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Variation in the Chemical Composition of *Saccharina Japonica* with Harvest Area and Culture Period

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Abstract

Saccharina japonica is commercially important marine brown algae which grow as a single blade (reaching 10 meters in length) with a short stipe. In this study, the edible brown weed *Sacchaina japonica* was assessed for nutritional composition. Samples were collected monthly from seaweed farms at Kijang and Wando on the south coast of the Republic of Korea, during the 2011 culture season. *S. japonica* in Kijang and Wando showed the highest crude protein content in February and the highest carbohydrate content in July. Monthly changes in sugar, fatty acid, mineral, and total amino acid contents observed from February to July 2011. Fucose was the most abundant and galactose the second most abundant in the monosaccharide composition profiles, while mannose, glucose, xylose, ribose, and rhamnose were present in low quantities and lactose, mannitol, and arabinose were not detected. Significant increases of the major fatty acids in Kijang (C18:2 n-6 and C20:4 n-6) and Wando (C18:3 n-6) were observed as the culture period progressed. The highest mineral content of both Kijang and Wando samples is potassium and followed by sodium, calcium, magnesium, and so on. In the total amino acid contents, Kijang samples increased from February to April but decreased from May to July, while Wando samples increased on March but decreased from April to July.

Keywords: *Saccharina japonica*; Brown algae; Harvest area; Culture period; Chemical composition

Introduction

China, Japan, and the Republic of Korea are the largest consumers of edible seaweeds [1]. Seaweeds include high alginic acid, fucoidan, and laminara contents, so it is effective for hematocele and lipid metabolism improvement such as lowering blood pressure and cholesterol in the blood, and anti-cancer [2]. According to a survey conducted on worldwide production of aquatic plants, there are approximately 16 million tons of annual aquatic plants, of which 14.9 million tons produced by aquaculture [3]. Algal production in Korea is mainly limited to *Porphyra tenera*, *Saccharina japonica*, and *Undaria pinnatifida*, which comprise 94% of the total harvested seaweed [4]. *S. japonica* is very popular as a healthy food because of low calorie and abundant vitamin, mineral, dietary fiber, calcium, potassium, magnesium, phosphoric acid, and microelements and high iodine content as compared with other seaweeds [5].

In recent years, many studies on macro-algae have carried out and their proximate composition differs according to species, geographic origin, and seasonal conditions [6,7]. Growth change of laminaria closely related with culture period, most researchers studied to determine correlation between growth and nitrogen concentration [8]. Moreover, growth and chemical composition are various in different environments such as current, nutrients supply, fresh water inflow, and water temperature. Perennial *Saccharina japonica* generates alternately, and grows at subantarctic zone as well as temperate climate regions [9]. Cosson [10] reported that survival rate of *Laminaria digitata* spores is substantially lowered at over radiation intensity (about 170 $\mu E \cdot m^{-2} \cdot s^{-1}$). Kang and Koh [11] found that optimal growth temperature and light intensity of *Laminaria japonica* sporophyte were at 10°C and 70 $\mu E \cdot m^{-2} \cdot s^{-1}$.

To our knowledge, detailed studies have not conducted to evaluate the effects of the culture period and harvest area on the chemical composition of *S. japonica*. This fundamental study performed to assess changes of proximate composition, sugar, fatty acid, mineral, and amino acid of *S. japonica* obtained from two sampling regions in Korea, Kijang and Wando, which had definitely different environment, and during the culture period from February to July.

Materials and Methods

Sampling

In order to observe variations in chemical composition during the harvest time, *S. japonica* was collected from an environmentally quite different seaweed farm at Kijang and Wando located on the southern coast of the Republic of Korea once a month from February to July 2011 (Figure 1). Both sporophytes of *S. japonica* transferred to the ocean at 0.5 m water depth in the same time (December 2010), and 5-20 individuals of whole *S. japonica* (blade, stem, and root) collected during the 2011 culture season. Freshly collected plants wrapped in paper towels with seawater, sealed in plastic bags, kept in an icebox, and transport to the laboratory where they washed with distilled water twice and freeze-dried. Each powered *S. japonica* (about 500 g) used for triplicate analysis.

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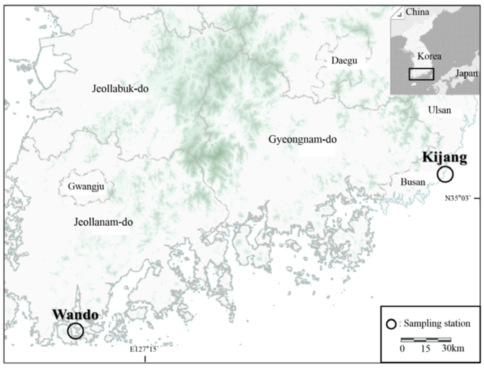

Figure 1: A map showing the site where *Saccharide japonica* were harvested during the 2011 culture period.

Condition	
Column	Shim-pack ISA-07 (4.0 mm×250 mm)
Mobile phase	A: potassium borate (pH 8.0) B: potassium borate (pH 9.0)
Flow rate	0.6 mL/min, gradient
Reagent	1% arginine in 3% boric acid (0.5 mL)
Reaction temperature	150°C
Detector	Fluorescence detector (Ex=320, Em=430)
Oven temperature	65°C

Table 1: HPLC operating conditions for component sugars.

General component analysis

Moisture, crude protein, crude lipid, and ash content were determined using the standard methods described by the Association of Official Analytical Chemists [12]. Protein content analyzed using the semi-Kjeldahl method. Lipids extracted with anhydrous diethyl ether using a Soxhlet apparatus. Moisture quantified by oven drying the samples at 105°C for 12 h. Ash was determined after incineration in a furnace at 550°C. Total carbohydrate content calculated by subtracting the sum of moisture, crude protein, crude lipid, and ash mass from that of the total sample [13].

