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Chemical composition of carrageenan blends determined by IR spectroscopy combined with a PLS multivariate calibration method

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Abstract

The content of *kappa*, *iota* and *lambda* carrageenan in mixtures was determined by application of FT-IR spectroscopy combined with partial least-squares multivariate regression (PLS). This method allows the determination of the relative amounts of the different carrageenans in a rapid and accurate manner.

Keywords: Carrageenan; Quantitative analysis; IR; ^{1}H NMR; PLS

1. Introduction

Carrageenan is a generic term for a group of commercially important galactan sulphates extracted from red seaweed. Carrageenans[1] are extensively used in the food and pharmaceutical industries as viscosity, gel or texture enhancers, stabi1isers, etc. These additives give textural properties and protective effects to a wide range of products such as frozen desserts, chocolate, cottage cheese, whipped cream, instant breakfasts, yoghurt, jellies, pet foods, relishes, sauces and syrups. The backbone of the polysaccharide is formed of (1→3)-linked-D-galactopyranose residues alternating with (1→4)-linked-D-galactopyranose residues that can carry various sulphated groups in varying amounts. They are classified according to the presence of the 3,6-anhydro-D-galactose on the (1→4)-linked residue and the position and the number of sulphate groups.[2] Greek letters have been assigned to various 'idealised' disaccharide repeat units, but natural carrageenans containing only one type of repeating disaccharide may not exist, but rather consist of molecular hybrids of two or more 'idealised' structures.[3] The properties of such hybrids should 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* (Fig. 1). *Kappa* (κ) and *iota* (ι) contain the 3,6-anhydrogalactose unit and are gelling polymers, but *lambda* (λ) carraggenan with only galactose residues, is a thickening polymer. The commercial forms of these three carrageenans are normally not pure forms, but contain varying amounts of the other carrageenan types. The exact amount of impurity depends on the seaweed source and the extraction procedure.

Because of the influence of carrageenan blend composition on their properties and functionality, different analytical methods have been applied.[1,4–6] These usually involve depolymerization, which is time consuming, labour intensive, and complicates accurate quantification of the different components in blends. More recently, techniques without depolymerization prior to qualitative or quantitative analysis[7–9] have been employed, but still there is a lack of adequate analytical techniques, suitable for industrial analytical routine operation, to quantify the relative concentrations of the different carrageenans in mixtures, and to determine the purity of these important components of modern food products.

In recent work[10] we applied ^{1}H NMR spectroscopy to determine the relative concentration of *kappa*, *iota* and

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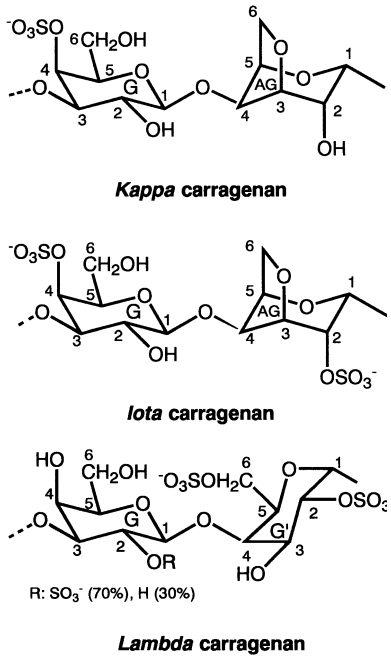

Fig. 1. Structures of the disaccharide repeat residues of the three principal carrageenans used in the food industry.

lambda carrageenan in standard mixtures samples and production batches on intact polymeric carrageenans. The results were especially successful for *kappa*–*iota* mixtures, but the accuracy of quantification decreases when the sample contains *lambda* carrageenan.

Infrared spectroscopy is one of the standard techniques used for characterisation of carrageenans and analysis of the spectra has been reported by a number of authors,[11–19] in which the different structural elements of carrageenan are assigned to different absorption bands. In recent years a powerful tool has been applied to resolve complex mixtures of compounds with similar spectral characteristics: the multivariate analysis method of Partial Least-Squares[20] (PLS). The aim of this paper was to investigate the ability of the PLS method to resolve a complex mixture of *kappa*, *iota* and *lambda* carrageenans by application to the IR spectra of carrageenan films, by developing a mathematical relationship between spectral measurements and the respective chemical compositions of the carrageenans. In addition, ^{1}H NMR was used to determine the real composition of commercial carrageenans used to prepare the calibration standards.

