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Structure and performance of commercial kappa-2 carrageenan extracts I. Structure analysis

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Abstract

Extracts of two Chilean carrageenophyte red seaweeds, *Sarcothalia crispata* and *Gigartina skottsbergii*, were prepared by each of two industrial processes: a mild alkaline extraction with aqueous sodium hydroxide and a more vigorous aqueous alkaline extraction with lime. The resulting extracts, recovered by precipitation with isopropyl alcohol, were separated into gelling and non-gelling fractions by leaching with 2.5% KCl. These processes were also applied to *Chondrus crispus* and to separated gametophyte and sporophyte samples of the Chilean seaweeds for comparative purposes. The carrageenan compositions of these extracts and fractions were determined using both chemical and spectroscopic techniques. In addition, a set of decision rules is proposed and applied to convert data from glycosyl linkage analysis to carrageenan composition. The gametophyte extracts contained mixtures/hybrids of kappa and iota carrageenans (and also mu and nu carrageenans, depending on the extraction conditions used). The tetrasporophyte extracts contained lambda carrageenan (and also theta carrageenan, depending on the extraction conditions used). Extractive fractionation of mixed life phase samples using 2.5% KCl yielded insoluble, 'gelling' carrageenans quite similar to those from a gametophyte extract of the same species, but the soluble, non-gelling fractions were not the same as the corresponding sporophyte extracts.

Keywords: Carrageenan; Kappa-2 carrageenan; *Sarcothalia crispata*; *Gigartina skottsbergii*; *Chondrus crispus*; Structure analysis

1. Introduction

Industrially important sulfated galactans, known as carrageenans, are found in numerous red seaweeds. Traditionally, Greek letters have been assigned to carrageenans comprised of certain idealised carrageenan disaccharide repeating units.[1] A summary of various carrageenan structures that have been assigned Greek letters was presented by Craigie (1990). However, native carrageenans often contain combinations of these idealised units, with variations in structure occurring not only between different species of seaweed but also within the different life-stages of a single species.

Kappa, iota, mu and nu carrageenans are found in the gametophytic life phase of various seaweed species in the family Gigartinaceae and the disaccharide repeat units of these carrageenans are shown in Fig. 1. Mu and nu carrageenans are the biochemical precursors of kappa and iota carrageenans. They both contain a sulfate ester group at position-6 of a 4-linked α-D galactosyl unit which affects the overall properties of the carrageenan by creating 'kinks' in the polymer chain that reduce its ability to gel. The tetrasporic life phase of Gigartinacean seaweeds contains a different type of carrageenan, most commonly lambda carrageenan (McCandless, Craigie, & Walter, 1973; Pickmere, Parsons, & Bailey, 1973; Matulewicz, Ciancia, Noseda, & Cerezo, 1989). Lambda is the precursor of theta carrageenan (Fig. 1) but theta does not occur predominantly in Gigartinacean seaweeds.

6-Sulfated galactosyl units are converted to the corresponding 3,6-anhydride (3,6AG) using hot alkali. The rate of conversion is dependent not only on temperature and alkali concentration but also on the ionic strength of the medium. Carrageenan structure is also important, with the conversion of lambda to theta being much slower than the other conversions shown in Fig. 1 (Ciancia, Noseda, Matulewicz, & Cerezo, 1993b).

The carrageenans that occur in Gigartinacean algae are important commercially as these particular mixtures or copolymers of kappa and iota carrageenan (referred to as

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[1] We employ the Greek letter designation of idealized carrageenan repeat units (Rees, 1972) rather than the newer and more versatile nomenclature of Knutsen, Myslabodski, Larsen, and Usov (1994). This article is aimed largely at industrial researchers who are more familiar with the Greek letter nomenclature.

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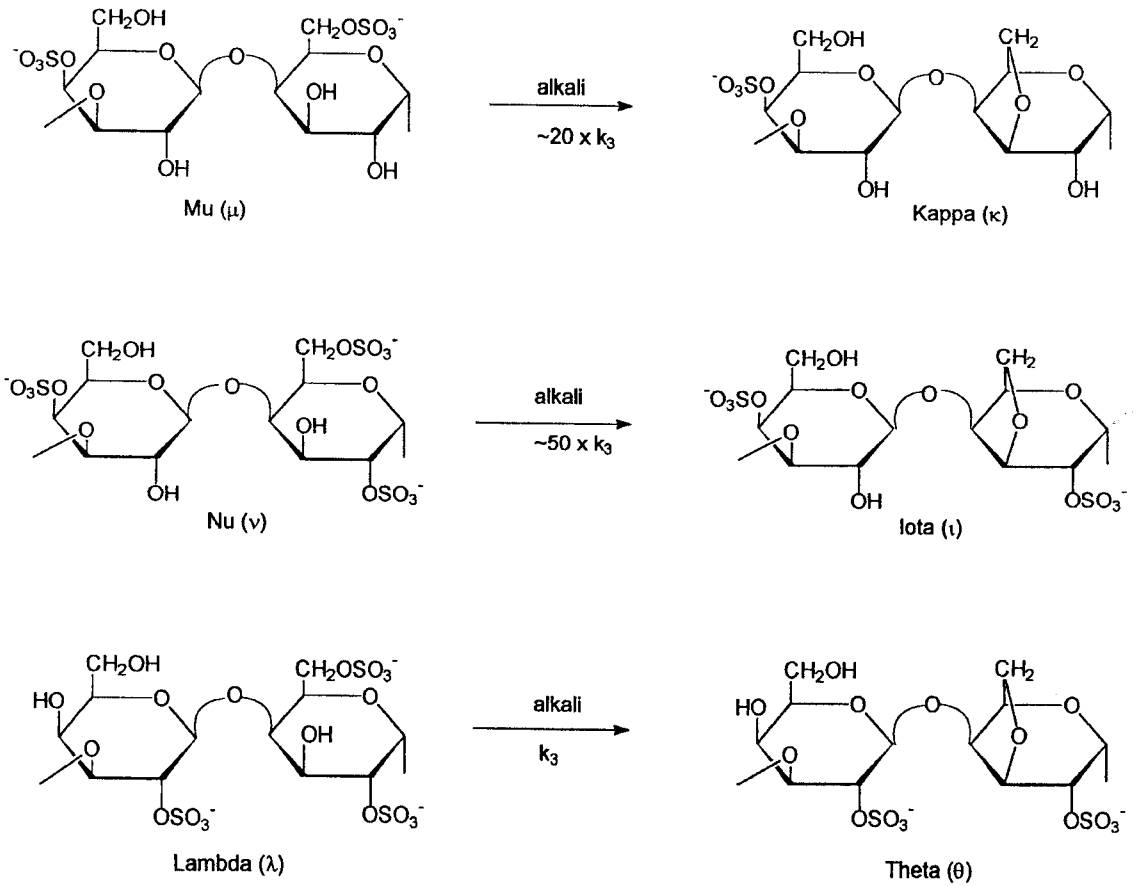

Fig. 1. Principal carrageenan structures showing conversions that occur on alkaline treatment. Relative rates of conversion of mu to kappa and nu to iota are given in terms of the rate constant, k_3, for conversion of lambda to theta.

