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Determination of the low molecular weight fraction of food-grade carrageenans

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Abstract

Recently there has been some debate regarding the presence and associated health risk of low molecular weight carrageenan in foodstuffs. Unfortunately measurement of the low molecular weight tail (LMT) of food-grade carrageenans (defined here as the carrageenan having relative molecular mass (Mr) below 50,000) is not trivial, largely due to its low abundance. So far methods employing light scattering have been unsuccessful in producing reproducible results, probably due to the poor detector response at low masses. In this work a method based on high performance size exclusion chromatography coupled to a refractive index detector (HPSEC-RI) has been used for the measurement of the LMT in food-grade carrageenan ingredients and in a carrageenan-containing finished product (a jelly). Over the course of half a year, 19 measurements were made on a reference carrageenan; the results demonstrated that the method had excellent reproducibility. Applied to a number of different carrageenan ingredients, it was found that, in general, the LMT represents less than 8% of the total carrageenan in ingredients, and under the correct conditions increases little during food processing. The data also indicated that pH appears to be a critical factor during food processing and pH levels below 4.0 should be avoided.
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Keywords: HPSEC; Poligeenan; Low molecular weight carrageenan; κ-Carrageenan; ι-Carrageenan; Food

1. Introduction

Red seaweeds have a long history (several centuries) of food use in the Far East and Europe. Today, they are an important source of carrageenans for the food and other industries. Carrageenans are polysaccharides composed of galactose and 3,6-anhydrogalactose, linked by alternating α1,3 and β1,4 glycosidic linkages. The monosaccharide residues may variously be sulphated, and differences in degree of sulphation and occurrence of the 3,6-anhydrogalactose residues give rise to the various forms of carrageenans. Of these forms, λ, κ and ι are probably the most important industrially. The carrageenans are exploited for their gelling, thickening and stabilising properties in a wide range of food products, from ice cream to pet food. They may be used individually, combined with other carrageenans or combined with other polysaccharides, proteins and salts to achieve a finished product with the desired physical properties [1,2].

The publication of a review article by Tobacman [3] in 2001 initiated a debate on the potential risks associated with the consumption of carrageenans in the diet [3–10]. In fact the debate revolves around degraded carrageenan (or poligeenan, as it has been named) which has a weight average molecular weight (Mw) of 10,000–30,000 [3,9,11] and has been shown to cause ulcerative colitis at high doses in experimental animals [12]. One camp argues that the presence of carrageenan in food may lead to health problems due to the presence of poligeenan [3]. The other camp argues that the amount of poligeenan in food-grade carrageenan and the consumption levels of carrageenan in the diet are such as to present no risk to human health [9]. In 2003 the European Scientific Committee on Food published the results of their review on this debate [13]. They proposed that "if feasible a limit of not >5% below 50 kDa should be introduced into the specification to ensure that the presence of any degraded carrageenan is kept to a minimum".

To monitor (and keep to a minimum) the amount of low molecular weight carrageenan in ingredients a method is needed

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for the analysis. A number of methods for the determination of carrageenan molecular weight and molecular weight distribution have been described [14–22]. To determine a distribution a method of separating the polysaccharides by molecular weight is required. This is most frequently performed by size exclusion chromatography (SEC) [14–21], but other techniques such as field flow fractionation (FFF) have also been applied [22]. Molecular weight can be determined by use of suitable detectors such as light scattering at low angle (LALS), right angle (RALS) or multiple angle (MALS) [15,16,18–20] and/or viscometry [21], or by calibration of the separation system with well-characterised standards [11,14]. Sedimentation analysis, osmometry and even gel breaking strength have also been applied to determine average molecular weights [15,23].

To respond to the needs of the industry the World Association of Seaweed Processors (Marinalg) initiated a study to find a suitable method for the screening of the low molecular weight tail (LMT) of carrageenan products [24]. In this study a number of labs were employed to measure the LMT content (defined as the % carrageenan with Mr below 50,000) of a number of carrageenan ingredients. Each lab employed its own method, in many cases the analysis was performed by high performance size exclusion chromatography (HPSEC) coupled to light scattering detectors, but some other techniques were also applied (e.g. using inductively coupled plasma as a detector [11]). The results from the different labs varied considerably, possibly due to the different methodologies employed, and certainly (as reported) the difficulty in making accurate light scattering measurements at low molecular mass and low concentration. In general the methodologies also required some quite specialised equipment and operators. The conclusion of the trial was that, at the time, a suitable validated method for determining the LMT did not exist.

