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Characterization of Commercial Carrageenans by Fourier Transform Infrared Spectroscopy Using Single-Reflection Attenuated Total Reflection

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The purity and composition of commercial carrageenans vary widely and, therefore, have to be checked prior to their use in the food industry. Infrared spectroscopy is an alternative method to the expensive and time-consuming wet chemical and NMR methods to characterize carrageenan samples. The use of an attenuated total reflection accessory coupled to a Fourier transform infrared spectrophotometer allows a direct analysis of the sample without any preparation step, which is an additional benefit for the rapid identification check of raw material at reception in an industrial environment. Using a set of calibration samples, three multivariate calibrations were developed to predict the total carrageenan content as well as the molar ratio of κ- and ι-carrageenans. A validation with an independent set of samples confirmed the robustness of the calibrations and the accuracy of the predictions. The accuracies of the calibrations given by their respective standard errors of prediction are 5.6 g/100 g, and 6.1 mol %, and 6.6 mol %, respectively, for the total carrageenan content and the molar ratios of κ- and ι-carrageenans. The total preparation and analysis time is <5 min per sample.

KEYWORDS: Carrageenan; identification; characterization; infrared; FTIR; ATR; multivariate calibration

INTRODUCTION

Carrageenans are sulfated galactans extracted from many species of red algae, the *Rhodophyceae*, composed of D-galactose residues linked alternately with α-(1→3) and β-(1→4) D-glycosidic linkages (*1*). The repeating units shown in **Figure 1** have been found to prevail in carrageenans, giving rise to three major fractions: κ, ι, and λ. The sulfate groups are covalently bound via ester linkages to the carbon atoms C-2, C-4, or C-6 of individual galactose residues. The positions and numbers of sulfate ester groups are important because they are, together with the anhydrogalactose bridge, responsible for carrageenan functionality.

κ- and ι-carrageenans contain the 3,6-anhydro units and are gelling polymers. λ-Carrageenan, with only sulfated galactose groups and no anhydrogalactose bridge, is a thickening polymer. Its structure varies considerably and is consequently more difficult to characterize by analytical methods.

Owing to the natural variation of seaweed stocks used as raw material and carrageenan extraction processes, the products present variable composition. Therefore, they are standardized by the addition of sugars, buffer salts, and gelling aids (such as potassium chloride) to give the required functionality for a specific application. Consequently, the composition of carrageenan raw materials is in many cases unknown to the suppliers and can vary considerably from batch to batch. This variability may have important consequences at the factory level such as inadequate texture or inconsistent quality of the finished products.

Therefore, carrageenan raw materials must be identified and characterized at reception in the production facilities.

Various approaches have been applied for the analysis of carrageenans. They include light microscopy, immunological detection electrophoresis, ^{1}H NMR, ^{13}C NMR, chromatographic methods such as GC, HPLC, or HPAE-PAD after enzymatic or chemical degradation, and colorimetric methods. A complete review of these techniques was published by Roberts and Quemener (*2*). Most of these methods are very expensive and time-consuming.

The approach developed in this study is to characterize the carrageenan raw materials in terms of purity (total carrageenan content) and composition (relative molar contents of κ- and ι-carrageenans) with a cheaper and faster alternative method.

Infrared (IR) spectroscopy is already used for the qualitative characterization of carrageenan: the specific infrared absorption bands of κ-, ι-, and λ-carrageenans are listed in the JEFCA specifications (*3*). Jacobson et al. (*4*) used dispersive IR spectroscopy and multivariate regression methods on selected peaks of the IR spectra for the quantification of individual carrageenans in KBr pellets. Hansen et al. (*5*) presented an IR method for individual characterization of purified carrageenan mixtures in KBr pellets using a partial least-square (PLS)

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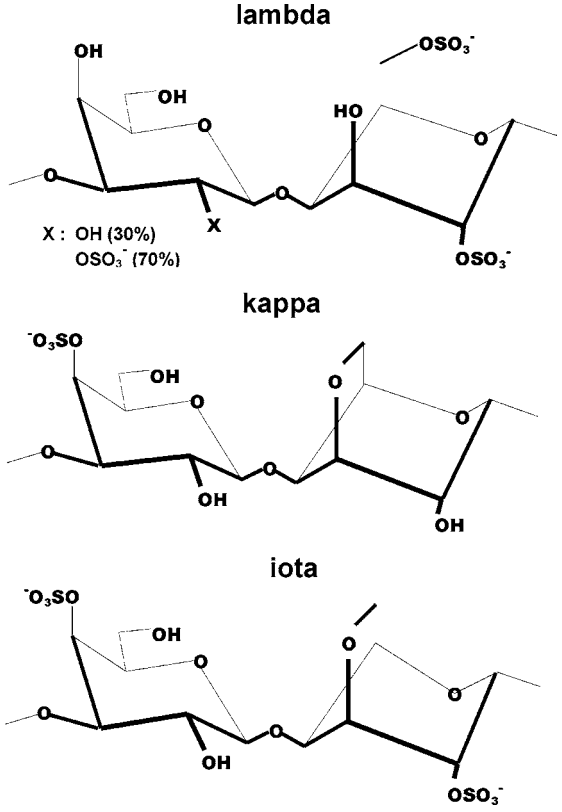

