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RESEARCH ARTICLE

Proximate Composition and Physico-chemical Properties of Dried *Pyropia acanthophora* in Sta. Praxedes, Cagayan, Philippines

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A B S T R A C T

Proximate composition and physicochemical properties to include mineral content, free amino acids, water retention capacity (WRC), and lipid adsorption capacity (LAC) of dried *Pyropia acanthophora* locally known as “Gamet” and endemic only in the northern Philippines were evaluated. Dried *Py. acanthophora* contained high amounts of crude protein (23.0±0.1%) followed by ash (16.7±0.1%), moisture (11.8±1.5%) and lipid (1.1±0.1%). Analysis of macrominerals using Inductively Coupled Plasma Optical Emission Spectrometry showed that dried *Py. acanthophora* have significant amounts of K, Na, Mg, P, and Ca in decreasing order. Twenty-four (24) free amino acids were also determined, and seven essential amino acids were identified including isoleucine, leucine, lysine, valine, phenylalanine, threonine, and tryptophan. A significant amount of taurine was also detected among the free amino acids. WRC of dried *Py. acanthophora* was determined to be 5.60 ±0.1g-H_2O.g^{-1}. LAC of dried *Py. acanthophora* were determined to be 2.88± 0.10g-oil.g^{-1}, 3.30± 0.21g-oil.g^{-1}, and 3.28±0.21g-oil.g^{-1} using sunflower oil, sesame oil and soybean oil, respectively. The high amounts of carbohydrate of dried *Py. acanthophora* determined by difference could have influenced its WRC and LAC. Based on the results of the study, *Py. acanthophora* should be promoted as health food and could be a potential material for the production of food supplements to meet the recommended intake of protein and other essential nutrients for humans.

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1. INTRODUCTION

Seaweed is an important component of the marine ecosystem along with the mangrove and coral reefs and can be viewed from two perspectives: its ecological value as well as its economic uses. Moreover, seaweed demonstrates original and interesting nutritional attributes and value depending on species and environmental conditions. Traditionally, seaweeds have been used as food, animal feed, fertilizer, and sources of traditional medicine in many Asian civilizations since ancient times (Radha 2018). Notably, they are marketed as “nutraceuticals” owing to their highly bioactive ingredients as well as food supplements in order to relinquish physiological conditions and resist diseases (Alwaleed 2019).

Seaweeds in the Philippines are highly diversified among the aquatic flora in the 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 economically important seaweeds are *Codium, Gelidiela racerosa, Halymenia, Sargassum* spp. and *Pyropia* spp. Among these seaweeds, *Pyropia acanthophora (*Zuccarello et al. 2022; Dumilag and Aguinaldo 2017), locally known as “Gamet,” is only gathered in northern Cagayan and Ilocos Norte, Philippines, during the northeast monsoon ushering cold weather beginning October to March (DA-BFAR RO2 2020). “Gamet” is formerly identified as *Phycocalidia acanthophora* (Santianez and Wynne 2020) and *Porphyra spp.* (Ame et al. 2010) until its recent identification as *Pyropia acanthophora* by Gluseppe Zuccarello of the School of Biological Sciences, Victoria University of Wellington, New

Zealand and Xinging Wen and Gwang Hoon Kim of the Department of Biological Sciences, Kongju National University, Korea in 2022 (Zuccarello et al. 2022).

Gathering "gamet" was considered an alternative source of livelihood for fisherfolk several years ago. This seaweed only grows during the lean months of fishing and is regarded as black gold. The latest recorded production of 10,350 kg was in the municipality of Sta. Praxedes, Cagayan, Philippines, as its major source (DA-BFAR RO2 2020).

Processing of this seaweed in other parts of the world comes in a variety of products, like in Korea, Japan, and China. In the Philippines, however, the "Gamet" industry remains underdeveloped, as raw materials are mainly gathered from the wild in the absence of available culture technology, and processing is only limited to drying (Ame et al. 2010). Moreover, the seasonality, risk of gathering, and low yield of dried products make this seaweed relatively expensive. Notably, information on the nutritional composition and properties of this seaweed resource is very limited. Providing information on the nutritional composition and its properties would guide the industry in product development from the resource and promote this commodity as a healthy food, thus, this study.