Component sugar analysis

In order to extract component sugar, a test sample (100 mg) mixed in the 15 mL test tube with 5 mL of 2M HCl. The oxygen in the test tube replaced by nitrogen gas, sealed, and placed in a heating mantle at 100°C for 5 h [14]. Hydrolyzed sample cooled, neutralized by adding 5 mL of 2M NaOH, and centrifuged at 650 g for 30 min. 3 mL supernatant filtered through a Millipore membrane (0.45 μm pore size), and analyzed by operating conditions (Table 1) using HPLC (Prominence HPLC, Shimadzu Co, Ltd. Kyoto, Japan).

Fatty acid composition analysis

Bligh and Dyer extraction was performed using the following method [15]: Briefly, lipids were extracted from 5-g samples by homogenization with 100 mL of chloroform and 200 mL methanol. The samples were then filtered and evaporated to remove solvent. Fatty acid methyl esters (FAME) were prepared using boron trifluoride (BF3) according to a method described by the AOAC [12]. Quantitative analysis of FAME was carried out on a GC-2010 gas chromatograph(Shimadzu Co., Japan) equipped with a split/splitless capillary inlet system and a flame ionization detector (FID) using SP-2560 capillary columns (0.20-μm stationary phase thickness, 100 mm (length)×0.25 mm (i.d.); Supelco, Inc., USA). The sample (0.5 μl) was injected in the split mode using an automatic injection system (AOC-20i, Shimadzu Co., Japan). The oven temperature was programmed to increase from 160 to 220°C at 1°C min^{-1} with an initial hold of 5 min and final hold of 40 min. The other operation parameters were as follows: injector temperature, 250°C; detector temperature, 250°C; helium carrier gas flow, 20 cm s^{-1}; split ratio, 1:50. The peak areas for the calibration curves and for calculation of fatty acid composition of oil samples were measured using a GC Solution system (Shimadzu Co., Japan).

Mineral contents

For the determination of mineral elements (calcium, copper, iron, potassium, magnesium, manganese, sodium, and zinc), samples were digested by dry ashing and dissolved in 1 M HCl [12]. The final diluted solution for calcium contained 1% lanthanum to overcome interferences. The concentration of the elements in *S. japonica* were determined with atomic absorption spectrophotometry (Perkin-Elmer, model 3110). Triplicate determinations for each element were carried out. The concentration of the elements were determined from calibration curves of the standard elements.

Amino-acid analysis

Samples (0.5 g) were acid-hydrolyzed with 3 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 121°C for 24 h. Tubes were cooled after hydrolysis, opened, and placed in a rotary evaporator at 50°C to remove HCl from the sample. The residue was then adjusted to pH 2.2 with 0.2 M sodium citrate loading buffer (pH 2.2), diluted to a final volume of 10 mL with water, filtered through a Millipore membrane (0.2 μm pore size), and analyzed for amino acids using an amino-acid analyzer (Pharmacia Biochrom 20, Biochrom Ltd., UK).

Statistical analysis

All mean values were analyzed by one-way analysis of variance (ANOVA, SPSS 1999). Values are expressed as mean ± standard deviation (SD; n=3 replicates). Group means were considered to be significantly different at $p<0.05$.

Results

Changes in proximate composition with harvest area and culture period

The proximate compositions of Kijang and Wando samples are shown in Tables 2 and 3. There was a high variation in moisture, crude protein, ash and crude lipid content with culture period and harvest area among the Kijang and Wando samples collected at different months from February to July. *S. japonica* in Kijang and Wando showed the highest crude protein content in February and the highest carbohydrate content in July. In the crude lipid content, February samples in Kijang and Wando generally tended to decrease until July. There was a high variation in ash content with culture period and harvest area, ranging from 14.29 ± 1.47% to 19.39 ± 0.75% (Tables 2 and 3).

Changes in component sugar and fatty acid composition with harvest area and culture period

Component sugar compositions of Kijang and Wando samples are shown in Tables 4 and 5. Fucose was the most abundant and galactose the second most abundant in the monosaccharide composition profiles. Mannose, glucose, xylose, ribose, and rhamnose were present at low

Component	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Moisture	10.55 ± 0.51[a]	10.67 ± 0.45[a]	10.25 ± 0.49[a]	10.31 ± 0.98[a]	10.45 ± 1.41[a]	10.41 ± 0.22[a]
Crude protein	9.39 ± 0.45[a]	8.54 ± 0.36[b]	7.61 ± 0.34[c]	7.27 ± 0.70[cd]	6.62 ± 0.51[d]	5.72 ± 0.51[e]
Crude lipid	1.69 ± 0.08[a]	1.43 ± 0.06[b]	1.35 ± 0.06[bc]	1.09 ± 0.12[d]	1.23 ± 0.14[cd]	1.17 ± 0.02[d]
Ash	15.11 ± 0.73[b]	17.88 ± 0.72[ab]	18.39 ± 0.75[a]	17.86 ± 2.58[ab]	17.35 ± 2.04[ab]	16.51 ± 0.34[ab]
Carbohydrate[b]	63.26 ± 3.02[a]	61.48 ± 2.59[a]	62.40 ± 2.61[a]	63.47 ± 6.78[a]	64.35 ± 5.62[a]	66.19 ± 1.23[a]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05). [b]Carbohydrate content (%)=100-(% moisture + % protein + % lipid + % ash).