2. Results and discussion

2.1. Infrared spectroscopy

The carrageenans studied in this work showed an absorbance band at 1250 cm^{-1} due to the total sulphate content, that decreased from *lambda* to *iota* and *kappa* carrageenan.[15] Other characteristic bands provided information about the sulphate positions: *kappa* and *iota* spectra display one band at 845 cm^{-1} arising from the galactose-4-sulphate;[14] *iota* carrageenan spectrum present other additional band at 805 cm^{-1} due to the 3,6-anhydrogalactose-2-sulphate;[17] and two bands appear at 830 and 820 cm^{-1} in the *lambda* spectrum corresponding to galactose-2-sulphate and to galactose-6-sulphate, respectively.[15]

2.2. ^{1}H NMR of starting carrageenans

The ^{1}H NMR spectra of carrageenans gave typical deshielded signals corresponding to the anomeric hydrogens. The chemical shifts of κ-, ι- and λ-carrageenan are given in Table 1. Assignments were based on close similarity with literature values[21,22] 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 at high temperatures. In this case only the signal corresponding to the anomeric proton G′1 can be unambiguously assigned.

The ^{1}H NMR spectra of the commercial carrageenans (*kappa*, *iota* and *lambda*) showed that they are not pure but composed of a dominant carrageenan contaminated with small and variable amounts of the other two, even after separation with KCl. 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.[10] Thus, integration of H-AG1 signals arising from *kappa* and *iota* allowed us to establish that commercial *kappa* is contaminated by 3.9–8.8% *iota*, and commercial *iota* is contaminated by 4.2–7.9% *kappa*, depending on the batch. After fractionation 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 fractionation with KCl. Thus, integration of H-AG1 arising from *kappa* and *iota* and H-G′1 arising

Table 1
Proton chemical shifts of *kappa*, *iota* (T^a 30 °C) and *lambda* (T^a 70 °C) carrageenan

		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
λ	G						
	G′	6.00					

from λ-carrageenan allowed us to detect impurities of 2.1% *kappa* and 1.1% *iota* in commercial λ-carrageenan previously treated with 0.3 M KCl.

2.3. Partial least-square method

A calibration set comprising 160 binary and ternary standard mixtures was constructed. An experimental design was set up to span all possible relative concentration ranges of each carrageenan form. Because all carrageenan forms contain some impurity of the others, no absolutely pure forms could be used. The IR spectra of the different calibration standard samples were recorded from 4000–400 cm^{-1}, but only the 1400–600 cm^{-1} region was employed for the multivariate studies. By application of the PLS-1 algorithm, a model was developed[23] and optimised using five factors for *kappa*, five factors for *iota* and three factors for *lambda* carrageenan. The correlation coefficients of the linear regression for the PLS-1 model obtained were: 0.993 for *kappa*, 0.992 for *iota*, and 0.995 for *lambda* carrageenan (Table 2).

The optimised PLS-1 model was assessed by resolution of artificial mixtures of carrageenans (randomly designed) prepared from commercial samples and also from industrial batches. The true concentration values were obtained by the ^{1}H NMR spectral method. IR films were prepared from the artificial mixtures after ethanolic precipitation without any other purification procedure. The predicted/true values ratios obtained are closed to 1 in all cases for the three carrageenans (Table 3). Although the PLS prediction for sample S8 is not good (−1, i.e., 0%), the true λ content is so low (3%) that all physical methods currently used in industrial laboratories would consider it as 0%. The predictive ability of the PLS-1 model therefore appears to be very satisfactory, with recovery values in the range of: 98–104% for *kappa*, 95–102% for *iota* and 98–102% for *lambda*.