'kappa-2', 'kappa/iota hybrid' or 'weak-gelling' kappa carrageenans) have different properties to simple mixtures of kappa and iota carrageenan from other sources. Long reaction times and highly alkaline conditions are used commercially to achieve a high level of conversion of precursors to kappa and iota carrageenan when gelling ability and/or protein reactivity of the carrageenan in foods are being maximised. Milder conditions that produce less conversion are used when cold solubility and viscosity are the desired results.

In this paper, the structural analysis of carrageenans from three temperate-water red carrageenophytes [*Sarcothalia crispata* (previously known as *Iridaea undulosa*), *Gigartina skottsbergii* and *Chondrus crispus*] is described. Several studies can be found on the structural analysis of the carrageenans from both hot and cold water extracts of individual life phases of these species (Dolan & Rees, 1965; McCandless et al., 1973; Matulewicz et al., 1989; Matulewicz, Ciancia, Noseda, & Cerezo, 1990; Ciancia, Matulewicz, Finch, & Cerezo, 1993a; Stortz & Cerezo, 1993; Stortz, Bacon, Cherniak, & Cerezo, 1994). However, the carrageenans analysed here were produced using commercial extraction procedures on mixed, as well as separated, life phase material, followed by simple fractionation with potassium chloride at one concentration to replicate an industry test procedure. In the following paper, the structures of these extracts are related to some of their commercial quality control and simulated dairy application properties.

2. Experimental procedures

2.1. Materials and methods

Two Chilean species, *G. skottsbergii* (known as 'pigskin') and *S. crispata* (from Chiloe Island and known as 'nama' or 'sandpaper') were used. The third species, *C. crispus*, was obtained from Western Nova Scotia, Canada. The samples were obtained from bags or bales of commercially dried and cleaned, mixed life phase seaweed stored in a Shemberg plant in Cebu, Philippines or in ISI's laboratory. Different samples of *G. skottsbergii* and of *S. crispata* were used to produce the high viscosity (HV) and high milk reactivity (HMR) process extracts. Dry weights were determined by drying samples at 105°C for 4 h. Clean weights were determined by removing surface sand and salt and extraneous matter. Kappa carrageenan from Sigma Chemical Co. was used as a reference material.

2.2. Extraction

The HMR process involved treatment with lime [0.1 M $Ca(OH)_2$], for 30–48 h at 95 ± 2°C. The HV process used a lower concentration of alkali (0.02 M NaOH) but the ionic strength was increased by addition of sodium chloride (0.1 M). This process was carried out for 4–5 h at 95 ± 2°C. Samples of carrageenan obtained from these extractions without any separation of seaweed life phases are denoted as 'mixed life phase' extracts and those without carrageenan fractionation as 'whole seaweed' extracts.

Three levels of extraction, differing primarily in batch size, were performed: (a) plant-scale using about 1000 kg of seaweed per batch (for HMR extraction of mixed life phase *G. skottsbergii* and HV extraction of mixed life phase *S. crispata*), (b) laboratory raw material testing scale using about 100 g seaweed (for HV extraction of mixed life phase *G. skottsbergii* and *C. crispus*, and HMR extraction of mixed life phase *S. crispata* and *C. crispus*), and (c) bench-scale using about 30 g of seaweed (for all extractions of separate gametophyte and tetrasporophyte samples).

In all cases, the slurry of seaweed solids and carrageenan solution was filtered with filter aid in a pressure filter. The resulting clear but slightly tan coloured solution was neutralised with HCl (4 M) to pH 8.0–8.5. In plant-scale extractions, the carrageenan was concentrated to about 2.5% w/v by ultrafiltration but this step was omitted in the smaller-scale extractions. Carrageenan was then coagulated by adding pure isopropyl alcohol, the coagulum was rinsed with fresh alcohol, pressed to remove as much liquid as possible, vacuum dried and powder milled (nominally 80 mesh).

2.3. Fractionation

In this work only a simple division of the carrageenan into gelling and non-gelling fractions was desired, so an extractive procedure using a relatively high concentration of KCl was used. Carrageenan powder (10 g) was dispersed in a 2.5% KCl (0.33 M) solution at a concentration of 1% w/v, and maintained at 25°C in a water bath with mild stirring for 12 h. Perlite filter aid was then added and the mixture filtered. The filter cake was washed with 2.5% KCl solution. The carrageenan in the filtrate was coagulated with isopropanol and recovered as a powder as described above. This carrageenan was designated the 'non-gelling' fraction. The undissolved carrageenan captured in the filter cake was reslurried in deionized (DI) water, and heated to 95°C. The hot solution was filtered, and the filter cake again washed with hot DI water. The carrageenan in the combined filtrates was recovered as above and designated the 'gelling fraction'.

2.4. Seaweed separation

Gametophytes and sporophytes of *G. skottsbergii* and *S. crispata* were separated from dry commercial weeds by visual inspection of rewetted fronds but this was not possible for *C. crispus*. Therefore, only the physically fractionated gelling and non-gelling fractions of extracts of this species could be studied, and the quantity of the latter was usually too small for any applications testing. It is well known that Western Nova Scotia populations of *C. crispus* contain very few sporophytes.

