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Protein Isolate from Brown Seaweed, *Sargassum tenerrimum*: Extraction and Characterization

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Abstract

The demand for plant-based raw materials as a protein source is experiencing a notable increase, and within this context, seaweed has emerged as a promising and sustainable alternative. However, seaweed resources remain under-exploited in spite of their abundance along the Indian shores. In this study, an attempt was made to isolate protein from brown seaweed, *Sargassum tenerrimum,* using the pH shift-based technique. The seaweed protein isolate (SPI) contained 3.19 ± 0.84 % moisture, 51.13 ± 0.66 % protein, 2.55 ± 1.11 % fat, 18.07 ± 0.64 % ash and 25.06 ± 3.45 % total carbohydrate. The physico-chemical and functional properties of seaweed protein isolate was also studied. From the results obtained, it is concluded that the locally available seaweed species such as *S. tenerrimum* could serve as a raw material for protein isolation. The protein isolate from *S. tenerrimum* can be used as an ingredient in protein-rich food formulations, including protein dietary supplements. However, it is necessary to make efforts towards improving the functional properties of the protein isolate to ensure its optimal suitability for a wide range of food applications.

Keywords: Seaweed protein, pH shift process, proximate composition, physio-chemical properties

Introduction

Recently, the consumption of plant protein has gained consumer preference as they are healthy natural foodstuffs and has sustainability credential (Fleurence, 1999). Seaweeds are known to be rich in minerals, vitamins, essential amino acids, carbohydrates, and dietary fibres. Moreover, seaweeds also contain bioactive compounds possessing antibacterial, antiviral, antifungal and antioxidant properties (Marinho-Soriano et al., 2006). Seaweeds have been used in food and pharmaceutical sectors for functional applications as phycocolloids, thickening and gelling agents (Fleurence, 1999).

Normally, type of species, site of collection and environmental factors etc. influence the protein content in seaweed (Fleurence et al., 2012). Generally, brown seaweeds contain lower protein (3-15 %) and green seaweed have moderate amount of protein (10-30 %) while red seaweeds contain the highest protein content (a maximum of 47 %) (Wong & Cheung, 2001). The presence of major pigmented proteins like phycobiliproteins, phycoerythrins, phycocyanins, and allophycocyanins is the reason for greater protein content in red seaweed (Mabeau & Fleurence, 1993). But studies on extraction and utilisation of protein from seaweed are limited (Vilg & Undeland, 2017). There are various techniques to recover the proteins from different biomasses including pH shift technique. The advantages of employing pH shift method include higher protein yields, reduced residual lipid in the end products and ease of operations and adoptability for large scale production and in certain cases it is found to improve the surface-active properties of protein isolates of plant origin (Jiang et al., 2018). The objective of this investigation was to develop a protein isolation process from brown seaweed, *Sargassum tenerrimum*. In addition, the protein isolate obtained under the developed process was characterized with reference to physico-chemical properties and functionalities. *S. tenerrimum* is available abundantly in east and west coast of India (Connan & Stengal, 2011). In addition, the low cost and availability of cultured raw material are the added advantages.

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Materials and Methods

The brown seaweed (*S. tenerrimum)* was collected from Ramanathpuram coast, Tamil Nadu, India with the help of local seaweed collectors. The seaweed was transported to the laboratory in semi-dried form.

Seaweed was subjected to soaking and washing in adequate amount of fresh water to remove extraneous matters. The washed seaweed samples were subjected to centrifugation in a basket type industrial centrifuge to remove excess water and dried at 50 °C in an electrical drier (Kraftwork drier-KSD 100, India) for 72 h and pulverized using 0.5-micron mesh (Maruthi pulveriser 12 Double 4000 rpm, India). The seaweed powder was packed in polythene pouches, sealed, and stored under desiccated conditions using an airtight container at room temperature (27 ± 2 °C) until further used.