In the work reported here it has been attempted to use a method based on HPSEC with refractive index (RI) detection for measuring the LMT and Mw of carrageenan and in addition to investigate the effect of processing conditions on the Mw and LMT in a finished food product (a jelly).

2. Materials and methods

All chemicals were from Merck or Sigma and of analytical grade or better except where indicated. κ-Carrageenan standards were originally obtained from Sanofi Bio-Industries, Carentan, France.

2.1. Production of low Mw carrageenan standard

κ-Carrageenan with Mw of 69,000 (Sanofi Bio-Industries, France) was dissolved in lithium nitrate solution (0.1 M) to a concentration of 10 mg/ml. Potassium citrate (50 mM, pH 3.0) was added to the solution until the final pH reached 3.1 then the solution was heated at 80 °C for 1 h. After hydrolysis the sample was neutralised with sodium hydroxide and dialysed (MWCO 3500) against deionised water for 28 h. The solution was then freeze dried and the dried powder stored at 4 °C until required. The Mw and number average molecular weight (Mn) were measured using HPSEC–MALS–RI and found to be 18,200 and 9700, respectively. After storage for some time it was noticed that black spots were appearing in the material. This was discovered to be due to autohydrolysis of the sample. A solution of the carrageenan was found to have a pH of 2.3. To prevent further hydrolysis the complete sample was re-dissolved and neutralised with sodium hydroxide to pH 7.6, filtered (to remove the black spots) and precipitated by adding four volumes of 2-propanol. The precipitate was washed with a solution of 2-propanol/water (4:1, v/v), dried in an oven at 40 °C then milled to give a powder. The Mw and Mn were once again measured and found to be 10,700 and 6700, respectively. The material was found to be stable and further hydrolysis was not observed.

2.2. Preparation of samples for analysis (ingredients and standards)

The carrageenan sample (50–300 mg) was dispersed in lithium nitrate solution (0.1 M, 70 ml) and left stirring for 20 min at 23 °C. The solution was then heated to 70 °C in a water bath for 30 min with constant stirring to achieve complete dissolution. Thereafter the sample was cooled to room temperature, transferred to a 100 ml volumetric flask and made up to the mark with lithium nitrate solution (0.1 M). For molecular weight measurements using MALS, the amount of carrageenan used was increased as the molecular mass decreased to compensate for the loss in sensitivity of the MALS detector at lower molecular weights.

2.3. Immobilisation of amyloglucosidase (AMG) on Eupergit C

AMG is used to remove glucose syrup from jelly samples. To prevent the enzyme from interfering with the analysis it is necessary to immobilise it on a solid support. To do so a solution of amyloglucosidase (*Aspergillus Niger*, E.C.3.2.1.3, Roche Diagnostics, Rotkreuz, Switzerland) was prepared in deionised water (1700 U/ml[1]). 3.0 g of Eupergit C (Fluka, Buchs SG, Switzerland) was weighed in to a 100 ml centrifuge tube. Phosphate buffer (1.0 M, pH 7.5, 17.46 ml) and the AMG solution (1700 U/ml, 540 μl) were added. The tube was sealed and left for 16 h at 23 °C with occasional mild agitation (not using a magnetic stirring bar, since this will damage the Eupergit beads). Warm (50 °C) sodium acetate solution (0.2 M, pH 6.0, 60 ml) was added and the tube shaken vigorously for 30 s. After centrifugation (720 × *g*, 20 °C, 2 min) the supernatant was removed using a Pasteur pipette and the beads were washed a further nine times with the warm sodium acetate solution. On the final washing stage the suspension was not placed in the centrifuge, but on a porous glass filter (porosity 2) and the liquid was allowed to pass through the filter under gravity. Thereafter the material was washed several times with demineralised water (total volume 600 ml) always on the porous glass filter under gravity. After

[1] One unit of the initial Roche enzyme was defined as the amount of enzyme that will release 1 μmol of glucose from glycogen in 1 min at 25 °C, pH 4.5.

all the water had passed through a vacuum was applied (using a water jet vacuum pump) for 70 s. Thereafter the wet beads could be accurately weighed without any residual water running out (note: 3 g of dry Eupergit C gave rise to approximately 10 g of wet beads). The activity of the immobilised enzyme was measured using starch as a substrate. One unit of immobilised enzyme was defined as the quantity of immobilised enzyme that would liberate 1 μmol of glucose in 1 min at 23 °C, pH 6.0 (typically the activity was in the range of 6–10 U/g).

