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Extraction and Characterization of Sodium Alginates Extracted from *Sargassum muticum* and *Turbinaria conoides*

Dina A Mohamed [1], Amal A Hassan[2], Manar T Ibrahim[3] and Marwa M Helmy[4]

[1,4] Food Engineering and Packaging Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt

[2,3] Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Abstract
This study was designed to investigate the physio chemical characterizes of Sodium alginates extracted from two species algae of brown algae using different extraction methods HCl, Hot, $CaCl_2$, and EDTA. The extraction yield, chemical components, gelling and melting point, viscosity, intrinsic viscosity, molecular weight and M/G ratio were determined. The obtained sodium alginates were characterized using FT-IR spectrophotometer and compared with commercial sodium alginates. The results indicated that Sodium alginates extracted from *Turbinaria conoides* had the highest yield 49.33% using EDTA method. The carbohydrates content ranged from 74.26 to 78.09% for *Turbinaria conoides* and 72.77 to 78.31% for *Sargassum muticum*. *Sargassum muticum* had the lower viscosity values compared with *Turbinaria conoides* which had the highest value of gelling point 46.6 ºC using $CaCl_2$ method. The melting point was between 80.60 to 90.67 ºC for both species. The highest intrinsic viscosity and molecular weight were 31.12 dl/g and 2.64×10^5 g/mol for *Turbinaria conoides*. The M/G ratio for sodium alginates extracted from *Turbinaria conoides* was higher than *Sargassum muticum* for all extraction methods
Keywords: *Sodium alginates, Sargassum muticum, Turbinaria conoides, intrinsic viscosity, molecular weight, M/G ratio.*

1. Introduction

Algae are photosynthetic organisms that differ in size, have high photosynthetic efficiency, grow rabidly and considered to be the main aquatic ecosystems producers. It has been used as a food, medicine and as a source for industrial, medicinal, and biopharmaceuticals products [1]. There are alginic acid, cellulose and other polysaccharides in the cell wall of algae. One of many solutions for improving process economics is the nutrient cost minimization nutrient as cellulose from algae was hydrolyzed to sugar and then fermented to ethanol that was not economic [2]. The natural polysaccharides of algae had rheological properties such as gelling, stabilization, viscosity of dispersions are used in various industries such as textile, paper, agri-food biomedical and pharmaceutical [3].

The common name given to a family Alginates of linear polysaccharides containing (1,4)-linked β-Dmannuronate (M) and its 1-4 epimer α-L-guluronate (G) residues arranged along the chain in non-regular blockwise order was alginate (salt of Alginic acid). The number and the distribution of each monomer including M/G ratio depend on the species of algae, seasons, the age of the algae, and the geographical variability of alginates for the same algae species [4], [5]. Within the alginate structure, the M and G monomers are sequentially assembled into either homopolymeric repeating (MMM or GGG) blocks or heteropolymeric alternating (MGMG) blocks. The physical properties of alginates depend on the relative proportion of those three block group [6]. Because of glycuronan as it has one of its main biofunctional properties, alginates have the ability to form viscous (gels) solutions in water in the presence of calcium ions, and hence are of great industrial interest, therefore alginates samples can be classified by intrinsic viscosity. Gel formation mainly relies on auto-cooperatively functions between chain regions enriched by GG-sequences [7]. Many factors such as pH, concentration, ions in and the presence of divalent ions affect the solubility of alginates. Alginates properties differ between speceies, the main commercial sources are species of *Durvillaea, Ascophyllum, Laminaria, Ecklonia, Lessonia, Sargassum, Macrocystis and Turbinaria*. The purpose of the extraction step is to convert the alginates to soluble form of extracted sodium alginates [8]. Algae are one of the most important classes of biopolymers for their film forming properties to produce edible films as food packaging and active ingredient carriers. Edible films are important part of food and can be consumed along with it, also it can be used to improve food quality such as deterioration changes, or chemical reactions. Film-forming materials utilized individually or as mixed composite blends [9].

The aim of the study was to investigate the physicochemical characteristics of sodium alginates extracted from *Sargassum muticum* and *Turbinaria conoides* using four different extraction methods then apply the best selected sodium alginates for food edible coating applications.

2. Materials and methods

2.1 Collection algae

Brown algae *Sargassum muticum* and *Turbinaria conoides* species were collected from Abukir, Alexandria coast and Guna, red sea coast, commercial alginates were purchased from Acmetic Company-Hong Kong (china).

2.2 Preparation of algae

To remove any impurities, the collected algae were washed, shade and dried at least 4 days and grounded into fine powder and stored at 4ºC for further use. The algae were identified at Ain Shames University (Plant Section).

2.3 Extraction and purification of Alginates

2.3.1 HCl extraction method

Alginates were extracted according to [10]. For the removal of phenolic compounds and pigments, 200 g of dried algae were immersed in 800 ml of 2% (v/v) formaldehyde during 24 h at room temperature. Samples were washed 3 times with deionized water, 800 mL HCl (0.2 M) was added and left for 24 h. Samples were washed again with deionized water before extraction with 2% Na_2CO_3 during 3 h at 100°C. The soluble fraction was collected by centrifugation (10,000 rpm, 30 min) and polysaccharides were precipitated by three volumes of ethanol (95%). Collected Sodium alginates were washed twice by 100 mL of acetone, dried at 65°C and dissolved in 100 mL of deionized water. It was then precipitated again with ethanol (v/v) and dried at 65°C.

