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Effect of acid hydrolysis on the molecular weight of kappa carrageenan by GPC-LS*

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Kappa carrageenan was subjected to acid-catalyzed hydrolysis under closely controlled pH and temperature conditions. The effect of acid hydrolysis was followed by measuring the change in molecular weight by gel permeation chromatography (GPC) coupled to a triple detector system consisting of a single (90°) angle laser light scattering (RALLS) detector, a viscometer and a refractometer. The changes in the weight average (M_w) molecular weight of kappa carrageenan can be described by a first-order random hydrolysis process involving selective attack at carrageenan glycosidic linkages. Kinetic parameters are also calculated and a general mathematical model is presented relating hydrolysis rate to pH and temperature. The model was used to compare carrageenan molecular weight change to published data on gel strength variation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Carrageenans are water soluble polysaccharides extracted from red seaweeds. These biopolymers consist of alternating 4-linked α-D-galactosyl- and 3-linked β-D-galactosyl residues. The commercial types include furcellaran, kappa, iota, and lambda carrageenans. Structurally, they differ from one another by the amount and location of ester sulfate, presence of pyruvate, or formation of a 3,6 anhydro derivative in the 4-linked sugar, which occurs either naturally or is formed chemically by alkali treatment of the native polysaccharide. These structural differences, along with variances in molecular weight, account for the gelling and other rheological properties that make carrageenans useful in food and other applications.

The functionality of carrageenans has traditionally been evaluated by Brookfield viscometers and by various gel testers (Marine Colloids, Stevens, etc.). These tests, although very useful, give only an indirect estimation of carrageenan molecular weight. A direct measurement of molecular weight will improve our understanding of the functionality of carrageenans. This will enable better process control in manufacturing and more effective product development.

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In this study, gelling carrageenans, kappa and iota, were subjected to acid hydrolysis under various pH and temperature conditions. The effect of acid hydrolysis of carrageenans was followed by measuring the change in molecular weight and intrinsic viscosity by gel permeation chromatography (GPC). The GPC unit was coupled to a triple detector system consisting of a single (90°) angle laser light scattering (RALLS) detector, a viscometer and a refractometer. In the present communication, we report the changes in the weight average (M_w) molecular weight of kappa carrageenan under closely controlled conditions of time, temperature and pH. A future report will deal with the results obtained with iota carrageenan.

MATERIALS AND METHODS

Chemicals

A sample of commercial kappa carrageenan (Gelcarin GP 911, Lot 861304) extracted from *Eucheuma cottonii* was obtained from FMC Corp. (Food Ingredients Division, Rockland, ME). Tri-potassium citrate monohydrate, citric acid monohydrate, potassium chloride, lithium nitrate, sodium azide and imidazole (all Purum grade) were obtained from Fluka Chemical Corp. Nitric acid (Reagent Grade) was obtained from VWR Scientific. All reagents were prepared in distilled (Barnstead) and polished water (Milli-Q System, Millipore Corp.)

and filtered through a 0.2 μm (Gelman VacuCap) bottle-top vacuum filter.

Hydrolysis reactor

Three (1 l) jacketed glass reactors were connected in series and temperature was regulated by a constant temperature circulator. The reactors were provided with high torque (Caframo) mechanical stirrers and condensers to prevent evaporation. Initial solution volume was 600 ml. At each sampling interval, 100 ml of solution were withdrawn.

pH was measured either on-line or at the time of sampling. In the first case, a DPAS (Ingold) electrode was mounted inside the reactor. In the latter case, a Ross Sure-Flow (Orion) electrode was used. In both cases, pH readings were corrected by an automatic temperature compensator probe attached to the pH meter (Orion 720A).

Hydrolysis procedure

The solution was formulated so it would be possible to study carrageenan hydrolysis without interference from carrageenan interaction with other components normally found in food systems (i.e. proteins). It was also required to quantitatively recover the carrageenans for further GPC analysis. These conditions restricted the formulation to a simple system, which consisted of 10 mM potassium citrate–citric acid buffer. Potassium ion concentration was maintained at a constant 30 mM by the addition of the necessary amount of potassium chloride. The potassium citrate level was that typically found in food dessert gels. Solutions were prepared at the required pH levels (room temp.) of 3, 4, 5, 6 and 7.

To minimize hydrolysis during sample dissolution (see Singh & Jacobsson, 1994), 0.3 g carrageenan was initially dispersed in 500 ml of potassium citrate solution and left to hydrate for 15 min. It was then rapidly dissolved by heating in a microwave oven with occasional stirring, and then added at temperature to the reactor containing the citric acid and potassium chloride solution. The contents were stirred for 1 min to stabilize temperature and the first aliquot was then retrieved. Subsequent samples were retrieved at the required times (see Table 1). Time, temperature and pH were recorded. The aliquot was split into two 50 ml sub samples (one retained, one processed) and quenched at −86°C (dry ice in acetone). All samples were stored at −20°C.

