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A Comparison Between the Hot and Cold Water Soluble Fractions of Two Locust Bean Gum Samples

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SUMMARY

Locust bean gum extracted from two carob flours from eastern and western Mediterranean sources were fractionated on the basis of their solubility in water. Weight-average molecular weights determined by sedimentation equilibrium were about 300 000 for both the hot water and cold water soluble fractions, whereas a commercial sample of guar gum had a molecular weight of 700 000. Their values were lower than would be predicted from Mark–Houwink relationships where molecular weights were originally determined by light scattering.

The hot water soluble fraction from the eastern Mediterranean flour showed unexpected rheological behaviour. It had an extremely high Huggins' constant and a different relationship between the coil overlap parameter and the zero shear rate viscosity compared with previously reported results for galactomannans. Both effects may be explained by the anomalously low intrinsic viscosity of this fraction when determined by a Huggins' extrapolation. The use of the Kraemer extrapolation gave significantly higher intrinsic viscosities for this particular sample. Gels formed from the two hot water soluble fractions with κ-carrageenan had similar rheological properties.

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INTRODUCTION

The galactomannans extracted from the *Leguminosae* share basic structural similarities in consisting of linear chains of 1,4-linked β-D-mannopyranose residues to which varying proportions of α-D-galactopyranosyl residues are linked at position 6 (Dea & Morrison, 1975). The two major galactomannans exploited commercially are locust bean gum and guar gum. These polysaccharides differ primarily in terms of their galactose content. Guar galactomannan contains around 39% galactose whereas locust bean gum has a galactose content of about 23%. As a consequence of its lower galactose content, locust bean gum is less soluble in water than guar gum.

Hui & Neukom (1964) demonstrated that locust gum could be fractionated on the basis of its solubility in water. They found that approximately 30% of the galactomannan was soluble in cold water with increasing amounts dissolving with increasing temperature. They reported that the cold water soluble material had a higher galactose content but a much lower number-average molecular weight than the hot water soluble material. A recent study by McCleary *et al.* (1985) is not in total agreement with this since it showed similar intrinsic viscosities for hot water soluble (HWS) and cold water soluble (CWS) fractions. This investigation determined the distribution of the galactose side chains along the mannose backbone using a method which involved characterisation of the products resulting from attack by well-characterised enzymes. It was concluded that the distribution of the galactose sidechains was non-regular with a high proportion of substituted couplets but an absence of blocks of substitution.

An additional consequence of the higher mannose/galactose ratio possessed by locust bean gum compared with guar gum is the ability of the former to form gels alone when subjected to freeze–thaw cycles and to interact with carrageenan to increase the strength of carrageenan gels (Dea, 1979). This property is shown to a much greater extent by the hot water soluble locust bean gum fraction because of its lower galactose content.

The viscosity of aqueous galactomannan solutions has recently been studied extensively (Doublier & Launay, 1981; Morris *et al.*, 1981; Robinson *et al.*, 1982). It has been shown that guar gum and locust bean gum show similar relationships between the zero shear rate viscosity and the product of the concentration and intrinsic viscosity (the coil overlap parameter). For equivalent values of the coil

overlap parameter at concentrations above that at which entanglements form, the galactomannans give higher viscosities than most other polysaccharides. This has been interpreted in terms of specific intermolecular interactions (hyperentanglements) (Morris *et al.*, 1981), although it is surprising that guar gum and locust bean gum show similar viscosities since locust bean would be expected to be far more susceptible to such interactions.

The original objective of the work described in this paper was to attempt to explain the different gel potentiating ability with carrageenan of two commercial carob flours, in terms of the proportion and properties of the HWS component of locust bean gum. The investigation did in addition reveal some results of interest with regard to the molecular weights and viscosities of the fractions.

MATERIALS AND METHODS

Materials

Two commercial grade carob flours, samples T and Y, were obtained from eastern and western Mediterranean sources, respectively. In commercial practice it was found that, when mixed with κ-carrageenan, sample Y gave significantly higher gel strengths than sample T. Galactomannans of known galactose:mannose (G:M) ratio (locust bean gum, guar gum and depolymerised guar gum) were a gift from Dr B. V. McCleary. κ-Carrageenan, another sample of guar, D(+)-mannose, D(+)-galactose and D(+)-xylose were purchased from Sigma Inc. (Poole, Dorset, UK) and used without further purification. GLC column packing material (0·2% polyethylene glycol succinate), 0·2% poly(ethylene glycol adipate) and 0·4% silicone XF 1150 (coated on Glas Chrom Q_1 100–120 mesh) were prepared and supplied by Phase Separations Ltd (Queensferry, Clwyd, UK). Silyl-8 column conditioner was obtained from Pierce Chemical Co. Ltd (Rockford, Illinois, USA).