The seaweed protein extraction was carried out as detailed here. The process flow of extraction of seaweed protein is represented in Fig. 1. The pulverized seaweed powder was suspended in 0.2 M EDTA and the suspension was subjected to stirring for one hour at room temperature (27 ± 2 °C) to remove the mineral content. Then, the suspension was filtered using a muslin cloth. The solid was collected and washed using potable water by stirring using overhead stirrer for one hour (3 washing, 20 min each). The solid obtained was dispersed in 9 % of H_2O_2 solution and kept in a water bath (Julabo TW20, Rose Scientific Ltd., Canada) at 65 °C for one hour for bleaching. After the bleaching treatment, the slurry was subjected to filtration and washed using potable water for 30 min (3 washing, 10 min each). The pH of residual mass was adjusted to 12 using 0.5 M NaOH and the extraction was carried out in a thermostatically controlled water bath at 80 °C for one hour under continuous stirring and the mixture was then filtered. The solid obtained was subjected to extraction of protein (2 cycles) at 80 °C for one hour and the supernatants from both cycles were combined and the pH was adjusted to 2 using 2 M HCl. After filtration, the precipitated samples were neutralized (pH 7) using 2 M NaOH, subjected to oven drying at 50 °C (Rotek hot air oven, India) and the dried mass obtained was thereafter referred as brown seaweed protein isolate. The protein was packed in a polythene pouch and stored under room temperature in a desiccator until further analysis.

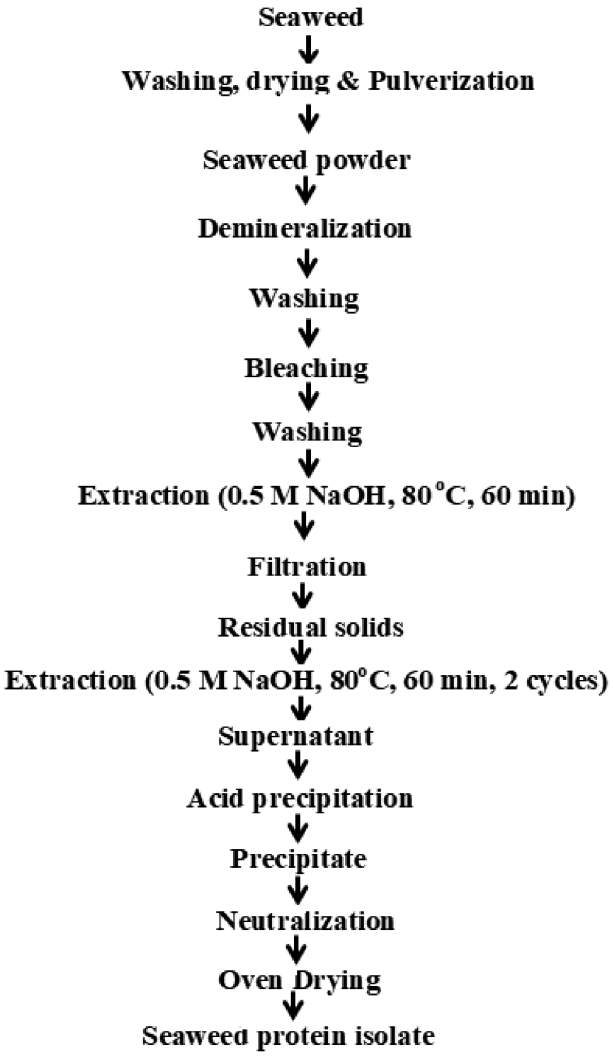

Fig. 1. Process flow for isolation of protein from brown seaweed (*S. tenerrimum*)

The proximate composition including moisture, protein, ash and lipid were analysed both for powdered seaweed used for protein extraction and the protein isolated according to the methods described in AOAC (2019).

The non-protein nitrogen (NPN) was determined using 10 % trichloroacetic acid (TCA) extract (AOAC, 2019).

Bulk density, particle density and porosity of seaweed powder and protein isolate were determined. Bulk density (ρb) was determined by weighing the sample in a 10 ml calibrated measuring cylinder and expressed in g cm^{-3} (Wang et al., 1993).

For particle density (ρs) estimation, known weight of sample was transferred to a 100 ml volumetric flask and the volume was made up using hexane. The sample mixture was subjected to continuous agitation under magnetic stirring. After 15 min of agitation, the weight of volumetric flask was taken and calculated using the equation given by Weindorf & Wittie (2003). The porosity was calculated using the formula of Lapple (1968).

$$\text{Particle density } (g/cm^3) = \frac{\rho h(Wc)}{Wc-(Wch-Wh)}$$

$$\text{Porosity } (\epsilon) = \frac{\rho s-\rho b}{\rho s} \times 100$$

Where, [ρh= Density of hexane, Wc= Wt. of seaweed, Wch= Wt. of seaweed and hexane (g), Wh= Wt. of 100 ml of pure hexane, ρs= particle density, ρb= bulk density]

The colour of the seaweed and seaweed protein isolate (SPI) were measured using ColorFlex EZ Spectrophotometer (HunterLab, USA). The white and black standards supplied with the equipment (HunterLab, USA) were used for calibration. The readings are reported in the CIE L^*, which indicates the lightness, a* indicates the redness or greenness and b* indicates yellowness or blueness. The hue angle (h), chroma (C*) and the distance between any two colours (ΔE) values are calculated according to the following equations.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h = \tan^{-1} (b^*/a^*)$$

$$\Delta E = \sqrt{100 - [(L_0^* - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2]}$$

(L_0^*, a_0^* and b_0^* are the colour attributes for standard and L_1^*, a_1^* and b_1^* the colour attributes of seaweed and protein isolate.)

