Journal of **A**pplied **B**iotechnology **R**eports

Original Article

Comparison of Extraction Different Methods of Sodium Alginate from Brown Alga *Sargassum* sp. Localized in the Southern of Iran

Ali Mohammad Latifi[1*], Ehsan Sadegh Nejad[1], Hamid Babavalian[1]

Abstract

The alginate was extracted with six different methods from Iran south seacoast algae, *Sargassum* sp, and the percentage yield of alginate was determined. We divided our methods to two groups including acidic extraction and non-acidic. In acidic methods, HCL and H_2SO_4 were used as a detergent in extraction process and $CaCl_2$ was exerted in non-acidic treatments. All treatments compared with each other and indicated an increasing in alginate yield when different methods used EDTA in extraction process. Finally, the main characteristics of sodium alginate were realized with FT-IR and H-NMR.

Keywords: Alginate; Brown Algae; Extraction; H-NMR; Polysaccharide

1. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

*** Corresponding Author**
Ali Mohammad Latifi
Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
E-mail: amlatifi290@gmail.com

Submission Date: 5/11/2015
Accepted Date:6/21/2015

Introduction

The brown alga *Sargassum* is usually the ecologically dominant species along tropical and subtropical coasts [1]. These algae are also found along the southern coasts of Iran and they play a remarkable economical role, used mainlyas source of alginate, a cell wall polysaccharide of brown seaweed with several industrial uses [2]. The quantity and quality of the alginates depend on the algae species, the type and age of the tissues used for alginate extraction [3]. Alginate is the major structural polysaccharide found in brown seaweeds (Phaeophyceae), that they constitute a family of linear copolymers of (1→4) α-L-guluronic acid (G) and (1→4) β-D-mannuronic acid (M) units (Fig. 1) [4, 5].

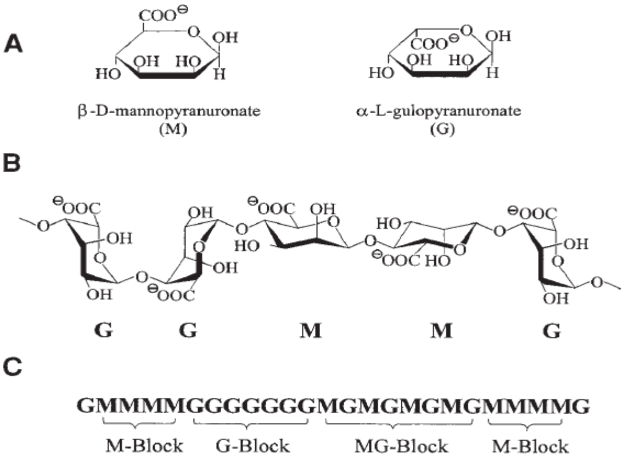


Figure 1. Alginate structural data: (A) alginate monomers (M versus G); (B) the macromolecular conformation of the alginate polymer; (C) chain sequences [18].

The biological source, growth, and stationary conditions are three important factors that they can be effective in the chemical composition and sequence of M and G units [4, 6]. Sodium alginate is a water-soluble polymer that it can ablethe gel formation in the presence of polyvalentcations, such as Ca^{2+} [7], although, gel properties depend on block structures (including M and G units) and the poly (β-D-mannuronate)/poly (α-L-guluronate) or M/G ratio. The lower the M/G ratiois caused stronggelation through the formation of "egg-box" structures by the poly (α-L-guluronate) blocks which strongly chelate Ca^{2+} (Fig. 2) [5, 8].

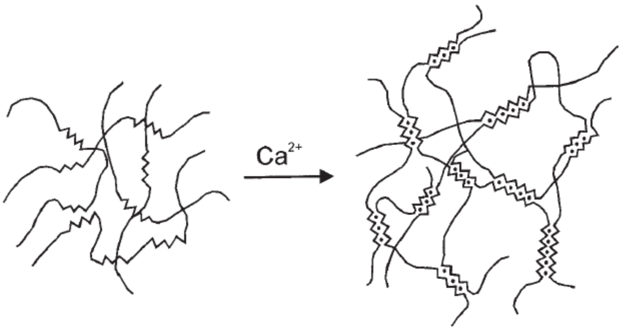


Figure 2. The “egg-box” structural model by the calcium-induced gelation of alginate [19].

