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Effect of high voltage electrode discharge on the physicochemical characteristics of alginate extracted from an Iranian brown seaweed (*Nizimuddinina zanardini*)

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Abstract

Alginic acids and alginates have important applications in food, medicine, pharmaceutical industry, dentistry and textile. The sodium alginate from *Nizimuddinia zanardini* (an Iranian brown seaweed) was extracted with high voltage electrical discharge to investigate the influence of this extraction method on its structural and physico-chemical characteristics. The extracted alginate had a M/G ratio of 1.22, a molecular weight of 119 kDa, a polydispersity index of 2.7 and an intrinsic viscosity of 170.8 mL/g. The rheological properties of an alginate solution (2 % w/v) indicated a Newtonian fluid type of behavior. The influence of pH on the flow behavior of this solution was investigated. The solution had a Newtonian behavior at pH 7.0, an intermediary behavior between Newtonian and non-Newtonian shear thinning at pH 6.0 and 5.0 and a clearly shear thinning behavior at lower pH values (4.5 and 3.0). Its antioxidant activity was tested by **diphenyl picrylhydrazyl** radical scavenging and hydroxyl radical-scavenging activity showing its potential for food preservation. High voltage electrical discharge improved the antioxidant properties explained by the co-extraction of phenolic compounds. The stability of emulsion increased with concentration of alginate and was reported at 94.7%±0.1 at 2 % (w/v) concentration. The stability of emulsions was not affected by temperatures up to 55 °C and was resistant to pH changes.

Keywords: *Nizimuddinia zanardini*; Alginate; High voltage electrode discharge; Rheology; Emulsion; Antioxidant

1. Introduction

Seaweeds are one of the renewable resources of marine environment, which have been used as a source of hydrocolloids and bio-compounds for centuries [1,2,3]. There are about 9,000 species of marine macroalgae, which are broadly classified in three major groups: brown, red, and green algae based on their pigment contents [4]. Brown algae is a rich source of alginic acid, laminarin and fucoidan with biological and physico-chemical activities. Each combination of these biopolymers has its own unique properties and characteristics [5]. Alginate is an anionic polysaccharide, a major structural compound in the cell wall of brown algae, which makes them flexible. It is a linear polymer of β -D-mannuronic acid (M with 4C_1 ring conformation) and α -L-guluronic acid (G with 1C_4 ring conformation) linked by (1,4) glycosidic bonds [6]. The two uronic acids are in pyranosic conformation. The arrangement of the monomers creates homogeneous MM and GG blocks only composed of one uronic acid species and heterogeneous MG (or GM) blocks where the two monomers are placed side by side [7,8]. The biological and physico-chemical properties of alginates depend on the number and the arrangement of these blocks in the polysaccharidic backbone, which varies depending on the species of seaweed, the type of tissues and the age of the algae [9]. Alginates with high M and MM blocks contents have a higher flexibility than those rich in GG blocks which are more rigid and give high viscous solution. This polysaccharide has a unique ability to selectively chelate divalent cations in the following order $Pb^{+2} < Cu^{+2} < Ba^{+2} < Sr^{+2} < Ca^{+2} < Mn^{+2} < Mg^{+2}$ to form hydrogels [10,11].

The well-known ability of alginates to form strong hydrogels in the presence of Ca^{+2} ions is related to GG blocks and well described in literature by the egg-box model [12]. Therefore, a high G/M ratio leads to an increase in gel strength, while a lower one produces a more

elastic gel stable even with high amounts of Ca^{2+} ions [13,14]. Alginic acid and its salts have many biological and industrial applications due to their carboxylate functional groups. They are used in food, pharmaceutical and health industries as hydrogels, thickeners, stabilizers and additives. This polymer has been widely used as a factor of increasing viscosity in the textile printing industry, for the use of wound dressings, dental molds and in the formulation of materials with the possibility of preventing gastric reflux [15,16,17]. High-power electrical energy (several tens of kilojoules) is used in high-voltage electrical discharge method. If the electric field is strong enough, an electron avalanche will be the starting point for the spread of the streamer from the high-voltage needle electrode to the plate electrode. Electrical decomposition is associated with secondary phenomena such as high-pressure shock waves, bubble cavitation, and fluid turbulence. These secondary phenomena cause partial decomposition and damage to the cell wall, which accelerates the extraction of biomolecules from cell cytoplasm [18]. In summary, in this method, a pulsed streamer discharge in solvents in the pool chamber is usually accompanied with production of localized high electric fields, shockwaves, cavitations, extreme turbulence in the fluid, releasing dense active radicals (ozone and hydroxide radicals), ultraviolet light, etc. These secondary phenomena cause a cell structure damage, such that the cell is severely destroyed and accelerate the extraction of active substance inside the cell which can diffuse into the solvent [19,20].

The use of renewable and biosourced emulsifiers is more interesting than their chemical counterparts because they are biodegradable. The biosourced emulsifiers are characterized by their functional characteristics and stability due to their wide variety in compositions

and structures. Accordingly, they have a wide range of applications, including in detergents, paints, cosmetics, medicines and food processes.

Nizamuddinina zanardinii is a brown seaweed belonging to the Sargassaceae family according to Algae Base (<https://www.algaebase.org/>). It is the only known species of *Nizamuddinina* genus. It grows in southwest Asia, Arabian Sea coasts (Oman, Yemen and Pakistan), Persian gulf (Qatar) and in the Oman Sea and Persian Gulf (Chabahar and Ghesm Islands, Iran). The first report on extraction, structural characterization, and shear flow properties evaluation of the alginate extracted from this macroalgae has already been published by our team [21]. The chemical composition and molecular structure of hydrocolloids often depend on the extraction process. The innovative technologies such as high voltage electric discharge (HVED) compared with the conventional methods can significantly enhance yields, improve efficiency, reduce solvent and energy consumption, and are environmentally friendly [20]. The technology of electrical discharges has been recently developed for enhancing extraction of biocompounds from different raw materials [19,22]. In this study, the effect of high voltage electrode discharge was investigated on the structural and functional properties of sodium alginate such as gelling properties and physical behaviors of gums, emulsifying agent and antioxidant.

