RESEARCH ARTICLE



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Characterization of sodium alginate extracted from brown seaweeds growing on Veraval coast, Gujarat

Kiran Dangar, Vaishali Varsani & Suhas Vyas^{*}

Department of Life Sciences, Bhakta Kavi Narsinh Mehta University, Junagadh 362 001, Gujarat, India *Email: vsuhas.13@gmail.com

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ABSTRACT

Marine environment is a major potential source of functional materials, including polysaccharides, vitamins, enzymes, oils, antioxidants and peptides. All these materials are extracted from different marine living organisms including microbes, plants and animals. Among these seaweeds or marine macroalgae are one of the important sources and they are a part of staple diet from time immemorial in the orient as they are nutritionally rich materials. Those species that adapted to these pressures will expand their living boundaries and higher potential of row material availability in industry like pharmaceutical market, textile, fertilizer and for animal and human consumption. The present study concerns about the specific brown seaweeds, which is suitable material for alginate and growing abundantly at seacoast of Gujarat. Out of many species of seaweeds growing on the coastline of Veraval, four species viz., *Sargassum tenerrimum, Dictyota dichotoma, Spathoglossum asperum, lyengaria stellata* were selected for alginate extraction. The focus of this study is to utilize natural resources as alternatives and sustainability of human health to use sodium alginate as novel polymer and which is also biodegradable. It may be associated to other biologically active molecules and has a wide range of physicochemical and biochemical properties. Because of amazing properties, alginate and its salts are used in drug delivery system.

Introduction

Seaweeds neither need fertile and productive land nor freshwater to flourish in oceans. They are attributed as primary producers, as they productively absorb inorganic compounds from the seawater and transform them to macronutrients, namely lipids, carbohydrates and proteins. Present scenario of research the scientist has been given more attention to seaweeds biomass and especially the brown seaweeds, which has a major source of bioactive polysaccharides such as fucoidan, laminaran, alginates and mannitol have been studied because of their productiveness as anticoagulant, anticancer, antitumor, antithrombotic, anti-inflammatory, contraceptive and antiviral agent (1). Alginic acid is a polysaccharide composed of uronic acids and obtained in the universe as one constituent of brown seaweeds and as capsular polysaccharides in soil born bacteria. Alginate is the most abundant marine biopolymer and occurs in the intracellular mucilage and algal cell wall (2). The utilization of alginate is based on three main properties. The first is their potentiality to dissolve in water and thicken the resulting solution. The second is to form gels when a calcium salt is added to a solution of sodium alginate in water (3). The third property of alginate is the ability to form films of sodium or calcium alginate and fibers of calcium alginate (4).

Materials and Methods

Study area:

Veraval chopati is located at 20.9° N Latitude and 70.37° E Longitude Gujarat coastline of Veraval is about 3.5 km long with rocky substratum, slightly muddy with abundance of coral species. At the intertidal zone of Veraval has great diversity of

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seaweeds, there are four species of brown seaweeds, which were collected from the study area and used as source of alginate.

Collection of seaweeds:

Four species of brown seaweeds (*Sargassum* tenerrimum, Dictyota dichotoma, Spathoglossum asperum and Iyengaria stellata) were collected from Veraval coastal area during (February / 2018). The collected seaweeds species were washed, identified taxonomically and classified according to seaweeds of India [4]. Selected species gradually treated with acid (HCl), alkali (NaOH) and formaldehyde. The species were oven dried and converted into fine powder and stored for further use.

Extraction of sodium alginate:

The method of extraction of sodium alginate that had been according to mentioned by Rinta Kusumawati [5]. In the current study, we have slightly modified the method, in which 100 gm sample (milled seaweeds) weighted and soaked in 4% CaCl₂ taken in air tight bottle for 2 hr, after 2 hr filtered out and residue transfer for acid treatment of 5% HCl for 20 min. In the third step, treatment with 40% formaldehyde for one hr and the sample was then washed with distilled water for 20 min and added a 1 μg of MgCO₃ to break down cell wall. Final residue was extracted with 80% iso-propane for overnight. The precipitate was washed with acetone and filtrate was bleached with 1%, 1.5% and 2% sodium hypo chloride. The solution was evaporated and dried overnight at 40 °C in hot air oven.

Physicochemical property of sodium alginate

pН

For estimation of pH 1 gm of product (sodium alginate) was dissolved in 100 ml of distilled water (5). The digital pH meter was used to determine pH of sodium alginate (Digital pH meter).

