



## Functional and Biochemical Properties of some Economically Important Edible Seaweeds

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### Abstract

We evaluated the functional properties and nutritional composition of six dried commercially valuable edible seaweeds *Porphyra* sp., *Undaria pinnatifida*, *Saccharinu* sp., *Hizikia fusiformis*, *Gracilaria* sp., and *Sargassum* sp. in the current study. The proximate composition of the dried seaweeds revealed that *Porphyra* sp had a high total crude protein content ( $38.58 \pm 0.16$  %) followed by *Undaria* sp. ( $23.03 \pm 0.30$ %), *Saccharina* sp. ( $11.39 \pm 0.09$ %), *H. fusiformis* ( $18.77 \pm 0.01$ %), *Gracilaria* sp. ( $18.30 \pm 0.13$ %), and *Sargassum* sp. ( $13.56 \pm 0.04$ %). Fatty acid profiling showed high MUFA content in *Sargassum* sp. (1.09 %); this seaweed also contained 0.84% saturated fatty acid and 0.48% PUFA. On the other hand, *U. pinnatifida* was rich in macro elements ( $297.57 \pm 11.09$  mg/100g) and *Gracilaria* sp. had high micromineral content ( $6397.35 \pm 89.42$   $\mu$ g/100g). Functional properties of the powdered seaweed were also evaluated. The water-holding capacity of *Porphyra* sp. ( $8.82 \pm 0.40$  g water/g algal sample) was better than *H. fusiformis* ( $6.22 \pm 0.30$  g water/g algal sample). Oil holding capacity of *Gracilaria* sp. ( $3.22 \pm 0.08$  g oil/g algal samples) was higher than *U. pinnatifida* ( $1.92 \pm 0.22$  g oil/g algal sample). Further, *H. fusiformis* had a good foaming capacity ( $38.0 \pm 2.0$  %). Based on the results obtained herein, it could be summarized that the seaweeds studied were nutritionally rich (containing minerals that are vital for human health), and could be used as a functional food and in various food formulations.



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### Introduction


Depleting natural resources and increasing population has awakened concerns towards human health globally. While population rise has caused

a hike in the demand for food grain production, the emerging climatic change and lack of availability of land, impede production of adequate terrestrial food crop. With the rise in consumer awareness,

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people seek a healthy, wholesome diet with optimum nutrition. This has led to an amplified interest in carrying out fundamental research to seek novel functional foods (i.e. foods that have health benefits apart from basic nutrition). In attempts to search for novel alternative foods, people have diverted their attention from terrestrial crops to seafood products. The sea offers a range of edible products ranging from fish to algae.

Seaweeds are a group of marine photosynthetic macroscopic algae that are abundantly found in the ocean. They are categorized into three main classes or phyla: Chlorophyceae (green algae), Phaeophyceae (brown algae), and Rhodophyceae (red algae).<sup>1</sup> Seaweeds are an integral part of the coastal ecosystems and support marine life. Due to their ubiquitous presence and nutritional benefits, seaweeds have been used in food, cosmeceutical, pharmaceutical, and nutraceutical industries. Out of the 630 species of marine algae available, nearly 250 seaweeds have been commercially used worldwide. However, approximately 150 species are favourably consumed by humans as food.<sup>2</sup> For example, seaweeds have been traditionally consumed as food or medicine in Asian countries like Japan, Korea, and China.<sup>3</sup> *Porphyra sp.*, *Laminaria sp.*, and *Undaria sp.* are the most recognized seaweeds used for human consumption.

Seaweeds produce a plethora of bioactive compounds including terpenes and phenolics.<sup>4</sup> They contain polysaccharides such as agar, alginates, and carrageenans. Apart from their low-calorie content, seaweeds (or macroalgae) are important marine bio-resources that contain proteins, peptides, beneficial amino acids, dietary fibres, health-promoting bioactive compounds, and polyunsaturated fatty acids (PUFAs).<sup>5-7</sup> Seaweeds are an outstanding source of vitamins A, B<sub>1</sub>, B<sub>12</sub>, and C; additionally, they also contain water-soluble and liposoluble vitamins such as  $\beta$ -carotene, riboflavin, thiamine, and tocopherol.<sup>8</sup> While their functional properties and biochemical composition make seaweed products nutritionally rich, their natural abundance makes them economic. Functional foods containing seaweed-derived components have several pharmacological properties (antibacterial, anticancer, antioxidant, gut health benefits, and neuro-protective effects) that are

reported to prevent or cure diseases.<sup>9,10</sup> Seaweeds are regularly exposed to sunlight in the marine environment, this explains the fact that they contain antioxidants including vitamins and pigments. The marine environment helps the seaweeds acquire their high content minerals (8–40% DW). Seaweeds are an abundant natural source of essential minerals, macronutrients and micronutrients such as sodium, calcium, magnesium, potassium, chloride, sulphate, phosphorus, iodine, zinc, iron, copper, selenium, molybdenum, fluoride, boron, and nickel.<sup>11</sup> Forming a significant part of daily food in Asia, seaweeds are particularly consumed in South Korea as they help fulfil the daily essential minerals and trace elements intake requirement.<sup>12,13</sup> Nevertheless, mineral composition of the seaweeds depends upon season, geographical conditions, species, sampling conditions, physiological variations, processing, etc.<sup>14,15</sup> Being a primary source of iodine, they could fulfil the daily iodine requirement (150 mg/day).<sup>16</sup> The enigma of obesity, as well as, vitamin and mineral deficiency has become a health rife in *Europe, USA, and Australia*.<sup>17</sup> Global dietary studies report that countries, where seaweed is consumed on a regular basis, have less diet-related diseases and obesity.<sup>18</sup>

Fundamental functional properties such as emulsification capacity, foaming capacity, as well as, oil and water holding capacity, maintain the texture modification, colour control, and water holding capacity of the ingredients, maintain the texture modification whipping properties of a food product. Seaweed biomass and protein offer various functional and nutritional properties that help its utility as a food ingredient or in functional foods. Functional property of a product (i.e. water/oil holding capacity, foaming capacity, nitrogen solubility, and emulsifying stability) determines its utility in the food, pharmaceutical or cosmetic industry. These functional properties however depend on various factors such as temperature, pH and salinity.<sup>19</sup> The objective of the present study is to investigate the functional and biochemical properties of the economically important seaweeds, considering their use as a functional food.

