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Thermal stabilities of polyphenols and fatty acids in *Laminaria japonica* hydrolysates produced using subcritical water

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Abstract−We investigated the thermal stability of polyphenols and fatty acids from *Laminaria japonica* powder by subcritical water hydrolysis among the range of the experimental conditions. The experiment was carried out at temperatures and pressures ranging from 200 to 280 °C and 13 to 60 bar, respectively for 28 to 42 min. Polyphenol and fatty acids in hydrolysates obtained from different conditions were analyzed by GC and UV-spectrophotometer. The results were compared with those obtained from *Laminaria japonica* oils extracted by supercritical CO_2. Polyphenol and several fatty acids in hydrolysates produced by subcritical water showed high thermal stability.

Key words: Fatty Acid, *Laminaria japonica*, Polyphenol, Subcritical Water Hydrolysis, Supercritical Carbon Dioxide, Thermal Stability

INTRODUCTION

Seaweed is a potential renewable resource in the marine environment. It provides an excellent source of bioactive compounds, such as carotenoid, dietary fiber, protein, vitamins, essential fatty acid, and minerals [1,2]. Interest in seaweed lipid has been on the rise owing to the recognition of important bioactive molecules like conjugated fatty acids, pigments that have profound physiological effects in the treatment of tumors and other cancer related problems [3-5]. In addition, polyunsaturated fatty acids (PUFAs) are reported to share more than 30% of the total fatty acid in diatom or brown algae [6]. *Laminaria japonica* is a sort of brown seaweed that contains carotenoids. It has the ability to rapidly colonize disturbed or new surfaces. This seaweed is widely used as a human food in different countries, especially in Korea and Japan.

Polyphenols of plant origin may act as antioxidants in human and animal diets, thereby reducing the risk of atherosclerosis and coronary heart disease. They can also protect against some forms of cancer [7]. It is widely accepted that significant antioxidant activity of food is related to high total phenolic content. Plants and foods contain a large variety of phenolic derivatives, including simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans, and lignins [8].

Currently, the most common method of extraction is liquid solvent extraction using many organic solvents. However, the conventional method involves the discharge of potentially hazardous solvents to the environment and can also damage the functional properties of the extracts by hydrothermal stress [9,10]. Therefore, alternative extraction techniques with better selectively and efficiently are sought. Supercritical fluid extraction (SFE) is an alternative separation technology. The extract obtained from SFE contains fewer polar impurities than the current organic liquid extract and so subsequent purification steps become easier [11]. The most commonly used supercritical fluid is supercritical carbon dioxide (SCO_2) because it has a favorable critical temperature and pressure (304.1 K and 73.8 bar) that enables heat labile materials such as biomolecules to be processed. In particular, SCO_2 has further processing advantages such as low viscosity, low surface tension, high diffusivity and good density, which play key roles in enabling the solvent to readily penetrate the solid biomass matrix as well as in extracting the solutes. SCO_2 is also non-toxic, nonflammable, inexpensive, widely available and chemically inert under many conditions [12-15].

Subcritical water (SW) is also an effective solvent for both polar and non-polar compounds. As the temperature of water increases, the polarity of water decreases. As a result, the solubility of non-polar organic compound increases, and the solubility of polar organic compound decreases [16]. By achieving low polarities at elevated temperatures, subcritical water hydrolysis (SWH) has proved high ability to produce high yields with short extraction time for a number of hydrophobic organic compounds [17]. For this reason, both SCO_2 and SW are considered very useful solvents for many industries such as the nutraceuticals and pharmaceuticals. The aim of the present work was to investigate the thermal stability of useful materials such as polyphenol and fatty acid from freeze-dried *Laminaria japonica* powder after subcritical water hydrolysis among the range of the experimental conditions.

MATERIALS AND METHODS

1. Materials

Laminaria japonica was collected from Guemil-eup, Wando-gun, Jeonnam, South Korea. Food grade carbon dioxide was pure in 99.9%. Gallic acid was purchased from Sigma Aldrich Co. (USA). All reagents were of analytical grade.

