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Physicochemical Properties and Quality Evaluation of *Porphyra acanthophora* in the Philippines

By

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A dissertation

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Abstract

Seaweeds and its hydrocolloids have captured the attention of various researchers in exploring its biologically active substances, mostly to develop foods and drugs beneficial to human health. Generally, major component of seaweeds are carbohydrates, proteins and the least are lipids which varies from species to species and environmental conditions.

Physicochemical properties and quality of *Porphyra acanthphora* were described in each chapter. Chapter 1 deals with the proximate composition, mineral content, total amino acids and some physicochemical properties, Chapter 2 deals with the antioxidant potential and Chapter 3 deals with the volatile compounds and compared in the different products of *Pophyra* by using headspace gas chromatography and mass spectrometry (GC-MS).

Proximate analysis revealed that *Porphyra acanthophora* contained 47.4±0.7 % of carbohydrate, 23.0±0.1% protein, 1.1%±0.1 crude lipid, 16.7%±0.1 ash and 11.8%±1.5 moisture. ICP emission spectrometry showed that macrominerals such as K, Na, Mg, P and Ca were present in the sample with corresponding amount, 29,291.0, 23,040.3, 5,350.0, 3,018.1, 2,218.7 in mg/kg respectively. Total amino acids determination showed that *Porphyra acanthophora* contained 24 amino acids. Seven among the 24 identified are

essential amino acids, which are threonine (30.6m/100g), valine (10.9 mg/100g), lysine (9.3 mg/100g), tryptophan (6.0 mg/100g), leucine (4.7 mg/100g), isoleucine (4.4 mg/100g) and phenylalanine (3.1 mg/100g). Meanwhile, physicochemical properties like water retention capacity and lipid adsorption capacity of *Porphyra acanthophora* were determined. Water retention capacity was 5.6g/water.g^{-1} dry sample and lipid adsorption capacity of 3.3±0.2, 3.3±0.2, 2.9± 0.1 g/oil.g^{-1} using soybean, sesame and sunflower oil respectively.

Five kinds of solvents such as distilled water, 70% aqueous acetone, ethanol, petroleum ether and chloroform were used to explore the antioxidant potential of *Porphyra acanthophora*. It was explored by screening phytochemicals, analyzing the phenol content and measuring the antioxidant activity such as DPPH radical-scavenging and Fe-chelating activity. Extraction of bioactive compounds from seaweeds depends on solvents used. DPPH radical-scavenging activities of the different extracts revealed significant differences as compared to control and scavenging activities ranged from 12.33% to 39.13%. Meanwhile, distilled water extract Fe-chelating activity was significantly different from the other four extracts while ethanol and ether, and chloroform and aqueous acetone were not significantly different. Fe chelating activities of the different extracts ranged from 60.3 % to 92.9 %. Total phenolic content (TPC) exhibited significant differences among extracts and TPC ranged from 15.0 to 38.5mg/GAE/g dry sample. Phytochemical screening showed that saponins, glycosides, triterpenes, phytosterols, alkaloids, protein and amino acids, diterpenes, phenols and tannins were present, while flavonoid was absent. With these findings, *Porphyra acanthophora* is a good source of natural antioxidants that is comparatively similar to terrestrial plant sources of natural antioxidants.

Volatile compounds of dried *Porphyra acanthophora* was determined using headspace Gas Chromatography-Mass Spectrometry (GC-MS). Compounds identified have taken into consideration the importance for flavor rather than their volatility. It was further compared among processed products *Porphyra* in Korea without oil and using different vegetable oil such as grapeseed, olive, sesame, and perilla. A total of 192 volatile compounds were identified and classified into aldehydes (16); alcohols (31); ketones (17); esters (19); ethers (3); nitrogen-containing substances (3); sulfur-containing substances (5); furans (7); other aromatic compounds(8); and other aliphatic hydrocarbons(83).

Introduction

In the recent years, marine resources such as seaweeds have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods. (Qi, Zhao, Zhang, Li, and Zhao, 2005). In particular, seaweeds are very important and commercially valuable resource for food, fodder, soil conditioners and pharmaceuticals (Yang, et al., 2006).

One of the widely studied among seaweeds are the different species of *Porphyra*. *Porphyra* is a red seaweed that grows in the intertidal zone throughout the world. It is considered as one of the most commercially important seaweeds in Asian countries. Locally known as "gamet" in the northern Philippines, "gim" in Korea, "zicai" in China, and "nori" in Japan.

In the northern part of the Philippines particularly in the province of Cagayan and Ilocos Norte, there were three (3) identified species thriving the coastal areas of said provinces out of the 133 reported species worldwide (Ame et al., 2010). *Porphyra* is seasonal and can only be gathered from the month October to March. It is being sold as either fresh or dried.

In Korea, China and Japan, various red and brown seaweeds are being processed. The most important among these are species of *Porphyra*.

Nutritional value of dried *Porphyra* that is being processed is almost as high as that of fresh as to protein levels, fats, and various essential minerals of dry matter. This means that *Porphyra* contains various biologically active substances beneficial to human health (Noda, 1993). As to the nutritional properties of *Porphyra* products, particularly in Japan, “nori” is processed under controlled conditions to prevent its breakdown. Protein levels are 25-50% of the dry weight, and various vitamins and essential minerals present in nori (Table 1).

Porphyra have compounds that contain polysaccharide called porphyran, which is a complex galactan. The physiological activity of porphyran in the animal body is not clear, but some studies have found excellent health benefits from it. It may inhibit growth of certain tumors. (Chen, 1999). Also, when nori powder was mixed with a basic diet at 2% concentration and fed to rats, it prevented a purposely induced carcinogenesis caused by the sulfation of the polysaccharide, which can enhance the effectiveness of it (Chen, 1999). Moreover, sulfated polysaccharides from marine algae are known to exhibit many biological and physiological activities including anticoagulant, antiviral, antitumor, anti-inflammatory and antioxidant

(Becker, Guimarães, Mourão, and Verli, 2007; F-Tischer, Talarico, Noseda, Guimarães and Duarte, 2006; Souza et al., 2007; Ye, Wang, Zhou, Liu, and Zeng, 2008). Antioxidants are substances that can delay or prevent oxidation of cellular oxidizable substrates (Wang, Zhang, Zhang, Zhang, and Li, 2009). Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the amount of reactive oxygen species (Li, 2007).

Seaweed protein is a source of all amino acids, especially glycine, alanine, arginine, proline, glutamic and aspartic acids. In algae, essential amino acids (EEAs) represent almost a half of total amino acids and their protein profile is close to the profile of egg protein. In case of non EEAs, all the three groups (green, brown and red seaweeds) contain the same amount. Red seaweed seems to be a good source of protein because of its value reaches up to 47%. (Cerna, 2011).With these, *Porphyra* and other seaweeds are the most available alternative source of protein.

Seaweeds can be a potential source of volatile compounds. In fact, several ready-to-eat foods like noodles comes into different flavors like chicken, beef, seafood and that includes seaweed. Researches in marine sediments

discovered that high amount of volatile compounds are found in macroalgae The types of compounds identified are alkanes, alkenes, aldehydes, ketones, furans, sulphides. Both the light hydrocarbons and volatile functionalized organic compounds are believed to originate from both biological and chemical low temperature reactions in the sediments (Whelan et al.,1982).

The aims of the study are to investigate the general composition and physicochemical properties of the material; explore the antioxidant potential; and to compare the volatile compounds of *Porphyra acanthophora* to the different processed "gim" products in Korea.

Objectives

Generally, the study was aimed to determine the physicochemical properties and quality evaluation of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines. Specifically, it aimed to determine the proximate composition, minerals, total amino acids, water retention capacity and lipid adsorption capacity; antioxidant potential and determine the volatile compounds of *Porphyra acanthophora* and compare with commercially available Korean "gim" products.

Chapter 1

Proximate composition, mineral content, total amino acids and some physicochemical properties of *Porphyra acanthophora* in the Philippines

Abstract

Proximate composition, mineral content, total amino acids, water retention capacity (WRC) and lipid adsorption capacity (LAC) of dried red seaweed (*Porphyra acanthophora*) gathered in Sta. Praxedes, Cagayan, Philippines were determined. *Porphyra acanthophora* contained high proportions of carbohydrates (47.4%) followed by protein (23.0%), ash (16.7%), moisture (11.8%) and lipid (1.06%) respectively. ICP emission spectrometry showed that *Porphyra acanthophora* contained high amount of macrominerals such as K, Na, Mg, P and Ca expressed in mg/kg. 29,291.0, 23,040.3, 5,350.0 3,018.1 and 2,218.7 respectively. Twenty four free amino acids were identified ranging from 0.6 to 881.5 mg/100g. Among 9 essential amino acids, seven were identified with corresponding contents expressed in mg/100g sample as follows: threonine (30.6), valine (10.9), lysine (9.3), tryptophan (6.0), leucine (4.7), isoleucine (4.4) and phenylalanine (3.1). Taurine, a sulfur-containing amino acid was also found (881.5 mg/100g). Other free amino acids identified were phosphoethanolamine (281.3), glutamic acid (261.5) phosphoserine (122.6), citrulline (130.0) aspartic acid (47.4), proline (24.7) glycine (15.0), serine (10.2), ornithine (4.8) α-amino-n-butyric acid (3.7), tyrosine (3.6), ethanol amine (1.9), arginine (1.9) and γ-amino-n-butyric acid (0.9). The high amount of carbohydrate attributes to LAC and WRC.

WRC of the seaweed was 5.60 ±0.1g/H_2O.g^{-1}. Three vegetable oils were used to determine the LAC and it showed that seaweed had 2.88± 0.1g/oil.g^{-1}, 3.30± 0.2 and 3.28±0.2 in sunflower, sesame oil and soybean oil respectively. With these, *Porphyra acanthophora* could be a potential material for the production of food supplement to meet the recommended intake of protein and essential minerals.

Introduction

Seaweed is an important component of the marine ecosystem along with the mangrove and coral reefs and can be viewed in two perspectives, from its ecological value as well as its economic uses. Moreover, seaweed demonstrates original and interesting nutritional attributes and value depending on species and the environment conditions. Protein algae ranges from 5-47% of dry basis and it contains all essential amino acids (EAAs) and all are available throughout the year, although seasonal variations in their concentrations are known to occur (Dawczynski et al., 2006).

Seaweeds in the Philippines are highly diversified among the aquatic flora in Asia-Pacific region. More than 800 species of seaweeds have been recorded in the country. The major commercial seaweeds in the Philippines are *Eucheuma, Kappaphycus, Gracilaria* spp. and *Caulerpa* lentillifera.

Other seaweeds with economic importance are *Codium, Gelidiela* acerosa*, Halymenia, Sargassum* spp. and *Porphyra* spp. (BFAR, 2013). *Porphyra acanthophora* is being gathered in Northern Philippines particularly in Burgos, Ilocos Norte and in Sta. Praxedes and Claveria ,Cagayan. *Porphyra* gathering has been considered as an alternative source of livelihood for fisherfolk several years ago for this seaweed grows during the lean months of fishing, from October to March.