2.4. Preparation of samples for analysis (jellies)

A sample amount containing approximately 50 mg of carrageenan was weighed in to a beaker. Warm (70 °C) sodium acetate solution (0.2 M, pH 6.0, 70 ml) was added and the sample stirred without further heating using a magnetic stirrer until complete dissolution (typically approximately 30 min). The sample was cooled to room temperature and quantitatively transferred to a 100 ml volumetric flask and made up to the mark with the same solution. An aliquot (4 ml) of the sample solution was transferred to a screw cap tube and 2 g of the immobilised enzyme (i.e. 12–20 U) were added. The sample was incubated at 37 °C for 45 min under constant rotary mixing (approximately 25 turns/min). After cooling the sample was centrifuged (720 × *g*, 20 °C, 20 min) and 2 ml of the supernatant was transferred to another screw cap tube. Four volumes of 2-propanol were added and mixed well, the sample was left for 2 min to allow the carrageenans to precipitate completely then centrifuged (2800 × *g*, 20 °C, 20 min). The supernatant was removed carefully using a Pasteur pipette, and the residue washed twice with 2-propanol/water (4:1, v/v). The residue was completely dried on a vacuum centrifuge (30 °C, 30 min). The dried residue was dissolved in the HPSEC mobile phase (lithium nitrate, 0.1 M, 2.0 ml) by stirring at room temperature for 30 min and transferred to a vial suitable for the HPLC autosampler.

2.5. Analysis by HPSEC-RI

HPSEC–RI was performed using a Waters Alliance 2695 separations system (Waters S.A., Montreux-Chailly, Switzerland) equipped with a Shodex RI-101 RI detector (Showa Denko Europe GmbH, Munich, Germany) operating at 40 °C. Separation was achieved on a series of columns composed of a TSKgel G6000PWxl (13 μm, 7.8 mm × 300 mm) and a G4000PWxl (10 μm, 7.8 mm × 300 mm) preceded by a PWxl guard column (6.0 mm × 40 mm) (Tosoh Bioscience, Stuttgart, Germany). The columns were all held at 60 °C, injection volume was 100 μl. Samples were eluted with a solution of lithium nitrate (0.1 M) flowing at 0.6 ml/min. Instrument control, data acquisition and analysis were made using Waters Empower software including the GPC add-on (Waters S.A., Montreux-Chailly, Switzerland).

2.6. Analysis by HPSEC-MALS-RI

HPSEC–MALS–RI was performed using a Waters 600E HPLC system controlled by Waters Empower Software (Waters S.A., Montreux-Chailly, Switzerland), attached were a Shodex RI-101 RI detector (Showa Denko Europe GmbH, Munich, Germany) and a Wyatt Dawn EOS MALS detector (Wyatt Technology Europe GmbH, Dernbach, Germany) or using a Viscotek GPCmax VE2001 HPLC system (Viscotek Europe, Irigny, France) attached to a Wyatt Optilab DSP RI detector and a Wyatt Dawn DSP MALS detector (Wyatt Technology Europe GmbH, Dernbach, Germany). Separation was achieved on a series of columns composed of a TSKgel G6000PWxl (13 μm, 7.8 mm × 300 mm) and a G4000PWxl (10 μm, 7.8 mm × 300 mm) preceded by a PWxl guard column (6.0 mm × 40 mm) (Tosoh Bioscience, Stuttgart, Germany). The columns were all held at 60 °C, injection volume was 200 μl. Samples were eluted with a solution of lithium nitrate (0.1 M) flowing at 0.6 ml/min. MALS control, data acquisition and analysis were made with Wyatt Astra software (Wyatt Technology Europe GmbH, Dernbach, Germany). Literature [18–21,23,25] was consulted to obtain appropriate dn/dc and A_2 values. A range of values can be obtained depending on which article is consulted. However, in general the values of Lecacheux et al. [20] seem to be generally accepted, thus we used the values dn/dc = 0.115 ml/g and $A_2 = 2.62 \times 10^{-3}$ mol ml/g^2.

