



Evaluation of the chemical composition of brown seaweed (*Saccharina japonica*) hydrolysate by pressurized hot water extraction



Periaswamy Sivagnanam Saravana^a, Jae Hyung Choi^b, Yong Beom Park^b, Hee Chul Woo^b, Byung Soo Chun^{a,*}

^a Department of Food Science and Technology, Pukyong National University, 45 Yongso-ro, Namgu, Busan 608-737, Republic of Korea

^b Department of Chemical Engineering, Pukyong National University, 365 Sinseon-ro, Namgu, Busan 608-737, Republic of Korea

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ABSTRACT

In this study, *Saccharina japonica* was treated with pressurized hot water extraction (PHWE) at a temperature of 180 °C–420 °C and pressure between 13 bar and 520 bar. The obtained hydrolysate was investigated for their yield, total organic carbon (TOC), pH, Maillard reaction products, viscosity, color, and amino acid, mineral, and monosaccharide contents. The extraction yield increased with an increase in temperature and varied from 72.21% to 98.91%. TOC, pH, and potassium and sodium content increased, whereas viscosity decreased, with an increase in temperature. Essential amino acids such as valine and lysine and non-essential amino acids such as aspartic acid, glutamic acid, glycine, and tyrosine recovered well at low temperature. The content of heavy metals such as arsenic, cadmium, mercury, and lead was very low in the obtained hydrolysate. The maximum amount of total amino acids was recovered at 180 °C/13 bar (761.95 ± 14.54 mg/g). The level of main monosaccharides such as glucose (6.70 g/L), fructose (8.40 g/L), and mannitol (17.50 g/L) was found to be very high at 180 °C/13 bar. The results indicated that the pressurized hot water extract of *S. japonica* has good potential for use in the fermentation industry and can be used as human food.

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1. Introduction

Saccharina japonica (Dashi kombu) is an aquatic class of Phaeophyceae (brown algae), a kind of kelp or seaweed, which is widely cultured in China, Japan, and Korea. It is an industrially orientated species. *S. japonica*, also known as “Kombu” (in China “Haidai,” in Korea “Dasima”), is a vital part of the diet for people in Japan. Bulky reaps are grown using rope farming, an easy way to cultivate macroalgae by hanging them to moving ropes along the sea. Recently, sulfated polysaccharides from the brown seaweed *S. japonica* were studied for their structures and functional materials [1]. A recent study showed that polysaccharides obtained from brown seaweeds were mainly composed of fucose, galactose, and sulfate groups, along with a trace amount of other monosaccharides [2]. Sulfated polysaccharides from *S. japonica* are widely explored because they vary from common polysaccharides, which mostly contain sulfated fucose. These sulfated galactofucans have an extensive range of biofunctional properties [3]. Recent reports are focused on algae species because of their potential as a source of nutrition and nutritional characteristics, including their composition of fatty acids [4], amino acids [5], and dietary fiber [6]. The health benefits of the intake of seaweeds have also been reported [7].

Water is a globally neutral solvent and nontoxic at room temperature, whereas H₂O molecule above its critical temperature and pressure, which is known as supercritical water, can behave as an organic solvent and acidic medium [8]. Because of the alteration in this condition, some physical properties of water beyond their critical points ($T_m > 374$ °C and $T_p > 220$ atm). It is an environmentally friendly processing agent and can also offer greater extraction yields from various samples [9]. Pressurized hot water extraction (PHWE) is performed by means of boiling water (from 100 to 374 °C) with a pressure (usually from 10 to 60 bar) to uphold the water in a liquid state. The key factor to study the various types of extraction procedures is the inconsistency of the dielectric constant with temperature. At room temperature, water is a polar solvent with a dielectric constant of 80. The dielectric constant significantly changes when the water is heated up to 250 °C, where the value will be 27, but the liquid state can be maintained by retaining a suitable pressure [10]. The equipment can be attached with a chilling machine for fast chilling of the product for immediately obtaining the pressurized hot water extract [11]. PHWE of biomass presents several advantages compared with traditional technologies (acid, alkali, and enzymatic hydrolysis). Its main advantage is that it does not use organic solvents, which is a factor of major importance in any process because organic solvents must be recycled, incinerated, or submitted to an appropriate unitary operation, resulting in a non-aggressive waste to the environment [12]. Moreover, it does not require biomass pretreatment; it is fast, presents lesser corrosion, lower residue generation, and lower sugar degradation than conventional hydrolysis methods [13].

* Corresponding author at: Department of Food Science and Technology, Pukyong National University, 599-1 Daeyeon-3dong, Nam-Gu, Busan 608-737, Republic of Korea.
E-mail address: bschun@pknu.ac.kr (B.S. Chun).

On the other hand, it has been reported that during PHWE of macroalgae [14], browning compounds, i.e., Maillard reaction products (MRPs), are formed due to the reaction of the carbonyl group of a reducing sugar with the free amino group of amino acids. It was identified to be useful as an antioxidant in herbal drugs and foods. In particular, it was reported that the antioxidant activity increased with the heating strength, in similar with color development and MRPs were responsible for the majority of the antioxidant activity, in hydrolysis macroalgae [14]. Therefore, it will be of good value to measure changes in MRP levels.

Thus, the aim of this study was to calculate the use of PHWE under different temperatures to identify the chemical composition of *S. japonica* like total organic carbon (TOC), pH, MRPs, viscosity, color, amino acids, minerals, and monosaccharides obtained. Our findings could relate the nutritional composition of *S. japonica* obtained by PHWE, which can be considered important in human health and also in the fermentation industry.

2. Materials and methods

2.1. Materials

The brown seaweed *S. japonica*, J.E. Areschoug, 1851, Lane, Mayes, Druehl, and Saunders was collected from Guemil-eup, Wando-gun, Jeollanam-do, Republic of Korea. High-purity nitrogen gas (99.99%) was supplied by KOSEM (Yangsan, Republic of Korea). All reagents used were of analytical or high-performance liquid chromatography (HPLC) grade, and galactose, glucose, fructose, arabinose, mannose, mannitol, sorbitol, xylitol, and xylose standards (purity > 98%) were purchased from Sigma-Aldrich (St. Louis, MI, USA).

2.2. Sample preparation

After washing fresh *S. japonica* samples with fresh water, unused materials, attached salts, and minerals were removed, and the samples were cut into small pieces. The pieces were dried at $-80\text{ }^{\circ}\text{C}$ for 3 days in a freeze dryer (Eyela FDU-2100, Tokyo Rikakikai Co., LTD, Japan) equipped with a square-type drying chamber (Eyela DRC-1000, Tokyo Rikakikai Co., LTD, Japan). The dried samples were collected into sealed plastic bags. The samples were then finely ground using a mechanical blender (PN SMKA-4000 mixer, PN Co., Ltd., Ansan-si, Korea) and were sieved through a 710- μm stainless steel sieving mesh.