Figure 1. Basic structures of λ-, κ-, and ι-carrageenan repeating units.

regression based on the IR spectra in the range of 1000−500 cm^{-1}. These studies were performed with "pure" carrageenans and mixtures of "pure" carrageenans. They were therefore not applicable to the analysis of all commercial carrageenans.

The FTIR−PLS method developed by Prado-Fernandez et al. (*6*) to quantify the different types of carrageenans in tertiary mixes and industrial blends was applicable to commercial carrageenans, but a tedious sample preparation was necessary to produce thin films of the product to be analyzed.

Any type of sample preparation can be avoided by directly collecting the spectra of the commercial carrageenans by attenuated total reflection (ATR). The aim of this work was therefore to develop a rapid method for the direct identification and characterization of carrageenans with regard to their purity and their relative κ- and ι-carrageenan content, using the specific advantage of a single-reflection diamond ATR. In this work, all samples used to develop and validate the calibrations were fully characterized by wet chemical and NMR methods, contrary to other studies previously published (*4*−*6*).

MATERIALS AND METHODS

Samples and Reference Values. Fifty-two samples of commercial refined carrageenans in a powder form (defined as "pure" or blends) were obtained from various suppliers (CNI, Danisco, FMC, Hahn, Hercules, Ingredient Solution, Marcel Carrageenan, Shemberg, and SKW). The samples were analyzed by classical wet chemistry methods for their moisture, proteins, free sugars, citrate, sodium, potassium, calcium, and magnesium contents.

Moisture determination was performed by oven-drying during 4 h at 102 °C according to IDF method 26A (1993). The nitrogen content was determined according to Kjeldahl following the method 22/3.1-(1985) of the *Manuel Suisse des Denrees Alimentaires*, and the corresponding protein content was calculated using a conversion factor of 6.25. Free sugars were quantified by high-pressure anion exchange chromatography (HPAE) on Dionex equipment following method 22/6.2(1991) of the *Manuel Suisse des Denrees Alimentaires*. The citrate content was determined using an enzymatic test kit following method 4/10.3(1998) of the *Manuel Suisse des Denrees Alimentaires*. The concentrations of sodium, potassium, calcium, and magnesium were determined by ion-coupled plasma atomic emission spectroscopy (ICP AES) on an ICP3000 instrument after microwave digestion of the sample according to AOAC method 984.27.

The total carrageenan content, expressed in grams per 100 g, was calculated by difference after subtraction of the contents of water, proteins, free sugars, citrate, sodium, potassium, calcium, and magnesium. The molar composition of κ-, ι-, λ- and other types of carrageenans was determined by ^{1}H NMR and ^{13}C NMR based on methods already described elsewhere (*7*, *8*). The results are expressed in mole percent (mol %; mol/mol). Four samples could not be described by the NMR methods due to the poor signal-to-noise ratio or the presence of interfering material in the sample. The composition of all samples used for calibration or validation is presented in **Table 1**.

The laboratory error [standard error of laboratory (SEL)] was estimated at 8 g/100 g for the "total carrageenan content", based on the cumulative error of the eight analytical results required to calculate it, according to the Eurachem/Citac procedure (*9*). The laboratory error of the "reference" NMR values for the molar ratio of κ- and ι-carrageenan was estimated from the known standard deviation of repeatability of the method, applying the factor 1.75 as suggested by Horwitz et al. (*10*), although it has been suggested that this ratio could vary between 1.6 and 2.6 (*11*). Ten replicate analyses of a sample containing 40 mol % of κ-carrageenan and 55 mol % ι-carrageenan (sample v8) were carried out, and the calculated standard deviation of repeatability was 1.5 mol % for κ-carrageenan and 1.4 mol % for ι-carrageenan. The corresponding SELs were 2.6 mol % for κ-carrageenan and 2.5 mol % for ι-carrageenan.

The calibration development focused on the prediction of the total carrageenan content expressed in grams per 100 g and the molar fraction of κ- and ι-carrageenans expressed in mole percent (mol/mol). The samples covered a wide analytical range: 35−85 g/100 g for total carrageenan content; 0−97 and 3−100 mol % for κ- and ι-carrageenan contents, respectively.