2. Materials and methods

Nizamuddinina zanardinii was collected in December 2015 from the shores of the Oman Sea in southern Iran (Chabahar Bay 25°20'53" N and 60°28'1" E) and its taxonomic characteristics were determined by the Off-Shore Fisheries Research Center. After washing

with fresh water and removing impurities, it was dried by sunlight. The dried algae was then ground and kept in sealed bags until use.

2.1. Extraction of alginate by High Voltage Electrode Discharge (HVED)

A needle and a plate electrode (20 mm diameter) were used prior to alginate extraction. Algae powder (5 gr) with a volume of hydrochloric acid (0.2 M) that completely covers the surface of the powder was placed in the chamber. The distance between the needle electrode and the surface of the solution was 2 mm and an electric current of 10 KV was used. The current was cut off and connected by the device button and manually to cause an electrical discharge. One second on and 1 second off, and 50 times electrical discharges was applied (the voltage type was DC). The voltage waveform is shown in Figure 1.

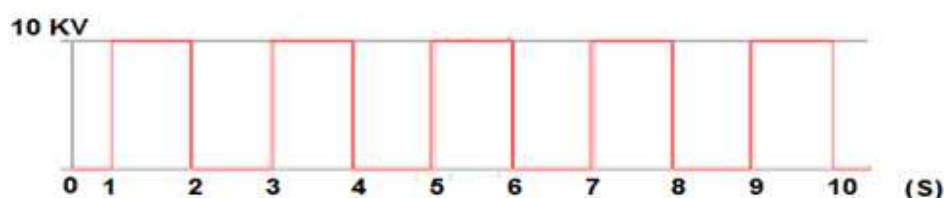


Fig. 1. The voltage waveform

The sample was then centrifuged at 10,000 g for 20 min at room temperature. The precipitate was washed again with deionized water. It was dried and kept in sealed bags until use. The extraction and purification of sodium alginate were then performed according to a method adapted to Abka Khajouei et al., 2018 [21]. Twenty-five grams of pre-treated dried algae were soaked in 800 mL of formaldehyde (2% v/v, 800 mL) at room temperature

and under stirring (200 rpm) for 24 h. The samples were then washed 3 times with MilliQ water to remove the remaining formaldehyde. After centrifugation (14000g, 20 min) and collecting precipitate, HCl (0.2 M, 800 mL) was added at 60 °C and the mixture was incubated under stirring (250 rpm) for three hours. The precipitate was then collected by centrifugation (10,000g, 20 min, 20 °C) and washed with water. Eventually, to dissolve alginic acid, an alkali treatment was performed adding 800 mL of 3% (w/v) sodium carbonate. After 2.5 h at 60°C under stirring (250 rpm) solubilized sodium alginate was separated by centrifugation at 10,000 g in 30 min and precipitated with 3 volumes of ethanol 96% (v/v). After 24 h, the precipitate was collected using vacuum filtration, dissolved with water (500 mL) and precipitated again with 3 volumes of ethanol to increase its degree of purity. Finally, the precipitate was collected as described above, and freeze dried.

2.2. Chromatographic analysis

2.2.1. Molecular weight determination

The weight-average molecular weight (M_w), number-average molecular weight (M_n), intrinsic viscosity, gyration radius (R_g) and hydrodynamic radius (R_h) were determined by High Pressure Size Exclusion Chromatography (HPSEC) fitted with three detectors: a Multi-Angle Laser Light scattering (MALLS), a He\Ne laser at 690 nm and a K5 cell (50 μ L) (HELEOS II Wyatt Technology Corp., USA), a Differential Refractive Index (DRI) (RID 10A Shimadzu, Japan) and a viscometer (Viscostar II, Wyatt Technology Corp., USA). The system included one OHPAK SB-G guard and two OHPAK SB 804 and 806 HQ columns eluted at a flow rate of 0.5 mL/min with LiNO₃ 0.1M degassed after passing

through a 0.1 μm filter. The samples at a concentration of 1 mg/mL were dissolved in the mobile phase for 24 h under stirring and then filtered (0.45 μm) before the injection (500 μL loop). The data collected from this system were analyzed by the Astra software 6.1.7.15 using a dn/dc of 0.15.

2.2.2. High pressure anions exchange chromatography

Hydrolyzed alginates were analyzed by High Pressure Anions Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). HPAEC-PAD analysis was performed with a Dionex ICS-3000 system including a SP- gradient pump, an ED-electrochemical detector working with a gold electrode, an Ag/AgCl reference electrode, and Chromeleon [version 6.5 \(Dionex Corp., Sunnyvale, CA\)](#). The separation of uronic acids was performed at 25 °C by Carbo PAC PA-I guard column (4 x 50 mm) and a column (4 x 250 mm) was connected to each other and eluted at 25°C with a flow rate of 1 mL/min. The eluant was composed of solutions A (100 mM sodium hydroxide (NaOH)) and B (100 mM NaOH and 1 M NaOAC) mixed together to form a linear gradient of solution B in A. Thus, at 0 min, the solution B was at 0% in the mixture and this percentage increased linearly during 60 min up to 100%. After each run, the column was washed for 10 minutes with 100% of B solution. The samples were injected at a concentration of 1 mg/mL through a 25 μL loop.