2.3. PHWE

PHWE was performed in a 200-cm³ batch reactor made of Hastelloy C276 (continuous-type supercritical water system, Phosentech, South Korea) with a temperature control (Fig. 1). A total of 6 g of sample material was loaded into the reactor with 150 mL of distilled water. The vessel was then locked and heated using an electric heater to the required temperature ($180\text{ }^{\circ}\text{C}$ – $420\text{ }^{\circ}\text{C}$). Pressures were determined on the basis of saturated steam to be between 13 bar and 520 bar for the temperature range studied. The temperature and pressure in the reactor were controlled using a temperature controller and pressure gauge, respectively. The sample was stirred using a four-blade stirrer at 150 rpm, and after reaching the desired temperature, 5 min of reaction time was maintained. Hydrolysate samples from the reactor were collected after reaching room temperature (within 1 or 2 h after stopping the reaction), filtered using a Whatman nylon membrane filter (0.45 μm), and stored at $4\text{ }^{\circ}\text{C}$. The residual samples recovered after PHWE were dried, and their weight was measured in grams (g).

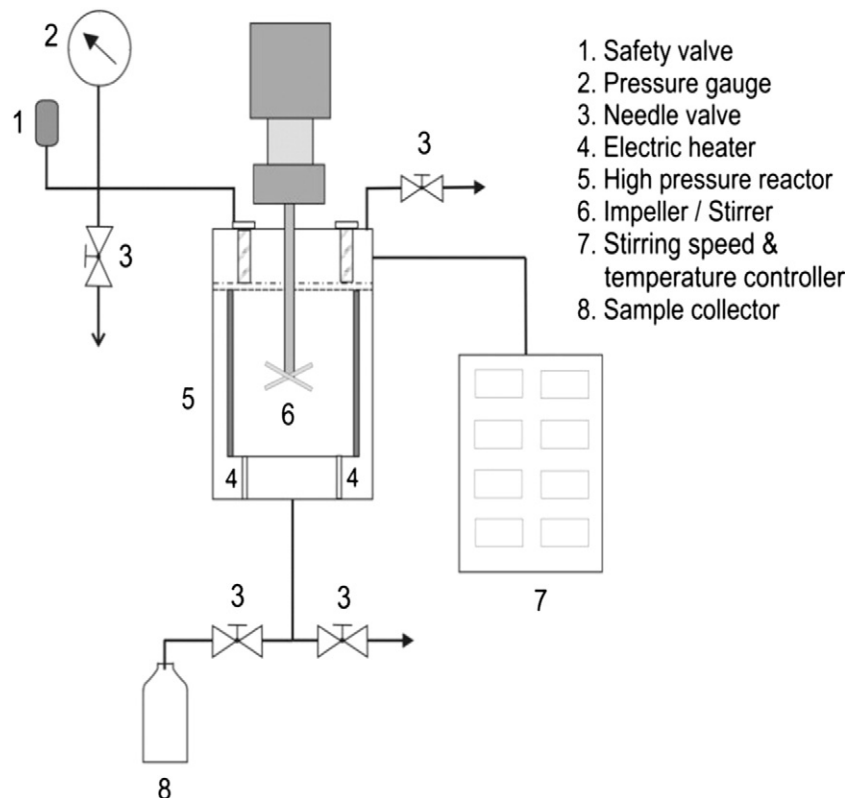


Fig. 1. Flow diagram of pressurized hot water extraction (PHWE).

2.4. Extraction yield

The extraction yield was calculated according to Plaza et al. [15] with a slight modification.

Extraction yield (%) = dry extract weight/initial dry sample weight (1)

The *S. japonica* hydrolysate was kept in an oven at 120 °C for 24 h to obtain the dry extract weight, and the initial dry sample weight of the freeze-dried *S. japonica* was calculated through the subtraction of water content with the total weight. The freeze-dried *S. japonica* was taken to dryness in an oven at 120 °C for 24 h. This process was conducted in triplicate. The percent of water content in the freeze-dried *S. japonica* was 10.69 ± 0.64%.

2.5. TOC

The carbon content of the *S. japonica* hydrolysate water was determined using TOC-Vcph, SSM (Shimadzu). The analysis was performed using 1000 ppm of hydrolysate water of each condition.

2.6. pH measurement

The pH of the *S. japonica* hydrolysate water was measured using a Mettler Toledo Five Easy Plus pH meter at 20 °C. Prior to the measurements, the pH meter was calibrated using technical buffer solutions of pH 4.01 ± 0.02, 7.00 ± 0.02, and 9.2 ± 0.02.

2.7. Determination of MRPs by absorbance measurement

The browning intensities of *S. japonica* hydrolysate were determined using MRPs (melanoidins). These extracts were filtered, and the browning intensity was directly measured at 360 nm and 420 nm [14]. It was expressed as arbitrary absorbance units (A.U.).

2.8. Viscosity measurement

A Brookfield DVII + Pro viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to measure the viscosity of *S. japonica* hydrolysate according to the method described by Ogawa et al. [16] with slight modifications. In total, 8 mL of 0.1% (w/v) sample in 0.1 M acetic acid was incubated at 25 °C for 10 min and then placed in a vessel. Spindle SC4-18 with agitation at 150 rpm was then used to measure viscosity, which was expressed as centipoise (cP).

2.9. Color measurement

The color of the *S. japonica* hydrolysate was studied in the CIELab color space using Minolta CM-2600d (Minolta Camera Co., Osaka, Japan) with illuminant D65, 10° observer, SCI mode, 11-mm aperture of the instrument for illumination and 8 mm for measurement. A low-reflectance glass (Minolta CR-A51/1829-752) was placed between the samples and equipment. The following color coordinates were determined: lightness (L^*), redness (a^* , ± red–green), and yellowness (b^* , ± yellow–blue). From these coordinates, hue (H^*) and chroma (C^*) were calculated as follows:

$$\text{Hue} = \frac{\tan^{-1}b^*}{a^*} \quad \text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

2.10. Amino acid composition analysis

The freeze-dried algae samples (400 mg) were hydrolyzed with 6 N hydrochloric acid in an ampoule containing 0.1% phenol (for the protection of tyrosine) for 24 h at 110 °C. After acid hydrolysis, 30 mL of citrate

buffer (pH 2.2) was added, and the pH was adjusted between 0.5 and 1 with 7.5 N NaOH and to pH 2.2 with 1 N NaOH. The sample obtained was diluted to 100 mL with citrate buffer after adding 1 mL of 50 μM norleucine solution (as an internal standard), while the *S. japonica* hydrolysate obtained by PHWE was filtered (0.22-μm cellulose acetate filter) and loaded onto a S430 (SYKAM) amino acid autoanalyzer for free amino acid analysis. A cation separation column LCA K07/Li (4.6 × 150 mm) with a column temperature of 37 °C–74 °C and buffer pH range of 2.90–7.95 was used for free amino acid analysis. The mobile phase was 5 mM of p-toluenesulfonic acid solution at a flow rate of 0.45 mL/min. A mixture of 5 mM p-toluenesulfonic acid, 20 mM of bis–tris, and 100 mM of EDTA was used as the post-column reagent at a flow rate of 0.25 mL/min. Excitation and emission wavelengths were kept at 440 and 570 nm, respectively. The individual amino acid stock solutions were prepared in 0.1 M HCl; tryptophan was prepared in water [17].

