Research Article

Yuchan Park, Samkelo Malgas, Rui W.M. Krause and Brett I. Pletschke*

Extraction and characterisation of sodium alginate from the Southern African seaweed *Ecklonia maxima*

https://doi.org/10.1515/bot-2024-0011
Received February 13, 2024; accepted May 29, 2024;
published online August 19, 2024

Abstract: *Ecklonia maxima* is an endemic South African seaweed with numerous commercially valuable compounds, including sodium alginate. This polysaccharide contains 1,4-linked β-D-mannuronic (M) and α-L-guluronic acid (G) residues, and has enormous health and food applications and benefits for biomedicine. In this study, acid extraction of sodium alginate from *Ecklonia maxima* was performed, and its chemical and physical properties were compared with commercial sodium alginate. A yield of 58.7 % (w/w of dry alga) sodium alginate was recovered from the seaweed. As expected, Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopic analyses of sodium alginate revealed the presence of M and G residues in the polysaccharide. Circular dichroism (CD) spectroscopy revealed an M/G ratio of 1.86 from the extracted sodium alginate. The viscosity average molecular weight of sodium alginate was 429 kDa using the Mark–Houwink–Sakurada equation. Three-dimensional conformational analysis by Congo red and CD spectroscopy suggested that sodium alginate exhibits a triple-helix conformation in solution. Furthermore, X-ray powder diffraction analysis confirmed that the alginate has an amorphous structure. Sodium alginate extracted from *E. maxima* found on the coasts of Southern Africa exhibits a high mannuronic content and high viscosity, potentially beneficial for drug delivery and biomedical applications.

Keywords: acid extraction; brown seaweed; intrinsic viscosity; polysaccharide

***Corresponding author: Brett I. Pletschke**, Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa, E-mail: b.pletschke@ru.ac.za. https://orcid.org/0000-0001-8117-1730
Yuchan Park, Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa, E-mail: g16p3593@ru.ac.za. https://orcid.org/0000-0002-1317-4072
Samkelo Malgas, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Hatfield 0028, South Africa, E-mail: samkelo.malgas@up.ac.za. https://orcid.org/0000-0003-0729-5393
Rui W.M. Krause, Department of Chemistry, Rhodes University, Makhanda 6140, South Africa, E-mail: R.Krause@ru.ac.za. https://orcid.org/0000-0001-6788-6449

1 Introduction

Seaweeds, or macroalgae, have traditionally been consumed in many countries for their high iodine content and health-promoting benefits. In recent years, they have become popular in many industries for their bioactive compounds for pharmaceutical and nutraceutical companies, and thickening and stabilising agents for the cosmetic and food industries (Cardozo et al. 2007; Holdt and Kraan 2011). Bioactive compounds in seaweeds have been reported to exhibit anti-inflammatory, antimicrobial, antioxidant, anticancer, anti-diabetes, and anti-HIV activities (Daub et al. 2020; Manilal et al. 2009).

Moreover, seaweeds are a valuable source of materials for human utilisation due to their rapid growth rates, ability to absorb large quantities of carbon and lack of requirements for fresh water and arable land (Lorbeer et al. 2015). Their economic potential has resulted in seaweeds being farmed and cultivated worldwide. The broad spectrum of bioactivities elicited by seaweed-derived compounds makes them valuable as pharmaceutical and nutraceutical products.

Among the many naturally occurring pharmaceutical compounds of interest, alginate, mainly extracted from brown seaweeds, is used extensively for many biomedical applications (Pal et al. 2014). Alginate is present in the intercellular space matrix of brown seaweeds, where it is responsible for the transportation of different salts (Rhein-Knudsen et al. 2015). As alginate can comprise up to 40–47 % of the dry mass of brown seaweed, this makes brown seaweeds an attractive source for extracting alginate (Pal et al. 2014; Rasmussen and Morrissey 2007).

As a result of its gelling properties, alginate is also used extensively in the food and beverage industries as a

thickening agent (Pal et al. 2014). In addition, alginate has found important applications in the pharmaceutical and cosmetics sectors since it has been shown to lower cholesterol levels, possesses antibacterial and anti-inflammatory activities, and prevents the postprandial increase of glucose, insulin, and C-peptide levels (Holdt and Kraan 2011; Torsdottir et al. 1991), to name but a few.

Alginate, however, cannot be readily extracted. It must be converted to alginate salt to solubilise in water, which assists with extraction. Sodium alginate, a form of alginate salt, is a linear polysaccharide containing 1,4-linked β-D-mannuronic and α-L-guluronic acid residues (Pal et al. 2014). In recent years, the use of various compounds from seaweeds, such as sodium alginate, found mainly in brown seaweeds, has received much attention as a biopolymer. Specifically, sodium alginate has been tested and commercialised as a food packaging alternative due to its gelling, anti-microbial, non-toxic and biodegradable properties. For example, sodium alginate and calcium alginate, another alginate salt, have been used in the vegetable, cheese and meat industries to extend their shelf life (Gheorghita et al. 2020; Kontominas 2020).

Seaweed compounds have been extracted using many different methods, including solvent to green extraction methods such as microwave- (MAE), enzyme- (EAE), and ultrasound-assisted extraction (UAE) processes. However, these green extraction processes are sometimes poorly understood, expensive, and/or characterized by low product yields that hamper their adoption for industrial use (Poojary et al. 2016; Saji et al. 2022). On the other hand, solvent extraction methods use acid to fractionate alginic acid, which is converted to soluble sodium alginate by neutralisation with sodium carbonate (Hahn et al. 2012; Lorbeer et al. 2015). However, the ease and use of low-cost reagents in the solvent extraction process make it more feasible than green extraction processes (Jacobsen et al. 2019; Poojary et al. 2016).

The endemic brown seaweed, *Ecklonia maxima*, grows extensively along the warm temperate waters of the south coast to the cool-temperate waters of the west coast of South Africa (Anderson et al. 2006; Rothman et al. 2006). *Ecklonia maxima* possesses numerous commercially valuable compounds, including sodium alginate. Since the 1950s, *E. maxima* beach casts have been exported to Europe, North America, and Asia for alginate production (Anderson et al. 1989). Examining the acid extraction of sodium alginate from the endemic South African brown seaweed, *E. maxima*, this study aimed to evaluate and compare the chemical and physical characteristics of the extracted sodium alginate against that of the commercially available sodium alginate.

