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Research Article

Biochemical composition and investigation on the economic feasibility of sodium alginate production of brown seaweed *Sargassum illicifolium* (Turner) C. Agardh, 1820 from Chabahar Bay (Gulf of Oman, Iran)

Hafezieh M.[1*]; Abkenar A.M.[2]; Jadgal S.[3]; Ajdari A.[3]

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Abstract

More than 3000 MT of *Sargassum illicifolium* ,annually washed-up from the Oman Sea (Sistan and Baluchestan province). , According to the estimates of the Iranian Fisheries Sciences Researchinstitiute.The brown seaweed biomass has been considered as one of the best free sources for production of sodium alginate. A key objective of this study was to determine the biochemical composition of *Sargassum illicifolium* collected from Chabahar Bay in November 2018 and to understand the economic potential and cost drivers of sodium alginate on basis of the present macroalgae. Alginates were purified by re-precipitation with ethanol and characterized by infrared spectroscopy. The results showed that the Chabahar *Sargassum* was characterized by total protein (TP), total lipid (TL) and carbohydrate as 9.8±0.8%, 4.4±0.2% and 33.2±4.1% dry weight, respectively. The ash content contained 41.6±2.3% DW. Moreover, the n-6/n-3 ratio was 2.62 and total essential amino acids and total minerals were 29.1±0.2 mg g^{-1} DW and 102. 2±0.6 mg g^{-1} DW, respectively. sodium alginate of *Sargassum illicifolium* was found to be high as 28.2% purification with molecular weight of 8.06×105 g mol^{-1}. Its total production price was evaluated 7.66 $ per kg sodium alginate, which is much cheaper than existing ones on the Iranian market.

Keywords: Alginic acid yield, Macro algae, Purification, Proximate composition, Chabahar coast, Sea of Oman

1-Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), P.O. Box: 15745/133. Tehran, Iran.
2 Department of Fisheries, Chabahar Branch, Islamic Azad University, Chabahar, Iran
3- Offshore Fisheries Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Chabahar, Iran
*Corresponding author's Email: jhafezieh@yahoo.com

Introduction

Seaweeds are potential renewable resource in the marine environment where about 6,000 species have been identified and grouped in various shades of green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae); among them, 221 species have commercial value and ten species are intensively cultivated (FAO, 2020FAO, 2020). These seaweeds, mainly the brown one, contribute greatly to the nutritional status of communities due to their rich composition of micro (I, Fe, Zn, Co, Se, Mo, Fl, Mg, Bo, Ni and Co) and macronutrients such as minerals (Na, Ca, Mn, K, Cl, S and in P), vitamins (B12, A and K), essential amino acids, fatty acids and pigments (FAO, 2020) destributed in the Persian Gulf and the Oman Sea (Karkhaneh Yousefi, 2020). One of the valuable compounds in seaweeds is hydrocolloids. There are agar and carrageenan in red seaweed and alginic acid in brown one.

Sodium alginate is the sodium salt of alginic acid, a natural polysaccharide presents in brown seaweed cell walls (Kloareg and Quatrano, 1988). In principle, the isolating process of alginates includes stages of pre-extraction with acid, washing, filtration and neutralization with alkali. Lastly, sodium alginate is precipitated from the solution by alcohol and re-precipitated in the same way. Sodium alginate is widely used as a stabilizer, thickener in agri-food industry and with viscosifying, rheological and gelling properties it is applied in various industries like textile cosmetic, biomedical and pharmaceutical (Draget *et al*., 2009). The properties of the alginate vary between species, so the choice of which seaweeds to harvest is based on both the availability of particular species and the properties of the alginate that they contain. Total global aquatic plant aquaculture in 2016 was more than 15 billion USD, approximately 30 million tones, most comprises human food products, some for carrageen, agar and alginate extraction (FAO, 2020).

The length of the Iran coastline, based on fractal scientists is less than 2440 kilometers along the Persian Gulf and Gulf of Oman (Wikipedia) which is propel for natural cultivated brown seaweed *Sargassum* spp. According to seaweed stock assessment projects were carried out by the Iranian Fisheries Sciences Research Institute, more than 3000 Mt of *Sargassum illicifolium* (Turner) C. Agardh is washed-up from the Oman Sea annually (Ajdari *et al.*, 2003; Gharanjic and Rohani, 2010; Hafezieh *et al.*, 2014). It is one of the best free resources for collecting/ harvesting and extracting hydrocolloids and sodium alginate based on previous laboratory analysis (Abkenar *et al.*, 2014).