2.5. ^{13}C NMR spectroscopy

Spectra were recorded on 3% w/v solutions in 1:1 v/v D_2O:H_2O at 90°C on a Varian Unity-500 spectrometer at a carbon frequency of 125 MHz, using a 10 mm broad band probe, acquisition time of 1.17 s, delay time of 0.8 s, and 80° pulse. Chemical shifts are quoted relative to internal Me_2SO at 39.47 ppm.

2.6. Constituent sugar analysis

Samples were reductively hydrolysed with *N*-methylmorpholine borane in aqueous trifluoroacetic acid then acetylated to prepare alditol acetate derivatives as previously reported (Stevenson & Furneaux, 1991), except that the initial hydrolysis at 80°C was carried out for 30 min to effect an improved recovery of the derivatives from 2-sulfated 3,6-anhydrogalactosyl (3,6AG) units (Jol, Neiss, Penninkhof, Rudolph, & DeRuiter, 1999). The alditol acetate derivatives were analysed by GLC using a Hewlett-Packard 5890 Series II chromatograph with flame ionisation detection and a Supelco SP-2330 column (15 m × 0.25 mm) at 220°C. Quantitation was based on experimentally determined response factors (Falshaw & Furneaux, 1994).

2.7. Glycosyl linkage analysis

Samples were converted to triethylammonium salts and methylated with $MeI/Me_2SO^-K^+$ (Stevenson & Furneaux 1991), purified by dialysis against $H_2O \times 1$; Et_3NHCl (0.1 M, pH 7) × 1; $H_2O \times 2$ and lyophilised. A second methylation and recovery was performed if, upon analysis of the samples, it was determined that the first methylation had been incomplete (by the presence of galactitol hexacetate). The methylated samples were reductively hydrolysed and acetylated as described above except that the initial hydrolysis time was 10 min. The resulting partially methylated alditol acetate derivatives were analysed by GLC as above but at 170°C (1 min) then 4°C/min to 220°C (5 min). To differentiate enantiomeric derivatives, C-1 deuterated alditol acetates were prepared from some methylated carrageenan samples by hydrolysis in TFA (2 M, 0.25 ml, 1 h, 120°C) followed by reduction with $NaBD_4$ (15 mg/ml in aqueous 1 M NH_4OH, 0.25 ml, 1 h, 25° C). After the reduction was quenched with acetone (0.5 ml), the sample was evaporated to dryness and acetylated by the method of Falshaw and Furneaux (1995), except that the organic layer was washed with water (4 ml), Na_2CO_3 (0.5 M, 4 ml) and water (4 ml). The partially methylated alditol acetate derivatives were analysed by

GLC, as above, and were also analysed by GLC-MS (Falshaw & Furneaux, 1994).

3. Results and discussion

3.1. Sample extraction

The yields of carrageenan obtained from HMR and HV extractions of gametophytic, sporophytic and mixed life phase seaweed are shown in Table 1, and are typical of commercial as-is yields. As-is yields are based on seaweed weight including moisture, sand, salt and foreign matter. The seaweeds under investigation were from high quality suppliers with moisture about 22% w/w and the other impurities 5% w/w or less.

3.2. Sample fractionation

The weight percent of the gelling and non-gelling fractions obtained using 2.5% KCl from a sample of each mixed life phase extract and the total recovery relative to the starting sample are tabulated in Table 1. Material balance closure ranged from 96 to 102%.

3.3. Chemical analysis

3.3.1. Constituent sugar analysis

3,6-Anhydrogalactose and galactose were the predominant sugars derived from all the samples. Small amounts of xylose (1%) and glucose (<5%) were also observed in some samples. More xylose (4%) was observed in the non-gelling fraction of the *C. crispus* HV extract and larger amounts of glucose (9 and 13%) in the non-gelling fractions of the HMR extracts of tetrasporic and mixed life phase *G. skottsbergii*, respectively. The xylose is most likely to occur as single branches on the 3-linked galactosyl units in carrageenans. The glucose is likely to be derived from floridean starch present in these samples. In order to compare the carrageenan compositions of the various fractions, the constituent sugar results were normalised to exclude glucose. The resulting 3,6AG contents of all the extracts and fractions analysed are shown in Table 1.

The level of 3,6AG was close to 50% for the gelling fractions of mixed life phase extracts of all three species extracted using the HMR process (and also the whole gametophyte extracts, where studied). This is to be expected for seaweeds that contain kappa, iota, mu and nu carrageenans where the mu and nu carrageenans have been converted to kappa and iota, respectively, during the long hot alkali conditions of the HMR process. The ratios of 3,6AG to galactose were less than 1:1 for the equivalent samples extracted using the HV process. The milder conditions used here evidently do not convert all the mu and nu carrageenans present in the original seaweed to kappa and iota carrageenans, respectively.

The whole tetrasporophyte extracts obtained using the HV process contained little or no 3,6AG, as expected for lambda carrageenan. The whole tetrasporophyte HMR extracts did contain significant amounts of 3,6AG but not close to the 50% expected if all the lambda carrageenan had been converted to theta carrageenan. This reflects the slower rate of conversion of lambda to theta (Fig. 1). Based on these values, the non-gelling fractions (for both the HV and HMR processes) of all the samples analysed contained more 3,6AG than would be expected if they contained just lambda and some theta carrageenans. The percent gelling fractions obtained for both HMR and HV extracts of *C. crispus* were higher than for *G. skottsbergii* which, in turn, were higher than for *S. crispata*. This progression would be expected from the 3,6AG levels in these samples (as well as from the relative performance of the extracts in the applications tests, see following paper).

The relatively large change in the amount of gelling fraction obtained for *S. crispata* in going from an HV extract to an HMR extract, compared with the smaller change for *G. skottsbergii* and the even smaller change for *C. crispus*, does not seem to coincide with the changes in 3,6AG content. For instance, it would be expected from the progressions in the yield of the gelling fractions that the 3,6AG increase in going from HV to HMR sample would be highest for *S. crispata* and lowest for *C. crispus*. However, from the results in Table 1, the 3,6AG changes are roughly the same for all three seaweeds (10–14 mole%). Most likely, this behaviour is related to where along the chains with gelling potential the 'dekinking' by 3,6AG formation took place. That is, a relatively small amount of 3,6AG formation could have resulted in the formation of large blocks of kappa and iota carrageenans on alkaline treatment of *S. crispata*; whereas for *C. crispus* large blocks of gelling structures evidently exist before alkaline treatment and are not much changed by the treatment.

