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Radical Depolymerization of Alginate Extracted from Moroccan Brown Seaweed *Bifurcaria bifurcata*

Bouissil Soukaina ¹, EL Alaoui-Talibi Zainab ¹, Pierre Guillaume ² , Rchid Halima ³,
Michaud Philippe ² , El Modafar Cherkaoui ¹ and Delattre Cédric ^{2,4,*}

¹ Laboratoire de Biotechnologie et Bioingénierie Moléculaire, Faculté des Sciences et Techniques Marrakech, Université Cadi Ayyad, Marrakesh 40000, Morocco; soukaina.BOUISSIL@etu.uca.fr (B.S.); zainab.elalaouitalibi@gmail.com (E.A.-T.Z.); elmodafar@uca.ac.ma (E.M.C.)

² Institut Pascal, Université Clermont Auvergne, CNRS, SIGMA Clermont, F-63000 Clermont-Ferrand, France; guillaume.pierre@uca.fr (P.G.); philippe.michaud@uca.fr (M.P.)

³ Laboratoire de Biotechnologies et Valorisation des Ressources Végétales, Faculté des Sciences, Université Chouaib Doukkali, El Jadida 24000, Morocco; rchidhalima@hotmail.com

⁴ Institut Universitaire de France (IUF), 1 Rue Descartes, 75005 Paris, France

* Correspondence: cedric.delattre@uca.fr

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Abstract: The degradation of alginate extracted from Moroccan *Bifurcaria bifurcata* has not been fully established to date. In this work, we report the extraction and the characterization of alginate (ASBB) from the brown algae *B. bifurcata*, as well as the production of oligo-alginates (OGABs) by using a green chemistry process. The depolymerization of ASBB was carried out by controlled radical hydrolysis through our green chemistry process using a hydrogen peroxide (H₂O₂) catalyst. The molecular weight (Mw) and degree of polymerization (DP) distribution of oligo-alginates (OGABs) obtained were then characterized by HPLC size exclusion chromatography (SEC) and high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Structural characterization revealed that after 6 h of depolymerization of ASBB, we obtained OGABs with Mw ≤ 5.5 kDa and 2 ≤ DP ≤ 24. These results highlight the effectiveness of the controlled radical hydrolysis of alginate to produce good yields of alginate fractions with controlled Mw with a known polymerization degree (DP) and without altering properties of oligo-alginates. *Bifurcaria bifurcata* can be a potential source of alginate and oligo-alginates given its abundance on the northwest Atlantic coast. The production and characterization of oligo-alginates promote their exploitation in the cosmetic, pharmaceutic, and agriculture fields.

Keywords: *Bifurcaria bifurcata*; alginate; oligo-alginates; Atlantic coast; hydrogen peroxide

1. Introduction

Among the main polysaccharides constituting brown algae, alginate represents 40% of their dry weight and it is mainly studied for its gelling properties [1]. The existence of alginate in the form of alginic acid substituted with mineral salts, notably calcium, magnesium and sodium, gives the brown algae a strong and flexible appearance [2]. At the molecular level, alginate is a linear anionic copolymer formed from two monomers— α -L-guluronic acid (G) and β -D-mannuronic acid (M)—linked together by (β -1 → 4) link and organized as homo and/or heteropolymeric blocks (F_{MM}/F_{GG} and F_{MG} blocks, respectively) [3]. About 30,000 tons of alginate are produced each year [4]. Therefore, alginate could be accounted as a copious resource for the development of biomaterials [5].

Due to its high viscosity, commercial applications of alginate are often limited in the food industry. Reducing the molecular weight of alginate is a good way to lower its viscosity and improve biological

properties through the generation of low molecular weight (LMW) of alginate and oligo-alginates. Generally, and based on the literature, the LMW is represented by the degree of polymerization (DP) from 11 to 30, while the oligosaccharides are oligomers with a DP from 2 to 10 [6]. Several strategies have been developed to produce oligo-alginates, mainly enzymatic depolymerization by alginate-lyases [7] and acid depolymerization [8]. Numerous studies published over the past decades have described the potential applications of oligo-alginates in therapeutics and in biotechnology [9–12]. It has been proven that oligo-alginates generated by enzymatic hydrolysis increase the proportion of *Lactobacilli* and *Bifidobacteria* in rats fed with alginate oligosaccharides (AOS) [13]. In addition, these oligo-alginates induce the secretion of cytokines from RAW264.7 cells of mouse macrophage cell line [10]. Previous studies have mentioned the elicitor effect of oligo-alginates, by the induction of the oxidative burst in *Laminaria digitata*, by causing an inhibitory effect against potentially harmful microorganisms [14]. In addition, oligo-alginates produced by gamma irradiation are growth promoters on *Amaranthus cruentus* [15]. On that account, this present work focuses on the production of oligo-alginates from alginate of Moroccan brown seaweed *Bifurcaria bifurcata* which has not been studied to date. Therefore, the aim of this work is to produce in good yields, the low molecular weight of alginate ($M_w \leq 5.5$ kDa) using for the first time a green chemistry process with hydrogen peroxide (H_2O_2), as well as study their structural characterization using different spectroscopic and chromatographic techniques.