FTIR Spectra Acquisition and Analysis. Mid-infrared spectra (4000−600 cm^{-1} at 1 cm^{-1} data intervals) were collected with a spectral resolution of 4 cm^{-1} on a Perkin-Elmer Spectrum 2000 FTIR spectrometer (Perkin-Elmer Corp., Norwalk, CT) equipped with an ATR system Specac MKII GoldenGate (Specac Inc., Smyrna, GA) positioned so that the incident angle was 45°. The spectrophotometer was fitted with a wire coil operated at 1350 K as IR light source, a potassium bromide beam splitter, and a DTGS detector.

Sample storage, sample preparation, and data acquisition were carried out at 25 °C. Data acquisition was performed over several days, and samples were taken in random order. After thorough mixing, a portion of the powder sample was positioned on the ATR diamond surface. A pressure of 11000 psi was applied on the sample by means of a pressure clamp. Four replicate spectra of each sample were collected on four different sample portions. Each spectrum represents the average of 16 scans ratioed against the background, which was collected with the empty ATR accessory under the same conditions at the beginning of each day of analysis. Between the samples, the ATR surface was thoroughly cleaned with water and alcohol. As no thermal equilibration was required, the whole spectra acquisition procedure took 5 min per sample.

Data analysis was carried out using Spectrum Quant+ version 4.51.02 (Perkin-Elmer Corp.). The reflectance spectra were transformed into absorbance and then normalized on the peak of maximum absorbance (peak in the range of 1024−1035 cm^{-1}) prior to their use for calibration development or validation.

RESULTS AND DISCUSSION

Analysis of the Spectra. The fingerprint region of typical spectra of κ- and ι-carrageenans is presented in **Figure 2**. The main spectral differences correspond to specific absorption bands of the fingerprint region described in the FAO Food and Nutrition Paper (*3*): 1220 cm^{-1} for the ester sulfate group,

Table 1. Carrageenan Samples Used for Calibration or Validation and Their Reference Values

sample[a]	moisture (g/100 g)	proteins (g/100 g)	minerals (Na, K, Ca, Mg) (g/100 g)	sugars (g/100 g)	carrageenan content (g/100 g)	molar ratio of the major types of carrageenans (mol %)		
						κ	ι	others
c1	12.8	0.3	6.9	ND[b]	80	91	4	5
c2	11.1	0.4	10	ND	79	1	88	11
c3	11.6	1.2	8.1	ND	79	41	31	28
c4	13.6	1.3	9.4	ND	76	53	40	7
c5	8.6	0.3	8.6	18.1[c] + 7.6[d]	57	32	31	37
c6	7.1	0.2	9.1	13.4[c] + 2.4[d]	68	13	76	11
c7	10.4	1.1	8.7	4.6[c] + 0.8[d]	74	45	24	31
c8	9.8	0.2	5.4	ND	85	97	3	ND
c9	8.4	0.2	9.3	11.0[c] + 2.7[d]	68	56	33	11
c10	10.2	1.3	7.8	14.2[c] + 2.3[d]	64	47	22	31
c11	8.6	0.6	7	16.8[c] + 1.7[d]	65	54	35	11
c12	7.5	0.3	10	25.5[c] + 1.0[d]	55	56	31	13
c13	10.2	1.2	7.2	16.9[c] + 1.6[d]	63	45	26	29
c14	11.3	0.3	9.6	ND	79	94	3	3
c15	7.9	1.2	6.3	36.6[e]	49	45	49	6
c16	7.1	0.3	17.4	3.4[c] + 0.5[d]	71	95	5	ND
c17	8.7	0.3	9.4	4.2[e]	77	6	89	5
c18	5.9	0.6	9	16.8[e]	68	57	36	7
c19	5.7	0.1	7	0.9[c] + 0.2[d] + 51.6[e]	35	77	18	5
c20	8.6	0.3	9.3	0.4[e]	81	ND	100	ND
c21	8.7	0.2	13.5	ND	78	92	6	2
c22	8.3	0.5	5.7	28.8[e]	59	74	19	7
c23	14.1	1.3	8.3	ND	76	49	36	15
c24	12.8	0.3	9.2	ND	78	1	96	3
c25	10.1	0.7	7.1	19.6[e]	63	62	25	13
c26	12.3	0.4	8.5	5.5[c] + 0.3[d]	73	89	8	3
c27	9.1	0.6	8.7	29.6[c] + 4.0[d]	48	55	37	8
v1	7.6	0.3	12.2	16.9[c] + 4.6[d]	58	55	35	10
v2	13.4	0.5	10.1	ND	76	2	91	7
v3	9.4	0.3	6.5	20.6[c] + 11.7[d]	52	45	34	21
v4	11.2	0.2	11.1	ND	77	2	94	4
v5	9.2	0.4	11	7.7[e]	72	83	14	3
v6	9.6	0.3	7.1	13.0[c] + 7.0[d]	63	66	25	9
v7	8.9	0.3	6.7	19.9[c] + 9.7[d]	55	40	34	26
v8	7.8	0.2	6.7	21.4[e]	64	40	55	5
v9	12.9	0.2	11.2	0.4[e]	75	5	90	5
v10	3.9	0.1	6	0.6[c] + 0.2[d] + 54.2[e]	35	75	20	5
v11	7.9	0.3	13.1	0.5[e]	78	88	12	ND
v12	9.7	0.3	8.7	13.4[c]	68	ND	100	ND
v13	13.3	0.3	7.6	ND	79	88	8	4
v14	9.8	0.2	12.2	ND	78	94	4	2
v15	11.8	0.3	9.9	ND	78	2	77	21
v16	11.8	0.3	9.6	ND	78	3	82	15
v17	9.8	0.3	8.1	ND	82	46	22	32
d1	12.4	1.22	8.5	ND	78	?	?	?
d2	9.5	0.17	15.2	ND	75	?	?	?
d3	4.4	ND	0.9	ND	?	?	?	?
d4	9.3	0.22	13.4	20[c]	?	?	?	?
d5	7.8	0.21	15.3	10.2[c] + 1.8[d]	65	43	52	5
d6	4.6	0.18	1.2	37[e]	23	71	29	ND
d7	6.6	27.74	4.6	1.3[c]	42	71	13	16
d8	5.2	0.19	5.8	41.6[e]	47	30	66	4

