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A comparative study on the compositions of crude and refined locust bean gum: In relation to rheological properties

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Abstract

In this study the composition of higher quality refined locust bean gum (rLBG) was compared with lower quality crude locust bean gum (cLBG) samples to understand the differences in functionality. M/G ratio has a great bearing on the viscosity and gelling properties of the material. The values obtained for M/G ratio of cLBG and rLBG samples range from 3.1 to 3.9, respectively, however high levels of arabinose were found in cLBG. This indicates that other polysaccharides, which could contain galactose or mannose, are present in the less refined materials. This would question the accuracy of using M/G ratio of cruder seed gums as a tool for predicting galactomannan content and so gelling properties. cLBG also contained higher levels of protein, fat, and ash which could also have an effect on functionality.

Keywords: Galactomannan; Locust bean gum; Composition; Crude; Refined

1. Introduction

Locust bean gum (LBG) is the preferred hydrocolloid for frozen desserts, cream cheese and other cultured dairy products. LBG, as a texturant, imparts a short, compact body to cream cheese giving superb "cuttability" and "mouthfeel". It is bland, very white in colour, and an excellent moisture binder in cream as well as other soft cheeses. In ice cream, LBG is unsurpassed for the control of heat shock, adds body to the mix, prevents lactose crystal formation (sandiness) and helps prevent formation of "sticky sugary syrup" when the ice cream is stressed.

Commercially available seed gums were found to differ significantly from the compounds which are normally used to study the viscosity and gelling properties of the gums. The behaviour, during thermal processing, of a higher quality refined locust bean gum (rLBG) was compared with a lower quality crude locust bean gum (cLBG) in an earlier study and published. The overall viscosity is a combination of solubilisation, degradation and temperature dependence of viscosity (Kök et al., 1996). The functionalities of the samples were greatly influenced by the non-galactomannan fraction. Studies also indicated that the thermal stability of the galactomannan fraction still differs between the refined and crude samples (Kök, Hill, & Mitchell, 1999).

The composition of locust bean gums plays an important role in their rheological properties however; they are often not commercially available in purified forms and contain significant amounts of non-galactomannan material. A current problem is a lack of research on the non-galactomannan fraction of commercially used gums.

To obtain seed gums the endosperm is removed from the husk and germ of a carob seed (*Ceratonia siliqua*). For this purpose the husk is softened and loosened with water, then endosperm removed by grinding to the required granulation. The fine powder produced is the seed gum. The typical composition of a high purity LBG material is moisture 10–13%, protein 5%, ash 1%, fibre 1% and the remainder is galactomannan 80–85% (Maier, Anderson, Karl, & Magnuson, 1993).

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The separation of the testa (husk) and endosperm is incomplete, therefore impurities are often found in the commercially sold gum. This determines the quality of the gum in terms of its functional properties in relation to viscosity and gelling (Glicksman, 1969; Fox, 1992). These impurities are reported to include 4% pentosan, 1% cellulosic material, 1% ash and 6% proteinaceous material. The latter is in part covalently bound to the polysaccharide. Major amino acids present in the proteinaceous material of LBG, in decreasing order, are glutamic acid, aspartic acid, glycine, arginine, alanine and serine (Anderson, 1985).

The aim of this research is to compare the composition of a higher quality rLBG with a lower quality cLBG.

2. Materials and methods

The LBG samples used in this study were obtained from various commercial sources. As well as testing the native powder samples the particulates, supernatants and ethanol precipitates of supernatants were also subjected to analysis. In addition a carob fruit sample was obtained, dried and ground, and sugar analysis conducted on the seed pod, the husk and the remainder of the seed (mainly endosperm). This fruit originated from the Mediterranean region of Turkey.

All the other chemicals used were from Fisons Chemicals Ltd., UK.

2.1. Sample preparation

2.1.1. Dispersion and solubilisation

Gums were dispersed in water, at ambient temperature using a high shear Silverson mixer and left over night to hydrate. Fresh samples were produced for each experiment and not used more than 24 h after being prepared. Longer hydration periods allow bacterial contamination. Dispersions were kept at 4 °C between measurements in order to protect from bacterial or enzymic activities. Samples were prepared at the desired concentration and mixed at high shear for 2 min. Solubilisation conditions were defined as an hour at 70 °C. This was achieved using a water bath with samples being continuously stirred by a bench mixer (Griffin and George Ltd., UK) at low speed. These conditions were chosen, as they were thought not to cause significant degradation.

2.1.2. Heat treatments

Heat treatments were carried out using the same equipment as that used for solubilisation, the exception being for those retorted. A small autoclave (Benchtop-50, Harvard/LTE Ltd., England) was used to achieve high temperature treatment up to 121 °C. Samples were contained in media bottles (20 or 250 ml). Time and temperature of heat treatments varied according to the experiment being conducted.

2.1.3. Separation of impurities from cLBG samples

After the normal dispersion process, samples were heat treated at the desired temperature and cooled to room temperature. Cooling was followed by centrifugation in a MSE 6–75 (Mistral 6L, England) High-Speed Centrifuge at a rate of 18,500*g* for 15 min. At the end of centrifugation supernatant (solubilised fraction) was recovered from non-soluble impurities (particulates). The dry weight of the supernatant and particulates were determined to indicate the level of impurity of the cLBG samples. Supernatants were also kept at 4 °C between the measurements in order to protect them from bacterial or enzymic activities.