2. Materials and Methods

2.1. Extraction and Purification of Sodium Alginate (ASBB)

Alginate extraction from *B. bifurcata* R. Ross seaweed was carried out using the method of Davis et al. [16]. First, 25 g of *B. bifurcata* powder were delipidated then depigmented before a double treatment with HCl (pH 2) for 2 h at 60 °C. The residue was then treated with 3% (*w/v*) of Na_2CO_3 solution at pH 11 and at 60 °C for 2 h under stirring (450 rpm). After centrifugation (5000 rpm, 15 min) the supernatants were precipitated with ethanol (96%) (3 Volumes). The precipitate was acidified to pH 3 with HCl solution at 6 M to obtain alginic acid. This latter was neutralized with (NaOH 1 M) to pH 7.5 after resuspending in ultra-pure water. The purification of ASBB was carried out three times by precipitation with ethanol 96%. The last precipitate obtained was lyophilized to get the sodium alginate of *B. bifurcata* (ASBB).

2.2. Chemical Analysis of Sodium Alginate (ASBB)

Total sugars were determined according to the method of Dubois et al. [17]. Uronic acids were determined using the Blumenkrantz method [18] and D-glucuronic acid was used as the standard. The sulfuric resorcinol method [19] was performed to quantify the neutral sugars taking L-fucose and D-glucose as standards. The sulfation degree was evaluated according to the turbidimetric method ($BaCl_2$ /gelatin) described by Dodgson and Price [20]. The protein content was quantified from a standard range of Bovine Serum Albumin (BSA) using the Bradford method [21]. According to the Folin–Ciocalteu method total phenolic compounds concentration was determined using a standard range of gallic acid [22].

2.3. 1H NMR Spectroscopy Analysis of Sodium Alginate (ASBB)

1H NMR spectroscopy analysis was carried out with a 400 MHz Bruker AVANCE spectrometer. Before analysis, 20 mg of ASBB was prepared three-fold in 0.5 mL of D_2O . The analysis of ASBB was performed at 60 °C. The signals obtained in 1H NMR spectra were used to determine the distribution of mannuronic (F_M) and guluronic (F_G) fractions, as well as the frequency of heterogeneous blocks (F_{MG} or F_{GM}) and homogenous blocks (F_{GG} and F_{MM}) [23].

2.4. Production of Low Molecular Weight (LMW) of Alginate

The depolymerization of ASBB to oligo-alginates (OGABs) was carried out by controlled radical hydrolysis using hydrogen peroxide (H_2O_2). A 2% (*m/v*) alginate solution was prepared in distilled

water; this solution was agitated at 300 rpm overnight, then heated to 70 °C. Temperature and agitation were maintained throughout the reaction. At the initial time of the reaction, 6.7 mL of H₂O₂ solution at 30% (*w/w*) were added to the alginate solution in order to achieve a mass ratio (alginate/H₂O₂:(*m*₁/*m*₂)) of 1:1. The study of the reaction was conducted over 10 h. Samples of 4.5 mL were taken at 30 min and then every hour, the fractions collected were inactivated for 30 min with 0.5 mL of ethanol 96%, and then lyophilized before they were analyzed by steric exclusion chromatography (CES) to determine their molar masses.

2.5. Reducing Sugars of Hydrolyzed Fractions (OGABs)

According to the Bicinchoninic Acid Assay (BCA) method [24], the reducing sugars were determined with slight modifications. Two assay solutions were prepared: solution A: in 200 mL of distilled water were dissolved 12.7 g of sodium bicarbonate (NaHCO₃), 4.84 g of sodium carbonate (Na₂CO₃), and 0.34 g of 2,2' biquinoline; and solution B: 0.25 g of cupric sulfate (CuSO₄, 5H₂O) with 0.25 g of L-serine were dissolved in 200 mL of distilled water. Then, 0.2 mL of OGABs at 0.2 g/L was added to 0.8 mL of distilled water and to 0.5 mL of solution A + solution B. After incubation for 15 min at 100 °C, reducing sugars content was quantified at 540 nm talking D-glucose as standard.

2.6. Molecular Weight by High-Performance Size Exclusion Chromatography (HPLC-SEC)

Analysis of molecular weight of oligo-alginates (OGABs) was executed by an Agilent 1100 Series using a Refractive Index Detector (RID). The separation was realized in two columns coupled in series: TSK G5000PWXL and TSK G3000PWXL. Sodium nitrate (NaNO₃) at 0.1 M was used as eluent with a flow rate of 1 mL/min. A range of Pullulan from 1.3 to 800 kDa was used as standard. Samples and standard were injected at 10 g/L in eluent after filtration through 0.45 and 0.22 µm, respectively.

