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Seaweed processing using industrial single-mode cavity microwave heating: a preliminary investigation

Sandra F. Uy,[a,*] Allan J. Easteal,[a] Mohammed M. Farid,[b] Richard B. Keam[c] and Gregory T. Conner[c]

[a]*Department of Chemistry, The University of Auckland, New Zealand*
[b]*Department of Chemical and Materials Engineering, The University of Auckland, New Zealand*
[c]*Keam Holdem Associates Ltd, Auckland, New Zealand*

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Abstract—A single-cavity microwave heating system has been designed and fabricated for microwave-assisted extraction of carrageenans from seaweed. The system comprises a single mode (TE_{101}) waveguide fitted with power and temperature controls, together with a continuous-flow-recycle reactor operating at atmospheric pressure. The system has been tested by extraction of *E. cottonii* and *E. spinosum* in aqueous organic solvents. Even without purification, the extraction products were found to have virtually identical FTIR and ^{13}C and ^{1}H NMR spectra to the reference samples of κ- and ι-carrageenan, respectively. The principal advantages of the microwave system are substantial reduction of extraction time and low consumption of organic solvents.

Keywords: Microwave; Extraction; Carrageenan; NMR; FTIR

1. Introduction

A microwave-assisted technique has been developed to carry out the extraction of ι-carrageenan from *Eucheuma denticulatum* (otherwise known as *E. spinosum*) and κ-carrageenan from *Kappaphycus alvarezii* (known as *E. cottonii* in the industry). Carrageenan is used in a wide variety of commercial applications such as gelling, thickening and stabilising agent in specialty food products, and it has further use in pharmaceutical formulations, cosmetics and industrial applications.

The conventional commercial method of producing carrageenan is extraction of the polysaccharide from the seaweed with 0.05–0.1 mol L^{-1} sodium hydroxide or calcium hydroxide at 90–100 °C for 3–5 h. The formation of 3,6-anhydro-α-D-galactose units from α-D-galactose-6-sulfate residues by alkaline treatment (Scheme 1) is an important and well-known reaction of carrageenans,[1,2] and is used to commercially enhance gelation behaviour.[3]

Typically, viscous materials such as carrageenan transfer energy poorly, and large thermal gradients can result in sub-optimum conversions and loss of product. When high temperatures are required, heat losses increase and conductive heating becomes inefficient. Under microwave conditions these problems are diminished.[4]

The method developed in this study is based on the use of extraction solvents that are fully or partially transparent to the microwaves as compared to the target material. In that way, selective and localised heating within the material can be accomplished without requiring excessive energy to heat the complete mixture.[5–10] The purpose of this communication is to report our initial results, which suggest that the microwave method has considerable promise as the basis for an efficient commercial method for extraction of carrageenan.

* Corresponding author. Tel.: +64 9 373 7599; fax: +64 9 373 7422; e-mail: s.uy@auckland.ac.nz

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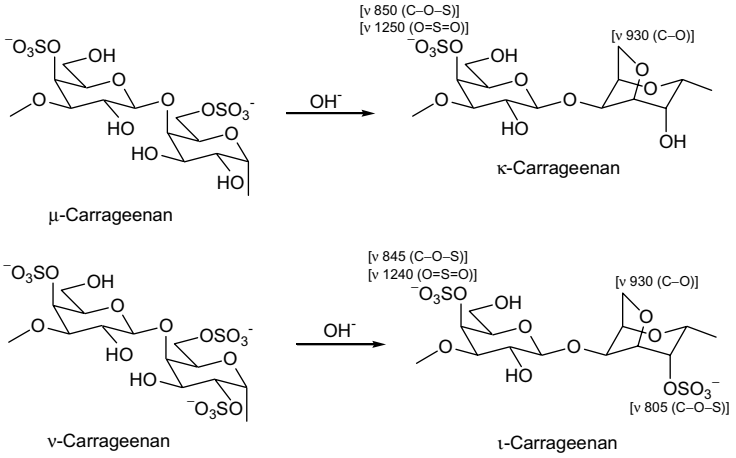

Scheme 1. Alkaline treatment of carrageenans.

