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# MOLECULAR WEIGHT DISTRIBUTION AND HYDROLYSIS BEHAVIOUR OF CARRAGEENANS

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## ABSTRACT

The molecular weight distribution of food-grade kappa- and iota-carrageenan was determined before and after limited hydrolysis. The hydrolysis parameters were selected to mimic gastric conditions, in order to estimate the degradation of ingested carrageenans. The molecular weight distribution of the samples was determined by molecular-sieving and light-scattering measurements.

## INTRODUCTION

The carrageenans constitute a group of galactans that can be extracted from some members of the class *Rhodophyceae* (red seaweed). The name "carrageenan" was originally used to designate extracts from *Chondrus* and *Gigartina* species, but galactans of similar structure, which have been found in related species such as *Furcellaria, Eucheuma, Hypnea, Iridea,* and *Polyides*, are also commercially named carrageenan. Common to the carrageenans is that the galactans contain alternately (1→3)-$\alpha$ and (1→4)-$\beta$ linkages and are often substituted with sulfate groups. Kappa- and iota-carrageenan have a double-helix structure depending upon an alternating sequence of D-galactosyl and 3,6-anhydro-D-galactosyl residues[1,2]. X-Ray studies have shown that the repeat distance in a complete turn of each single chain is 24.6 Å for kappa-carrageenan and 26.0 Å for iota-carrageenan[3].

The carrageenans are a selected group of food additives the safety of which is endlessly debated, despite a large number of recent toxicological investigations. From the standpoint of food safety, the E.E.C. directive[4] on emulsifiers, thickeners, and gelling agents and the Food Chemical Codex[5] have specified a minimum viscosity for carrageenans.

However, viscosity measurements do not give any accurate information about the molecular weight distribution of a polydisperse sample. Measurements of weight-average molecular weight are also insufficient for satisfactory characterisation of a sample. In both instances, the presence of a limited number of large molecules may obscure the fact that the sample is largely composed of material of low molecular weight.

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Therefore, we have used a method that permits a direct determination of the molecular weight distribution. Traditionally, a comparison of $\bar{M}_w$ and $\bar{M}_n$ is used to give information about molecular weight distribution[6]. The latter method is not very accurate and requires a reasonably well justified model for the distribution. The present method of choice is the combination of molecular-sieving chromatography and light scattering[7].

## EXPERIMENTAL

*General.* — The kappa- (batch 53 253-54) and iota-carrageenan (batch 32 367-69) used were gifts from Copenhagen Pectin Factory Ltd. All chemicals were of analytical grade. The molecular-sieving equipment comprised a Multiperpex 2115 peristaltic pump (LKB), UltroRac 7000 fraction collector (LKB), Uvicord III 2089 filter photometer (LKB), and an SR-25 water-jacketed, borosilicate glass, chromatographic column (Pharmacia) containing 465 mL of Sepharose CL-4B (Pharmacia).

*Light-scattering measurements.* — The measurements on kappa-carrageenan were carried out at 30°, in order to prevent the formation of double helixes, using a Chromatix KMX-6 laser light-scattering photometer equipped with a standard, aqueous-sample, cell accessory and a peristaltic pump. The light-scattering intensity was continuously recorded, in order to discriminate between true scattering and spurious signals due to dust particles and microbubbles. The samples were passed through a 1.2-$\mu$m Millipore filter and then through the cell at 0.1 mL/min. Light-scattering measurements on iota-carrageenan samples were not performed, because of thermostating problems at 60°. The refractive index increment for kappa-carrageenan in 0.2M LiCl was assumed to be 0.115 mL/g.

*Molecular-sieving chromatography.* — Sepharose CL-4B was adequate in both separation range and thermal stability. The strong polyelectrolyte nature of the carrageenans required an eluant of relatively high ionic-strength. However, high ionic-strength can cause salting out by increasing the tendency of the carrageenans to form double helixes. 0.2M Lithium chloride was used as a background electrolyte, since $Li^+$ has a low tendency to cause salting out.