Methods

Composition of carob flours

Total galactomannan content. The galactomannan content was determined by two methods. Crude carob flour (2 g) was extracted in

80% alcohol (40 ml g^{-1}) by heating for 13 min in a 95°C water bath as described by McCleary & Matheson (1975). After cooling in an ice/water bath, the carob flour was collected by centrifugation and suspended overnight in cold (4°C) distilled water (100 ml g^{-1}). The suspension was homogenised using an Ultra-turrax homogeniser and thereafter heated in a 95°C water bath such that the temperature of the sample was maintained above 80°C for 20 min. The solution was again homogenised, cooled in an ice/water bath, then centrifuged (19 200 *g*, 5°C, 45 min) to remove insoluble husk material. The supernatant was decanted and the dissolved galactomannan precipitated by pouring into a two volume excess of absolute ethanol. The precipitated material was then washed twice each with absolute ethanol, acetone and diethyl ether and dried off completely overnight *in vacuo* in the presence of silica gel. Once dry the galactomannan was milled to a white powder using a water-cooled Copley Mill.

The above procedure was repeated except that the 80% ethanol treatment and the overnight hydration were omitted. These samples were simply dispersed in cold water followed by heating at 85°C for 20 min and the galactomannan recovered as previously described.

Protein. Protein levels in the crude carob flours were estimated using the Kjeldahl procedure. Nitrogen concentrations were assayed calorimetrically by the formation of indophenol on a Technicon Auto-Analyser. A conversion factor of 6·25 was used to obtain the protein content from the nitrogen concentration.

Ash. Ash content was obtained after heating dried charred material in a muffle furnace at 550°C for 5 h.

Preparation of hot water soluble and cold water soluble fractions

Carob flour (20 g) was extracted in hot 80% ethanol, cooled and collected by centrifugation as described above. The carob flour was then suspended in 500 ml distilled water and allowed to hydrate for 30 min at 25°C. Undissolved material was removed by centrifugation (14 000 *g*, 50 min, 5°C) and the galactomannan collected from the supernatant by precipitation, followed by drying and milling as described above. This is the cold water soluble (CWS) locust bean gum fraction. The pellet obtained from centrifugation was re-suspended in distilled water and the hot water soluble (HWS) fraction

was extracted by heating to 90°C in a water bath and maintained above this temperature for 30 min followed by centrifugation and precipitation as described.

Temperature fractionation of carob flours

Each carob flour (6 g) was extracted in 300 ml of 80% ethanol by heating for 15 min in a 95°C water bath, then filtered on glass sinter and dried by washing with ethanol, acetone and diethyl ether. A 5 g sample of this flour was weighed into a 250 ml beaker, wetted with ethanol and suspended in 200 ml cold distilled water. The suspension was allowed to hydrate overnight at 4°C, then mixed using a Silverson homogeniser and heated to 25°C in a water bath and maintained for 15 min, then centrifuged (20 000 *g*, 40 min, 5°C). The supernatant was decanted and retained (25°C fraction); the pellet was resuspended in 200 ml distilled water, heated to 45°C in a water bath and maintained for 15 min. This procedure was repeated as above and at 75°C, 85°C and 100°C. The galactomannans extracted at each temperature were precipitated by pouring into a two volume excess of absolute ethanol and dried as previously described. The yield was recorded by weight in each fraction. The determination was repeated twice for each fraction.

Determination of galactose:mannose ratios

Galactose:mannose ratios (G:M) were calculated using an adaptation of the technique described by Albersheim *et al.* (1967). All evaporations were made using a stream of nitrogen at 40°C through a Techne Dry Block Sample Concentrator. Phosphorus pentoxide was used as the desiccant for all drying *in vacuo*. A 5 mg sample of a galactomannan was hydrolysed by 2 ml 2 M trifluoroacetic acid (TFA) in a sealed Sovirel tube at 120°C (1 h). The TFA was then evaporated, final traces being removed by drying overnight *in vacuo* in the presence of KOH. The resulting aldoses were reduced over 1 h at room temperature by the addition of 10 mg sodium borohydride dissolved in 1 ml M ammonium hydroxide. Excess sodium borohydride was decomposed by dropwise addition of glacial acetic acid; the resulting alditols were blown dry, followed by drying overnight *in vacuo*. The borate derived from decomposition of the sodium borohydride, which would otherwise interfere with acetylation, was removed by 6×1 ml successive washings with methanol (double

distilled), evaporating to dryness between each addition. Acetylation was then effected using 2 ml acetic anhydride, in the sealed Sovirel tube at 120°C (3 h). The alditol acetates were then evaporated to dryness using ethanol to assist evaporation and then dried overnight *in vacuo*. The dry residue was dissolved in dichloromethane and sodium acetate crystals filtered out using Pasteur pipettes plugged with non-absorbant cotton wool; 0·2–0·6 μl of this solution was injected onto the chromatography column. The GLC column used for all separations has a liquid phase of 0·2% polyethylene glycol succinate, 0·2% poly(ethylene glycol adipate) and 0·4% silicone XF 1150 coated onto Gas Chrom Q (100–120 mesh). This material was packed into a glass column (1·5 m × 4 mm i.d.) using vibration and a nitrogen pressure of 5 psi. The column was conditioned at 180°C for 24 h. All GLC work was done using a Pye 104 Series Gas Chromatograph fitted with a hydrogen flame ionisation detector. Peak areas were analysed on-line using a computing integrator (Supergrator, Columbia Scientific Industries, supplied by Kemtronix Ltd, Compton, Berks, UK). The column was operated isothermally (170°C) using a nitrogen carrier gas flow rate of 50 ml min^{-1}. The detector oven temperature was maintained at 375°C. The column was periodically conditioned using Silyl-8.