2. Materials and methods

2.1. Chemicals, reagents and carrageenans

Commercial carrageenans from *Eucheuma denticulatum* (C-4014 Type V) and *Kappaphycus alvarezii* (C1263 Type III), supplied by Sigma–Aldrich, were used as reference materials. Analytical Reagent grade solvents (methanol, acetone, ethanol and 2-propanol) were supplied by APS Chemicals (NZ) Ltd. Sodium hydroxide and potassium hydroxide were Scharlau Chemie S.A. (Barcelona, Spain) products. Plant materials (*E. cottonii* and *E. spinosum*) were kindly donated by FMC BioPolymer, Cebu City, Philippines. The dried seaweed was stored in a Votcsh humidity chamber at 35 °C and 60% relative humidity prior to use. The seaweed was washed with distilled water to eliminate residues from the thalli surface, cut and oven-dried at 60 °C before processing. Immediately prior to processing, the seaweed was pulverised to a fine powder using a Fritsch 'pulverisette 0' (Idar–Oberstein, Germany) vibratory micro mill.

2.2. Continuous microwave apparatus

A continuous microwave apparatus was designed and constructed. Its main features (see Fig. 1) include control of microwave power, measurement of absorbed and reflected microwave energy, a load-matching device to maximise heating efficiency, measurement of the reaction temperature and pressure, plumbing to facilitate sample introduction and withdrawal, and chemically inert surfaces and fittings.

Due to the nature of electromagnetic fields within the microwave cavity, it is difficult to obtain a uniform distribution of energy, and to monitor and control the temperature. In this experimental set-up, a single mode (TE_{101}) resonant cavity is tuned to the characteristics

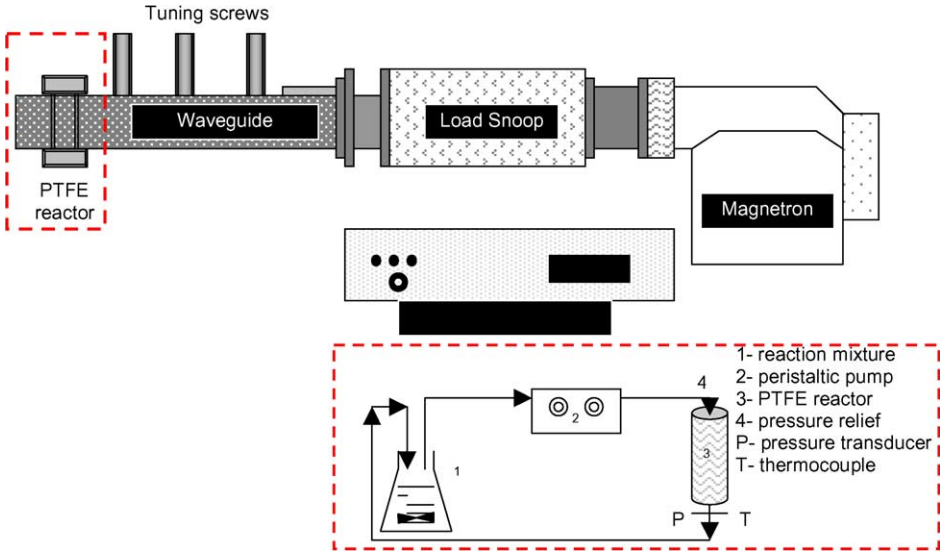

Figure 1. Schematic illustration of microwave extraction system.

of the material being heated by varying the position of tuning screws so that the cavity can be made to resonate at the working frequency. The LoadSnoop™ unit ensures a good impedance match between the waveguide and the cavity so that the energy is not reflected back towards the source. The efficiency of the matching is monitored using the software SnoopLink™. Fail-safe measures shut down the unit if the temperature exceeds the maximum allowable temperature, or in case of blockages if the pressure exceeds the pre-set limit of 0.5 bar. A PTFE reactor with a working volume of 35 mL (1.5 cm i.d. and 5.0 cm height) and a residence time of 1.4 min was used. Temperature was measured immediately after the exit point from the microwave zone. The exit temperature may not be necessarily the maximum temperature allowed by the reaction mixture, but since the reaction times are short, the reaction mixture should not have sufficient time to cool down appreciably before exiting the microwave zone. The exit temperature is consequently expected to be a good approximation to the maximum temperature in the reaction coil.