2.3. Complete acid hydrolysis of alginate

Complete hydrolysis of sodium alginates was performed according to the method described by Chandia et al., 2001 [23]. Ten milligrams of sodium alginate were mixed with 4.5 mL of

formic acid (90% (v/v) and heated in closed tubes for 6 h at 100 °C. The tubes were shaken every hour. The hydrolysate was then diluted with 20 mL of distilled water and heated in reflux for 2 h at 100 °C to complete the hydrolysis. After cooling, the solution was concentrated in a vacuum rotary evaporator to a final volume of 300 μ L.

2.4. Rheological measurements

The rheological properties of the extracted alginate (gel or solution) were measured with an AR-2000 rheometer (TA Instrument, Great Britain, Ltd) equipped with a Peltier temperature control system. First, a sodium alginate solution of 2% (w/v) was prepared and its rheological properties were determined at different pH (3, 4.5, 5, 6, and 7) using a concentric cylinders geometry and at pH 2 by a plate-plate geometry at two temperatures (5 and 20 °C). Viscosity and flow type experiments were performed on the solutions at different shear rates in the range of 10^{-2} to 10^{+3} s^{-1} . For rheological experiments on calcium alginate gel, 20 mL of sodium alginate solution 2% (w/v) were dialyzed using cellulose membranes with a molecular weight cut off of 10,000 Da against 0.1 mM calcium chloride solution for 24 h. After balancing in ion exchange and gel formation, a piece of gel with 4 mm thick was cut and its rheological properties were investigated using a plate-plate geometry. Dynamic tests were performed in linear viscoelastic range at two temperatures (5 and 20 °C). Dynamic modules (G' , G'') and complex viscosity (η^*) were determined as the applied frequency function (rad/s) by the Oscillation frequency sweep test. In this test, the frequency was increased under constant stress in the range 0-400 rad/s. Temperature ramp testing was performed to check the stability of the gel in the range 5- 90 °C. The possibility

of returning the gel texture to its original state was tested again by lowering the temperature from 90 to 5 °C.

2.5. Stability of emulsion

Stability of emulsion was determined by the adapted method of Cooper and Goldenberg, 1987[24]. Briefly, the sodium alginate solution of 2% (w/v) was homogenized in a test tube with an equal volume of canola oil using an ultra Turrax (T18 device IKA GmbH, Germany) at 2500 rpm for 2 min. After 24 h, the emulsion index was calculated as the ratio between the height of the emulsion layer and the initial height of the whole mixture. In order to investigate the effect of alginate concentration, pH and temperature, the stability of emulsion was measured at different concentrations (0.5, 1 and 2 % w/v), pHs (3, 5, 7, 9, and 11) and temperatures (25, 55 and 85 °C). To apply the thermal process, after the solution was homogenized with oil, we put the mixture in the thermostatic bath and kept at desired temperature for 30 min and then cooled the mixture to 20°C.

2.6. Total polyphenols assay

Total polyphenols were measured by the Gutfinger method [25]. The sodium alginate solution (0.1 mL, 0.5% w/v) was added to 4.9 mL of distilled water and then to 0.5 mL of Folin-Ciocalteu reagent. The mixture was stirred during 3 min and a sodium carbonate solution (1 mL, 35% w/v) and deionized water (3.5 mL) were added. The mixture was then stirred again vigorously. After one hour of incubation in dark at room temperature, the A_{725} was measured. Gallic acid was used as standard.

2.7. Antioxidant activities

2.7.1. Diphenyl picrylhydrazyl assay

The antioxidant activity of sodium alginate was determined by the method described by Yamaguchi et al., 1998 using diphenyl picrylhydrazyl (DPPH) [26]. Different concentrations of sodium alginate (0 to 10 mg / mL) were prepared in pure water. One mL of DPPH solution (0.1 mM in 99% ethanol) was added to 1 mL of sample or control solution. The solution was mixed vigorously and incubated in dark at 20°C for half an hour. The A_{517} was read and the DPPH inhibition percentage was calculated using the Equation 1.

$$DPPH\ inhibition(\%) = \left(1 - \left(\frac{A_{sample}}{A_{control}} \right) \right) \times 100 \quad (1)$$

Where A_{sample} is A_{517} of samples and $A_{control}$ is A_{517} of pure water.

Ascorbic acid was used as standard.

2.7.2. Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging activity of sodium alginate was determined as described by Chung et al. [27]. In a screw-capped test tube, 0.2 mL of different concentrations (0.25 to 5 mg/mL) of sodium alginate solution was added to a reaction mixture including 0.2 mL $FeSO_4 \cdot 7H_2O$ (10 mM), 0.2 mL of EDTA (10 mM) and 0.2 mL of D-deoxyribose (10 mM). The reaction mixture was then adjusted to 2 mL with 0.1 M phosphate buffer and 0.2 mL of H_2O_2 (10mM) was added. The mixture was incubated at 37°C for 1 h. Then 1 mL of a trichloroacetic acid (2.8%w/v) and a thiobarbituric acid (1.0% w/v) solution was added and put in boiling water for 10 min. After this incubation, A_{523} was read. Ascorbic acid was

used as standard. The hydroxyl radical-scavenging activity was calculated using Equation 2.

$$OH - scavenging\ activity\ (\%) = \left(1 - \frac{Ab_s - Ab_0}{Ab_c - Ab_0} \right) \times 100$$

(2)

Where Ab_0 is the absorbance with no treatment at 523 nm, Ab_c is the absorbance of treated control (containing all reagents except the sample) at 523 nm and Ab_s is the absorbance of treated sample.

2.8. Statistical analysis

All experiments were conducted as independent triplicates and all analytical measurements were done in triplicate. The results from the triplicates were compared using the ANOVA and Fisher's LSD test ($p \leq 0.05$) via SAS (version 9.4). The standard deviations obtained from the experimental replicates.