Viscosity measurements, using a Cannon–Fenske capillary tube viscosimeter (k 0.003), showed that a minimum temperature of 60° was necessary in order to suppress helix formation of iota-carrageenan. Molecular-sieving experiments were carried out at 60°. The available equipment could not be used at higher temperatures. The sample reservoir and the inlet tubing were heated by an I.R.-lamp in order to avoid sample gelation during the application step.

The sample volume was 25 mL and the flow rate was 0.8 mL/min. Viscosity problems limited the concentrations of kappa- and iota-carrageenan to 0.20 and 0.30%, respectively, which gave viscosities approximately twice that of the eluant at 60°.

Fractions were assayed enzymically for D-galactose, because of the lack of a

detector for direct determination of carrageenan. Fractions were hydrolysed[8] with 0.36M sulfuric acid boiling under reflux for 6 h. Only traces of anhydrogalactose survived the hydrolysis. Each hydrolysate was neutralised with $BaCO_3$ and then centrifuged, and the supernatant solution was decanted and filtered through paper.

The D-galactose assay[9] was based on the oxidation of β-D-galactose to D-galactonic acid by $NAD^+$ in the presence of β-D-galactose dehydrogenase. The amount of NADH formed, which is proportional to the D-galactose content, was measured spectrophotometrically at 340 nm. A Boehringer Mannheim kit was used with a maximum sample volume of 2 mL. Interfering opalescence occurred with sample volumes >0.8 mL, depending on the LiCl content. The sample solutions could be desalted and concentrated in one step by using an immersible molecular-filtration probe (Millipore PTCG 001 K1), whereby molecules having a nominal molecular weight of $<10^4$ are removed. A sample containing galactose from 6 μg of carrageenan resulted in an absorbance of 0.007 unit.

Partial hydrolysis of both kappa- and iota-carrageenan was performed at 37° in a solution containing 0.1M LiCl and 0.1M HCl. The hydrolysis was interrupted by neutralisation with solid LiOH, which made the final solution 0.2M with respect to LiCl.

## RESULTS

*Determination of the helix–coil transition temperature.* — Fig. 1 shows the relative viscosity as a function of temperature for kappa- and iota-carrageenan in 0.2M LiCl. Kappa-carrageenan showed a sharp transition at 23° and iota-carrageenan at 57°. The choice of temperatures for molecular-sieving and light-scattering measurements was based upon the viscosity measurements.

*Determination of molecular weight distribution.* — The Sepharose CL-4B elution-profiles for undegraded kappa- and iota-carrageenan, respectively, are shown

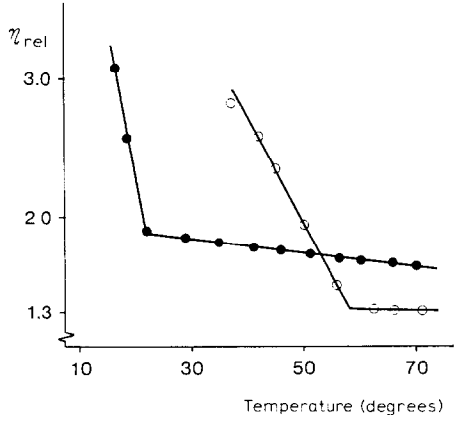


Fig 1. Relative viscosity as a function of temperature for kappa-(-●-) and iota-carrageenan (-○-).