[a] c1–c27, calibration samples; v1–v17, validation samples; d1–d8, samples discarded due to high residual ratio. [b] Not detected or below quantification limit. [c] Glucose. [d] Fructose. [e] Sucrose.

928 cm^{-1} for the 3,6-anhydrogalactose, 844 cm^{-1} for the galactose-4-sulfate, and 805 cm^{-1} for the 3,6-anhydrogalactose-2-sulfate.

A principal component analysis (PCA) was carried out with the recorded spectra of the 52 samples. The first 9 principal components (PC's) of this PCA represented 99.4% of the spectral variance. On the basis of on this PCA, the spectral residual *F* ratio of each spectrum was calculated, which is the ratio of the variance of the residuals between the measured spectrum and its calculated spectrum (reproduced from the selected factors) and the residual spectral variance of all spectra (*12*). A large value indicates that the residual spectrum contains features not modeled by the PCA. In such cases, the prediction results should be treated with caution and the features in the residual spectrum investigated. The spectral residual *F* ratio is calculated as

$$\text{spectral residual } F \text{ ratio} = \frac{(u - \hat{u})^T \times (u - \hat{u}) \times n}{\sum_{i=m-1}^{n} \lambda_i}$$

where u is a measured spectrum, $\hat{u}$ is the spectrum produced from the factors up to the cutoff point, λ_i is the eigenvalue of the eigenvector i, n is the number of spectra, and m is the number of factors up to the cutoff point.

The residual *F* ratio of the spectra can be used as a tool to ensure that the calibration models are suitable to accurately

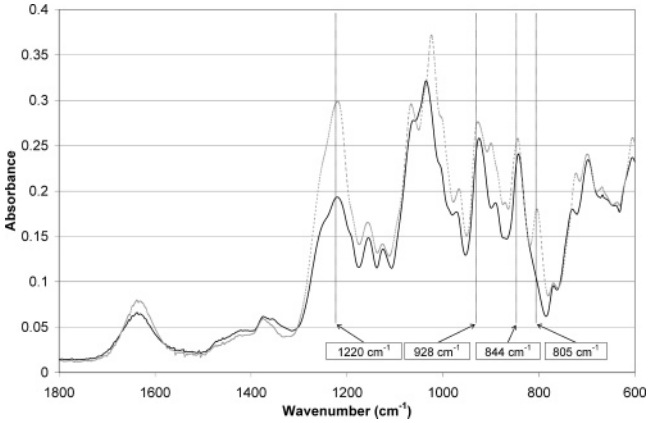

Figure 2. Fingerprint region of the FTIR spectra of typical κ-carrageenan (solid black line) and ι-carrageenan (dashed gray line) samples defined as pure, measured in absorbance on a GoldenGate ATR accessory.