2 Materials and methods

2.1 Materials and reagents

Commercial sodium alginate (Cat no. W201502) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemical reagents and kits were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), MERCK (Darmstadt, HE, Germany), and Megazyme™ (Bray, WC, Ireland).

2.2 *Ecklonia maxima* biomass

Mixed thalli of *E. maxima*, harvested from Port Nolloth (–29.253997, 16.867888), South Africa, were supplied by KelpX (Pty) Ltd. (Johannesburg, Gauteng, South Africa). Harvested thalli were first sundried, then cut into pieces (4–5 cm) before grinding to powder using a Platinum® coffee grinder (Model: JC-CG150W, Johannesburg, Gauteng, South Africa). The powder was stored in a dark, dry place until further use.

2.3 Extraction of sodium alginate from *E. maxima*

Sodium alginate was extracted according to a modified extraction process described by Lorbeer et al. (2015). Ground seaweed was depigmented in ethanol for 2 h with 99 % ethanol (1:2, solid:liquid) with gentle shaking; the process was repeated. The depigmented ground seaweed was dried at room temperature for 24 h, thereafter submerged in deionised water, and the pH adjusted to 1.0 using 2 M HCl in a solid-to-liquid ratio of 1:30 (w/v). This was left for 2 h at room temperature with a magnetic stirrer at 100 rpm on a magnetic stirrer plate. Thereafter, the supernatant and the residual biomass were separated through a cheesecloth, and the residual biomass was resuspended into a 0.5 M sodium carbonate solution at a solid-to-liquid ratio of 1:50. This solution was left overnight at a room temperature of 22 °C with a constant stirring with a magnetic stirrer at 100 rpm. The biomass was then sieved using a cheesecloth to separate it from the liquid containing the dissolved sodium alginate. This supernatant was made to a final ethanol concentration of 70 % (v/v) by adding 99 % ethanol and left for 1 h at room temperature to precipitate the sodium alginate. A sieve was used to separate the precipitated sodium alginate from the supernatant, which was washed with methanol and acetone (50 mL each) and then dried at room temperature of 22 °C overnight. The dried sodium alginate was stored in a closed container at room temperature until further use.

2.4 Sodium alginate purity analysis

2.4.1 Protein content determination

The Bradford assay was used to determine the contaminant protein content of the sodium alginate extracts, with bovine serum albumin as a suitable standard (Bradford 1976).

2.4.2 Phenolic content determination

The total phenolic content in the extracts was determined as described by Malgas et al. (2016) using the Folin-Ciocalteu method. Gallic acid was used as a suitable standard, and the phenolic content was expressed as % of gallic acid equivalents per g dry weight.

2.4.3 Total sugar determination

The total sugar content in the extracted sodium alginate was determined by performing the phenol-sulfuric acid method described by DuBois et al. (1956). The samples were made to 1 mg/mL using dH_2O, and 100 µL of this sample was added to 300 µL of concentrated sulfuric acid, followed by 50 µL of 5 % phenol (w/v). The samples were mixed, incubated at 90 °C for 10 min, and then cooled to room temperature. A volume of 200 µL of the sample was then transferred to a 96-well microtiter plate, and the absorbance readings were taken at 490 nm using a PowerWaveX™ spectrophotometer with KC Junior software® (Winooski, Vermont, USA). The total sugar content of sodium alginate was calculated from the phenol-sulfuric standard curve constructed using varying concentrations of commercial sodium alginate (0–1 mg/mL).

2.4.4 Total uronic acid determination

The total uronic acid content of the sodium alginate was determined by performing the 96-well carbazole assay method described by Cesaretti et al. (2003). Varying concentrations of sodium alginate samples (0–1 mg/mL) of 50 µL were placed in a 96-well plate. Sodium tetraborate in sulfuric acid, 200 µL, with a concentration of 25 mM, was added into the wells, followed by heating the plate at 100 °C for 15 min in an oven. A volume of 50 µL 0.125 % carbazole in absolute ethanol was added and then heated at 100 °C for 10 min in an oven. The plate was cooled for 15 min at room temperature and read using a PowerWaveX™ spectrophotometer with KC Junior software® (Winooski, Vermont, USA) at 550 nm. The total uronic acid content of sodium alginate was calculated from the mannuronic acid standard curve constructed using varying concentrations (0.1–1 mg/mL).

2.5 Structural characterisation of sodium alginate

2.5.1 Fourier-transform infrared (FTIR) spectroscopic analysis

FTIR analysis was used to characterise the sodium alginate samples using a Spectrum 100 spectrometer system (Perkin Elmer, Wellesley, MA, USA) with Spectrum™ One software. Baseline and ATR corrections for penetration depth and frequency variations were carried out using this program. The samples were pressed uniformly and tightly against the spring-loaded anvil, and the spectra were recorded from 4,000 to 600 cm^{-1}.

2.5.2 Nuclear magnetic resonance (NMR) spectroscopic analysis

The sodium alginate extracts (1 mg/mL) were dissolved in D_2O, followed by mixing. The deuterium-exchanged samples were subjected to ^{1}H-NMR analysis. Spectra were recorded at 70 °C using a Bruker 400 MHz spectrometer (Billerica, Massachusetts, USA) with Mnova 15.0.1 software (Mestrelab Research, Santiago de Compostela, A Coruña, Spain). The chemical shifts were expressed in ppm relative to tetramethylsilane (TMS) or the residual solvent signals.

2.5.3 Circular dichroism (CD) spectroscopy analysis

A sodium alginate solution of 0.25 mg/mL was scanned using a Chirascan spectrophotometer (Applied Photophysics Limited, Leatherhead, South East, United Kingdom) three times at 80 nm/min with 1 nm slit width and a time constant of 1 s. Data were collected from 190 to 350 nm at 1 nm intervals. The M/G ratio was calculated by the parameters described by Morris et al. (1980): mannuronate/guluronate ≈ 2.0(peak/trough), if peak/trough < 1 or % mannuronate ≈ 27(peak/trough) + 40, if peak/trough >1.