In order to determine total price production of sodium alginate extracted from *S. illisifolium*, this project was done in pilot commercial scale (100 kg wet seaweed *S. illisifolium*) to extraction, purification and price production determination of sodium alginate, in Offshore Fisheries Research Center, Chabahar, Sistan and Baluchestan province, Iran, 2018.

Materials and methods

Sampling and preparing seaweed

In order to avoid alginate destruction, brown seaweed must be collected, transported and dried as quickly as possible (Truus *et al.*, 2001). So, fresh samples of brown seaweed *S. ilicifolium* were collected from coastal line of Chabahar Bay, Oman Sea, Sistan and Baluchestan province, Iran (25°21'40"N 60°36'27"E), in November 2018 (Fig. 1). Then, they were cleaned and washed with sea water to remove impurities, transported to the Off-Shore Fisheries Research Center, Chabahar, rinsed with freshwater several times, dried under sun light, chopped and cut into the small pieces. Some parts of the samples were grinded to obtain powder with a particle size lower than 0.8 mm, using a mincer and passed through a 0.8 mm mesh sieve and then stored under vacuum in plastics bags at −20°C until analyzing of micro and macro nutritional compositions.

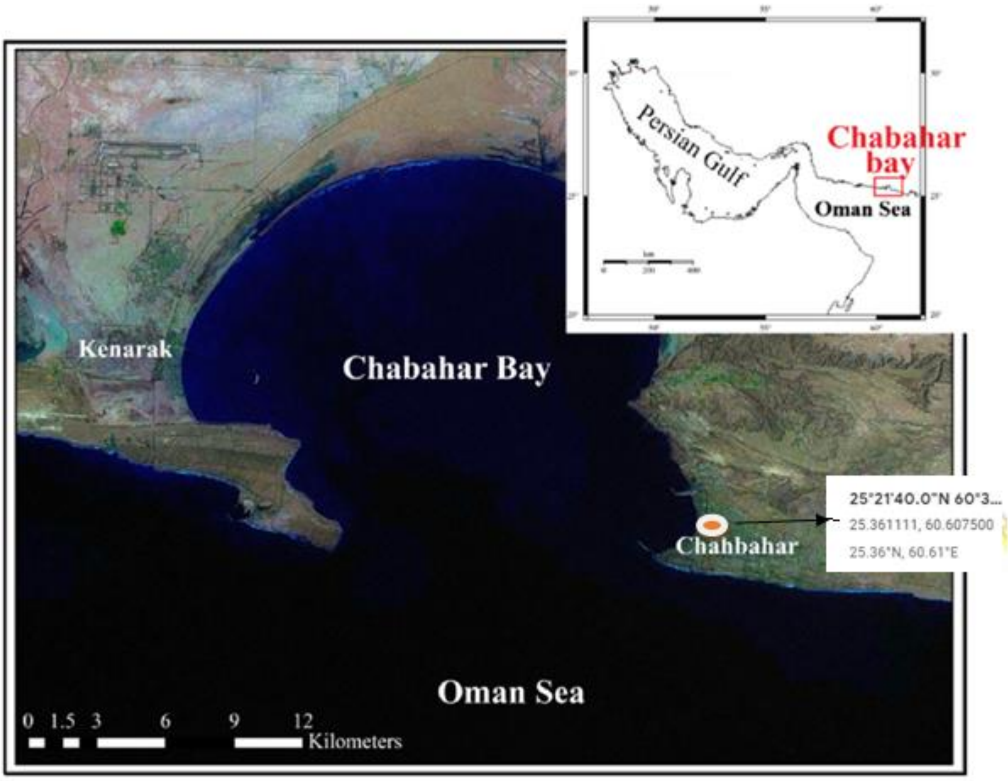

Figure1: Sampling station in Chabahar Bay, Sistan and Baluchestan Province.