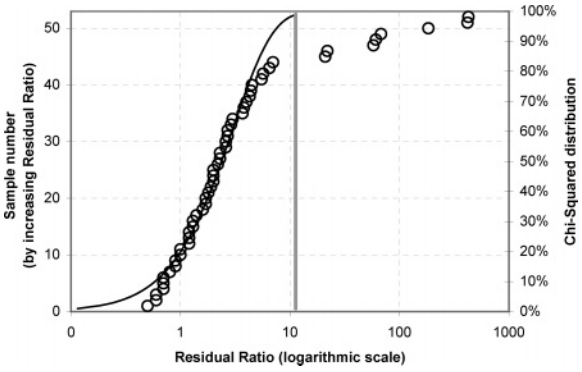

Figure 3. Graphical representation of the residual *F* ratio (○) of all samples in increasing order together with the chi-squared model (solid black line) and the 99% cutoff limit (gray line).

predict the total carrageenan content as well as the molar ratio of both κ- and ι-carrageenans of the unknown samples.

To determine the maximum acceptable residual *F* ratio, the residual *F* ratios of all samples were plotted by increasing order and a chi-squared function was modeled to best fit the data. The observed ratios as well as the chi-squared model are presented in **Figure 3**. The chi-squared model fitted well the data in the range of 20−80% and reached 99% at a residual ratio of 11.3, which can therefore be considered as the maximum residual *F* ratio, above which the spectra cannot be modeled accurately enough by the PCA. As a consequence, if the residual *F* ratio of an unknown spectrum is >11.3, then the predicted results should be evaluated with caution, because it may mean that the sample analyzed contains non-carrageenan compounds that can have an influence on the predicted results.

On the basis of the maximum acceptable residual *F* ratio, two distinct groups of samples could be identified. One group contained the first 44 samples, which have a residual *F* ratio between 0.5 and 6.5. The other group contained the remaining 8 samples, which display a residual *F* ratio between 22 and 427. This group contained the 4 samples (d1−d4 in **Table 1**) that could not be described by the NMR methods, and 4 other samples that were further investigated: 2 samples (d5 and d6) contained a large quantity of citrate (25 and 30 g/100 g), 1 sample (d7) contained 28 g/100 g of protein, and 1 sample (d8) contained carboxymethyl-cellulose.

The samples displaying a residual *F* ratio >11.3 were removed from the dataset. The remaining 44 samples were split manually into a calibration and a validation set, as presented in **Table 2**. Twenty-seven samples were selected for the calibration development, covering the whole concentration range for the three parameters of interest. The validation was carried out with 17 samples, also covering the whole concentration range for all parameters of interest.

Table 2. All Calibration and Validation Samples, with Their Reference Values and Predicted Values by FTIR for the Total Carrageenan Content and the Molar Ratio of κ- and ι-Carrageenans

		total carrageenan (g/100 g)		κ-carrageenan ratio (mol %)		ι-carrageenan ratio (mol %)	
sample[a]	residual ratio	ref	FTIR	ref	FTIR	ref	FTIR
c1	5.7	80	82	91	91	4	9
c2	1.6	79	79	1	1	88	86
c3	0.6	79	80	41	40	31	31
c4	1.3	76	78	53	50	40	44
c5	0.9	57	62	32	33	31	35
c6	1.3	68	59	13	25	76	66
c7	2.3	74	71	45	46	24	24
c8	1.7	85	79	97	92	3	9
c9	2.2	68	57	56	52	33	39
c10	1.2	64	61	47	46	22	25
c11	3.7	65	59	54	45	35	36
c12	1.8	55	51	56	52	31	40
c13	1.4	63	60	45	45	26	24
c14	0.6	79	77	94	95	3	–2
c15	1.7	49	47	45	53	49	33
c16	2.9	71	77	95	91	5	12
c17	2.0	77	72	6	6	89	84
c18	2.6	68	59	57	55	36	30
c19	0.9	35	46	77	72	18	19
c20	4.5	81	84	0	4	100	96
c21	3.0	78	79	92	95	6	10
c22	1.0	59	50	74	72	19	20
c23	1.0	76	76	49	48	36	42
c24	4.0	78	79	1	2	96	97
c25	5.5	63	55	62	63	25	30
c26	2.6	73	77	89	93	8	11
c27	1.2	48	48	55	55	37	40
v1	2.7	58	58	55	53	35	36
v2	0.8	76	81	2	–1	91	94
v3	0.7	52	58	45	32	34	25
v4	0.7	77	77	2	–3	94	93
v5	2.0	72	66	83	74	14	18
v6	1.2	63	61	66	51	25	26
v7	0.7	55	54	40	38	34	34
v8	2.3	64	61	40	44	55	44
v9	7.0	75	86	5	2	90	97
v10	0.5	35	41	75	73	20	18
v11	4.3	78	85	88	90	12	14
v12	2.0	68	74	0	7	100	84
v13	6.5	79	87	88	89	8	15
v14	1.9	78	80	94	96	4	6
v15	2.7	78	81	2	5	77	86
v16	3.8	78	85	3	2	82	94
v17	4.4	82	77	46	44	22	24

[a] c1–c27, calibration samples; v1–v17, validation samples.