The water activity (a_w) of the seaweed powder and isolated proteins (SPI) were determined at room temperature using AquaLab Water Activity System (Aqualab 4TE, India).

The hydrogen ion activity was measured by dispersing the dried seaweed powder and protein isolate in distilled water (1:10). The pH of the samples was analysed using pH meter (Labman pH meter, India).

The residual salt estimation by Mohr's method was carried out using the methodology of AOAC (2019). Accurately 2 g of pre-weighed sample was dispersed in 25 ml of water with an addition of 5 ml conc. HNO_3 and 40 ml of 0.1 N $AgNO_3$ and kept for boiling for 15 min. After cooling, the sample was titrated with 0.1 N ammonium thiocyanate using 1-2 drops of ferric alum indicator. The blank was run without sample. The result obtained was expressed in percentage (%).

The water holding capacity (WHC) and oil binding (OBC) capacity were determined as described by Suzuki et al. (1996). The protein isolate (0.5 g) was transferred into a pre-weighed centrifuge tube (15 ml) and 10 ml distilled water was added and kept at room temperature for 24 h and centrifuged at 4000 rpm for 30 min. The supernatant was decanted and the weight of centrifuge tube having sediment was recorded. The result was expressed as gram of water per gram of sample. Similarly, oil binding capacity (OBC) was analysed and expressed in g of oil/ g of sample.

Estimation of the mean and standard deviation was carried out using Excel programme of MS office. All the analyses were carried out in triplicate.

Results and Discussion

The proximate composition of seaweed (*S. tenerrimum*) and seaweed protein isolate (SPI) is presented in Table 1. The dried seaweed powder contained 10.93 ± 0.70 % moisture, 15.70 ± 1.74 % protein, 14.81 ± 0.27 % ash, 2.09 ± 0.01 % fat and 56.47 ± 1.26 % total carbohydrate. Seaweed from the same genus, *Sargassum crassifolium,* from south coast of Sri

Table 1. Proximate composition and Non-protein nitrogen content of dried seaweed and seaweed protein isolate (SPI)

Parameters	Dried seaweed	SPI
Moisture (%)	10.93 ± 0.70	3.19 ± 0.84
Protein (%)	15.70 ± 1.74	51.13 ± 0.66
Ash (%)	14.81 ± 0.27	18.07 ± 0.64
Fat (%)	2.09 ± 0.18	2.55 ± 1.11
*Total carbohydrate (%)	56.47 ± 1.26	25.06 ± 3.45
NPN (%)	0.77 ± 0.13	0.40 ± 0.00

*Total carbohydrate presented was estimated by the mass difference. The results are presented on dry weight basis as Mean ± SD (n=3)

Lanka was reported to have 10.28 % protein, 2.45 % fat and 3.52 % ash, which is similar to the values obtained in the present study (Maldeniya et al., 2020). The higher ash content indicates the presence of micro minerals with some trace elements (Mabeau & Fleurence, 1993). The biochemical composition of seaweed differs with species, season, geographical location and environmental factors such as temperature, salinity etc. (Fleurence, 1999). The seaweed protein isolate (SPI) contained 51.13 ± 0.66 % protein, 18.07 ± 0.64 % ash and 2.55 ± 1.11 % fat. The protein content of SP increased by 4-fold as a result of extraction process employed compared to the protein content of dried seaweed powder. Use of 2-mercaptoethanol with increasing pH upto 12 and precipitating with ammonium sulphate increased the protein concentration of *Enteromorpha compressa* from 17.48 ± 0.41 (raw seaweed) to 60.35 ± 2.01 %, in *E. tubulosa* from 19.09 ± 0.91 to 53.83 ± 0.70 % and in *E. linza* from 12.5 ± 1.26 to 33.36 ± 1.04 % and protein recovery was 6.48 %, 6.16 % and 5.71 %, respectively (Kandasamy et al., 2012). The protein isolated from brown seaweed, S. *crassifolium* collected from Sri Lankan coast was 71.43 ± 0.52 % after alkaline treatment (Maldeniya et al., 2020).