2.11. Mineral determination

The *S. japonica* hydrolysate and freeze-dried samples were prepared as described by Rocha et al. [18] with minor modifications. The hydrolysate was filtered by 0.45-μm syringe filters (Sartorius), immediately acidified with HNO₃ to a pH of <2 and stored in a precleaned (rinsed with 10% HNO₃ followed by rinsing with MilliQ water) high-density polyethylene vial. Filtration was done just before measurement to eliminate tiny materials present in the sample, which are undesirable for PerkinElmer Elan 6100 ICPMS (PerkinElmer, Waltham, MA, USA) measurement.

2.12. Monosaccharide composition

The levels of galactose, glucose, fructose, arabinose, mannose, mannitol, sorbitol, xylitol, and xylose were measured using HPLC with an evaporative light scattering detector. HPLC analysis was performed using a Jasco HPLC (Easton, USA) model 400 equipped with a ChromNav analysis software. High-purity nitrogen (99.99%) from KOSEM Co. was used as the carrier gas. A Shodex (Japan) sugar column (SP0810) of 300 mm, thermostated to 80 °C, was used to analyze monosaccharides. The water used for elution was filtered using a cellulose acetate filter (0.22 μm) and then sonicated. The flow rate of the eluent was maintained at 0.6 mL/min. Monosaccharides of freeze-dried algae samples (50 mg) were hydrolyzed with 1 M H₂SO₄ (3 mL) at 100 °C for 3 h. The reaction medium was then neutralized with 1 mL N₄OH (15 M), and 1 mL of 2-deoxy-D-glucose (1 mg/mL) was added as the internal standard, while the hydrolysate samples were diluted four fold using filtered and sonicated water (HPLC grade). The sample was filtered through a 0.2-μm nylon filter before it was analyzed by HPLC [19].

2.13. Statistical analysis

All mean values were analyzed by one-way ANOVA. Values are expressed as mean ± standard deviation. (SD; n = 3 replicates) (SPSS software; version 20 for windows, IBM, Chicago, IL, USA). Statistical analysis was performed using the Tukey test, and P < 0.05 was considered to be significant.

3. Results and discussion

3.1. Extraction yield

PHWE experiment temperatures ranged from 180 °C to 420 °C, and the time taken to reach the desired temperature varied from 30 min to 105 min. The pressure was monitored using the pressure gauge, and it ranged from 13 bar to 520 bar. Usually, the product acquired was a solid–liquid mixture. After waiting (until it reaches room temperature) for precipitation to occur, two layers of the hydrolysate were formed.

The upper part was an aqueous solution, which was clearer and less viscous, whereas the lower layer was a wet seaweed residue. Degraded products obtained from the hydrolysis of *S. japonica* appeared dark brownish and contained a blend of some liquid portion and little solid substances. The hydrolysate had a toasty aroma at low temperature, but it became more pungent with an increase in temperature. The samples were filtered using the Whatman nylon membrane filter (0.45 µm) and stored at 4 °C. The residual samples that were recovered after PHWE were dried, and their weight was measured in grams (Table 1). The residue content varied from 1.56 (180 °C/13 bar) to 0.59 (420 °C/520 bar). This clearly showed that a rise in temperature degrades the solid sample. Similar results were reported by Jung-Nam Park et al. [20].

The extraction yield of the *S. japonica* hydrolysate at different temperatures and pressures are shown in Table 1. They varied from 72.21 to 98.91 wt.% (dry weight). As can be seen in Table 1, temperature and pressure directly influenced the extraction yield. The effect of increasing yield with increasing temperature has been extensively observed in PHWE [15], and it is explained by the increasing mass transfer, lower surface tension, and higher solubility of numerous compounds. Watchararujji et al. [21] reported that PHWE of rice bran and soybean meal has a high hydrolysis yield because of strong accumulation through hydrophobic interactions; the resultant protein matter has low solubility in water at ambient temperature [22].

Corrosion in the PHWE environment is of concern. In particular, oxidizing and acidic environments can result in quick corrosion, and it will be more severe at subcritical conditions than at supercritical conditions owing to the relatively dense and polar character of subcritical water. The reactor frequently used for PHWE was made of nickel alloys such as Inconel 625 and Hastelloy C276; titanium alloys also have good resistance [23]. In our experiment, we used Hastelloy C276 as it has a good resistance to corrosion when used at high temperature conditions. The hydrolysate obtained from various PHWE conditions was examined for TOC, pH, MRPs, viscosity, color, and amino acid, mineral, and monosaccharide contents.

3.2. TOC analysis

The amounts of TOC in the *S. japonica* hydrolysate obtained in each temperature condition are shown in Table 1. The 180 °C/13 bar condition contained 18.64 ± 0.13 mg/L of carbon in the *S. japonica* hydrolysate, which essentially comprised sugars and organic acids. TOC of hydrolysate products obtained in each temperature condition varied. The amount of TOC (75.20 ± 1.69 mg/L) dramatically increased for the 420 °C/520 bar condition. Thus, the result showed that TOC increases with an increase in temperature. Similar results were reported by Garcia et al. [24]. Issara et al. [25] reported that the TOC content constantly increased due to the raise in temperature and that these type results are found in some food items when we cook. Further, there was a possible reduction of organic carbon into carbeneous gas products

because of oxidation. Although vaporous content was not investigated in this study, earlier studies described that some products released from the oxidation of biomass contain volatile carbon and water [26].

3.3. pH analysis

The pH of the obtained *S. japonica* hydrolysate was measured; the values are shown in Table 1. The pH values varied from 4.91 ± 0.00 (180 °C/13 bar) to 7.95 ± 0.02 (420 °C/520 bar) in the *S. japonica* hydrolysate, and the values were found to increase with an increase in temperature. The pH was increased at higher temperatures due to the formation of salts and degradation of all organic matter. The low pH was due to the degradation of sugars into organic acids, and these organic acids undergo a chain reaction, providing the acidity for increasing the speed of subsequent reactions as an autocatalytic process [27]. Gao et al. [28] reported that when sucrose was treated in subcritical water or water ethanol mixtures, the pH decreases due to the degradation of glucose and fructose products into acidic compounds. In subcritical water, the pH decreased further after the sucrose was completely hydrolyzed.

3.4. Absorbance measurement of MRPs

It was broadly recognized that the different intensities of brown color can be efficiently used to observe non-enzymatic browning reactions, including the Maillard reaction. Visual observation was one of the simplest ways to detect the presence of MRPs. For this purpose, the obtained MRP value has been frequently engaged as an indicator of a wide range of Maillard reactions for the occurrence of caramelization, also in foods. The increase in the browning effect is directly related to the advanced phases of the reaction. The absorbance at 360 nm and 420 nm is usually engaged to check the development of browning reaction of MRP [14]. The *S. japonica* hydrolysate had a significant content of MRPs based on the browning measurements at 360 nm and 420 nm (Table 1). The MRP content was high at the 420 °C/520 bar (4.00 ± 0.01 A.U. at 360 nm, 2.01 ± 0.00 A.U. at 420 nm) and very low at the 180 °C/13 bar (2.51 ± 0.00 A.U. at 360 nm, 1.90 ± 0.02 A.U. at 420 nm) conditions. These absorbance values were employed as indicators of caramelization and formation of brown advanced MRPs in thermally processed foods. Our data showed an increase in the formation of MRPs as the extraction temperature increased in PHWE of *S. japonica*. The formation of MRPs during PHWE of several samples has also been reported in other studies [14,29]. MRPs include a wide range of compounds of significant importance for the nutritional value of food and beverages. Some of these compounds have strong antioxidant activities, but others such as hydroxymethylfurfural can be toxic and mutagenic [30].