2.3.2 Hot extraction method

Samples of Air-dried seaweed (200 g) were immersed in 300 mL of 1% $CaCl_2$ solution at room temperature (27°C) overnight (about 18 h). Algae was washed with distilled water (3×300 mL), kept in 5% HCl solution for one hour, and then washed again with distilled water (3×300 mL). After that, the samples were kept in 300 mL of 3% Na_2CO_3 solution for three hours at 50°C. Water (250 ml) was added into the sample then left to stand overnight. A centrifuge was used at 14,000 rpm/1h to separate viscous mixture from its residue. The extracted sodium alginates were precipitated by adding an ethanol (96%)/water mixture (1:1, v/v) to the solution. The precipitate was filtered, washed with ethanol again and air dried, followed by oven drying at 50°C for 24 hours [11].

2.3.3. $CaCl_2$ extraction method

Samples of dried algae (200 g) were suspended in 2% $CaCl_2$ for two hours, washed using deionized water then treated with 40% formaldehyde for two hours to cross-link phenolic compounds. Alginates were extracted according to the method of [12] by adding 3% of an aqueous solution Na_2CO_3 for 48 h/ at 25°C, then filtered through muslin cloth and precipitated as sodium salt in ethanol**.** The samples were then washed using deionized water three times. The precipitate was separated by centrifugation 4000rpm/20min and left to dry overnight at 60°C.

2.3.4 EDTA extraction method

Alginates was extracted according to [13] by addition of aqueous solution of 3% Na_2CO_3 (1M) and 0.5 g of EDTA, pH of suspended was adjusted to [11]. Filtration through muslin cloth and precipitation was done as previously mentioned in $CaCl_2$ assay.

2.4 Extraction Yield

Extraction yield was calculated by equation (1) according to [14].

$$Alginate\ yield\ \% = \frac{extracted\ alginate\ weight(dry\ weight)}{intial\ algae\ weight} \times 100 \qquad (1)$$

2.5 Physiochemical properties of sodium alginates

2.5.1Chemical composition

Fat, protein, carbohydrates, moisture, ash and minerals were obtained by gravimetric method according to [14].

2.5.2 Gelling and Melting temperature

Alginates solution (five milliliter of (1.5%)) were poured into a test tube and gelatinized for 1 hr at 20°C. Liquefying point (melting point) of gel is the temperature at which a small lead ball (diameter l mm, 25 mg) sinks into the gel when heated by 1°C per minute [15].

2.5.3 Viscosity

Extracted alginates viscosity was measured using Brookfield Engineering labs DV-III Ultra Rheometer, USA, [16].

2.5.4 FT–IR spectrophotometer

Fourier-transform Infrared (FT–IR) studies of samples were carried out using a FT-IR spectrophotometer (4100 Jasco FTIR - Japan) to identify the functional groups.

2.5.6 Intrinsic viscosity

Ubbelohde glass viscometer with 0.5-mm capillary diameter was used to measure intrinsic viscosities. The solution flow time (t) was observed at 25°C±0.1°C relative to the solvent flow time (0.1 M NaCl) of 130s. After the viscometer measure 10 mL of dissolved sample in water, it was left in the water bath for around 10 minutes to reach thermal equilibrium. The solution was pumped above the upper indicator line and allowed to flow under gravity and time influence.

This was replicated three times, and flow time was taken as the average reading, the concentration of the sample was subsequently decreased with the addition of 1 mL of solvent, and dilution was replicated at least five times. The intrinsic viscosity was determined by plotting reduced viscosity, ηsp/c (Eq. 3), and inherent viscosity (Eq. 4), ln ηr/c, against concentration of the sample solution, (C).

Relative viscosity, [11]

$$\eta_r = \frac{t}{t_o} \qquad (2)$$

Specific viscosity

$$\eta_{sp} = \eta_r - 1 \qquad (3)$$

Reduced viscosity,

$$\frac{\eta_{sp}}{C} = \frac{\eta_r - 1}{C} \qquad (4)$$

Inherent viscosity,

$$\eta_{inh} = \frac{\ln \eta_r}{C} \qquad (5)$$

Where t is the flow time of solution and t_0 is the flow time of solvent.

2.5.7 Molcular mass

Molecular mass based on the intrinsic viscosity and the average molecular weight, M_W^a, was calculated using the Mark–Houwink–Sakurada equation:

$$[\eta] = kM_W^a \qquad (6)$$

Where (k) used was 0.00113 and a was 0.984, as stated ***by*** [17].

2.5.8 Block Composition and M/G Ratio

Sodium alginates block composition was determined using partial heterogeneous hydrolysis [18]. One gram of sample was properly weighed and hydrolysed in at 100°C for 2 hours in 100 mL (0.3 M) HC1 (36.5%). Centrifuge at 4000 rpm/20 min. was used to form a suspension and cooled to room temperature. The supernatant, referred to as heteropolymeric blocks

(F_{MG} blocks) fraction, was decanted off and the amount of alginates in the soluble fraction was determined by phenol-sulphuric acid reaction, using moisture known commercial samples as standard. A small amount of 0.1 M NaOH solution from above has been redissolved in and then diluted to 50 mL by adding 0.1 M NaCl. A (0.0025) M HC1 was added to adjuste pH to 2.85. A precipitate was formed and this was centrifuged at 4000 rpm. The supernatant was decanted and amounts of alginates in soluble fraction, referred to as fraction F_{MM} homopolymeric mannoronic blocks, and in the final residue, referred to as fraction F_{GG} homopolymeric guluronic blocks, were determined by the phenol-sulphuric acid reaction as mentioned earlier.