Determination of biochemical composition

Proximate composition of *Sargassum* meal including dry matter, crude protein and fiber, ash, neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 2009). Moisture, protein, and ash contents were determined following the ISO recommendations (ISO 936:1998). Dry matter (DM) was determined within 3 g weight change calculation before and after 105 °C drying, crude protein (CP) was determined by Kjeldahl total nitrogen method (total nitrogen content was multiplied by×6.25). For this, 500 mg of seaweed reacted with catalyst H_2SO_4 ($CuSO_4 \cdot 5H_2O$) in a digester, organic nitrogen was transformed into $(NH_4)_2SO_4$, and distilled in alkali condition. Amino acids were extracted following the method provided by determined by ethyl ether extraction (Soxhlet technique) and 50 mg of lipid extraction was used for fatty acids profile determination. Trans esterification (Domínguez *et al.,* 2015), GC equipment with a FAMEs expressed in g/100 g of FAME. Ash was measured after drying in muffle oven at 500°C. Minerals (Ca, Fe, K, Mg, Mn, Na, P, Zn and Cu) were measured using flame photometry.

Extraction of sodium alginate

Alginic acid is present in brown seaweeds mainly in the form of sodium and calcium salts. The purpose of the extraction step is to convert the alginate to the soluble form of sodium alginate and remove it from the algae (Hernández-Carmona *et al.,* 2002). Sodium alginate was extracted from chopped and cut seaweed, chemically at 40º, using 0.5% formalin for 2 hours, rinsed with freshwater then placed in 0.2 N sulfuric acid for 5 hours, rinsed again to obtain pH 7, and using 3% sodium carbonate for 6 hours, then it was filtered. After adding ethylic alcohol, the viscous mixture was separated from its residue by centrifugation at 14,000×g. A paste form sediment which has been dried to produce clod form, was powdered by grounder to obtain sodium alginate extraction (Larsen *et al.,* 2003; Torres *et al.,* 2007). The yield of alginate was extracted as percentage/ dry weight.

Purification monitoring by fluorescence spectroscopy

To follow the purification procedure, fluorescence spectroscopy was used. Alginates are strongly fluorescent due to small amounts of polyphenolic residues. This is a routine technique to measure these contaminants in a wide range of alginates. The spectra were obtained with USB2000-FLG spectrofluorometer following the method described by Klock *et al*. (1997).

Results

Biochemical Compositions of Sargassum illicifolium

The proximate chemical compositions of seaweed meal were determined through laboratory analysis as shown in Table 1. The minerals, essential amino acids and fatty acids profiles (mean± standard deviation values) (*n*=5, five replicates) are given in Table 2.

Table 1: Biochemical compositions of *S. ilicifolium* collected from Chabahar Bay in November 2018 (Mean ± SD, % dry matter, DM).

Parameter	Dry matter* (moisture)	Crude Protein	Ash	NDF	ADF	Crude lipid	Crude fiber
Sargassum meal	91.0±1/1 (9.0±0/1)	9.8±0.8	41.6±2.27	26.4±1.80	12.1±0.89	4.4±0.18	38.5±0.4

Note: * Values are in % DM; NDF, neutral detergent fiber; ADF, acid detergent fiber.

Table 2: Minerals (mg/g), essential amino acids and fatty acids profiles (mg/kg) of *S. illicifolium* collected from Chabahar Bay in November 2018 (Mean ± SD, *n* = 5, five replicate specimens).

Minerals	Ca	Fe	K	Mg	Mn	Na	P	Zn	Cu
	98.7 ± 47.2	13.3 ± 0.9	378.3 ± 13.4	86.8 ± 12.0	1.9 ± 0.7	457.7 ± 50.0	n.q.	n.q.	n.q.

Essential Amino Acids (2912.42 ± 204.93 mg/100 g DW)	Threonine	Valine	Methionine	Isoleucine	Leucine (% total AA)	Phenylalanine	Lysine	Histidine	Arginine
	363.2 ± 17.1	353.8 ± 32.9	147.5 ± 18.7	295.2 ± 25.7	537.3 ± 38.8	340.1± 17.74	431.7 ± 38.4	126.4 ± 10.6	316.7 ± 14.0

Fatty Acids	Saturated fatty acids	Mono-unsaturated fatty acids	Poly-unsaturated fatty acids	Omega 3 fatty acid	Omega 6 fatty acids	*n*-6/*n*-3
	25.1 ± 0.5	31.1 ± 0.2	43.5 ± 0.5	12.0 ± 0.1	31.4 ± 0.4	2.6 ± 0.0

Impurity monitoring by fluorescence spectroscopy

One of the most important characters of alginate extracted from seaweed is purification rate which is calculated after impurities determination. So, purification is crucial for alginate applications in the biomedical field, since this natural polymer is known to be largely impure alginates that can lead to the development of fibrotic cell over growth around alginate micro-capsules and be consequently responsible for side effects on humans. The principal alginate contaminants are polyphenols, endotoxins, and proteins. *S. illicifolium* alginate impurities was 71.80%, so its purification was 28.20%.

