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A simple ^{1}H NMR method for the quantification of carrageenans in blends

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Abstract

Traditional methods for the structural analysis of carrageenans require hydrolysis and derivation procedures followed by chromatographic analysis of the formed derivatives. These assays are time-consuming and do not adequately quantify the different components in blend samples. This paper describes a simple method for the quantitative determination of *kappa*, *iota* and *lambda* carrageenan in standard mixtures of samples and production batches on intact polymeric carrageenans using ^{1}H NMR spectroscopy.

Keywords: Carrageenans; Seaweed; Structural analysis; ^{1}HNMR spectroscopy

1. Introduction

The term 'carrageenan' describes a class of sulphated galactan polysaccharides that occur as cell wall constituents in numerous species of red seaweed (marine algae of the class Rhodophyceae). The backbone of the polysaccharide is formed of D-galactose units (G) linked alternately with α-(1 → 3) and β-(1 → 4) linkages. The carrageenans are classified according to the presence of 3,6-anhydro-D-galactose (AG) on the 1,4-linked residue and the position and number of sulphated groups (Usov, 1998). Greek letters have been assigned to various 'idealised' disaccharide repeating units. However, natural carrageenans containing only one type of repeating disaccharide may not exist but consist of molecular hybrids of two or more idealised structures (Greer & Yaphe, 1984). The properties of such hybrids depend very much on the distribution of the different disaccharide units along the polymeric chain. The three principal types of industrial importance are *kappa*, *iota* and *lambda* carrageenans (Fig. 1). *Kappa* (k) and *iota* (i) forms are gelling polymers, while *lambda* (λ) is a non-gelling, thickening agent. These three carrageenans are extensively used in the food, pharmaceutical and cosmetic industry as viscosity builders, gelling agents and stabilisers (De Ruiter & Rudolph, 1997). Their commercial forms are rarely pure and normally contain varying amounts of the other carrageenan types. The exact amount of impurity depends on the seaweed source and extraction procedure (Harris, 1990).

Because the influence of carrageenans blend composition on their properties, different analysis methods have been applied. These include chromatographic methods such as GLC, HPLC or HPAEC-PAD coupled to chemical or enzymatic depolymerization procedures (Roberts & Quemener, 1999; Quemener, Marot, Mouillet, Da Riz, & Diris, 2000), light microscopy (Flint, 1990), electrophoresis (Pechaneck, Blaicher, Pfannhauser, & Woidich, 1982; Roberts, Zhong, Prodolliet, & Goodall, 1998) and NMR spectroscopy (Turquois, Acquistapace, Vera, & Welti, 1996). However, usual methods for determination of the main constituents of carrageenan involve depolymerization, which is time-consuming, labour intensive, and complicates accurate quantification of the different components in blends. For these reasons techniques, which do not require depolymerization have to be developed. In the present work ^{1}H NMR spectroscopy is applied to intact high-molecular carrageenans to determine the proportion of k-, i- and λ-carrageenan in blends.

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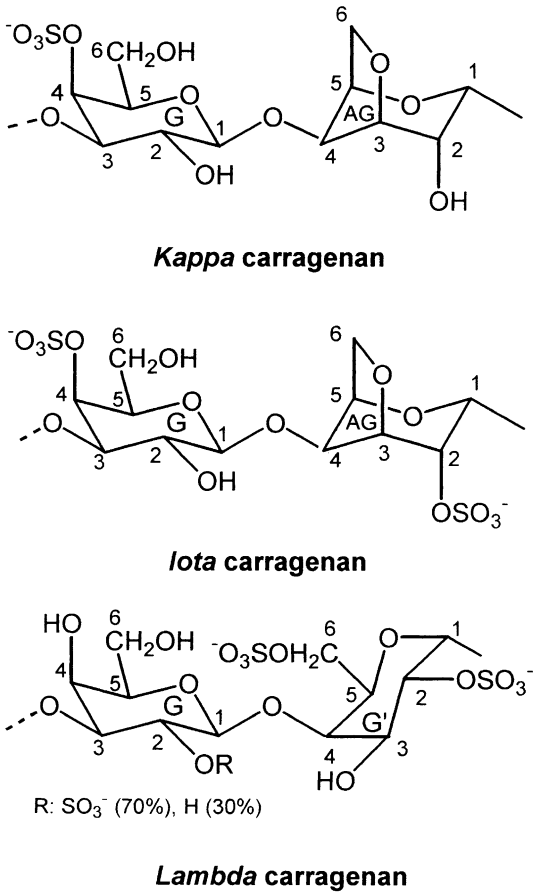

Fig. 1. Structures of the disaccharide repeat residues of the three principal carrageenans used in food: (a) *kappa*, (b) *iota* and (c) *lambda*.