GPC–triple detector system

The GPC system consisted of the following components: A 20 l glass reservoir containing the eluent, a four-channel in-line degasser (Hewlet Packard 1050 Series), an HPLC quaternary pump (Dionex Model 4500), a high-pressure pulse dampener (Dionex part No. 43945), a column oven (Perkin–Elmer Model LC100), a six-port sample injector (Valco Model C6W) with a 100 μl sample loop (Valco), and a Viscotek triple detector system. The injector was triggered by a microelectronic valve actuator (Valco). The columns' bank consisted of a TSK PW_{XL} column guard and a set of TSK-Gel Columns (300 × 7.8 mm connected in sequence): G6000 + G4000 PW_{XL} (TosoHaas). The triple detector system consisted of a right angle laser light scattering (Viscotek RALLS Model 600) unit, a viscometer (Viscotek Model H502B) and a differential refractometer (Knauer Model 298). All components were maintained at 60°C. Data were acquired with an A/D Data Manager (Viscotek Model 4000). Raw signals were processed with TriSEC GPC Software (Viscotek, Version 2.25). Samples were dialyzed to minimize ionic aggregation prior to chromatographic analysis. Polarimetry controls were examined to determine the onset of thermal aggregation. In order to determine sample loss during a chromatography run, eluent fractions were collected and analyzed for total anionic hydrocolloid content following an established procedure (Soedjak, 1994). Typical recoveries of about 90% were found. The development of the sample preparation protocol and the operational conditions during GPC will be published elsewhere.

Table 1. Sampling schedule (the times under 200 min include the lag period between sampling and final temperature quenching to stop the hydrolysis reaction)

Target temp. (°C)	Sample time			
	T0 (min)	T1 (min)	T2 (min)	T3 (min)
55	5	300	600	1200
75	5	64	94	184
95	5	9	14	24

Dialysis and chromatography eluent

Buffered 0.2 M $LiNO_3$ dialysis solution and chromatography eluent were prepared by dissolving 275.8 g of $LiNO_3$, 13.62 g of imidazole, and 5.0 g of NaN_3 (as preservative) in 2 l of water and diluting to 20 l. pH was adjusted to 7.5 with nitric acid. Eluent was stored in containers having a 0.2 g venting filter (PolyVent, Whatman) to prevent particulate contamination.

Dialysis procedure

In preparation for dialysis, samples were thawed, the gel was broken and a 5 ml sample was diluted with 5 ml of GPC eluent and transferred into a dialysis bag (Spectra/Por® 3,500 MWCO, Spectrum). The bag was then placed in a capped bottle containing 500 ml of eluent and equilibrated under constant stirring for a total of 4 days, with daily solution change. After dialysis, samples

were removed from the dialysis bag and stored in small glass vials at 4°C.

Samples were dialyzed to convert carrageenan to the lithium form. This eliminates gelling ions that would interfere in the determination of molecular weight. The use of the appropriate membrane resulted in a fast ion exchange without sample loss (Knutsen *et al.*, 1993). Potassium and magnesium concentrations (data not shown) were reduced to background level after a single exchange. Due to its high affinity with carrageenan, calcium required three exchanges for reduction to background levels.

Polarimetry

Carrageenan conformational changes as a function of temperature were measured by following changes in optical rotation of the polysaccharide (Perkin–Elmer Polarimeter Model 241 MC). Hot solution was transferred into the polarimetry cell (100 mm path length) and left to equilibrate for 15 min between measurements. The sample was prepared following the same procedure as for the GPC samples.

It was determined (data not shown) that the onset of thermal aggregation of kappa carrageenan in the GPC eluent occurs near 30°C. The same type of experiments were also carried out with iota carrageenan, which indicated that the GPC experiments had to be carried out at 60°C. To standardize the experiments, all GPC runs were conducted at 60°C.

GPC sample preparation

Dialyzed samples were heated to 80°C over a 10 min period under constant stirring and then successively filtered through two syringe filters, 1 μm (glass fiber, Puradisc 25GD, Whatman) and 0.45 μm (polysulfone, Puradisc 25 AS, Whatman); filtered solutions were stored in glass vials held at 65° C until chromatographed.

Determination of molecular weight

The determination of molecular weight by light scattering requires an accurate determination of the refractive index of the solvent. It also requires the measurement of the specific refractive index increment (dn/dc) of the solvent–polymer combination. The refractive index value for the present solvent was found to be 1.334. The dn/dc value was obtained by reference to the Pullulan value of 0.147 ml/g (Viscotek Corp., per. comm.). The average value obtained for kappa and iota carrageenan was 0.110 ml/g. In the calculation of molecular weight, the contribution of the second virial coefficient was neglected. In addition, the axial dispersion correction is included as an integral component in calculations with the Viscotek TriSEC software.