The study aimed to determine the proximate composition and some of the physico-chemical properties, including mineral content, free amino acids, water retention capacity (WRC), and lipid adsorption capacity (LAC) of dried *Py. Acanthophora,* endemic in Sta. Praxedes, Cagayan, Philippines.

2. MATERIALS AND METHODS

2.1 Materials and chemicals

Fresh seaweed *(Py. acanthophora)* (Figure 1) was gathered in Sta. Praxedes, Cagayan, Philippines (Figure 2). The product was sun-dried and vacuum-packed. Samples were brought to the laboratory of the Department of Food Science and Technology, Pukyong National University, Busan, South Korea, and stored at room temperature until used. Samples subjected to the different analyses were all pulverized before use. All chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated.

2.2 Proximate analyses and mineral content

Moisture, lipid, crude protein, and ash contents were determined using the Association of Official Analytical Chemists (AOAC) methods (2012). Carbohydrate content was calculated by difference, where all other components were subtracted from 100. Mineral content was analyzed using Inductively Coupled Plasm Optical Emission Spectrometry (ICP-OES) using a Perkin Elmer Optima 3100 XL spectrometer (Perez et al. 2007, Larrea-Marín et al. 2010).

Figure 1. A) Collection of fresh *Pyropia acanthophora*; B) Fresh *Py. acanthopohara*; C) *Py. aconthophora* before sun drying; D) Dried Py. *aconthophora* collected from Sta. Praxedes, Cagayan, Philippines.

2.3. Free amino acids determination

Five (5) grams of pulverized sample were used, and it was added with 3% trichloroacetic acid, mixed well, and diluted with distilled water to 10 mL. It was centrifuged at 8,000 rpm for 20 min, and its supernatant was collected. The supernatant was diluted with 0.02N hydrochloric acid and filtered at 0.45µm using a

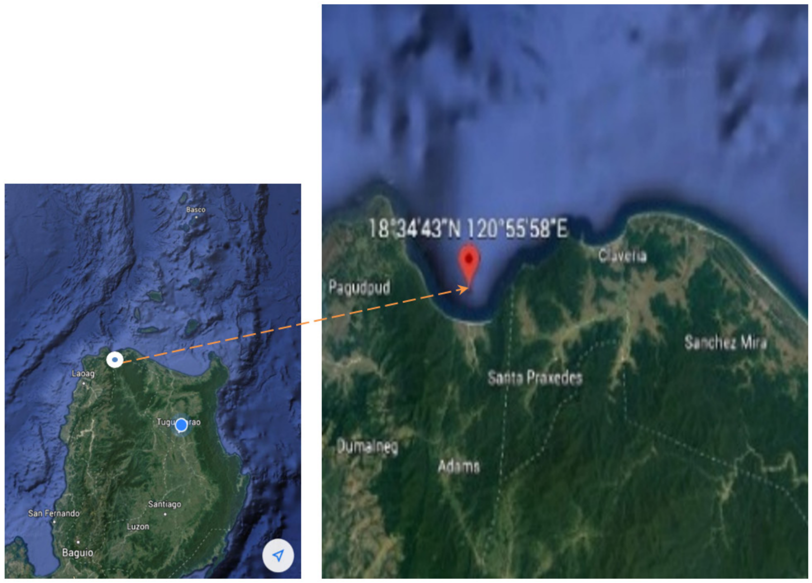

Figure 2. Location of study site—Sta Praxedes, Cagayan, Philippines.

syringe filter, and the solution in a vial was analyzed using a Hitachi L-8900 amino acid analyzer (Qu et al. 2001).

2.4 Determination of water retention and lipid adsorption capacities

Water retention capacity (WRC) was determined using the method of Rupérez and Calixto (2001) with slight modification. Ten (10) mL of distilled water was added to a 0.5-g sample in a centrifuge tube. The sample was agitated and left at room temperature for one hour and was centrifuged at 4,000 rpm for 20 min. The supernatant was discarded, and the residue weighed. The WRC was expressed as gram of water/g dry sample. Lipid adsorption capacity (LAC) was determined using the method of Carvalho et al. (2009) with slight modification. Three (3) grams of sample were added to 18 mL of three different oils, sunflower, sesame, and soybean, in each centrifuge tube. The sample was left at room temperature for 24 hours, and the mixture was centrifuged at 1,500 rpm for 10 min. The supernatant was discarded, and the weighed residue was expressed as LAC of oil/g dry sample.