The column was calibrated using solutions of galactose and mannose (5 mg ml^{-1}) which were prepared and then mixed to give solutions having a range of G:M ratios. These solutions were dried down then reduced and acetylated as described as above. G:M ratios calculated from peak areas on the Supergrator output and corrected to the nearest 0·5% corresponded very closely (within 1%) with the known values of G:M. Galactomannan standards (locust bean gum, guar and depolymerised guar of G:M ratios 24:76, 39:61 and 39:61, respectively) were also used; again calculated values were in close agreement with expected values. Thereafter, the column calibration was repeated daily using the galactomannan standards and sample G:M ratios were calculated directly from the Supergrator data output.

Preparation of solutions

Solutions for viscosity measurement and ultracentrifugation were prepared similarly. A known weight of galactomannan was wetted with a small quantity of absolute ethanol (~ 0·5 ml ethanol (100 mg galactomannan)$^{-1}$) to prevent aggregation and dispersed in distilled water for

5 min at room temperature using an Ultra-turrax mixer. The solution was then heated to approximately 80°C over a 10 min period using a steam bath. After this treatment no undissolved galactomannan was visible. The solution was cooled and stirred in an ice/water bath. For intrinsic viscosity measurements and ultracentrifugation the solution was dialysed against distilled water and the final concentration was determined by freeze drying an aliquot. In the case of 'concentrated solution' viscosity determinations, the solution was not dialysed and the concentrations quoted are based on the original galactomannan weight.

Analytical ultracentrifugation

Sedimentation equilibrium measurements were performed on a Beckman model E analytical ultracentrifuge employing Rayleigh interference optics and an RTIC temperature measurement system. The 'intermediate speed' method was employed (Creeth & Harding, 1982); in this method the speed is sufficiently low to allow adequate resolution of the fringes near the cell base. At equilibrium the concentration at the air/solution meniscus remains finite and is obtained by mathematical manipulation of the fringe data (Creeth & Harding, 1982). All determinations were made in 12 mm optical path length cells at the lowest possible concentration ($\sim 0{\cdot}8$ mg ml^{-1}) to minimise possible effects of thermodynamic non-ideality and/or associative phenomena. Partial specific volumes were evaluated from the carbohydrate composition. Since the $\bar{v}$ values of galactose and mannose residues are both 0·613 ml g^{-1} (Gibbons, 1972), the $\bar{v}$ values for the galactomannan (and guar) samples studied were all taken to be 0·613 ml g^{-1}.

Whole-cell weight-average molecular weights, M_w°, were extracted by using the limiting value at the cell base of a particularly directly determinable point average (the 'star' average, M^* (Creeth & Harding, 1982)) — an independent estimate for the initial concentration was not required. Point weight-average molecular weights, M_w, were obtained by using sliding-strip five-point quadratic fits to the observed fringe data.

Measurement of viscosity

Intrinsic viscosity. Measurements of dilute solution viscosity were made at a temperature of $25{\cdot}0 \pm 0{\cdot}1$°C with an Ostwald viscometer

which had a flow time for water of 286 s. The intrinsic viscosity was evaluated both from a Huggins' plot of the reduced viscosity versus concentration and a Kraemer plot of the natural logarithm of the relative viscosities against concentration.

To confirm that Newtonian behaviour was being achieved in the capillary viscometer, some measurements were also made using a Deer Rheometer equipped with concentric cylinder geometry ($r_1 = 2{\cdot}8$ cm, $r_2 = 2{\cdot}9$ cm) at shear rates less than 60 s^{-1}. The results from the two methods were in agreement within experimental error.

'Concentrated' solution viscosity. Viscosity measurements at higher concentrations were performed at $25{\cdot}0 \pm 1{\cdot}0$°C using three instruments.

(1) The Deer rheometer fitted with cone and place geometry with a cone angle of 4°. This covered the shear rate range 0·25–200 s^{-1}.

(2) A Weissenberg Rheogoniometer (model R-19) fitted with cone and plate geometry (2° cone angle) was used for shear rates from 200–1500 s^{-1}.

(3) For guar gum, measurements were made at high shear rates (2000–16 000 s^{-1}) using a slit viscometer described by Berrington (1985). In this instrument liquid is forced backwards and forwards across a slit by an oscillating air-pressure. Viscosity is calculated from the pressure difference between two points on the slit surface and the flow rate of the liquid. A Rabinowitch–Mooney correction was used to take account of non-Newtonian behaviour.

Measurement of gel properties

Preparation of gels. Gels were prepared from a mixture of the hot water soluble components Y and T with carrageenan in a 1:2 ratio (galactomannan:carrageenan) so as to give an overall concentration of 1·2 g of polysaccharide in 100 ml of mixed phosphate buffer (0·067 M KH_2PO_4, 0·067 M $Na_2HPO_4 . 2H_2O$, 0·2 M KCl, pH 7·0). The mixture was heated under reflux for 15 min at 90°C with stirring and then poured into glass moulds 1·2 cm in diameter and allowed to set for approximately 24 h in a refrigerator before use.

Storage modulus. The storage modulus (G') was measured at $25{\cdot}0 \pm 1{\cdot}0$°C and a frequency of 0·2 Hz using the Weissenberg Rheo-

goniometer equipped with parallel plate geometry. Measurements were made on discs of gel 0·4 cm in height which were glued to the plates using a cyano-acrylate ester adhesive (Superglue-3, Loctite Ltd, UK). It was demonstrated that determinations were being made within the linear viscoelastic region.