2. Sample Preparation

Fresh *Laminaria japonica* samples were thoroughly washed with water and their holdfasts and epiphytes were removed. The cleaned

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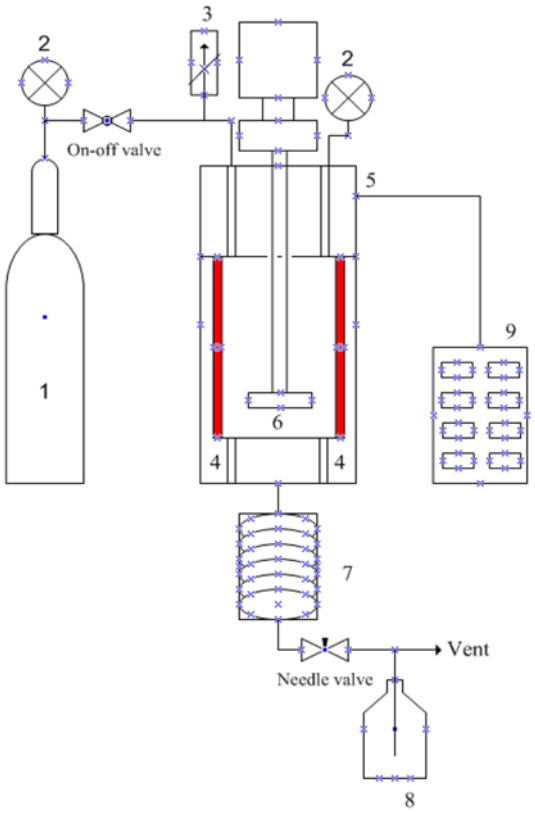

Fig. 1. Schematic diagram of subcritical water hydrolysis experimental apparatus.

1. Air tank
2. Pressure gauge
3. Safety valve
4. Electric heater
5. High pressure reactor
6. Impeller (stirrer)
7. Cooling bath
8. Separator
9. Stirring speed & temperature controller

samples were frozen and then dried in a freeze-drier for three days. The dried samples were ground by a mechanical blender and sieved (700 μm) by mesh and then stored at −20 °C.

3. Methods

3-1. Subcritical Water Hydrolysis

The SWH was carried out in 200 cm^3 of a batch reactor made of 276 Hastelloy with temperature control. Fig. 1 shows a schematic diagram of the SWH apparatus. 3 g of freeze dried samples were suspended separately in 150 ml of distilled water (material to water ratio of 1 : 50) and charged into the reactor. The reactor was then closed and heated by an electric heater to the desired temperatures at 200 °C, 220 °C, 240 °C, 260 °C, and 280 °C. The pressures were estimated based on saturated steam at 13 bar, 19 bar, 30 bar, 42 bar, and 60 bar for the temperature range studied. The temperature and pressure in the reactor of each experiment were measured by temperature controller and pressure gauge, respectively. The sample was stirred by stirrer at 150 rpm. The heat-up time for reaching the desired temperature took from 28 to 42 min (heat-up time is the amount of time that a device or system requires to go from a cold start to operating temperature). After rapid cooling to room temperature, the hydrolyzed samples from the reactor were collected and filtered using a filter paper (Advantec No. 5A). The liquid portion called hydrolysate was analyzed for polyphenol and fatty acids. All experiments were performed in duplicate.

3-2. SCO_2 Extraction

A laboratory scale SCO_2 extraction unit was used to extract oils including polyphenol and fatty acids. Schematic diagram of the SCO_2 extraction apparatus is shown in Fig. 2. *Laminaria japonica* powder (250 g) was packed into a stainless steel extraction vessel (extractor), which was 500 ml in volume. Before plugging with a cap, another layer of cotton was used at the bottom of the extraction vessel. Liquefied carbon dioxide was pumped to the extraction vessel by a high-