F_G guluronic and F_M mannoronic fractions can be estimated from the following relationships [19]:

$$F_G = F_{GG} + F_{MG} \quad (7)$$

$$F_M = F_{MM} + F_{MG} \quad (8)$$

3 Results and Discussion

3.1 Effect of extraction methods on the yield of sodium alginates

Extraction yield for two species of algae *Sargassum muticum* and *Turbinaria conoides* using different extraction methods $CaCl_2$, EDTA, HCl, and Hot were shown in Table (1). The percentage of extraction yield for *Sargassum muticum* at different methods were 24.66, 20.00, 26.33, and 32.33% for HCl, Hot, $CaCl_2$ and EDTA, respectively. While yield % *Turbinaria conoides* were 33.33, 32.00, 38.33, and 49.33%, respectively. The maximum yield value of sodium alginates was 49.33% for *Turbinaria conoides* with EDTA method extraction. The results indicated that using EDTA extraction method has an effect in sodium alginates yield for both two species of algae, this may be due to their thallus texture difference as previously reported by [11] , [20].

Table 1: Sodium Alginate yield as affected by different extraction methods

Algae species	Yield (%)			
	HCl	Hot	$CaCl_2$	EDTA
Sargassum muticum	24.66 ± 2.51^{c}	20.0 ± 2.0^{d}	26.33 ± 1.5^{b}	32.33 ± 2.51^{a}
Turbinaria conoides	33.33 ± 2.88^{c}	32 ± 2.5^{d}	38.33 ± 2.88^{b}	49.33 ± 1.15^{a}

Different superscripts within a row indicate significant differences among formulations ($p < 0.05$). Data shown is the mean ± SD, n=3.

3.2 Effect of extraction methods on chemical composition of extracted sodium alginates

The chemical compositions of alginates extracted from *Sargassum muticum* and *Turbinaria conoides* using four different extraction methods compared with commercial sodium alginates were determined as shown in Table (2).

Moisture content

Moisture content affects alginates shelf life, the lower moisture content caused longer its shelf life. The lower moisture content of sodium alginates powder from *Sargassum muticum* was 14.03±12.18% using Hot extraction method compared with moisture content of commercial alginates which was 15.61±0.28%. The lower moisture content of sodium alginates powder from *Turbinaria conoides* was 14.55±0.25% using $CaCl_2$ extraction method compared with commercial alginates 15.61±0.28%. It was concluded that the lower moisture content was for alginates extracted from *Sargassum muticum* using Hot extraction method this may be due to different in structure for both species. Also, sodium alginates extracted from two species *Sargassum muticum* and *Turbinaria conoides* had moisture content lower than commercial this meaning that those products might have long shelf life due to decrease microbial activity and growth of fungi [21].

Protein content

The protein content for sodium alginates extracted from *Sargassum muticum* ranged from 0.98 to 1.66% using different extraction methods. The maximum protein content 1.66±0.057% was found by using EDTA extraction method, followed by $CaCl_2$ 1.5±0.057%, HCl 1.23±0.05% and Hot extraction method 0.98±0.002% whereas Hot extraction method showed the minimum protein content. Moreover, sodium alginates extracted from *Turbinaria conoides* had the highest protein content 3.64±0.13% using EDTA extraction method which was higher than commercial sodium alginates followed by $CaCl_2$

3.1±0.59%, Hot 1.85±0.055% and HCl 1.7±0.005% methods. This may be due to that protein content change with different genera and species of the some genus [22].

Table 2: Chemical composition of sodium alginate using different extraction methods compared with commercial one

Parameters	*Sargassum muticum*				*Turbinaria conoides*				Commercial
	HCl	Hot	$CaCl_2$	EDTA	HCl	Hot	$CaCl_2$	EDTA	
Moisture %	17.90± 0.3^e	$14.0±12.18^b$	$18.08±0.44^f$	$19.0±1.20^g$	$18.02±0.45^f$	$16.6±0.26^d$	$14.55±0.3^b$	$13.73±0.3^a$	$15.61±0.28^c$
Protein %	$1.23±0.05^{ef}$	0.98±0 $.002^e$	$1.53±0.057^d$	$1.66±0.05^d$	$1.70±0.005^d$	$1.85±0.05^d$	$3.10±0.6^b$	$3.64±0.13^a$	$2.33±0.09^c$
Fat %	0.1±0 $.005^f$	$0.15±0.005^{ef}$	$0.21±0.01^{de}$	$0.2±0.011^d$	$0.86±0.057^a$	$0.20±0.1^{de}$	$0.46±0.05^b$	$0.01±0.0^g$	0.37 $±0.017^c$
Carbohydrate%	$72.77±0.5^c$	$78.31±2.03^c$	$72.68±0.47^d$	$74.83±1.0^d$	$74.26±0.13^c$	$75.82±0.5^b$	$76.96±0.3^c$	$78.09±0.4^a$	$78.09±0.60^a$
Ash %	$8.0±0.30^a$	$6.53±0.25^b$	$7.50±0.45^a$	$4.26±0.46^{ef}$	$5.16±0.55^{cd}$	$5.53±0.20^c$	$4.93±0.6^{ed}$	$4.60±0.10^e$	$3.60±0.1^f$
FIBRES	$7.47±0.38^d$	$2.28±0.19^g$	$8.98±0.086^c$	$10.24±0.2^b$	6.50± 0.200^e	$3.70±0.21^f$	$8.66±0.10^c$	$13.40±0.4^a$	$3.63±0.608^f$

Different superscripts within a row indicate significant differences among treatments ($p < 0.05$).
Data shown is the mean ± SD, n=3.

Fat content

Fat content for sodium alginates extracted from *Sargassum muticum* ranged from (0.1-0.25%). The maximum fat content 0.25±0.011% was recorded by using EDTA method followed by $CaCl_2$ 0.21±0.011%, Hot method 0.15±0.005% and HCl method 0.10±0.005%. However, sodium alginates extracted from *Turbinaria conoides* had the maximum fat content 0.86±0.057% using HCl method followed by $CaCl_2$ 0.46±0.57%, Hot method 0.2±0.10% and EDTA 0.01±0.0% compared with commercial sodium alginates 0.37±0.017% theses results may be due to different algae types.

