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Physicochemical, Physical Characteristics and Antioxidant Activities of Three Edible Red Seaweeds (*Kappaphycus alvarezii*, *Euचेuma spinosum* and *Euचेuma striatum*) from Sabah, Malaysia

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Abstract. *Kappaphycus alvarezii* (KA), *Euचेuma spinosum* (ESp) and *Euचेuma striatum* (Est) are tropical red seaweeds taken from Kunak, Sabah, contain essentials source of bioactive compounds to produce variety of functional foods. This study was intended to investigate the physicochemical, physical properties and antioxidant activities related to these edible seaweeds. Physicochemical properties value of swelling capacity (SWC) and oil retention capacity (ORC) in KA species showed the highest value ($p < 0.05$) compared to ESp and Est, and water retention capacity (WRC) in ESp species was significantly different than KA and Est ($p < 0.05$). As in physical properties, ESp exhibited higher in moisture content and opacity than KA and Est. KA showed the highest water solubility ($p < 0.05$) which associated to its carrageenan compound. The antioxidant activities; DPPH and TEAC assay with total phenolic content (TPC) of three seaweed species indicated that KA displayed the highest TPC (19.17 ± 0.04 mg GAE/g DW) and the lowest radical scavenging activity in both TEAC and DPPH assays. Hence, the findings disclose the physical and physiochemical properties and antioxidant values of these seaweeds application as new information and potential antioxidant sources in foods manufacturing commodities.

1. Introduction

Seaweed, one of marine macroalgae is widely spread around the world either cultivated or exist in the wild. They are categorized based on the pigmentation including red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta). In Asian countries, seaweeds have become sources for human and animal feed, herbal remedies, fertilizer, fungicides, herbicides, biopolymer and sources of pharmaceutical ingredients

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[1]. Researchers claimed seaweeds are nutritive food that contain vitamin, protein, mineral, fibre contents, polyunsaturated fatty acids, essential fatty acids as well as macro and trace elements which are beneficial to human and animal [1]–[3]. Food manufacturing industries utilised seaweeds as a source of phycocolloids such as agar, alginate, and carrageenan for stabiliser, thickening agent texture modifier in food products [4], [5]. Agar and carrageenan are extracted from various types of red seaweed species. There are several types of carrageenan, consisting of iota, kappa, lambda, Mu, Nu and Theta [6], [7], that vary by the different types of seaweeds species, seasons, geographic location and age of population. Red Seaweeds contain numerous amounts of bioactive compounds with rich pharmacological potential. Compounds such as sulphated polysaccharides including fucoidans from brown seaweeds, agars and carrageenan (sulphated galactans) from red and green seaweeds, and ulvans (sulphated glucuronoxylorhamnans) have shown pharmacological benefits as anti-inflammatory, antioxidant, antibacterial, and immunological activity [8]. While phlorotannins found in brown seaweeds and less in red and green seaweeds have been explored as functional food ingredients with many biological activities such as antioxidant, anti-inflammatory, antidiabetic, anti-tumor, antihypertensive, and antiallergic activities [9].

In Kunak Sabah, Malaysia, there are three types of red seaweed species which are widely cultivated for their variety of food functions, including *Kappaphycus alvarezii* (KA), *Euचेuma spinosum* (ESp) and *Euचेuma striatum* (ESt). *Kappaphycus alvarezii* is the most common species and widely farmed in Sabah besides *Euचेuma spinosum* and *Euचेuma striatum* in small percentage. KA species is cultivated mainly for the production of kappa carrageenan hydrocolloid, a thickening agent of foods such as yogurt, ice cream and pudding, and exhibited good gelling properties [9], [10]. The basic chemical composition of KA includes galactan, glucan, minerals, sulphate groups, and proteins [5], [11], [12]. *Euचेuma spinosum* (ESp) or known as *denticulatum* is also extracted as a source of carrageenan for thickening agent in foods. It contains high calcium, magnesium, potassium and sodium content, and no cadmium, lead, mercury, and arsenic detected which meet the food grade regulation. Meanwhile, *Euचेuma striatum* (ESt) species possessed high composition of galactose-6-sulfurylase, a key enzyme involved in the biosynthetic pathway of agars and carrageenan.

Red seaweeds become one of the main agricultural commodities in Sabah, Malaysia, yet, the information on the physical and physicochemical characteristic as well as the antioxidant potential especially *Euचेuma* species are very much circumscribed. Hence, the study aims to determine physical physicochemical properties of KA, ESp and ESt species by assessing the swelling capacity, water holding capacity, oil holding capacity, moisture content, opacity and water solubility. The antioxidant potential of those seaweeds was investigated by the analysis of DPPH scavenging activity, Trolox equivalent antioxidant activity and total phenolic content. Our findings could disclose the physical and physicochemical characteristic and antioxidant potential of the three common farmed seaweeds in Kunak, Sabah, Malaysia especially the new information of *Euचेuma* species as new sources of functional foods commodities.