## Materials and Method

### Sample Preparation

Two seaweed samples (*Gracilaria sp.*, *Saragassum sp.*) were collected from Mandapam coast (9.28°N 79.12°E) Tamil Nadu, while seaweeds *Undaria*

*pinnatifida*, *Hizikia fusiformis*, *Saccharina* sp and *Porphyra* sp. were purchased from a commercial market in Wando Island, South Korea. The dried seaweed samples that were brought to the laboratory, washed with distilled water to remove epiphytes, and re-dried under the sunlight. The sun-dried seaweeds were oven-dried (40° C, 24 h, or until they had a constant weight). After this, the dried moisture-free samples were pulverized and sieved to obtain a nearly uniform particle size (0.5 mm). The milled seaweed samples were then stored in an airtight container at room temperature and used for further experimentation.

#### Protein content

The percentages of crude protein in the milled seaweed biomass was calculated. For this, the nitrogen content (determined using a CHN elemental analyser, *Euro Vector EA 3000*) of each sample was multiplied by a factor of 6.25.

#### Water (WHC) And Oil-Holding Capacity (OHC)

Water-holding capacity (WHC) and oil-holding capacity (OHC) of the seaweed samples were determined according to the procedure of Kumar *et al.*<sup>19</sup> Here, 100mg seaweed powder was mixed with 1.5 mL of distilled water or coconut, mustard, sunflower oil respectively in pre-weighed tubes and centrifuged at 12000 x g for 30 min. The supernatants obtained were thereby decanted and the centrifuge tubes containing sediment were weighed. WHC and OHC were expressed as grams of water or oil held by 100 mg of algal biomass by using the formula mentioned below.

$$\text{WHC/OHC gH}_2\text{O or oil/g biomass} = \{(W_2 - W_1) / W_0\} \times 100$$

where,

$W_0$  is the weight of the dry biomass sample(g),  $W_1$  is the weight of the tube plus the dry biomass sample (g), while,  $W_2$  is the weight of the tube plus the sediment (g).

#### Foaming Capacity and Stability:

The foaming capacity and foaming stability of the seaweed biomass were determined by a modified

method of Nath *et al.*<sup>20</sup> For this, a 10 ml solution of the algal biomass (100 mg, 250 mg, 500 mg) was vortexed (5 min, 2000 rpm, room temperature) and transferred to a measuring cylinder. The increase in volume was expressed as percent foaming capacity.

$$\text{Foaming capacity (\%)} = (V_2 - V_1) / V_1 \times 100$$

where,  $V_1$  is the volume of biomass solution before whipping, and  $V_2$  is the volume of biomass solution after whipping.

The foam stability was determined by measuring the decrease in foam volume as a function of time at intervals of 30 min (up to a period of 90 min).

$$\text{Foam stability (\%)} = \text{Volume after standing} - \text{Volume before whipping} / \text{Volume before whipping} \times 100$$

#### Biochemical Composition of Seaweeds

##### Ash Content Analysis

The ash content was determined by placing the dried seaweed powder in an oven (80° C, 1 h). A gram of this powdered sample (accurately measured) was then placed in a pre-weighed crucible and ashed in muffle furnace (550° C, 6 h to a constant weight). The ash obtained was allowed to cool down to room temperature in a desiccator and subsequently quantified gravimetrically<sup>21</sup>.

##### Mineral Analysis Using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Dried seaweed (1g) was ashed and cooled (as mentioned above). The ash obtained was moistened with 10 drops of distilled (Milli-Q) water (in a crucible) and carefully dissolved in 3 ml  $\text{HNO}_3$  (1:1 v/v), followed by heating at 100–120° C until the solution totally evaporated. Thereafter, the crucible was again placed in a furnace for ashing for 1 h at 550°C, followed by which the samples in the crucible were cooled. Subsequently, the ash obtained was dissolved in 3ml of 10 M HCl (1:1 v/v), and the solution was filtered using a Millipore syringe filter (0.25 $\mu\text{m}$ ) into 50 ml volumetric flask and 2 ml 0.1N HCl was added to the filtrate and the final volume was made up to 50 ml using distilled water (Milli-Q).<sup>22</sup> This solution was sent for mineral analysis using inductively coupled plasma atomic mass spectroscopy (ICP-MS, Perkin-Elmer, Optima 2000).

### Fatty Acid Analysis

The fatty acid analysis was determined by using the fatty acids methyl esters (FAMES) extraction method of Levy *et al.*<sup>23</sup> Fat extraction was carried using the soxhlet method for oil extraction. Gas chromatography–mass spectrometry [Agilent:GC: (G3440A) 7890A.MS MS: 7000 Triple Quad GCMS] equipped with Mass spectrometry detector was used.

### Vitamin Analysis

#### Fat–Soluble Vitamins

In order to determine the fat-soluble vitamins, the following procedure was carried out. Exactly 2 g of dry seaweed sample was taken in a 250 mL flask followed by addition of 10 mL Vitamin C (10 mL of 1.5 g vitamin C in 0.1 L ethanol). This solution was mixed thoroughly. Thereafter, 2.5 mL of aqueous potassium hydroxide (i.e. 250 g solid KOH in 200 mL water) was added. The flask was placed in a water bath (53 °C ± 2 °C). After shaking and saponification for about 45 min, the flask was immediately cooled to room temperature. The saponification liquid along with a bit of water was transferred to a 500 mL separating funnel. Thereafter, 100 mL petroleum ether was added, followed by gentle shaking. The aqueous phase was transferred to another 500 mL separating funnel and the second extraction was carried out in the manner mentioned before. The ether was filtered and dehydrated with anhydrous sodium sulphate. Thereafter, it was evaporated to near dryness on a rotary evaporator at 40 °C ± 2 °C under nitrogen. Methanol was used to transfer the residue to a 10 mL volumetric flask. The residue was thereafter brought to a constant volume with methanol followed by thorough mixing (shaking). The solution was passed through a 0.22 µm filter and stored until further testing.

#### Water–Soluble Vitamins

Seaweed sample (10 gm) was weighed in a screw-capped conical flask and dissolved in 10–15 mL of water; the flask was covered with an aluminium foil. Thereafter, 10 mL of 0.1 N hydrochloric acid was added followed by thorough mixing. The sample was heated for half an hour over a boiling water bath and stirred frequently. The sample was then cooled in a basin of water and pH adjusted to 4–4.5 with 2.5M sodium acetate solution. The extract was then moved into a 100 mL volumetric flask

(the volume was adjusted using distilled water). After shaking well, a portion of the extract was filtered through 0.22 µm membrane filter. Nearly 20 µl of the filtrate was injected into a LC–MS. Known concentrations of a mixture of standards were used as for comparison.