Table 2: Seasonal variation of proximate composition (%) in the dried sea tangle (*S. japonica*) cultured at Kijang area[a]

Component	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Moisture	10.38 ± 0.47[a]	10.51 ± 0.45[a]	10.45 ± 0.50[a]	10.12 ± 0.96[a]	10.34 ± 1.18[a]	10.46 ± 0.19[a]
Crude protein	8.20 ± 0.36[a]	8.20 ± 0.40[a]	7.51 ± 0.31[a]	6.54 ± 0.71[b]	5.58 ± 0.80[c]	5.15 ± 0.14[c]
Crude lipid	2.00 ± 0.09[b]	2.35 ± 0.09[a]	1.56 ± 0.07[c]	1.37 ± 0.21[cd]	1.26 ± 0.10[d]	1.23 ± 0.02[d]
Ash	16.68 ± 0.77[ab]	17.35 ± 0.73[a]	17.86 ± 0.80[a]	15.82 ± 2.39[ab]	14.29 ± 1.47[b]	14.69 ± 0.29[b]
Carbohydrate[b]	62.74 ± 2.89[a]	61.59 ± 2.61[a]	62.62 ± 2.91[a]	66.15 ± 7.29[a]	68.53 ± 7.22[a]	68.47 ± 0.49[a]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05). [b]Carbohydrate content (%)=100-(% moisture + % protein + % lipid + % ash).

Table 3: Seasonal variation of proximate composition (%) in the dried sea tangle (*S. japonica*) cultured at Wando area[a]

Sugar	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Rhamnose	0.11 ± 0.00[a]	0.08 ± 0.00[b]	0.08 ± 0.00[b]	0.04 ± 0.00[c]	0.04 ± 0.00[c]	0.03 ± 0.01[d]
Ribose	0.19 ± 0.00[a]	0.10 ± 0.00[b]	0.10 ± 0.00[b]	0.01 ± 0.00[d]	0.02 ± 0.00[c]	0.01 ± 0.01[d]
Mannose	0.66 ± 0.01[b]	0.55 ± 0.01[d]	0.65 ± 0.01[b]	0.72 ± 0.02[a]	0.60 ± 0.01[c]	0.56 ± 0.02[d]
Fucose	3.32 ± 0.08[c]	4.17 ± 0.13[b]	4.84 ± 0.12[a]	2.68 ± 0.06[d]	2.27 ± 0.06[f]	2.52 ± 0.16[e]
Galactose	2.31 ± 0.06[a]	1.92 ± 0.04[b]	1.97 ± 0.06[b]	0.80 ± 0.02[d]	0.88 ± 0.02[c]	0.71 ± 0.11[e]
Xylose	0.35 ± 0.01[a]	0.24 ± 0.01[b]	0.16 ± 0.00[c]	0.06 ± 0.00[d]	0.05 ± 0.00[e]	0.05 ± 0.00[e]
Glucose	0.55 ± 0.01[d]	0.64 ± 0.02[c]	0.68 ± 0.02[b]	0.73 ± 0.01[a]	0.72 ± 0.02[a]	0.53 ± 0.12[d]
Total	7.48 ± 0.17[b]	7.70 ± 0.17[b]	8.47 ± 0.21[a]	5.03 ± 0.13[c]	4.56 ± 0.11[d]	4.40 ± 0.14[d]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05).

Table 4: Seasonal variation of component sugar in the dried sea tangle (*S. japonica*) cultured at Kijang area[a]

Sugar	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Rhamnose	0.12 ± 0.00[b]	0.13 ± 0.00[a]	0.07 ± 0.00[c]	0.03 ± 0.00[d]	0.02 ± 0.00[e]	0.02 ± 0.00[e]
Ribose	0.19 ± 0.01[a]	0.10 ± 0.00[b]	0.09 ± 0.00[c]	0.01 ± 0.00[e]	0.02 ± 0.00[d]	0.01 ± 0.01[e]
Mannose	0.75 ± 0.02[a]	0.67 ± 0.02[c]	0.74 ± 0.02[a]	0.70 ± 0.02[b]	0.59 ± 0.01[d]	0.47 ± 0.08[e]
Fucose	3.49 ± 0.09[a]	2.98 ± 0.06[b]	2.92 ± 0.08[bc]	3.01 ± 0.07[b]	2.83 ± 0.05[c]	1.85 ± 0.54[d]
Galactose	2.21 ± 0.05[a]	1.65 ± 0.04[c]	1.81 ± 0.04[b]	0.80 ± 0.02[d]	0.80 ± 0.01[d]	0.63 ± 0.09[e]
Xylose	0.43 ± 0.01[a]	0.28 ± 0.01[c]	0.30 ± 0.01[b]	0.06 ± 0.00[d]	0.07 ± 0.00[d]	0.05 ± 0.01[e]
Glucose	0.67 ± 0.02[b]	0.67 ± 0.02[b]	0.73 ± 0.02[a]	0.72 ± 0.01[a]	0.42 ± 0.01[c]	0.42 ± 0.01[c]
Total	7.87 ± 0.21[a]	6.48 ± 0.14[b]	6.65 ± 0.21[b]	5.34 ± 0.16[c]	4.76 ± 0.11[d]	3.45 ± 0.73[e]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05).

Table 5: Seasonal variation of component sugar in the dried sea tangle (*S. japonica*) cultured at Wando area[a].

quantities, and lactose, mannitol, and arabinose were not detected.