The procedure for using viscometric measurements to correct the scattering data to zero angle with the TriSEC software relies on an iterative process of calculation. An initial estimate of molecular weight, M_{est}, is first obtained from the Rayleigh equation,

$$M_{est} = R(\theta)/Kc \tag{1}$$

where $R(\theta)$ is the light scattering constant for the right angle light scattering detector, c is the injected concentration of carrageenan, and

$$K = 2\pi^2 RI^2/\lambda^4 N_A, (dn/dc)^2 \tag{2}$$

where λ is wavelength, RI is refractive index of the $LiNO_3$ solvent, N_A is Avogadros Number, (dn/dc) is the differential refractive index increment for carrageenan in solution. This estimate of molecular weight is inserted, along with the measured intrinsic viscosity, η, into the Flory–Fox equation to obtain an estimate of the radius of gyration, Rg_{est},

$$Rg_{est} = [1/(6)^{1/2}](\eta M_{est}/f)^{1/3} \tag{3}$$

where f is the Flory Constant equal to 2.86×10^{23}. The estimated radius of gyration is inserted into the Debye equation to estimate the angular scattering probability function $P(\theta)$,

$$P(\theta) = (2/x^2)(e^{-x} + x - 1) \tag{4}$$

where x is $(8/3)\ \{(\pi RI)/\lambda)Rg_{est}\sin(\theta/2)\}^2$ and θ is 90°. The estimated $P(90)$ is used in equation (5) below to calculate an improved estimate of molecular weight M:

$$M = M_{est}/P(90) \tag{5}$$

The molecular weight calculation is iterated, beginning with equation (3), until the values of M, Rg, and $P(90)$ converge.

RESULTS

Proper determination of the molecular weight of carrageenans requires conditions that avoid aggregation. Aggregation will result in artificially high molecular weight values. Aggregation is caused by intermolecular interactions that accompany conformational changes in carrageenans. These changes depend on the total ion content and on the temperature of the system. The choice of hydrolysis buffer therefore cannot be a fortuitous one made at random (see Perrin *et al.*, 1974; Gueffroy, 1975). The buffer should be fully characterized regarding its pK values, buffer capacity, dilution effects, temperature dependence and complexing association constants with different counterions.

The buffer should be an inert component in the system and must not interact with the reactants. It has to have enough buffering capacity to hold pH constant during long periods of hydrolysis. When

working with systems at varied temperatures, the change in buffer pH as a function of the temperature (ΔpH/°C) must be known. Some buffers are known to undergo large shifts (Gueffroy, 1975) due to changes in individual ion dissociation constants. The addition of the sample (carrageenan is a polyelectrolyte) and its accompanying salts will also cause shifts in the pH of the system. All of these facts underscore the necessity of fully characterizing the buffer to be used in hydrolysis studies.

When operating at multiple temperatures and with viscous samples, careful techniques must be used during the determination of solution pH (see Bates, 1964: Galster, 1991). Temperature compensated pH values obtained under operational conditions are critical if these values are to be used in the determination of acid hydrolysis reaction constants.

The citrate buffer employed in the present study (Table 2a) had minimal pH shifts due to changes in temperature and the addition of carrageenan. The largest shifts observed were between −0.22 and +0.27 pH units (Table 2b). Moreover, after a rapid initial stabilization period, pH values remained constant for all runs, even after 20 h.

The weight average molecular weight (M_w) (see Tanford, 1961, for a discussion of the different molecular weight averages and distributions; Schröder *et al.*, 1989 for the various experimental methods) of the different sample aliquots was obtained by GPC-light scattering (LS). An independent determination was conducted to obtain M_w for carrageenan prior to hydrolysis. The value found was 523 000 Daltons (Da) (M_w), which compares favorably with previously reported values determined by GPC-LS (340–575, 353 and 690 kDa) for commercial kappa carrageenans (Lecacheux *et al.*, 1985; Slootmaekers *et al.*, 1991; Rochas *et al.*, 1989, respectively). A typical elugram is shown in Fig. 1. The elugram shows the reduction in the molecular weight of the sample due to acid hydrolysis as seen by a larger retention volume as the hydrolysis proceeds. The M_w values for the different runs are summarized in Table 3a. It is noteworthy to observe the very small loss of molecular weight at pH 6. The changes at pH 7 were even smaller. Therefore, values obtained at pH 6 and 7 were not used in the subsequent data analysis. Statistical and kinetic analyses were performed with the JMP® statistical software package from the SAS Institute. The triple detector system used in this study also gives an independent measure of intrinsic viscosity, η, used to calculate Rg_{est}, as previously discussed. Values of η corresponding to each determined value of M_w are listed in Table 3b.