2.7. High-Performance Anion Exchange Chromatography (HPAEC) Analysis

In order to study the distribution of the degree of polymerization (DP) in the fraction (OGABs-6 h) generated after 6 h of hydrolysis of ASBB, an analysis by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was performed. The analysis was executed with a Dionex ICS-3000 system with version 6.5 of Chromeleon (Dionex Corp., Sunnyvale, CA, USA). The distribution of DP in the OGABs-6h fraction was carried out at 25 °C on a CarboPac PA-200 column (4 mm × 250 mm) fixed to a CarboPac PA-200 guard column (Dionex 4 mm × 50 mm). A gradient of A (NaOH at 100 mM) and B (NaOH at 100 mM and NaOAc at 250 mM) was used as eluent with a flow rate of 1 mL/min. Then, 20 µL of filtered samples at 1 g/L were injected.

3. Results

3.1. Chemical Analysis of ASBB

B. bifurcata was treated by alkaline solution of sodium carbonate (3% (*w/v*), pH 11.0). After drying, the extraction yield was estimated at 24% (*w/w*). To study the nature of the extracted polysaccharide, a biochemical characterization was carried out. The results mentioned in Table 1 show that the extract was mainly composed of uronic acids with few neutral sugars and traces of sulfate which may be due to the coextraction of low traces of fucoidans by diluted HCl.

Table 1. Biochemical characterization of alginate (ASBB).

Analytical Parameter	% (<i>w/w</i>)
Uronic acid	58.44 ± 0.55
Neutral monosaccharides	18.25 ± 0.85
Total sugar	48.61 ± 0.45
Protein	Traces *
Sulfate	1.78 ± 0.23

* Traces: % *w/w* ≤ 1 × 10^{−3}. Each value was the mean of 3 repetitions ± standard error.

3.2. Structural Characterization of ASBB by ^1H NMR

For the structural characterization of the alginate blocks, ^1H NMR spectroscopy was performed as a suitable method for the structural determination of uronic acid polymers. ^1H NMR spectrum, shown in Figure 1, indicates the signal areas of HG-1 (A_1), HM-1 + HGM-5 (A_2), and HG-5 (A_3). According to Grasdalen [23] the equations below were used to determine the characteristic blocks and sequences of ASBB.

$$F_G = \frac{A_1}{A_2 + A_3}$$

$$F_M = 1 - F_G$$

$$\frac{M}{G} = \frac{1 - F_G}{F_G} = \frac{F_M}{F_G}$$

$$F_{GM} = F_{MG} = F_G - F_{GG}$$

$$F_{MM} = F_M - F_{MG}$$

$$F_{GG} = \frac{A_3}{A_2 + A_1}$$

Figure 1. ^1H NMR spectra of ASBB.

The values obtained in Table 2 indicate that guluronic acid (F_G) was the main compound of ASBB. According to Grasdalen [23], the η parameter given by this equation ($\eta = F_{GM}/[F_M + F_G]$) allowed the determination of the homo or heteropolymeric block. In the case of $\eta < 1$, the homopolymeric block was abundant. ASBB was characterized by M/G ratio of 0.47 and $\eta < 1$ with abundance of homoguluronic blocks (F_{GG}).

Table 2. Structural characterization of *B. bifurcata* sodium alginate (ASBB) by ^1H NMR.

Composition	Frequencies
M/G	0.47
F_G	0.68
F_M	0.32
F_{MM}	0.09
F_{MG}^a	0.23
F_{GG}	0.45
H	0.23

^a $F_{MG} = F_{GM}$.

3.3. Molecular Weight Determination

For the first time, *Bifurcaria bifurcata* alginate was radically degraded according to a controlled radical hydrolysis by a green chemistry process using only hydrogen peroxide (H_2O_2) to produce homogenous LMW fraction of ASBB with an $M_w \leq 5.5$ kDa. For more information on the depolymerization process, a high-performance size exclusion chromatography (HPSEC) analysis was carried out on the fractions withdrawn upon kinetics after each 2 h of hydrolysis. Figure 2 represents the HPSEC spectra of ASBB. It was eluted after 14 min giving an average molecular weight (M_w) of 220,000 g/mol according to the calibration curves made with Pullulan standards. The average molecular weight profile of oligo-alginates (OGABs) (Figure 2) ranged from 220,000 to 3500 g/mol after 10 h of radical depolymerization of ASBB. These results show a very large decrease in the M_w of the native alginate ASBB (220,000 g/mol) just after 2 h of hydrolysis. For comparison, native ASBB molecular weight was 220,000 g/mol and after 6 h of hydrolysis we obtained a fraction of LMW (OGABs-6h) with an M_w of 5500 g/mol. This result demonstrates the quantitative conversion of alginate (ASBB) polymer to shorter chains (OGABs). After 8 h of hydrolysis, the developed process allowed us to generate a fraction with a level around 3700 g/mol without any post-purification of the LMW fractions.

Figure 2. HPLC-SEC chromatography spectrum of ASBB and hydrolyzed alginate oligosaccharides (OGABs) from 0 to 10 h.