Calibration Development. The calibrations were developed over the whole recorded spectral range with the four replicate spectra of each sample of the calibration set. This aimed at improving the repeatability and robustness of the calibration (*13*, *14*). The PCR+ algorithm (commercial name of principal component regression from Perkin-Elmer software) was used to develop the calibration models combining a maximum of nine principal components (PCs). The optimum number of PCs for each calibration was defined by leave-one-out cross-validation procedure: six PCs for the total carrageenan content, eight for the molar ratio of κ-carrageenan, and four for the molar ratio of ι-carrageenan.

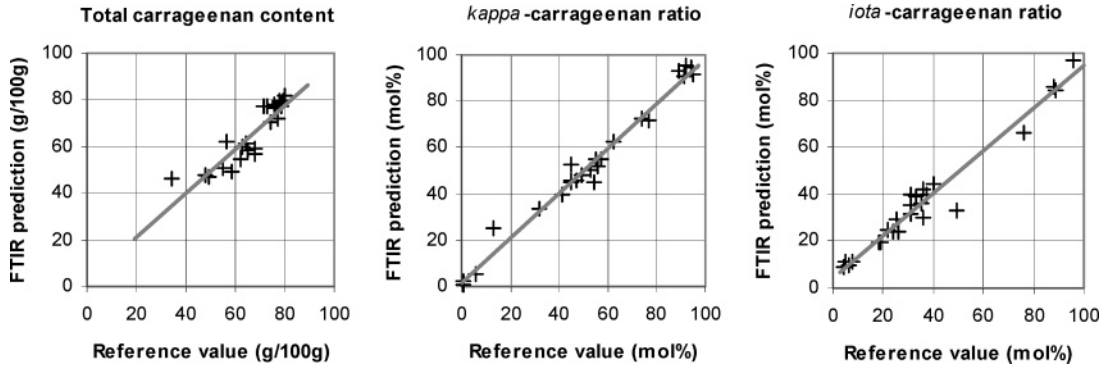

Figure 4. Regression graphs of the calibration models for the total carrageenan content and the molar ratio of κ- and ι-carrageenans obtained with calibration samples.

Table 3. Performance Characteristics of the Calibration Models for the Prediction of Total Carrageenan Content and the Molar Ratio of κ- and ι-Carrageenans

	total carrageenan	κ-carrageenan ratio	ι-carrageenan ratio
n	27	27	27
range	35–85 g/100 g	0–97 mol %	3–100 mol %
R^2	0.817	0.981	0.966
SEC[a]	7.0 g/100 g	5.2 mol %	7.0 mol %
suspect samples[b]		1 (c6)	1 (c15)

[a] Standard error of calibration. [b] Suspect samples are samples showing an absolute prediction error larger than the 95% confidence interval.

The whole statistical analysis is based on the average of the four predicted values for each sample. **Table 2** lists the predicted values obtained for the calibration samples, when using the four models developed. The regression graphs of reference versus predicted values for all calibration models are presented in **Figure 4**. Visual examination of the regression graphs tends to confirm the assumption of linearity.

Table 3 summarizes the performance characteristics of the calibration models for the prediction of total carrageenan content and the molar ratio of κ- and ι-carrageenans. A good correlation was obtained between the reference and predicted values for all three calibration models, as indicated by the coefficients of determination (R^2) >0.8. The obtained values of standard error of calibration (SEC) were considered to be acceptable, as they were all in the range of the estimated laboratory error of the reference values:

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{(n - m - 1)}}$$

y_i is the "reference" value for sample *i*, $\hat{y}_i$ is the "predicted" value for sample *i*, *n* is the number of calibration samples, and *m* is the number of factors used in the calibration.

Two samples were considered to be suspect as they displayed a difference between the reference and predicted values greater than the 95% confidence interval: sample c6 for its κ-carrageenan content and sample c15 for its ι-carrageenan content. As the two "suspect samples" displayed large differences for both κ- and ι-carrageenan content and presented a low residual ratio, their suspect behavior may be linked to the limitation of the NMR methods due to the presence of hybrid carrageenans, as suggested by Turquois et al. (*7*).