### Statistical Analysis

One-way analysis of variance (one-way ANOVA) was carried out to confirm significant differences in response at  $p < 0.05$ .

### Result and Discussion

Seaweeds are known to demonstrate intriguing nutritional characteristics depending on particularly on species and environmental conditions. The seaweeds investigated in our study had unique biochemical composition.

#### Total Protein Content

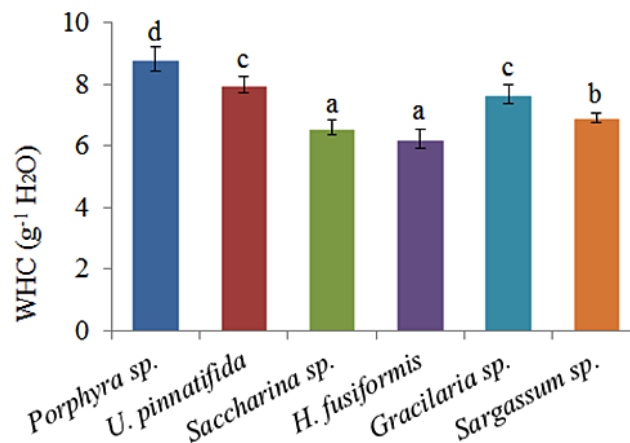
The protein content of seaweeds investigated ranged from 11.4–38.58 % DW (dry weight) and showed significant differences between species ( $P < 0.05$ ). Fluerence<sup>24</sup> reported a protein content of 10–47% DW in case of red and green seaweeds. In our investigation, *Porphyra sp.* (38.58 ± 0.16 %), *Undaria pinnatifida* (23.03 ± 0.30%), *Saccharina sp.* (11.39 ± 0.09%), *Hizikia fusiformis* (18.77 ± 0.01%), *Gracilaria sp.* (18.30 ± 0.13%) and *Sargassum sp.* (13.56 ± 0.04) had distinct total protein contents.<sup>25</sup> report the crude protein of dried *Porphyra sp.* was to be 42.99 ± 0.051% DW, which was slightly lower than that obtained for *Porphyra sp.* in our study (38.58 ± 0.16 %). As evidenced in our studies and in other reports, the protein content of seaweed vary with species; for example *Bryopsis corticolans* varies (38.20%), 38.20%), *Sirophysalis trinod is* (14.64%) and *Galaxaura rugosa* (17.82 %), *Kappaphycus alvarezii* (18.16 ± 0.03%), *Caulerpa veravelensis* (7.77 ± 0.59%), *Caulerpa scalpelliformis* (10.50 ± 0.91%), *Caulerpa paracemosa* (12.88 ± 1.17%), *Plocamium brasiliense* (15.72%), *Laminaria japonica* (9.1%), *Ochtodes secundiramea* (10.1%), *Palmaria palmate* (13.5%), *Sargassum oligocystum* (5.63%) and *Ulva rigida* (17.8%), have distinct protein content,<sup>26,27,19,28</sup> However, the total protein content of the same species collected at different locations and time also vary; for example, Garcia–Vaquero *et al.* (2016)<sup>29</sup> reported protein content of *H. elongata* to be 6.5 ± 0.7 % DW while Cofrades *et al.*<sup>30</sup> reported protein content of 5–11% DW for the same species.

Differences in seasonality and growth conditions in the environment<sup>31</sup> could be the cause of variation in protein content of seaweeds.

### Water - Holding Capacity (WHC)

At ambient temperature, the water-holding capacity of *Porphyra* sp. was  $8.82 \pm 0.40$  g water/g sample (Figure 1). This was higher than the *U. pinnatifida* ( $7.991 \pm 0.245$  g water/g sample), *Gracilaria* sp. ( $7.67 \pm 0.30$  g water/g sample), *Sargassum* sp. ( $6.91 \pm 0.15$  g water/g sample), *Saccharina* sp. ( $6.59 \pm 0.23$  g water/g sample) and *H. fusiformis* ( $6.22 \pm 0.30$  g water/g sample). It is essential to note that sparse researches have been conducted on seaweeds for their WHC,

especially whole seaweed powder. WHC values for certain powdered seaweeds reported previously include: *Hypnea charoides* (10.9–12.4 g water/g sample), *Hypnea japonica* (11.8–14 g water/g sample), *Ulva lactuca* (8.68–9.71 g water/g)<sup>32</sup> and *U. pinnatifida* (19–44 g water/g sample),<sup>33</sup> however, these values are higher than the values obtained in our study. The WHC of *Sargassum tenerimum*<sup>34</sup> is reported to vary with temperature (13.5g and 15.5g water/g dry matter at 25°C and 37°C respectively). However, WHC of the protein concentrate from *H. elongata*<sup>29</sup> has been reported to be  $10.27 \pm 0.09$  g water /g protein concentrate. *K. alvarezii* seaweed extracts have WHC value of  $2.22 \pm 0.04$  g water/g protein.<sup>19</sup>



**Fig.1: Water holding capacity of the seaweeds. Values are mean  $\pm$  standard errors. Different letters show significant difference ( $P < 0.05$ ) between means using Duncan's Multiple Range test.**

Water-holding capacity and oil-holding capacity of products determine their application in food products (e.g. as a texture enhancer); they also determine the suitability of a product to improve viscosity of food formulations. If a product has a capacity to hold water without dissolution of protein and provides thickening and viscosity, it is said to have a good WHC. A high WHC value aids in maintaining freshness and moist mouth-feel of baked foods, moreover, it is also associated with reduced moisture loss in packed bakery goods. Viscous food products, like sausages, custards, dough, and baked products, require high WHC values. Nevertheless, researchers<sup>35</sup> advocate that the physicochemical properties of seaweed powder could be assumed to reflect its fibre content. As the seaweed samples investigated here

demonstrated good WHC, they could be explored for their utilization in food products.