Table 1

Experimental summary of residue obtained after PHWE, yield, TOC, pH, Maillard reaction products (MRPs) and viscosity at different PHWE conditions.

Conditions (°C/bar)	Residue obtain after PHWE (g)	Yield (%)	TOC (mg/L)	pH	MRPs (A.U.)		Viscosity (c.p.)
					Absorbance at 360 nm	Absorbance at 420 nm	
180/13	1.56	72.21 ^a	18.64 ± 0.13 ^j	4.91 ± 0.00	2.51 ± 0.00 ^g	1.90 ± 0.02 ^e	6.80 ± 0.20 ^l
200/17	1.35	75.01 ^a	19.05 ± 0.00 ^j	4.94 ± 0.00	3.00 ± 0.00 ^f	1.95 ± 0.02 ^d	6.50 ± 0.15 ^k
220/25	1.23	78.10 ^b	20.01 ± 0.31 ^j	5.05 ± 0.00	3.21 ± 0.05 ^e	1.96 ± 0.01 ^c	6.10 ± 0.10 ^j
240/34	1.18	79.82 ^b	22.20 ± 0.13 ⁱ	6.03 ± 0.01	3.30 ± 0.01 ^d	1.97 ± 0.00 ^b	5.80 ± 0.05 ⁱ
260/49	1.03	81.37 ^c	25.53 ± 0.10 ^h	6.57 ± 0.00	3.40 ± 0.01 ^c	1.97 ± 0.02 ^b	5.50 ± 0.00 ^h
280/72	0.95	85.64 ^c	30.14 ± 0.06 ^g	6.75 ± 0.00	3.61 ± 0.01 ^b	1.97 ± 0.02 ^b	4.80 ± 0.45 ^f
300/100	0.87	90.20 ^c	39.45 ± 0.43 ^f	6.84 ± 0.03	3.61 ± 0.01 ^b	2.00 ± 0.01 ^a	4.50 ± 0.18 ^f
320/120	0.79	92.45 ^c	47.16 ± 0.22 ^e	6.96 ± 0.02	3.99 ± 0.11 ^a	2.00 ± 0.00 ^a	4.10 ± 0.90 ^e
350/150	0.75	94.04 ^c	56.50 ± 0.80 ^d	7.28 ± 0.01	4.00 ± 0.00 ^a	2.00 ± 0.01 ^a	3.90 ± 0.05 ^d
375/260	0.71	94.15 ^d	58.41 ± 0.21 ^c	7.45 ± 0.00	4.00 ± 0.00 ^a	2.01 ± 0.01 ^a	3.50 ± 0.00 ^c
400/400	0.64	95.67 ^d	61.19 ± 0.49 ^b	7.64 ± 0.01	4.00 ± 0.00 ^a	2.01 ± 0.00 ^a	3.10 ± 0.01 ^b
420/520	0.59	98.91 ^d	75.20 ± 1.69 ^a	7.95 ± 0.02	4.00 ± 0.01 ^a	2.01 ± 0.00 ^a	2.90 ± 0.05 ^a

Values are expressed as mean ± SD. Different letters indicate significant differences (P < 0.05) according to Tukey's multiple range test.

3.5. Viscosity

The viscosity of the obtained *S. japonica* hydrolysate was measured, and the values are shown in Table 1. The viscosity values varied from 6.8 ± 0.20 cP (180 °C/15 bar) to 2.90 ± 0.05 cP (420 °C/520 bar) in the *S. japonica* hydrolysate. The viscosities showed decreasing trends with an increase in temperature. Schrieber et al. [31] reported that the molecular weight of hydrolysates can influence the viscosity of solutions and that hydrolysates with low molecular weight have a tendency to produce a solution of low viscosity that was easier to process even at a relatively high concentration. Hawthorne and Opell [32] described that a decrease in viscosity and surface tension with an increase in temperature improves the mass transfer rates of compounds from plant materials. Therefore, viscosity is an important measurement for the industry to monitor and control hydrolysis.

3.6. Color

Color is one of the most important quality parameters in foods. With regard to color coordinates (Table 2), lightness (L^*) values ranged between 24.06 ± 3.12 and 37.10 ± 0.80 , obtained at different conditions of the hydrolysate. Lightness is influenced by the presence of pigments and hygroscopic substances, which when thermally treated increase their volume and reflected light. The lower lightness values observed in the hydrolysate at higher temperature could be associated with higher browning reactions that could take place in these samples. The coordinate redness (a^* , red–green) showed values ranging between 3.20 and 4.00, while the coordinate yellowness (b^* , yellow–blue) showed values ranging between 1.36 and 2.34. The redness and yellowness values of the hydrolysate obtained at the 420 °C/520 bar condition were higher than those at other conditions. The lower hue values (H) of the *S. japonica* hydrolysate could be related to browning reactions because lower hue values indicate that higher redness is present in the sample. The hue values increased with an increase in temperature. The chroma value (C) was in the range of 4.33 ± 0.14 to 6.41 ± 0.23 .

3.7. Amino acid composition

Amino acids play a key function in the human body to construct a mass of proteins and as intermediaries in metabolism. Various amino acids taste sweet and bitter and also give flavor to food. Therefore, hydrolysates rich in amino acids can be used as seasonings in foodstuff production [33]. The non-essential amino acid (NEAA) and essential amino acid (EAA) yields of the *S. japonica* hydrolysate generated by treatment at various temperatures are shown in Table 3. The NEAA yield was higher than the EAA yield in the extract. The highest yield of total amino acids of the *S. japonica* hydrolysate was found at 180 °C

(761.95 ± 14.54 mg/g). The amino acid yield was reduced at temperatures above 240 °C. All EAAs, except phenylalanine and tryptophan, were detected in the *S. japonica* extract. Among EAAs, threonine, valine, isoleucine, and leucine were abundant. The highest yields for individual EAAs were for threonine (6.15 ± 0.37 mg/g), valine (12.63 ± 0.38 mg/g), isoleucine (4.03 ± 0.48 mg/100 g), and leucine (12.24 ± 0.65 mg/g) at 180 °C. All NEAAs, except proline, were detected in the *S. japonica* extract. The highest yields for NEAA were found at 180 °C for serine (6.70 ± 0.36 mg/g), aspartic acid (280.13 ± 2.06 mg/g), glutamic acid (105.82 ± 1.54 mg/g), glycine (201.16 ± 7.14 mg/g), alanine (71.08 ± 0.86 mg/g), and arginine (61.66 ± 0.67 mg/g). As the temperature increased, the amino acid and total protein contents also decreased. At the 240 °C/34 bar condition, the total protein content was (274.8 ± 12.48 mg/g). EAAs such as threonine (3.11 ± 0.30 mg/g), valine (6.26 ± 0.25 mg/g), isoleucine (1.96 ± 0.30 mg/100 g), and leucine (3.90 ± 0.26 mg/g) and NEAAs such as serine (5.20 ± 0.01 mg/g), aspartic acid (51.33 ± 2.68 mg/g), glutamic acid (63.63 ± 2.41 mg/g), glycine (51.17 ± 3.02 mg/g), alanine (34.88 ± 1.44 mg/g), and arginine (53.27 ± 1.80 mg/g) were found at the 240 °C/34 bar condition. The freeze-dried *S. japonica* hydrolysate showed a total EAA content of 2533.61 ± 113.01 mg/g and total NEAA content of 4107.72 ± 169.96 mg/g.