Sodium alginate contents of S. illicifolium and production cost

Ninety percent of the wet seaweed disappeared after drying which was measured as moisture. From 100 g rest powdered seaweed, it can be extracted 28.2 g sodium alginate as bleached powder. Thus, from 100 kg wet seaweed, it can be obtained 10 kg DW and 2.82 kg sodium alginate. Total production cost for production one kg sodium alginate is detailed in Table 3.

Table 3: The final production price for one kg feed grade sodium alginate extracted from *Sargassum ilicifolium* collected from Chabahar Bay (Sistan and Baluchestan Province).

	Procedure	Amount	The cost $
1	Collecting, rinsing and drying	one kg DW seaweed	1.14 $
2	Chemical including formalin, sulfuric acid and sodium carbonate, ethylic alcohol and bleaching	2.5 lit.	3 $
6	Drying, powdering and packing	Produced one kg sodium alginate	1.2 $
7	Electricity	Produced one kg sodium alginate	0.66 $
8	Water supplying	Produced one kg sodium alginate	0.33 $
9	Workers	Produced one kg sodium alginate	1.33 $
10	Total production cost	Produced one kg sodium alginate	7.66 $
11	**Price in market (Chinese brand)**	**One kg**	**11.6 $**
12	Benefit of local production		4$

Discussion

Nine percent of the content of this seaweed was moisture and 91.0±1/1 DM, which is in close agreement with the data reported by Rodrigues *et al.* (2015), who also noticed that the moisture content of different edible seaweeds species ranged from 8 to 10%. Gómez-Ordoñez *et al.* (2010) also reported similar moisture contents (between 8.64% and 9.86%) in seaweeds from the northwestern Spanish coast. However, the differences in the result between this project with Chan and Matanjun (2017) in freeze-dried *Gracilaria changii* seaweed (lower moisture content, 5.32%) is due to difference in seaweed species.

S. illicifolium species presented total protein content of 9.8±0.8% DW, which is completely in agreement with the data reported by Fleurence (۱۹۹۹) (<15% DW in *F. vesiculosus*, *A. nodosum*, *Laminaria digitata* and *Himanthalia elongata*). Similar values were found by Gómez-Ordoñez *et al.* (2010) and Alves *et al.* (2016) in *B. bifurcata* (10.92% DW and 8.57% DW, respectively) and Chan and Matanjun (2017) in *G. changii* (12.57% DW), but it was lower than those obtained by Rodrigues *et al.* (2015) for brown (14.4–16.9% DW), red (20.2–23.8% DW) and green (18.8% DW) seaweeds and the observations by Fleurence (۱۹۹۹) in other seaweed species such as *Porphyra tenera* (47% DW) and *Palmaria palmata* (35% DW). On the contrary, Sánchez-Machado *et al.* (2004) obtained lower protein content (5.46% DW) in *H. elongata* dried seaweed. The protein level varied among different algal species, geographic areas, seasons, or environmental conditions (Denis *et al.*, 2010).

Ash contents of *S. illicifolium* was 41.6±2.27 % DW which is in agreement with the data reported as 40.58±2.10% DM by Alves *et al.* (2016) and 42.01±1.78% DM by Gómez-Ordoñez *et al.* (2010), but higher than that reported by Peinado *et al.* (2014) in *F. vesiculosus* (21–19% DW). It is known that high amounts of ash are linked with high levels of minerals. Mineral salts could be found on surface and in thallus. Conditions of hydrology and hydrochemistry on the habitat also influence the ash content.