A linear correlation was obtained between the reciprocal of the molecular weight and hydrolysis time ($1/M_w$ vs time). A typical result is presented in Fig. 2. This represents a first-order random hydrolysis process (Mark & Tobolsky, 1950). For such a reaction, the kinetics of hydrolysis can be described by equation (6) (Masson, 1955).

$$1/M_t - 1/M_o = kt/m \tag{6}$$

where M_t and M_o (kDa) are the molecular weights at time t and time zero, respectively, k (min^{-1}) is the first-order rate constant, t (min) is the reaction time, and m (kDa) is the molecular weight of the repeating

Table 2a. Buffer composition

pH	0.1 M Citric acid (ml)	2.0 M KCl (ml)	0.1 M Tripotassium citrate (ml)	Water (ml)
3	52.3	7.85	7.7	531.3
4	37.4	5.62	22.6	534.3
5	23.5	3.53	36.5	536.5
6	10.6	1.58	49.4	538.5
7	1.8	0.27	58.2	539.7

Table 2b. pH values for the 10 mM potassium citrate–citric acid buffer (constant potassium content). Buffer values recorded without carrageenan. System values recorded in the presence of carrageenan at operating hydrolysis temperature

Target pH	Buffer pH (25°C)	Buffer pH (75°C)	System pH at (temp. °C)	System pH at (temp. °C)	System pH at (temp. °C)
3.0	2.95	2.98	3.00 at 59	3.10 at 78	3.11 at 84
4.0	3.98	4.02	3.89 at 55	4.03 at 75	3.98 at 85
5.0	4.96	5.07	4.66 at 54	4.74 at 75	4.78 at 85
6.0	5.97	6.18		5.81 at 78	5.87 at 85
7.0	6.96	7.15		7.23 at 74	7.22 at 85

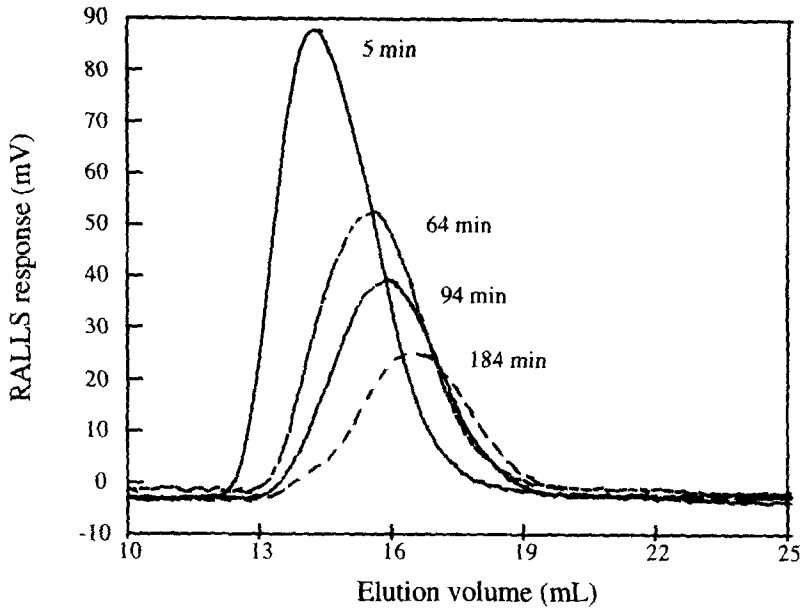

Fig. 1. GPC-RALLS elugram for the hydrolysis of kappa carrageenan at pH 4, 75°C. Sampling intervals at 5, 64, 94 and 184 min.

unit for kappa carrageenan (0.392 kDa). This equation holds very precisely for all but the very lowest degrees of polymerization (Mark & Tobolsky, 1950). The use of the dimer as the weight of the 'monomer' in the kinetic calculation is justified based on the very different response towards acid attack of the two bridging bonds in kappa carrageenan (see below).

Statistical data analysis indicated that pH and temperature were key independent variables affecting hydrolysis rate. The pH and temperature dependence of the first-order reaction rate constant can be expressed by equation (7):

$$\mathrm{Log}\,k(\mathrm{pH}, T) = \mathrm{Log}\,A' - B\,\mathrm{pH} - C/T \qquad (7)$$

which at constant pH can be reduced to equation (8):

$$\mathrm{Log}\,k(T) = \mathrm{Log}\,A - E/2.303RT \qquad (8)$$

which is the well-known Arrhenius equation, accounting for the effect of temperature on reaction rates. Figure 3 shows the good fit of the values from the present study to the Arrhenius relation. The lines having the same slope indicate that the activation energy, E, (30 kcal) is the same for the hydrolysis reaction at different pH values. The expected increase in hydrolysis rate with increase in temperature is clearly seen. The different intercepts on the Y axis correspond to the different values of the pre-exponential parameter, A, in