3. Results and Discussion

3.1. Sodium alginate extraction

High Voltage Electrode Discharge (HVED) was used to extract sodium alginate as described in the materials and methods section. The yield of alginate extraction was $27 \pm 0.5\%$. It is higher than that of $24\% \pm 0.3$ obtained previously without applying the high voltage [21]. This result can be explained by the positive impact of high voltage causing the break of cell wall, the destruction of the tissue, the penetration of the solution into the cell and the cell wall, thus the extraction percentage of alginate was increased. Researchers

have concluded that as a result of the electrical breakdown between the needle and plate electrodes, air bubbles and high-pressure shock waves (up to 10 kbar) are produced, which causes the cell wall to break down and the compounds can diffuse into the solvent [18,19,20,28]. Recent studies on the use of HVED have also shown that this method increases cell wall damages and further extraction of other intracellular compounds such as proteins, polyphenols and other bio-compounds [29,30].

3.2. Characterization of alginate in diluted regime

Molecular weight distribution of the extracted alginate was investigated by HPSEC-MALLS analysis and the weight-average molecular weight (M_w), number-average molecular weight (M_n), intrinsic viscosity ($[\eta]$), and hydrodynamic radius (R_h) were determined (**Table 1**). These parameters previously reported for M_w , M_n , $[\eta]$ and R_h of sodium alginate extracted from *Nizimuddinia zanardini* without pretreatment were respectively 1.03×10^5 g/mol, 0.84×10^5 g/mol, 342 mL/g and 17.3 nm [21]. The M_w of alginate extracted with the high voltage method was 1.19×10^5 g/mol. This value is not significantly different to that obtained with the classical method of alginate extraction, and is in accordance to those required for commercial alginates from brown algae between 0.32×10^5 to 4×10^5 g/mol [31].

The polydispersity index ($\mathfrak{D} = M_w/M_n$) for the alginate obtained with the high voltage extraction method was 2.7. This value indicates a high polydispersity of this alginate, higher to that of alginate extracted with conventional procedure ($\mathfrak{D} = 1.3$) [21]. This result clearly revealed the low degree of homogeneity of the extracted polysaccharide and that

alginate fragmentation has occurred. The lower M_n of alginate extracted with high voltage method also confirms this fragmentation. Moreover, its hydrodynamic radius is also lower.

Table 1.

Physico-chemical characteristics of alginates extracted from *Nizimuddinia zanardin*

Sample	M_w (g/mol)	M_n (g/mol)	R_h (nm)	Intrinsic viscosity	$\bar{D} = M_w/M_n$
				(mL/g)	
Normal*	103,000	84,000	17.3	342	1.22
HVED**	119,000	43,000	12	170.8	2.7

* Sodium alginate extracted without High Voltage Electrode Discharge **Sodium alginate extracted with High Voltage Electrode Discharge

Chromatograms of HPSEC-MALLS (**Fig. 2**) detected macromolecules not significantly concentrated but having a significant light diffusion ability proving their very high molecular weights corresponding probably to aggregates eluted in the void volume of the column. This confirms that the chain was broken into heterogeneous pieces, which is consistent with the \bar{D} .

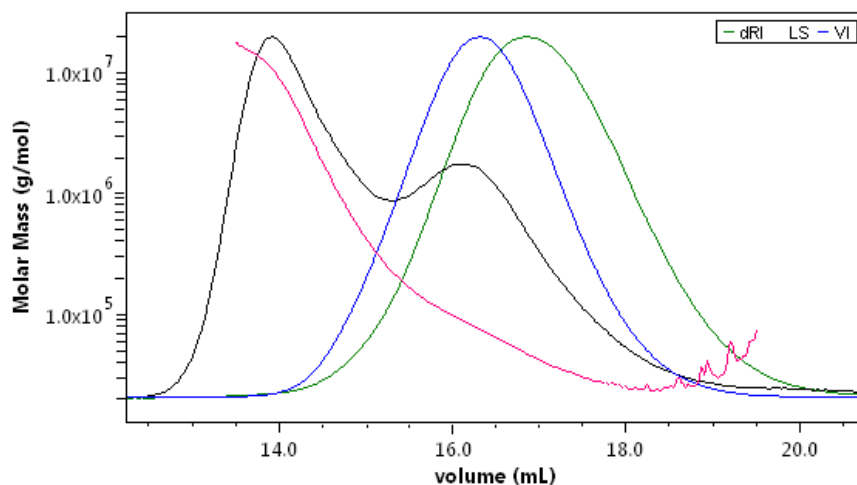


Fig. 2. SEC-MALLS chromatogram of sodium alginate from *Nizimuddinina zanardini*. Extracted by High Voltage Electrode Discharge. (blue line) intrinsic viscosity, (green line) refractive index and (black line) light scattering intensity at the angle 90°

3.3. Complete acid hydrolysis of sodium alginate

Complete hydrolysis was performed by formic acid on sodium alginate extracted from *Nizimuddinina zanardini* and hydrolysis products were analyzed by HPAEC to selectively recognize uronic acids. The results of chromatography showed sodium alginate from studied algae contained D-manururonic and L-guluronic acids with a M/G ratio equal to 1.22. By comparing the result of this sample with previous reports for the same alginate extracted without pretreatment (M/G 1.2) it was found that applying high voltage has logically no effect on the sequence ratio of this biopolymer [21].

3.4. Rheological properties

The study of rheological parameters of alginates are of first importance before using them in various fields, particularly in food industry, as gelling and stabilizing agents. To investigate the rheological properties of the extracted sodium alginate with HVED

pretreatment and to compare it with that from classical method of extraction, flow and oscillatory tests were performed on its solution and gel (2% w/v). The shear stress was drawn against the shear rate to get the best model that describes the behavior of alginate solution. Due to the linear relationship between these two parameters, alginate flow behavior was described using the equation 3 and power law model.

$$T = m\varepsilon^n \quad (3)$$

where T is the shear stress (Pa), ε is the shear rate (s^{-1}), m the consistency index ($Pa \cdot s^n$) and n the flow behavior index.