2.7. Data analysis

Mw and Mn values were calculated by the Wyatt Astra software using data from HPSEC-MALS-RI or by the Waters GPC add-on to Empower using data from HPSEC-RI. The LMT (defined as the % carrageenan having Mr below 50,000) was calculated by the Waters GPC add-on to Empower. All samples were analysed in duplicate and average values are reported herein. The standard deviation of the duplicates was calculated, divided by the average value and multiplied by 100 to give the percent coefficient of variation (%CV) as an indicator of repeatability.

3. Discussion

3.1. Choice of analytical approach

Carrageenans are gelling polysaccharides and quickly form viscous solutions or gels even at fairly low concentrations. In our experience this means working with typical food-grade carrageenans at concentrations of not much more than 1 or 2 mg/ml if problems with high viscosity are to be avoided during analysis. The measurement of LMT using light scattering techniques is challenging due to the problem of having to work at low concentration combined with the poor instrument sensitivity at low molecular mass. It was attempted to overcome this problem by isolating and pre-concentrating the LMT by fractional precipitation (using 2-propanol). Unfortunately despite trying many different precipitation conditions the LMT could not be quantitatively isolated from the rest of the carrageenan. Another approach to separate the LMT from the rest of the carrageenan was attempted by Titoria et al. [26]. In their research they attempted to separate differently sized carrageenans using ultrafiltration membranes. Although quite successful with partially hydrolysed carrageenans, when applied to native samples

Table 1
Molecular weight data for standards, as determined by HPSEC–MALS–RI

Carrageenan standard	Mw[a]	%CV[a]	Mn[a]	%CV[a]
1	10,700	4.6	6,700	9.6
2	54,200	1.6	32,300	2.4
3	68,300	0.2	42,400	1.2
4	151,500	1.4	89,300	1.5
5	301,000	0.5	184,000	1.9
6	514,000	0.8	197,000	2.9

[a] Mw and Mn are average values, $n = 2$, %CV = standard deviation/average × 100.

containing a small amount of LMT the approach did not work. In the end it was decided that it would be necessary to measure the LMT without pre-concentration and thus light scattering techniques were deemed unsuitable. The most obvious solution was to rely on HPSEC–RI using a suitably calibrated column set.

3.2. Calibration of HPSEC column set

Since HPSEC does not separate molecules purely on molecular mass, but rather on the volume and shape the molecules occupy in solution it is not appropriate to calibrate the column set with easily available calibration standards such as dextrans, or pullulans. In our laboratory several κ-carrageenans (previously obtained from Sanofi Bio-Industries) were available covering the molecular weight range from 69k to 550k. However the range did not include any standards with Mw below 50,000. Since this was the mass region of interest, an additional standard was prepared by partial mild hydrolysis of the lowest Mw carrageenan in the collection according to previously described methods [21]. A time course experiment (results not reported) was run to establish that (using the conditions described in Section 2) a 1 h hydrolysis time was required to produce a standard having Mw of approximately 20,000. A κ-carrageenan with Mw of 18,000 was produced (as measured by MALS), which unfortunately autohydrolysed during storage. This was rectified by redissolving the sample in water, neutralising with sodium hydroxide and finally precipitating and drying the carrageenan.

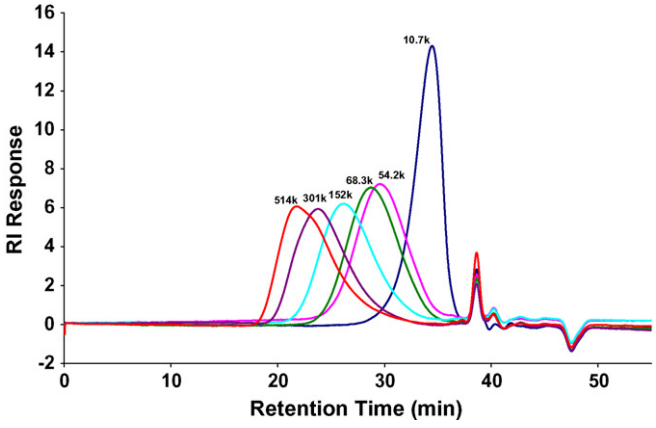

Fig. 1. Overlay of chromatograms of the six broad calibration standards (Mw labelled).