Carbohydrates content

Carbohydrates content of sodium alginates extracted from *Sargassum muticum* ranged from 72.68 to 78.31%, the maximum carbohydrates content recorded 78.31±2.03% using Hot method followed by EDTA 74.83±1.09%, HCl 72.77±0.57% and $CaCl_2$ 72.68±0.47% extraction methods. However, sodium alginates extracted from *Turbinaria conoides* had the maximum carbohydrates content 78.09±0.44% using EDTA extraction method followed by $CaCl_2$ 76.96±0.36%, Hot 75.82±0.59% and HCl 74.26±0.129% extraction methods compared with commercial sodium alginates 78.09±0.44%.

Ash content

Mineral salts of sodium alginates differ in each extraction methods and it could be found on surface or thallus of algae. Sodium alginates extracted from *Sargassum muticum* had the highest ash content 8.0±0.3% using HCl method followed by $CaCl_2$ 7.5±0.45%, Hot 6.53±0.25% and EDTA 4.26±0.46% extraction methods. The maximum ash content for sodium alginates extracted from *Turbinaria conoides* was 5.53±0.2% using Hot extraction method followed by HCl 5.16±0.55%, $CaCl_2$ 4.93±0.66% and EDTA 4.6±0.1% extraction methods compared with commercial sodium alginates 3.6±0.1%. The high percentage of ash indicated that samples were contaminated by calcareous organisms [23].

Fibers content

The maximum fibers content for sodium alginates extracted from *Sargassum muticum* was 10.24±0.25% using EDTA extraction method followed by $CaCl_2$ 8.98±0.086%, HCl 7.47±0.38% and Hot 2.28±0.19% extraction methods. However, the maximum value of sodium alginates extracted from *Turbinaria conoides* was 13.4± 0.44% using by EDTA method followed by $CaCl_2$ 8.66±0.1%, HCl 6.5±0.2% and Hot extraction method 3.7±0.208% compared with commercial sodium alginates 3.63±0.608%.

3.3 Effect of extraction methods on the minerals content of extracted sodium alginates

Seven minerals (Zn, K, Mg, Mn, Na, Ca, and P) were measured for sodium alginates extracted from *Sargassum muticum* and *Turbinaria conoides* compare with commercial sodium alginates as shown in Table (3). The amount of minerals (Zn, K, Mg, Mn, Na, Ca, and P) for commercial sodium alginates were (0.45, 0.40, 57.10, 0.08, 11.11, 44.03 and 0.34 g/Kg) respectively.

The amount of Zn for sodium alginates extracted from *Sargassum muticum* ranged from 0.27 to 0.40 g/Kg using different extraction methods, and for *Turbinaria conoides* ranged from 0.54 to 0.67 g/Kg. The highest amount of Zn 0.67g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using $CaCl_2$ extraction method.

The amount of K for sodium alginates extracted from *Sargassum muticum* ranged from 0.01 to 0.25 g/Kg using different extraction methods, for *Turbinaria conoides* ranged from 0.06 to 0.78 g/Kg. The highest amount of K 0.87 g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using EDTA extraction method.

While, the amount of Mg for sodium alginates extracted from *Sargassum muticum* ranged from 23.71 to 54.09 g/Kg using different extraction methods, and for *Turbinaria conoides* ranged from 34.85 to 81.07 g/Kg. The highest amount of Mg 81.07 g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using HCl extraction method.

The highest amount of Mn 0.82 g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using EDTA. The amount of Mn for sodium alginates extracted from *Sargassum muticum* ranged from 0.05 to 0.2 g/Kg using different extraction methods, for *Turbinaria conoides* ranged from 0.13 to 0.82 g/Kg.

The amount of Na for sodium alginates extracted from *Sargassum muticum* ranged from 8.33 to 15.05 g/Kg using different extraction methods, for *Turbinaria conoides* ranged from 11.20 to 14.72 g/Kg. The highest amount of Na 15.05 g/Kg was found in sodium alginates extracted from *Sargassum muticum* using $CaCl_2$ extraction method. The amount of Ca for sodium alginates extracted from *Sargassum muticum* ranged from 12.91 to 23.40 g/Kg using different extraction methods, for *Turbinaria conoides* ranged from 12.36 to 47.98 g/Kg. The highest amount of Ca 47.98 g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using EDTA extraction method.

The amount of P for sodium alginates extracted from *Sargassum muticum* ranged from 0.25 to 0.40 g/Kg using different extraction methods, for *Turbinaria conoides* ranged from 0.02 to 0.47 g/Kg. The highest amount of P 0.47 g/Kg was found in sodium alginates extracted from *Turbinaria conoides* using HCL extraction method.

The difference of minerals amounts may be due to the effect of geographical location and season of algae harvesting. The diversity in Mg amounts could be attributed to the fact that element has destroyed during processing. The differences in the Mn amounts could be traced to the possible indigestion during process [22].

Table 3: Minerals content of sodium alginate using different extraction methods compared with commercial one.