Rupture strength. Measurements of rupture strength were made at ambient temperature by compressing samples of gel, 1·2 cm in diameter and 2 cm in length, using an Instron 1140. The crosshead speed was 1 cm min^{-1} and the diameter of the compression anvil was greater than the diameter of the gel. The rupture strength was the force (N) corresponding to the height of the first peak.

RESULTS

Fractionation of galactomannans

The galactomannan components of the flour samples Y and T did not differ to a great extent. Sample Y contained slightly more galactomannan and somewhat less protein than T, but the G:M ratios of the total extracted material were similar (Table 1).

The results of the temperature fractionation shown in Table 2 again revealed little difference between the two materials, although there is some evidence that Y contained more of the high M:G ratio fraction extractable at 85°C.

TABLE 1
Composition of Carob Flours

Sample	*Galactomannan (%)*		*Galactose:mannose ratio*	*% Protein*	*% Ash*	*% Moisture*
T	58·7[a]	58·7[b]	21·5:78·5	10·3	0·6	6·8
Y	61·7[a]	59·9[b]	21·5:78·5	6·9	0·5	8·0

[a] Extracted by dispersing in cold water followed by heating at 85°C for 20 min in a boiling water bath.
[b] Extracted in ethanol (80%) and heated overnight.

TABLE 2
Temperature Fractionation of Carob Flours

Temperature of extraction (°C)	*T*		*Y*	
	Yield (% total gum extracted)[a]	*Galactose:mannose ratio*	*Yield (% total gum extracted)*	*Galactose:mannose ratio*
25	37·5	28:72	33·0	28·5:71·5
45	16·5	24·5:75·5	18·0	24:76
65	32·0	21:79	30·0	21:79
85	14·0	18:82	17·0	18:82

[a] Extraction above 85°C yielded only trace quantities of galactomannans.

Ultracentrifugation

Figure 1(a) shows a typical plot of the equilibrium distribution of a galactomannan (Y HWS) as represented by the logarithm of the absolute fringe concentration J versus the radial displacement parameter ξ, where ξ is a function of the radial displacement r:

$$\xi = \frac{r^2 - a^2}{b^2 - a^2} \tag{1}$$

a and b being the radial position of the meniscus and base, respectively. The downward curvature near the cell base is indicative of the presence of thermodynamic non-ideality, even at the low cell-loading concentrations used. No evidence of significant self association or polydispersity in the samples was evident from these plots — unlike, for example, mucus glycoproteins (Harding, 1984) — although such effects may be masked by the high non-ideality (see, e.g., Teller (1965) and Fujita (1975)).

The values of the whole-cell weight-average molecular weights, M_w°, corresponding to these plots for the HWS and CWS fractions and for guar gum, along with the measured G:M ratios, are given in Table 3. These values may be lower than the true 'ideal' molecular weights because of the non-ideality, even at the low cell loading concentrations ($c^\circ \approx 0{\cdot}8$ mg ml^{-1}) used. However, extrapolation of the point weight-average molecular weights (a typical plot of which is given in Fig. 1(b),

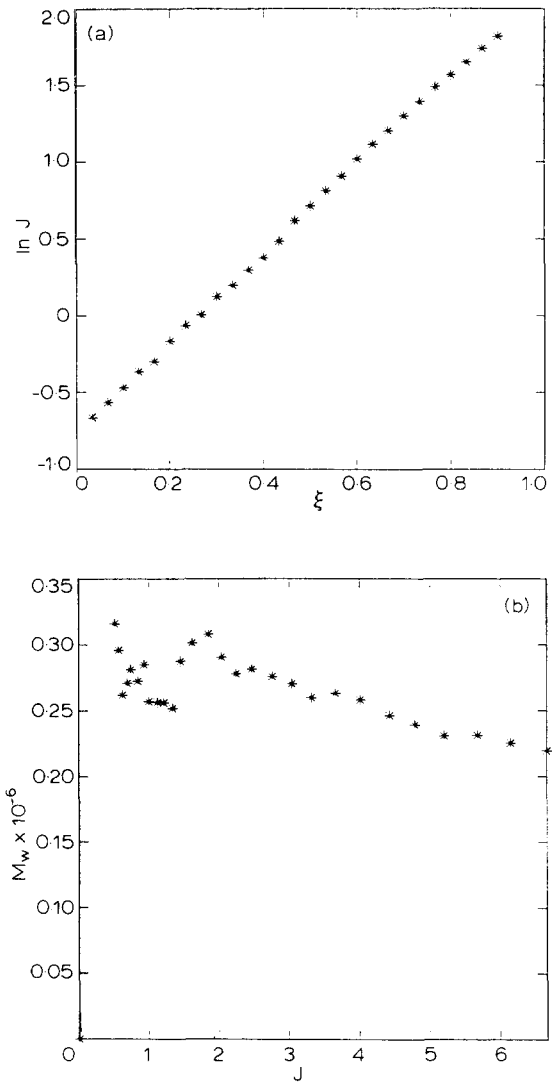

Fig. 1. (a) Plot of the logarithm of the absolute fringe concentration, J, against the radial displacement parameter ξ for sample Y HWS. (b) Corresponding plot of the point weight-average molecular weight M_w versus J. Because of the small fringe increment near the meniscus, points below $J \lesssim 1$ are less accurate. Rotor speed 5558·2 rev min^{-1}; temperature = 25·5°C. Initial loading concentration ≈ 1 mg ml^{-1}.