3.4. Osidic Composition of OGABs

To assess the radical depolymerization reaction of the native alginate ASBB, reducing sugars in the fractions obtained after 1, 3, 6, and 10 h of hydrolysis were quantified by colorimetric assay using

glucose as standard. The results obtained for the fractions are summarized in Figure 3. After 10 h of hydrolysis, the reducing sugars increased from 2.5% (*w/w*) in native ASBB to 20.10% (*w/w*) detected in the last fraction of OGABs.

Figure 3. Profile of reducing sugars of hydrolyzed alginate oligosaccharides (OGABs). Each value was the mean of 3 repetitions $\pm$ standard error.

3.5. Structural Characterization of OGABs by HPAEC-PAD

After the analysis of Mw of OGABs by HPSEC, the fraction of LMW with Mw ≤ 5.5 kDa was obtained after 6 h of hydrolysis (OGAB-6h). To get an idea of the distribution of the degree of polymerization (DP) in this fraction, an analysis by HPAEC-PAD was performed. Figure 4 shows the HPAEC-PAD spectrum of the OGABs-6h fraction. Taking into account the Mw of OGAB-6h (5500 g/mol) and the Mw of a sodium form of mannuronic acid (216 g/mol) we determined the maximum degree of polymerization (DP) in our fraction which varied from 2 to 24. The purification of the various DPs then analysis by LC-MS spectrometry are necessary to confirm the degree of polymerization of all the LMWs obtained in the fraction OGAB-6h.

Figure 4. Distribution of degree of polymerization (DP) in the OGAB-6h fraction by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

4. Discussion

Over the past decade, the newly observed functions of oligosaccharides have been responsible for their regulatory properties on different organisms [9]; hence the interest in developing efficient processes for the production of oligosaccharides. Therefore, algae polysaccharides were considered as a source of diverse oligosaccharide structures to the extent that oligosaccharides have demonstrated a role in many important industrial processes, such as brewing [25] and baking [26]. Moreover, it has been largely studied that the degradation products of algae polysaccharides have prebiotic activity [9,13] and are a potential elicitor of plants' growth and natural defenses [15,27]. It has therefore become interesting to develop a rapid process which generates with good yield, homogeneous fractions of a low molecular weight. There are many ways to achieve degradation of polysaccharides: enzymatic, physical or chemical [28]. Methods of depolymerization of algae polysaccharides include depolymerization of fucoidan by radical process [29], chemical hydrolysis of carrageenan [30], enzymatic degradation of glucuronan [31], thermal hydrolysis, and enzymatic degradation of alginate [7,32]. The water soluble alginate from *Bifurcaria bifurcata* was previously studied [33], and ¹H-NMR analysis of ASBB (Figure 1) reported a higher amount of guluronic acid, with abundance of homoguluronic blocks (Table 2). Compared to the alginates from other brown algae, the guluronic acid abundance in ASBB was higher than that observed in alginates of *Cystoseira* species [34–36], *F. vesiculosus* [37], *Sargassum* species [16,35,38–40], *L. japonica* and *L. digitata* [41,42]. Similar results are noted in alginates of *C. myrica* and *L. hyperborea* [23,35]. However some *Sargassum* species, notably *S. polycystum* and *S. filipendula*, were characterized by a higher amount of guluronic acid [39]. The uronic acids content and the proportions of the homoguluronic blocks determined the physicochemical properties of alginate [43]. Therefore, alginate with a M/G ratio value inferior to 1 form strong and rigid gels [44,45]. Based on these interpretations and our results (M/G < 1 and F_{GG} = 0.45), we can confirm that a gel can be formed using ASBB. Despite the gelling property of ASBB, it was recently applied as an elicitor of natural defense in date palm roots with interesting results [33].

In this context, the development of a rapid and efficient process for a large production of oligo-alginates (ASBB) was the objective of this work, which consisted of the exploitation of alginate of *B. bifurcata* to produce oligo-alginates (OGABs) with Mw ≤ 5.5 kDa. Several physico-chemical methods of degradation of alginate were already reported, including thermal degradation [32], microwaves [27], Gamma irradiation [46] and ultrasounds [15] for physical processes, as well as enzymatic [7,46] and radical depolymerizations for (bio)chemical strategies. Among these methods, we adopted a controlled radical hydrolysis using hydrogen peroxide (H₂O₂) in small quantities. Hydrogen peroxide has been reported to cause a scission of alginate, xyloglucan, dextran, and pectin [47]. Figure 2 shows that depolymerization in presence of H₂O₂ was rapid (occurred in 2 h), and after 4 h it remained nearly constant. This was expected since the concentration of H₂O₂ was exhausted as a function of the reaction time. A similar phenomenon was observed in other reported works [48]. The decrease in molecular weight can be explained by the manifestation of radical attacks at the glycosidic bond, associated with oxidative degradation [49]. When hydrogen peroxide (H₂O₂) was added to alginate solution, H₂O₂ molecules were dissociated to form •OH active free radicals which are powerful oxidants able to break the glycosidic bonds of alginate. The mechanism of the reaction of the degradation of alginate chains by hydrogen peroxide remains unclear. It was suggested that the attack of a hydrogen atom in position C1, C4 on (1 → 4)-linked polysaccharides caused a division of the glycosidic bond [50], and consequently the formation of carbonyl groups by reducing the end of C-4 (Figure 5a) and delta-lactone groups by reducing the end of C-1 (Figure 5d). In the Figure 5, we present the most probable mechanism of the action of •OH on a polysaccharide chain.

