Potential of Green Seaweed *Ulva rigida* in Thailand for Healthy Snacks

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ABSTRACT

Seaweed is an alternative food source with high nutritional value for health-conscious consumers. In this research, we focused on the development of a healthy Thai seaweed snack that provides high fiber and protein content from green seaweed *Ulva rigida*. The results showed that *U. rigida* contained approximately 43 % fiber, 32 % protein, 19 % minerals, and 2 % fat based on dry weight. The pretreatment of fresh seaweed by washing five times in clean water and 15 s blanching in boiling water resulted in stable green color and alleviated fishy odor. For the process of forming seaweed sheets, the addition of seaweed polysaccharide at the concentration of 0.25 % (w/v) assisted the bonding of seaweed, consequently facilitating sheet formation and reducing product fracture. In addition, the drying of seaweed sheets was done at 150 °C for 35 min. These conditions could enhance crispness and reduce moisture content of seaweed to approximately 2 %, which is comparable to commercial crispy seaweed snacks available in the market. After seasoning, this seaweed snack consisted of high fiber and protein with low fat content. The findings demonstrated that *U. rigida* has the potential to be further developed as a healthy snack.

Keywords: Drying, Fiber, Macroalgae, Polysaccharide, Protein, *Ulva rigida*

INTRODUCTION

Seaweeds are considered as essential future sources of bioactive compounds with a range of biological activities that have potential applications in functional foods and nutraceuticals. They consist of dietary fibers, proteins, polyunsaturated fatty acids including omega-3 fatty acids, and minerals as well as polyphenols, pigments (chlorophylls, fucoxanthin, phycobilins), and mycosporine-like amino acids (Holdt and Kraan, 2011). Apart from being a rich source of bioactive compounds, seaweeds have become an attractive source for commercial applications as they have fast growth rates and do not require arable land, fresh water, or fertilizer when compared with terrestrial plants (Lorbeer *et al*., 2013). Although the cultivation of seaweed in Thailand has been developed since 1986, the consumption and processing of seaweed is not widespread. The application of seaweed is limited to agar production, animal feed, and treatment of waste-water from shrimp ponds (Chirapart, 2006). *Ulva rigida* was selected as a raw material due to its abundance in the region, high potential for cultivation at an industrial scale, and because it contains nutrients of commercial interest which are good for human health. Increasing consumer awareness of the complex relationship between diet and health is resulting in demand for new and healthy products in the current market.

Snack products are popular, greatly appreciated by everyone and consumed throughout the world (Hossain and Shin, 2013). The change

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of people's lifestyles and demand for convenient foods help to increase the snack market. In Thailand, revenue from the snack food segment is an estimated US$137m in 2019. The market is expected to grow annually by 1.2 % according to Compound annual growth rate 2019-2023 (Statista, 2019). The main process of making snack products involves deep frying, which is dehydration at high temperature when the food pieces are immersed into hot oil. Today, processed foods prepared by frying are popular. The foods are not only rapidly cooked, but frying also provides unique sensorial attributes such as color, aroma, flavor and texture and improves overall palatability (Pedreschi *et al*., 2018). Snack foods (cookies, chips, and other salty snacks) tend to be energy-dense and low in nutritional value, as they are high in sugar, sodium, and/or saturated fat (Hess and Slavin, 2018). Moreover, carcinogens such as acrylamide usually form in the high temperature frying process of high carbohydrate snacks. Therefore, healthy consumer food trends are driven and new categories of healthy snacks with low fat or low calories are being developed instead of fried snacks, which are considered as concentrated sources of fat and energy.

Normally, the deep-fat frying process is applied to produce seaweed snacks in Thailand and other countries, both commercially and at the household level, as their specific organoleptic properties are difficult to achieve with other cooking methods. With regard to health concerns, there is an increasing interest in the development of alternative oil-free processes. Wang *et al*. (2019) evaluated that the most popular drying method is conventional hot-air drying. This process has no required special equipment and has been frequently used due to lower cost and reduction of overheated product compared to microwave ovens. However, only few studies have reported on oil-free drying methods used to develop innovative seaweed snacks for health-conscious consumers. Therefore, the purpose of this research was to examine the chemical composition of Thai green seaweed *U. rigida* and the potential of developing a seaweed snack using an oil-free drying process.