Moisture content

The mass of the sodium alginate on heating at 45 °C under the specified operating conditions (4).

Ash content

The ash content was obtained by gravimetric method by heating the sample in the crucible till the sample is converted into ash (6).

Solubility

Different organic solvents and acids are used to determine the solubility of sodium alginate. 0.1 gm of sodium alginate was dissolved in 1 ml of individual solvents such as water, acetone, ether, chloroform and acids such as hydrochloric acid (HCl) and sulphuric acid (H_2SO_4).

Determination of purity of sodium alginate (phytochemical analysis)

To determine the purity of sodium alginate, tests for alkaloids, carbohydrates, flavonoids, steroids, phlobatannins, glycosides, terpins, saponins, tannins and phenols were carried out using standard methodologies (7). Organoleptic evaluation is to find out the colour, odour, taste and texture of extracted sodium alginate.

Biochemical constituents of sodium alginate

Protein

The protein presence in sodium alginate was determined by the biurette method. Protein was calculated by using BSA as standard and expressed as mg/gm protein (8).

Estimation of carbohydrate

Total carbohydrate contain in alginate was carried out by anthrone method by referring to standard D-Glucose and results have been expressed as mg/gm sugar (9).

Results and Discussion

The different seaweed species showed variation in the yield of alginate after bleaching with 1-2% sodium hypo chloride showed the highest alginate extracted at 1.5% sodium hypo chloride solution from Dictyota dichotoma species (19.43%) and with the increasing concentration of hypo chloride solution the yield of sodium alginate was decrease. Usually the sodium alginate content of various seaweeds is varied much. Sargassum tenerrimum had 16.32% alginate content at 2% sodium hypo chloride, Spathoglossum asperum had 17.45% at 1.5% concentration and Iyengaria stellata had 16.63% at 1% sodium hypo chloride. Variation being depended on the alginate extraction method and also the variation in the species of seaweeds (Table 1).

Table 1.	Yield	of	sodium	alginate	which	expressed	as	weight
percentag	ge with	۱ dif	ferent c	oncentrat	ion of a	ılkali solutio	on.	

SI. N	lo. Name of species	Yield of alginate (%)	Concentration of sodium hypochloride (%)
	0	15.00	1
1	Sargassum - tenerrimum -	15.12	1.5
		16.32	2
		19.39	1
2	Dictyota dichotoma	19.43	1.5
		18.73	2
3	- Spathoglossum asperum	17.23	1
		17.43	1.5
		17.45	2
		17.64	1.5
	-	15.31	2
4		16.63	1
	Iyengaria stellata	16.46	1.5
	-	15.00	2

Yield of sodium alginate

The phytochemical analysis was carried out in the present study to determine the purity of the extracted sodium alginate. This result showed that the extracted sodium alginate of selective seaweed species pure form and it has no trace of any phytochemicals, but the only element carbohydrate and its derivative saponins were present, which confirmed its better purity (Table 2).

Table 2. Determination of purity of sodium alginate(phytochemical analysis)

Sl. No.	Tests	S. tenerrium	D. dichotoma	S. asperum	I. stellata
1	Carbohydrate	+	+++	++	+++
2	Alkaloids	-	-	-	-
3	Flavonoids	-	-	-	-
4	Steroids	-	-	-	-
5	Phlobatannins	-	-	-	-
6	Glycosides	-	-	-	-
7	Terpins	-	-	-	-
8	Saponins	++	+++	+	+
9	Tannins	-	-	-	-
		4.5	1. ()		

(+) = positive result, (-) = negative result, (++) = high content (+++) = very high content

Organoleptic characters evaluation

The organoleptic characters showed that all the seaweeds were noted to be odourless, whereas, *S. tenerrimum* was found to be salty in taste and rest of the other seaweeds were tasteless. Characters of sodium alginate from *S. tenerrimum* were noted to be yellowish grey, odourless, salty taste and was observed to be powdery in appearance. Sodium alginate from *D. dichotoma* were noted to be white, odourless, tasteless and granular in texture. Characteristics of sodium alginate from *I. stellata* were having characteristics of brownish black, tasteless, odourless and granular in texture (Table 3).