3.3.2. Glycosyl linkage analysis

In the samples where glucose was observed by constituent sugar analysis, the partially methylated alditol acetate species 4-Glc was observed, as expected if floridean starch were present. The data shown in Tables 2 and 3 was normalised to exclude 4-Glc in order to allow direct comparison of the carrageenan compositions of all the samples. The data for whole gametophyte and tetrasporophyte extracts are consistent with those obtained previously for these species (allowing for differences in sample origin, extraction conditions and analytical methods) (McCandless et al., 1973; Matulewicz et al., 1990; Ciancia et al., 1993a). The total 3,6AG contents were close to those obtained by constituent sugar analysis for HMR extracts but not for HV extracts as additional conversion of 6-sulfated 4-linked galactosyl units to the corresponding 3,6-anhydrides occurred in these samples during methylation. This was confirmed by the absence of 4,6-Gal (indicative of mu carrageenan) in all these samples. However, some 2,4,6-Gal (indicative of nu or lambda carrageenans) was observed. This reflects the

Table 1
Percentage yields and 3,6-anhydrogalactose contents of carrageenans obtained from seaweed extraction and 2.5% KCl fractionation (– = not determined)

Seaweed species and extraction scale[a]	Seaweed life phase	Extraction process	Carrageenan extract		Gelling fraction		Non-gelling fraction		Total recovery (%w/w)
			Yield[b] (%w/w)	3,6AG[c] (normalised mole%)	Yield[d] (%w/w)	3,6AG (normalised mole%)	Yield[d] (%w/w)	3,6AG (normalised mole%)	
C. crispus (L)	Mixed	HMR	32	*44*	94	47	6	nd	100
G. skottsbergii (P)	Mixed	HMR	42	*42*	83	48	13	16	96
S. crispata (L)	Mixed	HMR	42	*42*	79	48	17	24	96
C. crispus (L)	Mixed	HV	33	*34*	90	37	8	4	98
G. skottsbergii (L)	Mixed	HV	42	*32*	71	37	31	18	102
S. crispata (P)	Mixed	HV	44	*23*	50	34	50	11	100
G. skottsbergii (B)	Gametophyte	HMR	32	50	–	–	–	–	–
S. crispata (B)	Gametophyte	HMR	38	49	–	–	–	–	–
G. skottsbergii (B)	Sporophyte	HMR	41	13	–	–	–	–	–
S. crispata (B)	Sporophyte	HMR	31	12	–	–	–	–	–
G. skottsbergii (B)	Gametophyte	HV	36	35	–	–	–	–	–
S. crispata (B)	Gametophyte	HV	33	33	–	–	–	–	–
G. skottsbergii (B)	Sporophyte	HV	32	1	–	–	–	–	–
S. crispata (B)	Sporophyte	HV	33	0	–	–	–	–	–

[a] P, L and B refer to the plant, laboratory or bench scale of extraction respectively (see Section 2.1 for details).
[b] As-is basis (see Section 3.1 for explanation).
[c] Values (in italics) for mixed life phase extracts are calculated from the values obtained for the gelling and non-gelling fractions. The value for *C. crispus* HMR is based on the gelling fraction only.
[d] Bone dry weight basis.

Table 2
Glycosyl linkage analysis of whole gametophyte (G) extracts and gelling fractions (Gel) of mixed life phase (Mixed) samples of *C. crispus* (Cc), *G. skottsbergii* (Gs) and *S. crispata* (Sc). Numbers in bold relate to the major idealised carrageenan structures

		Normalised mole%									
Species		Cc	Cc	Gs	Gs	Gs	Gs	Sc	Sc	Sc	Sc
Life Phase		Mixed	Mixed	Mixed	Mixed	G	G	Mixed	Mixed	G	G
Extraction		HMR	HV	HMR	HV	HMR	HV	HMR	HV	HMR	HV
Fraction		Gel	Gel	Gel	Gel	Whole	Whole	Gel	Gel	Whole	Whole
Constituent sugar and deduced substitution[a]	Carrageenan type										
2,3-Gal	λ, θ	**1**	**1**	**2**	**1**	**0**	**0**	**2**	**0**	**1**	**0**
3,4-Gal	κ, ι, μ, ν	**46**	**47**	**39**	**45**	**46**	**44**	**49**	**43**	**46**	**47**
3-Gal	β, α, δ, γ	3	3	4	3	4	4	1	0	2	2
3,4,6-Gal[b]	π, pyruvated α	0	0	0	0	0	1	0	1	0	1
2,3,6-Gal[c]	λ-type	0	0	0	0	0	0	0	1	0	0
Total 3-linked units		*50*	*51*	*45*	*49*	*50*	*49*	*52*	*45*	*49*	*50*
4-AnGal	κ, (μ^d), β	**38**	**38**	**36**	**35**	**33**	**34**	**29**	**35**	**32**	**33**
2,4-AnGal	ι, θ, (ν^e), α	**12**	**9**	**17**	**10**	**16**	**10**	**17**	**11**	**17**	**11**
2,4,6-Gal[c]	λ, ν, γ	**0**	**2**	**1**	**6**	**0**	**5**	**1**	**5**	**0**	**5**
4,6-Gal	μ, δ	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
4-Gal		0	0	0	0	0	0	0	1	0	0
2,4-Gal	ξ	0	0	0	0	0	0	0	1	0	0
Total 4-linked units		*50*	*49*	*54*	*51*	*49*	*49*	*47*	*53*	*49*	*49*
2,3,4-Gal		0	0	1	0	1	1	1	1	2	1
2,3,4,6-Gal		0	0	0	0	0	1[f]	0	1[f]	0	0
Total ambiguous linkage units		*0*	*0*	*1*	*0*	*1*	*2*	*1*	*2*	*2*	*1*

[a] 2,4-Gal means a 2,4-disubstituted and/or linked galactopyranosyl unit, analysed as 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-galactitol etc.
[b] Assumed to be pyruvated [4,6-*O*-(carboxyethylidine)] 3-linked galactosyl unit.
[c] Enantiomeric partially methylated alditol acetates (2,3,6-Gal and 2,4,6-Gal) differentiated by deuterium labelling and determined by GC-MS analysis.
[d] Converted to κ-carrageenan during processing or glycosyl linkage analysis.
[e] Converted to ι-carrageenan during processing or glycosyl linkage analysis.
[f] A second methylation of these samples resulted in degradation of the sample.

slower conversion of lambda to theta and nu to iota than mu to kappa carrageenan.