Non-protein nitrogen concentration (NPN) varies widely among the species. The NPN content of seaweed (0.77 ± 0.13 %) and SPI (0.40 ± 0.00 %) revealed the presence of non-protein nitrogen. This may include impurities like chlorophyll, nitrite, nitrate, free amino acids, nucleic acids and ammonium compounds (Lourenco et al., 1998). Dawczynski et al. (2007) studied red and brown algae and reported that red algae *Porphyra* sp. contributed higher NPN fraction (4.40 g/100 g) than brown algae *Undaria pinnatifida* (0.4 g/100 g) due to the presence of higher chlorophyll, phycoerythrin and phycocyanin in red seaweed.

The physicochemical properties of seaweed powder and protein isolate are described in Table 2. Bulk and particle densities of powder are the important properties while considering the design of storage container and transportation. However, during product development, for example in protein paste preparation for people under medical treatment and children, the paste thickness is one important parameter to be considered (Ogunwolu et al. 2009). Protein isolates with higher bulk density was reported to be desirable as it reduces the paste thickness. Similarly, the porosity influences the wettability of the protein isolates. The bulk density

Table 2. Physico-chemical properties of dried seaweed powder and SPI

Parameters	Dried seaweed	SPI
Bulk density (g/cm^3)	0.65 ± 0.01	0.40 ± 0.01
Particle density (g/cm^3)	0.87 ± 0.01	0.76 ± 0.01
Porosity (%)	25.28 ± 0.01	47.36 ± 0.01
Water activity	0.58 ± 0.01	0.57 ± 0.01
pH	6.98 ± 0.20	6.26 ± 0.20
Salt content (%)	8.41 ± 0.29	3.19 ± 0.24

The results are presented on dry weight basis as Mean ± SD (n=3)

of powdered seaweed (*S. tenerrimum*) was 0.65 g/cm^3 whereas bulk density of SPI was 0.40 g/cm^3. The particle density of both seaweed powder and SPI were 0.87 g/cm^3 and 0.76 g/cm^3 whereas the porosity of seaweed powder and SPI were 25.28 % and 47.36 %, respectively. Raman & Doble (2014) reported the bulk density, particle density and porosity of seaweed, *Kappaphycus alverzii* collected from Tuticorin, Tamil Nadu, India. The bulk density of dried *K. alverzii* seaweed powder was 0.5 g/cm^3, the particle density was 0.03 g/cm^3 and the porosity was 90 %. The results of dried seaweed powder from *K. alverzii* was similar to the results of the present study.

The pH values of dried seaweed powder and the protein isolate (SPI) were 6.26 ± 0.20 and 6.98 ± 0.20 respectively, which is near neutrality. The salt content for seaweed powder was 8.41 ± 0.29 % and for SPI 3.19 ± 0.24 %. As per the FDA guidelines, the salt content of any formulated food products should be in the range of 5-20 %. The salt content for both seaweed and protein isolate were low and it could be incorporated into any functional food formulations. Seaweed powder and protein isolate were analysed for functional properties and the results are shown in Table 3. As the colour influences the consumer's acceptance, it is considered to be an important parameter during food incorporation (Jimenez-Aguilar et al., 2011). The change in food colour often affects the consumer's judgments about flavour identity (Spence et al., 2010). The lightness (L*) value of dried *S. tenerrimum* powder was 43.46 ± 0.01 and SPI was 23.08 ± 0.71. The powdered seaweed had more yellowness/blueness (b*) value (17.37 ± 0.03) than SPI (7.82 ± 0.55). The greenness /redness values (a*) of dried *S. tenerrimum* powder

Table 3. Functional properties of dried seaweed powder and SPI

Parameters	Dried seaweed	SPI
WHC (g of sample/g of water)	7.17 ± 0.13	1.13 ± 0.25
OBC (g of sample/g of oil)	0.74 ± 0.05	0.81 ± 0.19
Colour		
L*	43.46 ± 0.01	23.08 ± 0.71
a*	5.10 ± 0.01	-0.28 ± 0.09
b*	17.37 ± 0.03	7.82 ± 0.55
C*	18.15 ± 0.03	7.82 ± 0.55
H	73.68 ± 0.04	-87.88 ± 0.86
ΔE	46.91 ± 0.01	28.88 ± 0.67

