due to the differences in extraction methods and different equivalents used, the results from different studies were incomparable. For example, TPC of methanol extracts from S. horneri was 0.51 ± 0.02 mg GAE/g in Yin and Woo’s research, while the analysis of Generalic Mekinic showed 9.90 mg pyrocatechol equivalent/g in methanol extract of S. horneri. [18–21]. In our study, TPC and TFC of a variety of Sargassum species were analyzed and the highest concentrations were discovered in S. miyabei Yendo and S. hemiphyllum. Furthermore, by using the same equivalent, we found that our results suggest that flavonoids were the major phenolic contents in Sargassum species, which is corroborated by the strong Pearson correlation (R = 0.97) between TPC and TFC.

The total antioxidant capacities were analyzed by ABTS, DPPH, FRAP, and superoxide radical scavenging assays.
Tyrosinase is critical in the melanogenesis and enzymatic browning. Its inhibitors attract great attention in the cosmetic industry as depigmentation agents and in the food industry as antibrowning compounds. Phenolic compounds can inhibit tyrosinase competitively by mimicking the substrate of tyrosinase [7]. Our research indicated that S. fillicinum and S. yendoi, which contained moderate amounts of phenolics and flavonoids showed the strongest inhibition against tyrosinase. This suggests that the composition rather than the concentration of phenolics have a bigger influence on tyrosinase. In addition, nonphenolic compounds may also exert anti-tyrosinase activity. For example, astaxanthin and fucoxanthin, two carotenoids that occurring naturally in brown algae, had shown anti-tyrosinase activities [27,28]. Further studies are needed to identify the bioactive compounds in the extracts that exert strong anti-tyrosinase activity.

SCM and SHQA are known bioactive components in Sargassum species. The structure of SCM shows that SCM is a natural homologue of δ-tocotrienol with an additional carboxyl group in polyphenyl tail [29]. SHQA and SCM are two bioactive components isolated from various Sargassum species that have drawn growing attention. These two meroterpenoid compounds have shown various biological benefits. For example, SCM and SHQA extracted from S. serratifolium showed anti-inflammatory, anti-hyperpigmentation, and anti-obesity activities in vivo and in vitro studies, [30], [31]. Our research showed that only two species exhibited detectable amounts of SCM and SHQA among 11 Sargassum species. Further studies are needed to have a more comprehensive investigation of the health benefits of Sargassum species and maximize the commercial usage of these species.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was a part of the project titled "Future fisheries food research center", funded by the Ministry of Oceans and Fisheries, Korea, and was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science, ICT & Future Planning (MSIP)) (no. CD20200160).

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