Alginates were extracted from brown seaweed during several decades in order to obtain high yields and a controlled molecular weight for different applications [9, 10]. In this study, we investigated the different methods of alginate extraction and purified them with acetone.

Then sodium alginates obtained from *Sargassum* collected from southern coasts of Iran, were analyzed and characterized by FT-IR and H-NMR. Finally, the characteristic properties of extracted sodium alginates from different methods were studied and compared with each other.

Materials and Methods

Algae collection

Brown algae were collected from Persian Gulf in south coast of Iran. The species were in family Sargassaceae: *Sargassum* sp (SRG) (genus *Sargassum*). The crude algae are washed in distilled water, dried over night at 40-45ºC in an oven. The dry weights were gained after drying overnight at 105°C and the ash percentages were determined after burning the samples overnight at 400-450°C.

Extraction of alginate

$CaCl_2$ route (I)

The samples (2 g) were suspended in 2% $CaCl_2$ for 2 h, washed with deionized water and treated with 40% formaldehyde for 2 h to cross-link phenolic compounds. Alginate was extracted according to the method of Haug *et al*., [3]. The samples were then washed three times with deionized water and the alginate was extracted by addition of an aqueous solution of 3% Na_2CO_3 for 48 h. In other treatment, samples were also suspended in mixture of Na_2CO_3 1 M and 0.5 g of EDTA and the pH of the suspension adjusted to pH 11. This was then filtered through muslin cloth and precipitated in ethanol as sodium salt. The precipitate was separated by centrifugation and dried overnight at 60°C. The yield of alginate was expressed as percentage/dry weight.

HCl route (II)

The samples (2 g) of dry seaweed were moistened by addition 40% formaldehyde for 2 h to cross-link phenolic compounds. The samples were washed three times with deionized water and then a 0.1N HCl aqueous solution was added for 24 h, following a similar extraction method reported by Haug *et al*., [3]. After washing, the moistened seaweed was placed in a beaker with Na_2CO_3 1N solution (pH: 11) for 48 h. In other treatment, samples were also suspended in mixture of Na_2CO_3 1 M and 0.5 g of EDTA. This was then filtered through muslin cloth and precipitated in ethanol as sodium salt. The precipitate was separated by centrifugation and dried overnight at 60°C. The yield of alginate was expressed as percentage/dry weight.

H_2So_4 route (III)

The samples (2 g) of dry seaweed were moistened by addition 40% formaldehyde for 2 h to cross-link phenolic compounds. The samples were washed three times with deionized water and then a 1% H_2So_4 aqueous solution was added for 24 h. After washing, the moistened seaweed was placed in a beaker with Na_2CO_3 1N solution (pH: 11) for 48 h. In other treatment, samples were also suspended in mixture of Na_2CO_3 1 M and 0.5 g of EDTA.

This was then filtered through muslin cloth and precipitated in ethanol as sodium salt. The precipitate was separated by centrifugation and dried overnight at 60°C. The yield of alginate was expressed as percentage/dry weight.

Purification of sodium alginate extracts

Sodium alginate was purified according to the method of Gomez *et al*., [11]. Aqueous solution of sodium alginate obtained in the extraction step was directly precipitated, under stirring, by addition of ethanol until reaching a proportion 1:1 in volume, respectively. Thus, the insoluble polymer was separated and then exhaustively washed with acetone by sox let for 100 h. Finally, the biopolymer was dried at room temperature under vacuum until constant mass.

