

Toward the Recognition of Structure–Function Relationships in Galactomannans

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In this paper the determination of the physical/rheological characteristics is described for a series of commercial galactomannans of which the structural details have been reported previously. Both solubility of the galactomannans and rheological properties of galactomannan solutions and galactomannan/xanthan mixtures were determined. Using a statistical analysis approach an attempt was undertaken to recognize correlations between structural and rheological data. The best correlation found was between the abundance of galactose substituents at a regular distance (type of galactomannan) and the storage modulus (G') of mixed galactomannan/xanthan gels, underscoring the hypothesis that branching hinders the formation of a network with xanthan gum. Also, the G' for the group of locust bean gums correlated with the degree of blockiness, that is, the size and occurrence of nonsubstituted regions on the mannose backbone. In addition, galactomannans displayed an apparent decrease in gelling ability with increasing average molecular weight. That G' also relates to the type of galactomannan can therefore partly be attributed to differences in average molecular weight for the various galactomannan types. However, within the series of locust bean gums tested, also an increase of G' with molecular weight was observed. This can be explained by the decreasing number of loose ends of the polymers and the concomitant increasing efficiency in network participation with increasing molecular weight.

KEYWORDS: Guar; locust bean; tara; galactomannans; gums; polymers; structure–function relationship

INTRODUCTION

Galactomannans are plant reserve carbohydrates that are present in large amounts in the endosperm of the seeds of many Leguminosae (1, 2). During sprouting they are enzymatically degraded and used as nutrient. Galactomannans consist of a (1→4)- β -D-mannopyranosyl backbone to which single-unit (1→6)- α -D-galactopyranosyl residues are attached (2). The mannose/galactose ratio varies from about 10 to 1, depending on the source used. For locust bean, guar, and tara gums, mannose/galactose ratios of, respectively, 3.5, 1.5, and 3 are commonly found (1). The distribution of the galactose units over the mannan backbone is believed to be important for the functional properties of the polysaccharide when applied in food products, for example, for the effect of galactomannans on the gel-forming properties of κ -carrageenan, agar, and xanthan gum. The distribution of the galactosyl residues has been investigated by X-ray diffraction, NMR, and chemical and enzymatic

degradation studies (reviewed in refs 2 and 3). Recently we presented a method that enables rapid determination of the fine structure of galactomannans using enzymes (4), based on a strategy applied previously by our group to characterize pectins (5, 6). Digests of galactomannan polymers obtained by endo- β -mannanase treatment were analyzed by high-performance anion-exchange chromatography (HPAEC) and pulsed amperometric detection (PAD). In this way, information reflecting the galactosyl distribution(s) of a whole range of commercial galactomannan preparations (such as locust bean, guar, tara, and cassia gums) was obtained. Because dissimilarities in the distribution of galactose result in nonidentical degradation profiles, comparison of these degradation profiles reflects the (dis)similarity of the galactose distribution of the galactomannans studied. These differences were plotted in a similarity distance tree. In this Y-shaped tree three major groups could be discerned: (1) galactomannans that released hardly any or only a few substituted and nonsubstituted mannose oligomers after enzymatic degradation (i.e., guar gums and the highly substituted gum of *Prosopis juliflora*); (2) gums that released large amounts of substituted and some nonsubstituted oligomers (tara, locust bean gum, enzymatically degalactosylated guar, and cassia gum); and (3) polymers that mainly form nonsubstituted

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mono- to trimannose (linear mannan and coffee galactomannans). In addition to this more general differentiation, the absolute amount of nonsubstituted mannose released by the enzyme was found to enable more subtle distinctions on the distribution of galactose over the mannan backbones. All guar gums and the highly substituted gum of *P. juliflora* were found to have a blockwise distribution of galactose. For different batches of tara gum both random and blockwise distributions were found. Among batches of locust bean gum the greatest variation was observed: random, blockwise, and ordered galactose distributions were present. Cassia gum was found to have a quite regular distribution of galactose (4).

To verify the importance of the distribution of the galactose units over the mannan backbone for the functional properties of galactomannans, both the structural and physical properties need to be determined. Because of their high hydrodynamic volume, galactomannans are able to form highly viscous solutions even at low concentrations (1, 2, 7). McCleary and Neukom have shown that the intrinsic viscosities of galactomannan solutions treated with α -galactosidase were determined by the concentration of the mannan backbone and independent of the mannose/galactose ratio (8). The galactose content, however, determined the solubility. Galactomannans with a galactose content of <10% form precipitates at room temperature due to association of the nonsubstituted regions.

Galactomannans in combination with κ -carrageenan, agar, or xanthan gum display an interactive effect that, depending on the concentration, can result in strong gels. The distribution of the galactose units over the mannan backbone is suggested to be important for the gel-forming properties of galactomannans with xanthan gum (1, 2, 7). Xanthan gum is a heteropolysaccharide with a backbone of β -1,4-linked D-glucose which is substituted, through O-3, on every alternating glucose residue with a trisaccharide side chain consisting of 6-O-acetyl- β -D-mannose-(1,4)- β -D-glucuronic acid-(1,2)- α -D-mannose. The latter mannose can be substituted at the 4- and 6-positions with pyruvate residues (1). Aqueous solutions of xanthan gum are extremely viscous, and at higher concentrations the solution exhibits elastic properties on short time scales, but no real gel is formed. Models have been proposed to explain the interaction between xanthan gum and galactomannan and the synergistic increase in viscosity on mixing. In early models this effect was assumed to result from the interaction between the backbone of the ordered xanthan molecule and the nonsubstituted region of the galactomannan main chain (9, 10). The enhanced synergistic effect of deacetylated xanthan gum on gelation was explained by a lock-key binding mechanism between the side chains of the xanthan molecule and the backbone of the galactomannan (11). Evidence of such specific intermolecular binding was obtained from X-ray diffraction patterns (12, 13). Both heated and nonheated mixtures of xanthan gum and galactomannan can form gels. However, maximum synergism, resulting in stiffer gels, is obtained only after heating (14). A proposed mechanism involved the binding of disordered parts of xanthan gum with galactomannans for both hot- and cold-set gels (15). The latter would involve partial dissociation of the double strands at temperatures lower than the transition temperature (16). Another model suggested binding of the galactomannan chain with the ordered xanthan helix (17).