The model parameters for solution (2 % w/v) of the extracted alginates in different temperatures (5 and 20°C) are shown in **Table 2**. The values of flow behavior index (n) for the solutions of sodium alginate in water are closed to 1.0 in all samples meaning that the polymer exhibited almost Newtonian or very low shear thinning behavior at 2.0 % (w/v) concentration and the extraction method and temperature had no effect on the behavior of the flow (**Fig. 3**).

Table 2 shows that the flow index is independent of the temperature (20 °C vs 5 °C). However, the viscosity of the solution at 5 °C is higher than that obtained at 20 °C which proves that the polymer is sensitive to temperature and is fully compliant with the rules of polymers.

Table 2.

Consistency and flow behaviour index of solution of sodium alginate from *Nizimuddiniana zanardini* in MilliQ water. Data are expressed as the average \pm SD of three independent experiments ($n = 3$).

Sample	T (°C)	<i>m</i>	<i>n</i>	<i>R</i> ²
Normal*	5	0.092± 0.006	0.997± 0.001	0.995
	20	0.064 ± 0.005	0.995 ± 0.002	0.999
HVED**	5	0.071± 0.004	0.996± 0.002	0.997
	20	0.034 ± 0.006	0.995± 0.001	0.999

* Sodium alginate extracted without High Voltage Electrode Discharge ** Sodium alginate extracted with High Voltage Electrode Discharge

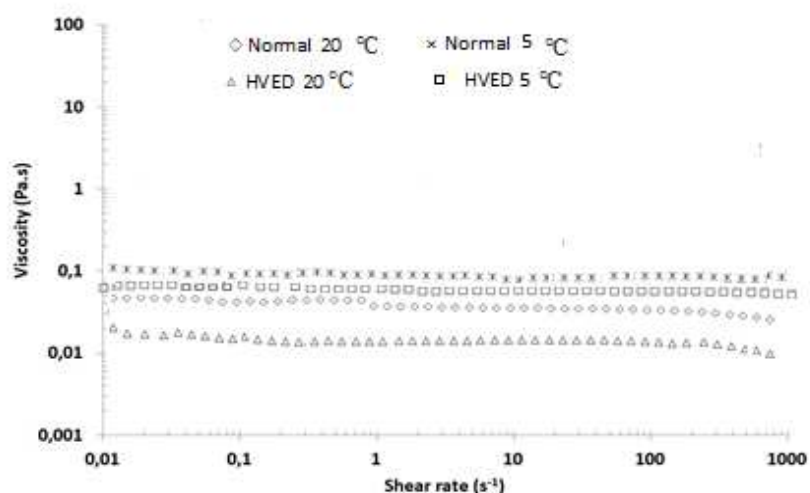


Fig. 3. Apparent viscosity vs shear rate for 2.0 % (w/v) of sodium alginate solutions extracted with High Voltage Electrode Discharge (HVED) pretreatment and without it (Normal) from *Nizimuddiniana zanardini* at 20 °C and 5 °C in MilliQ water. Data are expressed as the average ± SD of three independent experiments (n = 3).

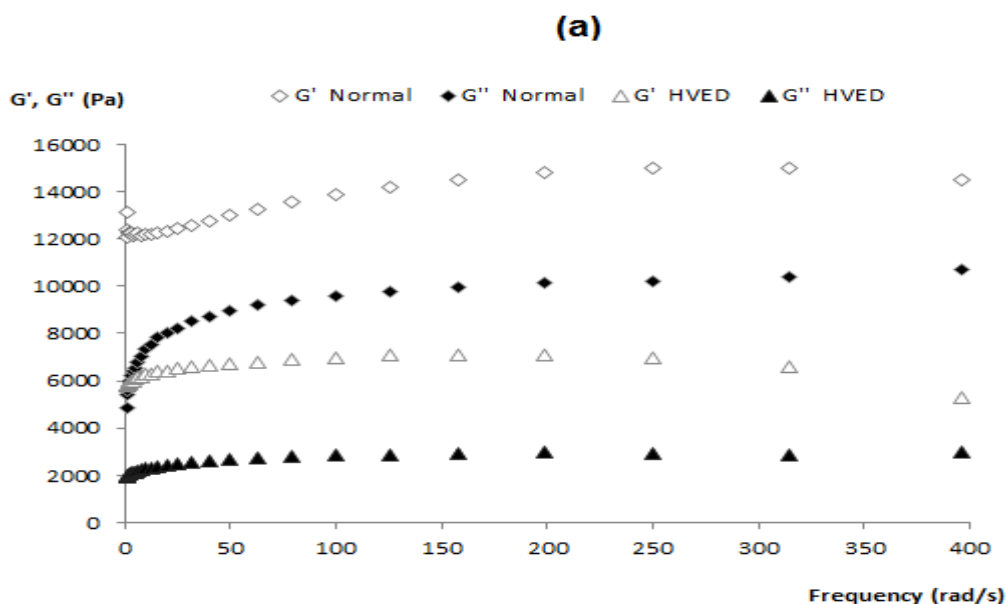
Viscosity analysis of 2% (w/v) sodium alginate solution at different pHs has already been published. In the 5 to 11 pH range, the relationships between shear stress and shear rate where linear viscosities were constant and all solutions had Newtonian behavior; at pHs 5