Moisture Content

Moisture content of sodium alginate was obtained by using Omar et al., [12] method. About 1 g sodium alginate prepared above was accurately weighed and dried in a vacuum oven at 70°C for 24 hours. The sample was allowed to cool to room temperature in a vacuum desiccator and re-weighed. Moisture content of the alginate sample was determined from the weight difference and expressed as percentage of original weight.

FT-IR

Fourier-transform IR (FT–IR) studies of samples were carried out using a FT-IR spectrophotometer from Alpha-Centauri (Nicolet Avatar 360 FTIR) to identify the functional groups. The dry sample was mixed with potassium bromide and pressed into plate for measurement. The FT-IR spectrum was then recorded.

Chemical analysis by NMR

Chemical composition of sodium alginates extracted and purified from *Sargassum* Sp. is recorded by NMR spectra. The freeze-dried sodium alginates were dissolved in D_2O with a concentration 6 g/L and dried several times prior to NMR spectrum acquisition. H-NMR experiments were performed using a Bruker AC-300 (Germany) spectrometer. Calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide was obtained from SDS (Vitry, France).

Results and Discussion

Brown seaweeds (*Sargassum* sp.) from Persian Gulf coast (Iran) were collected for extraction and purification of sodium alginate. The products were extracted by three ways, named $CaCl_2$ (I), HCl (II), and H_2So_4 (III) route. After that, the soluble polymer diffuses out of the seaweed under basic conditions, and then it is purified by acetone way (Fig. 3).

Figure 3. Schematic presentation from final product of alginate sodium.

The final products were analyzed and their chemical compositions as well as physical properties were compared.

Dry weight of sodium alginate

The yields of alginate obtained from algae harvested at the south sea coast of Iran (*Sargassum* sp.) reached amaximum in $CaCl_2$ (I) routes and minimum in acidic routes. These ranged from 12% to 16.5% in acidic routes and 25% to 30% in the $CaCl_2$ (I) routes (Fig. 4).

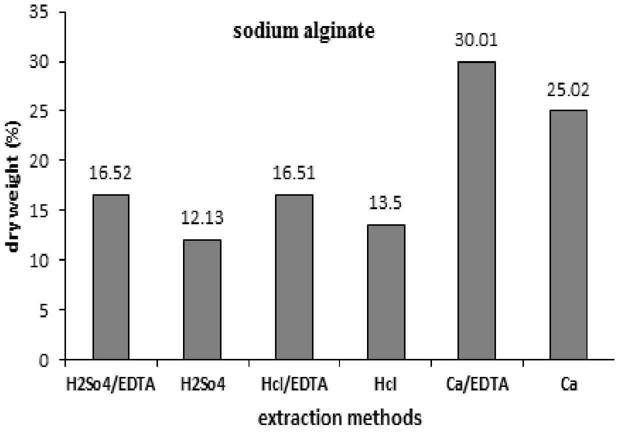


Figure 4. Percentage yield of sodium alginates obtained from extraction different methods.

We have found that, in absence of EDTA, the alginate yield is lower (4.5-5%), although, the presence of EDTA in all routes of extraction had the positive effective in dry weight of sodium alginate.

The yields of alginate from the $CaCl_2$ route (I) combined with EDTA of brown algae is in the same order of magnitude, around 30% that Chee *et al*., [13] and Mafra and Cunha, 2006 [14] reported on different *Sargassum*. In fact, technique used for isolation can be effective in the yields of alginate; especially, we have found that, in presence of EDTA, the alginate yield is higher (4.5-5%). In addition, studied results on the other brown algae collected from Madagascar [15] or *Macrocystis pyrifera* collected on Patagonian Argentine coast [11] were in agreement with these values.

Moisture content of sodium alginate

Figure 5 shows the percentage of moisture content of alginates extracted from the seaweeds samples by different methods mentioned above. Values pattern obtained for alginate moisture is the same in $CaCl_2$ route (I) and HCl route (II), although, it is vice versa in H_2So_4 route (III). It means that alginate obtained from extraction methods combined with EDTA had lower moisture content in $CaCl_2$ route (I) and HCl route (II), but this rate is high in H2So4 route (III) without EDTA (19.1) than this method combined with EDTA (16.9). There was no significant difference in the moisture content of alginate extracted in these methods.