Figure 5. Proposed action of $\bullet\text{OH}$ on alginate. Reducing end: (a) Pyranone groups, (d) Lactone groups, and non-reducing end: (b), (c) glycopyranose groups.

It has been reported that H_2O_2 treatment under extreme conditions can break the sugar cycle and that recombination reactions between the different radicals can occur [51]; and since the reaction system contained in the presence of excess H_2O_2 , the carbonyl group of the lactones group in the new reducing end generated can be largely oxidized to a carboxyl group [52] as illustrated in Figure 6.

Figure 6. Oxidation of the reducing end of most oligomers to a carboxyl group.

Nevertheless, very little degradation of the rings was observed by controlling the conditions of degradation (temperature and duration) [49]. It should be mentioned that the depolymerization reaction of ASBB after 6 h gave a fraction with $M_w < 5500$ g/mol, 40 times narrower than native alginate ASBB (220,000 g/mol). Chang et al. [53] reported that radical depolymerization of chitosan (383,000 g/mol) using 1.5% of H_2O_2 generated an oligo-chitosan fraction (10,400 g/mol) after 6 h of hydrolysis. Fry [47] reported that a very rapid scission occurred in the alginate when hydrogen peroxide was in the presence of ascorbate at pH 5.5. Hydrogen peroxide with Cu^{2+} and ascorbate were also adopted to produce a LMW of heparin [54]. The same mixture was applied to dermatan sulfate to generate molecular weight fractions of 25,000 to 2000 g/mol [55]. An oligo-fucoidan (32,000 g/mol) [29] was obtained after 6 h of radical hydrolysis of sulfated fucoidan (320,000 g/mol) in the presence of copper acetate II. Under the same reaction, sulfated oligo-galactans were generated with an M_w of 8500 g/mol after 3 h of depolymerization of sulfated galactans [30]. The main drawback of combining

H₂O₂ and copper acetate II is the need for post-purification of the LMW fractions to remove copper and metallic catalysts. In another study, the thermal degradation of alginate was also documented [32]. Oates and Ledward [56] mentioned that the treatment of alginate at temperatures above 250 °C lead to extensive degradation. In addition, a more recent study by Holme et al. [32] showed the possibility of degradation of alginate to moderate temperatures (20 to 60 °C). However, the harsh processes (high temperature and pressure) induced simple coloration by sugar degradations or reversion reactions and generated undesirable compounds such as monosaccharides or salts implying post degradation purification [57]. Enzymatic processes have also been reported as the most used method for producing oligo-alginates. Depolymerization of alginates was principally performed by alginate lyases produced by different organisms [7]. Due to the complex structural features of alginate, these organisms usually produce different alginate lyases with various enzymatic properties for alginate utilization [58,59]. A recent study reported the effectiveness of cellulase to generate oligo-alginates. This degradation resulted in an increase of reducing sugar from 0.105 mg/mL to 0.441 mg/mL [60]. Indeed, hydrochloric acid (HCl) is also the most used chemical reagent for alginate modification; for example, sodium alginate and alginic acid were efficiently depolymerized using aqueous HCl solutions ranging from 0.1 to 1.0 M with pH 2.8 to 4.5 [61].

Despite the fact that radical depolymerization seems to be random, this was suggested with the microbial polysaccharides and carrageenan models [57]. The process used in this study was simple, easy to control reaction conditions, and most importantly, allowed us to obtain a high amount of LMW with Mw of 5500 g/mol only with H₂O₂ and in the absence of metallic catalysts. This fraction of LMW obtained could be considered for an elicitation test of natural defense mechanisms in date palm and tomato (data not published yet), insofar as the native alginate ASBB has already shown a significant elicitor effect for these mechanisms [33].

5. Conclusions

A green chemistry depolymerization process using only hydrogen peroxide (H₂O₂) was developed for alginate extracted from *Bifurcaria bifurcata*, a Moroccan brown seaweed. This process generated a large number of homogenous oligo-alginates with Mw of 5500 g/mol and DP ≤24 after 6 h of a radical depolymerization process.