### Oil Holding Capacity (OHC)

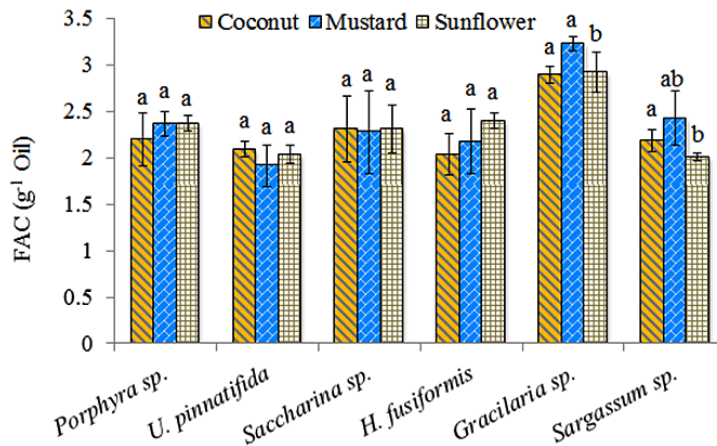
The oil holding capacity essentially determines the utility of food ingredients in formulated food and cosmetics. High OHC is essential for flavour retention, and improving the palatability of products.<sup>36</sup> In our study, the OHC of *Gracilaria* sp. was the highest ( $3.22 \pm 0.08$  g oil/g sample), while *U. pinnatifida* showed least OHC values ( $1.98 \pm 0.22$  g oil/g sample, Figure 2). Fat absorption capacity of *Sirophysalis trinodis* and *Polycladia myrica* extracts are reported to be  $2.26 \pm 0.22$  and  $2.65 \pm 0.11$  g oil/g sample<sup>37</sup> respectively. *K. alvarezii* powder TM is reported to have 329 is reported to have a OHC



of  $5.11 \pm 0.36$  g oil/g).<sup>38</sup> However, Kumar *et al.* (2014)<sup>19</sup> obtained OHC values for protein concentrate of *K.alvarezii* ( $1.29 \pm 0.20$  g oil/g). OHC of *H. elongata* protein concentrate<sup>29</sup> to be  $8.1 \pm 0.07$  g oil / g.

The OHC of the food ingredients that are incorporated in formulated food essentially determines its use. Appropriate interactions of water and oil with proteins

indirectly affect the flavour and texture of foods. Food processing methods have significant an influence on protein conformation and hydrophobicity. High oil absorption/holding is indispensable for the formulation of foods such as cake batters, sausages, salad, and mayonnaise.<sup>39</sup> The ability of a product to bind fat is very vital, especially if it is being used in meat replacers or extenders.<sup>40</sup>

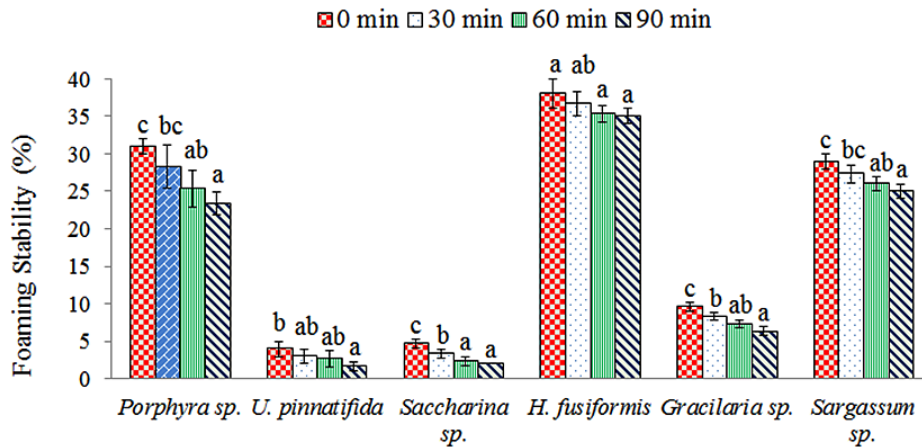


**Fig. 2: Oil holding capacity of the seaweeds. Values are mean  $\pm$  standard errors. Different letters show significant difference ( $P < 0.05$ ) between means by Duncan’s Multiple Range test.**

**Foaming Capacity and Stability**

The foaming capacity of the seaweeds examined in the present study, not only depended on the mass used but also on time (Figure 3). *Saccharina sp.* shows the lowest foaming capacity ( $2.66 \pm$

$0.57$  %), while highest FC found recorded in case of *H. fusiformis* ( $38.0 \pm 2.0$  %), followed by *Porphyra sp.* ( $31.0 \pm 1.0$  %), *Sargassum sp.* ( $29.0 \pm 1$  %), *Gracilaria sp.* ( $11.0 \pm 1.0$  %) and *U. pinnatifida* ( $9.6 \pm 0.5$  %) respectively.



**Fig. 3: Effect of different time interval on foaming stability of the seaweeds. Values are mean  $\pm$  standard errors. Different letters show significant difference ( $P < 0.05$ ) between means by Duncan’s Multiple Range test.**

The basic necessities for algal biomass to be a good foaming agent include: (i) the ability to undergo rapid conformational changes at the interface, and (ii) the ability to rapidly adsorb at the air–water interface during bubbling. Kumar *et al.*<sup>19</sup> observed that the protein concentrate *K.alvarezii* showed the lowest

FC ( $38 \pm 2\%$ ) at pH 6.0, and highest FC ( $53.33 \pm 2.31\%$ ) at pH 4.0; this value was slightly lower than that of *E. compressa* ( $55.0 \pm 2.6\%$ )<sup>41</sup> and much lower than the fenugreek protein concentrate i.e.  $89.5\%$ <sup>42</sup> which is much higher than FC of *E. linza* ( $33.3 \pm 5.7\%$ ) and *E. tubulosa* ( $31.9 \pm 2.7\%$ ).<sup>41</sup>

**Table 1: Macro (mg/100g DW), Micro and trace elements ( $\mu\text{g}/100\text{g DW}$ ) determined by ICP-MS; Below Detection Limit: the values that have a similar superscript in the same row are not significantly different ( $P > 0.05$ ).**