The fatty acid compositions of Kijang and Wando samples are shown in Tables 6 and 7. Lignoceric acid (24:0) was the most abundant fatty acid, followed by arachidonic acid (20:4 n-6), oleic acid (18:1 n-9), and palmitic acid (16:0). Polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) constituted about 54.9%, 52.3% of total fatty acids, and saturated fatty acids (SFA) represented 45.1%, 47.7% of the total fatty acids in the Kijang and Wando samples, respectively. The Kijang-Jul samples showed the highest PUFA composition (37.5%) among the samples, while Wando-Mar showed the lowest PUFA composition (30.1%), indicating that there was a high variation in fatty acid contents with the harvest area and culture period.

Changes in mineral content and total amino acid composition with harvest area and culture period

The mineral contents of Kijang and Wando samples are shown in Tables 8 and 9. The results show that *S. japonica* is rich in K and Na with moderate amounts of Ca and Mg whereas Cu, Fe, Mn, and Zn are present in small quantities. The total amino acid (TAA) compositions of Kijang and Wando samples are shown in Tables 10 and 11. Glutamic acid, aspartic acid, alanine, and leucine were the most common amino acids in all samples, while the percentage of cysteine was the lowest in the TAA profile. TAA of Kijang samples decreased during the harvest time from April to July while TAA of Wando samples decreased from March to July.

Fatty acid (%)	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
12:0	0.18 ± 0.01[c]	0.27 ± 0.01[a]	0.21 ± 0.00[b]	0.03 ± 0.00[f]	0.05 ± 0.00[e]	0.07 ± 0.00[d]
14:0	9.50 ± 0.21[a]	9.66 ± 0.19[a]	7.11 ± 0.15[c]	4.73 ± 0.15[e]	7.68 ± 0.15[b]	6.02 ± 0.12[d]
16:0	13.53 ± 0.31[d]	16.25 ± 0.35[b]	17.22 ± 0.22[a]	17.34 ± 0.40[a]	14.51 ± 0.33[c]	11.07 ± 0.27[e]
16:1 n-7	3.08 ± 0.08[c]	3.78 ± 0.12[a]	3.44 ± 0.08[b]	3.06 ± 0.07[c]	3.49 ± 0.09[b]	3.20 ± 0.09[c]
18:0	0.86 ± 0.02[c]	1.00 ± 0.02[b]	1.16 ± 0.03[a]	0.69 ± 0.02[d]	0.87 ± 0.02[c]	0.89 ± 0.02[c]
18:1 n-9	19.20 ± 0.40[a]	16.83 ± 0.42[c]	15.67 ± 0.42[d]	13.30 ± 0.40[e]	16.27 ± 0.39[cd]	17.75 ± 0.42[b]
18:2 n-6	5.58 ± 0.15[b]	6.79 ± 0.19[a]	6.78 ± 0.15[a]	6.93 ± 0.14[a]	6.95 ± 0.16[a]	7.03 ± 0.16[a]
18:3 n-6	1.78 ± 0.04[e]	2.17 ± 0.05[d]	2.90 ± 0.07[c]	2.94 ± 0.07[c]	3.97 ± 0.09[a]	3.76 ± 0.09[b]
18:3 n-3	7.38 ± 0.15[a]	6.54 ± 0.16[c]	6.79 ± 0.21[b]	3.84 ± 0.09[d]	2.96 ± 0.07[e]	2.97 ± 0.06[e]
20:0	0.40 ± 0.01[c]	0.50 ± 0.01[b]	0.50 ± 0.01[b]	0.26 ± 0.01[d]	0.51 ± 0.01[ab]	0.53 ± 0.01[a]
20:2 n-6	1.30 ± 0.03[c]	1.76 ± 0.04[a]	1.59 ± 0.05[b]	1.66 ± 0.04[b]	1.80 ± 0.04[a]	1.78 ± 0.04[a]
20:3 n-6	1.42 ± 0.03[e]	1.95 ± 0.04[a]	1.84 ± 0.05[b]	1.67 ± 0.04[c]	1.53 ± 0.03[d]	1.62 ± 0.05[c]
20:4 n-6	13.35 ± 0.30[d]	14.92 ± 0.40[c]	15.37 ± 0.32[c]	19.19 ± 0.45[b]	19.40 ± 0.21[b]	20.34 ± 0.45[a]
C24:0	22.44 ± 0.44[b]	17.58 ± 0.40[d]	19.42 ± 0.54[c]	24.36 ± 0.64[a]	20.01 ± 0.21[c]	22.97 ± 0.54[b]
Saturates	46.91 ± 1.07[ab]	45.26 ± 1.29[bc]	45.62 ± 1.11[abc]	47.42 ± 0.62[a]	43.64 ± 0.93[c]	41.55 ± 1.26[d]
Monoenes	22.27 ± 0.59[a]	20.62 ± 0.46[bc]	19.11 ± 0.61[d]	16.35 ± 0.49[e]	19.77 ± 0.45[cd]	20.95 ± 0.48[b]
Polyenes	30.81 ± 0.67[d]	34.13 ± 0.80[c]	35.27 ± 0.76[bc]	36.23 ± 0.81[ab]	36.60 ± 1.02[ab]	37.50 ± 0.45[a]
P/S	0.66 ± 0.02[d]	0.75 ± 0.02[c]	0.77 ± 0.02[c]	0.76 ± 0.02[c]	0.84 ± 0.02[b]	0.90 ± 0.02[a]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05).

Table 6: Seasonal variation of fatty acid composition (percentage of weight) in the dried sea tangle (*S. japonica*) cultured at Kijang area[a].