Processing of this seaweed in other parts of the world comes in the variety of products, like in Korea, Japan and China. In the Philippines however, the *Porphyra* industry remains to be underdeveloped, as raw materials are mainly gathered from the wild and processing is only limited to drying (Ame et al., 2010). Moreover, the seasonality, risk of gathering and low yield of dried product makes this seaweed relatively expensive. Gathering is said to be risky since this seaweed dwells on sharp- edged rocks. Coastal dwellers particularly in Sta. Praxedes and Claveria, Cagayan have low consumption of this seaweed, for they prefer to sell as fresh or dried in order to earn money. Hence, the absence of information on the nutritional composition and its property might be the reason of low consumption in the area and residents do not see the health benefits as food.

Table 1 shows the primary composition of *Porphyra* "nori" in Japan on dry weight basis and amino acid in Table 2. On the other hand, since *Porphyra acanthophora* is seasonal, researchers did not pay much attention to explore the potential of these seaweeds in human nutrition rather they studied on the social aspect of gathering as an alternative livelihood during lean months of fishing. The study aims to determine the proximate composition, free amino acid and some physicochemical properties of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines. It can be hypothesized that *Pophyra acanthophora* in the Philippines do not differ from those *Porphyra* in Japan, India and Spain as potential source of protein, vitamins, minerals, and other micronutrients the body needs. In addition, the physicochemical properties of *Porphyra* in Spain can be similar to the seaweed from the Philippines. Once the various components of this seaweed will be known, it will encourage people in northern Philippines to eat more *Porphyra* realizing that this seaweed can be a cheap alternative source of nutrients like protein and minerals and can help prevent malnutrition in the area. Furthermore, this can encourage stakeholders to invest in the culture of *Porphyra* and later on boost the seaweed industry in the northern Philippines.

Table 1. Nutritive composition of nori on a dry weight basis.

Primary Composition (%)		Mineral (mg, %)		Vitamin (mg, %)	
Protein	43.6	Ca	440	A potency	16000 (IU)
Lipid	2.1	P	650	B_1	12.9
Carbohydrate		Fe	13	B_2	38.2
-Non Fibrous	44.4	Na	570	B_6	1.04[d]
-Fiber	2.0	K	2400	B_{12}	0.029[d]
Ash	7.8	Mn	2[b]	Niacin	11.0
		Zn	10[b]	Choline	292.0[d]
				Inositole	6.2[d]
				C	112.5

[a] Anon, 1982 [b] Noda, 1971 & Noda et al., 1981 [c]Horiguchi et al., 1971 [d] Kanazawa, 1973

Table 2. Amino acid composition of protein, free amino acids and taurine in "hoshi-nori" on a dry weight basis

Amino acid	Bound form (%)	Free form (ng%)
Alanine	9.92	15280
Argine	5.92	150
Aspartic acid	8.48	3220
Glutamic acid	9.28	13300
Glycine	6.88	240
Histidine	1.18	100
Isoleucine	4	200
Leucine	7.68	310
Lysine	2.56	120
Methionine	3.36	20
Phenylalanine	5.28	70
Proline	4.64	40
Serine	4.8	370
Threonine	3.2	460
Tryptophan	1.1	trace
Tyrosine	2.4	130
Valine	9.28	150
Taurine	-	12100

* Calculated by assuming protein = total nitrogen x 6.25.

[a] Kanagawa, 1983,[b] Noda et al., 1975.

Materials and Methods

Seaweed sample

Porphyra acanthophora was gathered in Sta. Praxedes, Cagayan, Philippines during the last quarter of the year. The product was sun-dried, vacuum-packed, and stored at room temperature. Dried samples were grinded and subjected for the different analyses.

1. Proximate analysis

Proximate composition such as moisture content, crude lipid, crude protein and ash content of the sample was measured.

1.1 Determination of moisture content

Moisture content was determined using AOAC, oven method (2000) with slight modifications. Two grams of sample was accurately weighed and subjected for heating in an oven at 105˚C for 5 hrs. It was cooled for 30 min in a desiccator and weighed. It was re-dried for 1 hr and the process was repeated until constant weight has been achieved, i.e, change in weight between successive drying at 1 hr interval is < 5 mg.

The moisture content was calculated using the following formula:

$$\textbf{Moisture}(\%) = \frac{W_1 - W_2}{W} \times 100\%$$

Where, W_1 = initial weight (g) of sample and moisture dish; W_2= final weight (g) of sample and moisture dish after drying and W= initial weight of sample.

1.2 Determination of ash content

Using the method of AOAC, 0.5 grams of sample was accurately weighed in crucible. It was heated in oven at 100˚C for 24 hrs. Sample was transferred to muffle furnace and increased the temperature to 550 ˚C ± 5 ˚C. The temperature was maintained for 8-10 hrs until white ash is obtained. The sample was cooled in desiccators for 30 min and weighed. It was repeatedly heated until the weight become constant. The ash content was calculated using the following formula:

$$\textbf{Ash}(\%) = \frac{W_1 - W_2}{W} \times 100\%$$

Where, W_1 is the initial weight of crucible with sample (g); W_2 is the final weight of crucible with ash and W is the weight of the sample.

1.3 Determination of crude protein content

Crude protein was determined based on AOAC (2000) using Kjeldahl method. This method evaluates the total nitrogen content of the sample after it has been digested in sulphuric acid with selenium as catalyst. One gram of sample was loaded into Kjeldahl flask followed by the addition of 10 g potassium sulphate, 0.7 g mercuric oxide and 20 ml concentrated sulfuric acid. The flask was placed at an angle in the digester and brought to boiling point and retained until the solution was clear. The 90 ml of distilled water was added to cool the mixture followed by 25 ml sodium sulphate solution and stirred. One glass bead and 80 mL of 40% sodium hydroxide solution were added and the flask was kept tilted. The flask was rapidly connected to the distillation unit, heated and collected 50 mL of distillate containing ammonia in 50 ml of 4% boric acid with indicator solution. At the end of distillation, the receptor flask was removed, rinsed and titrated with the standard hydrochloric acid solution.. A blank was run to eliminate error for

contamination reagents. The crude protein contents were calculated using the equation below:

$$\textbf{Nitrogen in sample}(\%) = \{\frac{\text{A x B}}{\text{C}} \times 0.014\} \times 100$$

$$\textbf{Crude protein in sample} = \text{nitrogen content} \times 6.25$$

Where, A is the hydrochloric acid used in titration (ml), B is the normality of standard acid and C is the weight of the sample (g) 6.25 is the nitrogen conversion factor and 0.014 is the molar mass of nitrogen.

1.4 Determination of crude lipid

Crude lipid was conducted using AOAC method with slight modifications using Soxhlet system. Soxhlet boiling flask was dried for some time, cooled and balanced without touching it with fingers. Five grams of sample was put into thimble and placed in the extraction apparatus. Diethyl ether was loaded into flask at 2/3 of the total volume. The heater was raised into position leaving about ¼ inch gap between the beaker and the heating element followed by switching the water condenser and ether leakage was checked. It was extracted for 8-12 hrs, dried and weighed. The crude lipid content was calculated using the below equation:

$$\textbf{Crude Lipid}(\%) = \frac{W_1 - W_2}{W} \times 100$$

Where, W_1 is weight of flask with fat (g) W_2 is weight of clean dry flask (g) and W is the weight of the sample.

1.5 Calculation of carbohydrate content (by difference)

% Carbohydrates = 100- (% moisture) - (% protein) – (% lipid) – (%ash)

2. Mineral Content

Mineral content was analyzed by the method of Perez et al. (2007) using ICP-OES using a Perkin Elmer Optima 3100 XL spectrometer.

3. Total amino acids determination

Five grams of sample was used and it was added with 3% Trichloro-acetic acid, mixed well and diluted with distilled water to 10^{-1}. It was centrifuged to 8,000 rpm for 20 min and supernatant was collected. Supernatant was diluted with 0.02N HCl and filtered at 0.45μm using

syringe filter and solution in vial was analyzed using Hitachi L-8900 amino acid analyzer.

4. Water retention capacity

Water retention capacity was determined using the method of Rupérez et al., (2001) with some modifications. 10 mL of distilled water was added to 0.5 g sample in a centrifuge tube. The sample was agitated and left at room temperature for one hr. The mixture was centrifuged at 4,000 rpm in 20 minutes. The supernatant was discarded and the residue weighed. The water retention capacity (WRC) was expressed as gram of water/g dry sample.

5. Lipid adsorption capacity

Lipid adsorption capacity was determined using Carvalho et al., methodology (2008).Three grams of sample were added to 18 mL of three different oils- sunflower, sesame and soybean in a centrifuge tube. The sample left at room temperature for 24 hrs. Then, the mixture was centrifuged at 1,500 rpm for 10 min, the supernatant discarded and the residue weighed. The lipid adsorption capacity (LAC) was expressed as oil/g dry sample.

Statistical analysis

All analyses were performed in triplicate. The data were expressed as means (± S.D.) and reported on a dry matter basis. One-way analysis of variance (ANOVA) was carried out to assess any significant differences between the means. Differences between means at the 5% ($P<0.05$) level were considered significant.

Results and Discussion

1. Proximate analysis of *Porphyra acanthophora*

Proximate analysis was done to determine the general composition of *Porphyra acanthophora* such as moisture content, crude lipid, crude protein and ash content. The proximate composition of *Porphyra acanthophora* is shown in Table 2. As shown on the data of proximate analysis, carbohydrate found to be the highest component of *Porphyra acanthophora* approximately 47.4% and the least component is the lipid content approximately 1.8 %.

Generally, in countries like Korea, China and Japan, *Porphyra* are processed to prevent the breakdown of its products and its nutritive value.

Thus, processed *Porphyra* is almost as high as that of fresh in terms of nutritive value (Noda, 1993). Meanwhile, majority of marine macro algae are primarily high in terms of ash ranging from 8.4-43.6% dry weight and not so much with lipids which ranges from 0.92-5.2% (Prathami, 2014). Hence, proximate composition of seaweed of same species varies depending on the geographical location and season that is being harvested (Yuan, 2007).

As shown in Table 1, proximate composition of *Porphyra* in Japan is higher as to its protein and lipid content compared to *Porphyra* from the Philippines. However, carbohydrate content and ash content of the latter was higher. Algae are rich in a wide variety of minerals, but they are also rich in other components, such as dietary fiber and resistant protein that may pass through the intestine without being absorbed and can retain dietary mineral components (Holland, Unwin and Buss, 1991, Urbano et al., 2002).

Table 3. Proximate composition of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philipppines

Component	Amount
Moisture	11.8±1.5
Ash	16.7±0.1
Crude lipid	1.1 ±0.1
Crude protein	23.0±0.1
Carbohydrate*	47.4±0.7

Values are means± standard deviation of triplicates.(*) means taken by difference

2. Mineral composition

Inductively coupled plasma emission spectrometry of the sample in Table 3 showed that *Porphyra acanthophora* contained high amounts of macrominerals such as Na, K, Ca, Mg, and P which was also detected in the species of *Pophyra* in Japan by Noda (1993) and Rao et al. (2007) in India. However, Fe, Mn, Se and Zn that were found in Japan and India were not present in sample from the Philippines. Suprisingly, *Porphyra acanthophora* do not have similarity in essential mineral contents of *Porphyra columbina* in Argentina. Various researchers compared that macromineral contents of seaweeds like *Porphyra* are mostly higher than those of terrestrial plants like corn and some vegetables.