Independent Validation. Validation was performed with an independent set of samples. All predicted results listed in **Table 2** consist of the average of four replicate predictions. The regression graphs of reference versus predicted values for all calibration models are presented in **Figure 5**. Visual examination of the regression graphs tends to confirm the assumption of linearity.

Table 4 summarizes the performance characteristics obtained by the validation for the models developed to predict the total carrageenan content and the molar ratio of κ- and ι-carrageenans. A good correlation was obtained between the reference and the predicted values for all three validations, as indicated by the coefficients of determination (R^2) >0.85. As the obtained values for the standard error of prediction (SEP) were comparable to the corresponding SEC values, it can be assumed that the three models are robust. The ratio of SEP to SEC was below the limit defined by the AACC (*15*), where the recommended performance target is SEP/SEC < 1.2. SEP values were all in the range of the estimated laboratory error of the reference values:

$$\mathrm{SEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}}$$

y_i is the "reference" value for sample *i*, $\hat{y}_i$ is the "predicted" value for sample *i*, and *n* is the number of validation samples.

Further statistical analysis of the data was performed. The statistical analysis of the differences indicated that none of the calibration models showed any systematic error ($p < 0.05$). The regression analysis of the validation results of the three calibration models did not show any significant proportional error ($p < 0.05$).

Three samples were considered to be suspect as they displayed a difference between the reference and predicted values larger than the 95% confidence interval: samples v6 and v3 for their molar ratio of κ-carrageenan and sample v12 for its molar ratio of ι-carrageenan. Two of these "suspect samples" displayed large differences for both κ- and ι-carrageenan content and presented a low residual ratio; their suspect behavior may be linked to the limitation of the NMR methods due to the presence of hybrid carrageenans, as suggested by Turquois et al. (*7*).

Limit of Quantification. The limit of quantification can be estimated on the basis of method recovery. The recovery of each sample was calculated as the ratio of the predicted value to the reference value, expressed as a percentage. Graphs in **Figure 6** present the recovery as a function of the reference value for the three calibration models: total carrageenan content

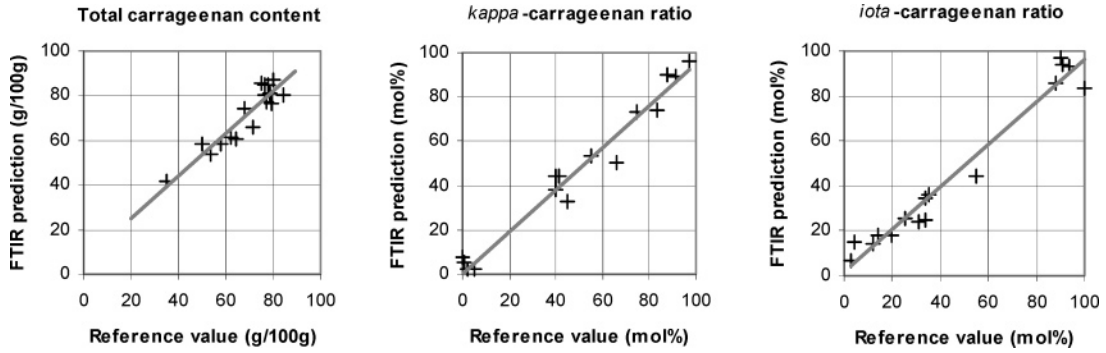

Figure 5. Regression graphs of the calibration models for the total carrageenan content and the molar ratio of κ- and ι-carrageenans obtained with validation samples.

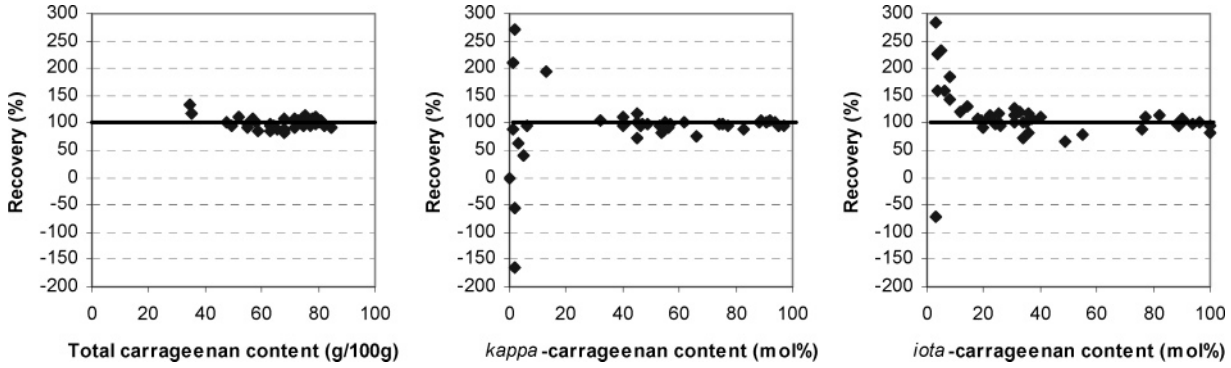

Figure 6. Recovery obtained for all samples as a function of the reference value for the three models developed: total carrageenan content and molar ratio of κ- and ι-carrageenans.