Asaduzzaman and Chun [17] reported that amino acids are converted into organic acids or volatile resources during hydrolysis, resulting in the substantial reduction of amino acid content using hydrolyzed processing of marine resources. Therefore, it can be assumed that high temperatures lead to the reduction of amino acids into organic acids or other byproducts. Cheng et al. [34] reported that the yields of most amino acids were high at reaction temperatures of 180 °C–220 °C. In the report by Mabeau and Fleurenc et al. [35], the aspartic and glutamic acid levels were high and were major factors for the taste and flavor of seaweed. The total amino acid content in *S. japonica* was reported by Shin et al. [36]; when compared with this result, even after hydrolysis, almost all amino acid contents were maintained until 240 °C, after which all amino acids were destroyed.

3.8. Mineral content

The dominant molecule in brown seaweed is a carbohydrate, with alginate identified as the major constituent. Alginate is found in most brown seaweeds as a physical constituent for the formation of cell wall, and it forms an insoluble salt, chiefly comprising calcium, with minor amounts of magnesium, sodium, and potassium [19]. In the present study, various mineral components such as macrominerals (calcium, magnesium, sodium, phosphorus, and potassium), microminerals (copper, iron, iodine, manganese, zinc, and aluminum), and heavy metals (arsenic, cadmium, mercury, and lead) were determined in the *S. japonica* hydrolysate (Table 4). The *S. japonica* hydrolysate contained

Table 2
Color properties of *S. japonica* at different PHWE conditions.

Conditions (°C/bar)	Color				
	L^*	a^*	b^*	C	H
180/13	37.10 ± 0.80^a	4.89 ± 1.09^a	1.36 ± 0.14^g	5.08 ± 0.05^b	15.54 ± 1.89^g
200/17	34.56 ± 5.35^b	4.78 ± 0.40^b	2.1 ± 0.32^f	5.22 ± 0.02^b	23.71 ± 2.07^f
220/25	32.68 ± 6.32^c	4.00 ± 2.14^c	2.34 ± 0.86^f	4.63 ± 0.17^c	30.32 ± 1.03^e
240/34	31.38 ± 7.85^d	3.82 ± 3.23^d	2.46 ± 0.53^f	4.54 ± 0.21^c	32.78 ± 5.00^e
260/49	30.97 ± 3.45^e	3.20 ± 0.10^d	2.91 ± 0.90^e	4.33 ± 0.14^c	42.28 ± 2.78^d
280/72	30.08 ± 6.00^f	2.89 ± 0.43^e	3.01 ± 0.25^d	4.17 ± 0.11^c	46.16 ± 3.94^d
300/100	29.10 ± 7.21^g	2.71 ± 0.71^e	3.6 ± 0.10^d	4.51 ± 0.05^c	53.02 ± 1.65^c
320/120	28.49 ± 5.10^h	2.56 ± 1.23^e	4.29 ± 0.77^c	5.00 ± 0.02^b	59.17 ± 0.77^c
350/150	27.99 ± 6.90^i	2.21 ± 0.13^e	4.56 ± 0.35^c	5.07 ± 0.02^b	64.14 ± 0.19^b
375/260	27.45 ± 4.50^j	2.01 ± 0.54^e	5.10 ± 0.02^b	5.48 ± 0.06^b	68.48 ± 1.80^b
400/400	25.12 ± 8.25^k	1.97 ± 0.24^f	5.48 ± 0.20^b	5.82 ± 0.04^b	70.22 ± 2.31^a
420/520	24.06 ± 3.12^l	1.89 ± 0.65^g	6.12 ± 0.15^a	6.41 ± 0.23^a	72.83 ± 2.07^a

L – lightness, a – redness, b – yellowness, C – chroma, and H – hue. Values are expressed as mean \pm SD. Different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple range test.

Table 3
Total amino acid yield from *S. japonica* at different PHWE conditions.