Most notably, the compositions of the non-gelling fractions of the mixed life phase HV extracts were distinctly different from those of the corresponding whole tetrasporophyte extracts, with significant amounts of 3,4-Gal (and, thus, kappa, iota, mu and/or nu carrageenans) present.

3.3.3. Data analysis

3.3.3.1. Rules for determining extract composition. Most of the derivatives observed in Tables 2 and 3 can be attributed to more than one of the idealised carrageenan disaccharide repeat units shown in Fig. 1. For example, 2,4,6-Gal can be derived from both lambda and nu carrageenans since lambda = 2,3-Gal + 2,4,6-Gal and nu = 3,4-Gal + 2,4,6-Gal. Obviously, there could be just lambda or just nu or a combination of both carrageenans present in any given sample depending on the relative amounts of 2,3-Gal and 3,4-Gal present. Numerous simultaneous equations can be constructed depending on the number and type of carrageenan structures defined.

All the samples examined here also contain some miscellaneous 3-linked or 4-linked monosaccharide units that do not correspond to any of those shown in the idealised carrageenan structures of Fig. 1. Some of the units, such as 3-Gal, can be attributed to a number of other, known carrageenan structures (such as alpha, beta, gamma or psi-carrageenans) (Craigie, 1990), while other units may be components of carrageenans with idealised structures that have not been assigned Greek letters. In addition, there are units with ambiguous linkage/substitution patterns. 2,3,4-Gal, for example, is derived from a galactopyranosyl unit that is either linked or substituted at the 2-, 3- and 4-positions. It could correspond to a 2,4-disulfated 3-linked galactopyranosyl unit or, a 2,3-disulfated 4-linked galactopyranosyl unit as observed in the polysaccharide from the red seaweed, *Champia novae-zealandiae* (Miller, Falshaw, & Furneaux, 1996). Since seaweeds are natural organisms there is always potential for structures that do not fit in a rigid naming system. These unusual monomers may not be contained in homogeneous polymers but scattered along a chain of other carrageenan structures and act as spacers where they may interrupt double helix formation, and thus prevent, or weaken gelation (Lawson, Rees, Stancioff, & Stanley, 1973; Penman & Rees, 1973).

The estimation of the relative compositions of complex polysaccharide mixtures can be conducted using sets of predefined decision rules [e.g. cell wall composition of grape berries (Nunan, Sims, Bacic, Robinson, & Fincher, 1998)]. Ciancia et al. (1993a) calculated the contribution of various carrageenan disaccharide repeat units to of three gametophyte

Table 3
Glycosyl linkage analysis of whole tetrasporophyte (T) extracts and non-gelling fractions (Non-Gel) of mixed life phase (Mixed) samples of *C. crispus* (Cc), *G. skottsbergii* (Gs) and *S. crispata* (Sc). Numbers in bold relate to the major idealised carrageenan structures

		Normalised mole%								
Species		Cc	Gs	Gs	Gs	Gs	Sc	Sc	Sc	Sc
Life Phase		Mixed	Mixed	Mixed	T	T	Mixed	Mixed	T	T
Extraction		HV	HMR	HV	HMR	HV	HMR	HV	HMR	HV
Fraction		Non-Gel	Non-Gel	Non-Gel	Whole	Whole	Non-Gel	Non-Gel	Whole	Whole
Constituent sugar and deduced substitution[a]	Carrageenan type									
2,3-Gal	λ, θ	**19**	**42**	**10**	**48**	**40**	**44**	**25**	**42**	**39**
3,4-Gal	κ, ι, μ, ν	**18**	**3**	**36**	**0**	**2**	**2**	**14**	**0**	**0**
2,3,6-Gal[b]	λ-type	**2**	**3**	**0**	**2**	**3**	**3**	**3**	**5**	**5**
3-Gal	β, α, δ, γ	11	1	3	0	2	1	4	0	1
Total 3-linked units		*50*	*49*	*49*	*50*	*47*	*50*	*46*	*47*	*45*
4-AnGal	κ, (μ[c]), β	**9**	**5**	**25**	**2**	**2**	**3**	**15**	**1**	**0**
2,4-AnGal	ι, θ, (ν[d]), α	**6**	**14**	**5**	**15**	**3**	**18**	**4**	**10**	**3**
2,4,6-Gal[b]	λ, ν, γ	**23**	**28**	**21**	**29**	**43**	**24**	**29**	**37**	**48**
4,6-Gal	μ, δ	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
4-Gal		3	0	0	0	0	0	1	0	0
2,4-Gal	ξ	2	0	0	0	0	0	0	0	0
Total 4-linked units		*43*	*47*	*51*	*46*	*48*	*45*	*50*	*48*	*51*
2,3,4-Gal		6	4	0	4	3	2	2	2	2
2,6-Gal		0	0	0	0	2	3	2	3	2
Terminal Xyl		1	0	0	0	0	0	0	0	0
Total ambiguous linkage units		*7*	*4*	*0*	*4*	*5*	*5*	*4*	*5*	*4*

[a] 2,4-Gal means a 2,4-disubstituted and/or linked galactopyranosyl unit, analysed as 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-galactitol etc.
[b] Enantiomeric partially methylated alditol acetates (2,3,6-Gal and 2,4,6-Gal) differentiated by deuterium labelling and determined by GC-MS analysis.
[c] Converted to κ-carrageenan during processing or glycosyl linkage analysis.
[d] Converted to i + carrageenan during processing or glycosyl linkage analysis.

fractions of *G. skottsbergii* but the process of calculation was not described. We propose here a set of decision rules, by which the proportions of various combinations of carrageenans under study here can be estimated, based on idealised carrageenan structures. While the derived conclusions suffer from certain systematic problems (as explained below), they can certainly be used for comparative purposes since they are calculated in a consistent manner. The following rules were applied to the data in Table 2:

1. the mole% lambda carrageenan was the mole% of 2,4,6-Gal (to the level of 2,3-Gal), plus an equal mole% of 2,3-Gal;
2. the mole% theta carrageenan was the mole% of 2,3-Gal remaining unassigned after application of Rule 1, up to the level of 2,4-AnGal, plus an equal mole% of 2,4-AnGal;
3. the mole% nu carrageenan was the mole% of 2,4,6-Gal remaining unassigned after application of Rule 1, up to the level of 3,4-Gal, plus an equal mole% of 3,4-Gal;
4. the mole% iota carrageenan was the mole% of 2,4-AnGal (remaining unassigned after application of Rule 2) up to the level of 3,4-Gal that remained unassigned after application of Rule 3, plus an equal mole% of 3,4-Gal;
5. the mole% mu carrageenan was twice the difference between idealised 3,6AG mole% (50%) and the actual 3,6AG mole% by constituent sugar analysis (if <48%, based on the value obtained for Sigma kappa carrageenan) minus the percentage of lambda and nu carrageenans already calculated from Rules (1) and (3) (as these do not contain 3,6AG either);
6. the total mole% of ambiguous/miscellaneous carrageenans was the mole% of 3-Gal + 3,4,6-Gal + 2,3,6-Gal × 2 (as this was always larger than the mole% of 4-Gal + 2,4-Gal), or the total ambiguous units × 2, whichever was the greater;
7. the mole% kappa carrageenan was 100% minus % lambda, % theta, % nu % iota, % misc. and % mu, if 3,4-Gal > 0.

This method tends to underestimate the % nu, due to alkali-modification during methylation and thus overestimate the % iota. If the % nu is underestimated, the % mu will be overestimated because of Rule 5. As an internal check, the mole% of 4-AnGal × 2, should equal the total % kappa + % mu (as mu is converted to kappa due to alkali-modification during methylation). In most samples this was the case.

3.3.3.2. Gametophyte extracts and gelling fractions. Using these rules, 100% of the units could be assigned in all the samples, Table 4. As expected, the whole gametophyte HMR extracts contained kappa and iota carrageenans. A small amount of theta carrageenan was also assigned to the whole gametophyte HMR extract of *S. crispata*. The gelling fractions of mixed life phase samples from HMR extracts were similar to the corresponding whole gametophyte HMR extracts, except that small amounts of

Table 4
Idealised carrageenan structures in gelling fractions and gametophyte extracts

Seaweed species	Seaweed life phase	Extraction process	Extractive fraction	lambda (mole%)	theta (mole%)	nu (mole%)	iota (mole%)	mu (mole%)	kappa (mole%)	misc. (mole%)	Total (mole%)
C. crispus	Mixed	HMR	Gelling	0	2	0	22	6	64	6	100
G. skottsbergii	Mixed	HMR	Gelling	2	2	0	32	0	56	8	100
G. skottsbergii	Gametophyte	HMR	Whole	0	0	0	32	0	60	8	100
S. crispata	Mixed	HMR	Gelling	2	2	0	32	0	62	2	100
S. crispata	Gametophyte	HMR	Whole	0	2	0	32	0	62	4	100
C. crispus	Mixed	HV	Gelling	2	0	2	18	22	50	6	100
G. skottsbergii	Mixed	HV	Gelling	2	0	10	20	14	48	6	100
G. skottsbergii	Gametophyte	HV	Whole	0	0	10	20	20	40	10	100
S. crispata	Mixed	HV	Gelling	0	0	10	22	22	38	8	100
S. crispata	Gametophyte	HV	Whole	0	0	10	22	24	38	6	100

theoretically non-gelling lambda/theta carrageenan were also present. Either this lambda/theta carrageenan exists in some kind of gelling molecule or some non-gelling material was retained in the gelling fractions. Similar observations have been made previously (Stortz & Cerezo, 1993). One consequence of setting defined rules is the creation of anomalies. Thus, because the observed mole% 3,6AG was slightly lower than expected, the data analysis indicates that the gelling fraction of the HMR extract of mixed life phase *C. crispus* contains 6% mu carrageenan. This, in fact, is unlikely, given the conditions used to extract the sample.

As expected, the whole gametophyte HV extracts contained kappa, iota, mu and nu carrageenans. Again, the gelling fractions of mixed life phase samples from HV extracts of the three species were similar to the corresponding whole gametophyte HV extracts, except that small amounts of theoretically non-gelling lambda carrageenan were sometimes present.

3.3.3.3. Tetrasporophyte extracts and non-gelling fractions. The results of the data analysis are shown in Table 5. For these samples, lambda was calculated as the mole% of 2,4,6-Gal plus an equal amount of (2,3-Gal + 2,3,6-Gal), if these were >0, in order to minimise the level of miscellaneous carrageenans. This is reasonable because lambda carrageenans from several species in the Gigartinaceae are known to contain 2,6-disulfated 3-linked units (Matulewicz et al., 1990; Stevenson & Furneaux, 1991; Falshaw & Furneaux, 1994). A proportion of the 3-Gal present should also be assigned to 'lambda' carrageenan since unsulfated 3-linked units are known to occur in the 'lambda' carrageenan from *C. crispus* (Dolan & Rees, 1965). However, without data on equivalent samples from gametophyte and tetrasporophyte extracts from *C. crispus*, it is impossible to calculate how much 3-Gal to assign to 'lambda' carrageenan. Therefore, it has all been considered as 'miscellaneous' even though this results in a large amount of miscellaneous units for the non-gelling fraction of the *C. crispus* HV extract. In some instances, the total calculated carrageenan, even including miscellaneous carrageenans, does not total 100%. This highlights another deficiency of applying rigid rules to such data.

As expected, the two whole tetrasporophyte HV extracts contained predominantly lambda carrageenan. Some of this lambda carrageenan was converted to theta carrageenan in the corresponding whole tetrasporophyte HMR extracts. The non-gelling fractions from the HMR extracts of mixed life phase *G. skottsbergii* and *S. crispata* were similar to the corresponding whole tetrasporophyte HMR extracts but contained small amounts of mu and/or kappa carrageenan. (There was insufficient non-gelling fraction from the HMR extract of mixed life phase *C. crispus* to be analysed.) Most notably, all the non-gelling fractions from the HV-processed mixed life phase seaweeds contained significant amounts of kappa, iota mu and nu carrageenans. The most plausible explanation for this behaviour is that the mu, nu, kappa, and iota hybrid polymers found in this fraction are less 'blocky', i.e. more randomly distributed and, thereby, more soluble in 2.5% KCl.