The results are presented on dry weight basis as Mean ± SD (n=3)

and SPI were 5.10 ± 0.01 and -0.28 ± 0.09 respectively. The SPI sample had low L* value (lightness). The chroma value (C*) reveals the degree of deviation from grey towards pure chromatic colour and hue angle (h°) value represents the relative amount of red, yellow, green and blue colour present in the sample. For the seaweed powder and SPI, the C* and h° values were found to be 18.15 ± 0.03 and 7.82 ± 0.55, and 73.68 ± 0.04 and -87.88 ± 0.86 respectively. The ΔE value for seaweed and SPI were 46.91 ± 0.01 and 28.88 ± 0.67 respectively. García-Vaquero et al. (2017) reported the colour values of freeze-dried protein powder from *Himanthalia elongate*, which had L*=59.36, a*=0.47, and b*=16.26, C*=16.26, h°=88.34 and ΔE=27.62. Seaweed is classified according to the colour of thallus (brown, red and green). So, it can be assumed that the protein isolates from particular seaweed may have the colour of raw material chosen. In our experiment, we used brown seaweed and the extracted protein powder obtained was brown in colour. From the results, it is also evident that the bleaching process employed did not yield the protein isolates with better lightness values.

Water-holding capacity is an essential parameter, which helps not only to maintain the freshness but also to keep the food moistened e.g., soups, custards, dough and baked products. This property is responsible for its ability to absorb water without dissolution of protein and imparts functionalities like viscosity (Cooper & Goldenberg, 1987; Kumar et al., 2014). WHC of seaweed *S. tenerrimum* powder was 7.17 ± 0.13 g of water/g of sample whereas the WHC for the extracted protein powder (SPI) was 1.13 ± 0.25 g water/g protein powder. The WHC of lyophilised protein concentrate prepared by pH shifting method from green seaweed, *E. compressa* (1.53 ± 0.07 g /g), *E. tubulosa* (1.32 ± 0.11 g/g) and *E. linza* (1.22 ± 0.06 g/g) (Kumar et al., 2014), were comparable with the present study. While comparing to the dried seaweed powder, the WHC of SPI is considerably lower, which could be due to the presence of insoluble polysaccharide and uronic acid content in seaweed powder (Sjamsiah et al., 2014). Further, it should be mentioned that there will be structural changes in proteins as the higher temperature (80 °C) employed during extraction process break the hydrogen bonds and destabilize the secondary structure. This leads to protein denaturation and aggregation. However, for isolation of proteins from seaweeds, heating at 80 °C under alkaline conditions mainly helps to break the cell wall components, which aids in better protein extractability. In general, the employment of pH shift techniques leads to protein denaturation. The initial raw material has different kind of polysaccharides including alginate. This could be the reason for higher water holding capacity of the raw material observed. On the other hand, as a result of the isolation process employed including drying, the protein molecules might have undergone denaturation and interaction with the other molecules present in the isolate, which could have resulted in lesser water holding capacity of protein isolates.

The oil binding capacity (OBC) is an another important property as it helps during development of formulated foods such as sausages, cake batters and mayonnaise (Chandi & Sogi, 2007). The OBC of seaweed powder was 0.74 ± 0.05 g /g of solid and for SPI, it was 0.81 ± 0.19 g of oil/g of solid. The oil absorption capacity of dried red seaweed *K. alvarezii* collected from off Tuticorin coast of Tamil Nadu, India was 1.00 ± 0.12 g oil/g of solid (Raman & Doble, 2014). The oil binding capacity of extracted protein powder of *K. alvarezii* collected from Okha, Gujarat, India was 1.29 ± 0.20 g oil/g of protein (Kumar et al., 2014). Kandasamy et al. (2012) reported the oil holding capacity of freeze-dried protein concentrates from *E. compressa (*1.34 ± 0.10), *E. linza (*1.05 ± 0.07) and *E. tubulosa* (1.08 ± 0.04), which are comparable with the results of the present study.

The plant protein isolates used in food industry are mostly from the sources such as soy, wheat, chickpea and cereals but these raw materials are expensive and their availability will be limited in future. Therefore, food manufacturers and consumers continue to search alternative sustainable protein sources, which are economically viable and available round the year. Seaweeds are widely available but the studies on seaweeds as source of proteins are limited. In the present study, *S. tenerrimum,* a brown seaweed has been explored as source of protein and the protein isolate produced had a protein content of 51.13 %. SPI produced from *S. tenerrimum* can be considered as an ingredient in protein rich food formulations including protein dietary supplements. Further, studies on developing food products and developing functional derivatives from seaweed protein isolate is under progress.

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