The dry brown algae collected were analyzed for dry weight and ash percentage (Table 1). We observed high figures for ash in all treatments of extraction.

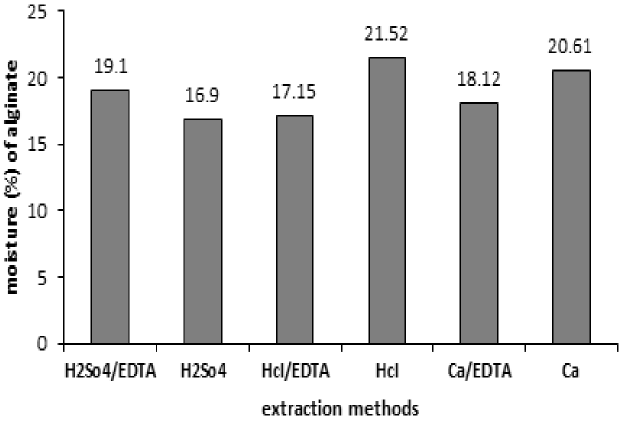


Figure 5. Percentage moisture content of alginates from extraction different methods.

The algae harvested from Egyptian Red Sea coast confirmed this characterization [16] and explained that the extensive washing is not effective for some of species contained calcareous animals. This rate was the high at $CaCl_2$ (I) route than other routes, although, it is non-significant.

FT-IR

FT–IR spectrum of sodium alginate is given in Figure 6. As the alginate is a carbohydrate, the characteristic peaks of alginate appeared at 3429, 1630 and 1428 cm−1, corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. However, the peaks are shifted; the carboxyl groups of alginate are shifted from 1428 to 1448 cm^{-1}. Our results indicated that these three peaks were appeared in FT–IR spectrum obtained from sodium alginate extracted from brown alga *Sargassum* sp. localized in the southern of Iran.

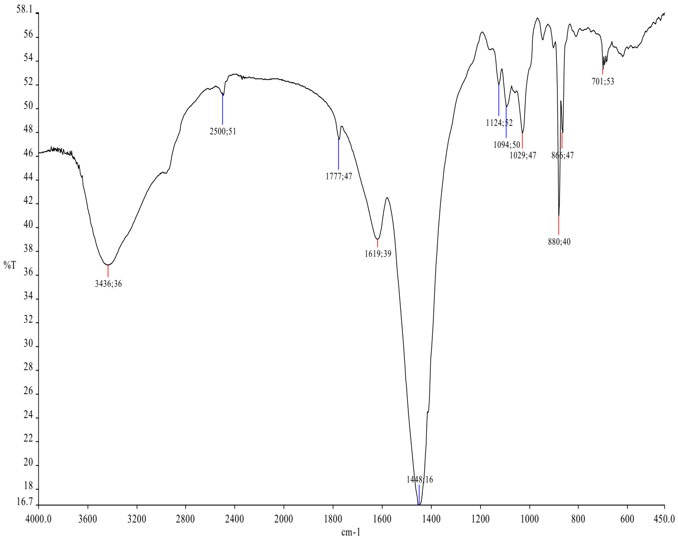


Figure 6. FT-IR analysis of sodium alginate extracted from brown alga *Sargassum* sp. localized in the southern of Iran.

H-NMR spectroscopy

The chemical composition of the extracted sodium alginate was determined using H-NMR spectroscopy (Fig. 7).