2. Materials and Method

2.1. Sample preparation

Fresh seaweed species were collected in the North Borneo coastal areas (Latitude: 4° 40' 59.99" N, Longitude: 118° 14' 60.00" E) specifically in Kunak, Sabah, Malaysia. The taxonomic identification of the scientific name of the selected seaweeds was conducted with the help of the Seaweed Research Centre, Universiti Malaysia Sabah (UMS) and the Department of Fisheries, Sabah, Malaysia. The seaweed species were identified as *Kappaphycus alvarezii*, *Euचेuma spinosum* and *Euचेuma striatum*. The seaweeds were extensively washed to extract salt, foreign materials such as sands, ropes and shells with running water and dried in oven, 50°C, 24 hr. The sample was grounded into fine powder through 0.5 mm sieves and kept at -80 °C until further analysis.

2.2. Physicochemical properties

Physicochemical properties analysis were involved swelling (SWC), water retention (WRC) and oil retention capacity (ORC). SWC analysis performed by measuring by the bed volume technique after the seaweed sample was being equilibrated in excess solvent [11]. 200 mg of sample was weighed and 20 ml of distilled water was added in. Then, the mixture was mixed vigorously and allows the mixture to settle for 24 hr. The SWC was occupied with the sample volume (ml) was expressed as ml/g of dry weight.

WRC and ORC were determined according to the previous method [27]. Two grams of samples were mixed with 30 ml of distilled water and the mixture of slurry was equilibrated at 10 min. The sample was then centrifuged at 2,000 rpm using a centrifuge (Kubota 5922, UK) for 15 min. The supernatant was discarded after centrifugation (Thermo Scientific, Malaysia) the weight of remaining precipitate was measured. ORC was performed similar to WRC except corn oil used to mixed with the sample. The final valued for both analysis were calculated as g/g DW.

2.3. Physical Properties

Three physical properties analysis were involved including moisture content, water solubility and opacity. Analysis of Moisture content was performed according to AOAC (2000)[27]. Fresh seaweed species (2 g) were transferred in to a crucible and left overnight at 105°C in a universal oven (Binder GmbH, Germany) until constant weights were obtained.

Water solubility measurement of red seaweed species was adapted from method [13]. T Two gram of seaweed were dehydrated in a industrial oven at 100°C, 24 h. Then, the sample was dissolved by 30 ml of water and mixed constantly at 25 °C, 24 hr in a water bath (Mettler, UK). The samples were filtered then, placed oven for 24 h at 100 °C and initial dry weight (W_o) and dry undissolved weight (W_f) were measured. The % water solubility ($WS\%$) was calculated as follows:

$$WS\% = \left(\frac{W_o - W_f}{W_o} \right) \times 100 \quad (1)$$

Two gram of sample was dissolved in ethanol and placed with cuvette to measure its opacity value according to [14]. The dissolved samples was measured at 600 nm using an UV spectrophotometer (HITACHI U-1800, Japan). The absorbance value was cpllected as Abs 600, and thickness of cuvette was measure as b (mm).The following equation has been performed to determine the opacity value

$$Opacity = \frac{Abs600}{b} \quad (2)$$

2.4. Total phenolic content and antioxidant analysis study

2.4.1. Extraction of red edible seaweeds. All samples were washed with 0.5 M ammonium formiate solution and rinsed with distilled water prior added with liquid nitrogen. The frozen samples was macerated in a mortar using pestle and left dried at 45 °C, 72 h. Then the samples was keep frozen at -20 °C for later analysis.

2.4.2. Total phenolic Content and DPPH antioxidant activity. Folin-Ciocalteu method was adapted to determine the total phenolic content of red seaweeds[17]. Total 1ml mixture solutions were prepare as follows: 10 µl sample, 50 µl 10% (v/v) Folin reagent, 150 µl of 7% (v/v) sodium carbonate solution, 790 µl MiliQ water. Sample were mixed and measured at 765 nm using UV-visible spectrophotometer The absorbance value were articulated as mg of Gallic acid equivalents/g DW (mg GAE/g DW).

DPPH antioxidant activity was performed according to method[17]. 0.6 mM of DPPH solution was prepared by diluting with ethanol, incubated in dark at 18 hours before used. The sample mixture, total of 1ml was formulated according to: 0.025 ml of samples mixed 0.975 ml of 0.6 mM DPPH and placed

to cuvette for 4 hours prior to the measurement of the absorbance. The absorbance at 585 nm were measured using UV-visible spectrophotometer after 4The results were calculated as % inhibition of DPPH radical.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay: The mixture containing 7mM of ABTS•- radical cation and 2.45 mM of potassium sulphate (2.45mM) prepared with PBS (10 mM) and incubated at 25 °C for 30 min. The initial reactive mixture must allowed between wavelength of 0.72 ± 0.2 nm using UV-visible spectrophotometer. Then, the 20 μ l was added into final solution and measured at 734 nm using UV-visible spectrophotometer for 20 min. The absorbance value was calculated as micromoles of Trolox equivalents per gram dried weight of sample (mM of TE/ gDW).