3. Total amino acids

Amino acids are building blocks of proteins while proteins are the building blocks of life. Proteins of *Porphyra acanthophora* contained a high level of essential amino acids. The composition of amino acids is the factor determining the quality of protein in foods (Lee et al., 2009).Twenty four amino acids were identified in the aqueous solution as showed in Table 3

and chromatogram on Figure 1. Total amino acids ranges from 0.6 to 881.5mg/100g dry sample. High amounts of alanine, phosphorethanolamine, glutamic acid, citrulline and phosphoserine were identified. Meanwhile, among the 9 essential amino acids, seven were found in *Porphyra acanthophora* expressed in mg/100g, theses are threonine (30.6), valine (10.9), lysine (9.3), tryptophan (6.0), leucine (4.7), isoleucine (4.4) and phenylalanine (3.1). These essential amino acids were also identified in *Porphyra* from Japan (Noda,1993; Fleurence, 1999). It was further confirmed the presence of these essential amino acids in the study of amino acid composition data base of Korean foods (Lee et al., 2009). Meanwhile, 2 EAAs such as methionine and histidine were not identified in the sample. Taurine content of 881.5 mg/100g was identified to be the highest in *Porphyra acanthophora* and the only sulfur containing amino acid (SAA). Taurine has tremendous health benefits and it is considered as the amino acid of anxiety control and stress management because it lowers cortisol (Poliquin group article, 2012). Alanine content of 303.3mg/100g was found to be the second highest amino acid content in the sample. It is a α-amino acid classified as non-essential and non-polar amino acid.

Table 4. Mineral composition of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philipppines.

Components	Amount (mg/kg)
Na	23,040.3±1267.0
K	29,291.0±3333.0
Ca	2,218.7 ±267.2
Mg	5,350.0±76.8
P	3,018.1±88.2

Values are means± standard deviation of triplicates.

Porphyra acanthophora also contained 281.3 mg/100g phosphoethanolamine. Though it is not essential amino acid, phospoethanolamine is an ethanolamine derivative that is used to construct sphingomyelins (SM). Glutamic acid was found in sample to have 261.5 mg/100g. Glutamic acid is another non-essential amino acid but it is considerably important in food industry as flavoring. Japanese, most especially believed that glutamate enhances the palatability of any food and have been used and known worldwide. The substantial amount of aspartic acid (47.4 mg/100g) is also important because both glutamic and aspartic acid are responsible for the special flavor and taste of seaweeds (Yaich et al., 2011).

On the other hand, β-alanine with 0.6mg/100g was found to be the least amino acid content of *Porphyra acanthophora.* Beta-alanine (β-alanine) is a naturally occurring beta amino acid in which the amino acid group is at the position from the carboxylate group. Beta-alanine is known to boost muscle performance. Research showed that athletes undergoing intense anaerobic exercise can blunt the effects of acid build-up in the muscles by taking a simple amino acid supplement called beta-alanine. It decreases fatigue and increases the total work output of muscles. (Nutrition express article, 2014).

Table 5. Total amino acid composition of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines

Amino acids	Amount (mg/100g)
Isoleucine*	4.4 ± 0.4
Leucine*	4.7 ± 1.2
Lysine*	9.3 ± 0.3
Valine*	10.9 ± 0.2
Phenylalanine*	3.1 ± 1.0
Threonine*	30.6 ± 2.7
Tryphtophan*	6.0 ± 4.2
Methionine	-
Histidine	-
Alanine	303.3± 5.7
Arginine	1.9± 1.3
Glycine	15.0 ± 4.4
Aspartic acid	47.4±4.4
Glutamic acid	261.5 ± 16.0
Proline	24.7 ± 7.5
Serine	10.2± 3.1
β-alanine	0.6± 0.1
Tyrosine	3.6± 0.5
Phosphoserine	122.6 ± 1.0
Citrulline	130.0± 10.4
Phospho ethanol amine	281.3 ± 1.0
a-Amino-n-butyric acid	3.7 ± 1.9
γ-amino- n-butyric acid	0.9 ± 0.1
Ethanol amine	1.9±0.6
Ornithine	4.8±0.7
Taurine	881.5± 1.2

Values are means ± standard deviation of duplicate. (-) not detected (*) EAAs

Phenylalanine and tyrosine were also identified which are characterized as aromatic amino acids found in the sample.

4. Water retention capacity

The results of this analysis following an *in vitro* methodology showed that the water retention capacity of *Porphyra acanthophora* is 5.6±0.1 g/water.g^{-1} dry sample. According to Robertson and Eastwood (1981), water in seaweeds exists in fiber in three forms: It is bound to hydrophilic polysaccharides, within the fiber matrix or it is trapped within the cell wall. Water retention capacity of some seaweed attributes to insoluble fiber, whereas others attribute to the high content of uronic acid-components of soluble fraction of dietary fiber (Femenia et al.,1997, Rupérez et al., 2001). This can be true due to the high carbohydrate composition of the sample. Nevertheless, researchers consensus about this property depends on the experimental conditions such as temperature, pH, time, centrifugation circumstances as well as sample preparation and particle size (Carvalho et al., 2008, Femenia et al.,1997, Suzuki et al., 1996, Michel et al.,1988).

5. Lipid adsorption capacity

Three different vegetable oils were used to determine the lipid adsorption capacity of the sample. Using sunflower oil, *Porphyra acanthophora* had LAC of 2.9±0.1g/oil.g^{-1} while sesame oil exhibited adsorption of 3.3±0.2 and soybean oil showed 3.3±0.2, lipid adsorption capacity. In the study of (Carvalho et al., 2008) using green seaweed *Ulva fasciata* Delile, lipid adsorption capacity of the seaweed was higher, compared to that of chitosan. Statistics showed that adsorption capacity of *Porphyra acanthophora* using sunflower oil was significantly different at 0.05 confidence level as compared to the adsorption of the material using sesame and soybean oil. On the other hand, *Porphyra acanthophora* adsorption capacity using sesame and soybean oil was significantly the same at 95% level of confidence.

The high Lipid adsorption capacity is an important characteristic of seaweed, since dietary fibers present in seaweed might absorb unnecessary fats which may help in the control of body weight and blood lipid profile abnormalities. Some authors proved (Ruperez and Calixto, 2001; Jimenez et al, 1999) that brown and red seaweeds contain high amounts of dietary fiber.

Conclusion

Proximate composition of *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines is a potential alternative cheap source of nutrients like protein (23.0%) and ash (16.7%). *Porphyra acanthophora* could also be used as a food supplement to help meet the recommended daily intake of protein and essential minerals. Though lipid content was minimal (1.1%), lipids in macroalgae are known to be polyunsaturated which is beneficial to human health. The lipid adsorption and water retention capacity of *Porphyra acanthophora* are important physicochemical properties of the seaweed that can be attributed to the high amount of carbohydrate. *Porphyra acanthophora* had high content of free amino acids. The notable amount of protein in the sample indicates the high levels of amino acids. The presence of essential amino acids such as isoleucine, leucine, lysine, phenylalanine, threonine, and valine is comparable to terrestrial foodstuffs and can be a cheap and potential alternative source of essential amino acids. The palatable taste of this seaweed is attributed to the high levels of glutamic and aspartic acid while the desirable seaweed flavor is due to the presence of aromatic amino acids like tyrosine and phenylalanine.

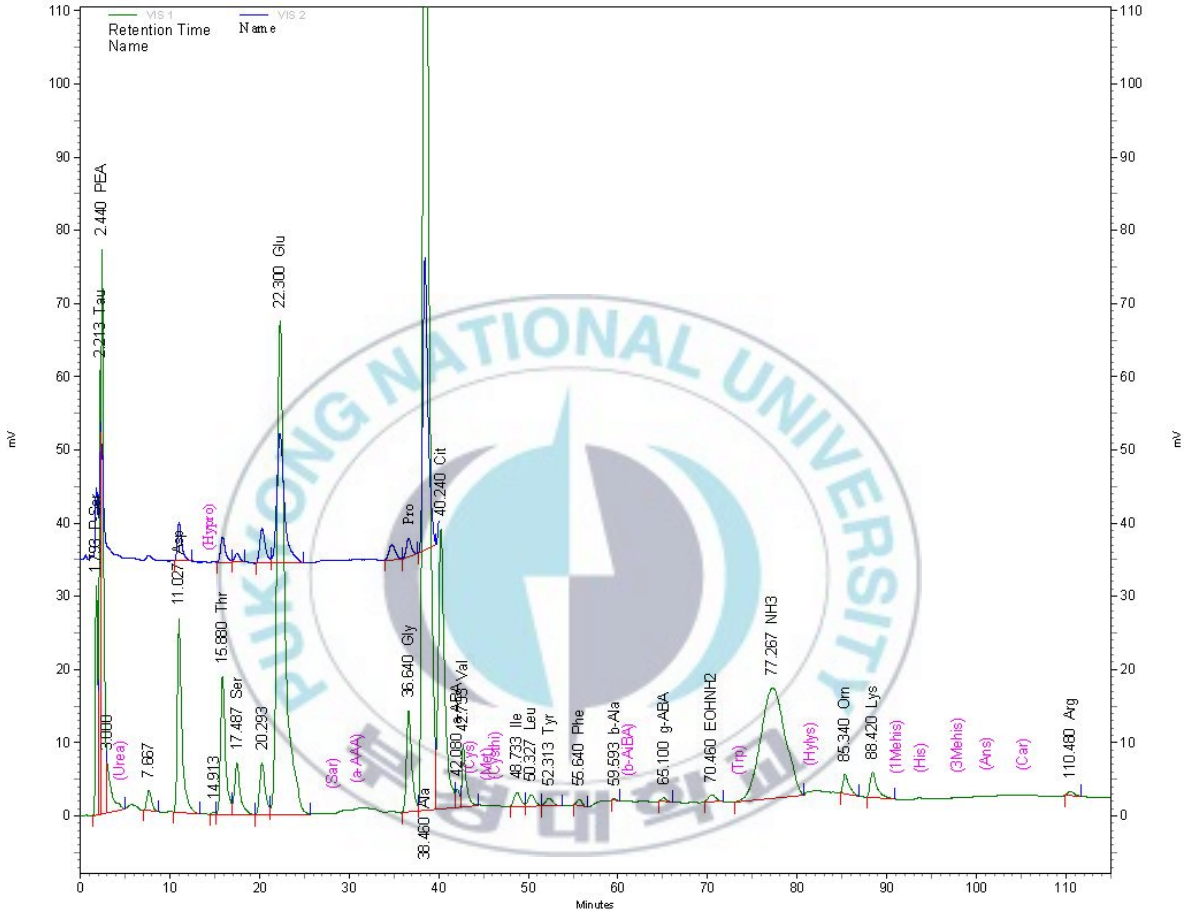

Fig. 1. Chromatograms of total amino acids identified in *Porphyra acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines.

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Chapter 2

Antioxidant potential of *Porphyra acanthophora* in the Philippines

Abstract

Five kinds of solvents (70% aqueous acetone, chloroform, distilled water, ethanol and petroleum ether) were used to explore the antioxidant potential of *Porphyra acanthophora.* It was explored by screening phytochemicals, analyzing the phenol content and by measuring the antioxidant activity such as DPPH radical-scavenging and Fe-chelating activity. Extraction of bioactive compounds from seaweeds depends on solvents used. . Phytochemical screening showed that saponins, glycosides, triterpenes, phytosterols, alkaloids, protein and amino acids, diterpenes, phenols and tannins were present, while flavonoid was absent. Total phenolic content (TPC) exhibited significant differences among extracts and TPC ranged from 15.0 to 38.5mg/GAE/g dry sample. DPPH radical-scavenging activities of the different extracts revealed significant differences as compared to control and scavenging activities ranged from 12.3% to 39.3%. Meanwhile, distilled water extract Fe-chelating activity was significantly different from the other four extracts while ethanol and ether, and chloroform and aqueous acetone were not significantly different. Fe chelating activities of the different extracts ranged from 60.3 % to 92.9 %. With these findings, *Porphyra acanthophora* is a good source of natural antioxidants that is comparatively similar to terrestrial plant sources of natural antioxidants.