Table 3a. Weight average molecular weight (M_w) changes due to acid hydrolysis. Temperature and pH measured under operational conditions

Time (min)	Weight average molecular weight (kDa)				
			Temp (°C)/pH:		
	59/3.0	55/3.9	54/4.7		
5	471	462	469		
300	90	280	420		
600	54	208	393		
1200	35	167	346		
			Temp (°C)/pH:		
	78/3.1	75/4.0	75/4.7	78/5.8	75/7.0
5	362	458	580	515	487
64	50	222	462	473	504
94	34	175	386	458	443
184	23	117	290	442	474
			Temp (°C)/pH:		
	84/3.1	85/4.0	85/4.8	85/5.9	85/7.0
5	230	407	461	480	507
9	159	307	446	469	474
14	98	251	402	482	513
24	76	196	312	483	475

Table 3b. Weight average intrinsic viscosity (η) changes due to acid hydrolysis. Temperature and pH measured under operational conditions

Time (min)	Weight average intrinsic viscosity (dl/g)				
			Temp (°C)/pH:		
	59/3.0	55/3.9	54/4.7		
5	5.8	5.8	6.1		
300	1.4	3.9	5.5		
600	0.9	3.1	5.2		
1200	0.6	2.6	4.7		
			Temp (°C)/pH:		
	78/3.1	75/4.0	75/4.7	78/5.8	75/7.0
5	4.9	5.9	6.5	6.3	6.5
64	0.9	3.2	5.4	6.3	6.7
94	0.6	2.6	4.9	6.0	6.0
184	0.4	1.8	3.9	5.8	6.3
			Temp (°C)/pH:		
	84/3.1	85/4.0	85/4.8	85/5.9	85/7.0
5	2.9	4.7	5.5	5.6	5.8
9	1.8	3.8	5.3	5.6	5.5
14	1.2	3.1	4.8	5.6	5.6
24	0.9	2.5	4.4	5.6	5.5

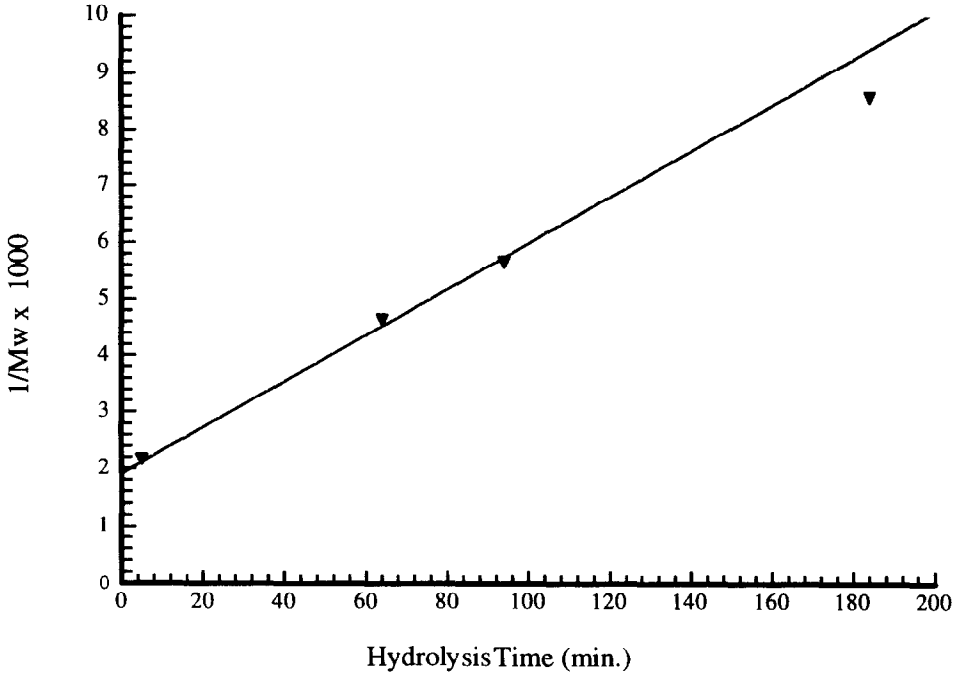

Fig. 2. Correlation between the reciprocal weight average molecular weight and time for the hydrolysis of kappa carrageenan at pH 4, 75°C. Points represent single GPC-LS runs.

the Arrhenius equation. From the statistical analysis it was determined that this parameter has a linear dependency on hydrolysis pH.