2.3. Extraction procedure

Preliminary work was carried out to establish operational parameters. Forward and reflected powers were measured within the waveguide, and reflected power was minimised by the load-matching device. Microwave energy is often guided to the material by a hollow rectangular metal pipe (waveguide). By inserting metal tuning rods into the waveguide to particular depths at intervals along the waveguide, interference between the reflected energy and the forward energy can be set up to minimise the reflected energy. Microwave-assisted extractions were performed at 38% of full power (800 W) and at a frequency of 2450 MHz at atmospheric pressure. The method of power control employed for the system was duty cycling, whereby for a cycle of 5 s duration, for example, for 50% power the magnetron was energised for 2.5 s. This parameter is stored in the controller configuration file.

Table 1 lists the water-miscible solvents with varying dielectric properties that were tested. Starting with the pure solvent in which carrageenan was insoluble, water was gradually added until the hydrocolloid just became soluble. The aqueous mixtures that were just able to dissolve the carrageenan were found to be methanol–water (45–55 wt %), ethanol–water (37–63 wt %), acetone–water (55–45 wt %) and 2-propanol–water (40–60 wt %).

Sodium hydroxide or potassium hydroxide was added to aqueous solvent mixture to make a 0.1 mol L^{-1} solution, and powdered, oven-dried seaweed was added to make a 6% (wt/wt) seaweed mixture. The resulting reaction mixture was recirculated through the microwave zone via a peristaltic pump for 30 min. After extraction, the reaction mixture was centrifuged, and the supernatant was removed. It was freeze dried for 48 h and pulverised prior to analysis. Extracts were analysed without further purification, and identification of carrageenan was confirmed by ^{13}C and ^{1}H NMR and FTIR spectroscopy, by comparison of the sample spectra with those of the reference samples. The spectroscopic data used are given in Tables 2–4.

Table 1. Selected data for solvents used[a]

Solvent	Dielectric constant[b], ε′	Dipole moment[c]	Dissipation factor, $\tan\delta$ ($\times10^{-4}$)	Boiling point[d] (°C)
Acetone	20.7	2.69		56
Ethanol	24.3	1.69	2500	78
Methanol	32.6	2.87	6400	65
2-Propanol	19.9	1.66	6700	82
Water	78.3	1.87	1570	100

[a] All data from Ref. 16.
[b] At 20 °C.
[c] At 25 °C.
[d] At 101.4 kPa.

2.4. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were acquired on a 9.4 Tesla Bruker AV400DRX (Bruker Biospin GMBH, Rheinstetten, Germany) instrument operating at 450 MHz for ^{1}H and 100 MHz for ^{13}C. Samples for ^{13}C NMR spectroscopy were prepared at 5% (wt/wt) and ^{1}H NMR samples at 0.5% (wt/wt) solutions in D_2O and freshly freeze dried samples using ultrasound for depolymerisation at 65 °C for both ^{1}H and ^{13}C NMR spectroscopy. For ^{1}H NMR spectroscopy, 128 scans were acquired and averaged in 2.6 s acquisition time after a 0.1 s relaxation delay and 30° pulse with a 6410 Hz sweep width resulting in 32K complex data points. The spectra were externally referenced using DSS ($\delta = 0.015$ ppm). For ^{13}C NMR spectroscopy, 29,000 scans were averaged with a 0.7 s acquisition time after a 1.0 s relaxation delay and 55° pulse with a 24,038 Hz sweep width resulting in 32K complex data points. The total measurement time was 16 h.

2.5. FTIR spectroscopy

For the IR studies, 5% (wt/wt) of ground, dried carrageenan were pressed in KBr discs. The FTIR spectra were recorded in the 1500–500 cm^{-1} spectral range using a Bio-Rad FTS 60 instrument. A total of 128 scans were averaged for each sample with a resolution of 2 cm^{-1}.