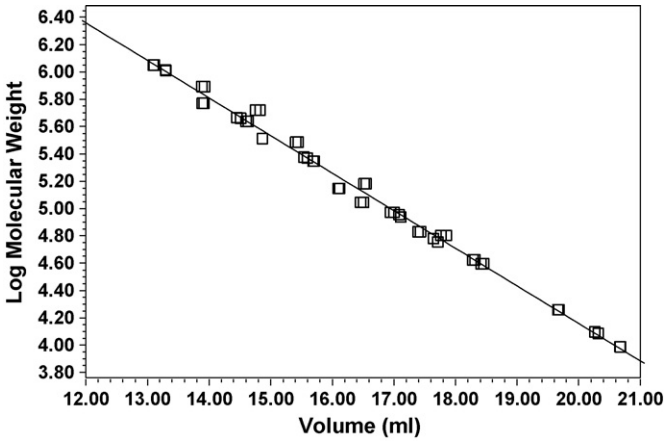

Fig. 2. Typical calibration curve for the HPSEC column set.

The new Mw was found to be 10,700. To avoid autohydrolysis in any future investigations the material should not be dialysed after preparation, but rather be precipitated and washed with alcohol [18]. In this state the dry powder was quite stable. The Mw and Mn of all the standards were determined using HPSEC–MALS (Table 1). The κ-carrageenans were then used as broad standards (Fig. 1) to prepare calibration curves (Fig. 2) using the GPC add-on to Waters Empower. A first-order linear fit was used, a typical equation describing the curve would be:

$$\text{Log Molecular weight} = 9.30 - 0.265V$$

where V represents the elution volume of the molecule, r^2 values were typically 0.997 and always greater than 0.994.

3.3. Partial validation of the analytical system

The system was used to analyse different carrageenan samples over the course of 6 months. Each time the columns were calibrated with the carrageenan standards before and after the samples and the calibration curves were prepared from both sets of data. During this time a κ-carrageenan from Sigma (C-1263) was always included in the analysis set as a reference sample. In total 19 separate measurements were made on the reference sample over the 6 months period. The average Mw for the carrageenan was 540,000 with %CV 1.1 (max. 556,000, min 531,000), the average content of LMT was 7.6% with a %CV of 2.9 (max. 8.0%, min 7.2%). These data demonstrate that the method was performing in a repeatable manner within our laboratory. An inter-laboratory study such as the one carried out by the Marinalg association would be required to ascertain that the system also gives reproducible results in other laboratories.

Carrageenan ingredients from a number of suppliers were analysed using the HPSEC–RI system (Table 2). Unfortunately the Mw of most of the samples was larger than the Mw of our largest standard (514,000). Since Mw measurement was not the primary goal of this work it was not attempted to address the problem. However, separate determination of Mw using HPSEC–MALS indicated that, in general, the Mw obtained by extrapolation of the calibration curve well predicted

Table 2
Mw and %LMT of some carrageenan ingredients measured by HPSEC–RI

Sample	Carrageenan type	Mw[d] (/1000)	%CV[d]	LMT[c,d] (%)	%CV[d]
A	ι	676[a]	0.1	5.5	0.3
B	κ/ι	889[b]	0.3	3.4	1.9
C	κ/ι	665[a]	0.1	4.1	0.9
D	ι	552[a]	0.6	6.2	4.0
E	λ/κ/ι	676[a]	0.1	4.6	0.5
F	κ	626[a]	0.2	4.1	1.6
G	κ/ι	566[a]	0.2	5.0	2.4
H	κ/ι	535[a]	0.2	7.9	0.5
I	ι	659[a]	1.8	6.9	9.0
J	κ	326	2.0	12	14

[a] Measured Mw is beyond the largest standard (514,000) used to make the calibration curve. However the measured Mw is in good agreement (less than 10% difference) with that measured by MALS.
[b] Measured Mw is beyond largest standard used to make the calibration curve and MALS indicates Mw is 1,410,000.
[c] LMT is defined as carrageenan with Mr < 50000. Data reported are average values from duplicate analyses.
[d] Mw and LMT are average values, $n = 2$, %CV = standard deviation/average × 100.