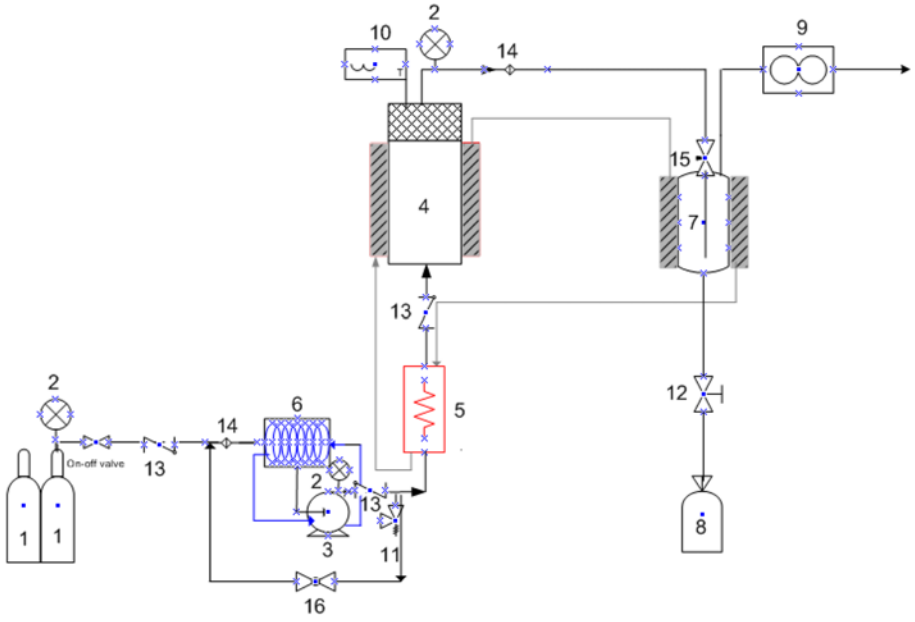

Fig. 2. Schematic diagram of supercritical carbon dioxide extraction apparatus.

1. CO_2 tank
2. Pressure gauge
3. High pressure pump
4. Extractor
5. Heat exchanger
6. Chiller
7. Separator
8. Sample collector
9. Flow meter
10. Digital thermometer
11. Safety valve
12. Needle valve
13. Check valve
14. Filter
15. Metering valve
16. BPR

pressure pump (pu-2-88, Jasco, Japan) up to the desired pressure which was regulated by a back-pressure regulator. The experiment was performed at temperature ranges of 35 to 55 °C and pressures from 150 to 250 bar for 2 h. The flow rate of CO_2 was kept constant at 26.81 g/min for all extraction conditions, and CO_2 volume passing through the apparatus was measured with a dry gas meter. The extracted oil was collected on the glass separation vessels and stored at −40 °C until further analysis.

3-3. Hexane Extraction

The extraction was carried out in a soxhlet apparatus with hexane as solvent. Two grams of freeze dried raw *Laminaria japonica* sample was placed into the extraction thimble and the extraction was run 24 h until the color of the condensed solvent at the top of the apparatus was clear. The extracted oil was collected on the vial and stored at −40 °C until further analysis.

3-4. Fatty Acids Analysis

Fatty acids compositions of *Laminaria japonica* obtained after SWH and SCO_2 extraction were determined by GC-flame ionization detector (FID) using a Agilent Technologies 6890N gas chromatograph (Agilent Technologies, USA). The fatty acid methyl esters (FAMEs) were prepared firstly and then separated using an SP™-2560, Fused silica capillary column (100 m length×0.25 mm internal diameter, 0.20 μm of film). Helium at a flow rate 0.9 ml/min was used as a carrier gas of fatty acid methyl esters. The split ratio was fixed at 10 : 1. The oven temperature was programmed starting at a constant temperature of 140 °C for 5 min, and then increased to 240 °C at a rate of 3.5 °C/min and hold at 240 °C for 15 min. Injector and detector temperatures were 250 °C. FAMEs were identified by comparison of retention time with standard 37 Component FAMEs mixture (Supleco™, USA).