3. Results and discussion

Depending on the flow rate, the residence time of the mixture varied from 0.58 to 3.5 min. The total residence

Table 2. Identification of the types of carrageenan according to the occurrence (+), or lack thereof (−), of certain peaks in the IR spectra[a]

Wavenumbers (cm^{-1})	Bond(s)/group(s)	κ	μ	ι	ν
1240	S=O of sulphate esters	+	+	+	+
930	C–O of 3,6-anhydro-D-galactose (DA)	+	–	+	–
845	C–O–S of axial secondary sulphate on C-4 of G4S	+	+	+	+
830	C–O–S of equatorial secondary sulphate on C-2 of D2S, 6S	–	–	–	+
820	C–O–S of equatorial primary sulphate on C-6 of D2S, 6S or D6S	–	+	–	+
805	C–O–S on C-2 of DA2S	–	–	+	–

[a] Ref. 17.

Table 3. Proton chemical shifts of κ- and ι-carrageenan[a]

		H-1	H-2	H-3	H-4	H-5	H-6
κ	G	4.70	3.68	4.15	4.94	3.89	3.89
	AG	5.17	4.22	4.61	4.73	4.68	4.30/4.16
ι	G	4.77	3.71	4.18	4.95	3.90	3.90
	AG	5.35	4.77	4.86	4.78	4.78	4.35/4.21

[a] Ref. 18.

Table 4. ^{13}C NMR spectral assignment of ι- and κ-carrageenan[a]

Carrageenan	Unit	C-1	C-2	C-3	C-4	C-5	C-6
ι	G4S	103.1	69.9	77.3	72.5	75.3	61.8
	DA2S	92.4	75.3	78.7	78.9	77.5	70.3
κ	G4S	103.1	70.2	79.1	74.4	75.3	61.8
	DA	95.5	70.2	79.7	78.9	77.3	70.0

[a] Ref. 18.

time is obtained by multiplying the residence time per pass by the number of passes made by the mixture through the reactor. For example, when the flow rate is 25 mL min^{-1}, the residence time is 1.4 min per pass. In 30 min the 500 mL of mixture makes $30 \times 25/500 = 1.5$ passes through the reactor, and the total residence time is $1.5 \times 1.4 = 2.1$ min. An extraction time of 2.1 min was found to be sufficient for the formation of 3,6-anhydro-α-D-galactose units from α-D-galactose-6-sulfate residues by alkaline treatment.

Refined carrageenan has been commercially produced by extracting the polysaccharide from the seaweed with 0.05–0.1 N sodium hydroxide or calcium hydroxide at 90–100 °C:[19] the dispersed solids are removed by centrifugation and/or filtration, then concentrated and precipitated with alcohol or potassium chloride. The filtration removes acid insoluble materials, mainly cellulose. Most of the carrageenan used in food is isolated from the liquid extract by selective precipitation of the carrageenan with 2-propanol, and the carrageenan is subjected to three washes in a solvent–water mixture. The weight ratio of solvent to water is typically from 25:75 to 99:1.[20] However, with the proposed microwave-assisted technique, for each solvent mixture used, no subsequent washing or precipitation was required, with consequent significant reduction of solvent requirement. It is evident from the FTIR and NMR spectra (see below) that even without further treatment or purification steps, the carrageenans derived from microwave heating were virtually identical to the reference materials.

It has been shown that NMR and FTIR spectroscopy are attractive tools for structural determination of carrageenans,[11] and we have, accordingly, adopted those techniques in the present work. The following discussion is focused on the infrared spectral range from 600 to 1500 cm^{-1} to avoid the strong water bending mode centred around 1640 cm^{-1} and the noisy low energy range below 560 cm^{-1}. It is also in this range that the more important information about the compounds can be obtained.[12]

κ- and ι-carrageenan contain 3,6-anhydro bridges in the α-(1→4)-linked D-galactopyranose residue. The non-gelling μ- and ν-carrageenans (carrageenan-6,4-disulfate and carrageenan 2,6,4-trisulfate, respectively) are the natural precursors that are present in the seaweeds that contain κ- and ι-carrageenans, respectively, and have a sulfate ester group at C-6 of the α-(1→4)-linked D-galactopyranose residue of the dimeric unit. The 3,6-anhydro bridges are formed by the elimination of the sulfate from the C-6 sulfate ester of the precursors and the concomitant formation of the 3,6-anhydro bridge.[2]