In this paper the determination of the physical/rheological characteristics is described for a series of galactomannans of which the structural details have been presented previously (4). Both the solubility of the galactomannans and rheological properties of galactomannan solution and galactomannan/

xanthan gels were determined. In view of previous successful attempts to relate structural and rheological properties by statistical analysis procedures (18), a comparable approach was undertaken here to determine possible correlations for a series of galactomannans.

MATERIALS AND METHODS

Materials. All guar gums were extracted from the endosperm of *Cyamopsis tetragonoloba* seeds, and all locust bean gums were derived from the endosperm of *Ceratonia siliqua* seeds. Diaguvar GH 200/5000 (G64A), Diaguvar CS (C12; from the endosperm of *Cassia tora/obtusifolia* seeds), Diaguvar 1.1 (H80; from the endosperm of *P. juliflora*), and tara gum (T35; from the endosperm of *Caesalpinia spinosa*) were obtained from BFGoodrich Diamalt GmbH (Munich, Germany). The locust bean gums SC1500-80 μ (L29A) and SC1500-250m (L30) and the guar OP 3309 (G64B) were provided by SKW Biosystems (Baupte, France). Supercol U guar (G62) was obtained from Aqualon (Wilmington, DE). The locust bean gum Genu Gum RL-200Z (L29B) was provided by Copenhagen Pectin A/S (Lilli Skensved, Denmark). The locust bean gum Meypro-LBG Fleur M-175 (L29C) and the processed guar gums Meypro 7 and 30 (G70 and G67A), Meypro 50 and 100 (G67B and G67C), and Meypro 500 (G66) were obtained from Meyhall Chemical AG (Kreuzlingen, Switzerland). The tara gum Ferwotar E417 (T36) was provided by Ferdiwo BV (Oudewater, The Netherlands). The locust bean gum Grindsted LBG 147 (L32) and Grindsted Guar 1400 (G65) were obtained from Danisco Ingredients (Brabrand, Denmark). The enzymatically processed guar Sherec QSG81 (G26) was provided by Quest International (Carrigaline, Ireland). Linear mannan (β -(1,4)-linked; M2) was isolated from ivory nut by Megazyme International (Wicklow, Ireland). The mannose and galactose content, the mannose/galactose ratio, and the degree of substitution (DS) of all galactomannans and mannan are listed in Table 1. Keltrol T xanthan was provided by Nutra Sweet Kelco Co. (Surrey, U.K.). For all experiments demineralized water was used. All galactomannans mentioned above have been studied previously in detail for their fine structural characteristics (4) except for the apparent molecular weights. These have been determined using high-performance size exclusion chromatography (HPSEC) performed on an SP8800 HPLC (Spectra Physics) equipped with three Bio-Gel TSK columns (each 300 \times 7.5 mm) in series (60XL, 40XL, 30XL; Bio-Rad Laboratories). Elution was at 30 $^{\circ}$ C with 0.4 M sodium acetate buffer (pH 3.0) at 0.8 mL/min. The eluents were monitored with a combined RI detector and viscometer (Viscotek, model 250), a right angle laser light-scattering detector (RALLS, Viscotek, LD600), and a UV detector (TSP UV 2000). Amounts of 100 μ L were injected. Apparent molecular weights were calculated using the light-scattering module of the Trisec software (Viscotek) with pullulan standards (Polymer Laboratories).

Sample Preparation. A xanthan gum solution (0.5% w/w) was prepared by dispersing the xanthan in 0.1 M NaCl at 80–90 $^{\circ}$ C. After stirring of the mixture for 30 min, the sample was cooled to room temperature. There are no indications of sample breakdown during this procedure as checked using gel permeation chromatography (not shown). A galactomannan solution (0.5 or 0.25% w/w) was prepared by wetting the galactomannan powder with 99% ethanol (a few drops), adding the required amount of 0.1 M NaCl, and stirring under heat until the boiling point for 4–5 min. The sample was allowed to cool to room temperature, and water was added to the sample to correct for losses due to evaporation of the solvent. All concentrations mentioned are on a weight/weight basis.

Rheological Measurements. Equal volumes of xanthan gum and galactomannan solutions were combined, resulting in samples containing 0.25 or 0.125% galactomannan and 0.25% xanthan gum. The mixture was transferred as quickly as possible to the rheometer and covered with soy oil to avoid evaporation. The G' , $\tan \delta$, and yield stress measurements were performed on a VOR (Bohlin) controlled-strain rheometer. A C14 geometry and a torsion bar of 3 g \cdot cm were used. The following measurements were performed (with the abbreviation used in parentheses):

- gel formation by the cold-mixed sample at 20 $^{\circ}$ C during 15 min (measurement A)