MATERIALS AND METHODS

Pre-treatment of fresh seaweed samples

The fresh green seaweed *Ulva rigida* collected from the Phetchaburi Coastal Aquaculture Research and Development Center, Thailand was washed five times with tap water at the ratio of 1:8 (w/v), then blanched in boiling water for 15 s, and immediately cooled with iced water.

Sample preparation and drying process

After cooling, the seaweed samples were weighed and mixed with water at a ratio of 1:10 (w/v) and ground for 40, 60, and 90 s using a blender (model BL3001, Tefal, France). Then, seaweed samples were filtered through a muslin cloth in order to collect the residue for drying. Seaweed polysaccharides dissolved in water at the concentrations of 0.125, 0.25, and 0.5 % (w/v) were added into filtered seaweed samples at a ratio of 2:3 (w/w). The samples were spread as a thin layer on heat-resistant Teflon film to form seaweed sheets (2 mm thickness). The seaweed samples were dried at 150 °C in a hot air oven (model KET-6-T, Cleveland, USA) for 5 to 45 min. After that they were coated with a seasoning mixture containing soy sauce, sugar, and flavoring, then dried at 130 °C for 8 min in a hot air oven to obtain the finished seaweed snack.

Determination of chemical and physical properties of seaweed samples

Composition analysis of fresh seaweed and seaweed snack

Analyses of moisture, protein, fat, ash, calories, dietary fiber (total, soluble, and insoluble fiber), amino acid profiles, minerals (calcium, chromium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, and zinc), and heavy metals (arsenic, cadmium, lead, mercury, and tin) of the fresh seaweed *U. rigida* were conducted by standard methods of Central

Laboratory (Thailand) Co., Ltd, mainly based on Association of Official Analytical Chemists (AOAC) methods (2010 and 2016), Compendium of Methods for Food Analysis Thailand (2003, 2004, and 2005), and the Official Journal of European Communikties, L257/16. The chemical composition of seaweed snack was evaluated by the standard methods as the fresh seaweed by SGS (Thailand) Limited.

Particle size analysis

The particle size of seaweed samples ground in water for 40, 60, and 90 s were measured by light microscope (Axio Lab.A1, Zeiss, Germany) and Vernier scale micrometer (530-312, Mitutoyo, Japan).

Color measurement

The colors of fresh seaweed and seaweed after washing and blanching were measured using a colorimeter (UltraScan Pro, HunterLab, USA). L*, a* and b* parameters were measured using specular reflectance including mode with light source D65 and observation angle of 10°. Parameter L*indicates the brightness of color (L*=0 indicates black and L*=100 indicates white). Parameter a* when negative indicates green color, while a* when positive indicates red color. Parameter b* when negative indicates blue color, while b* when positive indicates yellow color.

Moisture content measurement and drying curves

Moisture content of seaweed samples was determined by oven method at 105 °C (AOAC, 2005). All treatments were conducted in triplicate. The moisture content of samples was calculated applying Equation 1. The drying curves were plotted between moisture content of dry basis (%) and drying time.

$$\%M_{dry\ basis} = \frac{W_1 - W_2}{W_2} \times 100 \qquad (1)$$

where W_1 is seaweed sample weight (g) before drying and W_2 is seaweed sample weight (g) after drying.

Texture profile analysis

The dried seaweed sheets were analyzed for textural properties. A texture analyzer (TA-XT2, Texture Technologies Corp., USA) with crisp fracture support rig (HDP/BS) was used to measure the hardness. The compression speed for measurement was 2 $mm \cdot s^{-1}$. The samples were cut into thin square sheets of 6×6 cm^2 each for texture analysis. All quality measurements were conducted in triplicate.

Statistical analysis

Results are expressed as mean±SD from triplicate measurements. One-way analysis of variance (ANOVA) was used to compare the means. Differences in color and hardness were considered significant at $p<0.05$ by a Duncan's multiple range test using the software package of version 16.0 SPSS (IBM Corporation, USA).