The study of carrageenans by FTIR spectroscopy shows the presence of very strong absorption bands in the 1210–1260 cm^{-1} region (due to the S=O of sulfate esters) and 1010–1080 cm^{-1} region (ascribed to the glycosidic linkage) in all carrageenan types. The other chemical groups are characteristics of a given carrageenan type, namely 3,6-anhydro-D-galactose at 925–935 cm^{-1}, D-galactose-4-sulfate at 840–850 cm^{-1}, D-galactose-2-sulfate at 820–830 cm^{-1} and 3,6-anhydro-D-galactose-2-sulfate at 800–805 cm^{-1}. Both κ- and ι-carrageenan FTIR spectra show a band at 845–850 cm^{-1}, but the 800–805 cm^{-1} band is characteristic and distinctive of ι-carrageenan.[13,14]

The FTIR spectra of κ- and ι-carrageenan (reference and extracted materials) in Figures 2 and 3 show almost identical spectra of ι- and κ-carrageenan reference materials and the corresponding microwave-assisted carrageenan extracts from *Kappaphycus alverezii* and *Eucheuma denticulatum* without further treatment. Both spectra confirmed that these plants contained mainly carrageenans of the κ-type (bands at 933

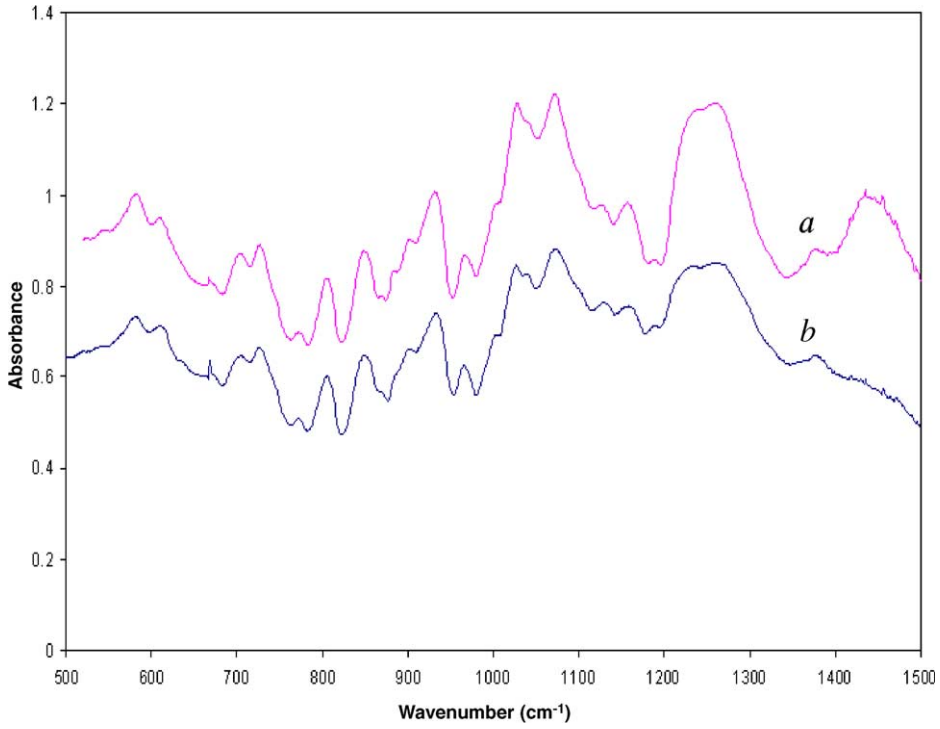

Figure 2. FTIR spectra of (a) MW *Eucheuma denticulatum (spinosum)* and (b) pure ι-carrageenan.