Minerals	Sargassum.sp				Turbinaria				Commercial
	HCl	Hot	CaCl2	EDTA	HCl	Hot	CaCl2	EDTA	
Zn	0.4±0.05^{e}	0.32±.01^{f}	0.27±0.02^{g}	0.35±0.01^{ef}	0.62±0.00^{b}	0.54±0.00^{c}	0.67±0.01^{a}	0.59 ±0.0^{b}	0.45 ±.02 d
K	0.18 ± 0.01^{e}	0.01± 0.0^{g}	0.25±0.01^{c}	0.17±0.00^{e}	0.23±0.00^{d}	0.06±0.00^{f}	0.24 ± 00^{cd}	0.78 ±0.01^{a}	0.40 ± .00^{d}
Mg	30.33± 0.47^{h}	32.54±1.3^{g}	23.71±1.82^{i}	54.09±1.54^{c}	81.07±0.44^{a}	34.85± 0.12^{f}	48.59±0.34^{d}	41.84±0.42^{e}	57.10± .47^{b}
Mn	0.2±0.05^{c}	0.16±0.05^{c}	0.09±0.0^{de}	0.05±0.00^{e}	0.27 ± 0.00^{b}	0.25±0.01^{b}	0.13± 0.00^{cd}	0.82±0.02^{a}	0.08± .01de
Na	13.5± 0.57^{c}	8.33±0.28^{e}	15.05±.04^{a}	14.23±0.63^{b}	11.22±.41^{ab}	11.2±0.1^{d}	15.12±0.11^{a}	14.72±0.36^{ab}	11.11±.20^{d}
Ca	15.89±0.09^{e}	23.40±2.90^{d}	13.46±0.4^{df}	12.91±0.78^{f}	12.63±.06^{f}	37.59±0.60^{a}	15.79 ±0.46^{e}	47.98±0.34^{b}	44.03±1.4^{c}
P	0.25 ±0.00^{d}	0.26±0.05^{d}	0.40±0.00^{b}	0.33±0.0^{c}	0.47±0.01^{a}	0.08 ± 0.00^{e}	0.02 ± 0.00^{f}	0.08 ± 0.02^{e}	0.34 ±.04^{c}

Different superscripts within a row indicate significant differences among treatments ($p < 0.05$). Data shown is the mean ± SD, n=3.

3.4 Effect of extraction method on physical properties of sodium alginates extracted from *Sargassum muticum* and *Turbinaria conoides*

Effect of different extraction methods on physical properties of extracted sodium alginates was shown in Table (4). The results indicated that the gelling point of sodium alginates extracted from *Sargassum muticum* was (35.66, 40.66, 46.6 and 34.65 °C) using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively.** However gelling point was (39.33, 30.66, 33.34 and 35.35 °C) for sodium alginates extracted from *Turbinaria conoides* using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively, compared with** commercial sodium alginates 41°C.

The melting point of sodium alginates extracted from *Sargassum muticum* was (85.62, 90.65, 85.60 and 88.60 °C) using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively.** However melting point was (80.66, 90.67, 92.60 and 85.33 °C) for sodium alginates extracted from *Turbinaria conoides* using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively, compared with** commercial sodium alginates 89.05°C.

Viscosity of sodium alginates extracted from *Sargassum muticum* was (31.66, 38.33, 48.35 and 58.32 cp) using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively.** Whereas viscosity of sodium alginates extracted from *Turbinaria conoides* was (60.33, 80.25, 100.53 and 90.66 cp) using **HCl, Hot, $CaCl_2$, and EDTA extraction methods, respectively.**

The viscosity of sodium alginates extracted from *Turbinaria conoides* was higher than that extracted from *Sargassum muticum* compared with commercial sodium alginates (97.66 cp). The higher viscosity was for sodium alginates extracted from *Turbinaria conoides* (100.53 cp) using **$CaCl_2$ extraction method which is higher than** commercial sodium alginates (97.66 cp), the low sodium alginates viscosity was caused by the low purity of sodium alginates produced. Viscosity of sodium alginate can also be influenced by aquatic conditions, pH, salinity, light, depth of the water from which the seaweed is extracted and nutrients.

Table 4: Physical properties of extracted sodium alginates using different extraction methods

Properties	*Sargassum muticum*				*Turbinaria conoides*				commercial
	HCl	Hot	$CaCl_2$	EDTA	HCl	Hot	$CaCl_2$	EDTA	
Gelling point (°C)	35.66 ±1.15^{c}	40.66 ±1.15^{b}	46.60 ±1.14^{a}	34.65 ±1.15^{d}	39.33 ±1.15^{b}	30.66 ±1.13^{e}	33.34 ±1.14^{d}	35.32 ±1.10^{cd}	41.00 ± 0.00^{b}
Melting point (°C)	85.62 ±1.15^{c}	90.65 ±1.14^{ab}	85.60 ±2.30^{c}	88.60 ±1.17^{d}	80.66± 1.18^{c}	90.67 ±1.15^{ab}	92.60 ±1.14^{a}	85.33 ±1.15^{c}	89.05 ±0.00^{b}
Viscosity (cp)	31.66 ±2.88^{g}	38.33 ±2.67^{f}	48.35 ±2.1^{e}	58.32 ±2.65^{d}	60.33 ±1.12^{d}	80.25 ±1.15^{c}	100.53 ±1.14^{a}	90.66 ± 0.57^{b}	97.66 ± 0.57^{a}

Different superscripts within a row indicate significant differences among treatments ($p < 0.05$). Data shown is the mean ± SD, n=3.

3.5 FT-IR of different extracted sodium alginates from *Sargassum muticum* and *Turbinaria conoides*

The quantitive information is very important to describe function groups and composition of sodium alginates. This parameter was analyzed using the FT-IR spectra for sodium alginates extracted from (*Sargassum muticum* and *Turbinaria conoides)* and commercial sodium alginates in the range 4000 - 400 cm^{-1} as shown in Fig. (1).