Introduction

Seaweeds are potential renewable resource in marine environment comprising about 6,000 species and are classified into three major varieties such as Chlorophyceae (green), Rhodophyceae (red) and Phaeophyceae (brown). Of the 6,000 species of seaweeds worldwide, there are more than 800 species found in the Philippines. Moreover, a number of investigators have found that traditional sources of food provide not only nutritional benefits, but also help fight diseases and contribute to the maintenance of good heath (Bhatia et al, 2011). Nowadays, antioxidative properties of natural compounds from marine plants like seaweeds are considered very important ones because they are not only use as food and cosmetics but also for medicine (Mishra et al., 2012).

In the recent years, numerous studies on natural oxidants have focused on terrestrial plants and their application in food systems to prevent oxidation. Due to scientific claims that synthetic food additives have adverse effects on human health, the demand of natural products in human diet is continuously increasing.

Studies proved that a great number of medicinal plants, aromatic herbs, fruits and leaves of some berry plants biosynthesize phytochemicals possessing antioxidant activity and maybe used as potential natural sources radical scavenging compounds (Baltrusaityteet.al., 2005; Sacchetti et al.,2005; Yu et al., 2005; Miliauskas et.al.2004 Wang and Lin, 2000).

In living systems, various metabolic processes and environmental stresses generate various reactive species. These are free radicals and mainly reactive oxygen species (ROS) Mishra et.al 2011. Increased level of ROS can damage structure of biomolecules and modify their functions and lead to cellular dysfunction and even cell death. The cumulative effect of increased ROS can increase oxidative stress in systemic level and it is manifested in the form of variety of health problems such as cancer, age related disease and cardiovascular diseases.

The aim of the study is to evaluate the antioxidant activity and to determine the phenolic content of *Porphyra acanthophora* using different solvent extracts.

Materials and Methods

1. Determination of phytochemicals

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction. (Tiwari et al., 2011). The following solvents were used for phytochemical screening: Distilled water; 70% aqueous acetone; chloroform; ethyl alcohol and petroleum ether.

1.1 Plant homogenization and extraction

Dried seaweeds were grinded in a blender to become fine particles and sieved in 450µm. Extracts was prepared using the method of (Wang, 2009) with slight modification. Five grams of powdered *Porphyra acanthophora* was mixed with 100 mL of the different solvents used and incubate for 24 hrs in shaking incubator with 25 ˚C temperature with 185 rpm. It was centrifuged at 3500 rpm for 10 min and filtered using Whatman no. 2 filter paper.

1.2 Phytochemical screening

1.) **Detection of alkaloids**- extracts were dissolved individually in dilute hydrochloric acid and filtered. Using Mayer's Test, filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicated the presence of alkaloids.

2.) **Detection of glycosides**- Extracts were hydrolyzed with diluted HCl then subjected to test for glycosides. Using modified Borntrager's Test, extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides. Legal's test will also be used to determine the presence of cardiac glycosides. Extracts will be treated with sodium nitropruside and sodium hydroxide. Formation of pink to blood red indicates the presence of cardiac glycosides.

3.) **Detection of saponins**- Using Froth test, extracts were diluted in 20 ml distilled water and will be mixed in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. Foam test was also used to confirm the presence of saponin by mixing 0.5 gram of extract mixed with 2 ml water. If foam produced persist for 10 minutes, it indicates the presence of saponins.

4.) **Detection of phytosterols**- Using Salkowski's test, extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes. Libermann Buchard's test was also used. Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

5.) **Detection of phenols**- Ferric Chloride test was used wherein extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

6.) **Detection of tannins-** Gelatin test was used. Extracts were treated with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.

7.) **Detection of flavonoids-** Alkaline reagent test was used. Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color which becomes colorless on addition of dilute acid indicates the presence of flavonoids.

8.) **Detection of proteins and amino acids-** Xanthoproteic test will be used to determine the presence of protein by adding extracts with few drops of concentrated nitric. Formation of yellow colour indicates the presence of proteins. The presence of amino acid will also be confirmed using Ninhydrin Test. Extract will be added with 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue color indicates the presence of amino acids.

9.) **Detection of diterpenes-** Copper acetate test will be used. Extracts will be dissolved in water and treated with 3-4 drops of

copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

2. Determination of total phenolic content

The total phenol content of the different extracts of *Porphyra acanthophora* was determined by the method of Folin-Ciocalteau reaction using gallic as standard (Lee et.al., 2009 and Kim et.al., 2013). To 200µL of the different extracts (200µg/mL), 200µL of FolinCiocalteau reagent was added and let it stand for 3 minutes then it was added with 10% Na_2CO_3 solution. Mixture was incubated at room temperature for 1 hour and the absorbance was checked at 725nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per sample.

3. Determination of 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

Different extracts used in total phenol content were also used in DPPH radical scavenging activity using the method of (Lee et al., 2009). A ratio of (16:100) 1,1Diphenyl-2-picrylhydrazyl (DPPH) and ethyl alcohol were mixed and placed in dark room for 1hr as blank sample. Absorbance was

checked using at 517nm.Ethanolic DPPH (4ml) was mixed with 1mL extract using 0.5- 5.0 g/ml concentration and placed in dark place for 1hr and absorbance was checked at 517nm. Percent absorbance was calculated using the following formula:

$$(\%)\mathbf{inhibition} = \frac{A_2 - A_1}{A_2} \times 100$$

Where, A_2 is the absorbance of blank sample and A_1 is the absorbance of the extract.

4. Determination of Fe-chelating activity

Different extracts used TPC and DPPH radical scavenging activity were also used in Fe chelating activity. Fe chelating was conducted following the method of (Kim, et al, 2012). Each sample extract (aqueous acetone, chloroform, distilled water, ethanolic and petroleum ether extract) was taken with 2.5 mL and mixed with 12.5 mL of methanol and centrifuged at 2,000 rpm for 20 min. A control was prepared by measuring 3mL of the supernatant liquid of methanol and sample mixed with 0.1 mL of 2mM $FeCl_2$ and 0.2 mL distilled water. A sample was prepared by taking 3mL

sample from the centrifuged extract mixed with 0.1 mL of 2mM $FeCl_2$ and 0.2mL 5mM ferrozine. A blank sample was prepared by mixing 3mL of methanol, 0.1mL of 2mM $FeCl_2$ and 0.2mL of 5mM ferrozine. All samples are wrapped with aluminum foil to avoid the penetration of light and left for 30 min. Absorbance was checked using 561nm and metal chelation was computed using the following formula:

$$\textbf{Metal chelation } (\%) = \{A_{blank} - (A_{sample} - A_{control})\} / A_{blank} \times 100$$

Results and Discussion

1. Preliminary phytochemical screening

Phytochemical screening proved that *Porphyra acanthophora* extracted from the different solvents had no flavonoids. However, it gave the confirmation of the presence of saponin, glycosides, triterpenes, phytosterols, alkaloids proteins and amino acids, diterpenes and tannins in some solvents (Table 1).

Table 6. Phytochemical tests of *P. acanthophora* by the different solvents

Phytochemicals	Distilled water	Aqueous acetone	Ethyl alcohol	Chloroform	Petroleum ether
Saponin	+	-	+	-	+
Glycosides	-	+	-	-	+
Triterpenes	-	-	-	+	-
Phytosterols	-	-	-	+	-
Flavonoids	-	-	-	-	
Alkaloids	-	-	-	+	+
Proteins & amino acids	+	+	+	-	+
Diterpenes	-	+	+	-	+
Phenols	-	+	+	+	+
Tannins	+	+	+	-	+

(+ present, - absent)

2. Extraction yield for TPC and DPPH radical scavenging activity.

Considerable variations in extraction yield were found in different extraction solvents (data not shown). The highest extraction yield recorded was the ethanol while the least was the distilled water extract and the most difficult to filtrate due to its viscosity. This was due to the high water holding capacity of the seaweed which was determined in chapter 1 and the high content of alginate in the extract.

3. Total phenol content

Antioxidant activity of natural products is due to the antioxidant principles like polyphenols, carotenoids, and vitamins C & E (Bhatia et al., 2011). It was also proved in the study of (Prathami, 2014) that antioxidant properties of hydrosylate water of brown seaweed from *Saccharina japonica* were affected by bioactive compounds such as phenolic, flavonoid, minerals and others. Total phenolic content of *Porphyra acanthophora* is shown in (Table 7). Ethanolic extract was found to be the highest (40.79 mg GAE/g sample) while aqueous acetone revealed the lowest content (15.92 mg GAE/g sample).

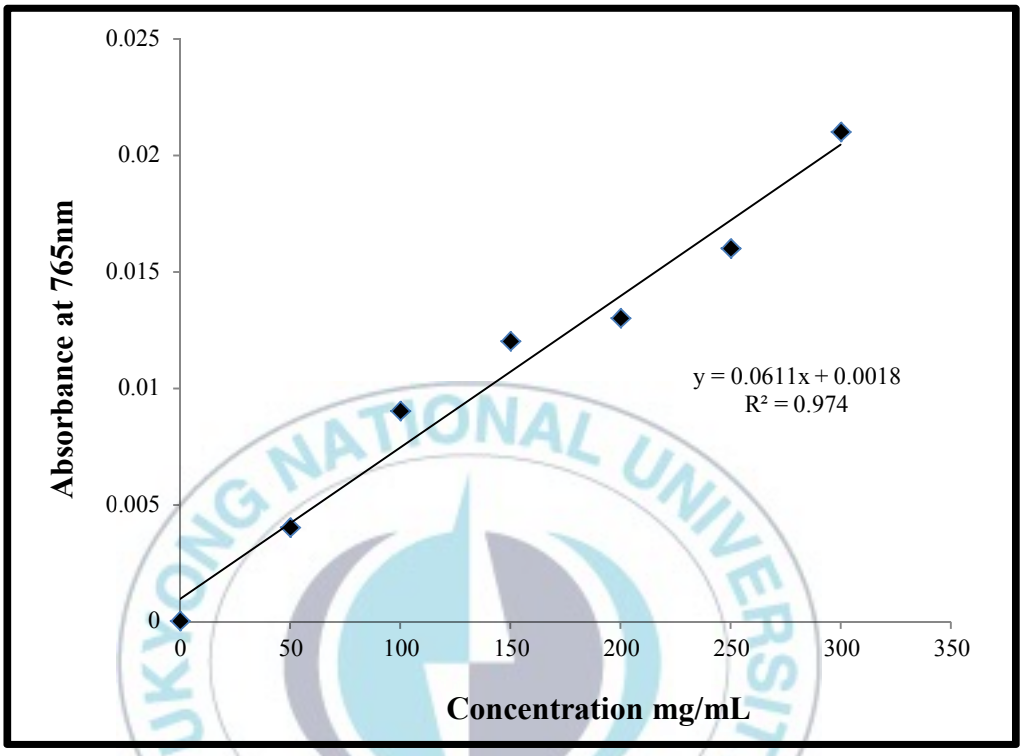

Fig. 2. Calibration curve of gallic acid standard for total phenolic content of *Porphyra* acanthophora.