(within 10%) the Mw of the samples measured by MALS (Table 2), giving some indication that the calibrated column set produces fairly accurate Mw data. In the case of only one sample (sample B) were the HPSEC–RI and MALS data different. According to MALS the sample had a Mw (1,410,000) almost three times higher than that of the largest standard and was outside the working range of the HPSEC–RI system.

It also appears that while the standards are composed of κ-carrageenan, the system works well for other types of carrageenan since the Mw measurements from MALS agree well with those from the HPSEC–RI across the different types tested. However, it should be noted that most of the types tested here were κ or ι (or mixtures of the two), this observation should be confirmed before applying to other carrageenan types. In the food industry the majority of the carrageenans used are of the ι- and κ-types thus the system developed here should be widely applicable.

3.4. LMT content of ingredients

The data obtained from the analysis of the carrageenan ingredients (Table 2) shows that the Mw and LMT content varies between different carrageenans and in half of the cases the LMT exceeds the 5% "if feasible" limit proposed by the European Scientific Committee on Food [13]. While it appears that the 5% limit is indeed feasible, a number of the carrageenans tested here would be outside the specification and several, which are within the specification, are quite close to the limit. Increasing the proposed limit to around 7 or 8% would probably allow carrageenan producers to more easily reach this target while keeping the amount of degraded carrageenan in the food chain to a minimum.

3.5. Analysis of finished products

Measurement of carrageenan LMT in a finished product was somewhat more challenging because of interferences from other ingredients. In this work the degradation of carrageenan during processing in a relatively simple model (a jelly) was studied. Jellies contain quite high amounts of carrageenan (1–3%) and during processing the carrageenans are exposed to high temperatures and low pH, ideal conditions for depolymerisation. Samples were prepared on a pilot plant using a normal recipe but adjusting exposure times (beyond normal) to high temperature and low pH.

The jellies were prepared according to a standard recipe which included glucose syrup as one of the ingredients. The glucose syrup turned out to be a challenging issue for the analysis of the LMT since the chromatographic peak from the glucose syrup overlapped with the LMT of the carrageenan (Fig. 3A) making it impossible to measure the amount of LMT present. It was attempted to isolate the carrageenan from the rest of the mixture using alcohol precipitation or by anion exchange chromatography. Unfortunately it was not possible to achieve a quantitative recovery of the carrageenan using either method. With precipitation, some glucose syrup always co-precipitated, and with anion exchange chromatography the carrageenan could not be completely released from the column (as previously observed [27]). Enzymatic hydrolysis of the high molecular mass fraction of the glucose syrup, followed by precipitation of the carrageenan was successful in removing the interference, but unfortunately the enzyme introduced a new interfering signal. To overcome this problem the enzyme was immobilised on a solid support (Eupergit C). This approach worked well, however a great deal of care had to be taken in the preparation of the immobilised enzyme. Particularly important was the washing of the solid support after immobilisation to ensure that there was no free enzyme left over. After numerous trials the protocol described in Section 2 was developed and the analyses could be performed without significant interference from the glucose syrup or enzyme (Fig. 3B).

Analyses of the carrageenans used in jelly production were performed at various stages of the process. Initially carrageenans

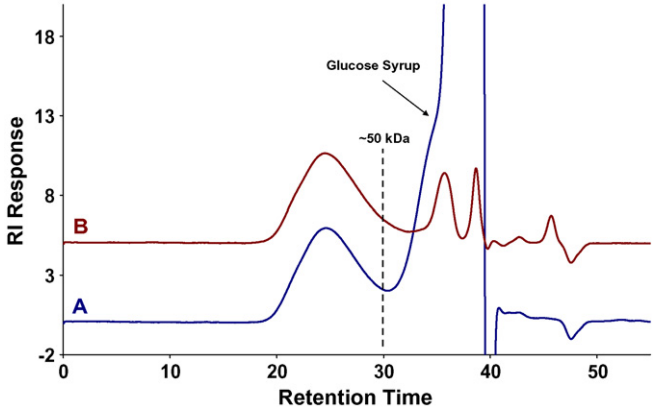

Fig. 3. Chromatograms of the carrageenan present in jelly samples: (A) without pre-treatment and (B) after glucose syrup removal with immobilised AMG.