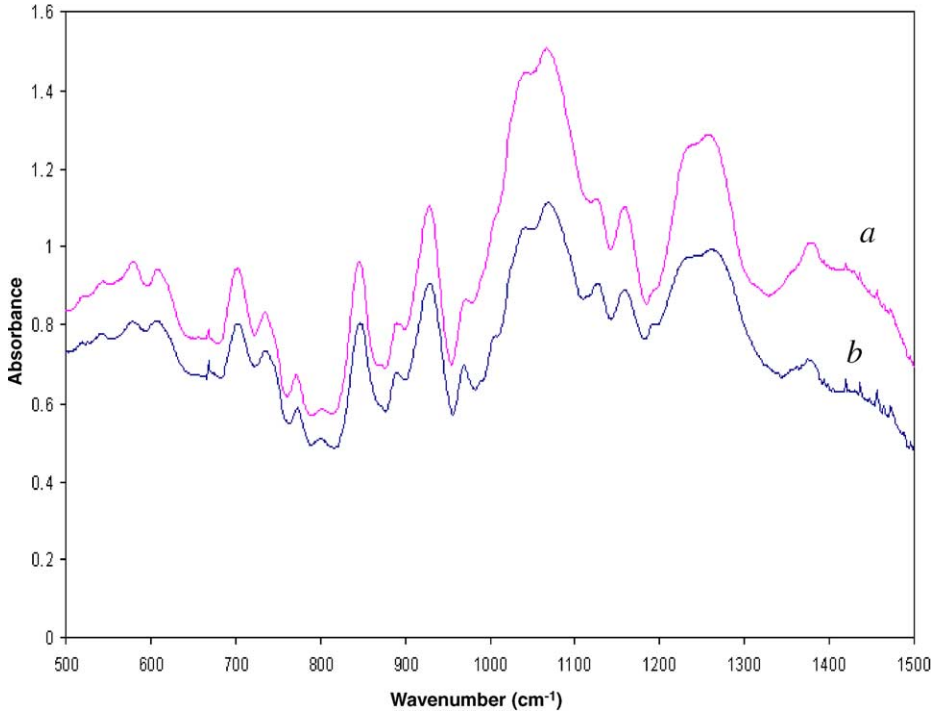

Figure 3. FTIR spectra of (a) MW *Kappaphycus alvarezii (Cottonii)* and (b) pure κ-carrageenan.

and 847 cm^{-1}) and ι-type (bands at 933, 847 and 805 cm^{-1}). The spectra in Figure 3 show a band at approximately 847 cm^{-1}, which is assigned to D-galactose-4-sulfate. The strong band at approximately 933 cm^{-1} indicates the presence of 3,6-anhydro-D-galactose. The FTIR spectra of ι-carrageenan also show bands at approximately 933 and 847 cm^{-1}. A band at 805 cm^{-1} indicates the presence of two sulfate ester

groups on the anhydro-D-galactose residues, characteristic of ι-carrageenan.

From the pioneering work of Usov and co-workers, NMR spectroscopy is now the preferred technique to determine and quantify the composition of carrageenan batches.[15] In the present workup, ^{13}C (Fig. 4) and ^{1}H (Fig. 5) NMR spectra of κ- and ι-carrageenan were recorded; the chemical shift data are summarised in Tables 3 and 4. The chemical shifts are in good agreement with those reported in the literature, although they were measured under different conditions. Ethanol was used to precipitate carrageenans from the extraction liquid, and an ethanol signal can be observed in the ^{1}H NMR spectra as a characteristic triplet at 3.65 ppm. The ^{1}H NMR