TABLE 3
Molecular Weights and G:M Ratios of Galactomannan Fractions

Fraction	*Whole cell average* (M_w°)	$M_w(J \rightarrow 0)$	*Galactose:mannose* ratio
T CWS	300 000 ± 10 000	340 000 ± 30 000	25:75
T HWS	270 000 ± 10 000	310 000 ± 30 000	19:81
Y CWS	320 000 ± 10 000	390 000 ± 30 000	28:72
Y HWS	266 000 ± 10 000	325 000 ± 30 000	19:81
Guar (Sigma)	630 000 ± 30 000	800 000 ± 60 000	39:61

for sample Y HWS) to zero (fringe) concentration yields a weight-average molecular weight ($M_w(J \rightarrow 0)$) that is less precise than the M_w° values, but not affected by non-ideality, or self-association phenomena (but will still be influenced by polydispersity if present). Such values are also given in Table 3).

Viscosity

Table 4 displays the intrinsic viscosities obtained by both Huggins' and Kraemer extrapolations, Huggins' coefficients and Huggins' plot slopes for the HWS and CWS locust bean gum fractions and for guar

TABLE 4
Dilute Solution Viscosity Parameters for Galactomannan Fractions

Fraction	*Intrinsic viscosity* ($dl\,g^{-1}$)		*Huggins' constant* (k')	*Slope of Huggins' plot* ($dl^2\,g^{-1}$)
	Huggins' extrapolation	*Kraemer extrapolation*		
T CWS	8·9	9·2	0·75	60
T HWS	6·0	8·9	5·05	185
Y CWS	10·3	10·3	0·73	78
Y HWS	13·8	14·7	0·80	154
Guar (Sigma)	11·5	12·7	1·04	138

gum. It can be seen that the HWS component of T behaves anomalously in two respects. First, the Huggins' and Kraemer intrinsic viscosities are significantly different and, secondly, the Huggins' coefficient (k^1) is extremely high. For the other locust bean gum fractions our values for k^1 are quite consistent with those reported by Doublier & Launay (1981). These workers found Huggins' constants ranging from 0·54 to 1·12 for locust bean gum fractions in water. The Huggins' plots for the hot water soluble fraction of sample T obtained using different stock solutions gave similar slopes but significantly different values of $[\eta]$. This was not found for the other galactomannan fractions. The data for the four determinations from which the mean values of $[\eta]$, k^1 and the Huggins' plot slope were determined are shown in Fig. 2.

The HWS component of T also behaved anomalously when the zero shear rate viscosity was plotted against the coil overlap param-

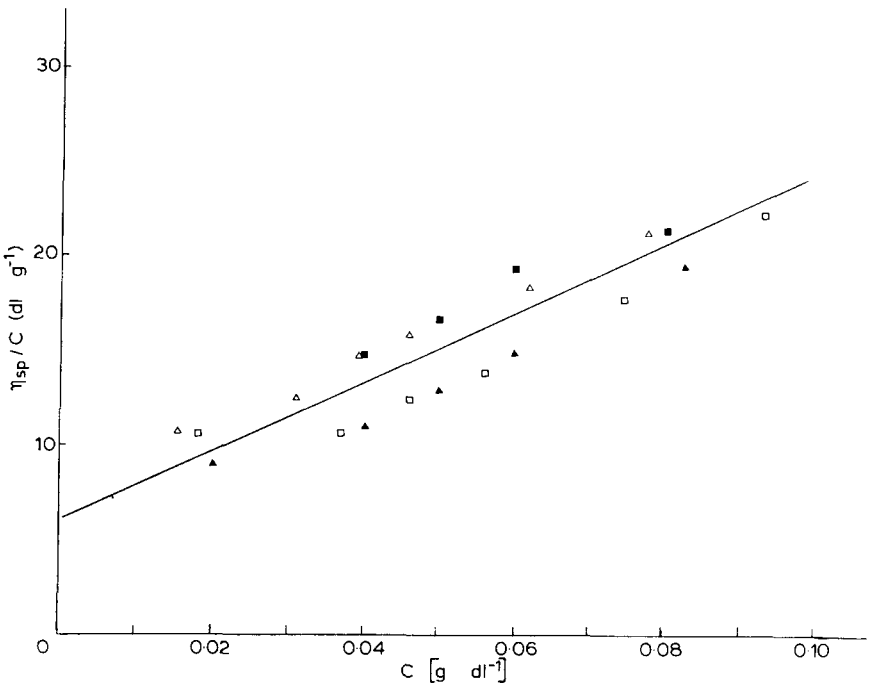

Fig. 2. Huggins' plots for T HWS. The points represent four determinations using separately prepared stock solutions: △, □, ▲ — results obtained using an Ostwald capillary viscometer; ■ — results obtained using a Deer Rheometer. Temperature of measurement 25·0°C.

eter, $c[\eta]$ (Fig. 3). The other materials showed reasonable agreement with the results of Morris *et al.* (1981), Doublier & Launay (1981), Robinson *et al.* (1982) and Sabaters de Sabates (1979). Better agreement was obtained for T HWS when the Kraemer rather than the Huggins' value for the intrinsic viscosity was used.