In the findings of (Bhatia et al., 2011), *Porphyra vietnamensis* phenolic content of the different extracts like porphyran, polar pigments and alcohol extract were 941.0, 712.0 and 513.0 mg GAE/100g sample respectively. Generally, phenolic compounds are more soluble in polar organic solvents than in water (Wang et al., 2009). For effective extractant, aqueous mixtures of methanol, ethanol and acetone were also recommended by (Waterman and Mole, 1994). However, findings showed that distilled water extract had higher phenol content (26.32 mg GAE/g) than acetone (15.92 mg GAE/g sample). This might be due to the known characteristics of seaweeds to contain high levels of water-soluble components such as polysaccharides, protein and peptides (Galland-Irmouli et al.,1999) which were not extracted properly by aqueous acetone. On the other hand, acetone as pointed out by (Wang et.al,2010 and Hagerman, 1988) has the ability to inhibit protein-polyphenol complex formation during extraction or even breakdown hydrogen bonds formed between phenolic group and protein carboxyl group.

4. DPPH radical-scavenging activity.

All extracts exhibited DPPH radical scavenging activity at 517 nm absorbance which is widely used in similar researches. Different extracts of

Porphyra acanthophora at concentrations of 0.05, 0.5, 1.0, 2.0 and 5.0 were recorded and all activities were relatively lower than the standard. Ethanolic and petroleum ether extract of *Porphyra acanthophora* significantly (P>0.05) scavenged DPPH in a concentration dependent manner while other extracts are not significantly different from other concentrations (Fig. 2). This means that ethanol was best among extraction solvent since petroleum ether cannot be used in foods which resulted to better radical-scavenging and Fe-chelating activity of *Porphyra acanthophora*. Antioxidant reaction with DPPH was observed through the color change from purple to colorless. Aqueous acetone extract percent inhibition means ranged from 18.0±1.3 to 27.2±0.5 while distilled water extract ranged from 17.4±1.27 and 20.60± 0.78 and ethanol extract ranged from 12.3±0.74 and 39.1±0.96. A number of authors pointed out that change in extractant polarity alters its efficacy to extract a specific group of antioxidant properties of the extracts. (Wang et al, 2009 and Zhou and Yu, 2004). Moreover, it was observed that the extract with high total phenol content had also high DPPH scavenging activity.

Table 7. Total phenolic content in the different extracts of *P. acanthophora*

Sample extract	Phenol content (mg GAE/g sample)
Aqueous acetone	15.0 ± 1.1^{d}
Chloroform	21.4 ± 2.3^{c}
Distilled water	24.9 ± 0.6^{bc}
Ethyl alcohol	38.5 ± 0.3^{a}
Petroleum ether	27.8 ± 4.0^{b}

Values are mean (± SD) (n=3). Values with different superscripts are significantly different at ($P<0.05$).

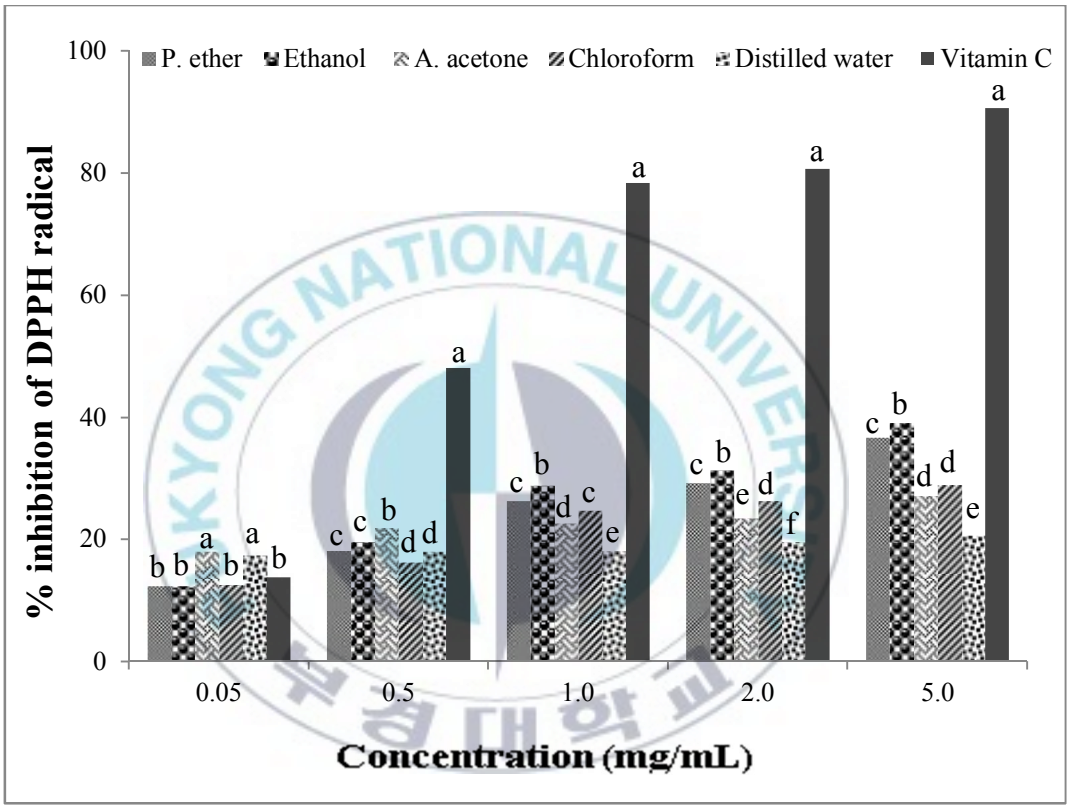

Fig. 3. Inhibition against DPPH by the different extracts from *Porphyra acanthophora*. Values of the same letter in every concentration are not significantly different at (P>0.05).

5. Fe-chelating activity

Extract used confirmed antioxidant activity of Porphyra acanthophora. Fe-chelation percentage was shown in Table 8. Petroleum ether extract exhibited highest chelating activity. However, it was not significantly different with ethanolic extract. Meanwhile, chloroform and aqueous acetone extract were not also significantly different while distilled water extract was significantly different in chelating activity. Interestingly, antioxidant activity percentage in this experiment was higher than DPPH radical scavenging activity while extracts containing high phenolic content were also potent radical scavengers and metal chelating activity. Metal chelating in seaweeds as pointed out by (Wang et al., 2009) is due to the high binding capacities to different heavy metals of algal dietary fibers such as alginate and fucoidan from brown algae while carrageenan and agar for red algae. Researches in brown seaweeds revealed that metal chelating capacities of polyphenols are potent ferrous ion chelators (Wang et al., 2009, Chew, Lim, Omar and Khoo, 2008, Senevirathne et al., 2006); and metal chelating potency of phenolic compounds are dependent upon their phenolic structure and the number and location of the hydroxyl groups (Farvin et al.,

Table 8. Fe-chelation by the different extracts of *Porphyra acanthophora*

Sample extract	% Metal chelation
Aqueous acetone	73.2%±2.2[b]
Chloroform	75.0%±2.2[b]
Distilled water	60.3%±7.0[c]
Ethyl alcohol	92.8%±2.4[a]
Petroleum ether	92.9%±1.0[a]

Values are mean (± S.D.)in triplicates. Values with different superscripts are significantly different at ($P<0.05$).

2013, Santoso, Yoshie-Stark, and Suzuki, 2004, Brand- Williams, Cuvelier and Berset, 1995).

Conclusion

Different species of *Porphyra* in other parts of the world were studied in terms of their antioxidant potential while *Porphyra acanthophora* in the Philippines is unexplored yet. *Porphyra acanthophora* gathered in the Philippines is equally a potential source of antioxidant which was proved in the phytochemical screening, total phenol content, DPPH radical scavenging and Iron (Fe) chelating activity. Phytochemical screening demonstrated that *Porphyra acanthophora* contained phytochemicals like saponins, glycosides, phytosterols, flavonoids, proteins and amino acids, diterpenes, phenols and tannns. Extraction of bioactive compounds from seaweeds depends on solvents used. Phenol content, DPPH radical-scavenging and Fe chelating activitycan be effectively determined using ethanol as extractant as compared to other solvents used. With these findings, *Porphyra acanthophora* is a good source of natural antioxidants that is comparatively similar to terrestrial plant sources of natural antioxidants.

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Chapter 3

Determination of volatile compounds in the different *Porphyra* products using Headspace Gas Chromatography Mass Spectrometry

Abstract

Aroma of any food comes from volatile compounds at different concentration present in the product because chemical compounds have smell or odor when they are sufficiently volatile. In this study, volatile compounds of the different products from *Porphyra* in Korea and Philippines were determined using headspace gas chromatography and mass spectrometry (GC-MS). Compounds identified have taken into consideration the importance for flavor rather than their volatility. Dried *Porphyra acanthophora* from the Philippines was compared to the processed products in Korea without oil and using different vegetable oil such as grapeseed, olive, sesame and perilla. A total of 192 volatile compounds were identified and classified into aldehydes (16); alcohols (31); ketones (17); esters (19); ethers (3); nitrogen-containing substances (3); sulfur-containing substances (5); furans (7); other aromatic compounds(8); and other aliphatic hydrocarbons(83). Nine volatile compounds were identified that were common to all samples namely: acetaldehyde, propanal, 3-methyl butanal, ethanol, ethyl ether, benzene, methyl benzene,1- propene and hexane. Products without the application of heat and oil found to contain more volatile compounds than the products subjected to high controlled temperature.

Introduction

Porphyra is a red seaweed that grows in the intertidal zone throughout the world. Locally known as “gamet” in the northern part of the Philippines, “gim” in Korea, “nori” in Japan, “zicai” in China, and “laver” in the west, it is considered as one of the most economically important seaweeds with great potential revenue both in local and international markets. This seaweed is also being cultured in Korea China, and Japan. For *Porphyra tenera* only, a reported production in 2012 was estimated to 691,428 metric tons (FAO, 2012) with value of 1,287,446 USD.

Different species of *Porphyra* is a part of staple diet in the orient as they are nutritionally rich materials. Seasoned roasted “gim” is one of the famous commercial commodities in Korea. It is being sold in big supermarkets as well as small stores in Korea. This product has several famous brands available and the product can be distinguished differently because of the oil being used. Different manufacturers used sesame, canola, perilla, soybean, corn, and olive oil. In the northern part of the Philippines, there is no other processing technique employed for *Porphyra acanthophora* except drying. Based on the packaging materials of said products, each product with or

without oil can only be kept for six months while raw-dried from the Philippines though it was not studied can last 8-10 months. Hence, it is interesting to find out the volatile compounds that can be responsible for the oxidation of products with vegetable oil and deterioration of processed dried *Porphyra.*

Fats and oils can enhance the flavor of any product. Moreover, oils can enhance the color, texture and a contributory factor for feeling of satiety and palatability to products and has been known significantly to affect food quality even they constitute as a minor component (Encarnacion, 2008).With this, nutritionists always recommend and encourage manufacturers to increase the use of polyunsaturated fats in foods versus saturated fats due to health reasons. Various medical researchers proved that polyunsaturated fatty acids help lower blood cholesterol at the same time reduce the risk of heart disease. Vegetable oils like canola and corn contain a high amount of polyunsaturated fats while olive oil contains monounsaturated fats which are also effective in lowering blood cholesterol but commands higher price compared to other kinds of oils.