Fatty acid (%)	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
12:0	0.28 ± 0.01[b]	0.58 ± 0.01[a]	0.09 ± 0.00[d]	0.04 ± 0.00[e]	0.16 ± 0.00[c]	0.08 ± 0.00[d]
14:0	10.47 ± 0.25[b]	10.92 ± 0.13[a]	8.70 ± 0.17[c]	6.72 ± 0.18[e]	7.09 ± 0.15[d]	5.74 ± 0.18[f]
16:0	16.08 ± 0.40[c]	16.94 ± 0.33[b]	17.13 ± 0.45[b]	18.07 ± 0.37[a]	11.25 ± 0.14[d]	10.58 ± 0.24[e]
16:1 n-7	2.92 ± 0.07[d]	3.10 ± 0.08[c]	3.45 ± 0.07[b]	3.15 ± 0.05[c]	3.79 ± 0.09[a]	3.20 ± 0.07[c]
18:0	1.25 ± 0.02[a]	1.14 ± 0.02[b]	0.82 ± 0.01[c]	0.81 ± 0.02[c]	0.82 ± 0.02[c]	0.81 ± 0.02[c]
18:1 n-9	17.26 ± 0.19[a]	17.07 ± 0.34[a]	14.95 ± 0.30[c]	15.41 ± 0.43[bc]	15.71 ± 0.42[b]	15.95 ± 0.48[b]
18:2 n-6	5.24 ± 0.05[d]	5.46 ± 0.17[d]	6.03 ± 0.17[c]	6.38 ± 0.13[b]	6.86 ± 0.15[a]	6.51 ± 0.13[b]
18:3 n-6	1.75 ± 0.04[f]	2.22 ± 0.05[e]	2.34 ± 0.06[d]	2.65 ± 0.07[c]	3.05 ± 0.08[b]	3.67 ± 0.09[a]
18:3 n-3	6.85 ± 0.16[a]	5.70 ± 0.13[c]	6.26 ± 0.14[b]	4.70 ± 0.13[d]	4.15 ± 0.13[e]	4.00 ± 0.09[e]
20:0	0.40 ± 0.01[d]	0.47 ± 0.01[b]	0.43 ± 0.01[c]	0.35 ± 0.00[e]	0.42 ± 0.01[c]	0.49 ± 0.01[a]
20:2 n-6	1.49 ± 0.04[b]	1.71 ± 0.03[a]	1.40 ± 0.04[c]	1.68 ± 0.05[a]	1.26 ± 0.04[d]	1.52 ± 0.04[b]
20:3 n-6	1.58 ± 0.03[b]	1.77 ± 0.04[a]	1.63 ± 0.04[b]	1.51 ± 0.03[c]	1.59 ± 0.04[b]	1.49 ± 0.03[c]
20:4 n-6	13.55 ± 0.32[e]	13.19 ± 0.41[e]	16.82 ± 0.39[c]	15.60 ± 0.37[d]	17.55 ± 0.36[b]	18.96 ± 0.45[a]
C24:0	20.89 ± 0.60[c]	19.73 ± 0.45[d]	19.95 ± 0.50[cd]	22.93 ± 0.24[b]	26.30 ± 0.74[a]	27.00 ± 0.71[a]
Saturates	49.37 ± 1.56[a]	49.78 ± 0.98[a]	47.12 ± 1.16[b]	48.93 ± 1.04[a]	46.04 ± 1.12[bc]	44.71 ± 0.58[c]
Monoenes	20.17 ± 0.46[a]	20.17 ± 0.46[a]	18.40 ± 0.32[c]	18.56 ± 0.43[c]	19.50 ± 0.62[ab]	19.15 ± 0.57[bc]
Polyenes	30.46 ± 0.69[d]	30.05 ± 0.80[d]	34.48 ± 0.62[b]	32.51 ± 0.90[c]	34.46 ± 0.74[b]	36.14 ± 0.81[a]
P/S	0.62 ± 0.01[d]	0.60 ± 0.02[d]	0.73 ± 0.02[b]	0.66 ± 0.01[c]	0.75 ± 0.02[b]	0.81 ± 0.02[a]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05).

Table 7: Seasonal variation of fatty acid composition (percentage of weight) in the dried sea tangle (*S. japonica*) cultured at Wando area[a].

Discussion

There are big environmental differences between Wando and Kijang. Wando is semi-closed sea, and affected by big tide and fresh water inflow from many rivers around. Kijang has a small tide, but high temperature high salinity Tsushima current and low temperature low salinity North Korea current meets in this area. To our knowledge, this is the first study that evaluated differences in the nutritional composition of *S. japonica* with harvest area and culture period. We found that protein content of *S. japonica* was highest in February and the carbohydrate content was highest in July for the Kijang and Wando samples over the culture period from February to July 2011. A similar pattern was previously reported for the collection of *Laminaria japonica* [16]. Rosemberg and Ramus [17] found inverse relationships between carbohydrate and protein content in the red seaweed *Gracilaria cervicornis* during collection from July 2000 to June 2001. The seaweed protein content was lowest when photosynthetic activity and carbohydrate synthesis were highest. Shin et al. [18,19] found that carbohydrate content of *Porphyra yezoensis* increased with late culture period: Dec (39.4%), Feb (47.2%). However, the protein content decreased with late culture period: Dec (39.4%), Feb (34.6%). Lipid content was not affected by culture period. A positive correlation was also detected between carbohydrate and temperature, along with correlations with salinity and solar radiation, which indicated that carbohydrate synthesis and protein concentration are affected by several seasonal factors, including water temperature, nitrogen content, and light intensity [16,18]. The lipid content was low relative to the other chemical constituents. However, the lipid content observed