Elements	<i>Gracilaria</i> sp.	<i>U. pinnatifida</i>	<i>Saccharina</i> sp.	<i>H. fusiformis</i>	<i>Porphyra</i> sp.	<i>Sargassum</i> sp.
mg/100 g						
Na	136.05 $\pm$ 1.96 <sup>e</sup>	234.57 $\pm$ 7.96 <sup>f</sup>	121.9 $\pm$ 10.48 <sup>d</sup>	74.4 $\pm$ 9.94 <sup>c</sup>	59.92 $\pm$ 2.58 <sup>b</sup>	20.17 $\pm$ 0.89 <sup>a</sup>
K	7.62 $\pm$ 0.21 <sup>b</sup>	1.25 $\pm$ 0.04 <sup>a</sup>	42.89 $\pm$ 1.12 <sup>e</sup>	78.84 $\pm$ 1.82 <sup>f</sup>	28.01 $\pm$ 0.22 <sup>d</sup>	13.25 $\pm$ 0.58 <sup>c</sup>
Ca	5.66 $\pm$ 0.05 <sup>e</sup>	0.49 $\pm$ 0.00 <sup>c</sup>	0.28 $\pm$ 0.01 <sup>b</sup>	1.99 $\pm$ 0.01 <sup>d</sup>	0.1 $\pm$ 0.00 <sup>a</sup>	12.28 $\pm$ 0.05 <sup>f</sup>
Mg	42.77 $\pm$ 0.69 <sup>b</sup>	54.12 $\pm$ 2.37 <sup>d</sup>	46.35 $\pm$ 1.44 <sup>b</sup>	50.72 $\pm$ 0.81 <sup>d</sup>	22.3 $\pm$ 0.36 <sup>a</sup>	107.16 $\pm$ 9.55 <sup>e</sup>
P	4.02 $\pm$ 0.07 <sup>a</sup>	7.14 $\pm$ 0.23 <sup>b</sup>	9.33 $\pm$ 0.12 <sup>c</sup>	16.12 $\pm$ 1.60 <sup>d</sup>	21.79 $\pm$ 0.21 <sup>e</sup>	4.74 $\pm$ 0.55 <sup>a</sup>
Total	196.12 $\pm$ 0.07	297.57 $\pm$ 11.09	220.69 $\pm$ 11.09	222.07 $\pm$ 14.18	132.12 $\pm$ 3.37	157.6 $\pm$ 11.62
$\mu\text{g}/100\text{g}$						
S	81.83 $\pm$ 1.08 <sup>c</sup>	8.57 $\pm$ 0.10 <sup>a</sup>	9.18 $\pm$ 0.17 <sup>a</sup>	103.03 $\pm$ 1.56 <sup>d</sup>	24.82 $\pm$ 0.25 <sup>b</sup>	101.84 $\pm$ 13.15 <sup>d</sup>
Cd	1.88 $\pm$ 0.48 <sup>d</sup>	1.21 $\pm$ 0.01 <sup>c</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	1.49 $\pm$ 0.01 <sup>c</sup>	1.05 $\pm$ 0.12 <sup>b</sup>	3.71 $\pm$ 1.04 <sup>e</sup>
Co	6.28 $\pm$ 0.03 <sup>f</sup>	0.34 $\pm$ 0.04 <sup>c</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	1.15 $\pm$ 0.00 <sup>d</sup>	0.29 $\pm$ 0.01 <sup>b</sup>	3.32 $\pm$ 0.04 <sup>e</sup>
Cr	79.38 $\pm$ 2.88 <sup>f</sup>	21.58 $\pm$ 1.79 <sup>a</sup>	24.46 $\pm$ 0.87 <sup>b</sup>	59.08 $\pm$ 0.51 <sup>e</sup>	36.49 $\pm$ 0.69 <sup>c</sup>	47.56 $\pm$ 3.09 <sup>d</sup>
Cu	39.77 $\pm$ 0.38 <sup>f</sup>	3.5 $\pm$ 0.20 <sup>b</sup>	1.26 $\pm$ 0.21 <sup>c</sup>	38.76 $\pm$ 1.28 <sup>e</sup>	12.58 $\pm$ 0.09 <sup>c</sup>	23.97 $\pm$ 0.11 <sup>d</sup>
Fe	5331.44 $\pm$ 78.09 <sup>f</sup>	412.32 $\pm$ 22.22 <sup>b</sup>	102.39 $\pm$ 11.73 <sup>a</sup>	2914.24 $\pm$ 19.56 <sup>e</sup>	513.38 $\pm$ 79.14 <sup>c</sup>	878.25 $\pm$ 22.62 <sup>d</sup>
Mn	769.04 $\pm$ 5.82 <sup>f</sup>	17.21 $\pm$ 0.82 <sup>b</sup>	9.6 $\pm$ 0.69 <sup>a</sup>	180.26 $\pm$ 2.59 <sup>e</sup>	64.09 $\pm$ 1.04 <sup>c</sup>	73.05 $\pm$ 1.26 <sup>d</sup>
Zn	83.76 $\pm$ 0.47 <sup>f</sup>	57.47 $\pm$ 0.61 <sup>c</sup>	7.78 $\pm$ 0.47 <sup>a</sup>	77.61 $\pm$ 0.35 <sup>e</sup>	51.06 $\pm$ 1.14 <sup>b</sup>	66.87 $\pm$ 2.32 <sup>d</sup>
Hg	-	-	-	-	-	-
Mo	1.03 $\pm$ 0.01 <sup>c</sup>	0.38 $\pm$ 0.04 <sup>b</sup>	0.20 $\pm$ 0.00 <sup>a</sup>	1.02 $\pm$ 0.02 <sup>c</sup>	1.63 $\pm$ 0.01 <sup>d</sup>	2.08 $\pm$ 0.11 <sup>e</sup>
Se	2.94 $\pm$ 0.18 <sup>f</sup>	0.15 $\pm$ 0.02 <sup>b</sup>	0.05 $\pm$ 0.01 <sup>a</sup>	0.45 $\pm$ 0.03 <sup>e</sup>	0.24 $\pm$ 0.01 <sup>c</sup>	0.38 $\pm$ 0.01 <sup>d</sup>
Total	6397.35 $\pm$ 89.42	522.73 $\pm$ 25.85	155.15 $\pm$ 14.16	3377.09 $\pm$ 25.91	705.63 $\pm$ 82.5	1201.03 $\pm$ 43.75

In our study, maximum foaming stability (FS) found in case of *Porphyra sp.* ( $28.33 \pm 2.89\%$ ), *H. fusiformis* ( $36.67 \pm 1.52\%$ ) and *Sargassum sp.* ( $27.33 \pm 1.15\%$ ), recorded after 30 min at pH 6.0, was higher than that reported for *E. tubulosa* and *E. linza* ( $16.7 \pm 1.5\%$  and  $4.4 \pm 2.0\%$  respectively).<sup>41</sup> Stable foams are vital for the production of a variety of foods. The foaming property usually depends on the source, method of foaming, temperature, pH, mixing time, processing parameters, etc. Among the various factors influencing foaming capacity (FC), the type of foaming equipment, method of agitation, and, speed of whipping, are also important.