With the exception of guar gum, our data on the non-Newtonian behaviour of these fractions do not extend to very high shear rates. When plotted against the generalised shear rate (β):

$$\beta = \dot{\gamma}(\eta_0 - \eta_s)\, M_w^\circ / cRT \qquad \text{(Graessley, 1974)}$$

(where η_0 is the zero shear rate viscosity, η_s is the solvent viscosity, $\dot{\gamma}$ is the shear rate, c is the concentration and M_w° is the whole-cell average molecular weight), the fractions do not show marked differences in behaviour (Fig. 4). Our results for guar gum are in quite good agreement with those of Robinson *et al.* (1982).

Gel strengths

The storage modulus and rupture strengths of mixed gels of the hot water soluble locust bean gum components and carrageenan are shown in Table 5. No significant difference was found between the two fractions.

DISCUSSION

Galactomannan contents

Our results for the galactomannan content of the flours and galactose:mannose ratios for both the total gum and the HWS fraction are in excellent agreement with the data of McCleary *et al.* (1985) obtained for four commercial flours. The proportion of CWS material (25°C, see Table 2) is somewhat higher for our flours than the average value found by McCleary *et al.*, although our solubility data are in good agreement with Hui & Neukom (1964).

Molecular weights

Mark–Houwink parameters have recently been published for guar gum by Robinson *et al.* (1982) and Doublier & Launay (1981). In both

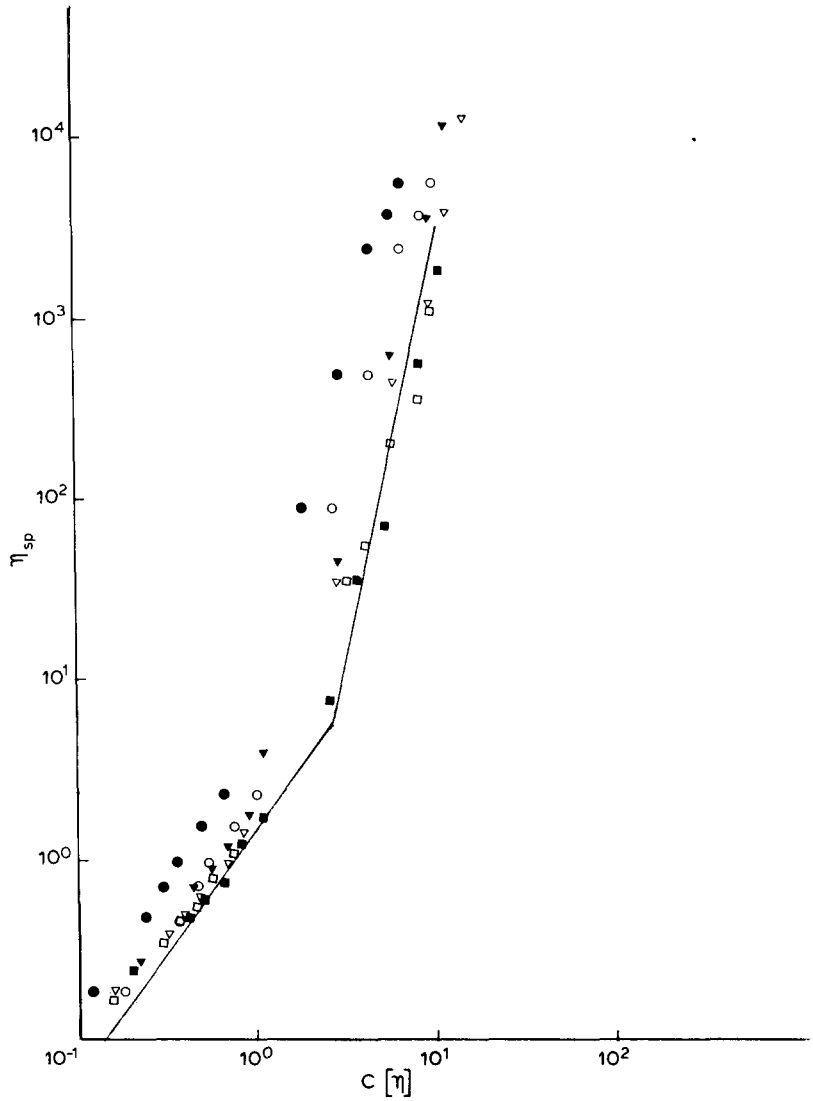

Fig. 3. Plots of zero shear rate specific viscosity against the coil overlap parameter ($c[\eta]$) for the galactomannan fractions: ●, T HWS; □, T CWS; ▽, Y HWS; ■, Y CWS; ▼, guar gum (Sigma) (intrinsic viscosities obtained from Huggins' extrapolation); ○, T HWS (intrinsic viscosity obtained from Kraemer extrapolation). Solid line represents the results of Morris *et al.* (1981) for guar gum and locust bean gum. Temperature of measurement 25°C.