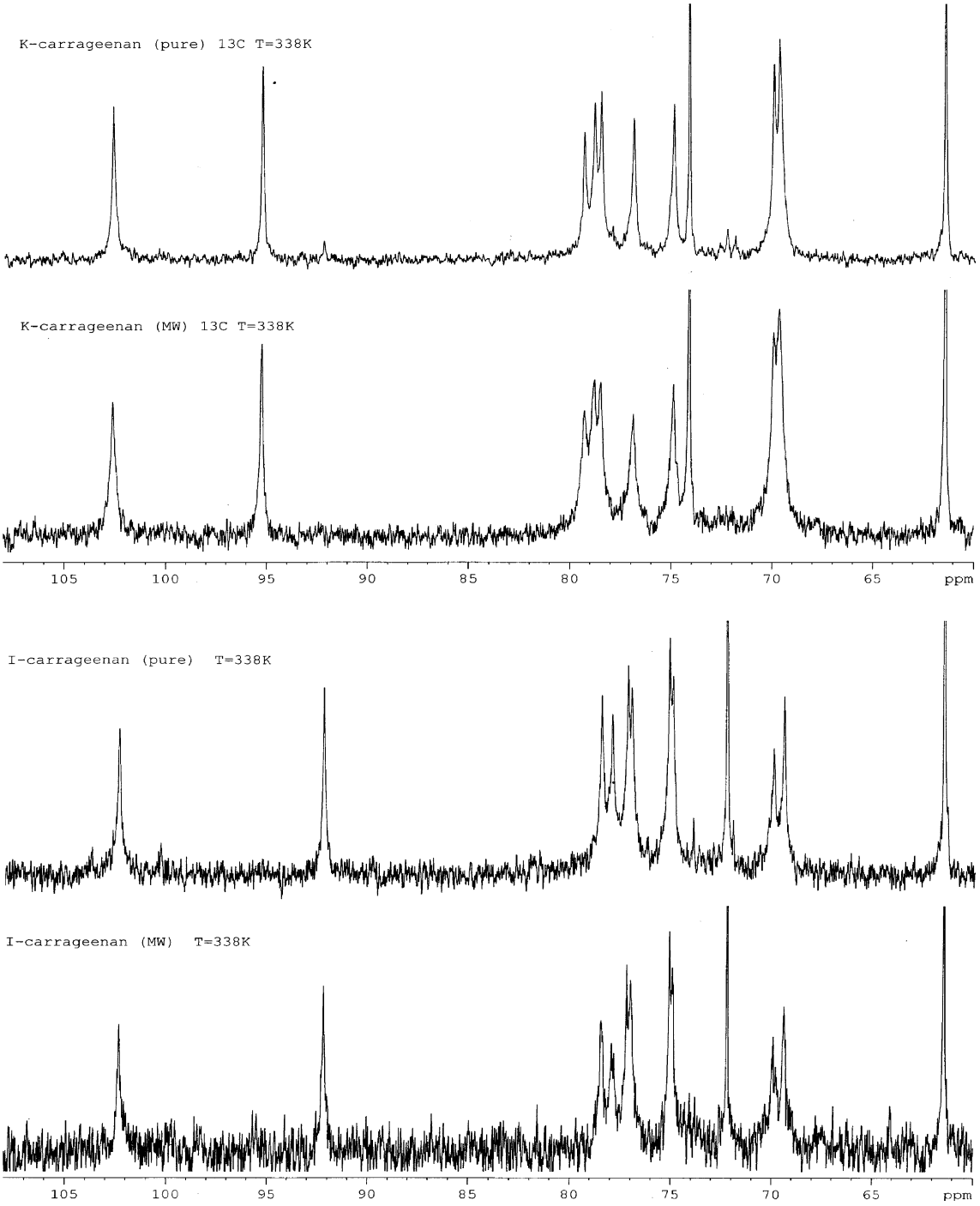

Figure 4. A comparison of ^{13}C NMR spectra for κ- and ι-carrageenan with (a) pure carrageenan and (b) carrageenan from microwave assisted solvent extraction.