Table 1. Mannose and Galactose Related Properties of a Series of Galactomannans

sample code	sample type	code type ^a	total sugar (w/w %)	Man + Gal (w/w %)	Man (w/w %)	Gal (w/w %)	Man/Gal ratio	DS ^b (%)	DB ^c (%)	di-/trimannose ratio ^d	subst vs nonsubst area ratio ^e	MW ^f (×1000)
M2	mannan	1	71	67.6	66.3	1.4	48.5	2.1	67.4	1.06	0.03	0.91
D1:1	diagum	7	72	87.1	48.3	38.8	1.2	7.0	6.4	5.5	3.37	1295
C12	cassia	3	85	78.1	69.8	8.3	8.4	11.9	42.2	1.1	0.56	884
L29A	LBG	4	96	95.1	73.8	21.3	3.5	28.9	48.0	1.0	1.40	429
L29B	LBG	4	94	92.5	71.8	20.8	3.5	28.9	32.9	1.1	1.23	492
L29C	LBG	4	89	85.4	66.4	19.0	3.5	28.7	46.0	1.0	1.25	594
L30	LBG	4	94	92.1	71.1	21.1	3.4	29.7	25.4	1.0	1.45	473
L32	LBG	4	83	62.3	45.0	17.3	3.2	31.6	36.3	1.1	1.38	632
T35	tara	5	85	82.7	61.3	21.4	2.9	34.9	29.6	1.06	2.98	582
T36	tara	5	79	87.2	64.9	22.3	2.9	34.3	41.1	1.2	2.19	640
G26	guar	6	91	86.5	52.6	33.8	1.6 ^g	64.3				440
G62	guar	6	84	78.8	48.8	30.0	1.6	61.6	18.3	1.6	2.50	1088
G64A	guar	6	89	85.4	51.9	33.5	1.6	64.5	15.3	1.1	1.93	1130
G64B	guar	6	91	86.0	52.5	33.5	1.6	63.7	14.9	0.9	2.01	1214
G65	guar	6	84	80.0	48.4	31.6	1.5	65.3	14.8	1.4	2.40	1356
G66	guar	6	82	78.3	47.1	31.2	1.5	66.2	17.3	1.4	2.11	1602
G67A	guar	6	71	67.5	40.3	27.2	1.5	67.4	11.5	2.1	3.02	251
G67B	guar	6	88	84.0	50.3	33.7	1.5	66.9	10.8	1.8	2.60	829
G67C	guar	6	83	79.5	47.7	31.8	1.5	66.6	11.6	1.9	2.58	1126
G70	guar	6	64	61.6	36.3	25.3	1.4	69.6	15.8	2.3	3.17	50

^a 1, mannan (ivory nut); 3, cassia; 4, locust bean gum; 5, tara; 6, guar; 7, *Prosopis juliflora*. ^b DS = degree of substitution. ^c DB = degree of blockiness. ^d For details and definitions see Daas et al. (4). ^e Average molecular weight has been determined by HPSEC. ^f Note that this sample does contain many free galactoses prior to dialysis.

- frequency sweep at 20 °C (measurement B)
- stress overshoot at 20 °C (measurement C)
- heating-cooling cycle from 20 to 80 to 20 °C at 2 K/min (measurement D); sample kept at 80 °C for 5 min
- frequency sweep on the hot-mixed sample at 20 °C (measurement E)
- stress overshoot at 20 °C (measurement F)

Although generally in the literature the materials are mixed at elevated temperatures, we decided to study also the effect of cold mixing, because the statistical analysis approach might be sensitive to such differences in preparation. Gel formation by cold-mixed samples and during the heating-cooling cycle (measurements A and D) was followed by dynamic oscillation measurements at 1 Hz. For samples with a galactomannan concentration of 0.25% the strain applied during oscillation was 0.1, and the frequency sweeps ranged from 0.001 to 1 Hz. For the samples with a galactomannan concentration of 0.125% the strain during oscillation was 0.03, and the frequency sweeps ranged from 0.01 to 1 Hz. During the stress overshoot experiments a constant shear rate of 0.01 s⁻¹ was applied, and the stress was measured in time until the gel was yielded. The maximum stress (the yield stress) and the accompanying strain were determined from the time curve. Although the stress overshoot can be strongly dependent on the applied strain rate, we chose to select here only a single rate in view of the scope of this work. All experiments were performed at least in duplicate.

Intrinsic Viscosity. A concentration range of 0.005–0.020% galactomannan was prepared by dilution either in water or in a 0.1 M NaCl solution (1–4 g of a 0.25% solution in 50 g of solvent). Intrinsic viscosities were determined by measuring the kinematic viscosity for a concentration range of the galactomannan by means of an Ubbelohde tube viscosimeter at 20 °C. The Ubbelohde viscometer was calibrated with water. The viscosity was determined as duplicates (i.e., repeating preparation of the sample and measurement). Prior to a viscosity determination, undissolved plant material was allowed to settle. The settlement constituted only a negligible amount. The fluid on top was used for the measurement. The Ubbelohde was rinsed twice with the sample prior to the actual measurement. All experiments were performed at least in duplicate. The intrinsic viscosity $[\eta]$ was estimated by using the Huggins equation for extrapolation, which reads as follows: $\eta - \eta_0/(\eta_0c) = [\eta] + k_H[\eta]^2c$, where k_H is the Huggins constant and c the galactomannan concentration.

Classification of Solubility. In addition to the intrinsic viscosity and the physical properties, the solubility of 0.5 and 0.25% solutions

(after stir-heating until the boiling point) of the galactomannans was inspected both visually and microscopically. Solubility was qualitatively expressed as “good” [clear transparent solutions are obtained (<20% nonsoluble material on weight basis)], “medium +” [clear solutions with a minor contribution of nonsoluble particles (20–40% on weight basis)], “medium –” [solution with significant nonsoluble particles (40–60% on weight basis)], and “bad” (<60% of material can be dissolved). “Nonsoluble” is defined here as being pelleted by 10000g at 20 °C. The samples are prepared at least in duplicate.

Statistical Analysis. For the determination of the correlation between the structural and physical data SPSS 9.0 (SPSS Inc., Chicago, IL) was used. Structural characteristics used were (1) galactomannan type, (2) neutral sugar content, (3) combined galactose and mannose, (4) galactose content, (5) mannose content, (6) mannose/galactose ratio, (7) degree of substitution, (8) degree of blockiness, and characteristics deduced from HPLC analysis of mannanase-treated samples such as the (9) dimer/trimer ratio, (10) ratio of total amount of substituted mannan oligomer versus unsubstituted oligomers, (11) areas of the mannose mono-, di-, and trimer peaks relative to substituted fractions defined as S4 through S8 (see ref 4), and (12) molecular weight. Physical characteristics of galactomannans determined were as follows: solubility of 0.5 and 0.25% solutions (after stir-heating until the boiling point), intrinsic viscosity in water and 0.1 M NaCl (both intercept height and the slope of the specific viscosity/concentration versus concentration), and the physical properties of both 0.25 and 0.125% galactomannan and 0.25% xanthan gum mixtures. The latter measurements included sinusoidal oscillation (G' , $\tan \delta$ at 1 Hz), frequency sweep (G' at 0.001, 0.01, and 1 Hz), and stress overshoot (viscosity and maximal strain) of the cold-mixed sample (20 °C). After the sample had been heated to 80 °C followed by cooling to 20 °C, the same measurements were repeated. A Microsoft Excel spreadsheet program was employed for all calculations. A more detailed description of the analysis procedure is provided under Results and Discussion.