3-5. Total Polyphenol Analysis

The total polyphenol content (TPC) was determined by the Folin-Ciocalteau method [18]. The extraction method of polyphenols described by the ISO 14502-1 was used [19]. Briefly, 0.200±0.001 g of each sample was weighted in an extraction tube, and 5 ml of 70% methanol at 70 °C was added. The extract was mixed and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 200 g for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated twice. Both extracts were pooled and volume adjusted to 10 ml with cold 70% methanol. 300 μl of saturated Na_2CO_3 were mixed with 20 μl of *Laminaria japonica* liquid extracts and added 1.58 ml of distilled water and 100 μl of 2 N Folin-Ciocalteau reagent. Absorbance was measured at 765 nm in a UV-spectrophotometer (UVIKON 933, USA) after 30 min. Gallic acid was used as a standard and the total phenolic content was expressed as gallic acid equivalents. To evaluate the thermal stability of polyphenols, TPC extracted by SWH was compared with that extracted by SCO_2 and with that extracted using methanol (70%) extraction from raw *Laminaria japonica*. The methanol extract was concentrated by vacuum evaporator, then TPC from SCO_2 extracted oil and from the concentrated methanol extract were analyzed by the same method mentioned above.

RESULTS AND DISCUSSIONS

1. Subcritical Water Hydrolysis Yield

The hydrolysis efficiency of *Laminaria japonica* at different temperatures after SWH is shown in Fig. 3. The residual *Laminaria japonica* powder recovered after SWH was dried and weighed [W (g)], Conversion yield of *Laminaria japonica*, X, was evaluated from the weight change of *Laminaria japonica*, as:

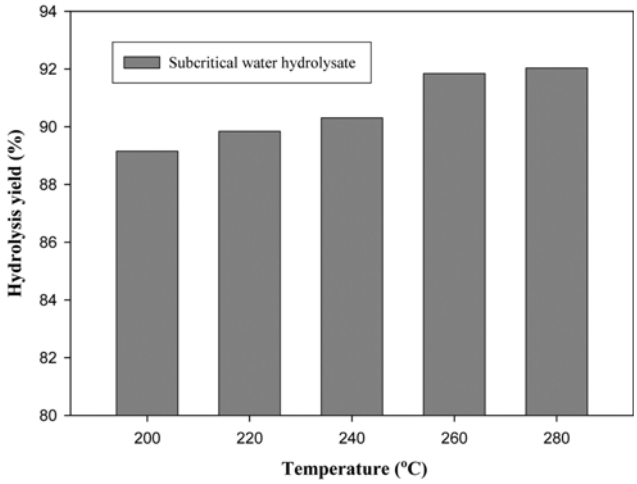

Fig. 3. Comparison of hydrolysis yield of *Laminaria japonica* obtained by subcritical water at different conditions.

$$X = \frac{W_0 - W}{W_0} \times 100$$

Where, W_0 is the total amount of *Laminaria japonica* introduced in reactor, which is nearly 3 g.

The hydrolysis yield increased with the increase in temperature in the vessel (high pressure reactor). The highest hydrolysis yield in *Laminaria japonica* was 92.03% at 280 °C. Similar results were reported by Watchararuji et al. [20] for subcritical water hydrolysis of rice bran and soybean meal. In fact, because of its strong aggregation through hydrophobic interactions, protein usually has low solubility in water at ambient temperature. However, the solubility of new materials such as protein in water increases at higher temperature. In addition, at high temperature the hydrolysis yield increased due to the increased rate of hydrolysis caused by the raise in water ionization constant.

2. Oil Yield by SCO_2 Extraction

The highest oil yield obtained by SCO_2 extraction from *Laminaria japoncia* was 2.59 g/250 g at temperature 55 °C and pressure 250 bar. The applied pressure and temperature variation greatly affected the oil solvating power of SCO_2 and hence the amount of yield. The yield of extracted oil was increased with the increasing of CO_2 mass, depending on the pressure and temperature. At constant temperature, the amount of extracted oil from *Laminaria japonica* was increased with the increase in pressure. The effect of pressure can be attributed to the increase in solvent power and by the rise of intermolecular physical interactions [21]. Similar results were found in the extraction of oil from green coffee [22] and boiled anchovy [23].