2.5.4 Determination of the intrinsic viscosity and molecular weight

A 10 mg/mL sodium alginate stock solution was prepared in 100 mM saline water, and 0.1–10 mg/mL dilutions of the stock solution were prepared. After vortexing the solution

for 5 s, 1 mL of each was loaded on a semi-micro viscometer (Cannon Instrument Company, State College, PA, USA). The flow time of the solution from the top to the bottom meniscus was measured. The intrinsic viscosity was calculated by using the formulae:

$$\eta_r = \eta / \eta_s \quad (1)$$

$$\eta_r = 1 + [\eta]C \quad (2)$$

where η_r is the relative viscosity, η is the sodium alginate solution viscosity (mPa s), η_s is the solvent viscosity, $[\eta]$ is the intrinsic viscosity (L/g), and C is the sodium alginate concentration (g/L).

The following formula estimated the molecular weight of sodium alginate by intrinsic viscosity, where k and a are sodium alginate-specific constants (Halabalová et al. 2004).

$$[\eta] = k[M_w]^a \quad (3)$$

The parameters of k and a were substituted with the Mark–Houwink–Sakurada (MHS) parameters of Vold et al. (2006):

when I = 0.1 M,

$$20{,}000\ \mathrm{g/mol} < M_w < 10{,}000\ \mathrm{g/mol}: [\eta] = 0.0054 \times M_w^{1.00} \quad (4)$$

$$100{,}000\ \mathrm{g/mol} < M_w < 1{,}000{,}000\ \mathrm{g/mol}: [\eta] = 0.071 \times M_w^{0.89} \quad (5)$$

where $[\eta]$ is mL/g and M_w is g/mol.

2.5.5 Congo red polysaccharide folding analysis

A modified Congo red assay described by Yang et al. (2021) was performed. A 2 mg/mL concentration of sodium alginate was prepared. Congo red was prepared to 80 µmol/L, which was dissolved in different concentrations of NaOH ranging from 0 to 0.5 mol/L (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/L) to be mixed in a volume ratio of 1:2. The mixture was incubated for 1 h at 25 °C. Distilled water was used for the control. The total absorbance was scanned from 450 to 510 nm using a PowerWaveX™ spectrophotometer with KC Junior software.

2.5.6 X-ray powder diffraction analysis

The crystallinity of the sodium alginate was measured using a Bruker D2 2nd Gen Phaser (Billerica, Massachusetts, USA). Samples were scanned from 2θ (Theta) of 5–60° with a step size of 0.02° per second. The relative crystallinity of the polysaccharide was calculated by dividing the area of the diffraction peak at 2θ by the total area of 17–21°.

2.5.7 Thermogravimetric analysis of sodium alginate samples

Commercial sodium alginate and sodium alginate extracted from *E. maxima* samples were subjected to thermogravimetric analysis (TGA). TGA was conducted with a PerkinElmer® (Pyris Diamond model) thermogravimetric analyser (Waltham, USA) following the method of Mabate et al. (2021). The samples were placed in an alumina crucible and heated from 30-700 °C with a heating rate of 10 °C min^{-1} whilst continuously flushing the apparatus with argon gas at a 60 mL/min atmospheric pressure flow rate.

2.6 Statistical analysis

Triplicate data sets were used in all experiments. Unpaired *t*-tests were used to determine differences between data means. Data were considered statistically significant at $P < 0.05$. Data analyses were performed using GraphPad Prism software version 9.0.0 (GraphPad Inc.). Data are expressed as means ± standard deviations (SD).

3 Results and discussion

3.1 Chemical composition of sodium alginate

The yield of sodium alginate from *E. maxima* was 55.47 ± 5.21 % (w/w of dry alga). This is on the higher end of sodium alginate yields reported in the literature for brown seaweeds. Lorbeer et al. (2015) reported a yield of 44 % (w/w of dry alga) from *Ecklonia radiata*, while Sugiono and Ferdiansyah (2019) reported a yield of 40.6 % (w/w of dry alga) from *Sargassum cristaefolium*. The composition of sodium alginate extracted from South African *E. maxima* is shown in Table 1, along with commercial sodium alginate, which was included as a positive control for compositional analysis. The extracted sodium alginate from South African *E. maxima* had a total sugar content of 67.94 ± 1.20 % (w/w), which was not statistically different from that of the total sugar content of the commercial sodium alginate, which was 72.62 ± 5.30 % (w/w) (P = 0.1855). No protein contaminants were observed in either sodium alginate sample. Phenolic contents were higher in the extracted sodium alginate from South African *E. maxima* (P = 0.0075), with 0.84 ± 0.68 % (w/w) and 1.14 ± 0.66 % (w/w) for the commercial sodium alginate and the sodium alginate extract, respectively. Sulphate contents were similarly higher in the extracted sodium alginate from *E. maxima* (P = 0.0001). In general,

Table 1: Chemical profile of the sodium alginates [as a percentage composition of sodium alginate (dry w/dry w)].

Sodium alginate source	Protein (%)	Phenolic (%)	Sulphate (%)	Ash content (%)	Total sugar (%)	Uronic acid (%)
Commercial sodium alginate	0	0.84 ± 0.064	0.05 ± 0.025	22.38	72.62 ± 5.30	119.89 ± 2.38
Ecklonia maxima sodium alginate	0	1.14 ± 0.066	0.84 ± 0.076	30.58	67.94 ± 1.20	126 ± 6.80
P-Value[a]	–	0.0075	< 0.0001	–	0.1855	0.2195

[a]Considered statistically significant if <0.05.

sodium alginate from *E. maxima* showed similar chemical characteristics to commercial sodium alginate, including total sugar and monosaccharide composition. Both sodium alginates contained mainly sugar, 72.62 ± 5.30 % (w/w) for the commercial sodium alginate and 67.94 ± 1.20 % (w/w) for the extracted sodium alginate; however, the commercial sodium alginate had fewer contaminants, such as sulphates and phenolics. The commercial and extracted sodium alginates also contained monosaccharides, which were negligible (data not shown). The uronic acid contents of the sodium alginates were calculated using the carbazole assay with mannuronic acid as a standard. Similar to the total sugar content, the uronic acid values between the commercial sodium alginate (119.89 ± 2.38 %, w/w) and the sodium alginate extracted from *E. maxima* (126 ± 6.80 %, w/w) were not statistically different (P = 0.2195). The chemical profiles confirmed that, generally, there were no significant chemical differences between the commercial sodium alginate and the sodium alginate extracted from *E. maxima* using the optimised method, except for contaminants such as phenolics and sulphate.