RESULTS AND DISCUSSION

Details on the fine structure of a whole series of galactomannans from various origins were presented in previous work (4). Here, a statistical analysis procedure is used to derive relationships between measured physicochemical properties of this series of carbohydrates and their previously reported fine

Table 2. Qualitative Assignment of Dissolving Properties of Galactomannans at Concentrations of 0.5 and 0.25% in 0.1 M NaCl^a

sample code	concn of stock solution		remarks on stock solution of 0.25%
	0.50%	0.25%	
mannan	bad	bad	many blobs
D 1:1	medium –	medium +	small fragments of cellular material
C12	medium –	medium +	small fragments of cellular material
L29A	good	good	
L29B	good	good	
L29C	medium –	medium +	small fragments of cellular material
L30	good	good	
L32	medium –	medium +	small fragments of cellular material
G26	medium –	medium –	few blobs
G62	medium –	medium –	few blobs
G64A	medium –	medium –	few blobs
G64B	medium –	medium –	few blobs
G65	medium –	medium –	few blobs
G66	medium –	medium –	many blobs
G67A	medium –	medium –	few blobs
G67B	medium –	medium –	few blobs
G67C	medium –	medium –	few blobs
G70	medium –	medium –	few blobs
T35	medium –	medium +	small fragments of cellular material
T36	medium +	good	

^aVisually inspected samples are classified according to “bad”, “medium –”, “medium +”, and “good” (see definitions under Materials and Methods). Boldfaced entries indicate samples selected for determination of physicochemical properties.

structure. This approach could contribute to the predictability of galactomannan functionality applied in food technological processes.

Dissolving Properties. The appearance of the solution and the dissolving properties for the series of galactomannans in 0.1 M NaCl tested are presented in **Table 2**. The samples were analyzed qualitatively only, because quantitative methods proved to be unreliable. Dissolving the galactomannans at a concentration of 0.5% appeared to be difficult, but not unexpected in view of the high content of mannose and galactose in these samples. Many samples still contained particles of swollen material. Dilution to a concentration of 0.25% resulted for a number of samples in a dissolution of the blobs. In some samples also traces of nonsoluble plant material could be identified. It is important to note that within groups of galactomannans (guar gum, LBG, etc.) large differences in solubility can occur between various suppliers [see, e.g., L29C and L30 (**Table 2**)]. For the rheological measurements and for the determination of the intrinsic viscosity only those samples were used that had solubility classified as “good” when dissolved at a concentration of 0.25%. Consequently, on the basis of the data presented in

Table 2 all guar gums and the mannan sample were excluded from further physicochemical measurements.

Rheological Data of 0.125% Galactomannan/0.25% Xanthan Gels. A summary of the results of the rheological measurements on the 0.125% galactomannan and 0.25% xanthan mixtures is presented in **Table 3**. For the cold gel formation experiments G' and $\tan \delta$ are given after an aging time in the rheometer of 15 min. Both for the cold and heated mixtures G' values are presented only for oscillation frequencies of 0.1 and 1 Hz. The reproducibility of the data is clearly illustrated by comparison of the G' -values of experiment A/D with those of measurement B/E at 1 Hz frequency. For the stress overshoot experiments (measurements C and F) the maximum stress and the accompanying strain are given. For measurement D the G' at 20 °C is presented after completion of a heating cycle and a delay time of 5 min at 80 °C. The maximum strain during oscillation was found to be 0.03. According to the literature this should be well in the linear region (18–21). The variation in the data of the overshoot experiments (measurements C and F) is larger than for the other measurements. This might be attributed to defects in the gel structure caused, for example, by entrapped air bubbles formed during the preparation of the mixtures that were unable to escape from the sample due to the high viscosity of the solution. Such defects have a much stronger effect on large deformation and fracture properties of gels than on small deformation properties (22). Also, plant insoluble material present in some of the galactomannans (**Table 2**) could contribute to formation of defects in the gel. For the cold mixtures LBG gels generally gave the highest G' values, whereas the lowest were observed for Diagam (D1:1). For the stress overshoot data no clear relationship with the source of galactomannan could be observed. Within the group of LBG significant variation in the G' values was found; especially L32 gave lower values for G' than the other LBGs. When the galactomannan/xanthan gum mixture was prepared at elevated temperature, (1) all G' values were higher compared to those for the same sample prepared at low temperature and (2) cassia gum behaved more like LBG, whereas the tara gums did not. The overshoot measurement of both the cold- and hot-mixed samples with Diagam did not show a conventional fracture stress–strain curve. The mixture appeared as a viscous slurry. These samples also exhibited a high value for $\tan \delta$.

Rheological Data of 0.25% Galactomannan/0.25% Xanthan Gels. Generally, G' values for 0.25% galactomannan/xanthan gel samples (not shown) were lower than those reported for the 0.125% galactomannan samples, and the difference between the various samples was less obvious. However, we

Table 3. Summarized Rheological Data of 0.125% Galactomannan + 0.25% Xanthan Gels^a