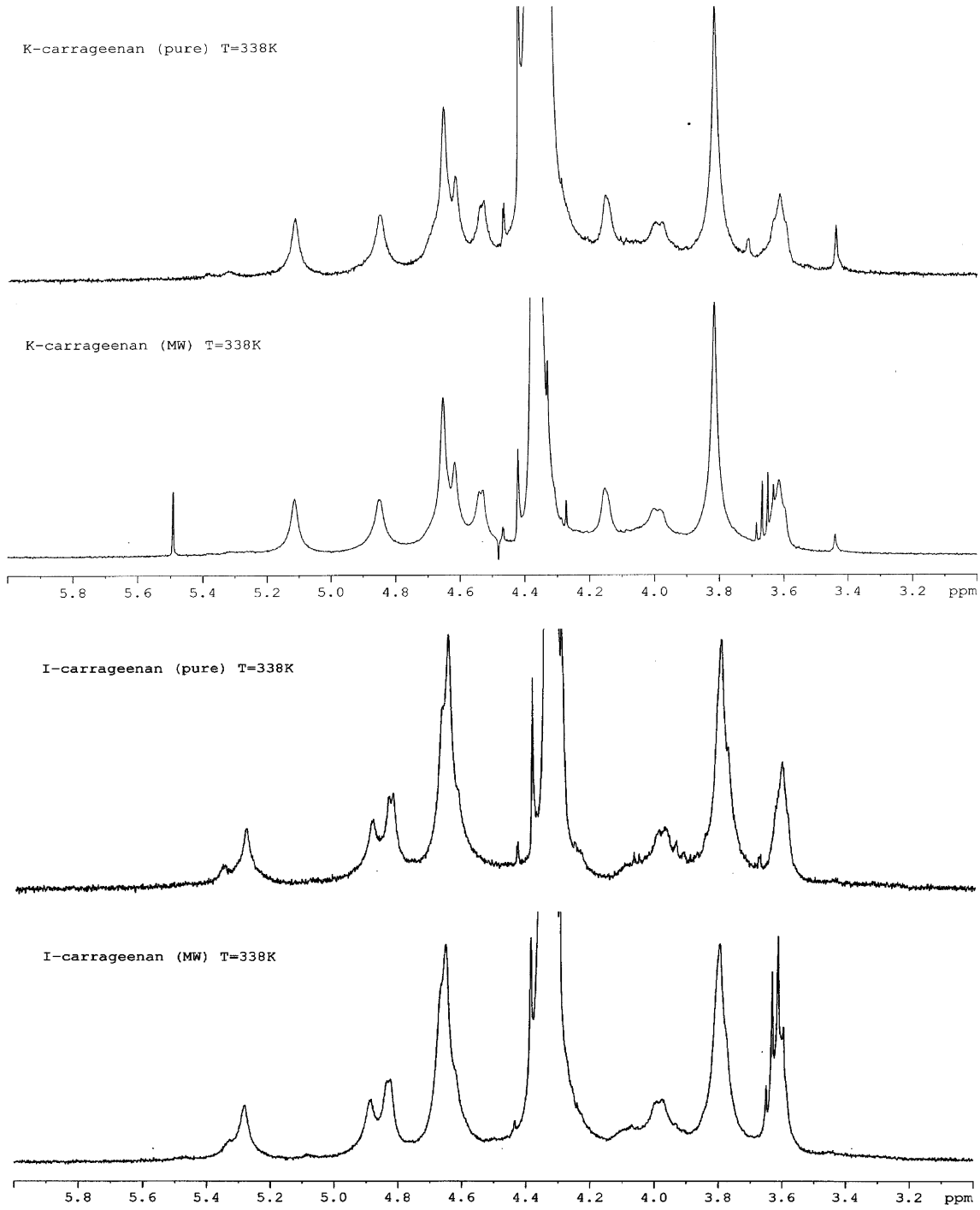

Figure 5. A comparison of ^{1}H NMR spectra for κ- and ι-carrageenan with (a) pure carrageenan and (b) carrageenan from microwave assisted solvent extraction.

spectra have significantly larger signal-to-noise ratio due to the larger sensitivity of ^{1}H NMR spectroscopy despite the acquisition time of 16 h of the ^{13}C NMR spectra.

The anomeric carbon signals at 104.7 and 97.4 ppm are characteristic of κ-carrageenan, and the corresponding signals at 104.4 and 94.3 ppm are characteristic of ι-carrageenan. As expected, very few differences associated with the method of heating were found, except for an additional signal at the 3.65 ppm in the spectra of microwave-extracted κ-carrageenan. Because of the signal of the anomeric proton 5.45 ppm, the signal may be due to the presence of floridean starch, which can accompany carrageenans in the extraction and precipitation steps. Floridean starch is mainly a branched α-glucan similar to amylopectins of plants, and red seaweeds differ considerably in its content.

4. Conclusions

A laboratory-scale continuous-flow microwave reactor that has been developed for microwave-assisted organic extraction under controlled conditions, has provided a potentially useful method for extracting ι- and κ-carrageenans from the seaweeds *E. spinosum* and *E. cottonii*. FTIR and NMR spectra of the extracted carrageenans are virtually identical with reference samples of the carrageenans, indicating that the carrageenans can be extracted in high purity, without the need for the purification procedures used in conjunction with the conventional extraction of carrageenans. The main advantages of the proposed procedure are the reduced consumption of solvents and extraction time.

In this work, both ^{13}C and ^{1}H NMR spectra of κ- and ι-carrageenan were recorded. The chemical shifts are in good agreement with those reported in the literature, although they were measured under different conditions. As expected, very few differences associated with the method of heating were found.

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