Whatever the explanation, it is clear that the extractive fractionation procedure used here does not produce a non-gelling fraction identical in composition to the carrageenan present in the sporophyte of the same weed. It does, however, yield a fraction whose structural analysis is of industrial significance and whose makeup is important in interpreting certain applications data (see following paper).

3.3.3.4. Total extracts. The data in Tables 1, 4 and 5 were used to estimate the carrageenan compositions of total extracts from mixed life phase seaweeds, Table 6. For the HMR extractions, the compositions of *G. skottsbergii* and *S. crispata* carrageenans are quite similar. On this basis, they would be expected to perform similarly in product applications. The HV-extracted *S. crispata* carrageenan contained much less kappa, and much more lambda than the equivalent HV-extracted *G. skottsbergii* carrageenan or the HMR-extracted *S. crispata*. This is most likely due to different proportions of gametophytic and tetrasporic seaweed present in the various raw materials used. The performance of these extracts would be expected to be different. The

Table 5
Idealised carrageenan structures in non-gelling fractions and tetrasporophyte extracts (nd = not determined)

Seaweed species	Seaweed life phase	Extraction process	Extractive fraction	lambda (mole%)	theta (mole%)	nu (mole%)	iota (mole%)	mu (mole%)	kappa (mole%)	misc. (mole%)	Total (mole%)
C. crispus	Mixed	HMR	Non-gelling	nd	nd	nd	nd	nd	nd	nd	nd
G. skottsbergii	Mixed	HMR	Non-gelling	56	28	0	0	6	2	8	100
G. skottsbergii	Sporophyte	HMR	Whole	58	30	0	0	0	0	8	96
S. crispata	Mixed	HMR	Non-gelling	48	36	0	0	0	6	10	100
S. crispata	Sporophyte	HMR	Whole	74	20	0	0	0	0	10	104
C. crispus	Mixed	HV	Non-gelling	42	0	4	12	7	13	22	100
G. skottsbergii	Mixed	HV	Non-gelling	20	0	22	10	2	40	6	100
G. skottsbergii	Sporophyte	HV	Whole	86	0	0	4	0	0	10	100
S. crispata	Mixed	HV	Non-gelling	56	0	2	8	6	18	10	100
S. crispata	Sporophyte	HV	Whole	88	0	0	0	0	0	8	96

Table 6
Estimated idealised carrageenan structures in whole seaweed extracts

Seaweed species	Seaweed life phase	Extraction process	Extractive fraction	lambda (mole%)	theta (mole%)	nu (mole%)	iota (mole%)	mu (mole%)	kappa (mole%)	misc. (mole%)	Total (mole%)
C. crispus[a]	Mixed	HMR	Whole	0	2	0	22	6	64	6	100
G. skottsbergii	Mixed	HMR	Whole	9	5	0	26	1	46	8	96
S. crispata	Mixed	HMR	Whole	10	8	0	25	0	50	3	96
C. crispus	Mixed	HV	Whole	5	0	2	17	20	46	7	98
G. skottsbergii	Mixed	HV	Whole	8	0	14	17	11	47	6	102
S. crispata	Mixed	HV	Whole	28	0	6	15	14	28	9	100

[a] Based on gelling fraction only.

consequences of the different compositions of these materials are discussed in the following paper.

3.4. ^{13}C NMR spectroscopy

Ciancia et al. (1993a) tentatively assigned minor ^{13}C NMR signals in carrageenan from gametophytic *G. skottsbergii* to a number of carrageenan structures (most without designated Greek letters), based on chemical shifts calculated from a model of idealised disaccharide units (Stortz & Cerezo, 1992). However, more recent, experimentally-derived results indicate deficiencies in this model (Falshaw & Furneaux, 1994; Stortz et al., 1994; Falshaw, Furneaux, Wong, Bacic, Liao, & Chandrkrachang, 1996). The limit of detection is now generally considered to be around 5%, so minor components are often undetected. Also, lambda-type carrageenans can be virtually invisible in mixtures with other types of polysaccharides, due to unfavourable spin relaxation properties of highly viscous samples. However, ^{13}C NMR spectroscopy provides the opportunity to independently check the major carrageenan compositions calculated above.

3.4.1. Gelling fractions and gametophyte extracts

The whole gametophyte extracts and gelling fractions of mixed life phase samples of the three species extracted using the HMR process produced ^{13}C NMR spectra showing signals corresponding to those expected for kappa and iota carrageenan (Usov & Shashkov, 1985). There was no evidence of mu/nu carrageenan in the spectrum of the HMR-extracted mixed life phase *C. crispus*, suggesting that the mu carrageenan in Table 6 is an anomaly resulting from application of the rules set out in Section 3.3.3.1. The equivalent HV-processed samples contained signals corresponding to those expected for kappa, iota and mu/nu carrageenans (Ciancia et al., 1993a). Mu and nu carrageenans are not resolved using this technique. The lambda and/or theta carrageenans present in some of these samples were at too low a level to be detected.

3.4.2. Non-gelling fractions and tetrasporophyte extracts

Spectra of the whole tetrasporophyte HV extracts of *G. skottsbergii* and *S. crispata* were both poorly resolved but showed signals characteristic of lambda carrageenan (Falshaw & Furneaux, 1994). Surprisingly, a signal at 72.0 ppm was observed in the spectrum of the whole tetrasporophyte HV extract of *G. skottsbergii*. This signal is characteristic of iota carrageenan (Usov & Shashkov, 1985) but would not be expected in a sample from only tetrasporic seaweed. However, the calculated carrageenan composition does include a small amount of iota carrageenan. The origin of this iota carrageenan is unclear but could have arisen from less than perfect separation of gametophytic and tetrasporic plants. Spectra of the whole tetrasporophyte HMR extracts of *G. skottsbergii* and *S. crispata* were also poorly resolved, but showed signals characteristic of lambda and theta carrageenan, as expected (Falshaw & Furneaux, 1994). The spectrum for the whole