Aroma of any food comes from volatile compounds at different concentration present in the product. The presence of oil in food products if

not stored properly and packed in appropriate packaging material will lead to oxidation. Oil oxidation will result to off-flavor like rancidity which makes the product unacceptable by the consumers.

Rancidity is caused mainly by the presence of volatiles from lipid oxidation. Volatiles have an impact on flavor even at extremely low concentrations (even below 1ppm) (Frankel, 2005). The low molecular weight components responsible for flavor and odor in many foods are formed via decomposition of lipid hydroperoxides by different mechanisms. Example of which is hexanal, a very common aldehyde flavoring component formed by the destruction of a 13-hydroperoxide fatty acid derivative (Perkins, 1992). Another classic example is the formation of 2, 4-decadienal by cleavage of the 9-hydroperoxide (Frankel, 2005). Further decompositions of hydroperoxides are responsible for the formation of complete homologous series of saturated and unsaturated hydrocarbons, saturated and unsaturated aldehydes, ketones, alcohols, and carboxylic acids (Frankel 2005).

The use of vegetable oil such as sesame, canola, perilla, soybean, corn and olive in different products by food manufacturers has attracted public attention due to its health benefits. Sesame oil contains high amount of lignin compounds (1034 ppm) such as sesamol as well as tocopherols (44

ppm) having good antioxidant activities (Lee et al., 2008). As a food source, the presence of high amount of linoleic and linolenic acids are another merit of sesame oil (Lee et al., 2011).

The determination of volatile compounds usually includes an extraction step, which is followed by the analytical quantification of the individual compounds using gas chromatography-mass spectrometry (GC-MS) (Du et al., 2013). Gas chromatography-mass spectrometry is analytical techniques that are combined to form a single method of analyzing mixtures of chemicals qualitatively and quantitatively. It is used to separate mixtures of chemicals into individual components. Once isolated, the components can be evaluated individually. In all chromatography, separation occurs when the sample mixture is introduced (injected) into a mobile phase. In liquid chromatography (LC), the mobile phase is a solvent while gas chromatography (GC) is an inert gas such as helium. In mass spectroscopy (MS) as the individual compounds elute from the GC column, they enter the electron ionization (mass spec) detector. (www.gmu.edu, 1998). Over the past decades, the conventional extraction methods applied by different researchers are vacuum hydrodistillation (Jumtee et al.,2011), dispersive

liquid-liquid microextraction (Sereshti et al.,), distillation liquid/liquid extraction (Gu et al., 2010), hydrodistillation (Tontul et al., 2012).

Some compounds tend to be absorbed and decomposed on the columns or injector and to exhibit non- reproducible peak areas, height and shapes because of polarity of many substances. Thus, this study aims to determine the volatile compounds and their peak areas present in the different products of *Porphyra* applied with different vegetable oils from Korea, processed without oil as well as dried raw from the Philippines and compare their differences. Moreover, the determination of volatile compounds present in these products will be able to give us information about the compounds that will be responsible for the oxidation of oiled products and will make the product unacceptable.

Materials and Methods

1. Samples

Six *Porphyra* products were used in this study. Five commercially available from Korea and one from the Philippines. Four samples from Korea are seasoned roasted ("gim") in different oils (grapeseed, olive, sesame and

perilla); and one “gim” sheets without oil that is used for sushi . Sample from Philippines was raw, sun-dried and vacuum packed.

2. Adsorption and desorption of headspace volatile compounds

One gram of samples was put into 250 ml amber screw cap bottles and tightly sealed. It was put in dry oven at 60˚C for 30 min. and it was cooled at room temp. for 1 hour. The headspace volatile compounds were adsorbed for five (5) minutes in stainless steel desorption pipe (Agilent,USA) of 90 cm of the compacted Tenax-TA by using vacuum pump (VPC-10, Shimadzu, Japan) and mass flow controller (MFC).

The desorption step of the stainless steel pipe was carried out by using automatic thermal desorber (ATD 400, Perkin Elmer, UK) and it was desorbed in the opposite direction of adsorption direction. The desorption condition of ATD was 350˚C for 4 min. The desorbed gas was focused at -30 ˚C for 1 min. Desorbed flow was maintained to be 50.2ml/min.

3. Operation condition of Gas Chromatography-Mass Spectrometry

The desorbed volatile compounds were automatically injected into gas chromatography (Shimadzu 2010, Japan) by using automatic thermal desorber, separated and identified by mass selective detector (Shimadzu QP-2010 plus, Japan).

The used GC column was AT-1 (60m x 0.32mm x 1.0μm, Altech, USA). The temperature condition of oven was divided into four steps. For the first step, the oven temperature was kept at 35˚C for 10 10 min. The second step was to increase the temperature up to 120˚C at the rate of 8˚C per min. and maintained for 10 min. The third step was to increase the temperature up to 180˚C at the rate of 12˚C per min and kept for seven 7 min and the final step was to increase the temperature up to 230˚C at the rate of 15˚C per minute and maintained for 10 min. The temperature of the GC interface was kept at 230˚C. Carrier gas is helium with purity of 99.9999 percent. The temperature of MSD was 250˚C. Mass range was set 20-350m/z. Ionization voltage was 70eV. Carrier gas was helium with purity of 99.9999 percent.

The other analysis conditions are the same as those of gas chromatography (GC).

The compounds of each peak on total ionization chromatogram (TIC) by GC-MSD were identified by comparing its mass spectrum with mass spectral databases of Wiley 211 and Nist 107 mass spectral databases (John Wiley and Sons, Inc.).It was also compared to Kovats retention index. The qualitative analysis of the identified volatile compounds was carried out with the relative ratios, based on 100% of total peak area for all the identified compounds.

Results and Discussion

Volatile composition is also influenced by process parameters such as moisture and pH as Maillard reaction is related to temperature and water activity during the drying step (Bermis-Young, Huang and Bernhard, 1993). A total of 193 volatile compounds identified in the different processed *Porphyra* products broken down as follows; 16 aldehydes, 31 alcohols, 17 ketones, 19 esters,3 ethers, 3 nitrogen-containing substances,5 sulfur-

containing compounds, 7 furans, 8 other aromatic compounds, and 83 other aliphatic hydrocarbons, (Table 9). Samples contained high amount of aliphatic hydrocarbons followed by alcohols and least of furans. Meanwhile, aliphatic hydrocarbons and alcohols which found to be abundant are not greatly affected to flavor and contribute very low effect to food quality (Arachchi, 2014). Eight volatile compounds were detected by headspace GC-MS common to all the samples like acetaldehyde, propanal, 3 methyl butanal, n-Hexanal, ethanol, hexane, benzene and methyl benzyne. Among these compounds, aldehydes are the most important because they are used for flavoring in foods but it is characterized to be easily oxidized. It is also used as disinfectant and dying items. In some studies, some aldehydes, alcohols and furans are investigated as potential markers derived from Maillard reaction or thermal degradation of lipid.

End product characteristics are not only related to raw materials but also to manufacturing conditions. Indeed, throughout the transformation process, several factors can affect both the cooking performance as well as nutritional and organeloptic properties of the final product (Gianetti et.al, 2013). Detection of volatile compounds showed that processed products without the application of oil and were not subjected in high-controlled

temperature found to contain more volatile compounds. This is due to the destruction of compounds during roasting of the products using high temperature before the application of different vegetable oil or exposure to air before packaging. Like aldehydes, they degrade in air via the process of autoxidation.

Among aldehydes, propanal showed the highest peak while 2-methyl-2-pentenal found to be the lowest. Generally, propanal also known as propionaldehyde gives a fruity odor in foods while 2-methyl-2-pentenal is a foodgrade and halal food flavor ingredient which was only detected in processed raw dried sample from the Philippines.

On the other hand, the presence of alcohols confirmed the presence of 1-penten-3-ol which exhibited the highest peak while ethanol which was identified in all samples found as the second highest peak and 3- methyl-1-butanol carbonate revealed the lowest peak. Alcohols are not greatly affected to food flavor because of high value of flavor threshold (Karadian et al.,1989). The higher retention of alcohols in product which were not thermally heated might be due to the lower processing temperatures used for the2 samples without oil application. This is also similar to the findings of

(Aguayo et.al, 2010) that the loss of aroma-related volatile compounds could be induced by volatilization during heat processing.

Among ketones, methyl ethyl ketone showed the highest peak while 3-methylene-2,6-heptadion found to be the lowest peak. Ketone compounds have important physiological properties. Ketones are mostly found in fruit products like raspberry. It has a natural phenolic and aroma compound.

In esters, ethyl acetate showed the highest peak particularly in products applied with olive and sesame oil. Low peak of oxalic acid, butyl-6- ethyl-octyl ester exhibited in processed *Porphyra* sheets. Ethyl acetate is important flavor compound characterized with sweet smell and it is used in decaffeinating tea and coffee.

There were only 3 nitrogen-containing substances found in samples and were limited to dried sample from the Philippines with 2,6-dimethyl pyrazine and revealed the highest peak while processed samples without oil and roasted in grapeseed oil contained minimal amount of 2,5 dimethyl pyrazine and 2-pyridinamine, respectively.

Other aromatic compounds like methyl benzene and benzene were detected in all samples. Methylbenzene is an aromatic compound that is widely used as an industrial feedstock and as a solvent. It is a typical smell of paint thinners. Methyl benzene when exposed to air evaporates. Ethyl benzene found to be the lowest peak among aromatic compounds. On the other hand, benzene is the simplest organic, aromatic hydrocarbon and considered parent compound of numerous important aromatic compounds.

Sulfur-containing volatile compounds are a major aroma class found in vegetables, cooked meat, and other foods. These compounds were also found in samples. Out of the 5 identified compounds, 3 compounds were identified in samples using grapeseed oil. The other 2 compounds were found in product using perilla oil. Sulfur-containing compound like dimethyl sulfide is characterized with disagreeable odor in foods.

Aliphatic hydrocarbons which have the most numbered volatile compounds revealed that 2,4-dimethyl hepatane showed the highest peak while 3-methylene-2,6-heptadione was the lowest in peak.

Conclusion

Several factors affect the volatile compounds present in food, such as source of raw material, ingredients and the processing techniques employed. Numerous volatile compounds were detected by headspace gas chromatography but are not of significant for flavor in foods. The study confirmed the presence of aldehydes, alcohols, ketones, esters, ethers, nitrogen and sulfur-containing substances, aliphatic compounds. For better extraction of volatile compounds in samples, heating at 60˚C for 30 minutes is necessary. Processed products which were not subjected to high controlled temperature better retained their volatile compounds. Regardless of the methods on processing of these products, acetaldehyde, propanal, 3-methyl butanal, ethanol, hexane, benzene and methyl benzene found to be common in all samples.