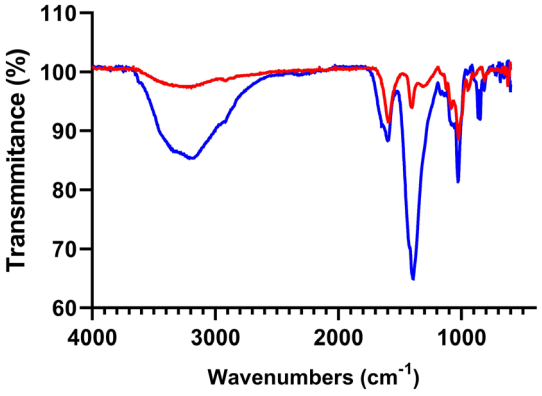

Figure 1: Fourier-transform infrared spectra of sodium alginate samples. The red line represents the commercial sodium alginate, and the blue line represents the sodium alginate extracted from *Ecklonia maxima* using the optimised method.

3.2 Structural characterisation of the sodium alginates

3.2.1 Functional group determination of sodium alginates by FTIR spectroscopy

FTIR spectra in the 4,000–600 cm^{-1} range were collected for the commercial and extracted sodium alginates (Figure 1). Typical sodium alginate spectra were shown for both sodium alginates. Extracted sodium alginate showed all the typical polysaccharide bands of O–H stretching vibrations at 3,300 cm^{-1}, asymmetric stretching of carboxylate O–C–O vibration at 1,600 cm^{-1}, and symmetric stretching vibration of the carboxylate group at 1,400 cm^{-1} (Fertah et al. 2014; Silverstein et al. 1991). The absorption bands in the 950 cm^{-1}, 900 cm^{-1}, and 810 cm^{-1} regions indicated the C–O stretching vibration of uronic acid residues, Cl–H deformation vibration of β-mannuronic acid residues, and characteristics of mannuronic acid residues, respectively (Belattmania et al. 2020; Fertah et al. 2014). Peaks at 1,230–1,280 cm^{-1} (attributable to sulphated polysaccharides such as fucoidan) were absent, indicating highly purified sodium alginate (Rashedy et al. 2021). In addition, there were no peaks in the 1755 cm^{-1} region (an indication of stretching of the protonated carboxylic group). Instead, the peaks appeared at 1,600 cm^{-1}, indicating asymmetric and symmetric stretching vibration of the free carboxyl group of sodium alginate, which showed the displacement of the proton with a sodium ion (Taha et al. 2005). FTIR analysis was not able to identify the presence of guluronic acid residues. NMR was subsequently used to confirm the presence of mannuronic and guluronic acid residues in the sodium alginate extracts.

3.2.2 Linkage analysis of the sodium alginates by ^{1}H NMR

The three key signals, the anomeric proton of guluronic acid at 5.1–5.2 ppm, the anomeric protons of mannuronic acid, the H-5 of alternating blocks at 4.7–4.9 ppm, and the homopolymeric G blocks H-5 of guluronic acid residues at 4.5–4.6 ppm, are frequently used in the literature to identify

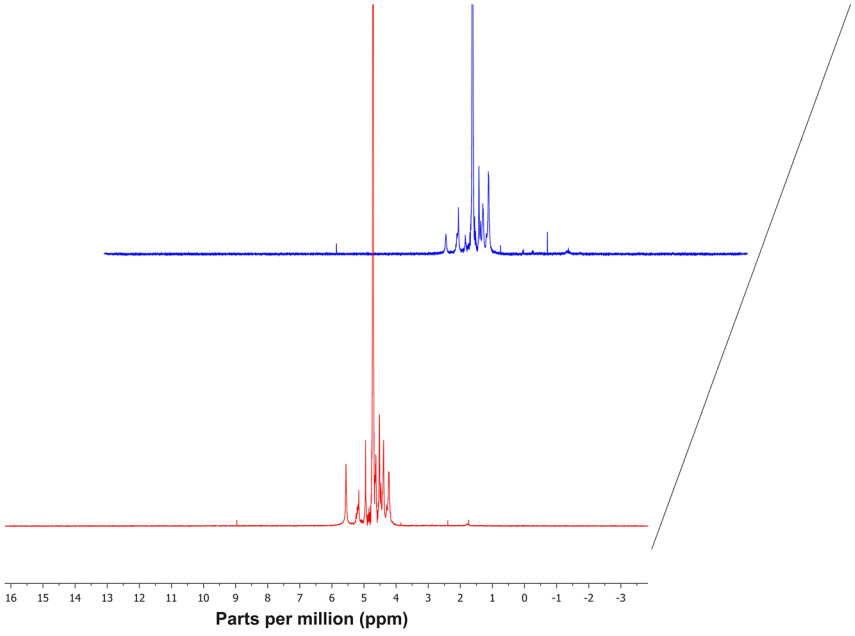

Figure 2: Nuclear magnetic resonance spectra of sodium alginate samples. The red line represents the commercial sodium alginate, and the blue line represents the sodium alginate extracted from *Ecklonia maxima* using the optimized method.

and report on the components in sodium alginates (Belattmania et al. 2020; Fertah et al. 2014). The three key signals for sodium alginates were present in the ^{1}H-NMR spectra generated from the *E. maxima* extracts and the commercial sodium alginate samples (Figure 2). However, the solvent peak of D_2O was shown around the 4.75 ppm region, which overlapped with the mannuronic acid protons and the H-5 of alternating blocks. Although running the NMR at a temperature of 70 °C was used to overcome this problem, the sodium alginate peaks shifted with the solvent peak. However, the maximum intensity at 4.7–4.9 ppm still revealed the presence of a D_2O solvent peak for both the commercial sodium alginate and the extracted sodium alginate. On the other hand, both sodium alginates showed peaks at 4.5–4.6 ppm, indicating the presence of homopolymeric guluronic blocks. A peak at around 5.1–5.2 ppm was also observed for both the commercial and extracted sodium alginates, indicating the presence of guluronic acid units, with extracted sodium alginate with higher intensity than the commercial sodium alginate. Although the NMR revealed the building blocks of commercial and extracted sodium alginate, the M/G ratio could not be identified due to the high solvent peak. Therefore, CD spectroscopy was used to identify the M/G ratios of the sodium alginates.