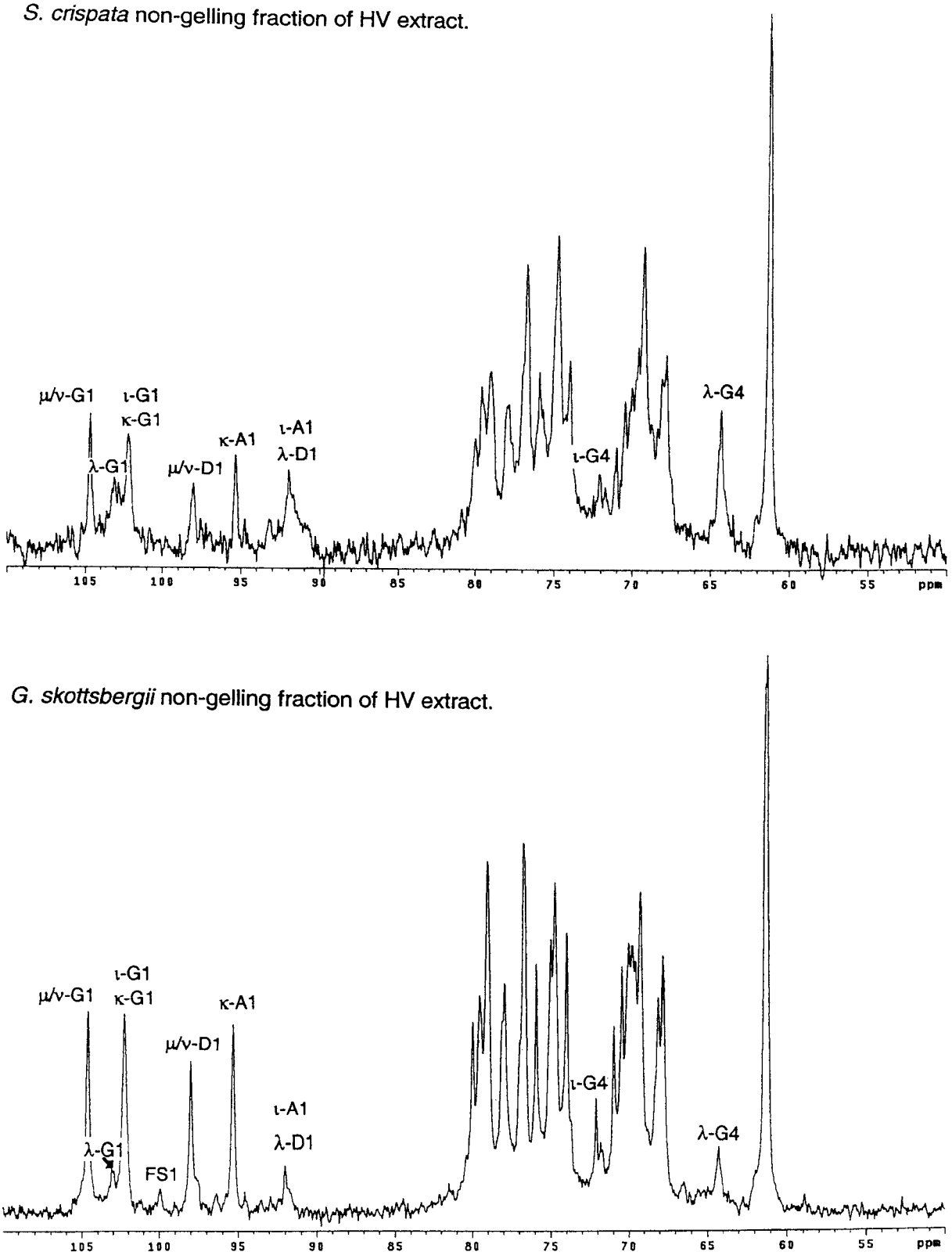

Fig. 2. ^{13}C NMR Spectra (50–110 ppm region) of *G. skottsbergii* and *S. crispata* non-gelling fractions of HV extracts. Key signals are labelled (G = 3-linked β-D-galactopyranosyl, D = 4-linked α-D-galactopyranosyl units, A = 3,6AG units and FS = floridean starch).

tetrasporophyte HMR extract of *G. skottsbergii* also contained signals characteristic of floridean starch (Falshaw, Furneaux, & Stevenson, 1998), as expected from constituent sugar analysis of this sample.

Spectra of the non-gelling fractions of HMR extracts from mixed life phase *G. skottsbergii* and *S. crispata* were reasonably well resolved and showed signals characteristic of lambda and theta carrageenan, as expected. However, the resolution was insufficient to conclusively assign signals to the C-1 of 4-linked units in kappa carrageenan (95.3 ppm) as opposed to theta carrageenan (95.6 ppm) or iota carrageenan (91.9 ppm) cf. lambda carrageenan (91.7 ppm). The spectrum of the non-gelling fraction of HMR extracted *G. skottsbergii* also contained signals characteristic of floridean starch, as expected from constituent sugar analysis. The spectrum of the non-gelling fraction of HMR extracted *S. crispata* contained an unassigned signal at 99.9 ppm. This was distinct from the C-1 of 3-linked units in theta carrageenan (100.3 ppm). A signal at 99.9 ppm is characteristic of the C-1 of 4-linked β-D-glucopyranose units in floridean starch but no glucose was observed by constituent sugar analysis of this sample.

Spectra of the non-gelling fractions of HV extracts from mixed life phase *G. skottsbergii* and *S. crispata* were reasonably well resolved, but complex (Fig. 2). Signals characteristic of lambda, kappa, iota, and mu/nu carrageenans were all observed but not those for theta carrageenan in both cases. This is, in fact, consistent with the calculated carrageenan compositions. A signal at 99.9 ppm characteristic of floridean starch was also observed in the spectrum of non-gelling HV extracted *G. skottsbergii*. Glucose (2 mole%) was observed by constituent sugar analysis of this sample. There was insufficient non-gelling HV *C. crispus* extract on which to record a ^{13}C NMR spectrum of this material.

4. Conclusions

A range of carrageenan samples has been prepared by extraction using typical commercial processes and subjected to structural analysis. It was shown that the extractive fractionation of mixed life phase samples using 2.5% KCl yields gelling carrageenans quite similar to those from a gametophyte extract of the same species. However, the non-gelling fractions are not the same as the corresponding sporophyte extracts. In general, calculated carrageenan compositions were consistent with those evident from ^{13}C NMR spectroscopic examination of the corresponding samples.

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