Table 9. Volatile compounds identified from the different *Porphyra* products by means of headspace gas chromatography and mass spectrometry

Chemical name	RT	RI	Area 10^5					
			D	NO	G	O	S	P
Aldehydes								
Acetaldehyde	3.40	>500	20.8	29.8	48.2	8.8	9.9	11.4
Propanal	4.24	>500	131.5	141.2	21.9	11.1	16.1	17.1
2-methyl propanal	5.39	545	3.6	9.4	3.1	1.4	1.2	1.1
n-Butanal	6.06	564	5.9	4.7	-	-	-	-
3-Methyl butanal	9.00	617	6.8	21.3	7.6	1.5	0.9	0.9
2 Methyl butanal	9.50	631	2.7	10.0	2.2	0.4	-	-
n-Pentanal	11.60	693	-	18.6	18.4	2.7	2.3	2.4
2- Methyl-2-butenal	14.10	746	5.9	-	-	-	-	-
n-Hexanal	16.30	776	60.5	45.7	-	3.7	4.7	5.4
2-Methyl -2- pentenal	17.50	831	0.1	-	-	-	-	-
2-Hexanal	18.10	847	11.3	-	-	-	-	-
n-Heptanal	19.60	887	12.7	5.1	-	-	-	-
Benzaldehyde	21.40	939	9.6	10.0	-	-	-	-
Nonanal	26.10	1078	-	4.2	-	-	-	-
3-Methyl heptanal	26.20	1080	-	-	-	-	2.3	0.2
2-heptanal	28.70	1137	-	0.6	-	-	-	-
Alcohols								
Methanol	3.50	>500	-	68.5	-	-	-	-
Ethanol	4.00	>500	73.2	45.8	91.3	62.9	74.1	86.7
1,2- Propanediol	4.50	503	-	2.9	-	-	-	-
2- Methyl-2 propanol	5.00	527	1.4	-	-	-	-	-
1-Propanol	5.80	561	41.6	-	-	-	-	-
2-Butanol	8.50	613	7.4	-	-	-	-	-
n-Butanol	10.80	627	6.6	1.7	-	-	-	-

1-Methoxy-2-propanol	11.20	628	-	17.6	-	-	-	-
1-Penten-3-ol	11.50	632	104.3	-	-	-	-	-
3-Methyl-1pentanol	12.90	703	-	-	-			
Heptanol	12.90	715	2.8	-	-	-	-	1.3
3-Methyl-1-butanol	14.20	754	13.2	1.8	-	-	-	-
2-Methyl-1-butanol	14.50	756	14.3	-	-	-	-	-
Pentanol	15.70	784	16.0	-	-	-	-	-
1-Hexanol	19.00	873	40.7	-	-	-	-	-
5-Methyl-2-Hexanol	19.90	894	2.2	-	-	-	-	-
2-Methyl-3-pentanol	20.50	911	1.2	-	-	-	-	-
5-Methyl-2-heptanol	21.60	947	1.5	-	-	-	-	-
5-Methyl-1-hexanol	21.79	952	1.7	-	-	-	-	-
Heptanol	21.80	953	6.4	-	-	-	-	-
cis-Hep-4-enol	21.85	954	39.2		-	-	-	-
1-Octen-3-ol	22.00	958	99.3	-	-	-	-	-
4-Ethyl cyclohexanol	22.40	971	15.0	-	-	-	-	-
Cyclohexanol	22.50	972	-	-	-	-	-	0.7
2-Ethyl-4-methyl-1 pentanol	23.60	1004	-	4.6	-	-	-	-
2-Ethyl hexanol	23.60	1005	-	4.6	-	-	-	-
3-Methyl-1-butanol carbonate	23.70	1008	-	-	0.5	-	-	-
1-Octanol	25.10	1053	19.0	-	-	-	-	-
Neomenthol	25.65	1067	10.1	-	-	-	-	-
1-Hexadecanol	25.74	1069	-	-	-	1.3	-	-
3,4 Dimethyl cyclohexanol	27.00	1103	-	3.1	-	-	-	-
Ketones								
4-hydroxy-3-propyl-2 Hexanone	4.26	>500	43.3	-	-	-	-	-
2-Propanone	4.30	>500	-	-	-	-	-	6.1
2,3 Butanedione	6.10	573	-	1.2	-	-	-	-
Methyl ethyl ketone	6.50	588	-	46.8	-	-	-	1.0
Methyl propyl ketone	11.30	630	0.7	-	-	-	-	-
3-Hydroxy butanone	12.50	706	6.3	-	-	-	-	-
2-Butanone	12.60	701	-	1.0	-	-	-	-

Cyclopentanone	15.9	791	-	-	-	0.7	-	-
3-Hydroxy-3-methyl-2-butanone	16.4	799	2.3	-	-	-	-	-
2-Heptanone	19.3	881	3.3	-	-	-	-	-
3-Methyl-2-Heptanone	20.7	896	3.9	-	-	-	-	-
4-Methyl-2-Heptanone	20.7	918	-	0.6	-	-	-	-
2-Octanone	21.1	932	4.3		-	-	-	-
2.6-Dimethyl-4-heptanone	21.7	947	-	1.5	28.3	-	-	10.8
4-6-Dimethyl-2-heptanone	22.2	964	-	4.6	13.5	-	2.5	-
3,5- Octadiene-2-one	25.7	1073	14.4	-	-	-	-	-
3-Methylene-2,6-Heptadione	27.5	1113	-	0.1	-	-	-	-
Esters								
Methyl formate	3.7	>500	1.5	-	-	-	-	-
Ethyl acetate	7.46	609	-	35.8	-	103.8	101.9	10.5
Acetic acid, ethyl ester	7.6	616	3.7	-	8.3	-	-	-
Phosphorous acid, tributyl ester	13.46	706	-	0.4	-	-	-	-
Propanoic acid, 2-methyl, 3-methyl butyl ester	15.09	716	-	-	-	-	-	1.1
2-Methoxy ethyl ester	16.8	811	0.7	-	-	-	-	-
Ethyl benzoic acid ester	16.81	812	2.6	-	-	-	-	-
Octanoic acid ethyl ester	18.03	845	-	-	3	-	-	-
3, Methyl butyl decanoate	18.04	847	-	-	-	-	2.3	-
3-Methyl butyl dodecanoate	18.05	847	-	-	-	-	2.3	5
Oxalic acid dineopentyl ester	22.28	966	-	-	-	-	0.6	-
Oxalic acid,2 ethylhexyl hexyl ester	23.3	994	-	-	-	-	1.1	1.2
Oxalic acid ,butyl-6-ethyl-octyl ester	24.23	1062	2.1	0.1	-	-	-	
Carbonic acid, butyl ester octyl ester	25.07	1053	13	-	-	-	-	-
2-Ethyl hexyl methacrylate	25.87	1072	-	4.3	-	-	-	-
n-Octyl acetate	25.9	1073		-		14,5	-	2.7
Acetic acid, octyl ester	25.91	1074	-	-	3.8	-	-	-
Sulfurous acid, 2-ethylhexyl nonyl ester	27.41	1102	15	-	-	-	-	-
Sulfurous acid, 2-pentyl undecyl ester	29.89	1107	30	-	-	-	-	-

Ether								
Ethyl ether	4.59	508	7.4	4.4	9.8	6.9	6.8	-
Butyl isopentyl ether	18.04	846	-	-	-	-		5.1
n-Hexyl ether	24.64	1036	-	6.4	-	-	-	-
Nitrogen-containing substance								
2,6 Dimethyl pyrazine	20.2	903	30.1	-	-	-	-	-
2,5 Dimethyl pyrazine	20.3	903	-	0.7	-	-	-	-
2-Pyridinamine	23.9	1012	-	-	0.6	-	-	-
Sulfur-containing compounds								
Sulfur dioxide	3.2	>500	-	-	7.2	-	-	3.3
2-Aminoethyl hydrogen sulfate	3.23	>500	3.2	-	-	-	-	-
Dimethyl sulfide	4.65	513	-	-	7.2	-	-	-
Methyl sulfide	4.7	513	-	-	-	-	-	3.1
Dihexyl sulfide	24.21	1023	-	-	0.8	-	-	-
Furans								
2-3-dihydro furan	1.46	>500	-	1.7	-	-	-	-
Dihydro-3-3-dimethyl-2 (3H)-Furanone	9.23	650	-	-	2.5	-	-	-
2-Ethyl furan	12.2	635	2	4.1	-	-	-	-
Ethyl furan	12.25	699	-	-	0.6	-	-	-
Dihydromethyl furanone	20.8	922	3.4	-	-	-	-	-
5-Methyl-2-ethyl furan	26.19	1088	7.2	-	-	-	-	-
4-Methyl-3(2-furanyl) pentanal	28.39	1130	-	0.8	-	-	-	-
Other aromatic compounds								
Methyl cyclopentane	8.6	616	-	2.3	-	47.4	31.4	16.2
Benzene	9.9	640	9.3	7.3	9.9	6.7	7.4	8
Cyclohexane	9.87	623	-	-	-	10.6	-	1.5
Methyl benzene	16.14	751	5.6	18	122.9	19	13.5	11
Xylene	19.42	870	1.3	1.7	-	-	-	1.8
1,2 Dimethyl benzene	19.34	868	-	4.8	2.4	-	2.1	-
Ethyl Benzene	19.67	889	0.8	-	-	-	-	-
Cyclopentane	21.1	930	8.6	-	-	-	-	-

Other aliphatic hydrocarbons								
1-Propene	3.2	>500	2.2	2.8	1.8	1.3	1.8	1.9
3-Hydroxy propanenitrile	3.47	>500	-	-	26.7	-	-	-
2- Methyl propene	3.48	>500	20.3	-	-	-	20.2	21.5
Methyl propene	3.5	>500	-	-	-	16	-	-
n-Pentane	4.44	500	-	-	21.9	2.5	4.5	-
2-Methyl butane	4.5	507	0.6	-	-	-	-	3.6
2,3-Dimethyl-1-butene	5.9	567	6.4	-	-	-	-	-
2-Methyl pentane	6	564	-	-	32.9	22.9	13.8	8
3-Methyl pentane	6.3	581	-	-	57.7	20.2	12.5	8.5
2-Methyl-1-pentene	6.5	587	-	-	-	0.8	-	-
Hexane	7.1	600	13.6	18.1	64.5	19.4	19.6	18.6
2,2 Dimethyl pentane	7.9	609	1.8	-	-	-	-	1.1
3-Methyl-1-pentene	10	622	-	0.5	-	-	-	-
2-4-Dimethyl pentane	10.4	625	-	1.8	-	-	-	2.1
3-Methyl hexane	10.9	629	-	1.3	-	0.6	-	1
3,3,4-Trimethyl Hexane	11.01	630	-	-	-	-	-	1.8
2,2 Dimethyl hexane	11.8	633	-	-	-	-	-	1.2
n-Heptane	12.28	700	1.6	1.9	3	-	-	3.7
2,2 Dimethyl butane	12.3	701	-	-	-	0.8	1	-
3,5-Dimethyl-1-Hexene	12.8	703	-	8.6	-	-	-	-
5-Methyl-1 -hexene	12.84	714	-	-	1.5	-	-	-
Trimethyl pentane	13.05	715	-	0.7	-	-	-	-
3-Methyl pentane	13.9	737	1.5	-	0.5	-	-	-
2,5 Dimethyl hexane	14	743	-	-	1.1	-	-	-
3,4-Dimethyl-1-hexene	14.8	767	-	3.3	-	1.2	1.4	-
2,3,4- Trimethyl pentane	15.08	769	-	-	1.8	1.2	-	-
2,3 Dimethyl hexane	15.1	770	-	-	1.3	-	1.8	-
4-Methyl heptane	15.52	779	-	-	35.7	16.2	20.9	-
3-Methyl heptane	15.7	784	-	-	-	-	1.1	-
2,4 Dimethyl hexane	15.77	785	3.7	-	11.7	2.2	3.2	3.6
3,3 Dimethyl-1-pentene	15.8	786	-	-	0.2	-	-	-