sample code	cold mix at 20 °C							hot mix after cooling to 20 °C					
	measurement A		measurement B,		measurement C		measurement D		measurement E,		measurement F		
	G' (Pa), 1 Hz	$\tan \delta$	G' (Pa)		stress (Pa)	strain	G' (Pa), 1 Hz	$\tan \delta$	G' (Pa)		stress (Pa)	strain	
		0.01 Hz	1 Hz					0.01 Hz	1 Hz				
D1:1	2.3 ± 0.1	0.55 ± 0.02	0.3 ± 0.1	2.2 ± 0.1	NR ^b	NR	2.1 ± 0.1	0.63 ± 0.04	0.2 ± 0.1	2.1 ± 0.1	NR	NR	
C12	9.2 ± 0.5	0.20 ± 0.02	5.7 ± 0.2	9.42 ± 0.5	1.0 ± 0.2	0.46 ± 0.13	37.7 ± 1.0	0.10 ± 0.01	36.1 ± 0.7	37.1 ± 0.9	12.0 ± 0.3	0.46 ± 0.02	
L29A	20.1 ± 0.1	0.10 ± 0.01	15.0 ± 0.1	20.4 ± 0.2	2.2 ± 0.1	0.57 ± 0.24	35.1 ± 0.6	0.07 ± 0.01	30.4 ± 1.2	32.6 ± 0.4	9.9 ± 0.5	0.62 ± 0.01	
L29B	19.0 ± 1.8	0.14 ± 0.05	14.7 ± 1.1	20.2 ± 0.8	2.3 ± 1.3	0.63 ± 0.31	40.4 ± 1.3	0.04 ± 0.01	37.1 ± 1.3	40.4 ± 1.5	14.8 ± 4.3	0.69 ± 0.13	
L29C	19.4 ± 0.2	0.09 ± 0.01	15.2 ± 0.2	19.7 ± 0.1	2.5 ± 0.2	0.37 ± 0.04	42.4 ± 4.1	0.02 ± 0.01	39.4 ± 3.7	42.2 ± 4.3	17.4 ± 0.3	0.49 ± 0.04	
L30	19.9 ± 0.2	0.11 ± 0.01	14.7 ± 0.5	20.2 ± 0.3	2.6 ± 0.7	0.50 ± 0.12	35.9 ± 0.6	0.06 ± 0.01	32.5 ± 0.6	35.6 ± 0.4	10.7 ± 1.8	0.58 ± 0.03	
L32	15.4 ± 0.3	0.14 ± 0.01	10.6 ± 0.4	15.6 ± 0.3	3.3 ± 0.6	0.54 ± 0.08	43.8 ± 0.3	0.03 ± 0.01	40.6 ± 0.3	43.5 ± 0.1	34.8 ± 9.5	0.76 ± 0.01	
T35	8.3 ± 0.5	0.26 ± 0.03	8.5 ± 0.4	8.5 ± 0.4	4.0 ± 0.1	0.86 ± 0.09	15.9 ± 0.2	0.12 ± 0.01	11.5 ± 0.2	15.9 ± 0.2	12.8 ± 3.9	0.98 ± 0.04	
T36	8.6 ± 0.4	0.26 ± 0.01	4.5 ± 0.5	8.7 ± 0.4	3.4 ± 0.5	0.66 ± 0.12	17.8 ± 0.9	0.12 ± 0.01	13.3 ± 0.1	17.9 ± 1.1	28.9 ± 8.8	0.89 ± 0.38	

^aThe type of measurement has been defined under Materials and Methods. ^bNR = determined but not included in table due to experimental uncertainties.

Table 4. Intrinsic Viscosity and Huggins Constants of Various Galactomannan Solutions^a

sample code	absence of salt		presence of 0.1 M NaCl	
	intrinsic viscosity (dL/g)	k_H	intrinsic viscosity (dL/g)	k_H
C12	3.4 ± 0.3	9.6 ± 0.9	2.1 ± 0.3	37.4 ± 3.8
L30	10.8 ± 1.2	1.0 ± 0.1	10.2 ± 0.2	0.7 ± 0.1
L29A	9.8 ± 0.1	1.1 ± 0.1	7.9 ± 0.6	3.0 ± 0.2
L29B	12.1	0.6	12.3 ± 0.1	0.6 ± 0.1
L29C	10.7	1.2	10.4 ± 0.1	1.3 ± 0.1
T36	12.1 ± 0.2	0.9 ± 0.1	12.1 ± 0.2	0.9 ± 0.2
L32	12.9 ± 0.9	0.3	9.8 ± 1.1	1.3 ± 0.2
D1:1	9.3 ± 0.6	1.5 ± 0.2	6.9 ± 0.7	6.3 ± 0.6
T35	10.9	1.3	10.1 ± 0.7	1.4 ± 0.3

^a Intrinsic viscosity ($(\eta - \eta_0)/(\eta_0 c)$) is determined by extrapolation to infinitely low galactomannan concentration, whereas the Huggins constant is calculated from the slope $(d\eta/dc)/[\eta]^2$.

considered the rheological measurements on the 0.125% galactomannan/xanthan gels (**Table 3**) to be more reliable, because it appeared to be difficult to select one condition under which all mixed gels displayed a linear behavior up to the maximum oscillation strain. Moreover, many of the galactomannans dissolved better at lower concentrations. It should be noted that the applied maximum strain of 0.1 should be in the linear region according to the literature, but this was only the case for part of these samples.

Intrinsic Viscosity Measurements. The intrinsic viscosities of the galactomannan solutions in the absence and presence of 0.1 M NaCl are listed in **Table 4**. The intrinsic viscosity reveals information on the hydrodynamic volume and molecular shape of the polymer in solution, whereas the slope of the plot of the specific viscosity versus the polymer concentration is related to intermolecular interactions. Lower values for the intrinsic viscosity are found for almost all samples in the presence of NaCl, except for L29B. Apparently, ionic interactions are involved in the viscosity behavior of the galactomannan solutions tested, which is unexpected for polymers reported to be solely composed of (neutral) mannose and galactose units (1, 2) (e.g., L29B consisting of >94% of galactose and mannose). However, in the presence of salt the intrinsic viscosities of solutions of the locust bean gums L30, L29B, and L29C and tara gums do not differ greatly, whereas cassia gum displays a clearly lower viscosity. D1:1, L29A, and L32 show an intermediate behavior.

Table 4 also presents Huggins constants for the various samples. In theory, higher values may indicate a stronger tendency of the macromolecules to aggregate by intermolecular interactions. The exceptional value found for L32 in the absence of salt might be artificial, in view of the “normal” behavior of this sample in the presence of salt. For most samples the presence of NaCl results in higher Huggins values. Such difference could qualitatively be interpreted as an increased intermolecular interaction of the polymers caused by a lowered solvent quality.