2.5. Statistical analysis

All experiments were performed in triplicate analysis. Data each measurement were collected and calculated statistical analysis with ANOVA (SPSS 9.1) by Duncan Multiple Test using SAS 9. Each result were represented significantly difference at p-value less than 0.05.

3. Result and discussion

3.1. Physicochemical properties

Seaweed is known for containing high amount of dietary fibre which determine their hydration properties. Swelling capacity (SWC), water retention capacity (WRC) and oil retention capacity (ORC) were analysed to determine the physicochemical properties of the red seaweeds species as shown in Table 1.

Table 1. The swelling, water and oil retention capacity of *K. alvarezii*, *E. spinosum* and *E. striatum*

Seaweeds	SWC (ml/g DW)	WRC (g/g DW)	ORC (g/g DW)
<i>K. alvarezii</i>	34.19 \pm 0.72 ^a	10.03 \pm 0.09 ^{ab}	3.07 \pm 0.11 ^a
<i>E. spinosum</i>	20.63 \pm 0.38 ^c	13.43 \pm 2.57 ^a	2.34 \pm 0.22 ^b
<i>E. striatum</i>	22.17 \pm 0.15 ^b	8.45 \pm 0.09 ^b	2.84 \pm 0.06 ^{ab}

^{abc} mean value with different superscript within the same column is different (p<0.05)

The SWC of the seaweeds were significantly different with KA shown the highest (34.19ml/g DW) than ESt (22.17ml/g DW) and followed by ESp (20.63ml/g DW). The SWC results obtained from this type of seaweeds were similar to some seaweed species such as *U. pertusa* and *U. intestinalis* [15]. There were also some studies reported that the SWC values will be different among seaweeds or food products because of their variable sample sizes, sample preparations and experimental conditions [16]. Besides swelling capacity, water retention capacity (WRC) of ESp was the highest followed by those of KA and ESt with 13.43, 10.03 and 8.45 g/g DW, respectively. SWC and WRC properties of seaweeds are related to their polysaccharide characteristics which indicated that this type of seaweeds may be used as functional ingredient that will contribute in improving physical properties of food products.

Other than hydration characteristics which are SWC and WRC, seaweed species has also oil retention capacity (ORC). In this study, *K. alvarezii* showed the highest value of ORC with 3.07 g/g DW followed by *E. striatum* (2.84 g/g DW) and ESp (2.34 g/g DW). This result was comparable to KA powder (2.14 g/g DW) and *Gracillaria spp.* (1.79-2.23g/g DW) [17]. The high ORC reported in this study suggested its ability to stabilize food emulsion. Therefore, those red seaweeds could be a good alternative as stabilizers in formulate food products and probably in animal feed pellet production. Those red seaweeds values of SWC, WRC and ORC suggested that it can be potentially used as modifier of texture and viscosity in food industries. To the best of our knowledge, physicochemical properties of ESp and ESt seaweeds species is the first time reported in the study.

3.2 Physical Properties

Table 2 shows the moisture content, opacity, and water solubility of those three red seaweeds species in Sabah, Malaysia. The moisture content of the red seaweeds ranged between 76.56% and 81.13%. ESp was found to have the highest ($p < 0.05$) moisture content (81.13%) while ESt was observed to have the lowest moisture content (75.56%) which in agreement with previous the report [18] As one of marine microalgae, seaweeds contain high amount of water in nature, with the average of 75-85% water and 15-25% organic components and minerals [19].

Table 2. Moisture Content, Opacity and Water Solubility of Seaweed of *K. alvarezii*, *E. spinosum* and *E. striatum*

Seaweeds	Moisture Content (%)	Opacity (mm^{-1})	Water Solubility, (WS%)
<i>K. alvarezii</i>	79.89±0.13 ^a	9.39±0.47a	53.91±0.12 ^a
<i>E. spinosum</i>	81.13±0.01 ^b	11.70±0.60b	51.37±0.10 ^b
<i>E. striatum</i>	76.56±0.09 ^c	11.13±0.4b	50.08±0.03 ^c

^{abc} mean value with different superscript within the same column is different ($p < 0.05$)