Conditions (°C/bar)													
	Freeze dried <i>S. japonica</i>	180/13	200/17	220/25	240/34	260/49	280/72	300/100	320/120	350/150	375/260	400/400	420/520
<i>Essential amino acids (EAA)</i>													
Threonine	432.17 ± 10.02 ^{a,b}	6.15 ± 0.37 ^a	5.69 ± 0.12 ^a	5.22 ± 0.20 ^b	3.11 ± 0.30 ^c	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	451.07 ± 18.24 ^{a,b}	12.63 ± 0.38 ^a	11.14 ± 0.31 ^b	11.88 ± 0.46 ^b	6.26 ± 0.25 ^f	4.18 ± 0.25 ^c	3.00 ± 0.34 ^d	1.02 ± 0.11 ^e	0.11 ± 0.02 ^g	N.D.	N.D.	N.D.	N.D.
Methionine	201.15 ± 14.17 ^{b,c}	0.03 ± 0.01 ^a	0.02 ± 0.01 ^b	0.01 ± 0.01 ^c	0.01 ± 0.01 ^c	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Isoleucine	301.78 ± 20.41 ^b	4.03 ± 0.48 ^a	3.27 ± 0.40 ^b	3.26 ± 0.30 ^b	1.96 ± 0.30 ^c	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Leucine	419.19 ± 4.74 ^{a,b}	12.24 ± 0.65 ^a	9.74 ± 0.37 ^b	8.25 ± 0.30 ^c	3.90 ± 0.26 ^d	2.25 ± 0.25 ^e	1.02 ± 0.01 ^f	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Histidine	320.19 ± 26.06 ^a	0.05 ± 0.00 ^c	0.05 ± 0.00 ^c	0.02 ± 0.00 ^d	0.08 ± 0.00 ^a	0.06 ± 0.00 ^b	0.06 ± 0.00 ^b	0.06 ± 0.00 ^b	0.05 ± 0.00 ^c	N.D.	N.D.	N.D.	N.D.
Lysine	408.06 ± 19.37 ^{a,b}	0.09 ± 0.00 ^c	0.05 ± 0.00 ^b	0.02 ± 0.00 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total EAA	2533.61 ± 113.01	35.22 ± 1.89	29.96 ± 1.21	28.66 ± 1.27	15.32 ± 1.12	6.49 ± 0.50	4.08 ± 0.35	1.08 ± 0.11	0.16 ± 0.02	N.D.	N.D.	N.D.	N.D.
<i>Non-essential amino acids (NEAA)</i>													
Serine	400.10 ± 39.10 ^{a,b}	6.70 ± 0.36 ^b	5.78 ± 0.14 ^a	5.51 ± 0.26 ^a	5.20 ± 0.01 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aspartic acid	1040.22 ± 12.50 ^a	280.13 ± 2.06 ^a	226.82 ± 1.16 ^b	87.88 ± 1.44 ^c	51.33 ± 2.68 ^d	41.84 ± 0.08 ^e	30.33 ± 1.35 ^f	9.49 ± 0.27 ^g	N.D.	N.D.	N.D.	N.D.	N.D.
Glutamic acid	987.41 ± 17.86 ^a	105.82 ± 1.54 ^a	83.24 ± 2.25 ^b	77.34 ± 1.02 ^c	63.63 ± 2.41 ^d	22.29 ± 1.32 ^e	10.05 ± 0.23 ^f	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glycine	519.20 ± 26.89 ^{a,b}	201.16 ± 7.14 ^a	177.34 ± 2.07 ^b	83.98 ± 1.77 ^c	51.17 ± 3.02 ^d	38.12 ± 1.02 ^e	35.26 ± 1.55 ^e	10.05 ± 0.73 ^f	N.D.	N.D.	N.D.	N.D.	N.D.
Alanine	508.47 ± 30.35 ^{a,b}	71.08 ± 0.86 ^a	61.82 ± 0.62 ^b	50.03 ± 0.60 ^c	34.88 ± 1.44 ^d	20.67 ± 0.54 ^e	5.00 ± 0.23 ^f	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cysteine	98.76 ± 11.09 ^c	0.10 ± 0.01 ^a	0.05 ± 0.01 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tyrosine	143.25 ± 13.88 ^c	0.08 ± 0.01 ^a	0.05 ± 0.01 ^a	0.01 ± 0.01 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Arginine	410.31 ± 18.29 ^{a,b}	61.66 ± 0.67 ^a	59.82 ± 0.24 ^b	58.38 ± 0.12 ^b	53.27 ± 1.80 ^c	50.47 ± 0.64 ^d	49.82 ± 0.76 ^e	30.42 ± 0.72 ^f	10.51 ± 0.15 ^g	N.D.	N.D.	N.D.	N.D.
Total NEAA	4107.72 ± 169.96	726.73 ± 12.65	614.92 ± 6.50	363.13 ± 5.22	259.48 ± 11.36	173.39 ± 3.60	130.46 ± 4.12	49.96 ± 1.72	10.51 ± 0.15	N.D.	N.D.	N.D.	N.D.
Total amino acid	6641.33 ± 282.97	761.95 ± 14.54	644.88 ± 7.71	391.79 ± 6.23	274.8 ± 12.48	179.88 ± 4.1	136.37 ± 4.47	51.04 ± 1.83	10.67 ± 0.17	N.D.	N.D.	N.D.	N.D.

Values are expressed as mean ± SD and in mg/g. Different letters indicate significant differences (P < 0.05) according to Turkey's multiple range test. N.D., not detected. Phenylalanine, tryptophan and proline were not detected in all the conditions.

Table 4
Macro-minerals, micro-minerals and heavy metal contents obtained from *S. japonica* during different PHWE conditions.

Conditions (°C/bar)													
	Freeze dried <i>S. japonica</i>	180/13	200/17	220/25	240/34	260/49	280/72	300/100	320/120	350/150	375/260	400/400	420/520
<i>Macrominerals</i>													
Calcium	4570.41 ± 40.80 ^c	0.32 ± 0.01 ^g	0.60 ± 0.03 ^{e,f}	0.55 ± 0.00 ^f	0.70 ± 0.05 ^e	0.68 ± 0.01 ^e	1.31 ± 0.05 ^d	1.48 ± 0.02 ^c	1.59 ± 0.04 ^{b,c}	1.59 ± 0.05 ^b	1.60 ± 0.02 ^b	1.60 ± 0.02 ^a	1.71 ± 0.04 ^b
Magnesium	4190.38 ± 30.06 ^c	0.57 ± 0.02 ^g	0.60 ± 0.02 ^g	0.56 ± 0.00 ^{f,g}	0.89 ± 0.05 ^f	0.30 ± 0.02 ^e	1.47 ± 0.03 ^d	2.01 ± 0.02 ^c	2.23 ± 0.12 ^c	2.20 ± 0.05 ^b	2.30 ± 0.02 ^b	2.37 ± 0.03 ^b	2.46 ± 0.02 ^a
Phosphorus	1500.26 ± 10.47 ^d	0.52 ± 0.02 ^a	0.35 ± 0.00 ^b	0.30 ± 0.01 ^b	0.23 ± 0.01 ^c	0.22 ± 0.01 ^c	0.08 ± 0.00 ^d	0.09 ± 0.04 ^d	0.09 ± 0.00 ^d	0.09 ± 0.00 ^d	0.09 ± 0.00 ^d	0.01 ± 0.00 ^e	0.01 ± 0.00 ^e
Potassium	35,870.47 ± 19.21 ^a	4.92 ± 0.22 ^k	6.59 ± 0.06 ^j	6.55 ± 0.05 ^j	9.18 ± 0.09 ⁱ	9.89 ± 0.26 ^h	10.35 ± 0.10 ^g	13.19 ± 0.09 ^f	16.35 ± 0.07 ^e	16.48 ± 0.07 ^d	17.70 ± 0.28 ^c	20.48 ± 0.10 ^b	24.34 ± 0.18 ^a
Sodium	9980.24 ± 37.89 ^b	1.34 ± 0.01 ^l	1.71 ± 0.01 ^k	2.41 ± 0.11 ^j	3.14 ± 0.08 ⁱ	4.25 ± 0.13 ^h	5.00 ± 0.14 ^g	6.23 ± 0.12 ^f	7.00 ± 0.14 ^e	8.23 ± 0.12 ^d	10.10 ± 0.05 ^c	10.19 ± 0.09 ^b	12.65 ± 0.08 ^a
<i>Microminerals</i>													
Iron	2.14 ± 0.10 ^l	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Iodine	30.60 ± 0.48 ^e	0.03 ± 0.01 ^b	0.02 ± 0.01 ^a	0.02 ± 0.01 ^{b,c}	0.02 ± 0.01 ^{b,c}	0.01 ± 0.01 ^c	0.09 ± 0.01 ^a	0.07 ± 0.01 ^a	0.08 ± 0.01 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a	0.09 ± 0.01 ^a	0.08 ± 0.01 ^a
Manganese	1.98 ± 0.50 ^l	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a
Zinc	10.80 ± 0.21 ^g	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aluminum	31.46 ± 0.17 ^e	0.36 ± 0.05 ^a	0.20 ± 0.07 ^b	0.20 ± 0.01 ^b	0.20 ± 0.01 ^b	0.21 ± 0.01 ^b	0.19 ± 0.01 ^b	0.18 ± 0.01 ^b	0.18 ± 0.01 ^b	0.18 ± 0.01 ^b	0.18 ± 0.01 ^b	0.18 ± 0.01 ^b	0.18 ± 0.01 ^b
<i>Heavy metals</i>													
Arsenic	20.89 ± 0.10 ^f	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a	3.25 ± 0.01 ^a
Cadmium	0.50 ± 0.01 ^l	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a
Mercury	5.06 ± 0.01 ^h	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Lead	0.35 ± 0.01 ^k	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a