Statistical Analysis of Structure—Function Relations in Galactomannans. The structural characteristics used for the correlation studies of the structure and physical properties of galactomannans are listed in **Table 1**. The statistical analysis program SPSS enables the search for correlations between numerical data sets. For example, from the data of **Table 1** it can be observed that by selecting a certain type of galactomannan (e.g., guar gum or LBG) not only is a polymer of a certain degree of substitution (DS) chosen (DS of about 65 and 30%

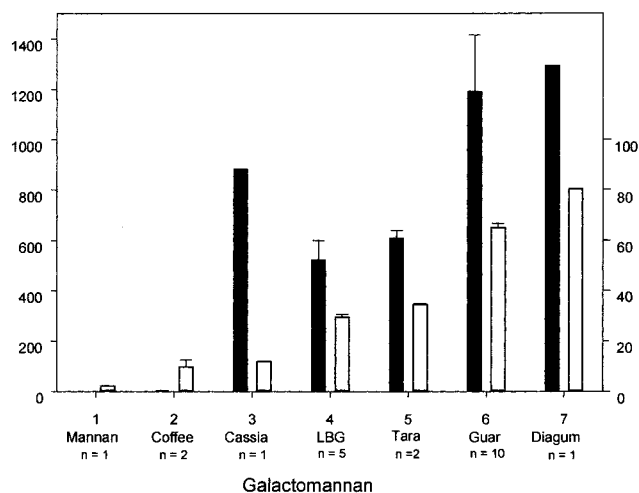


Figure 1. Degree of substitution (DS, %; white bars; right axis) and molecular weight (MW, ×10³; black bars; left axis) range for each type of galactomannan studied (n = number of samples).

for guar gum and LBG, respectively) but also polymers of a certain molecular weight (MW) range are chosen (average MW of 1200 and 500 kDa for guar gums and LBGs, respectively). Typical combinations of DS and MW for each type of galactomannan are illustrated in **Figure 1**. As a result of these two combined properties, the type of galactomannan employed can be expected to greatly affect the rheological properties of each of the samples studied.

For the data on the structural properties and for the physicochemical properties presented here Pearson and Spearman correlation coefficients were calculated. Pearson correlation reflects the linear association between two variables, whereas Spearman correlation is a nonparametric measure of the correlation between two series of ranked variables. Because of this feature, Spearman correlation is more sensitive in the differentiation of nonlinearly related variables (23). To enable a proper comparison, the correlation coefficients were converted to their absolute values. Next, the data sets with the 10 best Pearson correlation coefficients, including all fits >0.8, were ordered from high to low (not shown). From these data it can be observed that a number of correlations could be identified between the structural and physical characteristics determined. Because relationships between different *structural* characteristics are not the topic of this study, these correlations were ignored and will not be discussed in the remainder of this paper. Next, SPSS was used to produce scatter plots of these data sets to visually inspect the underlying relationships (not shown). In addition to this, “cross-correlation studies” were performed on the physical properties correlated with each structural characteristic to verify the relationship among those physical properties. **Table 5** shows the correlations identified after this analysis.

The best correlation between the structural and physical data of all galactomannans was found for the total substituted oligomer and nonsubstituted peak area ratio (GM/M ratio) and the G' at a frequency of 0.01 Hz of the heated mixture of 0.125% galactomannan and 0.25% xanthan gum (measurement E). Additional correlations were obtained for the G' at the end of the oscillation after the heating—cooling cycle (measurement D) and the G' at a frequency of 1 Hz (measurement E). These relationships are plotted in **Figure 2A**. From this figure it is apparent that a high GM/M ratio is correlated with a low G' . This can be explained by the fact that a high GM/M ratio is indicative of a large abundance of galactose substituents (branches) at a regular distance (4). Such structures will likely

Table 5. Best Absolute Pearson Correlation Coefficients after Visual Inspection of Scatter Plots and Cross-Correlation Studies of the Physical Data of Galactomannan Samples^a

measurement	GM/M ratio	measurement	code type	measurement	MW
E (0.01 Hz)	0.927	C (stress)	0.910	A (tan δ)	0.876
D (G')	0.918	D (G')	0.896	C (stress)	0.822
E (1 Hz)	0.913	E (0.01 Hz)	0.891	A (G')	0.820
C (stress)	0.895	E (1 Hz)	0.891		
F (strain)	0.890	F (strain)	0.876		
C (strain)	0.841				

^a All physical data are determined on 0.125% galactomannan samples.

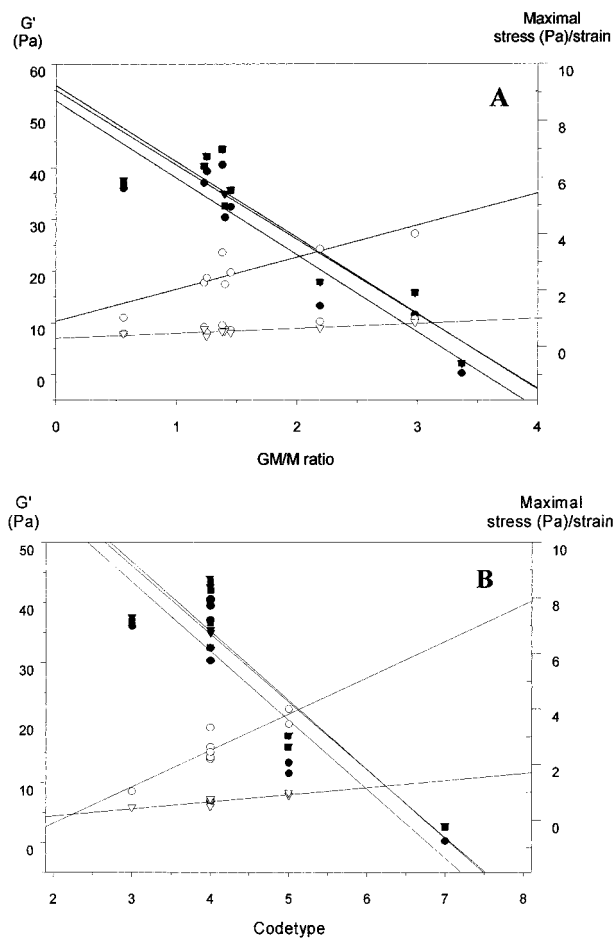


Figure 2. Physical properties correlated with total substituted oligomer and nonsubstituted peak area ratio (GM/M ratio; A) and the code type (B). Solid symbols correspond to G' and open symbols to maximal stress/strain: (●) measurement E, 0.01 Hz; (▼) measurement D, G' ; (■) measurement E, 1 Hz; (○) measurement C, stress; (▽) measurement F, strain.

prevent interaction with xanthan gum and, as such, disturb the formation of a network. In addition to this, the maximum stress of the cold mixture (measurement C) and the maximum strain of the heated mixture (measurement F) appeared to correlate with the GM/M ratio (Table 5 and Figure 2A).