and 6, the behaviors were between Newtonian and non-Newtonian shear thinning. At lower pHs (4.5 and 3) the non-Newtonian thickening behavior was observed and alginic acid precipitated at pH below 3 [21,32]. With alginate from this study (extracted by HVED pretreatment), the behavior in all tests was the same than that previously described above. However, as it can be seen in **Table 3** the consistency index (m) was reported lower. The reason for this decrease caused by the low hydrodynamic radius of the polymer and the fragmentation of the alginate chain. In order to obtain information on the viscoelastic properties of gel (in the presence of calcium) obtained with HVED alginate and compare it with the usual method of extraction, variable frequency oscillation tests were performed. G' (storage or elastic modulus) and G'' (viscosity or loss or plastic modulus) as a function of frequency are given in **Figure 4a**. Over a frequency range of 0 to 400 rad/s, the modulus of elasticity (G') is higher than the viscosity modulus (G''). Regarding the relationship between gel properties and polymer chain, it can be seen that the viscoelastic properties are related to both chemical composition and molecular mass of the polymer. Research has also shown that hydrophobic compounds, such as phenolic compounds, reduce the content of effective alginates and they may prevent the release of alginate chains into the solution and thus interact with calcium [33]. The gel was exposed to a temperature range of 5 to 90 °C. The results showed that as the temperature increases, the elasticity of the gel decreases and the gel becomes more and more viscous and it can be said that the gel is moving towards liquefaction. From 70 °C and higher temperatures, the gel has completely lost its elasticity (**Fig. 4b**). In both methods, the gel was destroyed by the heating till 90°C and did not regenerate by re-cooling and reducing the temperature from 90 to 5 °C which indicates that the gel was not a stable thermal product.

Table 3.

Influence of pH on the consistency and flow behaviour index of solutions of sodium alginate from *Nizimuddinia zanardini* at 2% (w/v) in MilliQ water at several pH with High Voltage Electrode Discharge (HVED) pretreatment and without it (**Normal**). Data are expressed as the average \pm SD of three independent experiments (n = 3).

pH	Sample	m	N	R ²
7	HVED	0.034 \pm 0.001	0.995 \pm 0.001	0.999
	Normal	0.064\pm0.005	0.995\pm0.002	0.999
6	HVED	0.036 \pm 0.002	0.992 \pm 0.004	0.999
	Normal	0.067\pm0.006	0.895\pm0.024	0.991
5	HVED	0.038 \pm 0.001	0.982 \pm 0.001	0.999
	Normal	0.068\pm0.021	0.899\pm0.021	0.993
4.5	HVED	1.704 \pm 0.012	0.436 \pm 0.014	0.989
	Normal	2.535\pm0.015	0.441\pm0.017	0.982
3	HVED	2.044 \pm 0.011	0.273 \pm 0.008	0.995
	Normal	4.560\pm0.018	0.343\pm0.0121	0.989



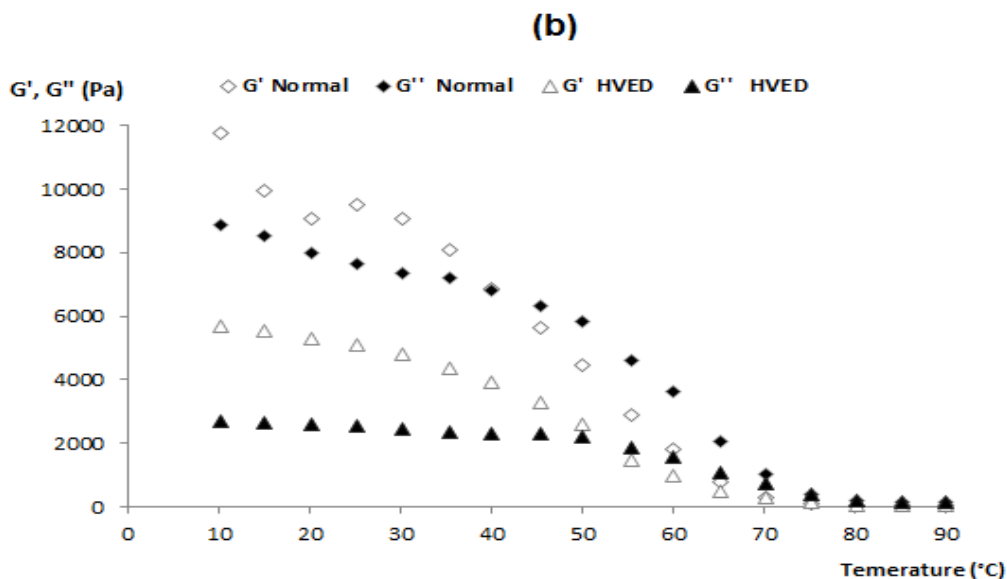


Fig. 4. Elastic and viscous modulus at different frequency of 2% calcium gels (w/v) of sodium alginates extracted with **High Voltage Electrode Discharge (HVED) pretreatment and without it (Normal)** from *Nizimuddinia zanardini* (a) and at different temperature (b). Data are expressed as the average \pm SD of three independent experiments (n = 3).

3.5. Emulsification properties

The emulsion stability of sodium alginate solutions at different concentrations (0.5-2% w/v) was studied using canola oil and the results are shown in **Table 4**. The stabilities of the emulsion with normal and HVED sodium alginates increased with increasing the concentrations of alginate and were reported at $97.9\% \pm 0.1$ and $94.7\% \pm 0.2$ respectively at 2 % (w/v) concentration. It has previously been reported that the viscosity of sodium alginate solutions from this algae increased by increasing the concentration of sodium alginate [21], thus, the reason for increasing the emulsion stability is the increase in viscosity. Dickinson reported that changing the rheology of the continuous phase, hydrocolloids usually reduce or even prevent creaming. The difference in effectiveness of different hydrocolloids

polymers that are used as emulsion stabilizers on emulsion stability, is mostly due to differences in their chemical compositions and structures [34]. At all concentrations, the stabilities of emulsions supplemented with HEVD sodium alginates were lower than those obtained with sodium alginate classically extracted, probably due to its lower viscosity at equal concentration.

Table 4.