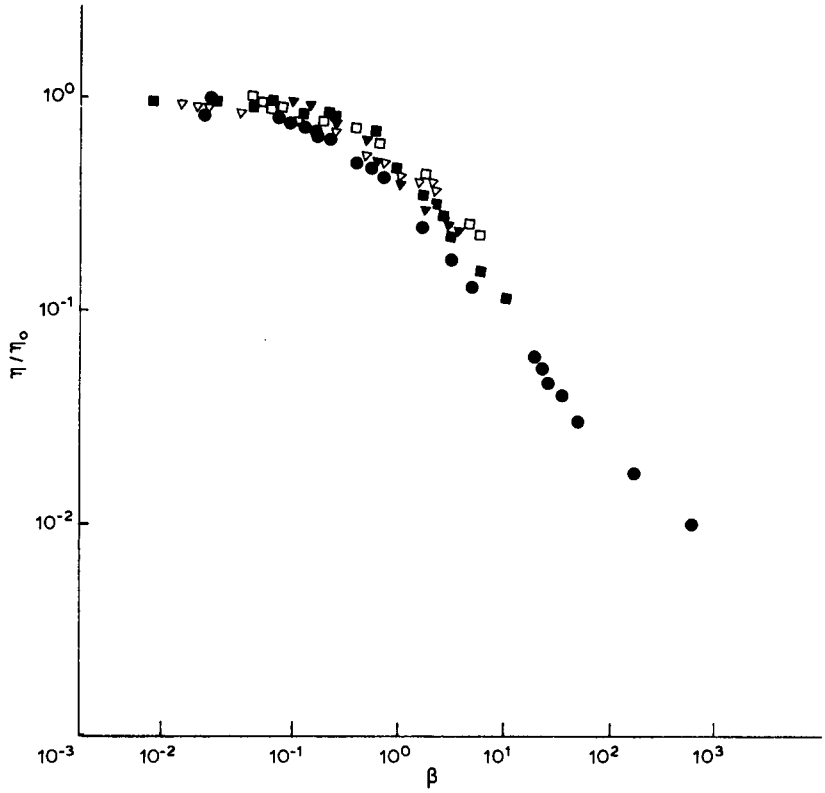

Fig. 4. Plots of generalised shear rate (defined in text) against η/η_0 where η_0 is zero shear rate viscosity and η apparent viscosity: □, T HWS; ▽, T CWS; ■, Y HWS; ▼, Y CWS; ●, guar gum (Sigma).

investigations weight-average molecular weights were determined by light scattering. The authors comment on the difficulties in applying this technique to the galactomannans because of the highly curved Zimm plots obtained. This is a consequence of the presence of very high molecular weight but compact particles that cannot be removed by filtration. The particles are probably incompletely dispersed galactomannan. Although various methods can be used to try and correct for errors due to this problem, both groups consider that it is possible that the molecular weights obtained are too high. Light scattering seems even less appropriate for locust bean gum, since it is more difficult to disperse than guar gum because of its higher

TABLE 5
Rheological Properties of Mixed Gels with Carrageenan

Fraction	*Storage modulus G' (Nm^{-2})*	*Rupture strength (N)*
T HWS	30 050 ± 1 900	80·2 ± 15·2
Y HWS	31 900 ± 2 500	77·2 ± 7·6

mannose:galactose ratio. Doublier & Launay (1981) and more recently Tako *et al.* (1984) have calculated molecular weights for locust beam gum from Mark–Houwink parameters obtained for guar gum. To do this, it is necessary to take into account the different mannose:galactose ratios of the two polysaccharides since the intrinsic viscosity depends on the length of the mannose backbone not the concentration of the galactose sidechains (Doublier & Launay, 1981; McCleary *et al.*, 1981). Thus the Mark–Houwink relationship of Doublier & Launay (1981) took the form

$$[\eta] = 11{\cdot}55 \times 10^{-4}((1-x)\,\bar{M}_w)^{0{\cdot}98} \qquad (1)$$

where

$$x = \text{galactose}/(\text{mannose} + \text{galactose})$$

If we substitute our values for the intrinsic viscosity into this equation we obtain molecular weights considerably higher than those obtained by ultracentrifugation. For example, for guar gum and the HWS component of T, eqn (1) would predict a value for $\bar{M}_w$ of $2{\cdot}16 \times 10^6$ and $8{\cdot}39 \times 10^5$, respectively (using the Huggins' intrinsic viscosity). The other locust bean gum components would give an even greater discrepancy between the predicted and measured molecular weights. The Robinson *et al.* relationship predicts $1{\cdot}58 \times 10^6$ for our sample of guar gum. Some support for our data comes from the study of Sharman *et al.* (1978) who obtained weight-average molecular weights from sedimentation velocity and diffusion experiments (using the Svedberg equation) for guar gum and locust bean gum of $6{\cdot}57 \times 10^5$ and $3{\cdot}19 \times 10^5$, respectively. These results agree within experimental error with our data for guar and the locust bean gum fractions. The intrinsic viscosities reported by Sharman *et al.* are con-

siderably higher than those found by other workers but, as suggested by Robinson *et al.* (1982), this is probably due to the unusual extrapolation method they used.