Similar relationships were found between the physical data and the type of galactomannan (code type) employed (Table 5 and Figure 2B). This relationship is rather obvious because the GM/M ratio and the code type are also very strictly correlated ($r = 0.928$). These results indicate a good relationship between G' after heating and the average galactose distribution found for each of the types of galactomannans tested. Apparently, the

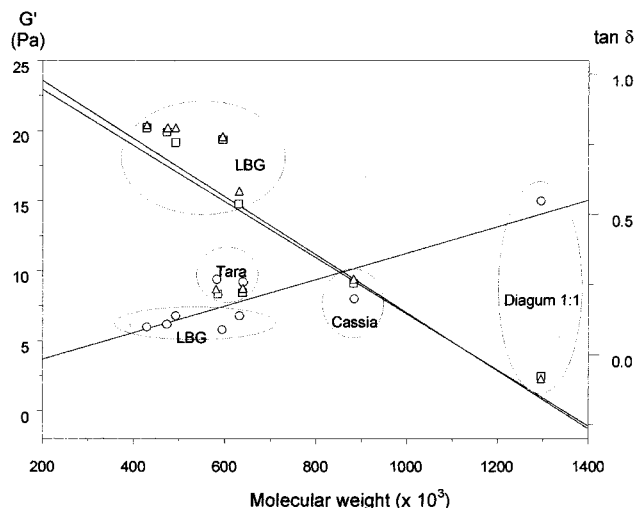


Figure 3. Physical properties correlated with molecular weight. Circles reflect measurement A (tan δ), squares measurement A (G'), and triangles measurement C (1 Hz).

dissimilarities that exist between the galactose distributions of different galactomannans of the same type (e.g., LBG) and their physical characteristics are small in comparison to the differences in the physical properties among the different galactomannan types. The fact that a correlation was found between the physical properties and the type of galactomannan indicates that the combined structural properties (e.g., DS and MW, as illustrated in Figure 1) of the galactomannan are responsible for its overall rheological behavior.

Correlations were also found between the MW and G' of the cold aged mixture (measurement A) and G' at a frequency of 1 Hz (measurement B) for the cold mixture of 0.125% galactomannan with 0.25% xanthan gum. These relationships are plotted in Figure 3. These data indicate that an increase in molecular weight results in a decrease in gel strength. This is unexpected. However, subdivision of the molecular weight data revealed that the type of galactomannan used was the underlying cause for the relationship between MW and G' values (Figure 3). Consequently, the molecular weight dependence found is only apparent. Also, a similar relationship between tan δ (measurement A) of the cold mixture of 0.125% galactomannan and 0.25% xanthan gum and MW was found. These results had the same subdivision, and it can therefore be assumed that the true effect is similar to the one described above. The smaller variation in tan δ for a certain class of the samples—in comparison to the G' data—presumably contributed to the somewhat higher correlation between the former and the molecular weight data.

At this point we want to stress that the molecular weights used in this work for correlation with rheological data are based on size exclusion chromatography. Consequently, the data may be flawed by differences in form factors of the different polymers. However, alternative molecular weight determination using a Viscotek setup produced only slightly different values but with similar trends between the different galactomannans (not shown). Re-evaluation of the statistical analysis using this latter data set as input for the statistical correlation analysis produced only small differences in Pearson correlation coefficients and does not affect the conclusions drawn here.

Statistical Analysis of Structure—Function Relationships in Locust Bean Gums. The effect of the structural characteristics on the physical properties of a single type of galactomannan was studied in more detail for the locust bean gum samples.

Table 6. Best Absolute Pearson Correlation Coefficients after Visual Inspection of Scatter Plots and Cross-Correlation Studies of the Physical Data of Locust Bean Gum Samples

measurement ^a	DB	measurement ^b	MW	area	visc slope (= $k_{sp}/[\eta]^2$)
A (G')	0.941	D (G' , 1 Hz)	0.936	monomer	0.995
B (G' , 1 Hz)	0.910	E (G' , 0.01 Hz)	0.936	dimer	0.968
visc slope	0.874	E (G' , 1 Hz)	0.918	trimer	0.915
B (G' , 0.01 Hz)	0.852	D ($\tan \delta$)	0.915	S4	0.908
				S5	0.907

^a Determined on 0.25% LBG samples. ^b Determined on 0.125% LBG samples.