The acid hydrolysis of carbohydrates is carried out through attack of the glycosidic linkage by the hydronium ion. The rate of acid hydrolysis is thus proportional to the pH of the system. The pH of the system is a direct measure of the hydrogen ion activity (not its concentration). This underscores the necessity of measuring pH under actual operating conditions, thus avoiding error in the true activity of hydrogen ion taking part in the reaction.

DISCUSSION

Carrageenans are biosynthesized with a common backbone of alternating 4-linked α-D-galactosyl (D) and 3-linked β-D-galactosyl (G) residues (see Knutsen *et al.*, 1994 for notation). These units are present in a strictly

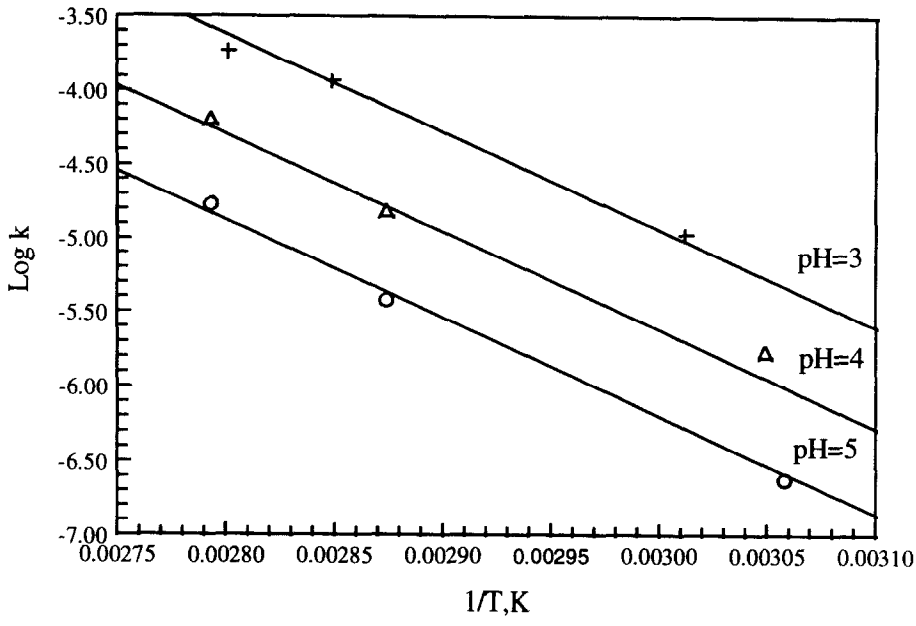

Fig. 3. Correlation between the first order rate of hydrolysis and absolute temperature for this study (see Tables 2 and 3 for individual data points).

alternating fashion, $[\text{-G-D-G-D-}]_n$. (Myslabodski, 1990). The commercial gelling carrageenans have most of their D units in the anhydro form (DA). In the case of kappa and iota, practically all the G units are sulfated at position 4 (G4S). Thus, we can represent the repeating dimer as DA-G4S for kappa and DA2S-G4S for iota, which has an extra sulfate at the 2 position. The glycosidic linkages present in carrageenan do not behave equally towards acid attack.

Early work (Haworth *et al.*, 1940; Rao & Smith, 1944) with model compounds showed that the introduction of the anhydro ring resulted in a strained pyranosyl structure, resulting in a less stable molecule toward acid attack. This causes the DA unit to hydrolyze at a rate closer to furanosyl than to pyranosyl sugars. The authors also found the beta anomer to be more stable than the alpha anomer, the one present in carrageenans.

Turvey (1961) studied the acid hydrolysis of a closely related compound, methylated agar LA-G-LA-G6M (the 4-linked unit belongs to the L-sugar series). Under conditions that hydrolyse all LA-G bonds, only 4% of the G-LA bonds were cleaved.

Lawson and Rees (1968) used acid hydrolysis to produce carrageenan oligomers for structural analysis. They determined DA-G as the bond that is hydrolyzed. They could not detect hydrolysis products from the G-DA bonds. More recently, Stanley (1970) found that the rate of acid hydrolysis of carrageenan has a linear relation to the DA content.

The difference in the hydrolysis rates for the two bonds (DA-G and G-DA) is so great that the rate of hydrolysis of the DA-G bonds determines the overall rate of acid hydrolysis of carrageenans.

Of the commercial gelling carrageenans, furcellaran (DA-G-DA-G4S), kappa (DA-G4S) and iota (DA2S-G4S), all contain DA units. Lambda carrageenan (D2,6S-G2S-D2S), being devoid of DA units, hydrolyses at much lower rates. It is known that iota is more stable than kappa carrageenan towards acid hydrolysis. Its hydrolysis rate is about half that of kappa. This stability is attributed to the presence of the extra sulfate (DA2S) (Guiseley *et al.*, 1980). Therefore, kappa is the carrageenan displaying the highest reaction rates for acid hydrolysis among the commercial carrageenans.