RESULTS AND DISCUSSION

Chemical composition of Ulva rigida

Given the high potential for cultivation at an industrial scale, as well as the content of micro- and macronutrients reported in green seaweeds and their potential applications in functional foods and nutraceuticals, the composition of *Ulva rigida* was analyzed in order to assess its suitability as a model species. The results of the compositional analyses are shown in Table 1. Total dietary fiber, proteins, and minerals of *U. rigida* were the main components, accounting for approximately 43 %, 32 %, and 19 % of dried weight (DW), respectively, while low fat content (2 %) and calories were also observed. The composition of *U. rigida* was compared with two other seaweeds, *Gracillaria fisheri* (red seaweed) and *Ulva intestinalis* (green seaweed) (Benjama and Masniyom, 2011 and 2012), which also have high potential for cultivation at an industrial scale in Thailand. Although total dietary fiber of *U. rigida* was lower than the other two seaweeds, its protein content was approximately 2-3 times higher. This result agreed with the previous report by Fleurence *et al*. (2018), stating that green and red seaweeds had protein content within the range 9-47 % DW.

The protein content of *U. rigida* was comparable to the whole seed of soybean known as high protein plant-based diet, accounting for approximately 40 % DW (Liu, 1997), and also higher than most plant-origin foods (<12 % DW) (Wu, 2019). *G. fisheri* contained the highest total dietary fiber content, but *U. rigida* and *U. intestinalis* contained higher soluble dietary fiber. *Ulva* species has soluble dietary fiber as sulfated ulvan, which is considered to have influential roles in several physiochemical and biological processes, and is therefore of potential interest for food, pharmaceutical, agricultural, and chemical applications (Wijesekara *et al*., 2011). The content of dietary fiber in *U. rigida* (28-30 % DW) was higher than in vegetables and fruits (1-3.5 % DW) commonly known as fiber sources (Rodríguez *et al*., 2006). The levels of minerals as ash content were similar among these three seaweed species. Therefore, *U. rigida* was selected as a model species in this study for its high protein, soluble dietary fiber, and mineral content.

The heavy metal content in fresh seaweed *U. rigida* based on DW are presented in Table 2. Importantly, the selected seaweed species in this study must meet the requirements of consumer safety regulations. The safety assessment for seaweed consumption is generally focused on potential critical parameters, particularly heavy metals. Results showed that all heavy metals were at levels that complied with international regulations.

In terms of mineral content (Table 3), *U. rigida* showed high Ca, Fe, Mg, Mn, P, K, and Na. Compared with the mineral content of other green seaweeds *U. pertusa* and *U. intestinalis* reported in the literature (Ca 6,694-10,478; Cu 9-10; Mg 30,981-36,700; P 1,770-2,719; K 12,241-25,386; Na 3,767-10,645; Zn 8-15 $mg \cdot kg^{-1}$ DW) (Benjama and Masniyom, 2011). *U. rigida* showed higher Ca, Mg, and P, but lower K, Na, Zn, and Cu. In addition, relatively high amounts of Ca, Mg, and K were detected in the seaweed samples compared

Table 1. Composition of fresh seaweed *Ulva rigida* compared with two other seaweed species.

Seaweed species	($g \cdot 100g^{-1}$ DW)						($Kcal \cdot 100g^{-1}$ DW)	
	Fat	Protein	Ash	Dietary fiber			Calories	Calories from Fat
				Total	Soluble	Insoluble		
*Ulva rigida**	2.31	32.34	19.06	43.28	23.63	19.65	335.31	20.78
*Gracillaria fisheri***	2.2	11.6	21.2	60.7	17.5	43.2	-	-
*Ulva intestinalis****	8.0	17.9	27.6	56.7	32.4	24.3	-	-

Note: * Values are means of analytical duplicate analyses (n=2); ** Results from Benjama and Masniyom (2012); *** Results from Benjama and Masniyom (2011).

Table 2. Heavy metal content of fresh seaweed *Ulva rigida.*

Heavy metals	($mg \cdot kg^{-1}$ DW)	Maximum level* ($mg \cdot kg^{-1}$ DW)
Arsenic	1.17	≤3.0 (Inorganic arsenic)
Cadmium	<0.01	≤3.0
Lead	0.22	≤10
Mercury	Not detected	≤0.1
Tin	Not detected	≤5.0

Note: Values are means of analytical duplicate analyses (n=2); *Mabeau and Fleurence (1993); EU (2008); Holdt and Kraan (2011); CEVA (2014); Charoensiddhi *et al*., (2017).