Influence of concentration on the index of sodium alginate emulsion from *Nizimuddinia zanardini* with High Voltage Electrode Discharge (HVED) pretreatment and without it (**Normal**) at 20 °C. Data are expressed as the average \pm SD of three independent experiments (n = 3).

Concentration (%)	Sample	E ₂₄ (%)
0.5	HVED	65.7 \pm 0.2
	Normal	77.3 \pm 0.1
1	HVED	72.6 \pm 0.2
	Normal	93.5 \pm 0.1
2	HVED	94.7 \pm 0.2
	Normal	97.9 \pm 0.1

In many industrial processes, emulsifiers are exposed to extreme temperatures and pH, therefore, emulsions were prepared with canola oil using normal and HEVD sodium alginates at 2% (w/v) and exposed to various temperatures (25, 55 and 85 °C) and pHs (3 to 11). The stabilities of the emulsions with the two sodium alginates were not affected by temperatures up to 55 °C but were reduced at 85 °C. The decrease of the emulsion stability

at this temperature was explained by the reduction of viscosity during heating indicating that the oil-alginate emulsion is not thermally stable (**Table 5**).

Table 5.

Influence of temperature on the index of sodium alginate emulsion (2% w/v) from *Nizimuddinia zanardini* with High Voltage Electrode Discharge (HVED) pretreatment and without it (**Normal**). Data are expressed as the average \pm SD of three independent experiments (n = 3).

T (°C)	Sample	E ₂₄ (%)
25	HVED	94.7 \pm 0.2
	Normal	97.9\pm0.1
55	HVED	92.3 \pm 0.2
	Normal	97.9\pm0.1
85	HVED	90.2 \pm 0.2
	Normal	91.4\pm0.1

The same experiments done with the two alginates at various pHs indicated systematically a lower stability of the emulsions obtained with HEVD alginate (**Table 6**). Dickinson reported that the large size of the macromolecules and the predominance of hydrophilicity of a polysaccharide emulsifier, forms a thick stabilizing layer that ables the protection of drops against aggregation in a wide range of adverse conditions, such as heat shock and acidification process. In contrast, protein stabilizing emulsions due to their low surface coating, is very sensitive to adverse environmental conditions and exposes emulsions to instability [34]. With the classical extraction method, the larger size of the molecules than the one from high voltage process was reported by size exclusion chromatography which is consistent with the results in this section. Also the highest stability of the two sodium alginate emulsions at pH equal to 3 may be explained by the increase of the alginate

viscosity (formation of acidic gel stabilized by hydrogen bonds) and can be due to the protonation of carboxylic acid groups and the reduction of water solubility. Researchers have reported that the viscosity of the alginate solution is approximately constant between pH 6 and 8 but the viscosity increases at pH below 4.5 and reaches a maximum at about 3-3.5. The formation of alginic acid gel is due to the predominance of hydrogen bonds over electrostatic repulsion at pH 3 [31,32,35].

Table 6.

Influence of pH on the index of sodium alginate emulsion (2% w/v) from *Nizimuddinia zanardini* with High Voltage Electrode Discharge (HVED) pretreatment and without it (**Normal**) at 20 °C. Data are expressed as the average \pm SD of three independent experiments (n = 3).

pH	Sample	E ₂₄ (%)
3	HVED	100 \pm 0.0
	Normal	100\pm0.0
5	HVED	92.4 \pm 0.2
	Normal	97.9\pm0.1
7	HVED	94.7 \pm 0.2
	Normal	97.9\pm0.1
9	HVED	93.4 \pm 0.2
	Normal	97.2\pm0.1
11	HVED	93.6 \pm 0.2
	Normal	97.9\pm0.1

This sodium-oil alginate emulsion was slightly sensitive to temperature changes up to 55 °C and was stable at acidic pH, therefore, its use as a strong stabilizer and emulsifier in food products is appropriate [34]. In contrast, Gutierrez reported that acidic conditions reduced the emulsion capacity of some commercial polysaccharides, such as xanthan and arabic gum [36].

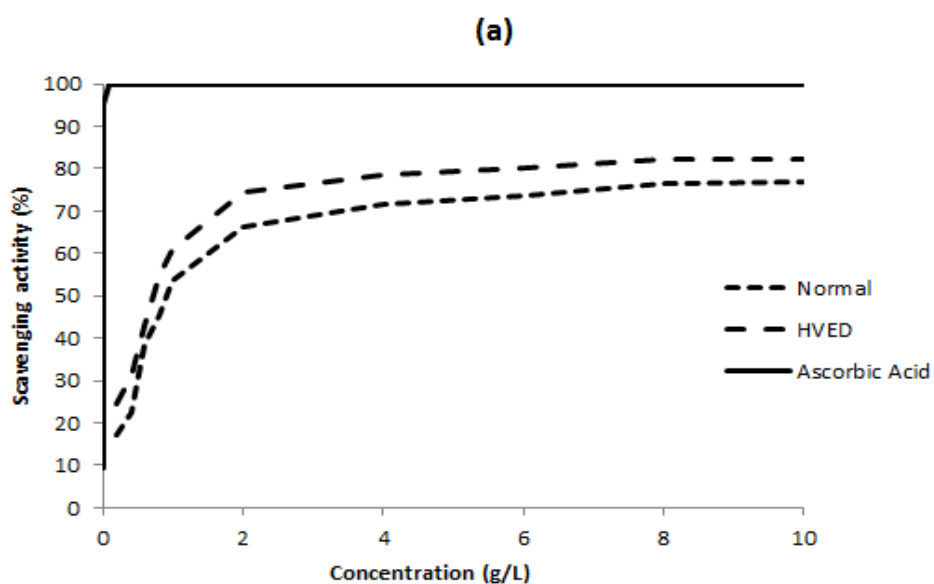
3.6. Total phenolic compounds

The amount of phenolic compounds in HEVD sodium alginate fraction was 2.6 ± 0.1 . This value was higher than that found in alginate extracted with classical method (1.1 ± 0.2 %). On the one hand, the high voltage has broken the cell walls and tissues releasing more phenolic compounds compared to classical extraction. On the other hand, high voltage method is mostly used to extract phenolic compounds which is consistent with the results obtained in this study [29,37]. For example, in 2015, Barba et al. concluded that the application of cellular disruption technologies like high voltage, is a powerful strategy for obtaining antioxidant compounds from stevia leaves [38].