3.2.3 Characterisation of the sodium alginate M/G ratio by CD spectroscopy

CD spectroscopy was performed to determine the conformational changes in the polysaccharides' secondary and local tertiary structures and to determine the mannuronic and guluronic ratios. Carbohydrates do not form defined structures like the α-helices and β-sheets in proteins, but they may be in the form of a disordered chain, an extended rigid chain, or a collapsed, flexible and helix-like chain (Sun et al. 2018). As shown in Figure 3, sodium alginate exhibited positive and negative Cotton effects at 199 and 213 nm, respectively, indicating the presence of helix-like and sheet-like structures (Sun et al. 2018). However, the peaks at 199 nm and the troughs around the 213 nm region displayed different values between the commercial and extracted sodium alginate samples, indicating different structural conformations. The mannuronate and guluronate ratios of the commercial and extracted sodium alginate were determined by a parameter described by Morris et al. (1980). The

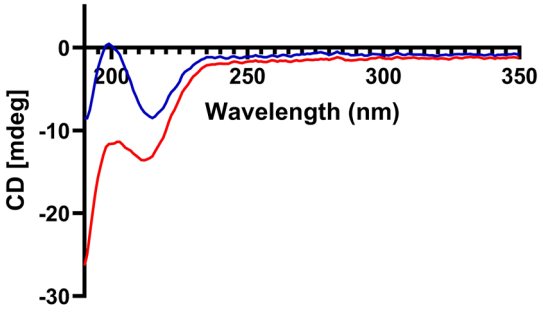

Figure 3: CD analysis of commercial sodium alginate samples. The red line represents the commercial sodium alginate, and the blue line represents the sodium alginate extracted from *Ecklonia maxima* using the optimized method.

commercial sodium alginate peaked at around −5.63, and the trough was around −9.08. Using the first parameter, the M/G ratio of the commercial sodium alginate revealed 43 % mannuronate and 57 % guluronate content. The extracted sodium alginate peaked at around 0.48, and the trough was around −8.49; therefore, the M/G ratio was 1.86, indicating 65 % mannuronate and 35 % guluronate content. The M/G ratios of commercial and extracted sodium alginate were 0.76 and 1.89, respectively. Although these values did not correspond to the chemical profiles reported for a typical sodium alginate, Saji et al. (2022), also using CD spectra, reported M/G ratios from different seaweed species in the range of 0.43–2.52.

M/G ratios obtained for different species of brown seaweeds and different commercial sodium alginates varied (Table 2). The results of our study showed that the commercial sodium alginate contained comparatively less mannuronic acid; the *E. maxima* extracted sodium alginate contained more mannuronic acid, but less compared to what has been reported in other commercial sodium alginates (Belattmania et al. 2020), or brown seaweeds (Rashedy et al. 2021), 15.58 % and 30.11 % less, respectively. Our results similarly showed that the commercial sodium alginate had a comparatively low M/G ratio; the *E. maxima* extracted sodium alginate had a higher M/G ratio, but much less compared to what has been reported in other commercial sodium alginates (Belattmania et al. 2020), or brown seaweeds (Rashedy et al. 2021), 77.78 % and 94.14 % less, respectively. The M/G ratio of alginate has been found to affect its gel strength and permeability. A high M/G ratio results in a weaker structure but a more permeable gel matrix, while a low M/G ratio leads to a stronger structure that is less permeable (Khanna et al. 2010; Ramos et al. 2018). Thus, the desired M/G ratio ranges intermediately between permeable and less permeable, which balances permeability and strength for general use. However, it should be dependent on the application of the sodium alginate gel. Extracting sodium alginate from an endemic South African brown seaweed to produce a desirable biopolymer with both permeable and strong gel characteristics could be explored. The endemic brown seaweed, *Ecklonia maxima*, may prove to be a promising candidate.

3.2.4 Molecular weight and intrinsic viscosity of the sodium alginates

The viscosity average molecular weights of the commercial and extracted sodium alginates were calculated from the Mark-Houwink-Sakurada (MHS) equation following the method of Vold et al. (2006). Their intrinsic viscosities were calculated by equations (1)–(3). The average intrinsic viscosity of the commercial sodium alginate was 5,728.19 mL/g, and for the extracted sodium alginate, 7,307.02 mL/g. The molecular weights of the commercial and extracted sodium alginates were calculated according to equations (4) and (5) with the MHS parameters of Vold et al. (2006). Using these equations, the average molecular weight of the commercial sodium alginate was calculated to be 326 kDa, while that of the extracted sodium alginate was 429 kDa. These molecular weights were higher compared to those of another brown seaweed, *Laminaria digitata*, reported by Vauchel et al. (2008, 105 kDa) and Fertah et al. (2014, 114 kDa). The extracted sodium alginate was shown to have a more comparable molecular weight to *E. radiata* from South Australia, which showed a molecular weight range of 373–986 kDa (Lorbeer et al. 2015).

3.2.5 Characterisation of sodium alginate structure by Congo red

A Congo red test determined whether the commercial and extracted sodium alginates exhibit a triple helix

Table 2: Mannuronic and guluronic compositions of sodium alginate extracted from *Ecklonia maxima* and commercial sodium alginate (this study) compared to one other commercial sodium alginate and two other brown seaweeds via NMR spectroscopy.

Species	F_G	F_M	M/G	F_{MM}	F_{GG}	$F_{GM/MG}$	Reference
Ecklonia maxima	0.35	0.65	1.86	–	–	–	This study
Laminaria digitata	0.47	0.53	1.12	0.47	0.41	0.06	Fertah et al. (2014)
Sargassum aquifolium	0.07	0.93	12.96	0.87	0.01	0.06	Rashedy et al. (2021)
Sigma-Aldrich Na-alginate	0.23	0.77	3.42	0.68	0.14	0.09	Belattmania et al. (2020)
Commercial Na-alginate	0.57	0.43	0.76	–	–	–	This study

Where F_G is the guluronic acid fraction, F_M is the mannuronic acid fraction, M/G is the mannuronic/guluronic acid ratio, F_{MM} is the mannuronic homopolymeric blocks, F_{GG} is the guluronic homopolymeric blocks, and $F_{GM/MG}$ is the heteropolymeric blocks of mannuronic and guluronic acids.