2. Materials and methods

2.1. Materials

Carrageenans were commercial samples supplied by SIGMA-ALDRICH as: k-carrageenan, type III (isolated from *Eucheuma cottoni*); i-carrageenan, type IV (isolated from *E. spinosa*; and λ-carrageenan, type IV (isolated from *Gigantinae aciculaire* and *G. pistillata*).

2.2. Preparation of samples for ^1H NMR

In order to avoid dealing with highly viscous solutions, which creates problems both in transferring the sample to the NMR tube and in achieving good resolution on the recorded spectrum, samples were prepared as follows. Carrageenans were precipitated from the aqueous commercial polysaccharide solutions by adding three volumes of ethanol. The precipitate was separated, washed twice with ethanol/water solutions of 80, 90% and absolute ethanol and dissolved in water. The solution was dialysed 15 times against Milli-Q quality water at room temperature for a total of 30 h. Only the standard type membrane, Medicell International visking tubing 27/32 was used. After dialysis

Table 1
Proton chemical shifts (δ ppm) of *kappa, iota* and *lambda*-carrageenan; T^a : 30 °C for *kappa* and *iota*, 70 °C for *lambda*

		H-1	H-2	H-3	H-4	H-5	H-6
Kappa	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
Iota	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
Lambda	G	–	–	–	–	–	–
	G′	6.00	–	–	–	–	–

the solution was finally lyophilised and the purified carrageenan was stored under inert atmosphere (Ar) to prevent decomposition. This treatment allowed us to get solutions of low viscosity even without heating (20 °C).

When previous separation of λ-carrageenan was needed, the commercial sample was treated with KCl as follows before precipitation with ethanol. The powdered

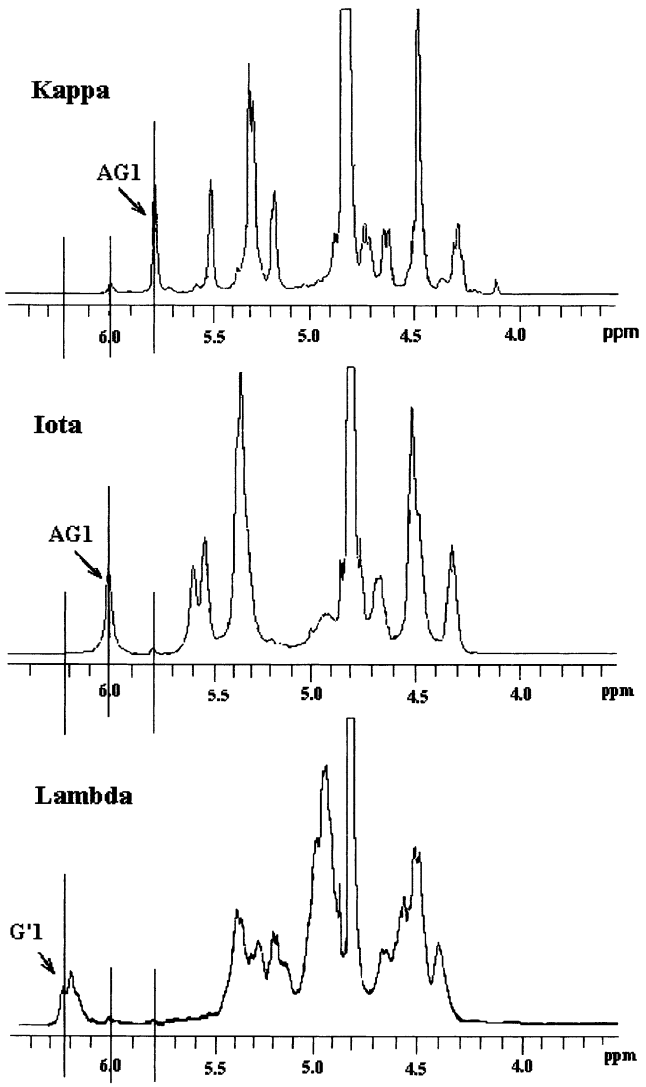

Fig. 2. ^1H NMR spectra of commercial *kappa*, *iota* and *lambda* carrageenans after treatment with KCl; $T^a = 90$ °C.