Carrageenan which is hydrophilic in nature that existed in KA and ESp resulted in higher amount of moisture compared to ESt species. Opacity is one of important traits measured by food industry during seaweed processing, favourable to low opacity value. High value of opacity exhibited less transparent traits of the seaweed species (Table 2). KA showed lowest opacity value, whereas ESp and ESt were observed with no significant difference in their opacity ($p > 0.05$). Opacity of seaweed were associated with the red pigment colour found in the seaweed species. Solubility of the red seaweeds mainly associated to the carrageenan compound, largely found in KA and ESp species. KA (53.91%) displayed the highest water solubility followed by ESp (51.37%) and ESt (50.08%). Carrageenan are associated with the moisture content and water solubility properties of seaweeds. However, the existence of hydrophilic sulphates compounds induces polysaccharide undergo conformational transitions which are conducive to the gelation properties, that reduce the solubility of seaweeds. Seaweeds used as thickening and stabilising agent were processed to exhibit higher solubility value whereas, higher gelling properties of seaweed was potentially used as biopolymer. However, hydrolysis of the hydrocolloids with specific enzymes such as agarose and its derivatives capable to degrade the hydrocolloid polysaccharides and thereby change the solubility and gel strength. To our knowledge, no attempts to analyse opacity and water solubility from red seaweed have been reported before.

3.3 Total phenolic content

Quantification of total phenolic compounds (TPC) and free radical scavenging ability of DPPH are presented in Table 3. Many authors reported the influence of polyphenols on antioxidant activity [20], [21], whereas the scavenging behaviour of the seaweed extracts are relevant to the seaweed species that have the antioxidant properties. Meanwhile, the DPPH assay is one of the fast evaluations of antioxidant activity because of its stability and long live radical and the simplicity of the assay. It can be seen that KA showed the highest value of phenolic compounds and scavenging activity compared to ESt and ESp species. There was positive correlation between TPC value with DPPH assay where the higher phenolic compounds showed higher antioxidant activity in the seaweed extract (Table 3).

Table 3. Total phenolic content and antioxidant activities of *K. alvarezii*, *E. spinosum* and *E. striatum*

Seaweeds	TPC (mg GAE/g DW)	% DPPH	TEAC (mM of TE/g DW)
<i>K. alvarezii</i>	19.17 ± 0.04 ^a	31.24% ^a	1.96±0.04 ^a
<i>E. spinosum</i>	15.41 ± 0.13 ^c	26.77% ^b	1.81±0.01 ^b
<i>E. striatum</i>	12.33 ± 0.20 ^b	22.34% ^c	1.77±0.02 ^c

^{abc} mean value with different superscript within the same column is different (p<0.05)

KA extracts possess excellent antioxidant and scavenging activities as analysed in the DPPH assay, reducing power, ferrous ion-chelating activity and antioxidant property in the linoleic acid system [22]. Carrageenan as the major compound in KA may contribute to the antioxidant activity potential besides the presence of ascorbic acid, vitamin A and various phenolics in the extract [23]. Meanwhile, Matanjun et al 2008 found strong correlation of total polyphenol content of ESp species with FRAP assays, which may be relevant to the polyphloroglucinol phenolics (phlorotannin) compounds in the red seaweed extracts [24]. In TEAC assay, KA extracts displayed higher antioxidant value than ESt and ESp similar to DPPH assays (p<0.05). The assay indicted the seaweed potency and potential use as a source of antioxidants based on the ability of antioxidants compound to scavenge the long-life radical cation ABTS⁺. This assay is also relevant to insoluble and water-soluble antioxidants existed in seaweed extracts, mostly related to the existence of phenolic hydroxyl group. From literature, green seaweeds, the green seaweeds *Caulerpa lentillifera* and *C. racemosa* had greater antioxidant activities compared to the brown and red seaweeds [24]. Flavanoid is one of common phenolic compounds found in microalgae is phlorotannin, which are polymers of phloroglucinols (1,3,5-trihydroxybenzene) that may be relevant to the antioxidant activities values shown in Table 3. Gonzalez et al reported carotenoid contained in red seaweed species as red pigmentation contributes to the antioxidant activity and commonly used as food colorants, feed supplements, and nutraceuticals in industry [26]. Assessment of polyphenols and active compounds that are relevant to the antioxidant activity of ESp and ESt has not been fully determined. TEAC and DPPH scavenging activities of ESt and ESp extracts are reported for the first time in this paper.

4. Conclusion

KA, ESp and ESt are those edible red seaweed that highly cultivate in Sabah for various food additive functions in manufacturing industries. The results from red edible seaweeds (KA, ESp and ESt) on their physicochemical properties in terms of the swelling capacity, water and oil retention capacity suggested that KA has highest potential to be functional ingredients in food industry. It is important as additive in the food industry to control moisture, texture and to stabilize foods. This study also presented that KA extracts showed highest value in antioxidant activity with 31.24% and 1.96±0.04 mM of TE/g DW. Further study concerning on the identification and characterization of specific compound responsible for the high antioxidant activity in these seaweeds is needed.

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