Table 3. Mineral content of fresh seaweed *Ulva rigida.*

Mineral	(mg·kg^{-1} DW)
Calcium (Ca)	13,974.84
Chromium (Cr)	0.35
Copper (Cu)	5.22
Iron (Fe)	434.90
Magnesium (Mg)	42,244.72
Manganese (Mn)	155.18
Phosphorus (P)	5,136.87
Potassium (K)	18,464.85
Selenium (Se)	0.67
Sodium (Na)	2,422.83
Zinc (Zn)	11.86

Note: Values are means of analytical duplicate analyses (n=2).

with typical values for grains, vegetables, and fruits (Ca 80-5,100; Mg 200-3,700; and K 700-24,900 mg·kg^{-1} DW, as reported by Marles (2017)). Although relatively high Na content was detected in *U. rigida*, it can be noted that Na/K ratio of the green seaweed (0.13) was lower than for spinach (0.8) (Bhattacharjee *et al*., 1998) and olives (43.6) (Rupérez, 2002), but higher than for bananas (0.02) (Leterme *et al*., 2006). A high dietary Na/K ratio has been more strongly correlated to high blood pressure and cardiovascular disease than either sodium or potassium individually (Rupérez, 2002; Iwahori *et al*., 2019).

The nutritional quality of protein was evaluated by quantity, proportion, and availability of essential amino acids (EAA); the amino acid profiles of the *U. rigida* samples are shown in Table 4. A total of 20 amino acids were analyzed. The EAA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan), which are important to human nutrition and control of cell regulatory functions (Uribe *et al*., 2018) represent almost half of total amino acids. EAA were found in *U. rigida* at approximately 43 % of total amino acid content. This seaweed has similar EAA content to other protein sources such as casein (43.6 %), leguminous plants (45.4 %) and ovalbumin (52.4 %) (Paiva *et al*., 2014). *U. rigida* also exhibited high levels of non-EAA, particularly alanine, aspartic acid, and glutamic acid which have been attributed to giving seaweeds their characteristic flavor (Admassu *et al*., 2018).

Color measurement

The color values of seaweed samples are given in Table 5. The color and taste of seaweed samples was significantly influenced by pre-treatment using washing and blanching techniques. The blanched seaweed presented a much higher intensity of green and yellow color than fresh and washed seaweed. Therefore, blanching was the main factor to increase green and yellow color of seaweed samples. In general, the main purposes of pre-treating foods by blanching are to inactivate enzymes, reduce the initial microbial load, remove pesticide residues and toxic constituents, enhance drying rate and product quality, expel air in plant tissues, decrease undesirable smells as well as improve color, flavor, and texture (Xiao *et al*., 2017; Gomes *et al*., 2018). In this case, the color change of blanched seaweeds may due to the inactivation of enzymes such as

chlorophyllase, polyphenol oxidase, and peroxidase, which are responsible for deterioration, causing undesirable color and texture, off-flavors, odors, and nutrient breakdown (Xiao *et al*., 2017). Fortea *et al*. (2011) extracted peroxidase from alga *Mastocarpus stellatus* and investigated its kinetic parameters and thermal stability in order to reduce the economic and nutritional loss induced by this enzyme during storage or processing of seaweed. Another reason for the increase in green and yellow color was air expulsion from plant tissues during blanching. After expelling air, plant tissue was flatter and denser, leading to higher concentration of color pigments. Also, a washing step should be applied in seaweed sample preparation to reduce fishy odor and subsequently enhance customer acceptability.

Table 4. Amino acid profile of fresh seaweed *Ulva rigida.*

Amino acid	**(mg·100g^{-1} DW)**
Alanine	2,415.79
Arginine*	1,747.64
Aspartic acid	3,639.16
Cystine	543.78
Glutamic acid	3,086.36
Glycine	1,908.91
Histidine*	527.16
Hydroxylysine	638.68
Hydroxyproline	<577.17
Isoleucine*	1,108.40
Leucine*	2,012.19
Lysine*	1,342.29
Methionine*	444.45
Phenylalanine*	1,746.85
Proline	1,426.46
Serine	1,760.20
Threonine*	1,391.24
Tyrosine	1,024.66
Valine*	2,078.64
Tryptophan*	314.69

Note: Values are means of analytical duplicate analyses (n=2); *Essential amino acid (EAA).