carrageenans (1 g) were added to a 0.3 M KCl solution (1 L) at room temperature with continuous stirring for 1 h, and then left to stand for two more hours. The solution was centrifuged and the precipitated washed with 0.3 M KCl solution to obtain λ-carrageenan. The aqueous supernatants containing *kappa* and *iota* were precipitated with ethanol and treated again with KCl.

2.3. NMR Spectroscopy

The 400 MHz ^{1}H NMR spectra were recorded with a BRUKER ARX400 spectrometer at different temperatures (20–90 °C). The parameters were as follows: pulse angle, 30°; acquisition time, 8.16 s; relaxation delay, 2 s; number of scans, 256. Two-dimensional spectra were obtained at 30 °C using standard Bruker software, with an acquisition time of 510 ms, and relaxation delay of 2 s, during which the residual HOD was presaturated. Solutions (20–30 mg/ml) for the NMR experiments were prepared directly in the NMR tube (Wilmad 527-PP-7, 5 mm) using D_2O (99.9%, 1 ml) and freshly lyophilised freeze-dried samples. Solutions with mixtures of two or three carrageenans were prepared by weighing and transferring the samples under an inert gas blanket. Chemical shifts (δ) were given in ppm relative to that of the residual HOD signal at 4.82 ppm.

3. Results and discussion

3.1. ^{1}H NMR of the starting carrageenans

The ^{1}H NMR spectra of carrageenans present typical deshielded signals corresponding to the anomeric hydrogens. The chemical shifts of k- i- and λ-carrageenan are given in Table 1. Assignments were based on the close similarity with literature values (Stortz, Bacon, Cherniak, & Cerezo, 1994; Usov, 1984) and confirmed by HMQC 2D NMR experiments for *kappa* and *iota*. Due to the irregular and hybrid structure of this polysaccharide, ^{1}H NMR spectrum of λ-carrageenan is composed of very broad envelopes rather than clearly defined signals, even when hot. In this case only the signal corresponding to the anomeric proton G′1 can be unambiguously assigned.

The ^{1}H NMR spectra of the starting commercial carrageenans (*kappa*, *iota* and *lambda*) showed that they are composed of a dominant carrageenan contaminated with small and variable amounts of the other two, even after separation with KCl (Fig. 2).

In order to determine the optimum condition to obtain the spectra, several ^{1}H NMR experiments of *kappa*, *iota* and *lambda* carrageenan were performed using different temperatures (Fig. 3). The effect of the temperature on the anomeric chemical shifts values is given in Table 2. It can be observed that an increase in temperature gives rise to a downfield shift of all resonances. Good quality spectra were