Comparing the present work to previous acid hydrolysis studies (see Table 4), it became apparent that the comparison has to consider the different materials and conditions employed during the various experiments. Data found in the literature are summarized in Table 5. None of the authors indicated the temperature at which the pH determination was made. Some studies were carried out at 130°C. This will not affect the determination of the activation energy (E), but certainly could affect the determination of the hydrolysis rate, if there was a large difference between the measured and the actual pH value.

Previous studies, with the exception of Singh and Jacobsson (1994), used a natural blend of two different carrageenans, the major component being kappa (DA-G4S), along with some lambda (D2,6S-G2S-D2S). The presence of lambda carrageenan would result in slower hydrolysis rates due to a lower overall content of DA units.

Masson *et al.* (1955) observed a slower reaction when oxygen was excluded from the system. The presence of oxygen would increase the overall hydrolysis rate by causing concurrent oxidative–reductive de-polymeriza-

Table 4. Comparison of Arrhenius coefficients for the first-order rate of hydrolysis of kappa carrageenan

pH	A (1/min)	E k cal	k_{35} (1/min)	k_{122} (1/min)	Author
7	4.6×10^{11}	29.2	5.5×10^{-10}	2.0×10^{-5}	Masson (1955)
7	7.3×10^{15}	38.5	2.2×10^{-12}	2.3×10^{-6}	Desai and Hansen (1986)
7	1.1×10^{9}	25.0	2.0×10^{-9}	4.1×10^{-5}	Bradley and Mitchell (1988)
7	7.9×10^{11}	30.2	3.1×10^{-10}	1.6×10^{-5}	This work
2	2.5×10^{13}	27.0	7.8×10^{-7}	1.3×10^{-2}	Singh and Jacobsson (1994)
2	4.6×10^{15}	30.2	1.8×10^{-6}	9.2×10^{-2}	This work

Table 5. Synopsis of the materials and conditions employed in previous studies of carrageenan hydrolysis

Authors	Masson, (1955).
Material	Low molecular weight extract (*Chondrus crispus*), kappa and lambda carrageenan.
Make-up	1% carrageenan converted to the sodium salt.
Mol. weight	75 000 Da (M_n) by osmometry.
Methods	Changes measured by viscosity and reducing-end titration
System	Phosphate buffer (M/30) at pH 7. Open system heated to 60–101°C, 82–478 h.
Findings	Loss in viscosity due to drop in pH in non-buffered systems. Air accelerates reaction
Authors	Masson *et al.* (1955).
Material	Fractionated extract (*Chondrus crispus*), enriched in kappa carrageenan.
Make-up	Carrageenan converted to the sodium salt, at 0.13%.
Mol. weight	2 500 000 Da (M_n) by osmometry.
Methods	Changes measured by viscosity.
System	Phosphate buffer (M/30) at pH 7. Oxygen-free system heated to 90°C up to 300 h.
Findings	Oxygen accelerates reaction over 60°C. Carrageenan stable at pH 9 with salts.
Authors	Badui *et al.* (1978).
Material	Commercial carrageenan, Sea-kem-2 (natural blend of kappa and lambda).
Make-up	Carrageenan without further treatment at 0.5%. Mainly calcium salt.
Mol. weight	230 000 Da (M_w) by ultracentrifuge.
Methods	Changes measured by ultracentrifuge, viscosity and reducing-end titration.
System	Milk-salt solution (J and K) at pH 6.7. Closed system heated to 122°C for 20 min.
Findings	Buffer not stable. pH drops to 5.5 accelerating the reaction.
Authors	Desai and Hansen (1986).
Material	Commercial carrageenan, Sea-kem-2 (natural blend of kappa and lambda).
Make-up	Carrageenan without further treatment at 0.4%. Mainly calcium salt.
Mol. weight	215 000 Da (M_w) by ultracentrifuge as apparent molecular weight.
Methods	Changes measured by ultracentrifuge.
System	Na-cacodylate, 0.05 M, pH 7, +0.2 M NaCl. Sealed, 100–130°C, up to 50 h.
Findings	High ionic strength stabilizes the carrageenan.
Authors	Bradley and Mitchell (1988).
Material	Commercial carrageenan (natural blend of kappa and lambda).
Make-up	Carrageenan without further treatment at 1.5–2.5%. Mainly calcium salt.
Mol. weight	Not given
Methods	Changes measured directly in a high-temperature slit viscometer.
System	NaCl (0.2 M) + EDTA (0.005 M), pH 7. Open system heated to 80–100°C.
Authors	Singh and Jacobsson (1994).
Material	Commercial kappa carrageenan from *Eucheuma cottonii*.
Make-up	Carrageenan used without further treatment.
Mol. weight	504 000 Da (M_w) by GPC-LALLS.
Methods	Changes measured by GPC-LALLS, gel strength and low-shear viscosity.
System	LiCl-HCl buffer (0.09–0.01 M), pH 2. Sealed and heated at 35, 45 and 55°C for 5 h.

tion (ORD), or by partially oxidizing the carrageenan, thus facilitating the acid hydrolysis process. Studies 3, 4, 5 and 6 (Table 5) were conducted with sealed systems in which the oxygen initially present would cause an increase in apparent hydrolysis rate. Once oxygen was exhausted, the reaction would continue as in an oxygen-free system.