2,2,4,4-Tetramethyl pentane	15.90	789	1.1	-	-	-	-	-
2-Ethyl-1-hexene	16.30	796	-	-	4.1	-	-	-
Octane	16.40	800	-	2.1	-	-	-	-
4-Methyl octane	16.70	801	-	5.0	-	-	-	-
2,4 -Dimethyl hexane	16.72	809	-	-	1.2	1.1	1.8	3.6
5-Methyl-2-hexene	17.09	819	0.6	-	-	-	15.4	-
2,4 Dimethyl-3-ethyl pentane	17.27	823	-	-	1.8	-	-	-
3,4,5 Trimethyl heptane	17.30	826	-	-	24.2	-	-	9.0
2,3,3,4-Tetramethyl pentane	17.39	829	-	-	-	11.5	-	-
2,3,3-Trimethyl hexane	17.40	830	-	-	-	6.5	6.8	4.2
2,4 Dimethyl undecane	17.59	832	-	-	217.3	-	80.0	-
2,4 Dimethyl-1-heptene	18.21	842	-	2.5	55.2	23.9	24.5	17.8
2,4 -Dimethyl heptane	18.32	851	-	9.8	116.5	112.5	79.3	100.2
5,6 Dimethyl decane	18.67	865	-	-	20.0	10.0	12.5	10.7
2,5,5-Trimethyl-1,3- hexadiene	18.83	867	0.5	-	-	-	-	-
1-Ethenyl-3-methylene-cyclopentene	19.69	890	-	-	-	1.6	1.7	-
2,7- Dimethyl octane	19.89	895	-	-	1.3	-	-	-
Nonane	20.17	900	-	60.4	-	-	-	-
4,4 Dimethyl undecane	20.45	909	-	1.1	-	1.5	-	-
2,4,6-Trimethyl heptane	20.46	911	-	-	2.5	-	-	-
Octylacetylene	20.85	922	-	1.7	-	-	-	-
2,3,3-Trimethyl butane	20.88	924	0.7	-	-	-	-	-
2,4- Nonadiene	20.93	925	-	0.2	-	-	-	-
3-Methyl undecane	21.00	928	0.8	-	2.1	-	-	-
4-Methyl nonane	21.68	948	-	-	-	0.8	-	-
3-Methyl decane	21.70	949	-	-	-	0.9	-	-
3,4,5 Trimethyl-1-hexene	21.80	951	-	4.3	-	-	-	-
3,5,5 Trimethyl-2-hexene	21.90	953	-	1.3	-	-	-	-
3-Methyl undecane	22.10	960	-	-	-	-	1.3	-
2,5-Dimethyl nonane	22.11	961	-	-	2.3	-	-	-
2,4,6 Trimethyl octane	22.12	962	-	-	-	1.1	-	-
2,2,4,6 6 Pentamethyl heptane	22.80	979	-	81.6	28.2	-	-	-

2,5,5-Trimethyl heptane	23.1	988	-	1.7	2.5	-	-	-
3,3,8-Trimethyl decane	23.12	989	-	-	-	2.9	1.3	-
3,3,5 Trimethyl heptane	23.14	990	-	-	-	1.4	-	-
3-Ethyl-3-methyl heptane	23.24	992	-	-	-	2.6	-	-
3,3 Dimethyl octane	23.25	993	-	-	-	-	2	-
2,2,6-Trimethyl octane	23.4	997	-	2.4	-	-	-	-
3,3,6-Trimethyl heptane	23.41	998	-	-	1.4	-	-	-
4-Methyl decane	23.5	1000	-	-	-	1.7	-	-
5-Methyl-5 -propyl nonane	24.67	1037	-	-	6.2	2.9	4.1	-
3-Ethyl-3-methyl heptane	24.81	1041	-	105.6	80.1	-	-	-
2,2,3 Trimethyl nonane	24.97	1048	11	-	-	-	-	-
5,2-Methyl propyl nonane	25.05	1049	-	35.2	6.2	-	-	-
2,4-Dimethyl-1-decene	25.7	1069	-	-	3.1	-	1.7	-
8-Methyl-2-decene	25.91	1074	-	-	1.7	-	-	-
2,3,6,7- Tetramethyl octane	26.45	1091	-	-	-	5.5	-	-
n-undecane	26.6	1100	3.8	-	-	-	-	6
5-Butyl nonane	26.62	1095	-	-	-	24.2	-	-
3,8-Dimethyl undecane	26.64	1101	-	-	-	17.4	19.4	-
5,6 Dimethyl decane	28.1	1125	-	0.5	-	-	-	-
3,3,4 Tetramethyldecane	29.4	1150	-	0.9	-	-	-	-

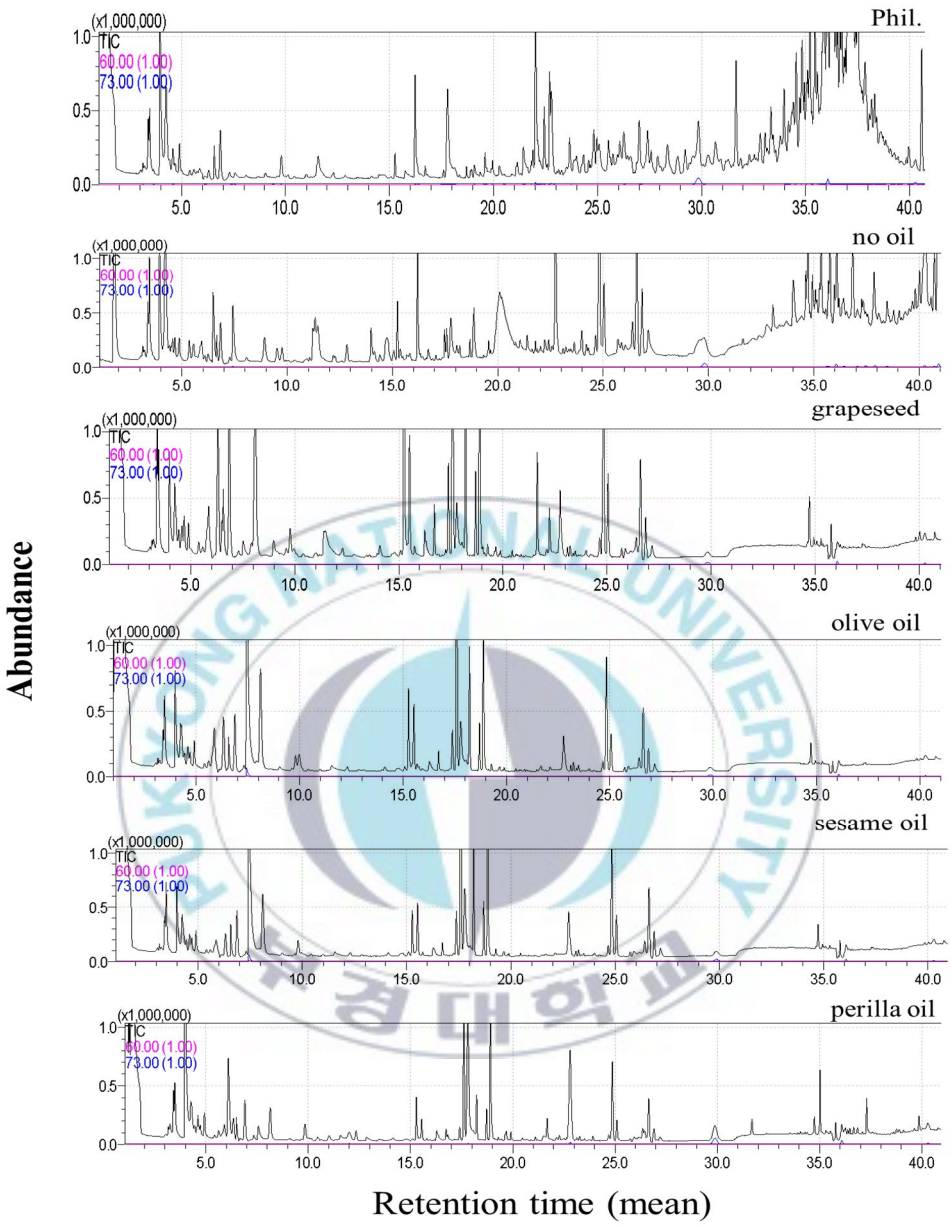

Fig. 4. Chromatograms of volatile compounds identified in the different *Porphyra* products.

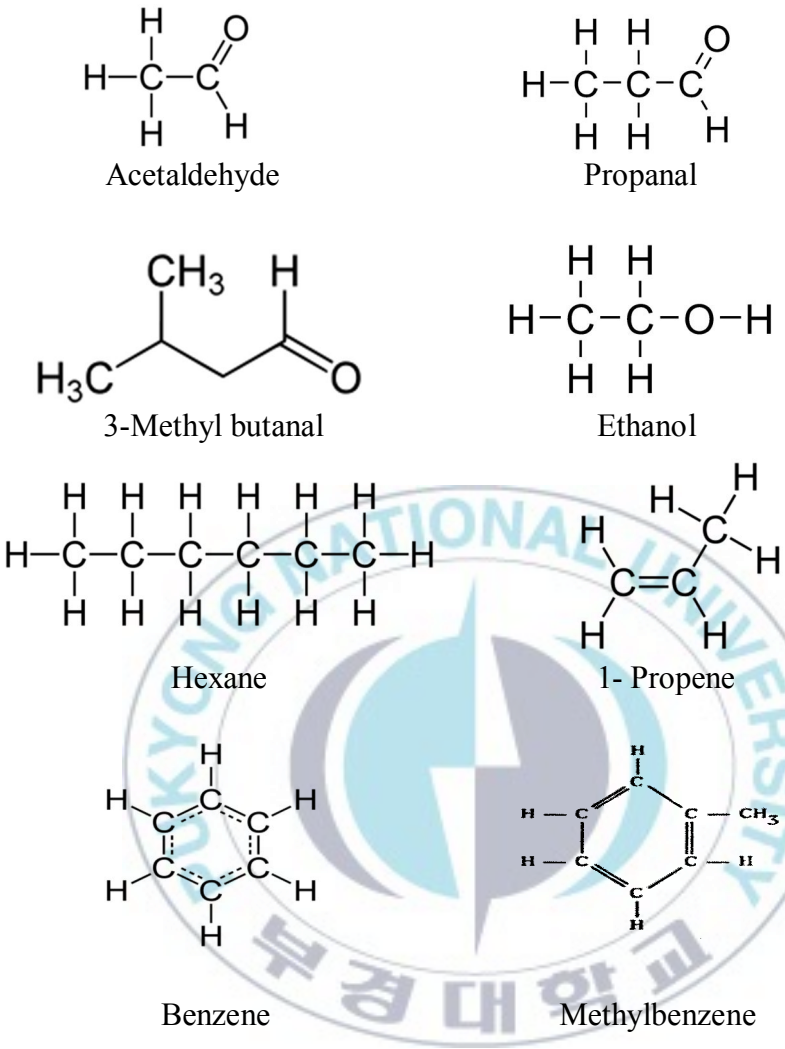

Fig. 5. Chemical structures of common volatile compounds identified in all samples.

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