Eight characteristic bonds were found in commercial sodium alginates as shown in Fig. (1-i). 1619.91 cm^{-1} referred to carboxylic ester band with symmetric stretching [24]. According to [25], 3442.31 cm^{-1} is assigned to stretching vibrations of O-H bonds and C-H stretching vibrations are assigned 2930.31 cm^{-1}. The two bands 1093.44 cm^{-1} and 1029.8 cm^{-1} may be corresponded to homopolymannuronate which has been attributed to pyranosyl ring and C-O stretching vibration [25]. The band 943.985 cm^{-1} is assigned to C-O stretching vibration of uronic acid residues [26], 877.452 cm^{-1} is assigned to the C1-H deformation vibration of β-mannuronic acid residues [24]. Finally, the band 815.742 cm^{-1} is characteristic of mannuronic acid residues [27].

The FT-IR spectra of sodium alginates extracted from *Sargassum muticum* with four different extraction methods shown in Fig. (1 a-d). Sodium alginates extracted using HCl method, presented seven characteristics bands in Fig. (1-a). The band 3462.56 cm^{-1} is assigned to O-H bonds stretching vibrations, and 2926.45 cm^{-1} are assigned to C-H stretching vibrations. 1624.73 cm^{-1} corresponds with symmetric stretching carboxylic ester band. The two bands 1093.44 cm^{-1} and 1027.87 cm^{-1} may be corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-O stretching vibrations, [25]. The bands 875.524 cm^{-1} and 849.49 cm^{-1} are characteristics of mannuronic acid residues [27].

Sodium alginates extracted using Hot method, showed five characteristics bands in Fig. (1-b). The band 3438.46 cm^{-1} is assigned to O-H bonds stretching vibrations, 2926.45 cm^{-1} is assigned to stretching vibrations of aliphatic C-H. 1624.73cm^{-1} corresponds to a symmetric stretching carboxylic ester band [24], and the band 1030.77 cm^{-1} corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-O stretching vibrations, [25]. The band 876.488 cm^{-1} is characteristic of mannuronic acid residues [27].

Sodium alginates extracted using $CaCl_2$ method, presented two characteristics bands in Fig. (1-c). 1687.41 cm^{-1} corresponds to a symmetric stretching carboxylic ester band [24] and 3462.56 cm^{-1} is assigned to O-H bonds stretching vibrations [25].

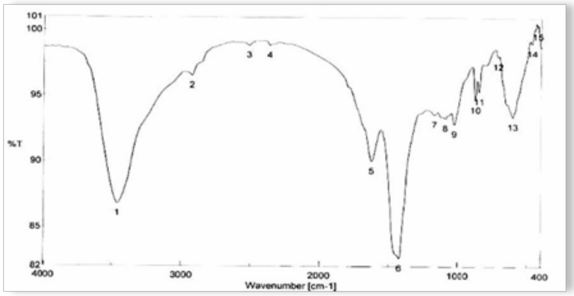
Sodium alginates extracted using EDTA method, the extracted sodium alginates presented six characteristics bands in Fig. (1-d). According to [25] Two bands 3469.31 cm^{-1} and 3063.37 cm^{-1} are assigned to O-H bonds stretching vibrations, 1458.89 cm^{-1} is assigned to symmetric stretching vibration of carboxylate salt ion, and the band 1019.19 cm^{-1} may be corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-O stretching vibrations. 1695.12cm^{-1} is assigned to a symmetric stretching carboxylic ester band [24]. The band 852.382 cm^{-1} is characteristic of mannuronic acid residues [27].

Sodium alginates extracted from *Turbinaria conoides* with four different extraction methods presented in Fig. (1 e-h). Sodium alginates extracted using HCl method presented four characteristics bands in Fig. (1-e). According to [25] the band 3463.53 cm^{-1} is assigned to O-H bonds stretching vibrations, 1633.41 cm^{-1} corresponds to a symmetric stretching carboxylic ester band [24]. The two bands 1064.51 cm^{-1} and 1028.84 cm^{-1} may be corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-Ostretching vibrations, [25].

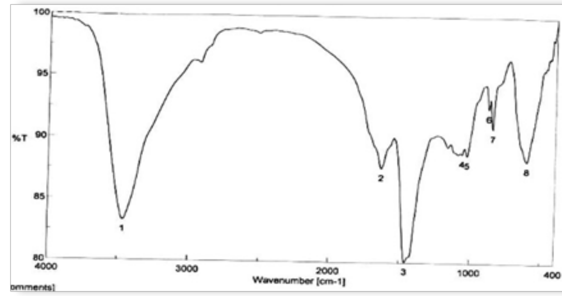
Sodium alginates extracted using Hot extraction method presented four characteristics bands in Fig. (1-f). The band 3438.6 cm^{-1} is assigned to O-H bonds stretching vibrations, and 2925.48 cm^{-1} is assigned to C-H stretching vibrations. 1621.84 cm^{-1} corresponds to a symmetric stretching carboxylic ester band [24]. The band 1030.77 cm^{-1} is corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-Ostretching vibrations, [25].

Sodium alginates extracted using $CaCl_2$ method presented five characteristics bands in Fig. (1-g). 1590.02 cm^{-1} corresponds to a symmetric stretching carboxylic ester band [24] and 3434.6 cm^{-1} is assigned to O-H bonds stretching vibrations and 2931.27 cm^{-1} is assigned to C-H stretching vibrations [25]. The band 1419.35 cm^{-1} is assigned to a symmetrical stretching of COO [28]. The band 1030.77 cm^{-1} is corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-Ostretching vibrations, [25].