3. Experimental

3.1. Materials

Carrageenans used as calibration standards were prepared from commercial samples supplied by Sigma–Aldrich as: κ-carrageenan, type III (isolated from *Eucheuma cottoni*); ι-carrageenan, type IV (isolated from *Eucheuma spinosa*); and λ-carrageenan, type IV (isolated from *Gigantinae aciculaire* and *Gigantinae pistillata*). Industrial production batches of carrageenans with unknown composition supplied by Compañía de Algas marinas S.A. (CEAMSA) were also used with the optimised PLS-l model.

Table 2
Linear regression for the individual components

Component	Equation	Correlation coefficient
Kappa	$y = 0.638 + 0.975x$	0.993
Iota	$y = 0.646 + 0.980x$	0.992
Lambda	$y = 0.146 + 0.998x$	0.995

3.2. Preparation of samples for ^{1}H 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 aqueous solutions of the commercial polysaccharides by the addition of three volumes of EtOH. The precipitate was separated, washed twice with EtOH–water solutions (80 and 90%) and absolute EtOH, then dissolved in water. The solution was dialysed 15 times against Milli-Q quality water at room temperature for a total of 30 h, using a standard-type membrane (Medicell International visking tubing 27/32). After dialysis, the solution was lyophilised and the purified carrageenan was stored under inert atmosphere (Ar) to prevent decomposition. This treatment produced solutions of very reduced viscosity even without heating (20 °C). When previous fractionation of λ-carrageenan was needed, the commercial sample was treated with 0.3 M KCl.

3.3. NMR spectroscopy

The ^{1}H NMR spectra were recorded with a BRUKER ARX400 spectrometer. In order to determine optima conditions, several ^{1}H NMR experiments on *kappa*, *iota* and *lambda* carrageenan were performed using different temperatures.[10] Good quality spectra were obtained even at 20 °C, but a temperature of 60 °C was selected for quantitative purposes in order to shift the anomeric signals away from the residual water resonance. The parameters were as follows: pulse angle, 30°; acquisition time, 8.16 s; relaxation delay, 2 s; number of scans, 256. 2D spectra were obtained at 30 °C using standard Brucker 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 (Wildmad 527-PP-7, 5 mm) using D_2O (99.9%, 1 mL) and freshly 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) are reported in ppm relative to that of the residual HOD signal at 4.82 ppm.

Table 3
Simultaneous determination of κ-, ι- and λ-carrageenan in artificial mixtures

Sample	κ			ι			λ		
	TV	PV	PV/TV	TV	PV	PV/TV	TV	PV	PV/TV
S1	41	40	1.0	18	21	1.1	41	38	0.9
S2	50	49	1.0	26	27	1.0	24	26	1.1
S3	13	13	1.0	71	72	1.0	16	17	1.1
S4	36	33	0.9	19	20	1.0	45	47	1.0
S5	19	20	1.0	16	15	0.9	64	66	1.0
S6	18	21	1.1	79	79	1.0	3	3	1.0
S7	31	34	1.1	61	63	1.0	6	5	0.8
S8	73	75	1.0	23	25	1.1	3	−1	
S9	13	16	1.2	9	8	0.9	78	74	1.0
S10	13	12	1.1	6	8	1.3	82	77	0.9

TV, true value (%); PV (%), predicted value.

3.4. Preparation of calibration standard samples

A calibration set of 160 standard mixtures (randomly selected) of κ-, ι- and λ-carrageenans was constructed by weighing under an inert and dry atmosphere, appropriate amounts of each purified carrageenan previously analysed by ^{1}H NMR. Standard mixtures were dissolved in ultrapure water (no more than 6 mg/mL) and films for infrared analysis were obtained by drying (using an air-convection oven) 5 mL of the solutions in a 6 cm diameter polystyrene petri dish at 50 °C for 1.5–2 h.

3.5. IR spectroscopy

A Bruker FT-IR IFS 28 Equinox IR-TF spectrophotometer was employed. The OPUS/IR 2.0 software was used for data acquisition. The Mattson Instruments' PLS Quantitative Analysis software package was used for statistical treatment of the data and PLS-1 method application.

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