2.5 Statistical analysis

All analyses were performed in triplicate. The data were expressed as means ± standard deviations 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.

3. RESULTS

3.1 Proximate analysis and mineral content of dried *Py. acanthophora*

Table 1 shows the proximate composition of Py. acanthophora. The dried seaweed contains a high amount of protein, about 23%, followed by an ash content of 16.7+0.1%, a moisture content of 11.8+1.5%, and 1.1+0.1% lipid. By contrast, the carbohydrate content is 47.4+0.7%. Meanwhile, the sample also contained significant amounts of macrominerals such as potassium, sodium, calcium, magnesium, and phosphorus, as shown in Table 2.

3.2 Free amino acids of dried *Py. acanthophora*

Twenty-four (24) free amino acids were identified in the aqueous solution, as shown in Table 3. High amounts of taurine were detected together with alanine, phosphoethanolamine, glutamic acid, citrulline, and phosphoserine. In addition, seven essential amino acids were found in the sample, including isoleucine, leucine, lysine, valine, phenylalanine, threonine, and tryptophan.

Table 1. Proximate composition of dried *Pyropia acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines.

Component	**Amount (%)**
Moisture	11.8±1.5
Ash	16.7±0.1
Lipid	1.1 ±0.1
Crude Protein	23.0±0.1
Carbohydrate	47.4

Values are means± standard deviation of triplicates.

Table 2. Mineral composition of dried *Pyropia acanthophora* gathered in Sta. Praxedes, Cagayan, Philippines.

Component	**Amount (μg/g)**
Na	23,040.3±1267.0
K	29,921.0±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.

Table 3. Free amino acid composition of dried *Pyropia acanthophora* in Sta. Praxedes, Cagayan, Philippines and essential amino acid daily requirement for adults (World Health Organization 2002).

Amino Acid	***Pyropia acanthophora*** **Amount (mg/100g)**	**Essential Amino Acid Adult Daily Requirement Amount (mg/100g)**
Isoleucine*	4.4 ± 0.4	2.0
Leucine*	4.7 ± 1.2	3.9
Lysine*	9.3 ± 0.3	3.0
Valine*	10.9 ± 0.2	2.6
Phenylalanine*	3.1 ± 1.0	2.5 (+ tyrosine)
Threonine*	30.6 ± 2.7	1.5
Tryptophan*	6.0 ± 4.2	0.4
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	
Phosphoethanolamine	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.; (*) Essential Amino Acid

3.3. Water retention and lipid adsorption capacity of dried *Py. acanthophora*

The results of the analysis showed that the water retention capacity of *Py. acanthophora* is 5.60±0.1g-water.g^{-1} while lipid adsorption capacity is 2.88±0.10g-oil.g^{-1}, 3.30±0.21g-oil.g^{-1}, and 3.28±0.21g-oil.g^{-1} using sunflower oil, sesame oil, and soybean oil, respectively.