#### Ash Content

Sea vegetables have high amount of ash as compared to terrestrial vegetables.<sup>43</sup> The ash content of a sample generally reflects its mineral content, Mushollaeni (2011)<sup>44</sup> particularly stated that the presence of ash content in the seaweed indicated the presence of mineral salt in the sample. The ash content of the investigated seaweeds ranged from  $10.33 \pm 0.58$  to  $50.33 \pm 0.58$  mg/100g DW. This high ash could be associated with high mineral elements. Highest ash content was observed in *H. fusiformis*  $50.33 \pm 0.53$  mg/100g followed by *Sargassum sp.*  $40.33 \pm 0.58$  mg/100g and least in *Gracilaria sp.*  $7.33 \pm 0.58$  mg/100g. As edible seaweeds rich in minerals have significant food utility, the investigated samples do promising applications.

#### Mineral Content

Table 1 compares the mineral content of the seaweeds *Gracilaria sp.*, *U. pinnatifida*, *Saccharina sp.*, *H. fusiformis*, *Porphyra sp.* and *Sargassum sp.* studied herein, as shown, 16 minerals were analysed. *Gracilaria sp.* (Table 1) had high Na ( $136.05 \pm 1.96$  mg/100g DW) and low Mo content ( $1.03 \pm 0.01$  µg/100g DW). *U. pinnatifida* also had high Na ( $234.57 \pm 7.96$  mg/100g DW) content, but it had low Se value ( $0.15 \pm 0.02$  µg/100g). *Sargassum sp.* recorded high Mg ( $107.16 \pm 9.55$  mg/100g DW) content and low Se ( $0.38 \pm 0.01$  µg/100g DW) content. The Na, K, Ca, Mg and P content however differed significantly among all seaweeds studied. A comparison of macro-elements revealed that *U. pinnatifida* contained high amount of minerals (Na+K+Ca+Mg+P) ( $297.57 \pm 11.09$  mg/100g) than *H. fusiformis* ( $222.07 \pm 14.18$  mg/100g), *Saccharina sp.* ( $220.69 \pm 11.09$  mg/100g), *Gracilaria sp.* ( $196.12 \pm 0.07$  mg/100g), *Sargassum sp.* ( $157.6 \pm 11.62$

mg/100mg) and *Porphyra sp.* ( $132.12 \pm 3.37$  mg/100g). Highest Na content was observed in *Undaria sp.* ( $234.57 \pm 7.96$  mg/100g) and least in *Sargassum sp.* ( $20.17 \pm 0.89$  mg/100g). The K content was higher in *H. fusiformis* ( $78.84 \pm 1.82$  mg/100g) and least in the *U. pinnatifida* ( $1.25 \pm 0.04$  mg/100g).

Micro minerals namely Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, S, Se, and, Zn, although needed only in trace amounts, are significant in regular functioning of the human body. Among the seaweeds studied, Fe content was significantly higher in *Gracilaria sp.* ( $5331.44 \pm 78.09$  µg/100g), followed by *H. fusiformis* ( $2914.24 \pm 19.56$  µg/100g), *Sargassum sp.* ( $878.25 \pm 22.62$  µg/100g), *Porphyra sp.* ( $513.38 \pm 79.14$  µg/100g), *U. pinnatifida* ( $412.32 \pm 22.22$  µg/100g), and *Saccharina sp.* ( $102.39 \pm 11.73$  µg/100g). Zn content of the seaweeds varied from  $83.76 \pm 0.47$  µg/100g (*Gracilaria sp.*) to  $7.78 \pm 0.47$  µg/100g (*Saccharina sp.*). The Se content of the six seaweeds varied from  $0.05 \pm 0.01$  µg/100g to  $2.94 \pm 0.18$  µg/100g, with highest content in *Gracilaria sp.* ( $2.94 \pm 0.18$  µg/100g); the lowest Se content was observed in *Saccharina sp.* ( $0.05 \pm 0.01$  µg/100g).

All the six seaweeds evaluated were rich in Mn (ranging from  $9.6 \pm 0.69$  µg/100g in *Saccharina sp.* to  $769.04 \pm 5.82$  µg/100g in *Gracilaria sp.*). The selected micronutrients (Cd+Co+Cr+Cu+Fe+Hg+Mn+Mo+S+Se+Zn) in *Gracilaria sp.* ( $6397.35$  µg/100g) were higher than land plants and vegetables like apple, brinjal, broad beans, cabbage, cauliflower, Lettuce, lichi, mangoripe, papaya ripe, potato, spinach, and, wood apple.<sup>45</sup> The tolerable daily intake of these elements that were established by the FAO/WHO Expert Committee<sup>46,47,48</sup> has been provided in table 2. It should be noted that Cd, Cu, Cr, Mo, Co and Zn are relevant elements that need to be tested when it comes to edible seaweeds, due to its potential toxicity to living organisms, absence of Hg in the seaweed is vital. The recommended daily intake of seaweeds *Porphyra sp.*, *U. pinnatifida*, *Gracilaria sp.*, *Saccharina sp.*, *H. fusiformis* and *Sargassum sp.* were calculated and provided in Table 2 (depending upon the permissible daily dose outlined for different toxic elements<sup>46,49</sup>). Based on toxicity limits of the mentioned elements, it is recommended that not more than 12.56 g DW of *Gracilaria sp.*, 204 g DW of *U. pinnatifida*, 15g DW of *Saccharina sp.*, 230 g DW of *H. fusiformis*, 72.5 g DW of *Porphyra sp.* and 215.8 g DW of *Sargassum*



sp. be used. These values were determined as per the tolerable daily intake of these elements provided by the FAO/WHO Expert Committee.<sup>46,47,48</sup> The results indicate the possibility of using

of seaweeds as a condiment in food supplements to improve the nutritive value of food in the human diet.