3. Comparison of Oil Yield by SCO_2 and Organic Solvent Extraction

The oil yield obtained by hexane from *Laminaria japonica* was about 1.33% (w/w in freeze-dried raw sample). On the other hand, the highest yield in SCO_2 extraction was about 1.04% for the experiment conducted at 55 °C and 250 bar. By considering that the extrac-

Table 1. (a) Identification and percentage of fatty acids from *Laminaria japonica* oil extracted by SCO_2 at different temperatures and 150 bar

Compound name	R.T	Area% 150 bar		
		35 °C	45 °C	55 °C
Caporic aicd (C6:0)	12.372	ND	ND	20.40
Myristic acid (C14:0)	23.213	8.81	8.57	6.96
Pentadecanoic acid (C15:0)	25.046	0.51	0.48	0.35
Palmitic acid (C16:0)	26.987	21.94	22.21	15.28
Palmitoleic acid (C16:1)	28.344	2.90	3.10	2.55
Heptadecanoic acid (C17:0)	28.746	8.68	8.72	7.21
Heptadecenoic acid (C17:1)	30.026	0.17	0.18	0.15
Stearic acid (C18:0)	30.464	2.94	3.25	1.18
Oleic acid (C18:1)	31.818	30.87	31.49	20.07
Linolelaidic acid (C18:2)	33.067	0.28	0.25	0.24
Linoleic acid (C18:2)	33.463	6.54	6.69	5.83
Arachidic acid (C20:0)	33.773	0.72	0.62	0.48
γ-Linolenic acid (C18:3)	34.749	1.75	1.63	2.39
Linolenic acid (C18:3)	35.434	1.96	1.93	2.29
Eicosadienoic acid (C20:2)	36.833	2.46	2.32	3.64
Eicosatrienoic acid (C20:3)	38.104	0.35	0.32	0.27
Arachidonic acid (C20:4)	39.241	5.82	5.20	7.75
Lignoceric acid (C24:0)	40.316	0.23	0.21	0.20
Eicosapentaenoic acid (C20:5)	41.594	3.07	2.82	2.78

ND, no detect

Table 1. (b) Identification and percentage of fatty acids from *Laminaria japonica* oil extracted by SCO_2 at different temperatures and 200 bar

Compound name	R.T	Area% 200 bar		
		35 °C	45 °C	55 °C
Myristic acid (C14:0)	23.213	7.98	8.04	8.52
Pentadecanoic acid (C15:0)	25.046	0.45	0.45	0.45
Palmitic acid (C16:0)	26.987	22.82	24.62	19.97
Palmitoleic acid (C16:1)	28.344	2.55	2.48	3.04
Heptadecanoic acid (C17:0)	28.746	8.65	9.16	8.71
Heptadecenoic acid (C17:1)	30.026	0.16	0.15	0.17
Stearic acid (C18:0)	30.464	3.32	3.46	1.98
Oleic acid (C18:1)	31.818	31.15	29.71	26.61
Linolelaidic acid (C18:2)	33.067	0.28	0.26	0.29
Linoleic acid (C18:2)	33.463	6.28	6.03	7.07
Arachidic acid (C20:0)	33.773	0.89	0.87	0.67
γ-Linolenic acid (C18:3)	34.749	1.62	1.55	2.68
Linolenic acid (C18:3)	35.434	1.81	1.76	2.63
Eicosadienoic acid (C20:2)	36.833	2.34	2.26	4.04
Eicosatrienoic acid (C20:3)	38.104	0.39	0.37	0.36
Arachidonic acid (C20:4)	39.241	6.01	5.65	9.08
Lignoceric acid (C24:0)	40.316	0.24	0.23	0.26
Eicosapentaenoic acid (C20:5)	41.594	3.08	2.95	3.48

ND, no detect

Table 1. (c) Identification and percentage of fatty acids from *Laminaria japonica* oil extracted by SCO_2 at different temperatures and 250 bar