Values are expressed as mean ± SD and in µg/g in DM (dry matter). Different letters indicate significant differences (P < 0.05) according to Turkey's multiple range test. N.D., not detected. Copper was not detected in all the conditions.

significant amounts of macro- and microminerals. Magnesium, calcium, potassium, and sodium contents were increased with an increase in temperature. The amount of each mineral between the 180 °C/13 bar and 420 °C/520 bar conditions was as follows: calcium [0.32 ± 0.01 µg/g in dry matter (DM) to 1.71 ± 0.04 µg/g in DM], magnesium (0.57 ± 0.02 µg/g in DM to 2.46 ± 0.02 µg/g in DM), potassium (4.92 ± 0.22 µg/g in DM to 24.34 ± 0.18 µg/g in DM), and sodium (1.34 ± 0.01 µg/g in DM to 12.65 ± 0.08 µg/g in DM). The most abundant element in the *S. japonica* hydrolysate was potassium, and the sodium content strongly increased with an increase in temperature. In contrast, the aluminum content decreased with an increase in temperature from 180 °C/13 bar (0.36 ± 0.05 µg/g in DM) to 420 °C/520 bar (0.18 ± 0.01 µg/g in DM). Iron, iodine, manganese, and zinc were found in trace amounts from 0.01 ± 0.01 µg/g in DM to 0.03 ± 0.01 µg/g in DM in all hydrolysis conditions. The increase in sodium and potassium and decrease in aluminum contents clearly show that the decomposition of the seaweed solid matter occurred very rapidly at high temperature. Heavy metals are considered as the major component in mineral composition. These heavy metals are considered as one of the important causes for polluting the environment due to their toxicity, tenacity, and ability to bioaccumulate [37]. The heavy metal composition of the *S. japonica* hydrolysate (arsenic, cadmium, mercury, and lead) is shown in Table 4. The heavy metal content of the *S. japonica* hydrolysate did not vary in any of the conditions. Arsenic was the most abundant heavy metal (3.25 ± 0.01 µg/g in DM); other heavy metals were found in very trace amounts in the *S. japonica* hydrolysate. The freeze-dried *S. japonica* showed 20.89 ± 0.10 µg/g in DM.

Taking these results into consideration, we can say that the contribution of aluminum from this seaweed hydrolysate is well below the specified provisional tolerable weekly intake (PTWI). The World Health Organization (WHO) technical report series 959 has reported that worldwide, the total arsenic value ranges up to 236 ppm for 953 food samples (WHO, 2011). In addition, the European Commission (EC) has mentioned in food surveys conducted in Denmark and UK that more than 50% of arsenic in daily diet comes from seafood. The PTWI values specified by both the Joint Expert Committee on Food Additives (JECFA) and EC for Pb and Cd are 7 ppb and 25 ppb, respectively (EC, 2004; JECFA, 2003). The JECFA has recommended a PTWI value of 6 ppm for arsenic in foods (JECFA, 2010). Khan et al. [38] reported that Al and As were found in high amounts in *S. japonica* (4.89 and 3.04 ppm, respectively), Hg content was very low (only 0.006 ppm), whereas the total arsenic content was below 4.49 ppm. Considering the various research findings and guideline values, the concentration of heavy metals was very low and their contribution to the total intake was very small. Thus, all *S. japonica* hydrolysates that were studied could be declared safe.

3.9. Monosaccharide composition

The PHWE processes can be used to break down the polysaccharide polymer in *S. japonica*. The extracts obtained were analyzed for their contents of sugars (Table 5). This table shows that various types of monosaccharides were identified such as galactose, glucose, gulose, fructose, arabinose, mannose, mannitol, sorbitol, xylitol, and xylose. Previously, in a study conducted at our laboratory, Meillisa et al. [19] stated the presence of mannose and gulose at the subcritical level, which also has been included in Table 5. Therefore, we continue to conduct research at high temperature conditions to check the degradation ability of brown seaweeds. The sugar content was changed in each condition. Glucose, gulose, fructose, mannitol, and xylose were the most abundant monosaccharides found in the *S. japonica* hydrolysate. The amount of galactose (2.33 ± 0.25 g/L), glucose (6.70 ± 0.00 g/L), gulose (9.80 ± 0.40 g/L), fructose (8.40 ± 0.18 g/L), arabinose (1.50 ± 0.13 g/L), mannose (1.50 ± 0.20 g/L), mannitol (17.50 ± 0.07 g/L), sorbitol (1.30 ± 0.05 g/L), xylitol (3.48 ± 0.33 g/L), and xylose (5.30 ± 0.31 g/L) were high at the 180 °C/13 bar condition. The recovery of sugars was

Table 5
The monosaccharide content from *S. japonica* at different PHWE conditions.