3.7. Antioxidant properties

The antioxidant capacity of polysaccharides, such as alginates, is an important feature for their use in food and pharmaceutical industries [39,40]. According to previous reports, functional groups of polysaccharides such as sulfate, amino, hydroxyl and carboxylic ones but also the availability of hydroxyl groups, monosaccharide compounds, molecular hydrogen bonds and molecular weight affect antioxidant activities of polysaccharides. Alginates have been identified as strong radical inhibitors [41,42]. Several studies have reported that some antioxidant molecules, such as phenolic compounds, are sometimes co-extracted with alginates modifying its antioxidant activity [43,44]. The DPPH method was used to determine the radical release of sodium alginates from *Nizimuddinina zanardini*. As seen in **Figure 5a**, the antioxidant activity of HEVD alginate was logically higher than that of the normal alginate due to its higher phenolic compound content. The 50% inhibitory

capacities were calculated at 0.9 and 0.7 mg/mL for normal and HEVD sodium alginates respectively versus 0.005 mg/mL for ascorbic acid. A hydroxyl radical test was also performed to confirm this result (**Fig. 5b**). The 50% inhibitory capacity was calculated at 1.3 and 0.8 mg/mL for respectively normal and HEVD sodium alginates. The inhibitory capacity at 2 mg/mL concentrations of normal and HEVD sodium alginates were respectively of 65.7% and 82.1% confirming the previous results.



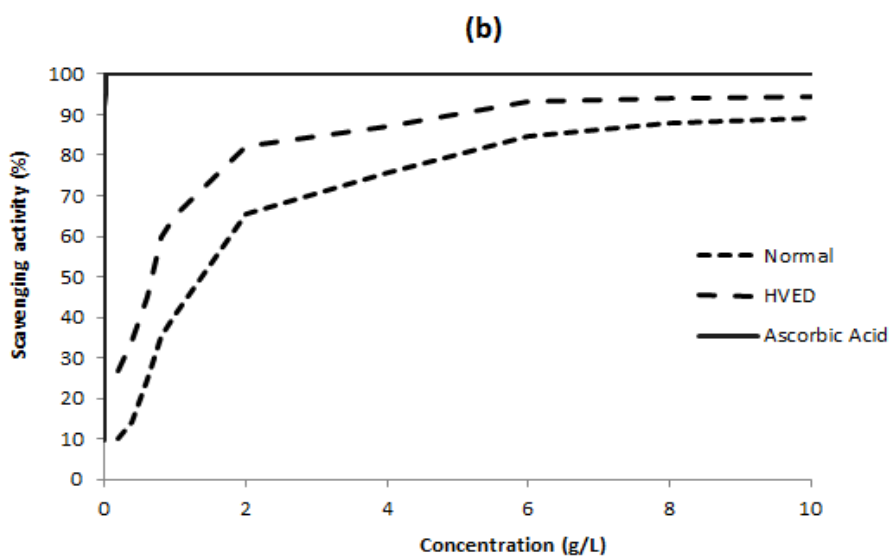


Fig. 5. Scavenging effects on **diphenyl picrylhydrazyl** radical (a) and Hydroxyl radical (b) for sodium alginate **with High Voltage Electrode Discharge (HVED) pretreatment and without it (Normal)**. Data are expressed as the average \pm SD of three independent experiments (n = 3).

4. Conclusion

The M/G ratio, calculated after alginate hydrolysis, was 1.22 indicating that the polysaccharide extracted from *Nizimuddinia zanardini* when applying high voltage was composed mostly of manururonic acids and was no different to that extracted using conventional procedure without pretreatment. Its M_w and the polydispersity index (\mathcal{D}) were 1.19×10^5 g/mol and 2.7, respectively. These higher values than those previously published for the alginate extracted from the same algae with conventional method showed that, despite the extraction of polysaccharides with high molecular weight, some degradations occurred leading to heterogeneity in M_w distribution. The rheological behavior of the alginate extracted with high voltage was the same to that of the alginate classically extracted without pretreatment, but the viscosity or consistency index (m) was much lower

for sodium alginate extracted with high voltage. In the variable frequency test, the modulus of elasticity was higher than the viscosity one in all measured frequency ranges and the gel had elastic properties, but if the thermal process was applied from 70 °C and above, the gel lost irreversibly its elasticity. The emulsion stability was proportionally increased with alginate concentration. The emulsion was resistant to pH changes and temperature up to 55 °C. The sodium alginate extracted with high voltages had higher antioxidant activity compared to those of alginate extracted with classical method without pretreatment.

CRedit Author Contributions Statement

Roya abka khajouei: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, review and editing, **Javad Keramat:** Writing - review and editing, **Nasser Hamdami:** Writing - review and editing, **Alina-Violeta Ursu:** Formal analysis, Data curation, Writing - review and editing, **Cedric Delattre:** Methodology, **Christine Gardarin:** Software , Methodology, **Didier Lecerf:** Software , Methodology, **Jacques Desbrières:** Software , Methodology, **Gholamreza Djelveh:** Data curation, **Philippe Michaud:** Formal analysis, Data curation, Writing - review and editing.

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Statement of Informed Consent, Human/Animal Rights

No conflicts, informed consent, or human or animal rights are applicable to this study

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