NDF (26.4±1.80% DM) and ADF (12.1±0.89% DM) were obtained in *S.illicifolium* fiber analysis. Similarly, Peinado *et al.* (2014) recorded NDF and ADF in *Sargassum tenerrium* as 27.90±1.09% DM and 11.0±1.10% DM and also Alves *et al.* (2016) in *B. bifurcata* (25.78±1.26% DM and 12.98±0.27% DM, respectively)

Seaweeds exhibit low fat content (bellow 4%) (Herbreteau *et al.*, 1997), and varies significantly through the year (Manivannan *et al.*, 2008). Our value (4.4±0.18 % DW) was similar to those reported by Peinado *et al.* (2014) from 3.95 to 4.64% DW in *F. vesiculosus* at different seasons and by Gómez-Ordoñez *et al.* (2010) and Alves *et al.* (2016), who observed fat levels of 5.67% DW and 5.81% DW in *B. bifurcata*, respectively. The World Health Organization (WHO) (2007) recommended a n-6/n-3 ratio below 10. In our study, we observed n-6/n-3 ratio of 2.62 placing this studied brown seaweed according to WHO recommendations. This outcome is in agreement with those reported by other authors (; Alves *et al.*, 2016; Chan. and Matanjun 2017) who found n-6/n-3 ratios between 4.1 and 0.02.

The carbohydrate content or crude fiber of dried brown seaweeds ranged from 21.93% to 56.75%. In *S. illicifolium* collected from Chabahar Bay it was 38.5±0.4% DW. The maximum carbohydrate content was recorded in *Colpomenia implexa* and *Lobophora variegata* found the minimum content. Similarly, Peinado *et al.* (2014) recorded high carbohydrate in *Sargassum tenerrium* as 67.90% DW.

Seaweeds, especially brown ones are usually eaten whole plants as a good source of minerals. In this research, Ca (98.7±47.2), Fe (13.3±0.9), K (378.3±13.4), Mg (86.8±12.0), Mn (1.9±0.7) and Na (457.7±50.0) were measured in *S.illicifolium* based on mg/g but P, Zn and Cu were not detected. Kasimala and Coworkers (2015) revealed that *Hypneu pannosa* had Na content (127.65 mg/g), and *Padina tenuis* had Ca (48.00 mg/g), Mg (44.13 mg/g), and Fe (6.64 mg/g). The differences between minerals contents of seaweeds mainly referred to species, the habitatswhere they grow and the water content of minerals (Mæhre *et al.*, 2014). The total EAAs content of 2912.42±204.93 mg/100 g DW for *S. illicifolium*, accords with other finding (3000.81±194.67 mg/100 g DW) by Chan and Matanjun (2017).

In fatty acids profile of the seaweed studied, polyunsaturated fatty acids (PUFAs) were the most abundant (43.47% for the *S. illicifolium*), which is in line with the data reported by the other authors (Cofrades *et al.*, 2010; Alves *et al.*, 2016; Chan and Matanjun,

2017) who found that PUFAs were the main fatty acids (more than 40%) in seaweeds. However, Peng *et al.* (2013) and Maehre *et al.* (2014) observed higher saturated fatty acids (SFA) content in different seaweed species.

Sodium alginate content of *S. illicifolium* collected from Chabahar Bay has 28.2% purity; regularly this active compound has purities ranging from 20 to 35% in different brown seaweed due to seasonal harvesting, water temperature and other physico-chemical parameters of surrounded water (Alves *et al.,* 2016). According to Viswanathan and Nallamuthu (2014) the purification of sodium alginate in *P. gymnospora* and *Colpomenia implexa* was 23.01% and 21.53%, respectively.

Sargassum illicifolium alginate impurities was 71.20% which this result is consistent with the values reported by Orive *et al.* (2002) for *Sargassum illicifolium* sodium alginate (63%) and Torres *et al.* (2007) for *Sargassum vulgarea* brown algae for which the intensity was reduced by 52.7%. Klock *et al.* (1994) noted that the remaining contaminants detected in the fluorescence spectra of alginates from *Durvillaea potatorum* (brown algae) could not be identified. The in vitro and in vivo biocompatibility tests showed that these impurities did not initiate a foreign body reaction (Klock *et al.*, 1997)

Conclusions

In this study, *S. illicifolium* brown seaweed was collected from the Oman Sea, Chabahar Bay, prepared for determination chemical composition and extraction of sodium alginate in the laboratory condition. We found that this species had 9.8±0.8, 41.6±2.27, 26.4±1.80, 12.1±0.89, 8.4±0.38 % DW of CP, ash, NDF, ADF, and CF, respectively. From one kilogram cleaned seaweed after proportional dehydration only 10% DW obtained and 28.2 g sodium alginate can be extracted. Thus, from 100 kg wet seaweed, it can be obtained 10 kg seaweed DW and 2.82 kg sodium alginate. Total economical production cost is estimated 7.66 $, compared to imported Chinese brand 11 $ based on data from imported

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