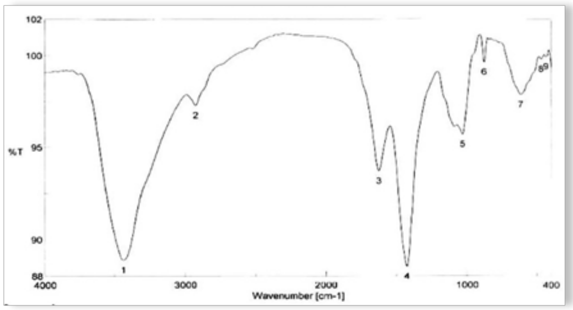
Extracted sodium alginates using EDTA method presented seven characteristics bands in Fig. (1-h). According to [25] The band 3467.38 cm^{-1} is assigned to O-H bonds stretching vibrations, 1455.99 cm^{-1} is assigned to symmetric stretching vibration of carboxylate salt ion, and the bands 1182.15 cm^{-1} and 1116.58 cm^{-1} may be corresponded to homopolymannuronate which was attributed to the C-O stretching vibration of pyranosyl ring and the C-Ostretching vibrations. 1635.34 cm^{-1} is assigned to a symmetric stretching carboxylic ester band [24]. 1024.02 cm^{-1} is assigned to C-O-C bonds stretching high guluronic acide. The band 849.49 cm^{-1} is characteristic of mannuronic acid residues [27].



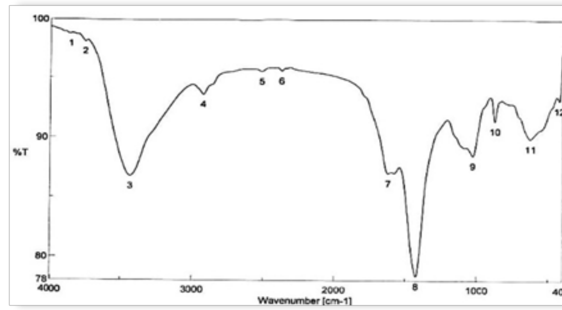
a- *Sargassum muticum* HCl



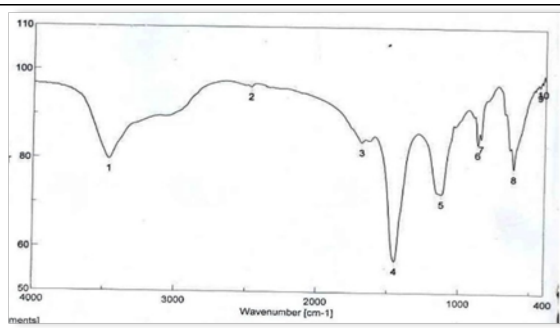
e- *Turbinaria conoides* HCl



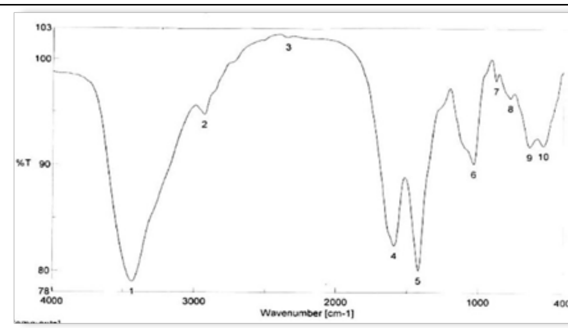
b- *Sargassum muticum* Hot



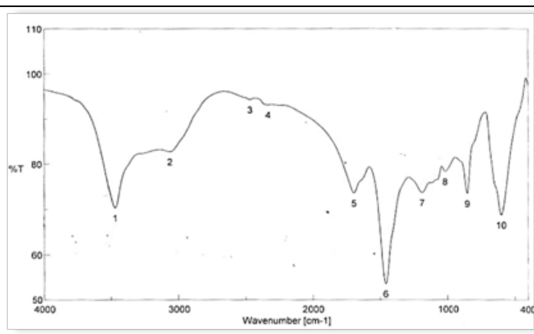
f- *Turbinaria conoides* Hot



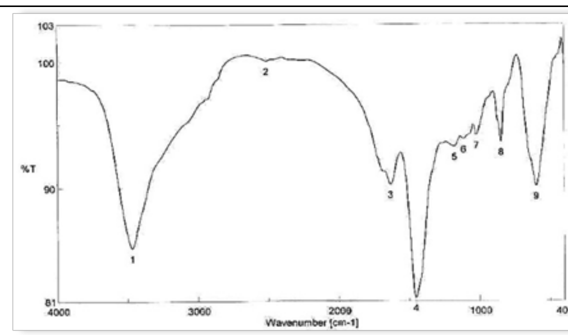
c- *Sargassum muticum* $CaCl_2$



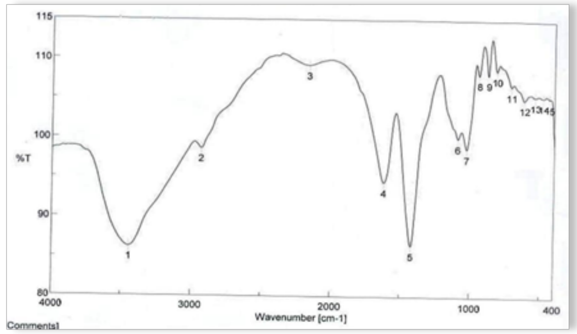
g- *Turbinaria conoides* $CaCl_2$



d- Sargassum muticum EDTA



h- *Turbinaria conoides* EDTA



i- Commercial sodium alginates

Fig.1 FT-IR for sodium alginates using different extraction methods compared with commercial one.

3.7 M/G ratio of alginates

The M/G ratio is an important value for the nature gel formed from alginates [19] and this property became very important in industrial applications. Table (5) illustrated the blocks composition and M/G ratio of alginates extracted from *Sargassum muticum* and *Turbinaria conoides* using four extraction methods (HCl, Hot, $CaCl_2$ and EDTA) and commercial sodium alginates.