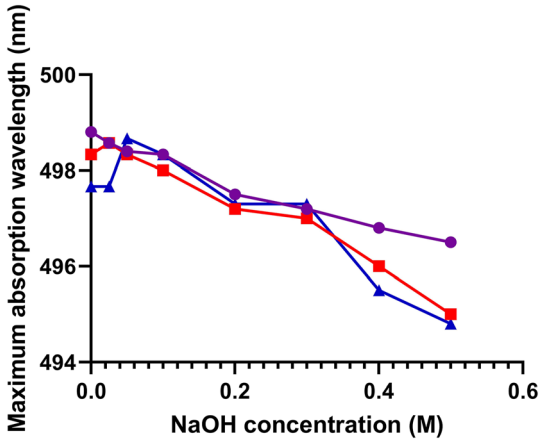

Figure 4: Congo red spectroscopy analysis of the sodium alginate samples. The purple line represents the Congo red standard, the red line represents the commercial sodium alginate, and the blue line represents the sodium alginate from the extracted *Ecklonia maxima* using the optimized method.

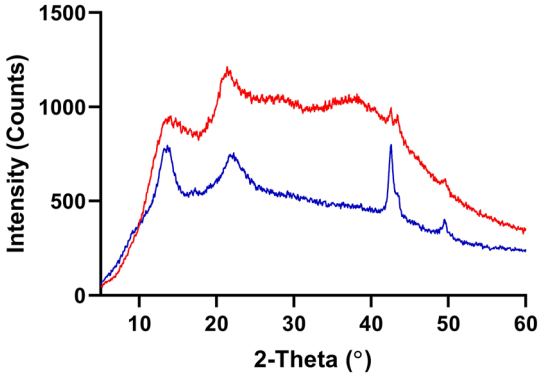

Figure 5: X-ray diffraction analysis of the sodium alginate samples. The red line represents the commercial sodium alginate, and the blue line represents the sodium alginate extracted from *Ecklonia maxima* using the optimized method.

conformation. Congo red can form complexes with the helical conformation of polysaccharides (Guo et al. 2018). The maximum absorption wavelength λ_{max} of polysaccharides will generate a bathochromic shift in the wavelength range of 400–600 nm, as opposed to no bathochromic shift for pure Congo red (Guo et al. 2018). The commercial and extracted sodium alginates showed a bathochromic shift with Congo red compared to pure Congo red (Figure 4). This phenomenon suggested that the commercial and extracted sodium alginates are in a triple-helix structure form (Yang et al. 2021). It has also been reported that the λ_{max} of the polysaccharide complex with helical conformation can also show a change to a longer wavelength at low concentrations of NaOH and decrease with increasing NaOH concentration (Guo et al. 2018).

3.2.6 Characterisation of sodium alginate morphology by X-ray powder diffraction

X-ray powder diffraction is a technique widely used to determine the structure of different substances. Repeating unit polysaccharides can be arranged in an orderly structure within their molecule structure and thus give a certain diffraction pattern when irradiated with X-rays on their surface (Qian et al. 2009; Wang et al. 2014). Usually, polysaccharides lead to 'bun-shaped' XRD curves due to poor crystallisation capacity (Wang et al. 2014). The XRD spectra of the commercial and extracted sodium alginates exhibited 'bun-shaped' diffraction peaks when 2θ (2-Theta) was about 17–20° (Figure 5), indicating that both the commercial and extracted sodium alginates had an amorphous structural makeup; this has been reported for sodium alginate before (Helmiyati and Aprilliza 2017).

3.2.7 Thermogravimetric analysis of the sodium alginates

Thermogravimetric (TGA) analysis and derivative thermogravimetry (DTG) analysis showed that both commercial and extracted sodium alginate samples lost weight slightly at approximately 50–250 °C (Figure 6A). The weight loss could be due to moisture loss and volatile compounds in the samples (Gufe et al. 2023). The TGA analysis showed greater weight loss between 250 and 300 °C and slower weight loss from 300 to 750 °C. The DTG analysis concurred with the TGA curve, showing a similar exothermal peak at 250–300 °C for the commercial and extracted samples (Figure 6B), reaching 50 % of their original weight. This was attributed to sample degradation, resulting in the formation of water and CH_4 and the release of CO_2 (Salisu et al. 2015). The last weight loss happened at around 300–750 °C, to approximately 20 % and 30 % of the ash content for the commercial and the extracted sodium alginates, respectively (Table 1). The difference between the commercial and the extracted sodium alginate could be due to the difference in the inorganic compounds or the sodium carbonate remaining in the *E. maxima* samples after extraction. However, the *E. maxima* samples showed a typical sodium alginate TGA profile and only contained a few contaminants compared to the commercial sodium alginate.

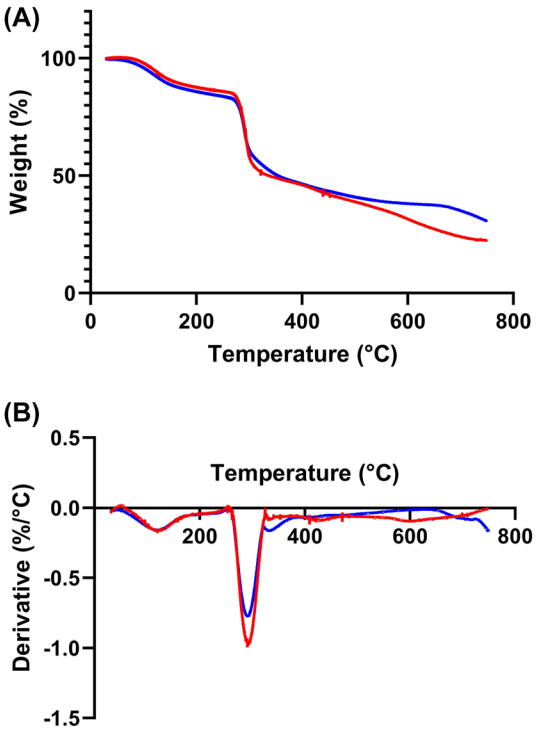

Figure 6: Thermal decomposition characteristics of sodium alginates. (A) Thermogravimetric analysis and (B) derivative thermogravimetry analysis of the sodium alginate samples. The red line represents the commercial sodium alginate, and the blue line represents the sodium alginate extracted from *Ecklonia maxima* using the optimized method.

4 Conclusions

Sodium alginate from *E. maxima* found widely along the South African west and south coasts, was extracted using a solvent extraction technique. Analyses of the chemical and physical properties of the extracted sodium alginate, compared against a commercially available counterpart, suggested that using an optimised extraction method, sodium alginate extracted from *E. maxima* was chemically comparable to the commercially available sodium alginate. The physical properties of the *E. maxima* extracted and commercial sodium alginates, however, exhibited different M/G ratios and molecular weights; that of *E. maxima* was 1.86 and 429 kDa, respectively, while that of the commercial sodium alginate, was 0.946 and 326 kDa. The high mannuronic acid content and high viscosity make sodium alginate extracted from *E. maxima* attractive for thickening and gelling agents, stabilisers, and emulsifiers in different industries, including in biomedical applications and drug delivery. With the appropriate modifications, the extraction process developed in this study may be applied to other value-added products using a range of similar brown seaweeds.