Table 2: Daily recommended dose of seaweeds based on permissibility of intake of toxic elements.<sup>46,49</sup>

Elements	Permissible daily dose (µg)	Recommended daily intake g per day					
		<i>Gracilaria sp.</i>	<i>U. pinnatifida</i>	<i>Saccharina sp.</i>	<i>H. fusiformis</i>	<i>Porphyra sp.</i>	<i>Sargassum sp.</i>
Cd	50-150	112.8	72.6	14.4	74.5	63	110.5
Co	200-1300	12.56	204	15	230	72.5	215.8
Mo	2-50	12.36	3.8	10	40.75	40.75	10.4
Cr	5.5-45	40.38	35	35	40.5	45	10.12

### Fatty Acid Composition

The seaweed samples contained saturated, monounsaturated and poly unsaturated fatty acids. The total fat content of the seaweeds varied from 0.36 % to 2.33% (Table 3). The total saturated fatty acids ranged from 0.34 to 0.84%. The total saturated

fatty acids (0.84%) content *Sargassum sp.* recorded in this study was lesser than the value reported by Kumari *et al.* (2013)<sup>50</sup> (i.e. 49.6%).

The total fatty acid content was expressed as g/100g total fat of each sample, *Porphyra sp.*, *U. pinnatifida*, *Saccharina sp.*, *H. fusiformis*, *Gracilaria sp.*, and *Sargassum sp.*, had a fatty acid contents of  $0.71 \pm 0.1$ ,  $0.36 \pm 0.015$ ,  $1.04 \pm 0.020$ ,  $0.76 \pm 0.043$ ,  $1.23 \pm 0.030$ ,  $2.33 \pm 0.01$  g/100g respectively. The main saturated fatty acids (SFA) observed were Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Lauric acid (C12:0), Arachidic acid (C20:0), Behenic acid (C22:0), Cis-10 Heptadecenoic acid (C17:0). Palmitic acid was the most dominant saturated fatty acid, lowest amount of palmitic acid were recorded in *U. pinnatifida* (0.14%) and *Saccharina sp.* (0.24%), while the rest of the species had higher amounts of palmitic acid. The results obtained herein are in agreement with that of Gressler *et al.*<sup>51</sup> who observed palmitic acid as the dominant saturated fatty acid in seaweeds. Arachidic acid was only found in *Saccharina sp.*, *Sargassum sp.*, *Gracilaria sp.* and *H. fusiformis*. The differences in the fatty acids among the seaweeds are associated with species and habitat.<sup>52</sup>

*Sargassum sp.* had the maximum amount (1.09%) of total mono-unsaturated fatty acids (MUFA) and *U. pinnatifida* had the least amount of total MUFA (0.12%) ( $P < 0.001$ ). The total MUFA in *Sargassum sp.* was lower than the range reported for seaweeds (3-56%).<sup>53</sup> The variation in MUFA content could be as a result of exclusion of palmitoleic and eicosenoic fatty acids. In case of monounsaturated fatty acids (MUFAs), Cis-Oleic Acid (C18:1) was detected in *U. pinnatifida*, *Saccharina sp.*, *H. fusiformis*, *Gracilaria sp.*, and *Sargassum sp.* Palmitoleic acid (C16:1) was found in *Saccharina sp.*, *H. fusiformis*, and *Gracilaria sp.*

Nevertheless, Erucic acid (C22:1) was observed in *H. fusiformis* and *Gracilaria sp.*, Cis-10 Heptadecenoic acid (C17:1) was observed in *Saccharina sp.* in this study.

**Table 3: Fatty acid composition (%) of seaweeds; SFA: saturated fattyacids; MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; Values with the similar superscript in the same row are not significantly different (P > 0.05).**

Fatty acid (wt%)	<i>Gracilaria</i> sp.	<i>U. pinnatifida</i>	<i>Saccharina</i> sp.	<i>H. fusiformis</i>	<i>Porphyra</i> sp.	<i>Sargassum</i> sp.
SFA	0.76 ± 0.01	0.34 ± 0.17	0.44 ± 0.02	0.40 ± 0.02	0.51±0.12	0.84 ± 0.01
Myristic acid(C14:0)	0.02 ± 0.003 <sup>a</sup>	0.03 ± 0.004 <sup>a</sup>	0.14 ± 0.005 <sup>c</sup>	0.05 ± 0.005 <sup>c</sup>	0.02±0.009 <sup>a</sup>	0.15 ± 0.001 <sup>b</sup>
Palmitic acid (C 16:0)	0.65 ± 0.002 <sup>d</sup>	0.14 ± 0.004 <sup>a</sup>	0.24 ± 0.009 <sup>d</sup>	0.33 ± 0.004 <sup>d</sup>	0.47±0.10 <sup>b</sup>	0.57 ± 0.004 <sup>f</sup>
Steric acid(C18:0)	0.03 ± 0.001 <sup>b</sup>	0.02 ± 0.001 <sup>a</sup>	0.04 ± 0.002 <sup>b</sup>	0.011 ± 0.003 <sup>b</sup>	0.02±0.007 <sup>a</sup>	0.06 ± 0.001 <sup>d</sup>
Lauric acid(C12:0)	-	0.15 ± 0.16 <sup>a</sup>	-	-	-	0.01 ± 0.001 <sup>b</sup>
Archidic acid (C20:0)	0.04 ± 0.001 <sup>c</sup>	-	0.02 ± 0.003 <sup>a</sup>	0.004 ± 0.002 <sup>a</sup>	-	0.01 ± 0.001 <sup>b</sup>
Behenic acid (C22:0)	0.02 ± 0.002 <sup>a</sup>	-	-	0.006 ± 0.001 <sup>ab</sup>	-	0.03 ± 0.001 <sup>c</sup>
MUFA	0.36±0.02	0.12±0.004	0.46±0.04	0.29 ±0.01	-	1.09 ± 0.00
Cis-Oleic acid (C18:1)	0.20 ± 0.01 <sup>c</sup>	0.12 ± 0.004	0.40 ± 0.030 <sup>c</sup>	0.22 ± 0.002 <sup>c</sup>	-	1.09 ± 0.00
Palmitoleic acid (C16:1)	0.02 ± 0.003 <sup>a</sup>	-	0.05 ± 0.004 <sup>b</sup>	0.06 ± 0.003 <sup>b</sup>	-	-
Erucic acid (C22:1)	0.14 ± 0.002 <sup>b</sup>	-	-	0.01 ± 0.001 <sup>a</sup>	-	-
Cis-10 Heptadecenoic acid (C 17:1)	-	-	0.01 ± 0.004 <sup>a</sup>	-	-	-
PUFA	0.12±0.002	0.02± 0.003	0.18±0.009	0.06±0.003	0.12±0.002	0.48±0.002
Cis-Linoleic acid(C18:2)	-	0.02 ± 0.003	0.12 ± 0.004 <sup>c</sup>	0.06 ±0.003	0.12±0.002	-
γ Linoleic acid (C18:3)	0.09 ± 0.001	-	0.05 ± 0.003 <sup>b</sup>	-	-	0.09 ± 0.001
Cis-11,14,17-Eicosatrienoic acid (20:3)	0.03 0.001	-	0.01 ± 0.002 <sup>a</sup>	-	-	0.39 ± 0.001
Total Fat	1.23 ± 0.030	0.36 ± 0.015	1.04 ± 0.020	0.76 ± 0.043	0.71±0.1	2.33 ± 0.01