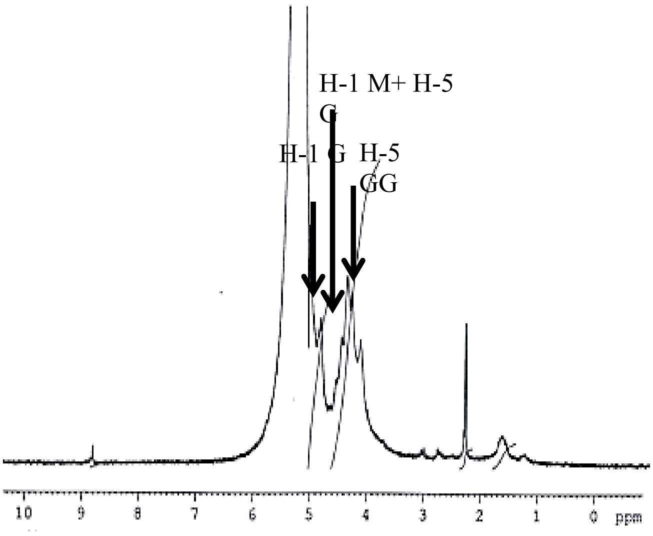
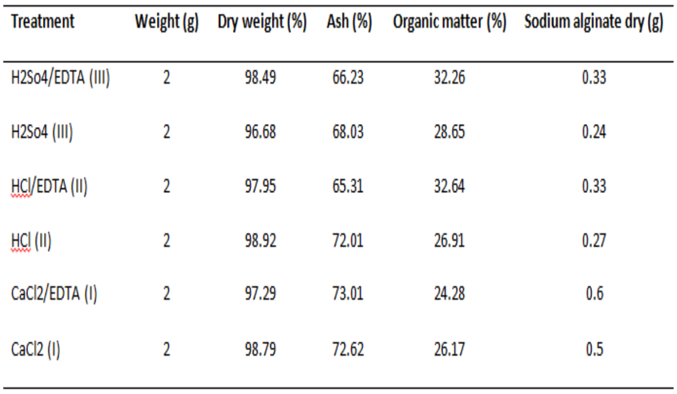


Figure 7. H-NMR spectra of the sodium alginates from brown algae Sargassum sp. localized in the southern of Iran.

We have assigned the signal at 4.95 or 5 ppm corresponds to the H-1 of guluronic units (H-1-G), whereas the H-5 of guluronic units in GGblock (H-5-GG) appears at 4.31 ppm, and the large signal between 4.45 and 4.7 ppm belongs to H1 of mannuronic units and H5 of guluronic units in GM moieties (H-1-M+H-5-GM) as assigned previously [17].

Table 1. Analysis of dry weight and ash percentage from the dry brown algae *Sarggasum* sp.



Treatment	Weight (g)	Dry weight (%)	Ash (%)	Organic matter (%)	Sodium alginate dry (g)
H2So4/EDTA (III)	2	98.49	66.23	32.26	0.33
H2So4 (III)	2	96.68	68.03	28.65	0.24
HCl/EDTA (II)	2	97.95	65.31	32.64	0.33
HCl (II)	2	98.92	72.01	26.91	0.27
CaCl2/EDTA (I)	2	97.29	73.01	24.28	0.6
CaCl2 (I)	2	98.79	72.62	26.17	0.5

Conclusion

In this paper, characterization related to brown algae *Sargassum* sp. localized in the southern of Iran was realized. The different methods of sodium alginate fractionation from *Sargassum* sp. were evaluated. Our data has been shown that EDTA treatment in extraction different methods of sodium alginate of mature *Sargassum* sp. increased the rate of alginate (30% W). In total, alginates obtained from acidic methods had a lower molecular weight average than that from non-acidic methods. These studies also showed that *Sargassum* sp. which is found in abundance in southern regions waters of Iran, could become a commercial source of alginate.

Acknowledgements

We sincerely thank all past and current members of the Applied Biotechnology Research Center of the Baqiyatallah University of Medical Sciences for their continuous efforts to unravel the new light of biology science.