Carrageenan hydrolysis can, therefore, be considered to occur through more than one depolymerization process. In open systems, with constant air supply, the ORD process could become the main depolymerization route.

Reported activation energies and depolymerization rate constants are similar to those found in the present study, with the exception of study four. That study reported an activation energy of 38.5 kcal and a reaction

rate approximately one order of magnitude slower than that found in the other studies. The authors attributed the improved stability to changes in carrageenan conformation in a solution containing high levels of inorganic salts and to the absence of oxygen. The presence of salts will screen the charges present in the carrageenan molecule (ionized sulfate groups), enabling the molecule to become more compact and therefore increasing its stability. This effect is not salt-specific. Even non-gelling cations like sodium will cause the carrageenan to coil.

Another explanation for the increased stability found in study 4 could lie in the composition of the carrageenan used in that study. The material used contained a considerable portion of lambda fraction, which is much more stable towards acid hydrolysis.

Since carrageenam is generally used for its gelling properties, it is of utmost importance, for economic reasons, to retain maximum gel strength in a given end-use application. As a guideline to food processors, FMC Corporation has published a table [based on specific viscosity measurements (FMC, 1981)] showing maximum processing times under a given set of pH and temperature conditions that should result in no more than a 25% estimated loss in gel strength of kappa carrageenan. With this control in gel strength loss, no significant reduction in carrageenan functionality can be expected. Tables 6a and b compare processing times shown in this previous work with processing times calculated from Equation (2) for lowering the weight-average molecular weight of kappa carrageenan by 25%. Lowering pH by one unit causes a 10-fold reduction in the time to reduce gel strength, while the time for reduction in molecular weight is only reduced by a factor of 6. With respect to temperature, an increase of 10°C causes an approximate 3-fold decrease in time for gel strength loss. The effect of temperature on molecular weight loss follows a gradient function. The higher the temperature, the lower the relative reduction in time for molecular weight loss.

The information presented in Tables 6a and b emphasize the importance of proper control of pH, temperature, and time in food processing to avoid loss of carrageenan functional properties.

CONCLUSIONS

A model system has been developed for determining the rate of hydrolysis of kappa carrageenan under varying conditions of time, temperature and pH. The model system shows that at lower pHs (< 4), where hydrolysis is much faster, there is good agreement between the time to reduce gel strength and molecular weight. Thus, under commercial formulation conditions, which are designed to maintain the gel strength of kappa carrageenan, there should be little loss (probably less than 25%) in molecular weight. The challenge exists in relating this observation to industrial situations where the systems are not fully characterizable, or where, as in most food systems, the carrageenan cannot be quantitatively recovered to be analyzed by GPC-LS.

Work continues in obtaining relationships between carrageenan molecular weight obtained by GPC-LS (a time consuming and difficult technique) and rheological parameters such as gel strength and viscosity, as recommended by Singh and Jacobsson (1994). The ideal is to find a reliable way to link results from basic science (e.g. M_w) to simple in-process monitoring tools available to both carrageenan manufacturers and end-use industries. This accomplishment would allow in-process optimization of carrageenan properties to provide the specific functionality required in a given end-use application.

The determination of true hydrolysis rates of carrageenan requires a full description of the system environment. Although solutions of gelling carrageenans hydrolyze faster than do other polysaccharides in the presence of acid, it is possible to mitigate molecular weight loss by proper control of processing conditions, with primary emphasis on time and temperature.

Table 6a. Time required for 25% reduction in carrageenan molecular weight

Temperature (°C)	pH 6	pH 5	pH 4	pH 3
50	12 days	2 days	8 h	1.4 h
70	16 h	3 h	30 min	5 min
90	1.4 h	15 min	3 min	28 s

Table 6b. Time required for 25% reduction in carrageenan gel strength[a]

Temperature (°C)	pH 6	pH 5	pH 4	pH 3
50	60 days	6 days	15 h	1.5 h
70	6 days	15 h	1.5 h	10 min
90	15 h	1.5 h	10 min	60 s

[a]Gel strengths were calculated based on a previously established correlation between Brookfield viscosity measurement and gel strength. A 50% reduction in viscosity yields an approximate 25% reduction in gel strength.

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