Acknowledgments: The authors thank KelpX for providing algal samples. The reviewers are thanked for their comments on and suggestions to earlier versions of this manuscript.

Research ethics: Not applicable.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission. Conceptualisation: BIP and YP; data curation: YP and SM; formal analysis: BIP, RWMK, YP, and SM; funding acquisition and supervision: BIP; writing - original draft: YP; writing – review and editing: BIP, SM, and YP.

Competing interests: The authors declare that they have no conflicts of interest.

Research funding: This study was supported by the Industrial Biocatalysis Hub (IBH), funded by the Department of Science and Innovation (DSI) and Technology Innovation Agency (TIA), and managed by the Council for Scientific and Industrial Research (CSIR) of South Africa. The financial support from Rhodes University (RRRG and RC grants) is also gratefully acknowledged. This work is based on the research supported in part by the National Research Foundation of South Africa (Grant number: MND190621449698).

Data availability: The raw data can be obtained on request from the corresponding author.

References

Anderson, R., Simons, R., and Jarman, N. (1989). Commercial seaweeds in southern Africa: a review of utilisation and research. *South Afr. J. Mar. Sci.* 8: 277–299.

Anderson, R.J., Rothman, M.D., Share, A., and Drummond, H. (2006). Harvesting of the kelp *Ecklonia maxima* in South Africa affects its three obligate, red algal epiphytes. *J. Appl. Phycol.* 18: 343–349.

Belattmania, Z., Kaidi, S., El Atouani, S., Katif, C., Bentiss, F., Jama, C., Reani, A., Sabour, B., and Vasconcelos, V. (2020). Isolation and FTIR-ATR and ^{1}H NMR characterisation of alginates from the main alginophyte species of the Atlantic coast of Morocco. *Molecules* 25: 4335.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.

Cardozo, K., Guaratini, T., Barros, M., Falcão, V., Tonon, A., Lopes, N., Campos, S., Torres, M., Souza, A., Colepicolo, P., et al (2007). Metabolites from algae with economical impact. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* 146: 60–78.

Cesaretti, M., Luppi, E., Maccari, F., and Volpi, N. (2003). A 96-well assay for uronic acid carbazole reaction. *Carbohydr. Polym.* 54: 59–61.

Daub, C., Mabate, B., Malgas, S., and Pletschke, B. (2020). Fucoidan from *Ecklonia maxima* is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. *Int. J. Biol. Macromol.* 151: 412–420.

DuBois, M., Gilles, K., Hamilton, J., Rebers, P., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350–356.

Fertah, M., Belfkira, A., Dahmane, E., Taourirte, M., and Brouillette, F. (2014). Extraction and characterisation of sodium alginate from Moroccan *Laminaria digitata* brown seaweed. *Arabian J. Chem*. 10: S3707–S3714.

Gheorghita Puscaselu, R., Gutt, G., and Amariei, S. (2020). The use of edible films based on sodium alginate in meat product packaging: an eco-friendly alternative to conventional plastic materials. *Coatings* 10: 166.

Gufe, C., Thantsha, M.S., and Malgas, S. (2023). Recovery of xylan from *Acacia mearnsii* using ultrasound-assisted alkaline extraction. *Soc Ind. Chem.* 17: 976–987.

Guo, Y., Cong, S., Zhao, Jun., Dong, Y., Li, T., Zhu, B., Song, S., and Wen, C. (2018). The combination between cations and sulfated polysaccharide from abalone gonad (*Haliotis discus hannai* Ino). *Carbohydr. Polym.* 188: 54–59.

Hahn, T., Lang, S., Ulber, R., and Muffler, K. (2012). Novel procedures for the extraction of fucoidan from brown algae. *Process Biochem*. 47: 1691–1698.

Halabalová, V., šimek, L., Dostál, J., and Bohdanecký, M. (2004). Note on the relation between the parameters of the mark-houwink-kuhn-sakurada equation. *Int. J. Polym. Anal. Charact.* 9: 65–75.

Helmiyati, H. and Aprilliza, M. (2017). Characterization and properties of sodium alginate from brown algae used as an ecofriendly superabsorbent. *IOP Conf. Ser. Mater. Sci. Eng*. 188: 12–19.

Holdt, S. and Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *J. Appl. Phycol.* 23: 543–597.

Jacobsen, C., Sørensen, A., Holdt, S., Akoh, C., and Hermund, D. (2019). Source, extraction, characterisation, and applications of novel antioxidants from seaweed. *Annu. Rev. Food Sci. Technol.* 10: 541–568.

Khanna, O., Moya, M., Opara, E., and Brey, E. (2010). Synthesis of multilayered alginate microcapsules for the sustained release of fibroblast growth factor-1. *J. Biomed. Mater. Res., Part A* 95A: 632–640.

Kontominas, M.G. (2020). Use of alginates as food packaging materials. *Foods* 9: 1440.

Lorbeer, A., Lahnstein, J., Bulone, V., Nguyen, T., and Zhang, W. (2015). Multiple-response optimisation of the acidic treatment of the brown alga *Ecklonia radiata* for the sequential extraction of fucoidan and alginate. *Bioresour. Technol.* 197: 302–309.

Mabate, B., Daub, C., Malgas, S., Edkins, A., and Pletschke, B. (2021). Fucoidan structure and its impact on glucose metabolism: implications for diabetes and cancer therapy. *Mar. Drugs* 19: 30.

Malgas, S., van Dyk, J., Abboo, S., and Pletschke, B. (2016). The inhibitory effects of various substrate pre-treatment by-products and wash liquors on mannanolytic enzymes. *J. Mol. Catal. B: Enzym.* 123: 132–140.

Manilal, A., Sujith, S., Kiran, G., Selvin, J., Shakir, C., Gandhimathi, R., and Lipton, A. (2009). Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. *Ann. Microbiol.* 59: 207–219.

Morris, E.R., Rees, D.A., and Thom, D. (1980). Characterisation of alginate composition and block-structure by circular dichroism. *Carbohydr. Res.* 81: 305–314.