Further, the total poly unsaturated fatty acids (PUFA) included Linoleic acid and Linolenic acid. Although *Sargassum* sp. had the most amount of PUFA (0.48%), it was lower than the range (34- 74%) reported by Dawczynski *et al.* (2007).<sup>31</sup> It has been reported that when there is a decrease in environmental temperature, algae normally accumulate PUFAs.<sup>54</sup> Therefore, the variation in PUFA could be attributed to environmental temperature.

Incorporation of seaweed in low salt meat emulsion models is reported to increase the PUFA content,<sup>55</sup> this shows the potential of seaweeds as a substrate in food processing. In our study, estimations of PUFA revealed the following facts. Cis-Linoleic acid (C18:2) was found in *Porphyra* sp., *U. pinnatifida*, *Saccharina* sp., and *H. fusiformis*, further,  $\gamma$ -Linoleic acid (C18:3) was found in *Saccharina* sp., *Gracilaria* sp., and *Sargassum* sp. and Cis-11, 14, 17-Eicosatrienoic acid (20:3) was found in *Saccharina* sp. and *Sargassum* sp.

Fatty acids are of great prominence in human nutrition and health. Low intake of saturated fat and an increased PUFA-to-SFA ratio is associated

with a lower risk of human coronary heart disease. Nonetheless, red seaweed *Champia parvula* showed long chain saturated fatty acid like eicosanoic acid in its structure<sup>56</sup> which is also seen in *Saccharina* sp., *Gracilaria* sp. and *Sargassum* sp.

#### Vitamin Content

In the marine environment, the habitat of seaweeds usually varies from species to species, many of them are often exposed to direct sunlight. Consequently, seaweeds contain many forms of antioxidants (including vitamins and protective pigments). Seaweeds contain both water- and fat-soluble vitamins (Table 4). Water soluble vitamins (Vitamin B2, Vitamin B3, Vitamin B9) and fat soluble vitamins (Vitamin E) were found in *Porphyra* sp., *U. pinnatifida*, *Saccharina* sp., and *Sargassum* sp. The Vitamin E content of *Porphyra* sp. and *Sargassum* sp. was  $0.09 \pm 0.01$  and  $0.63 \pm 0.01$  (mg/100g) respectively. The water soluble vitamins, vitamin B2 was found in *Porphyra* sp. ( $0.01 \pm 0.001$ mg/100g) and *Undaria*  $0.01 \pm 0.004$ mg/100g). Vitamin B3 was detected in *U. pinnatifida* ( $0.02 \pm 0.004$  mg/100g) and *Saccharina* sp. ( $0.01 \pm 0.001$  mg/100g), while Vitamin B9 found in *Porphyrasp.* ( $0.006 \pm 0.001$  mg/100g).

**Table 4: Vitamin composition of seaweeds in mg/100g**

Vitamins Profile	Seaweeds					
	<i>Gracilaria</i> sp.	<i>Undaria</i> sp.	<i>Saccharina</i> sp.	<i>Hizikia</i> sp.	<i>Porphyra</i> sp.	<i>Sargassum</i> sp.
Fat Soluble Vitamins						
Vitamin E	-	-	-	-	$0.09 \pm 0.01$	$0.63 \pm 0.01$
Water Soluble Vitamin						
Vitamin B2	-	$0.01 \pm 0.004$	-	-	$0.01 \pm .001$	-
Vitamin B3	-	$0.02 \pm 0.004$	$0.01 \pm 0.001$	-	-	-
Vitamin B9	-	-	-	-	$0.006 \pm 0.001$	-

Based on previous reports, it could be said that the vitamin content of seaweeds vary based on their occurrence and species (thus values for *Porphyra* sp., *U. pinnatifida*, *Saccharina* sp., *Gracilaria* sp. and *Sargassum* sp. would vary). *G. edulis* reported to contain  $2.14 \pm 0.17$  mg/g vitamin A and  $1.34 \pm 0.10$  mg/g vitamin E.<sup>57</sup> These values are lower than that obtained by us in this study. The variation in vitamin content could be due

to certain environmental factors e.g. atmospheric temperature, salinity, seasonality, methods of preservation and processing. Considering that Ortiz *et al.*<sup>58</sup> suggested that the daily vitamin requirements of the human body could be met by consuming 100 g of seaweeds, it is recommended to consume seaweeds to meet the daily nutritional requirement.

### Conclusion

Seaweeds are nutritionally rich as they possess protein, fatty acids, minerals, and vitamins that are essential for human and animal consumption. Seaweeds also a good source of various bioactive compounds. They have therefore been used for various food applications and as a functional food. The value of the global seaweed market in 2015 was \$10.4 billion and has now reached \$ 14.7 billion in 2021. Being rich in minerals, fatty acids, vitamins, and proteins, edible seaweeds are low caloric food supplements that serve as a perfect food supplement, they could be incorporated in the daily diet to make it balanced. As compared to the terrestrial food crops, seaweeds are abundant with trace elements, minerals, and PUFA, moreover they do not require terrestrial space for growth. These attributes, make seaweeds a healthy, consumer-friendly food commodity. They SFA/PUFA ratios lower than one which gives them an added advantage. These valuable under utilized resources present a Seaweeds are valuable and under-utilized resources, that present a variety biochemical composition (based on species).

However various factors such as temperature, light, salinity, pH, seasonal variation, geography, etc. determine their biochemistry. Popularizing the utility of this product would mean providing a new opportunity for employment and income for the coastal dwellers, and thereby prove to be socioeconomically beneficial.

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### Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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