Table 3. Organoleptic characters evaluation of sodium alginate

Sl. No.	Characters	S. tenerrimum	D. dichotoma	S. asperum	I. stellata
1	Colour	Yellowish grey	White	Greyish green	Brownish black
2	Taste	Salty	Tasteless	Tasteless	Tasteless
3	Odour	seaweed order	Odourless	Odourless	seaweed odour
4	Texture	Powder	Granular	Smooth powdery	Granular

Estimation of pH and moisture

The pH of 1% sodium alginate of selective species range 9.6–8.2. Maximum pH observed was in *S. tenerrimum*, whereas in *I. stellata* was noted to be minimum. The moisture content of sodium alginate observed ranged 16.52–11.87% maximum in *I. stellata* and minimum in *S. asperum*. Other than that, the *S. tenerrimum* and *D. dichotoma* had observed 12.65% and 14.72% respectively (Table 4).

Solubility

The solubility of sodium alginate showed that it was readily soluble in hydrochloric acid and sulphuric acid however, it was insoluble in all the other trailed solvents such as distilled water, ethanol, methanol, acetone, ether and chloroform (Table 5).

Ash content

The result of water-soluble ash content in *Sargassum tenerrimum* was noted to be higher (14.40%) than the other species. Water soluble ash content in *Spathoglossum asperum* (11.31%) and *Iyengaria stellata* (11.52%) was having marginal variations.

Table 4. Estimation of pH value of sodium alginate

Sl. No.	Name of species	Value of pH	Moisture (%)
1	Sargassum tenerrimum	9.6	12.65
2	Dictyota dichotoma	8.9	14.72
3	Spathoglossum asperum	9.5	11.87
4	Iyengaria stellata	8.8	16.52

Table 5. Ash content of sodium alginate

Sl. No.	Name of species	Water soluble Ash content	Acid insoluble ash content
1	S. tenerrimum	14.40%	3.63%
2	D. dichotoma	10.86%	5.71%
3	S. asperum	11.31%	2.06%
4	I. stellata	11.52%	3.85%

Species of *Dictyota* was noted to be lower in ash content (10.86%) compared to that of other seaweeds. Unlike to water soluble ash content, the acid soluble ash content was found to be having a noticeable range in all the seaweed species. The maximum acid soluble ash was observed in *D. dichotoma* (5.71%) and minimum acid soluble ash content was noted in *S. asperum* (2.06%) (Table 5).

Biochemical properties

Carbohydrates

Important biomolecules found to be present in the seaweeds, which creates their biological importance. Results of carbohydrate as a major source of energy and nutrition in sodium alginate was recorded to be in the range of $48.09-32.76 \ \mu g/ml$. Maximum concentration of carbohydrate was noted in *S. tenerrimum* and minimum in *S. asperum*. The values of protein content were interestingly found to be low than that of carbohydrates in different species of seaweeds. Range of protein content was observed to be 10.33 (*S. asperum*) to 29 $\mu g/ml$ (*D. dichotoma*) (Table 6).

Table 6. Mean value of triplicate observation Carbohydratesestimation from sodium alginate

Sl. No.	Name of species	Carbohydrate µg/ml	Protein µg/ml
1	S. tenerrimum	48.09	25.60
2	D. dichotoma	43.98	10.33
3	S. asperum	32.76	29.00
4	I. stellata	41.74	13.3

Conclusion

The major purpose of the study on seaweeds is for their utility in many industrial applications. Many countries utilise these seaweeds for extraction of valuable compounds used in companies of cosmetics, paints, food, medicine etc. From the current study, it can be derived that many biologically active compounds from the phytochemical study of seaweed can be helpful in the industries. It can be concluded that there is remarkable variation in the concentration of two important biomolecules i.e. carbohydrates and protein. All the seaweed species taken for the study showed variation in the production of sodium alginate and hence all these species are of having good potential as economical value. Seaweeds protein is used as an alternative source of dietary protein. The study of their physicochemical properties and biochemical composition discloses their appropriateness to be a beneficial source of food for human utilisation and consumption. There are several seaweeds with economic potential throughout the coastal belt of Gujarat coast. In the present study, it is indicated that all the four different seaweeds would be a good source of raw materials of sodium alginate. The exploitation of seaweed and other marine resources should be sustainable and needed to be balanced with the establishment of their growth. From the data of the present study, we can strongly reveal that seaweed culture should be extensively promoted for sustainable development in the coastal region of the country. Marine agronomy is to be developed and enhanced for the mass production of such species of seaweed for finding new natural resources.

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Authors' contributions

KD performed the practical and analytical portion of the experiments conducted during the study. VV carried out the collection of seaweed species. SV interpreted the data obtained and drafted the manuscript and coordination. All authors read and approved the final manuscript.

Conflict of interests

Authors do not have any conflict of interests to declare.

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