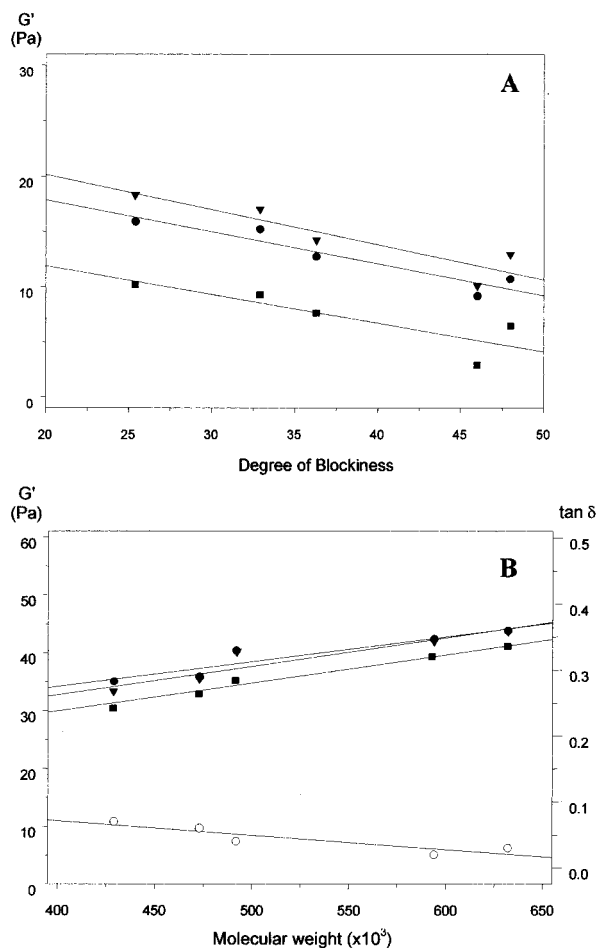


Figure 4. Physical properties of LBGs correlated with the degree of blockiness for a cold mixed 0.25% LBG (A) and molecular weight for hot mixed 0.125% LBG (B): (●) measurement A or D (G'); (▼) measurement B or E (G' at 1 Hz); (■) measurement B or E (G' at 0.01 Hz); (○) measurement D ($\tan \delta$).

This part of the study focuses on the correlation between differences in the distribution of galactose and the physical properties of the same type of galactomannan; any other relationship will not be discussed in the remainder of this paper with the exception of the effect of molecular weight. Careful inspection of all data in scatter plots and cross-correlation studies according to the procedure described above resulted in the relationships listed in **Table 6**.

The best correlation between the structural and physical data of the LBGs was found for the degree of “blockiness” (DB) and G' of the cold mixture of 0.25% galactomannan and 0.25% xanthan gum (measurement A). Additional correlations were found for the G' at frequencies of 0.001 and 1 Hz (measurement

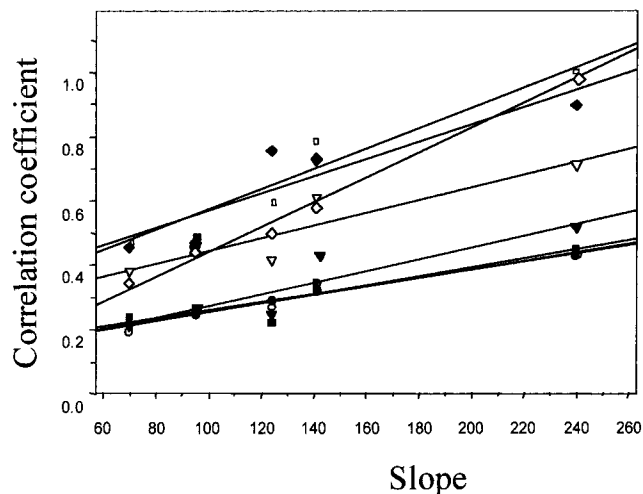


Figure 5. Structural properties of LBGs correlated with the slope of the specific viscosity/concentration versus the LBG concentration in 0.1 M NaCl: (▲) DB ratio (divided by 100); all other symbols reflect different integrated peaks of the elution profiles of hydrolyzed LBG samples [for details see Daas et al. (4)]; area of the (●) mannose monomer; (○) mannose dimer; (▼) trimer; (▽) galactose-substituted tetramer; (■) substituted pentamer; (□) substituted hexamer; (◆) substituted heptamer; (◇) substituted octamer.

B) of the 0.25% galactomannan mixture. These relationships (plotted in **Figure 4A**) indicate that an increase in the DB is coupled to a decrease in the G' of the cold-mixed gel. Because a high DB is indicative of the occurrence of large amounts of nonsubstituted regions in galactomannans, the latter relationship is not expected. When only one cross-link per nonsubstituted region would be possible, the formation of weaker gels with lower G' values could be explained by the lower absolute number of junction sites as a result of a higher DB. Also, a correlation between the slope of the specific viscosity/concentration versus concentration plot of the gums diluted in 0.1 M NaCl (visc slope) and the DB was observed (**Table 6** and **Figure 5** (gray triangles)). The nature of this relationship will be discussed below.

A correlation was also observed between the MW and the G' after a heating–cooling cycle (measurement D) of the mixture of 0.125% galactomannan and 0.25% xanthan gum. Additional correlations were obtained for the G' at frequencies of 0.01 and 1 Hz (measurement E) and the $\tan \delta$ (measurement D) of these mixtures. These relationships (plotted in **Figure 4B**) indicate that an increase in MW resulted in an increase in G' and a decrease in $\tan \delta$. This relationship can be explained by the fact that concomitant with an increase in MW the possible sites for xanthan–galactomannan interaction also increase, because the total number of “loose” ends in the sample decreases.

Finally, correlations exist between the enzymatic degradability of the LBG (the mono-, di-, tri-, and S4–S8 peak areas) and the slope of the specific viscosity/concentration versus concentration plot of the gums diluted in 0.1 M NaCl (visc slope). These relationships (plotted in **Figure 5**) show that an increase in the degradability of the sample is coupled to a increase in the slope of the specific viscosity/concentration versus concentration (visc slope). Because this slope is indicative of the effect of the increase in the concentration of the sample on the viscosity of the solution, it can be suggested that better degradable LBGs exhibit stronger mutual interactions than LBGs that are not well degraded. The latter demonstrates that the enzyme degradable

sequences in LBGs are very likely the same regions that are responsible for chain–chain interaction in these biopolymers.

Conclusions. During this study it became clear that it is a difficult and laborious task to establish reliable correlation between structural and physical data of galactomannans. However, careful comparison and analysis of “cross-correlations” of all relationships using statistical analysis software revealed a number of valid correlations. All correlations could be understood and explained with current insights, except for the inverse relationship between the DB and the gel strength of the locust bean gums. The unexplained correlation of the DB and gelling strength in LBGs is intriguing and requires additional studies. Because the physical properties of guar gums could not be reliably determined (20), structure–function correlation studies could not be performed for this type of galactomannan. Because the approach described in this work does yield relevant structure–function relationships, future work will certainly focus on determining reliable rheological properties of all guar gums and compare these data with the structural data of these samples and the physical and structural properties of other galactomannans.

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