Compound name	R.T	Area% 250 bar		
		35 °C	45 °C	55 °C
Myristic acid (C14:0)	23.213	8.64	7.96	8.07
Pentadecanoic acid (C15:0)	25.046	0.44	0.47	0.47
Palmitic acid (C16:0)	26.987	16.64	24.04	24.30
Palmitoleic acid (C16:1)	28.344	3.19	2.46	2.52
Heptadecanoic acid (C17:0)	28.746	9.05	9.54	8.96
Heptadecenoic acid (C17:1)	30.026	0.17	0.15	0.15
Stearic acid (C18:0)	30.464	1.57	3.46	3.45
Oleic acid (C18:1)	31.818	26.17	29.88	29.88
Linolelaidic acid (C18:2)	33.067	0.31	0.26	0.26
Linoleic acid (C18:2)	33.463	7.42	6.07	6.09
Arachidic acid (C20:0)	33.773	0.65	0.90	0.88
γ-Linolenic acid (C18:3)	34.749	3.05	1.55	1.57
Linolenic acid (C18:3)	35.434	2.84	1.75	1.79
Eicosadienoic acid (C20:2)	36.833	4.64	2.27	2.33
Eicosatrienoic acid (C20:3)	38.104	0.38	0.37	0.37
Arachidonic acid (C20:4)	39.241	10.70	5.70	5.72
Lignoceric acid (C24:0)	40.316	0.29	0.23	0.23
Eicosapentaenoic acid (C20:5)	41.594	3.87	2.96	2.97

ND, no detect

tion of oil using hexane was complete, the highest yield by SCO_2 extraction was almost 70%. Similar results were observed in the oil extraction yield form peach seed [24].

4. Thermal Stability of Fatty Acids

SCO_2 extraction showed high efficiency to extract almost all *Laminaria japonica* oils. Table 1(a)-(c) shows fatty acids composition of SCO_2 extracted oils. Total of 19 fatty acids were identified in the different extracts analyzed. Within saturated fatty acids, palmitic acid (C16:0) was present in the highest concentration, ranging from 15.28 to 24.62% of total identified fatty acids. Among monounsaturated fatty acids, oleic acid (C18:1) was also found in substantial amounts ranging from 20.07 to 31.49% of total identified fatty acids. Linoleic acid (C18:2) and Arachidonic acid (C20:4) in *Laminaria japonica* oil were present in higher amounts compared to other polyunsaturated fatty acids (PUFAs). The percentage of Eicosapentaenoic acid (C20:5) in total identified fatty acids ranged from 2.78 to 3.87%. By comparing with Table 1(a)-(c) and 2, the thermal stability of fatty acids in hydrolysates from *Laminaria japonica* obtained at different experimental conditions was observed. Due to high temperature reaction, almost all fatty acids in hydrolysates were not detected by GC-FID. This may be due to degradation of fatty acids by hydrolysis at high temperature. Similar findings were observed in the previous studies [25-29], but several fatty acids such as palmitic acid, oleic acid, linoleic acid and arachidonic acid showed high thermal stability.

5. Thermal Stability Analysis of Polyphenol

The TPC from *Laminaria japonica* using SWH and SCO_2 extraction are shown in Fig. 4 and Fig. 5, respectively. The TPC ranged

Table 2. Identification and percentage of fatty acids from *Laminaria japonica* extracted by SWH at different temperatures

Compound name	R.T	Area%				
		200 ºC	220 ºC	240 ºC	260 ºC	280 ºC
Caporic aicd (C6:0)	12.372	ND	61.83	ND	ND	ND
Palmitic acid (C16:0)	26.987	1.85	0.81	1.88	3.58	3.25
Heptadecanoic acid (C17:0)	28.746	96.58	36.54	96.30	89.50	86.77
Oleic aicd (C18:1)	31.818	1.57	0.82	1.82	3.96	3.86
Linoleic acid (C18:2)	33.463	ND	ND	ND	1.79	1.52
Arachidic acid (C20:0)	33.773	ND	ND	ND	ND	3.55
Arachidonic acid (C20:4)	39.241	ND	ND	ND	1.18	1.04