Conditions (°C/bar)	Galactose	Gulose	Glucose	Fructose	Arabinose	Mannose	Mannitol	Sorbitol	Xylitol	Xylose
Freeze dried <i>S. japonica</i>	12.68 ± 1.81 ^b	N.D.	8.15 ± 1.17 ^c	N.D.	12.15 ± 0.70 ^b	4.21 ± 0.52 ^a	89.47 ± 12.04 ^b	N.D.	N.D.	6.90 ± 0.10 ^d
180/13	2.33 ± 0.25 ^a	9.80 ± 0.40 ^a	6.70 ± 0.00 ^a	8.40 ± 0.18 ^a	1.50 ± 0.13 ^a	1.50 ± 0.20 ^a	17.50 ± 0.07 ^a	1.30 ± 0.05 ^a	3.48 ± 0.33 ^a	5.00 ± 0.31 ^a
200/17	2.17 ± 0.02 ^b	8.30 ± 0.13 ^a	6.50 ± 0.12 ^{ab}	8.00 ± 0.05 ^{ab}	1.25 ± 0.1 ^b	1.20 ± 0.15 ^a	15.90 ± 0.20 ^b	1.25 ± 0.02 ^a	3.10 ± 0.31 ^b	5.30 ± 0.05 ^a
220/25	1.87 ± 0.03 ^c	9.00 ± 0.36 ^{ab}	6.30 ± 0.00 ^b	7.90 ± 0.15 ^{bc}	1.05 ± 0.05 ^{bc}	1.10 ± 0.20 ^{bc}	13.85 ± 0.42 ^c	1.19 ± 0.04 ^{ab}	2.85 ± 0.02 ^b	5.30 ± 0.17 ^a
240/34	1.74 ± 0.03 ^c	8.50 ± 0.07 ^b	6.30 ± 0.00 ^b	7.50 ± 0.09 ^c	0.99 ± 0.06 ^{bc}	1.20 ± 0.02 ^{abc}	11.15 ± 0.10 ^d	1.19 ± 0.05 ^a	2.54 ± 0.10 ^{bc}	5.30 ± 0.10 ^a
260/49	1.56 ± 0.07 ^d	9.10 ± 0.12 ^b	6.25 ± 0.02 ^b	7.00 ± 0.36 ^c	0.97 ± 0.01 ^{bc}	1.00 ± 0.12 ^{bcd}	8.75 ± 0.37 ^e	1.14 ± 0.16 ^a	2.16 ± 0.05 ^{de}	4.80 ± 0.11 ^{ab}
280/72	1.43 ± 0.03 ^d	6.00 ± 0.25 ^c	5.85 ± 0.16 ^c	6.40 ± 0.04 ^d	0.92 ± 0.02 ^c	0.95 ± 0.02 ^{cde}	6.20 ± 0.10 ^f	1.10 ± 0.26 ^a	2.10 ± 0.11 ^{bc}	4.50 ± 0.05 ^{bc}
300/100	1.20 ± 0.05 ^e	5.26 ± 0.13 ^d	5.50 ± 0.15 ^c	6.35 ± 0.01 ^{de}	0.87 ± 0.01 ^c	0.85 ± 0.00 ^{def}	4.75 ± 0.32 ^g	0.98 ± 0.01 ^{abc}	2.00 ± 0.02 ^{de}	4.20 ± 0.30 ^c
320/120	1.14 ± 0.05 ^e	4.16 ± 0.09 ^e	5.00 ± 0.10 ^c	6.30 ± 0.13 ^{de}	0.88 ± 0.04 ^d	0.81 ± 0.01 ^{defg}	3.16 ± 0.47 ^h	0.95 ± 0.02 ^{abc}	1.99 ± 0.05 ^{de}	3.85 ± 0.23 ^d
350/150	1.01 ± 0.02 ^f	3.83 ± 0.21 ^e	4.80 ± 0.10 ^c	5.80 ± 0.10 ^d	0.44 ± 0.01 ^e	0.76 ± 0.08 ^{defg}	2.41 ± 0.20 ⁱ	0.89 ± 0.06 ^{abc}	1.74 ± 0.02 ^e	3.62 ± 0.28 ^{de}
375/260	0.85 ± 0.04 ^g	2.52 ± 0.18 ^f	4.50 ± 0.25 ^d	5.40 ± 0.10 ^e	0.35 ± 0.02 ^e	0.69 ± 0.02 ^{efg}	1.85 ± 0.13 ^j	0.87 ± 0.05 ^{b,c}	1.20 ± 0.09 ^f	3.36 ± 0.18 ^{de}
400/400	0.77 ± 0.01 ^g	1.82 ± 0.04 ^f	4.00 ± 0.01 ^d	4.30 ± 0.12 ^f	0.34 ± 0.03 ^e	0.60 ± 0.03 ^{fg}	1.45 ± 0.02 ^j	0.85 ± 0.02 ^c	0.88 ± 0.05 ^g	3.04 ± 0.02 ^f
420/520	0.31 ± 0.03 ^h	0.51 ± 0.01 ^f	3.80 ± 0.15 ^e	3.00 ± 0.18 ^g	0.13 ± 0.04 ^f	0.50 ± 0.01 ^g	1.04 ± 0.03 ^j	0.79 ± 0.05 ^c	0.64 ± 0.03 ^g	2.57 ± 0.05 ^f

Values are expressed as mean ± SD and in g/L. Different letters indicate significant differences ($P < 0.05$) according to Turkey's multiple range test. N.D., not detected.

high at the 180 °C/13 bar condition, but when the temperature and pressure were increased up to 420 °C/520 bar, the concentration of sugars in the *S. japonica* hydrolysate gradually decreased: galactose (12.68 ± 1.81 g/L), glucose (8.15 ± 1.17 g/L), arabinose (12.15 ± 0.70 g/L), mannose (4.21 ± 0.52 g/L), mannitol (89.47 ± 12.04 g/L), and xylose (6.90 ± 0.10 g/L). Therefore, we can say that monosaccharides are not stable at higher temperature and pressure. The freeze-dried sample showed galactose (2.33 ± 0.25 g/L), glucose (6.70 ± 0.00 g/L), gulose (9.80 ± 0.40 g/L), fructose (8.40 ± 0.18 g/L), arabinose (1.50 ± 0.13 g/L), mannose (1.50 ± 0.20 g/L), mannitol (17.50 ± 0.07 g/L), sorbitol (1.30 ± 0.05 g/L), xylitol (3.48 ± 0.33 g/L), and xylose (5.30 ± 0.31 g/L). From our result, it seems that the monosaccharide content decreased as a function of temperature. Probably caramelization and MRPs are being promoted by the increase in temperature.

Our *S. japonica* hydrolysate showed very high content of mannitol (17.50 ± 0.07 g/L) at the 180 °C/13 bar condition. In *S. japonica*, the mannitol content is approximately 5% of its dry weight, but it increases in summer up to >30% of its dry weight when the growth of kelp is maximum [39]. Ito and Hori [40] reported that 24.3% of dry weight is because of mannitol in *S. japonica*.

Mannose is a type of sugar that is most commonly used as a source for bioethanol production [41]. Mannose is reduced to mannitol, which has various industrial uses and is mostly used to make tablets or medicine [42]. Mannitol is classified as a sugar alcohol. Further, byproducts of sugar alcohols consist of xylitol and sorbitol. These two are isomeric sugars; the lone variance is the positioning of the hydroxyl group on carbon [43]. The WHO has listed mannitol as one of the important drugs; it was also listed as a very vital medicine for the well-being of humans. The global market for sugar alcohols in 2000 was \$1.3 billion. The largest sugar alcohol in terms of volume and dollar sales was sorbitol. The bulk prices for liquid and crystalline sorbitol are approximately \$0.55–0.65 per kg and \$1.61–2.26 per kg, respectively. The annual mannitol market was estimated at approximately 30,000 tons. However, according to a recent issue of the Chemical Market Reporter, the bulk price of mannitol (powdered) is \$7.32 per kg. For most sugar alcohols, the market is mature, and volume growths are expected to follow the trends of large-scale consumer products in which sugar alcohols are used [44].

4. Conclusion

This study showed that PHWE of *S. japonica* affects the yield, TOC, pH, MRPs, viscosity, and amino acid, mineral, and monosaccharide contents during different temperature conditions. High temperature causes TOC, pH, and the MRP content to increase. EAAs were recovered well at 180 °C than at other conditions. The contents of minerals such as calcium, magnesium, potassium, and sodium were found to be increased with an increase in temperature, whereas cadmium, mercury, arsenic, and lead were found in trace amounts under all conditions. The monosaccharide profile showed that the increase in temperature decomposes sugars. The mannitol content (17.5 g/L) was very high at 180 °C compared with other sugars. It can be concluded that 180 °C is the optimum condition to have a good nutritional composition of amino acids, minerals, and sugars. At high temperature conditions, all chemical compositions were degraded in the brown seaweed. Therefore, the pressurized hot water extract of *S. japonica* can be used as a good source of bioenergy, raw material source in the fermentation industry, and human food. Further research is ongoing to isolate and detect the structure of monosaccharides such as gulose and mannitol from *S. japonica* extracts.

Author contributions

All the experimental work was conducted by P.S. Saravana, J.H. Choi, Y.B. Park, and H.C. Woo. Manuscript was prepared by P.S. Saravana, B.S.

Chun supervised this work and provided all experimental and analytical equipment. All authors read and approved the final manuscript.

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