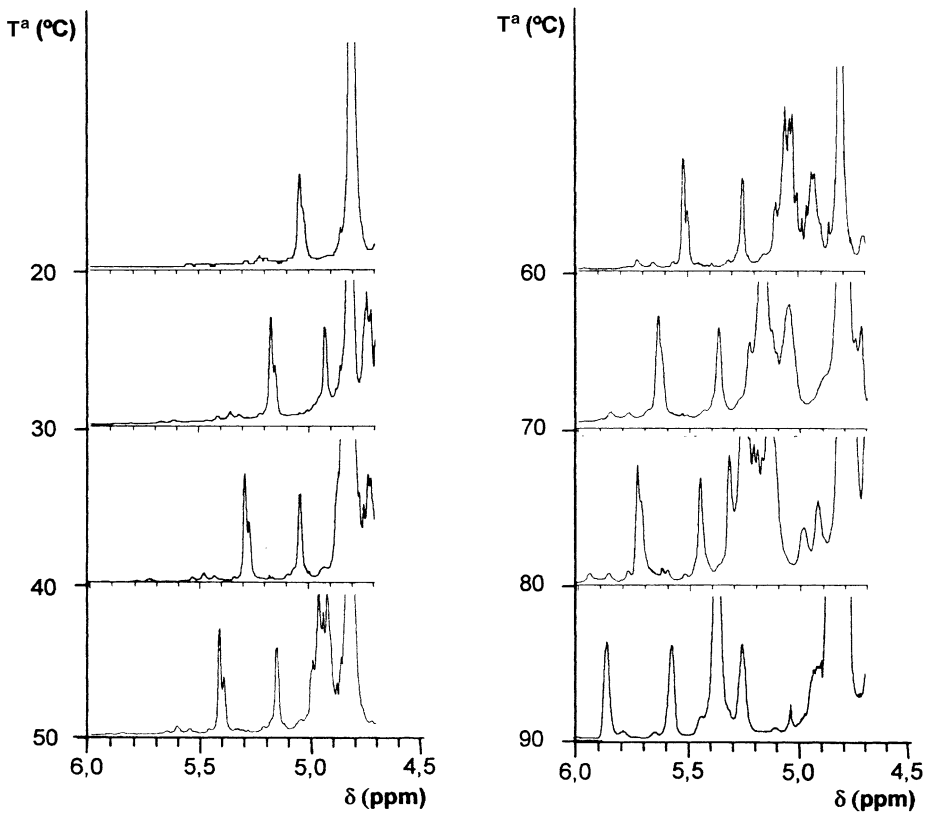

Fig. 3. Variation of *kappa* carragenan ^{1}H NMR spectra with temperature.

Table 2
Chemical shift variations of anomeric hydrogens AG1 (for *kappa* and *iota*) and G′1 (for *lambda*) with temperature

T^a (°C)	*Kappa*	*Iota*	*Lambda*
20	5.04	5.22	5.40
30	5.17	5.35	5.53
40	5.29	5.48	5.65
50	5.41	5.61	5.78
60	5.52	5.73	5.89
70	5.64	5.85	6.00
80	5.73	5.97	6.09
90	5.81	6.04	6.26

obtained even at 20 °C, but a temperature of 60 °C was selected for quantitative purposes in order to shift the anomeric signals to an area away from the residual water resonance.

In the absence of pure samples, the composition of k-, i- and λ-carrageenan must be carefully established before their mixtures can be studied. Percentages of the different carrageenans in samples were calculated by integration of the anomeric hydrogen peaks AG1 in *kappa* and *iota* and G′1 in *lambda*-carrageenan. The integration of H-AG1 signals arising from kappa and iota allowed us to establish that the commercial *kappa* batch used in this work is contaminated by 8.8% *iota*, and commercial *iota* is contaminated by 7.9% kappa. After separation with KCl no λ-carrageenan was detected in these samples.

However, small amounts of *kappa* and *iota* were detected in the ^{1}H NMR of *lambda* carrageenan, even after separation with KCl. Integration analysis of H-G′1 signal arising from *lambda* revealed that in this case the integral does not reflect the real proportion of the carrageenan, which is probably due to the width of the peak. In order to establish relationships between integral measurements and composition for heterogeneous carrageenan samples containing λ-carrageenan, several blends with different known proportions of k-, i-, and λ-carrageenan were prepared. From the spectroscopic analysis it was inferred that the *lambda* H-G′1 experimental integral (I_e) is lower than the theoretic one (I_t) by a factor of 1.8, so $I_t = 1.8\, I_e$. Taking into account this result, the proportion of *lambda* H-G′1 integrated signal may be also used as an analytical tool. Thus, integration of H-AG1 arising from *kappa* and *iota* and H-G′1 arising from λ-carrageenan allowed us to detect impurities of 2.1% *kappa* and 1.1% *iota* in commercial λ-carrageenan. However, it is important to point out that the percentage of impurities in commercial carrageenan can change depending on the Sigma batch origin.

3.2. ^{1}H NMR of known carrageenan blends

The method was assessed by measuring the integral values of each component in mixtures containing a known amount of added k-, i-, and λ-carrageenan. Several carrageenan blends directly prepared from the individual commercial polysaccharides freshly lyophilised were studied by ^{1}H NMR at 60 °C. Real weights of each component were calculated taking in account the percentages of impurities of other carrageenans previously established by integral measurements. Representation of integral ratio of *kappa–iota* (*I*k/*I*i) versus real weighs ratio (*W*k/*W*i) showed a very good linearity, with a correlation coefficient of 0.997. Similar representations for *kappa–lambda* and *iota–lambda* applying the corrective factor for *lambda* integral gave a correlation coefficient of 0.995 for *kappa–lambda* and 0.985 for *iota–lambda*. The correlation equations are shown in Fig. 4.