References

1. Prince, J.S., O'Neal, S.W., The ecology of Sargassum pteropleuron Grunow (Phaeophyceae, Fucales) in the waters off South Florida: I. Growth, reproduction and population structure. *Phycologia*, 1979, Vol. 18, pp. 109-114.
2. Blanshard, J.M.V., Mitchell, J., Polysaccharides in food. 2013: Elsevier.
3. Haug, A., Larsen, B., Smidsrød, O., Uronic acid sequence in alginate from different sources. *Carbohydr Res*, 1974, Vol. 32, pp. 217-225.
4. UG, A.H., LARSEN, B.R., Quantitative determination of the uronic acid composition of alginates. *Acta chem scand*, 1962, Vol. 16, pp. 1908-1918.
5. Penman, A., Sanderson, G., A method for the determination of uronic acid sequence in alginates. *Carbohydr Res*, 1972, Vol. 25, pp. 273-282.
6. Atkins, E., Nieduszynski, I., Mackie, W., Parker, K., Smolko, E., Structural components of alginic acid. II. The crystalline structure of poly α-L-guluronic acid. Results of X-ray diffraction and polarized infrared studies. *Biopolym*, 1973, Vol. 12, pp. 1879-1887.
7. Draget, K.I., Skjåk-Bræk, G., Smidsrød, O., Alginate based new materials. *Int J Biol Macromol*, 1997, Vol. 21, pp. 47-55.
8. Morris, E.R., Rees, D.A., Thom, D., Boyd, J., Chiroptical and stoichiometric evidence of a specific, primary dimerisation process in alginate gelation. *Carbohydr Res*, 1978, Vol. 66, pp. 145-154.
9. Carmona, G.H., Higuera, D.L.A., Montesinos, E.R., Efecto del tipo de precipitación en el proceso de obtención de alginato de sodio: Método de alginato de calcio y método de ácido alguinico. *Ciencias Marinas*, 1997, Vol. 32, pp. 195-207..
10. Hernández-Carmona, G., Casas Valdez, M.M., Fajardo León, C., Rodríguez-Montesinos, E., Sánchez-Rodriguez, I., Evaluación de *Sargassum* spp. en la Bahía de la Paz, BCS, México. 1990, Vol.6, pp. 1-9.
11. Gomez, C.G., Lambrecht, M.V.P., Lozano, J.E., Rinaudo, M., Villar, M.A., Influence of the extraction–purification conditions on final properties of alginates obtained from brown algae (Macrocystis pyrifera). *Int J Biol Macromol*, 2009, Vol. 44, pp. 365-371.
12. Omar, S., Ahmad, N., Ahmad, F., Composition of Aiginates from Brown Seaweeds, *Sargassum* and *Padina* spp. *Pertanika*, 1988, Vol. 11, pp. 79-85.
13. Chee, S.-Y., Wong, P.-K., Wong, C.-L., Extraction and characterisation of alginate from brown seaweeds (Fucales, Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. *J Appl Phycol*, 2011, Vol. 23, pp. 191-196.
14. Mafra Jr, L., Cunha, S., Sargassum cymosum (Phaeophyceae) in southern Brazil: Seasonality of biomass, recovery after harvest and alginate yield. *J Coastal Res*, 2006, Vol. pp. 1847-1852.
15. Andriamanantoanina, H., Rinaudo, M., Characterization of the alginates from five madagascan brown algae. *Carbohydr Polym*, 2010, Vol. 82, pp. 555-560.
16. Larsen, B., Salem, D.M., Sallam, M.A., Mishrikey, M.M., Beltagy, A.I., Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydr Res*, 2003, Vol. 338, pp. 2325-2336.
17. Rahelivao, M.P., Andriamanantoanina, H., Heyraud, A., Rinaudo, M., Structure and properties of three alginates from Ma-

dagascar seacoast algae. *Food Hydrocoll*, 2013, Vol. 32, pp. 143-146.

18. Draget, K., Taylor, C., Chemistry and physical properties of alginates. *Food Hydrocoll*, 2011, Vol. 25, pp. 251-256.

19. Christensen, B.E., Indergaard, M., Smidsrød, O., Polysaccharide research in Trondheim. *Carbohydr Polym*, 1990, Vol. 13, pp. 239-255.