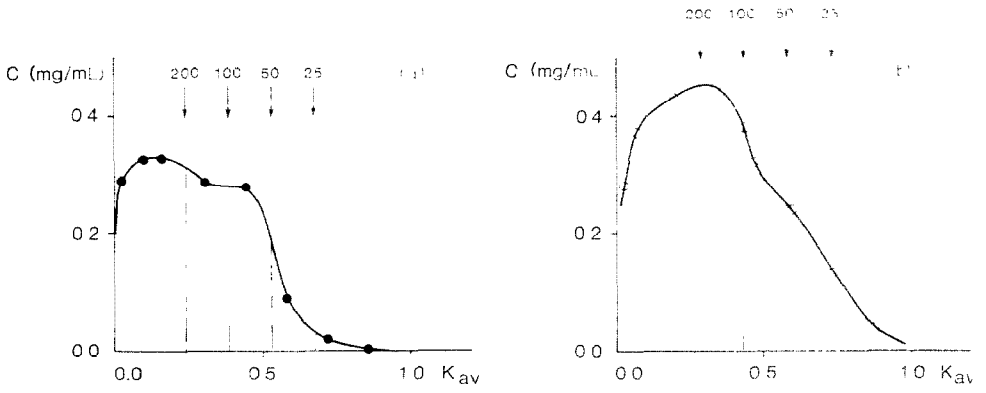


Fig. 2. Elution profiles for undegraded kappa-carrageenan (a) and undegraded iota-carrageenan (b). The arrows indicate elution positions corresponding to 25, 50, 100, and 200 kD.

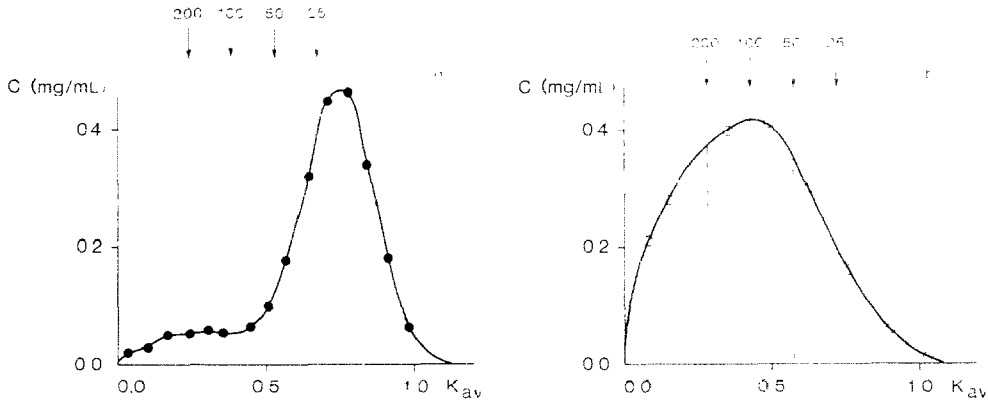


Fig. 3. Elution profiles for kappa-carrageenan (a) and iota-carrageenan (b) after hydrolysis for 6 h at 37° and pH 1. The arrows indicate elution positions corresponding to 25, 50, 100, and 200 kD.

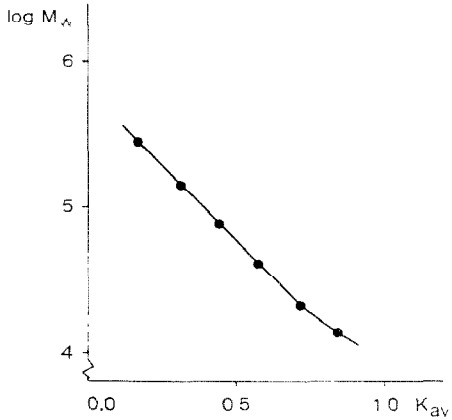


Fig. 4. Plot of log $M_w$ against $K_{av}$ for kappa-carrageenan.

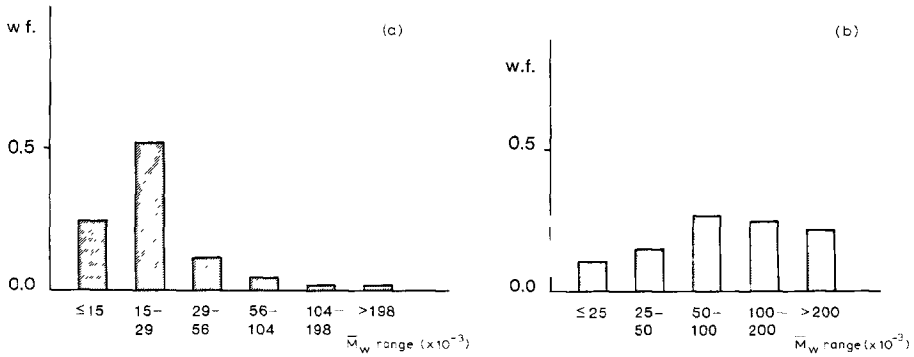


Fig. 5. Molecular weight distribution for kappa-carrageenan (a) and iota-carrageenan (b) after hydrolysis for 6 h at 37°and pH 1.