Table 5. Color of seaweed samples before and after pre-treatment.

Treatment	**L***	**a***	**b***
Fresh seaweed	19.58^{a}±1.22	-2.94^{a}±0.11	2.88^{a}±0.35
Washed seaweed	22.63^{b}±0.28	-3.17^{a}±0.25	3.61^{a}±0.63
Washed and blanched seaweed	19.18^{a}±0.12	-5.21^{b}±0.39	5.30^{b}±0.78

Note: Values are means±SD of five replicate analyses. Means in a column with different superscripts indicate significant differences ($p<0.05$).

Particle size analysis

The particle sizes of seaweed samples after blending are shown in Table 6. Results indicated that particle size decreased with increasing blending time, affecting the sensory evaluation of final prototypes. In case of too small a particle size (blending time at 90 s), panelists detected seaweed particles in the final prototype coating their throats. On the other hand, if the particle size was too large (blending time at 40 s), seaweed sheet formation was difficult due to fragility and easy fracture of the final prototype. Therefore, the blending time of 60 s was selected in this study, as the final seaweed prototype was deemed acceptable when evaluated for sensory characteristics by trained panelists (n=10).

Effect of seaweed polysaccharide on drying process

In order to improve bonding of the seaweed and reducing product fracture, seaweed polysaccharides were added to the ground seaweed during sheet formation. Then, the seaweed sheet was dried using hot air oven, as this drying method is a practical approach, low cost, and has shorter operation time compared to other drying methods such as freeze drying (Guine and Barroca, 2012). The moisture content by dry basis of all seaweed sheets (Figure 1) showed a linear relationship, which dramatically decreased during the first 35 min. Then, moisture content values were stable during 35 to 45 min. It was also noticed that there was no significant difference in drying curves between the control and seaweed polysaccharide treatments at concentrations of 0.125-0.5 % (w/v). Therefore, the suitable drying time chosen for this study was 35 min, resulting in a moisture content of the seaweed sheet of approximately 2 %, which is comparable to the commercial crispy seaweed products available in the market.

Additionally, the hardness values of both crispy seaweed with 0.5% (w/v) added seaweed polysaccharide and commercial seaweed product were comparable and significantly higher than for other samples (Table 7). However, crispy seaweed supplemented with 0.25% (w/v) seaweed polysaccharide provided the best sensory evaluation results because they had more appropriate thickness (approximately 0.34 mm) and with a decrease in product fracture. The added seaweed polysaccharide could improve adhesion linked to its gel-forming properties (Varela and Fiszman, 2011), leading to stronger seaweed structure. Also, their unique gelling abilities at low temperature alongside good heat stability of polysaccharides make them ideal to use as thickeners and stabilizers for a variety of applications in the food industry (Saha and Bhattacharya, 2010).

Chemical composition of seaweed snack

The composition of the seaweed snack after being coated with seasoning was investigated in order to confirm their potential use as a healthy food. Results in Table 8 show that seaweed snack contained dietary fiber of 14.8, protein of 13.4, and fat of 4.9 g·100 g^{-1}. According to the Thailand Notification of the Ministry of Public Health (No. 182) B.E. 2541 (1998) Re: Nutrition Labelling, a 30 g serving of this product could be declared as having high fiber, a good source of protein, and low in fat.

Table 6. Mineral content of fresh seaweed *Ulva rigida.*

Blending time (s)	Side	Particle size (mm.)
40	width	2.42±0.77
	length	3.04±0.91
60	width	0.64±0.16
	length	0.92±0.23
90	width	0.50±0.19
	length	0.75±0.26

Note: Values are means±SD of fifteen replicate analyses.

Table 7. Hardness of seaweed sheets supplemented with different polysaccharide concentrations (%w/v).