ND, no detect

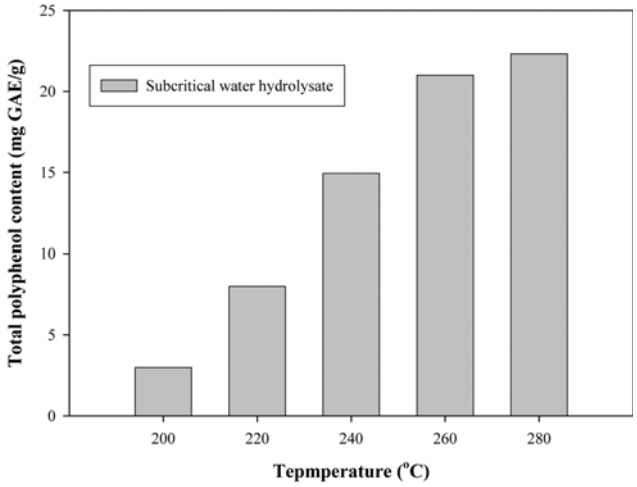

Fig. 4. Total polyphenol contents in SW hydrolysates at different experimental temperatures from *Laminaria japonica*.

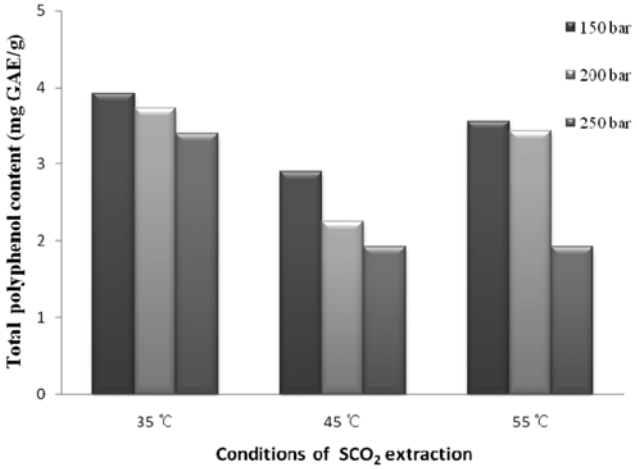

Fig. 5. Total polyphenol contents in oil extracted by SCO_2 from *Laminaria japonica* at different experimental conditions.

between 2.99 mg GAE/g at 200 ºC and 22.32 mg GAE/g at 280 ºC. TPC produced from *Laminaria japonica* increased with the increase of temperature. The higher temperatures led to improved production efficiency of polyphenol. Similar observation for TPC from Black rice bran has been reported by Chan-Ick Cheigh et al. [30]. These results demonstrate high thermal stability of polyphenolic compounds from *Laminaria japonica* after SWH. This is in agreement with previous works [31,32]. TPC from *Laminaria japonica* by methanol (70%) and SCO_2 extraction (at 150 bar and 35 ºC) were 1.06 and 3.92 mg GAE/g dried material, respectively. The efficiency of polyphenol production by SWH was higher than that extracted by other extraction methods. This showed that SWH is more efficient than different extraction methods such as the organic solvent and SCO_2 extraction method. Fig. 4 and Fig. 5 show that subcritical water hydrolysis is a suitable method for producing polyphenol from *Laminaria japonica* and an alternative separation technology as compared with different extraction methods.

CONCLUSIONS

Thermal stability of polyphenol and fatty acids from freeze-dried *Laminaria japonica* powder after SWH was investigated. The highest hydrolysis yield after SWH was about 92% at high temperature and pressure. The oil content of *Laminaria japonica* extracted by SCO_2 was 2.59 g at temperature 55 ºC and pressure 250 bar, and the major fatty acids were myristic acid, palmitic acid, oleic acid, linoleic acid and eicosapentaenoic acid in different extraction conditions. The TPC from *Laminaria japonica* using SWH was found to be increased with the increase of temperature and the highest yield was 22.32 mg GAE/g. Polyphenol and several fatty acids in hydrolysates produced by subcritical water showed high thermal stability. These results suggest that polyphenol and fatty acids content should be considered as an important feature of *Laminaria japonica*, as some of its nutritive and pharmacological effects could be attributed to their presence in seaweeds. The effect of temperature and pressure on the thermal stability of other biomaterials obtained by hydrolysis should be considered in a further study.

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