Table 4. Performance Characteristics Obtained by the Validation of the Calibration Models for the Prediction of Total Carrageenan Content and the Molar Ratio of κ- and ι-Carrageenans

	total carrageenan	κ-carrageenan ratio	ι-carrageenan ratio
n	17	17	17
range	35–82 g/100 g	0–94 mol %	4–100 mol %
R^2	0.863	0.973	0.964
SEP[a]	5.6 g/100 g	6.1 mol %	6.6 mol %
SEP/SEC	0.79	1.18	0.95
bias[b]	2.6 g/100 g[c]	–2.1 mol %[c]	0.7 mol %[c]
suspect samples[d]		2 (v3 and v6)	1 (v12)

[a] Standard error of prediction. [b] Bias is calculated as the average of the differences of predicted values minus reference values. [c] Bias not statistically significant (95% confidence level). [d] Samples showing an absolute prediction error larger than the 95% confidence interval.

and molar ratios of κ- and ι-carrageenan. From the graphs it can be estimated that the limit of quantification for total carrageenan is the value of the lowest calibration standard, that is, 35 g/100 g. For the molar ratio of κ- and ι-carrageenans, the quantification limit should be set at 15 mol %, a value below which the relative error can be >50%. This value is comparable to 2 × SEP, which could have been another way to estimate the limit of quantification.

Repeatability of the Method. The repeatability of the three calibration models was evaluated on the basis of 10 analyses of the same sample, each result being the average of 4 predictions. It was carried out with two different samples: sample v14, a κ-carrageenan defined as "pure", and sample c13, defined as a blend of κ- and ι-carrageenans with added sugars. For both products tested, the standard deviation of repeatability (SDr) was below 1.5 g/100 g and below 1.5 mol % for the total carrageenan content and the molar ratio of κ- and ι-carrageenans, respectively (see **Table 5**).

The calculated ratios of SDr/SEP were found below the maximum limit of 0.33 set by the AACC (*15*) for all three calibrations. The repeatability of the method can therefore be considered as good: the prediction results are not significantly affected by the homogeneity of the sample or by the repeatability of the FTIR measurement.

Conclusions. Carrageenans can be characterized without any sample preparation by FTIR spectroscopy based on a direct measurement of the powdered sample with a single-reflection diamond ATR (GoldenGate accessory). The accuracies of the calibrations given by their respective SEPs are 5.6 g/100 g for

Table 5. Repeatability of the FTIR Prediction of Total Carrageenan Content and Molar Ratio of κ- and ι-Carrageenan Obtained on Two Samples

	total carrageenan		κ-carrageenan ratio		ι-carrageenan ratio	
sample	v14	c13	v14	c13	v14	c13
mean value[a]	83.0 g/100 g	63.0 g/100 g	95.4 mol %	46.0 mol %	7.9 mol %	27.1 mol %
SDr[b]	0.4 g/100 g	1.1 g/100 g	1.0 mol %	0.6 mol %	0.7 mol %	1.2 mol %
SDr/SEP[c]	0.07	0.20	0.16	0.10	0.10	0.18

[a] Mean value of 10 measurements obtained under repeatability conditions. [b] Standard deviation of repeatability. [c] Ratio of standard deviation of repeatability to standard error of prediction.

total carrageenan content and 6.1 and 6.6 mol % for the molar ratios of κ- and ι-carrageenans. These values are higher than the estimated laboratory error of the reference methods but acceptable for raw material characterization.

Due to the fact that the method was developed with commercial carrageenans, defined as "pure" or blends, the spectral variability from non-carrageenan compounds is corrected by the PCR calibration models. The method can therefore be considered as suitable for all commercial carrageenans, "pure" blends or with small additions of other constituents. However, the computed residual F ratio has to be checked for each new prediction. A value >11.3 would mean than the unknown sample contains features not modeled during calibration and, therefore, the prediction results should be treated with caution.

The main advantages of this new approach are that no sample preparation is necessary and that the analytical results are available within 5 min. This technique is therefore suitable for the rapid characterization of raw materials at reception in an industrial environment.

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