TABLE I

MOLECULAR WEIGHT DATA FOR KAPPA-CARRAGEENAN FRACTIONS

| Elution volume | 220 | 260 | 300 | 340 | 380 | 420 |
|---|---|---|---|---|---|---|
| $K_{av}$ | 0.16 | 0.30 | 0.44 | 0.58 | 0.72 | 0.86 |
| $\bar{M}_w$ (×10$^{-3}$) | 278 | 141 | 76 | 41 | 21 | 15 |

in Fig. 2. Elution positions corresponding to masses of 25, 50, 100, and 200 kD are indicated. Column calibration data for iota-carrageenan were calculated on the assumption that kappa- and iota-carrageenan chains having the same number of units have the same $K_{av}$ value. The molecular weight ratio between iota- and kappa-carrageenan chains having the same number of units was estimated to be 1.22. Each carrageenan had a broad distribution of molecular weight. Fig. 3 shows the corresponding chromatograms for materials after hydrolysis. The light-scattering experiments on which column calibration was based were performed on the fractions shown in Fig. 3a. Table I shows the values for the fractions analysed, and the plot of log $\bar{M}_w$ against $K_{av}$ (Fig. 4) is smooth and virtually linear. Fig. 5 shows the molecular weight distribution for hydrolysed kappa- and iota-carrageenan.

## DISCUSSION

The data presented herein show that commercial samples of carrageenan are highly heterogeneous with respect to molecular weight. Therefore, the parameters previously used for classification of carrageenans, namely. weight-average molecular weight and specific viscosity, are poorly suited. Carrageenans of low molecular weight are generally assumed to be more harmful when ingested than their counterparts of high molecular weight. Based on this assumption, international regulations which define the requirements for food-grade carrageenans in terms of the parameters mentioned above have been laid down.

The weight-average molecular weight cannot reveal, unambiguously, whether the sample is essentially free from components of low molecular weight. The presence of a small proportion of material of very high molecular weight will result in a weight-average molecular weight that is not truly representative of the sample. Thus, there is a need for a method which gives a more accurate indication of the molecular weight distribution.

The results in Fig. 5 show that kappa-carrageenan is more sensitive than iota-carrageenan to low pH at 37°, probably because the latter retains a double-helix structure, whereas the former is present as a fully unwound, flexible coil as indicated by the viscosity data in Fig. 1. The molecular weight distribution of iota-carrageenan was shifted considerably towards smaller molecules after treatment for 6 h at pH 1 and 37°. Digested carrageenans are probably fragmented after ingestion, to an extent which cannot be predicted. The pH in the gastric tract can vary considerably, as can the time of residence, depending upon the amount, type, and water content of the ingested food-stuffs. The formation of undesirable fragments of low molecular weight from well-defined material of high molecular weight may therefore vary, but is likely to occur during normal conditions. Shortly following the onset of non-specific acid hydrolysis, every sample will contain material covering the entire range of molecular weight from polymer to oligosaccharide, and it is not possible to define conditions which could guarantee that potentially hazardous fragments are not formed after ingestion of carrageenan-containing foods. A special-risk group in this situation must be those who have hyperchlorhydria.

It seems likely that other types of carrageenan will behave on hydrolysis in a manner similar to that of kappa- or iota-carrageenan. Those present as flexible chains under the conditions used here should behave as do kappa-carrageenans, whereas materials involving regular oligo- or multi-chain arrangements are likely to behave more like iota-carrageenans.

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