Treatment	Average force (N)
Seaweed control	0.88^{a}±0.08
Seaweed + 0.125% polysaccharide	1.09^{b}±0.04
Seaweed + 0.25% polysaccharide	1.74^{c}±0.03
Seaweed + 0.5% polysaccharide	1.95^{d}±0.05
Commercial seaweed	2.02^{d}±0.03

Note: Values are means±SD of quadruplicate analyses with different superscripts indicating significant differences ($p<0.05$).

Table 8. Chemical composition of seaweed snack.

Analysis items	Composition ($g \cdot 100g^{-1}$)
Carbohydrate	70.1
Dietary fiber	14.8
Protein	13.4
Ash	6.0
Moisture	5.6
Fat	4.9

Note: Values are means of analytical duplicate analyses (n=2).

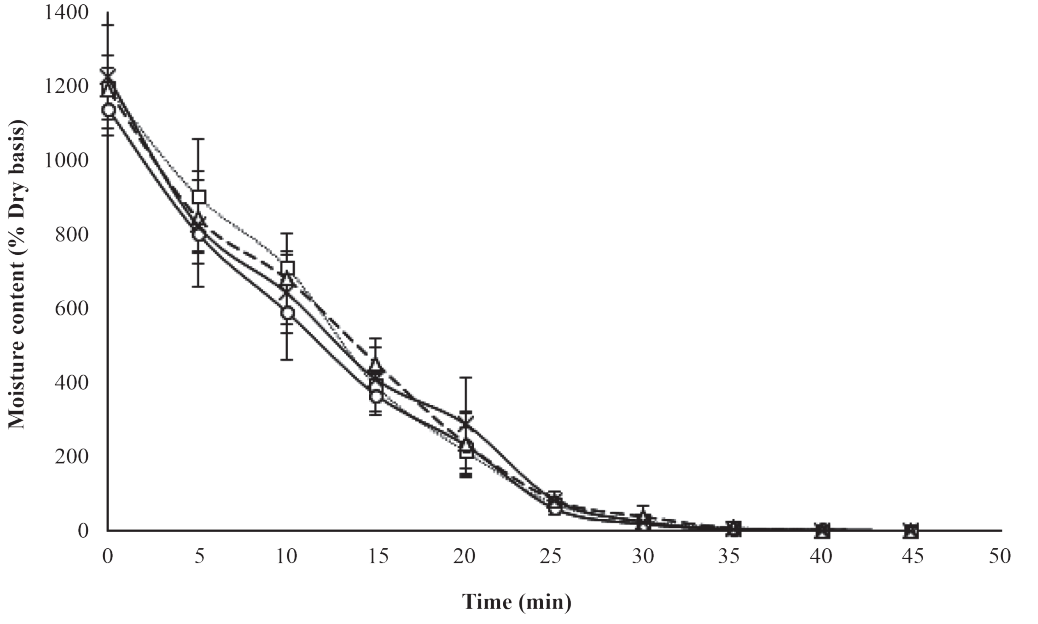

Figure 1. Drying curves of seaweed sheets by dry basis; Control seaweed sheet (□), Seaweed sheets supplemented with polysaccharide at 0.125 (○), 0.25 (Δ), and 0.5 (×) % (w/v). Values are means±SD of triplicate analyses.

CONCLUSION

Ulva rigida contains compounds of commercial interest, especially with regard to its dietary fiber and protein. The seaweed used in this study met the requirements of consumer safety regulations for heavy metals. The potential process of non-fried seaweed snack production for further development as a healthy product includes the pre-treatment of seaweed materials by both washing and blanching, sheet formation by adding seaweed polysaccharide at 0.25 % (w/v), and drying by hot air oven at 150 °C for 35 min. The seaweed snack after coating with seasoning consisted of high fiber and protein with low fat content. The findings demonstrated that *U. rigida* has the potential to be further developed as a healthy snack. The scope of this study was simply to examine the chemical compositions of *U. rigida* and the potential of developing a seaweed snack using an oil-free drying process. Further investigations for recipe development, consumer testing, and nutrition labeling similar to current commercial products are required to ensure the feasibility and availability of this product in the market. This work can develop a new platform for utilization of Thai seaweeds to create higher-value products which will serve social demand for health awareness and support economic development.

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