M/G ratio can differ in brown algae alginates from 0.5 to 2.5 due to heterogeneity between chain length and distribution of G and M blocks [29]. Mannuronic to guluronic acid ratio is an indicator of the nature of gels produced and these different M/G ratios mean that alginates obtained from different algae and extraction methods would have different physicochemical properties. It was found that alginic acid with a low M/G ratio (<1) and a large proportion of guluronic acid blocks, forms a strong and rigid gel [3]. On the other side, alginates with a low number of guluronic acid blocks and a high M/G ratio (>1) has soft and elastic gel [30]. This heterogeneity of alginates can be useful in many food and non food industrial applications [26]. The results indicated that sodium alginates extracted from *Turbinaria conoides* was higher than from sodium alginates extracted *Sargassum muticum* for all extraction methods used, so it is recommended to use the highest value of M/G ratio for edible coating of food. The results of *Turbinaria conoides* were correlated with substantially higher levels of MM blocks compared to GG ones as the level of FMG was very low. The low amount of alternating blocks (MG and GM) was in agreement with alginates from *Sargassum vulgare* and *Sargassum latifolum* [23], [21].

Table 5: Compositional data of sodium alginates using different extraction methods compared with commercial one.

METHODES	Sargassum muticum						Turbinaria conoides					
	F_M	F_G	M/G	F_{MM}	F_{MG}	F_{GG}	F_M	F_G	M/G	F_{MM}	F_{MG}	F_{GG}
HCl	0.52	0.46	1.13	0.47	0.05	0.41	0.58	0.43	1.35	0.57	0.01	0.42
Hot	0.42	0.58	0.72	0.41	0.01	0.57	0.53	0.50	1.06	0.50	0.03	0.47
$CaCl_2$	0.41	0.59	0.69	0.33	0.08	0.51	0.59	0.41	1.44	0.49	0.10	0.31
EDTA	0.45	0.56	0.80	0.44	0.01	0.55	0.63	0.35	1.80	0.61	0.02	0.33
Commercial	0.64	0.42	1.52	0.58	0.06	0.36						

F_G guluronic fraction, F_M mannoronic fraction, F_{MG} heteropolymeric blocks, F_{MM} homopolymeric mannoronic, F_{GG} homopolymeric guluronic blocks

3.6 Intrinsic viscosity and molecular weight

Viscosity measurement is important for determination of the molecular weight of alginates. The intrinsic viscosity is dependent on the method of extraction. Table (6) shows that the sodium alginates extracted from *Turbinaria conoides* had higher intrinsic viscosity for all extraction methods compared with sodium alginates extracted from *Sargassum muticum*.

The highest intrinsic viscosity was for sodium alginates extracted from *Turbinaria conoides* using EDTA method, this may be due to that different extraction methods affects in the intrinsic viscosity of extracted alginates [4].

Table 6: Intirsinc viscosity and molecular weight of sodium alginates using different extraction methods compared with commercial one.

parameters	Intrinsic Viscosity { µ }(dL/g)				Molecular weight M_w ×10^5 (g/mol)			
	HCl	Hot	$CaCl_2$	EDTA	HCl	Hot	$CaCl_2$	EDTA
Extracted sodium alginates *Turbinaria conoides*	18.649	27.321	29.377	31.12	2.79	2.45	2.64	1.67
Extracted sodium alginates *Sargassum muticum*	6.8033	3.8082	3.0471	9.0688	0.81	0.34	0.27	0.61
Commercial sodium alginates	18.241				1.64			

The molecular weight of sodium alginates extracted from *Sargassum muticum* and *Turbinaria conoides* were estimated from intirsinc viscosity using equation (6) as shown in Table (6). The molecular weight for sodium alginates extracted from *Sargassum muticum* were lower than the values for *Turbinaria conoides* and the highest value obtained from *Turbinaria conoides* using HCL method. Molecular weight of sodium alginates extracted from brown algae was between 0.032×10^5 and 4 ×10^5 gm/mol [21].

4. Conclusion

The sodium alginates were extracted from *Sargassum muticum* and *Turbinaria conoides* were collected from Mediterranean sea and Red sea. The sodium alginates was extracted and purified using four extraction methods (HCl, Hot, $CaCl_2$ and EDTA). The extraction yield, chemical components, melting point, gelling point, viscosity, intrinsic viscosity, molecular weight, M/G ratio were determined and characterized by FT-IR spectrophotometer and compared with commercial sodium alginates. The maximum yield of extracted sodium alginates from *Turbinaria conoides* using EDTA method was 49.33%. The gelling point for sodium alginates extracted from *Sargassum muticum* using four extraction methods ranged from 34.65 to 46.60 ºC, melting point ranged from 85.6 to 90.65 ºC and viscosity ranged from 31.66 to 58.32 cp. On the other hand sodium alginates extracted from *Turbinaria conoides* had gelling point ranged from 30.66 to 39.33 ºC, melting point ranged from 80.66 to 92.60 ºC and viscosity ranged from 60.33 to 100.53 cp. The M/G ratio for sodium alginates extracted from *Turbinaria conoides* was higher than *Sargassum muticum* for all extraction methods. The highest intrinsic viscosity was 31.12 dL/g for sodium alginates extracted from *Turbinaria conoides* using EDTA method. The molecular weight for sodium alginatess extracted from *Sargassum muticum* ranged from 0.27×10^5 to 0.81×10^5 g/mol and for *Turbinaria conoides* ranged from 1.67×10^5 to 2.79×10^5 g/mol.

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