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References

1. Bouissil, S.; Pierre, G.; el Alaoui-Talibi, Z.; Michaud, P.; el Modafar, C.; Delattre, C. Applications of algal polysaccharides and derivatives in therapeutic and agricultural fields. *Curr. Pharm. Des.* **2019**. [CrossRef]
2. Jiao, G.; Yu, G.; Zhang, J.; Ewart, H.S. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Mar. Drugs* **2011**, *9*, 196–233. [CrossRef]
3. Draget, K.I.; Smidsrød, O.; Skjåk-Bræk, G. Alginates from Algae. In *Biopolymers Online*; Wiley: Hoboken, NJ, USA, 2005. [CrossRef]
4. Falkeborg, M.; Cheong, L.Z.; Gianfico, C.; Sztukiel, K.M.; Kristensen, K.; Glasius, M.; Xu, X.; Guo, Z. Alginate oligosaccharides: Enzymatic preparation and antioxidant property evaluation. *Food Chem.* **2014**, *164*, 185–194. [CrossRef]

5. Tai, C.; Bouissil, S.; Gantumur, E.; Carranza, M.S.; Yoshii, A.; Sakai, S.; Pierre, G.; Michaud, P.; Delattre, C. Use of anionic polysaccharides in the development of 3D bioprinting technology. *Appl. Sci.* **2019**, *9*, 2596. [\[CrossRef\]](#)
6. Delattre, C.; Vijayalakshmi, M.A. Monolith enzymatic microreactor at the frontier of glycomics toward a new route for the production of bioactive oligosaccharides. *J. Mol. Catal. B Enzym.* **2009**, *60*, 97–105. [\[CrossRef\]](#)
7. Wong, T.Y.; Preston, L.A.; Schiller, N.L. Alginate Lyase: Review of Major Sources and Enzyme Characteristics, Structure-Function Analysis, Biological Roles, and Applications. *Annu. Rev. Microbiol.* **2000**, *54*, 289–340. [\[CrossRef\]](#)
8. Asilonu, E.; Bucke, C.; Keshavarz, T. Enhancement of chrysogenin production in cultures of *Penicillium chrysogenum* by uronic acid oligosaccharides. *Biotechnol. Lett.* **2000**, *22*, 931–936. [\[CrossRef\]](#)
9. Courtois, J. Oligosaccharides from land plants and algae: Production and applications in therapeutics and biotechnology. *Curr. Opin. Microbiol.* **2009**, *12*, 261–273. [\[CrossRef\]](#)
10. Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Iwamoto, Y.; Muramatsu, T. Structure-activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells. *FEBS Lett.* **2005**, *579*, 4423–4429. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Yamamoto, Y.; Kurachi, M.; Yamaguchi, K.; Oda, T. Stimulation of multiple cytokine production in mice by alginate oligosaccharides following intraperitoneal administration. *Carbohydr. Res.* **2007**, *342*, 1133–1137. [\[CrossRef\]](#)
12. Bland, E.J.; Keshavarz, T.; Bucke, C. The influence of small oligosaccharides on the immune system. *Carbohydr. Res.* **2004**, *339*, 1673–1678. [\[CrossRef\]](#)
13. Wang, Y.; Han, F.; Hu, B.; Li, J.; Yu, W. In vivo prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. *Nutr. Res.* **2006**, *26*, 597–603. [\[CrossRef\]](#)
14. Küpper, F.C.; Kloareg, B.; Guern, J.; Potin, P. Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiol.* **2001**, *125*, 278–291. [\[CrossRef\]](#)
15. Mollah, M.Z.I.; Khan, M.A.; Khan, R.A. Effect of gamma irradiated sodium alginate on red amaranth (*Amaranthus cruentus* L.) as growth promoter. *Radiat. Phys. Chem.* **2009**, *78*, 61–64. [\[CrossRef\]](#)
16. Davis, T.A.; Ramirez, M.; Mucci, A.; Larsen, B. Extraction, isolation and cadmium binding of alginate from *Sargassum* spp. *J. Appl. Phycol.* **2004**, *16*, 275–284. [\[CrossRef\]](#)
17. Dubois, M.; Gilles, K.; Hamilton, J.K.; Rebers, P.A.; Smith, F. A Colorimetric Method for the Determination of Sugars. *Nature* **1951**, *168*, 167. [\[CrossRef\]](#)
18. Blumenkrantz, N.; Asboe-Hansen, G. New Method for Quantitative Determination of Uronic Acids. *Anal. Biochem.* **1973**, *54*, 484–489. [\[CrossRef\]](#)
19. Monsigny, M.; Petit, C.; Roche, A.C. Colorimetric determination of neutral sugars by a resorcinol sulfuric acid micromethod. *Anal. Biochem.* **1988**, *175*, 525–530. [\[CrossRef\]](#)
20. Dodgson, K.S.; Price, R.G. A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem. J.* **1962**, *84*, 106–110. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Bradford, M.M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#)
22. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [\[CrossRef\]](#)
23. Grasdalen, H. High-field, ¹H-NMR spectroscopy of alginate: Sequential structure and linkage conformations. *Carbohydr. Res.* **1983**, *118*, 255–260. [\[CrossRef\]](#)
24. Doner, L.W.; Irwin, P.L. Assay of reducing end-groups in oligosaccharide homologues with 2,2'-bicinchoninate. *Anal. Biochem.* **1992**, *202*, 50–53. [\[CrossRef\]](#)
25. Letters, R. Origin of carbohydrates in beer sediments. *J. Inst. Brew.* **1969**, *75*, 54–60. [\[CrossRef\]](#)
26. Rouau, X. Investigations into the Effects of an Enzyme Preparation for Baking on Wheat Flour Dough Pentosans. *J. Cereal Sci.* **1993**, *18*, 145–157. [\[CrossRef\]](#)
27. Singh, V.; Sethi, R.; Tewari, A.; Srivastava, V.; Sanghi, R. Hydrolysis of plant seed gums by microwave irradiation. *Carbohydr. Polym.* **2003**, *54*, 523–525. [\[CrossRef\]](#)
28. Uffen, R.L. Xylan degradation: A glimpse at microbial diversity. *J. Ind. Microbiol. Biotechnol.* **1997**, *19*, 1–6. [\[CrossRef\]](#)
29. Chandía, N.P.; Matsuhira, B. Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *Int. J. Biol. Macromol.* **2008**, *42*, 235–240. [\[CrossRef\]](#)