Table 3
Changes in Mw and LMT during processing

Time at pH 4.6 (min)	Mw[b]	%CV[b]	LMT[a,b] (%)	%CV[b]
0	653,000	0.2	5.8	1.8
5	564,000	6.9	6.4	6.7
10	531,000	2.3	6.5	3.1
15	533,000	1.3	6.5	0.7
20	522,000	1.1	6.9	1.8
30	481,000	1.3	7.5	4.3
60	475,000	1.2	7.8	0.0

[a] LMT is defined as carrageenan with Mr < 50,000.
[b] Mw and LMT are average values, $n = 2$, %CV = standard deviation/average × 100.

were held at pH 6.5 and 95 °C for 1 h (much longer than would be expected during normal manufacturing). Samples were taken at time 0, after 30 min and after 1 h. The average Mw of the carrageenan appeared to decrease slightly from 659,000 to 633,000, however the LMT remained steady at 5.8%. Addition of acidic and flavouring components decreased the pH to 4.6. Samples taken just 5 min after the addition of the pH lowering ingredients showed a marked decrease in Mw (from 653,000 to 564,000) and a concomitant increase in the LMT from 5.8 to 6.4%. The mixture was held at this low pH at 95 °C for 1 h and samples were taken after 5, 10, 15, 20, 30 and 60 min (Table 3). The data clearly shows at this lower pH degradation takes place at a faster rate than at pH 6 and hence processing conditions should be adjusted to keep exposure time to this low pH to a minimum. Additional samples were prepared under typical processing conditions using more challenging pHs (4.1 and 3.8). Analysis of these samples revealed further degradation of the carrageenan material (particularly at pH 3.8). Combining these data along with average values from the timed trials (Fig. 4), it appears that pH values lower than 4 should certainly be avoided during processing as below this pH there appears to be a somewhat increased rate of hydrolysis of the carrageenan. This is in agreement with previous studies which also indicated that carrageenan hydrolysis was increased at pH below 4.0 [21,28]. Carrageenan suppliers currently recommend that the pH should be kept above 4.0, thus it should already be the case that processing conditions are controlled as to avoid such situations. The combined data

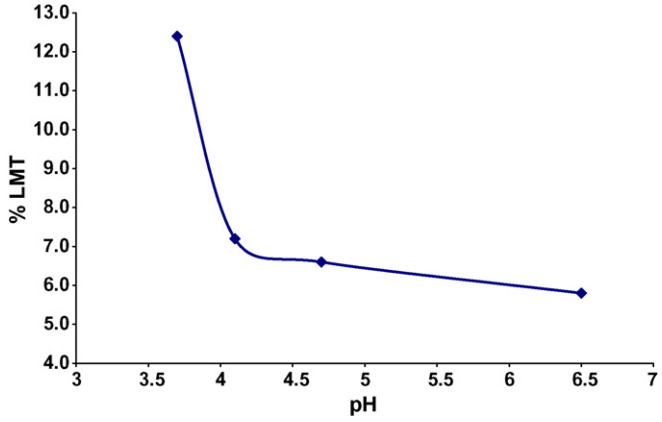

Fig. 4. Effect of pH on LMT during processing.

demonstrates that with good control of pH, i.e. avoiding that the pH drops below 4.0, and by minimising the exposure time to low pH levels during processing, excessive production of carrageenan LMT can be prevented (from the trials conducted here it was possible to prevent the amount of LMT from increasing by more than 15% of the amount contained in the original ingredient, i.e. if the original ingredient contained 10% LMT, the carrageenan in the finished product would not consist of more than 11.5% LMT).

4. Conclusions

An HPSEC–RI method has been used to measure the LMT fraction of carrageenan in ingredients and jellies. Mw measurements based on the HPSEC–RI method agreed well with measurements using the same HPSEC system coupled to MALS detection giving an indication that the column set is well calibrated. Multiple analyses of a reference material indicated that the day to day reproducibility of the method (in a single lab) is excellent. Determination of LMT in the food-grade carrageenans tested here indicates that around half are within the proposed 5% limit, but that almost all contain below 8%. It has also been shown that carrageenan degradation can be minimised during processing by keeping the pH above 4.0 and by minimising exposure times to low pH conditions.

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