Intrinsic viscosities

McCleary *et al.* (1985) reported that the intrinsic viscosities of a number of HWS and CWS fractions of locust bean gum were in the range 12 ± 2 dl g^{-1} and 10 ± 2 dl g^{-1}. Expressing the results in terms of the concentration of the mannan backbone gave 'theoretical' intrinsic viscosities of 15 dl g^{-1} for both the HWS and CWS fractions, from which it was concluded that the two fractions had similar molecular weights. This does not agree with Hui & Neukom (1964) who obtained very much higher number average molecular weights for the HWS fraction.

Our results are generally in agreement with McCleary *et al.* and our ultracentrifugation data confirm that the molecular weights of the CWS and HWS fractions are similar. The only puzzling point is the intrinsic viscosity measurement for the HWS fraction of T. Even the value for intrinsic viscosity obtained from the Kraemer extrapolation seems low when considered in conjunction with the measured molecular weight and the measured viscosity of the 'concentrated' solutions (Fig. 3). If the intrinsic viscosity of this fraction was comparable to HWS Y then the relationship between the zero shear rate specific viscosity for T HWS and the coil overlap parameter, $c[\eta]$, would be comparable to the other galactomannans. The most likely explanation is therefore that the degree of dispersion of this fraction was poor and the sample contained a higher proportion of very compact associates which make a small contribution to the intrinsic viscosity. The high Huggins' constant, solution viscosity at higher concentrations and difference between Kraemer and Huggins' extrapolation could be explained by the tendency for these associates to aggregate with increasing concentration to give rod-shaped particles. The ultracentrifugation studies showed no evidence for a concentration dependent association but, as discussed earlier, this could have been masked by non-ideality. A possible alternative explanation is that the polysaccharide is completely dispersed but the coil is very compact, i.e. water is a particularly bad solvent for this fraction. As the concentration increases intermolecular associations replace intramolecular

interactions. Although this idea does not seem too unreasonable it is not consistent with McCleary *et al.* (1981) who reported that the intrinsic viscosity of guar gum from which galactose had been enzymically removed remained constant when expressed in terms of mannose backbone concentration even down to a galactose:mannose ratio of 15:85. Thus the coil conformation does not change with a reduction in the content of galactose sidechains even though water becomes a poorer solvent as the galactose content of the polysaccharide decreases.

Gel strengths

The original objective of this work was to attempt to explain the higher gel strengths obtained when flour Y as opposed to flour T was mixed with carrageenan. The data in Table 5 suggest that this is not due to differences in the nature of the HWS component of the flours. Instead it may be due to a combination of three factors: (a) the slightly higher content of galactomannan in Y compared with T; (b) the higher proportion of HWS material in the locust bean gum extracted from Y; (c) the higher protein content in T since protein may interfere with the gelation process by interacting with carrageenan.

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REFERENCES

Albersheim, P., Nevins, D. J., English, P. D. & Karr, A. (1967). *Carbohyd. Res.* **5**, 340.

Berrington, D. (1985). Viscosity measurement of food systems at high temperatures, PhD Thesis, University of Nottingham, Faculty of Agricultural Science, UK.

Creeth, J. M. & Harding, S. E. (1982). *J. Biochem. Biophys. Meth.* **7**, 25.

Dea, I. C. M. (1979). In *Polysaccharides in food*, ed. J. M. V. Blanshard & J. R. Mitchell, Butterworths, London, p. 229.

Dea, I. C . M. & Morrison, A. (1975). *Adv. in Carb. Chem. Biochem.* **31**, 241.

Doublier, J. L. & Launay, B. (1981). *J. Texture Studies* **12**, 151.
Fujita, H. (1975). *Foundations of ultracentrifugal analysis*, Wiley, New York, pp. 342–4.
Gibbons, R. A. (1972). In *Glycoproteins*, ed. A. Gatschalk, Elsevier, Amsterdam, Part A, p. 24.
Graessley, W. W. (1974). *Adv. Polym. Sci.* **16**, 1.
Harding, S. E. (1984). *Biochem. J.* **219**, 1061.
Hui, P. A. & Neukom, H. (1964). *Tappi* **47**, 39.
McCleary, B. V. & Matheson, N. K. (1975). *Phytochemistry* **14**, 1187.
McCleary, B. V., Amado, R., Waibel, R. & Neukom, H. (1981). *Carbohyd. Res.* **92**, 269.
McCleary, B. V., Clark, A. H., Dea, I. C. M. & Rees, D. A. (1985). *Carbohyd. Res.* **139**, 237.
Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A. & Price, J. (1981). *Carbohyd. Polym.* **1**, 5.
Robinson, G., Ross-Murphy, S. B. & Morris, E. R. (1982). *Carbohyd. Res.* **107**, 17.
Sabaters de Sabates, A. (1979). Contribution a l'etude des relations entre caracteristiques macromoleculaires et proprieties rheologiques en solution aqueuse concentree d'un epaississant alimentaire: la gomme de caroube, PhD Thesis, l'Universite de Paris XI (Centre d'Orsay), France.
Sharman, W. R., Richards, E. L. & Malcolm, G. N. (1978). *Biopolymers* **17**, 2817.
Tako, M., Asato, A. & Nakamura, S. (1984). *Agric. Biol. Chem.* **48**, 2995.
Teller, D. C. (1965). PhD Thesis, University of California, Berkeley, USA.