30. Zúñiga, E.A.; Matsuihiro, B.; Mejías, E. Preparation of a low-molecular weight fraction by free radical depolymerization of the sulfated galactan from *Schizymenia binderi* (Gigartinales, Rhodophyta) and its anticoagulant activity. *Carbohydr. Polym.* **2006**, *66*, 208–215. [\[CrossRef\]](#)
31. Redouan, E.; Cedric, D.; Emmanuel, P.; Mohamed, E.G.; Bernard, C.; Philippe, M.; Cherkaoui, E.M.; Josiane, C. Improved isolation of glucuronan from algae and the production of glucuronic acid oligosaccharides using a glucuronan lyase. *Carbohydr. Res.* **2009**, *344*, 1670–1675. [\[CrossRef\]](#)
32. Holme, H.K.; Lindmo, K.; Kristiansen, A.; Smidsrød, O. Thermal depolymerization of alginate in the solid state. *Carbohydr. Polym.* **2003**, *54*, 431–438. [\[CrossRef\]](#)
33. Bouissil, S.; El Alaoui-Talibi, Z.; Pierre, G.; Michaud, P.; El Modafar, C.; Delattre, C. Use of alginate extracted from Moroccan brown algae to stimulate natural defense in date palm roots. *Molecules* **2020**, *25*, 720. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Hentati, F.; Delattre, C.; Ursu, A.V.; Desbrières, J.; Le Cerf, D.; Gardarin, C.; Abdelkafi, S.; Michaud, P.; Pierre, G. Structural characterization and antioxidant activity of water-soluble polysaccharides from the Tunisian brown seaweed *Cystoseira compressa*. *Carbohydr. Polym.* **2018**, *198*, 589–600. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Larsen, B.; Salem, D.M.S.A.; Sallam, M.A.E.; Mishrikey, M.M.; Beltagy, A.I. Characterization of the alginates from algae harvested at the Egyptian Red Sea coast. *Carbohydr. Res.* **2003**, *338*, 2325–2336. [\[CrossRef\]](#)
36. Zrid, R.; Bentiss, F.; Attoumane Ben Ali, R.; Belattmania, Z.; Zarrouk, A.; Elatouani, S.; Eddaoui, A.; Reani, A.; Sabour, B. Potential uses of the brown seaweed *Cystoseira humilis* biomass: 1- Sodium alginate yield, FT-IR, 1 H NMR and rheological analyses. *J. Mater. Environ. Sci.* **2016**, *7*, 613–620.
37. Rioux, L.E.; Turgeon, S.L.; Beaulieu, M. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydr. Polym.* **2007**, *69*, 530–537. [\[CrossRef\]](#)
38. Fenoradosoa, T.A.; Ali, G.; Delattre, C.; Laroche, C.; Petit, E.; Wadouachi, A.; Michaud, P. Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow. *J. Appl. Phycol.* **2010**, *22*, 131–137. [\[CrossRef\]](#)
39. Davis, T.A.; Llanes, F.; Volesky, B.; Mucci, A. Metal Selectivity of *Sargassum* ssp. and Their Alginates in Relation to Their α -L-Guluronic Acid Content and Conformation. *Environ. Sci. Technol.* **2003**, *37*, 261–267. [\[CrossRef\]](#)
40. Torres, M.R.; Sousa, A.P.A.; Silva Filho, E.A.T.; Melo, D.F.; Feitosa, J.P.A.; de Paula, R.C.M.; Lima, M.G.S. Extraction and physicochemical characterization of *Sargassum vulgare* alginate from Brazil. *Carbohydr. Res.* **2007**, *342*, 2067–2074. [\[CrossRef\]](#)
41. Zheng, N.-Y.; Zhang, Y.-X.; Fan, X.; Han, L.-J. Effects of composition and structure of alginates on adsorption of divalent metals. *Chin. J. Oceanol. Limnol.* **1994**, *12*, 78–83. [\[CrossRef\]](#)
42. Fertah, M.; Belfkira, A.; Dahmane, E.M.; Taourirte, M.; Brouillette, F. Extraction and characterization of sodium alginate from Moroccan *Laminaria digitata* brown seaweed. *Arab. J. Chem.* **2017**, *10*, S3707–S3714. [\[CrossRef\]](#)
43. Xiao, Q.; Tong, Q.; Zhou, Y.; Deng, F. Rheological properties of pullulan-sodium alginate based solutions during film formation. *Carbohydr. Polym.* **2015**, *130*, 49–56. [\[CrossRef\]](#)
44. Venugopal, V. Polysaccharide from seaweed and microalgae. In *Marine Polysaccharides: Food Applications*; Taylor and Francis Group: Boca Raton, FL, USA, 2011; pp. 111–122.
45. Murillo-Álvarez, J.I.; Hernández-Carmona, G. Monomer composition and sequence of sodium alginate extracted at pilot plant scale from three commercially important seaweeds from Mexico. *J. Appl. Phycol.* **2007**, *19*, 545–548. [\[CrossRef\]](#)
46. Wasikiewicz, J.M.; Yoshii, F.; Nagasawa, N.; Wach, R.A.; Mitomo, H. Degradation of chitosan and sodium alginate by gamma radiation, sonochemical and ultraviolet methods. *Radiat. Phys. Chem.* **2005**, *73*, 287–295. [\[CrossRef\]](#)
47. Fry, S.C. Oxidative scission of plant cell wall polysaccharides by ascorbat-induced hydroxyl radicals. *Biochem. J.* **1998**, *332*, 507–515. [\[CrossRef\]](#)
48. Mao, S.; Zhang, T.; Sun, W.; Ren, X. The depolymerization of sodium alginate by oxidative degradation. *Pharm. Dev. Technol.* **2012**, *17*, 763–769. [\[CrossRef\]](#)
49. Sarbu, A.; Gonçalves, F.; De Pinho, N.M. Radical initiated oxidative degradation of oat-spelts xylan. *Carbohydr. Polym.* **2003**, *53*, 1–8. [\[CrossRef\]](#)