4. DISCUSSION

Proximate analysis of *Py. acanthophora* revealed that crude protein content is high and the lipid content is the least component. However, higher crude protein content was reported in *Porphyra tenera* (36.88±0.90%) and *P. haitanensis* (32.16±1.21%) in Korea and China (Hwang et al. 2013). Meanwhile, the carbohydrate content and ash contents of *Py. acanthophora* are higher than the aforementioned *Porphyra* species, with around 10.30% ash and 36.82% carbohydrate contents only (Admassu et al. 2018). Notably, the proximate composition of seaweed species varies depending on the geographical location and season that is being harvested (Yuan 2008; Kaori et al. 2011). On minerals, some researchers compared that macromineral contents of seaweeds, like *Porphyra,* are mostly higher than those of terrestrial plants like corn and some vegetables (Subba Rao et al. 2007; Ruperez 2002). In this study, *Py. acanthophora* contained significant amounts of macrominerals such as K, Na, Ca, Mg, and P. These were also detected in the species of *Porphyra* "nori" in Japan by Noda (1993) and Subba Rao et al. (2007) in India. However, Fe, Mn, Se, and Zn, which were found in Japan and India, were not present in *Py. aconthopora* from the Philippines. Surprisingly, *Py. acanthophora* do not have any similarity in essential mineral contents of *Porphyra columbina* of Argentina (Perez et al. 2007). High amounts of alanine, phosphoethanolamine, glutamic acid, citrulline, and phosphoserine were determined in *Py. aconthopora* to include seven essential amino acids, such as isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine. These essential amino acids were also identified in *Porphyra* "nori" from Japan (Noda 1993; Fleurence 1999) and Korea (Lee et al. 2012). Notably, alanine was found to be the highest amino acid in *Py. acanthaphora*. The seaweed sample also contained phosphoethanolamine, an ethanolamine derivative used to construct sphingomyelins (Gupta and Ghannam 2011; Cerna 2011). In addition, glutamic acid was found in the sample, another non-essential amino acid that is considerably important in the food industry as a flavoring. It is believed that, most especially among Japanese people, glutamate enhances the palatability of any food and has been used and known worldwide (Nagahama et al. 2009; Prabhasankar et al. 2009). The substantial amount of aspartic acid is also important because both glutamic and aspartic acids are responsible for the special flavor and taste of seaweeds (Yaich et al. 2011). On the other hand, β-alanine was found to be the least amino acid content in *Py. acanthophora*. A significant amount of taurine was also identified in *Py. acanthophora* and the only sulfur-containing amino acid identified in the sample. Taurine is notably the most abundant amino acid in red algae, especially *Porphyra* species. The *Porphyra tenera* and *P. haitanensis* contained high levels of taurine, 975.04 mg and 645.55 mg in 100 g DW, respectively (Hwang et al. 2013). This amino acid plays a role in neurotransmission in the brain, where it appears to be involved in many functions from conception onward. Because *de novo* synthesis of taurine is relatively low in the brain, exogenous taurine is needed for appropriate development and adult brain functions. Notably, taurine supplementation prevents or improves some neurological disorders (Roysommuti and Wyss 2015). Moreover, taurine is a main ingredient of bile and aids in the digestion of fats and the absorption of vitamins that are fat-soluble, as cited by the report of Roysommuti and Wyss (2015). Also, increased dietary intake of taurine may have beneficial effects on the heart and may help battle diabetes and hypertension (Roysommuti and Wyss 2015). Meanwhile, phenylalanine and tyrosine, which are characterized as aromatic amino acids, were also found in the sample. It has been reported that the water retention capacity of some seaweed is attributed to insoluble fiber and to the high content of uronic acid-components of soluble fraction of dietary fiber (Femenia et al.1997; Rupérez and Calixto 2001). 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. 2009. Meanwhile, the lipid adsorption capacity of *Py. acanthophora* showed that using sunflower oil was significantly different ($p < 0.05$) compared to the adsorption of the material using sesame and soybean oil. In the study of Carvalho et al. (2009) using green seaweed *Ulva fasciata* Delile, the lipid adsorption capacity of the seaweed was higher compared to that of chitosan. 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 (Ciana et al. 2014). Some authors proved that brown and red seaweeds contain high amounts of dietary fiber essential to human health (Ruperez and Calixto 2001; Jiménez-Escrig and Sánchez-Muniz 2000; Fleury and Lahaye 1991; Urbano and Goňi 2002). Overall, it should be noted that variation in nutritional and functional values among species and product types makes product-specific nutritional analysis a prerequisite for accurate prediction of health benefits (Cho and Rhee 2019).

5. CONCLUSION

Py. acanthophora in Northern Philippines is a potential alternative cheap source of nutrients like protein and minerals based on the proximate composition analysis. It also contains high amounts of free amino acids. The presence of essential amino acids, such as isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine, is comparable to terrestrial food products and can be a cheap and potential alternative source of such essential amino acids. The palatable taste of this seaweed can be 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. Meanwhile, the water retention and lipid adsorption capacities of *Py. acanthophera* as important physico-chemical properties of the seaweed can be attributed to its high amounts of carbohydrate. As such, *Py. acanthophora* should be promoted as health food and could be used as raw material for food supplements to help meet the recommended daily intake of essential nutrients for humans.

CONFLICT OF INTEREST

There is no conflict of interest among authors, institutions and individuals mentioned above in the conduct of this study and the preparation and submission of this manuscript.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

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