Mineral	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Ca	567.11 ± 16.71[e]	972.86 ± 23.27[a]	858.81 ± 16.54[b]	745.45 ± 21.30[d]	783.84 ± 17.89[c]	741.41 ± 18.08[d]
Cu	0.29 ± 0.01[e]	0.46 ± 0.01[b]	0.34 ± 0.01[c]	0.47 ± 0.01[b]	0.67 ± 0.01[a]	0.31 ± 0.01[d]
Fe	8.15 ± 0.18[a]	3.70 ± 0.08[d]	3.16 ± 0.04[f]	6.20 ± 0.14[b]	3.39 ± 0.08[e]	4.46 ± 0.09[c]
K	3325.83 ± 83.43[c]	3516.51 ± 111.07[b]	4158.54 ± 99.29[a]	3554.55 ± 80.98[b]	3578.28 ± 95.21[b]	3165.47 ± 67.11[d]
Mg	630.63 ± 17.51[c]	592.81 ± 11.93[d]	606.55 ± 17.32[cd]	887.89 ± 20.04[a]	821.58 ± 17.91[b]	794.79 ± 12.45[b]
Mn	0.44 ± 0.01[d]	0.70 ± 0.02[b]	0.69 ± 0.02[b]	0.68 ± 0.02 [b]	0.86 ± 0.02[a]	0.55 ± 0.01[c]
Na	1209.21 ± 32.09[d]	1440.73 ± 39.94[a]	1361.45 ± 30.09[b]	1285.19 ± 26.22[c]	1253.65 ± 28.78[cd]	1204.93 ± 29.30[d]
Zn	1.65 ± 0.04[e]	2.34 ± 0.05[c]	2.19 ± 0.05[d]	2.76 ± 0.07[b]	0.37 ± 0.01[f]	3.04 ± 0.04[a]
Total	5,743.31 ± 120.58[c]	6,530.11 ± 156.09[b]	6,991.73 ± 211.17[a]	6,483.19 ± 154.05[b]	6,442.64 ± 151.48[b]	5,914.96 ± 126.98[c]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly ($p<0.05$).

Table 8: Seasonal variation of mineral contents in the dried sea tangle (*S. japonica*) cultured at Kijang area[a].

Mineral	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Ca	900.91 ± 22.15[a]	913.15 ± 17.93[a]	913.58 ± 23.49[a]	789.90 ± 18.30[b]	730.30 ± 13.90[c]	771.72 ± 14.14[b]
Cu	0.56 ± 0.01[c]	0.40 ± 0.01[d]	0.94 ± 0.02 [a]	0.90 ± 0.02[b]	0.22 ± 0.00[e]	0.12 ± 0.00[f]
Fe	2.89 ± 0.06[e]	2.48 ± 0.06[f]	3.55 ± 0.10[d]	5.52 ± 0.15[a]	4.92 ± 0.05[c]	5.24 ± 0.15[b]
K	3683.32 ± 84.26[c]	4020.03 ± 114.39[a]	3847.32 ± 93.92[b]	3643.64 ± 47.59[c]	3260.86 ± 69.51[d]	3182.01 ± 41.93[d]
Mg	644.72 ± 17.15[d]	723.22 ± 15.96[c]	593.00 ± 18.77[e]	836.84 ± 25.04[ab]	852.96 ± 19.57[a]	820.82 ± 17.93[b]
Mn	0.77 ± 0.02[a]	0.62 ± 0.01[d]	0.73 ± 0.02[b]	0.47 ± 0.01[e]	0.65 ± 0.02[c]	0.31 ± 0.00[f]
Na	1378.52 ± 32.66[b]	1435.11 ± 43.64[b]	1613.96 ± 36.48[a]	1173.17 ± 25.60[d]	1141.14 ± 33.21[d]	1286.39 ± 25.75[c]
Zn	1.44 ± 0.03[f]	2.13 ± 0.06[d]	2.35 ± 0.05[c]	2.53 ± 0.08[b]	3.04 ± 0.06[a]	1.69 ± 0.05[e]
Total	6613.13 ± 165.73[b]	7097.14 ± 138.26[a]	6975.43 ± 183.16[a]	6452.97 ± 132.95[b]	5,994.09 ± 84.62[c]	6068.3 ± 96.61[c]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly ($p<0.05$).

Table 9: Seasonal variation of mineral contents in the dried sea tangle (*S. japonica*) cultured at Wando area[a].