50. Hou, Y.; Wang, J.; Jin, W.; Zhang, H.; Zhang, Q. Degradation of *Laminaria japonica* fucoidan by hydrogen peroxide and antioxidant activities of the degradation products of different molecular weights. *Carbohydr. Polym.* **2012**, *87*, 153–159. [[CrossRef](#)]
51. Ovalle, R.; Soll, C.E.; Lim, F.; Flanagan, C.; Rotunda, T.; Lipke, P.N. Systematic analysis of oxidative degradation of polysaccharides using PAGE and HPLC–MS. *Carbohydr. Res.* **2001**, *330*, 131–139. [[CrossRef](#)]
52. Yang, Z.; Li, J.P.; Guan, H.S. Preparation and characterization of oligomannuronates from alginate degraded by hydrogen peroxide. *Carbohydr. Polym.* **2004**, *58*, 115–121. [[CrossRef](#)]
53. Chang, K.L.B.; Tai, M.C.; Cheng, F.H. Kinetics and products of the degradation of chitosan by hydrogen peroxide. *J. Agric. Food Chem.* **2001**, *49*, 4845–4851. [[CrossRef](#)] [[PubMed](#)]
54. Liu, Z.; Perlin, A.S. Evidence of a selective free radical degradation of heparin, mediated by cupric ion. *Carbohydr. Res.* **1994**, *255*, 183–191. [[CrossRef](#)]
55. Volpi, N. Dermatan sulfate from beef mucosa: Structure, physicochemical and biological properties of fractions prepared by chemical depolymerization and anion-exchange chromatography. *Carbohydr. Res.* **1994**, *255*, 133–144. [[CrossRef](#)]
56. Oates, C.G.; Ledward, D.A. Studies on the effect of heat on alginates. *Top. Catal.* **1990**, *4*, 215–220. [[CrossRef](#)]
57. Delattre, C.; Michaud, P.; Courtois, B.; Courtois, J. Medicinal Plants for Biotechnology and Biomedicine Oligosaccharides engineering from plants and algae. *Minerva Biotechnol.* **2005**, *17*, 107–117.
58. Xu, F.; Dong, F.; Wang, P.; Cao, H.Y.; Li, C.Y.; Li, P.Y.; Pang, X.H.; Zhang, Y.Z.; Chen, X.L. Novel molecular insights into the catalytic mechanism of marine bacterial alginate lyase AlyGC from polysaccharide lyase family 6. *J. Biol. Chem.* **2017**, *292*, 4457–4468. [[CrossRef](#)]
59. Xu, F.; Wang, P.; Zhang, Y.Z.; Chen, X.L. Diversity of three-dimensional structures and catalytic mechanisms of alginate lyases. *Appl. Environ. Microbiol.* **2018**, *84*, 1–12. [[CrossRef](#)]
60. Wardhani, D.H.; Cahyono, H.; Ulya, H.N.; Rahmawati, A.; Sugiarto, T.V.K. Viscosity of degraded alginate using cellulase. *AIP Conf. Proc.* **2020**, *2197*, 1–6. [[CrossRef](#)]
61. Liu, J.; Yang, S.; Li, X.; Yan, Q.; Reaney, M.J.T.; Jiang, Z. Alginate Oligosaccharides: Production, Biological Activities, and Potential Applications. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 1859–1881. [[CrossRef](#)]

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