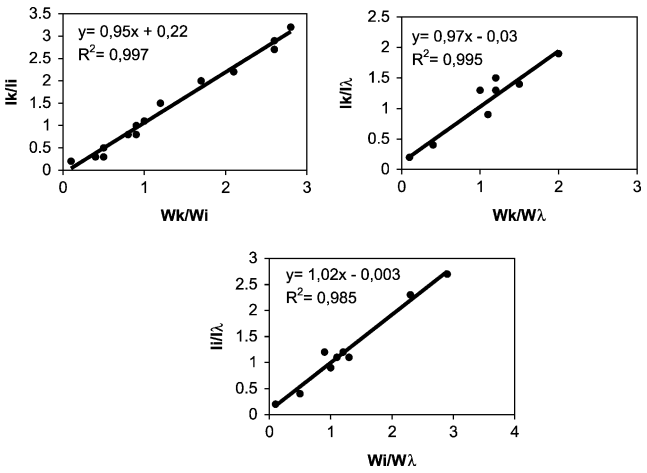

Fig. 4. Representation of integral ratios versus real weigh ratios for *kappa–iota*, *kappa–lambda* and *iota–lambda* carrageenans.

4. Conclusions

A relatively simple, rapid method has been developed for the determination of carrageenans in blends by ^{1}H NMR spectroscopy. The major advantages of the method over those previously established are that it does not involve tedious work-up and that carrageenans can be determined on the intact polysaccharide, with no destruction of the sample as a result of hydrolysis reactions.

^{1}H NMR spectroscopy measurements are successfully used to determine the carrageenan composition of several commercial samples. The precision of quantification of all components in blends depends on their composition. The best results are obtained for mixtures of *kappa* and *iota* carrageenans.

References

De Ruiter, G. A., & Rudolph, B. (1997). Carrageenan biotechnology. *Trends in Food Science Technology*, *8*, 339–429.

Flint, F. O. (1990). Micro-technique for the identification of food hydrocolloids. *Analyst*, *115*, 61–63.

Greer, C. W., & Yaphe, W. (1984). Hybrid (iota-nu-kappa) carrageenan from *Eucheuma nudum* (Rodophyta, Solieriaceae), identified using iota and kappa carrageenases and ^{13}C NMR. *Botanica Marina*, *27*, 479–484.

Harris, P. (1990). *Food Gels*. Oxford: Elsevier.

Pechaneck, U., Blaicher, G., Pfannhauser, W., & Woidich, H. (1982). Qualitative and quantitative analysis of gelling and thickening agents. *Journal of the Associate office of Analytical Chemistry*, *65*, 745–752.

Quemener, B., Marot, C., Mouillet, L., Da Riz, V., & Diris, J. (2000). Quantitative analysis of hydrocolloids in food systems by methanolysis coupled to reverse HPLC. Part 1. Gelling carrageenans. *Food Hydrocolloids*, *14*, 9–17.

Roberts, M. A., & Quemener, B. (1999). Measurement of carrageenans in food: challenges, progress, and trends in analysis. *Trends in Food Science and Technology*, *10*, 169–181.

Roberts, M. A., Zhong, H.-J., Prodolliet, L., & Goodall, D. M. (1998). Separation of high-molecular-mass carrageenan polysaccharides by capillary electrophoresis with laser- induced fluorescence detection. *Journal of Chromatography A*, *817*, 353–366.

Stortz, C. A., Bacon, B. E., Cherniak, R., & Cerezo, A. S. (1994). High-field N. M. R., spectroscopy of cystocarpic and tetrasporic carrageenans from *Iridaea undulosa*. *Carbohydrate Research*, *261*, 317–326.

Turquois, T., Acquistapace, S., Vera, A. F., & Welti, D. H. (1996). Composition of carrageenan blends inferred from ^{13}C NMR and infrared spectroscopic analysis. *Carbohydrate Polymers*, *31*, 269–278.

Usov, A. I. (1984). NMR Spectroscopy of Red Seaweed Polysaccharides: Agars, Carrageenans, and Xylans. *Botanica Marina*, *27*, 189–202.

Usov, A. I. (1998). Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids*, *12*, 301–308.