Pal, A., Kamthania, M., and Kumar, A. (2014). Bioactive compounds and properties of seaweeds – a review. *OALib* 01: 1–17.

Poojary, M., Barba, F., Aliakbarian, B., Donsì, F., Pataro, G., Dias, D., and Juliano, P. (2016). Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. *Mar. Drugs* 14: 214.

Qian, J.-Y., Chen, W., Zhang, W.-M., and Zhang, H. (2009). Adulteration identification of some fungal polysaccharides with sem, XRD, IR and optical rotation: a primary approach. *Carbohydr. Polym.* 78: 620–625.

Ramos, P., Silva, P., Alario, M., Pastrana, L., Teixeira, J., Cerqueira, M., and Vicente, A. (2018). Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. *Food Hydrocolloids* 77: 8–16.

Rashedy, S., Abd El Hafez, M., Dar, M., Cotas, J., and Pereira, L. (2021). Evaluation and characterisation of alginate extracted from brown seaweed collected in the Red Sea. *Appl. Sci.* 11: 6290.

Rasmussen, R. and Morrissey, M. (2007). Marine biotechnology for production of food ingredients. *Adv. Food Nutr. Res.* 52: 237–292.

Rhein-Knudsen, N., Ale, M., and Meyer, A. (2015). Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. *Mar. Drugs* 13: 3340–3359.

Rothman, M.D., Anderson, R.J., and Smit, A.J. (2006). The effects of harvesting of the South African kelp (*Ecklonia maxima*) on kelp population structure, growth rate and recruitment. *J. Appl. Phycol.* 18: 335–341.

Saji, S., Hebden, A., Goswami, P., and Du, C. (2022). A brief review on the development of alginate extraction process and its sustainability. *Sustainability* 14: 5181.

Salisu, A., Sanagi, M., Abu Naim, A., Abd Karim, K., Wan Ibrahim, W., and Abdulganiyu, U. (2015). Alginate graft polyacrylonitrile beads for the removal of lead from aqueous solutions. *Polym. Bull.* 73: 519–537.

Silverstein, R., Webster, F., and Kiemle, D. (1991). *Spectrometric identification of organic compounds*. John Wiley & Sons, Hoboken, NJ.

Sugiono, S. and Ferdiansyah, D. (2019). Biorefinery sequential extraction of alginate by conventional and hydrothermal fucoidan from the brown alga, Sargassum cristaefolium. *Biosci. Biotechnol. Res. Commun*. 12: 894–903.

Sun, T., Zhang, X., Miao, Y., Zhou, Y., Shi, J., Yan, M., and Chen, Anjin. (2018). Studies on antiviral and immuno-regulation activity of low molecular weight Fucoidan from *Laminaria japonica*. *J. Ocean Univ. China* 17: 705–711.

Taha, M., Aiedeh, K., Al-Hiari, Y., and Al-Khatib, H. (2005). Synthesis of zinc-crosslinked thiolated alginic acid beads and their in vitro evaluation as potential enteric delivery system with folic acid as model drug. *Pharmazie* 60: 736–742.

Torsdottir, I., Alpsten, M., Holm, G., Sandberg, A., and Tölli, J. (1991). A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *J. Nutr.* 121: 795–799.

Vauchel, P., Kaas, R., Arhaliass, A., Baron, R., and Legrand, J. (2008). A new process for extracting alginates from Laminaria digitata: reactive extrusion. *Food Bioproc. Tech*. 1: 297–300.

Vold, I., Kristiansen, K., and Christensen, B. (2006). A study of the chain stiffness and extension of alginates, in vitro epimerised alginates, and periodate-oxidised alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules* 7: 2136–2146.

Wang, K., Wang, J., Li, Q., Zhang, Q., You, R., Cheng, Y., Luo, L., and Zhang, Y. (2014). Structural differences and conformational characterisation of five bioactive polysaccharides from *Lentinus edodes*. *Food Res. Int.* 62: 223–232.

Yang, X., Wei, S., Lu, X., Qiao, X., Simal-Gandara, J., Capanoglu, E., Woźniak, Ł., Zou, L., Cao, H., Xiao, J., et al. (2021). A neutral polysaccharide with a triple helix structure from ginger: characterisation and immunomodulatory activity. *Food Chem.* 350: 129261.

Bionotes

Yuchan Park
Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa
g16p3593@ru.ac.za
https://orcid.org/0000-0002-1317-4072

Mr. Yuchan Park's current research focuses on the large-scale co-extraction of fucoidan and sodium alginate from *Ecklonia maxima*. This includes using acid extraction to obtain the highest simulataneous yields of fucoidan and sodium alginate with characteristics that are suitable for many different industrial uses.

Samkelo Malgas
Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Hatfield 0028, South Africa
samkelo.malgas@up.ac.za
https://orcid.org/0000-0003-0729-5393

Dr. Samkelo Malgas is a senior lecturer in the Department of Biochemistry, Genetics, and Microbiology at the University of Pretoria, South Africa. His research focuses on biomass pretreatment and the use of enzymes for the recovery of valuable co-products from agro-industrial derived lignocellulosic waste feedstocks. Furthermore, he evaluates these agroindustrial waste-derived products as prebiotics, antioxidants, and antidiabetic and anti-obesity leads to managing lifestyle diseases and overall human health.

Rui W.M. Krause
Department of Chemistry, Rhodes University, Makhanda 6140, South Africa
R.Krause@ru.ac.za
https://orcid.org/0000-0001-6788-6449

Prof. Rui W.M. Krause is a Professor of Organic and Medicinal Chemistry in the Department of Chemistry at Rhodes University, in Makhanda, South Africa. He is an organic and medicinal chemist, with an interest in natural products and in drug delivery. The use of biopolymers such as alginates feature strongly in his work at Rhodes University, particularly for antimicrobial and anti-infective therapies. He has published more than 120 papers in peer-reviewed international journals.

Brett I. Pletschke
Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa
b.pletschke@ru.ac.za
https://orcid.org/0000-0001-8117-1730

Prof. Brett I. Pletschke is a Professor of Biochemistry. His current research interests lie in the fields of enzymology and enzyme biotechnology, with a particular focus on the enzymatic extraction of bioproducts and bioactives from kelp and seaweed biomass for application in the nutraceutical and biomedical industries. He has published more than 130 papers in international journals and books. He is a fellow of the African Academy of Sciences (AAS) and serves on the editorial boards of several journals.