Amino acid	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Aspartic acid	1574.47 ± 46.38[a]	1422.98 ± 34.04[b]	1415.36 ± 27.26[b]	1250.12 ± 35.73[c]	1087.68 ± 21.32[d]	1032.76 ± 25.39[d]
Threonine*	693.70 ± 15.40[a]	721.08 ± 14.38[a]	718.71 ± 15.30[a]	636.35 ± 20.0[b]	526.62 ± 14.11[c]	446.02 ± 9.84[d]
Serine	773.96 ± 17.51[a]	677.69 ± 14.53[b]	674.86 ± 8.57[b]	604.14 ± 13.78[c]	515.94 ± 3.45[d]	487.02 ± 9.61[d]
Glutamic acid	1718.74 ± 43.11[a]	1623.86 ± 51.29[b]	1615.31 ± 38.57[b]	1502.92 ± 34.24[c]	1223.20 ± 9.64[d]	1089.29 ± 23.09[e]
Proline	676.85 ± 18.80[a]	565.19 ± 11.37[b]	562.15 ± 16.05[b]	542.35 ± 12.24[b]	497.45 ± 9.05[c]	400.25 ± 6.27[d]
Glycine	895.55 ± 18.66[a]	821.67 ± 20.32[b]	817.98 ± 21.71[b]	765.58 ± 22.93[c]	622.11 ± 8.24[d]	536.65 ± 14.07[e]
Alanine	1105.96 ± 29.35[a]	995.41 ± 27.59[b]	990.79 ± 21.90[b]	724.24 ± 14.78[c]	602.70 ± 10.81[e]	666.54 ± 16.21[d]
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine*	592.06 ± 12.43[c]	773.56 ± 18.45[a]	769.75 ± 24.25[a]	694.82 ± 15.83[b]	557.45 ± 0.26[d]	384.18 ± 10.56[e]
Methionine	345.06 ± 10.97[a]	321.79 ± 7.45[b]	319.66 ± 7.33[b]	275.21 ± 5.95[c]	210.27 ± 2.02[d]	188.26 ± 3.41[e]
Isoleucine*	435.32 ± 9.14[d]	623.87 ± 14.91[a]	620.55 ± 18.74[a]	586.15 ± 13.93[b]	496.92 ± 4.37[c]	424.00 ± 9.11[d]
Leucine*	1094.16 ± 26.91[b]	1174.31 ± 23.05[a]	1168.03 ± 30.03[a]	997.35 ± 23.10[c]	842.37 ± 5.94[d]	656.88 ± 12.04[e]
Tyrosine*	366.60 ± 8.37[a]	299.66 ± 8.00[b]	297.48 ± 6.15[b]	285.19 ± 6.73[c]	272.69 ± 2.88[d]	222.90 ± 5.42[e]
Phenylalanine*	636.80 ± 12.54[b]	697.82 ± 15.93[a]	693.92 ± 19.42[a]	585.45 ± 15.41[c]	512.50 ± 4.42[d]	393.85 ± 11.04[e]
Histidine	325.11 ± 7.44[c]	394.50 ± 11.23[a]	394.62 ± 9.63[a]	342.34 ± 4.47[b]	304.46 ± 3.28[d]	279.06 ± 3.68[e]
Lysine*	653.32 ± 17.38[b]	700.03 ± 15.45[a]	696.74 ± 22.06[a]	513.45 ± 15.36[c]	480.60 ± 6.28[d]	430.39 ± 9.40[e]
Arginine	511.63 ± 11.16[c]	660.01 ± 15.48[a]	658.02 ± 14.11[a]	641.75 ± 14.39[a]	544.90 ± 3.66[b]	482.10 ± 5.36[d]
Total	12399.29 ± 293.74[a]	12473.43 ± 379.31[a]	12413.91 ± 280.61[a]	9986.94 ± 217.90[b]	9217.87 ± 129.32[c]	7783.17 ± 155.80[d]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly ($p<0.05$).
*Essential amino acid

Table 10: Seasonal variation of total amino acid contents in the dried sea tangle (*S. japonica*) cultured at Kijang area[a].

in this study was similar to the content observed in other seaweeds, comprising from 1% to 3% of dry matter [20,21]. The ash content varied from 14.3% to 18.4% in our samples. It has been reported that the ash content fluctuates depending on the species, geographical location, and season investigated [22,23].

Component sugar compositions of Kijang and Wando samples were high in the following order: fucose, galactose, glucose, mannose, and so on. Polysaccharide of seaweed generally classified into cytoskeleton, intercellular mucoid, and storage polysaccharides, most of 2M HCl hydrolyzed polysaccharide from *S. japonica* in this study originated from storage polysaccharide [24]. In the Kijang and Wando samples, there was a variation in the sugar content depending on culture period ($P<0.05$). Galactose content of Kijang samples were higher than that of Wando in the all culture period ($p<0.05$).

Major fatty acid of Kijang and Wando samples is myristic acid (14:0), palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2),

Amino acid	Culture period					
	Feb	Mar	Apr	May	Jun	Jul
Aspatic acid	1311.08 ± 30.09[a]	1370.98 ± 38.01[a]	1350.66 ± 28.74[a]	1248.37 ± 37.12[b]	1197.13 ± 32.41[c]	987.30 ± 29.07[d]
Threonine*	662.58 ± 15.35[a]	686.76 ± 15.65[a]	685.58 ± 15.73[a]	593.84 ± 11.69[b]	519.70 ± 12.70[c]	403.39 ± 3.13[d]
Serine	622.40 ± 14.98[b]	648.74 ± 7.75[a]	643.88 ± 12.84[ab]	584.90 ± 15.87[c]	539.02 ± 13.64[d]	460.76 ± 11.59[e]
Glutamic acid	1490.24 ± 37.35[b]	1563.56 ± 30.46[a]	1541.39 ± 40.47[ab]	1490.61 ± 30.71[b]	1257.52 ± 8.65[c]	1170.02 ± 18.63[d]
Proline	518.72 ± 12.20[bc]	544.67 ± 13.83[a]	536.49 ± 10.88[ab]	511.15 ± 8.81[c]	485.48 ± 13.65[d]	384.76 ± 7.06[e]
Glycine	754.48 ± 14.36[b]	787.88 ± 16.64[a]	780.47 ± 12.97[a]	686.67 ± 14.51[c]	544.26 ± 9.64[d]	526.76 ± 12.53[d]
Alanine	913.89 ± 10.07[b]	955.28 ± 19.29[a]	945.36 ± 19.00[ab]	867.07 ± 24.20[c]	726.10 ± 12.95[d]	718.06 ± 19.23[d]
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine*	710.09 ± 15.14[b]	743.50 ± 16.17[a]	734.50 ± 17.42[ab]	581.42 ± 15.16[c]	515.00 ± 13.11[d]	458.48 ± 11.91[e]
Methionine	295.23 ± 6.77[b]	312.42 ± 7.13[a]	305.21 ± 6.73[ab]	227.70 ± 6.28[c]	211.71 ± 6.35[d]	194.27 ± 3.56[e]
Isoleucine*	572.64 ± 15.91[b]	601.11 ± 12.59[a]	592.24 ± 14.98[ab]	524.88 ± 6.90[c]	510.59 ± 6.78[c]	443.58 ± 5.14[d]
Leucine*	1077.85 ± 31.36[b]	1131.59 ± 21.26[a]	1114.74 ± 32.66[ab]	941.52 ± 28.54[c]	828.06 ± 12.26[d]	626.19 ± 19.45[e]
Tyrosine*	274.85 ± 5.60[b]	292.06 ± 6.82[a]	284.09 ± 6.35[ab]	224.07 ± 4.65[c]	193.09 ± 2.31[e]	205.54 ± 5.27[d]
Phenylalanine*	640.38 ± 14.90[b]	673.29 ± 21.10[a]	662.28 ± 15.18[ab]	551.35 ± 13.22[c]	490.96 ± 9.25[d]	368.61 ± 7.66[e]
Histidine	363.34 ± 10.38[b]	368.28 ± 8.41[ab]	376.21 ± 9.25[a]	313.23 ± 8.08[c]	283.56 ± 5.28[d]	241.80 ± 4.95[e]
Lysine*	642.57 ± 20.25[b]	671.81 ± 13.22[a]	664.74 ± 14.67[a]	542.88 ± 11.71[c]	493.55 ± 12.33[d]	431.80 ± 9.64[e]
Arginine	606.52 ± 13.83[a]	627.61 ± 14.36[a]	627.62 ± 12.39[a]	529.72 ± 14.13[b]	515.74 ± 14.81[b]	458.52 ± 13.99[c]
Total	11456.88 ± 261.03[b]	13776.53 ± 366.56[a]	11845.47 ± 251.11[b]	10498.01 ± 363.89[c]	9862.18 ± 7.70[d]	7609.83 ± 206.81[e]

[a]Values represent means ± standard error (n=3). Mean values in the same row followed by different letters differ significantly (p<0.05).

[*]Essential amino acid

Table 11: Seasonal variation of total amino acid contents in the dried sea tangle (*S. japonica*) cultured at Wando area[a]

α-linolenic acid (18:3), arachidonic acid (20:4), and lignoceric acid (24:0). Many researches on seaweed fatty acid composition have been reported [16,25-34], but there have been various fatty acid contents since which one is chosen for analysis among about 50 selling fatty acid standards. Moreover, fatty acid compositions of the seaweed are generally varied by analyzing its sampled part. In all the data, most fatty acid composition showed a variation with harvest area and culture period. Low fatty acids, such as lauric acid (12:0), stearic acid (18:0), and arachidic acid (20:0), showed different compositions on harvest area and culture period without tendency. These results are similar with previous report [16]. Linoleic acid, γ-linolenic acid, and arachidonic acid in Kijang samples and γ-linolenic acid, arachidonic acid, and lignoceric acid in Wando samples increased with culture period, whereas α-linolenic acid in Kijang samples and stearic acid in Wando samples decreased. Both Kijang and Wando samples decreased with culture period in saturates, while those of polyenes increased.

In the mineral contents of Kijang and Wando *S. japonica* samples, the results show that *S. japonica* is rich in K and Na with moderate amounts of Ca and Mg whereas Cu, Fe, Mn, and Zn are present in small quantities.

Major amino acid of Kijang and Wando *S. japonica* samples are glutamic acid, aspartic acid, leucine, alanine, glycine, valine, phenylalanine, but cystine was not detected. Glutamic acid and aspartic acid occupied over 20% in the total amino acid. It is known that amino acid of seaweed is generally composed of high contents in neutral and acidic amino acids such as alanine, aspartic acid, glycine, and proline [24], but *S. japonica* contained low glycine and proline contents. Sulfur amino acid, cysteine and cysteine, was not detected, methionine, histidine, and tyrosine were included in small amount. The average percentages of essential amino acids (EAA) in Kijang and Wando *S. japonica* samples were 39.5%, 37.8%, which is higher than the EAA requirement (32.3%) suggested by the Food and Agriculture Organization [35]. The amino acid composition observed in this study was similar to previous studies [36], where the sum of the average percentage of three amino acids, glutamic acid (13.5%), aspartic acid (11.9%), and alanine (7.9%), comprised the greatest proportion (33.3%) of TAA composition. Noda [37] suggested that the former three amino acids (glutamic acid, aspartic acid, and alanine) might produce the flavors specific to Nori (Porphyra). TAA content decreased at the end of the culture period. This phenomenon has also been observed in other seaweeds such as *Enteromorpha prolifera*, *C. fulvescens*, and *Codium fragile* [38].

In conclusion, we have ascertained that the monthly nutritional composition of *S. japonica* affected by harvest area and culture period from February to July 2011. *S. japonica* in Kijang and Wando showed the highest crude protein content in February and the highest carbohydrate content in July. Fucose was the most abundant and galactose the second most abundant in the monosaccharide composition profiles. Significant increases of the major fatty acids in Kijang (C18:2 n-6 and C20:4 n-6) and Wando (C18:3 n-6) were observed as the culture period progressed. The highest mineral content of both Kijang and Wando samples is potassium and followed by sodium, calcium, magnesium, and so on. In the total amino acid contents, Kijang samples increased from February to April but decreased from May to July, while Wando samples increased on March but decreased from April to July.

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