2.1.4. Ethanol precipitation

LBG (10 g) was dissolved in 0.5 l distilled water at room temperature. A water bath was used to heat samples at 70 °C for 1 h, whilst they were stirred continuously. Samples were left over night to cool and then centrifuged (18,500*g*, for 2 h). Supernatant was recovered by gravity draining overnight. An equal volume of pure ethanol (98%) was added to the supernatant, precipitant was freeze dried, and ground for 30 s in a Moulinex Grinder to achieve a fine powder. The yields from initial samples were recorded.

2.2. Analysis of composition

2.2.1. Moisture content

In order to calculate moisture content, native samples of 1 g were held in an oven at 105 °C and weighed regularly until readings stabilised.

2.2.2. Protein analysis

Protein analysis was undertaken in liaison with the Applied Biochemistry Section at Nottingham University and a commercial food laboratory. The amount of nitrogen was measured in approximately 0.25 g of sample using the Kjeldahl technique and converted into percentage of protein using the conversion factor of 6.25.

2.2.3. Fat analysis

Soxhlet analysis was undertaken in liaison with the Applied Biochemistry Section at Nottingham University and was used to determine the fat in the samples. Petroleum ether (40–60 °C) was used as a solvent to extract the fat in approximately 10 g of native sample, at 100 °C for 10 h (5 + 5 h).

2.2.4. Ash analysis

Ash analysis was also carried out in liaison with the Applied Biochemistry Section at Nottingham University. The samples were burned to ash for 10 h (5 + 5 h) in a muffle furnace at 550 °C.

2.2.5. Total sugar analysis of galactomannans

The quantity of total sugar was estimated as the remainder of the sample after other substances (protein, fat, ash, and moisture) were accounted for.

A standard GLC method was carried out routinely in this study in order to establish the nature of the sugar distribution. This was conducted in liaison with the Applied Biochemistry Section at Nottingham University. A paper chromatographic method was also used, in liaison with the Institute of Chemistry, Slovak Academy of Science.

2.2.5.1. GLC technique. Neutral sugars were measured using gas liquid chromatography (GLC) as alditol acetates following hydrolysis by sulphuric acid as described by Englyst, Wiggings, and Cummings (1982).

Galactomannan samples, 20–50 mg were dissolved in 25 ml of 1 M H_2SO_4 and boiled at 100 °C for 2 h (except where indicated) in a water bath. After cooling 3 ml of hydrolysate was removed and mixed with 0.5 ml internal standard (at a concentration of 1 mg allose/ml in 50% saturated benzoic acid).

Ammonium hydroxide, 1.2 ml of 12 M was added to 3.5 ml (3 + 0.5 ml) solution and mixed with 0.4 ml of freshly prepared solution of 3 M ammonium hydroxide (containing 50 mg of sodium borohydride/ml). This mixture was left for 1 h in a water bath at 40 °C. Glacial acetic acid (0.5 ml) was added to the solution and mixed again. A 0.5 ml aliquot of acidified sample was removed and 0.5 ml of 1-methylimidazole as well as 5 ml of acetic anhydride added.

The solution was mixed and left for 10 min. After this holding period 0.6 ml ethanol (96%) was added, it was mixed again and left for 5 min; 5 ml of distilled water was added, mixed and left for a further 5 min. Indicator solution, 0.5 ml (bromophenol blue 0.04%) was added and the sample placed in cold water to aid dispersal of heat and 5 ml of 7.5 M potassium hydroxide was added. A few minutes later a further 5 ml of 7.5 M potassium hydroxide was added. The tubes were capped, mixed by inverting and left until the mixture had separated into two phases. Some of the clear upper phase was pipetted out ensuring that none of the blue lower phase was included. The pipetted upper phase was transferred to a small vial for GLC analysis. The Standard was prepared with 0.5 ml each of 2.5 mg/ml glucose, arabinose, mannose, galactose, rhamnose, and xylose solution, mixed with 0.5 ml internal standard and treated as the gum sample before being passed through GLC.

GLC analysis was carried out by a 0.5 μl of sample being injected into a Supelco SP2330 column (30 m × 0.75 mm). Initial temperature of 200 °C in the system was increased to 240 °C at a rate 4 °C/min. The carrier gas used was Helium with a flow rate of 5 ml/min. Recoveries were expressed as a percentage of the dry weight of material hydrolysed. Peak assignments were confirmed by comparison with the appropriate monosaccharide.

2.2.5.2. Paper chromatography technique. The assay was carried out in liaison with the Institute of Chemistry, Slovak Academy of Science. Samples of six different sources of cLBG samples were examined for total sugar content as well as sugar distribution. cLBG6 (2%) was also tested in its heat-treated form at 25, 70, 90, 121 °C for an hour and 90 °C for 2.5 h. Their supernatants were recovered by centrifugation as described in Section 2.1.4. Supernatants and particulates were dried at 100 °C over night and ground to a fine powder for the assay.

The method used was as described by Fengel, Wegener, Heizmann, and Przyklenk (1977). Hydrolysis of 5 mg samples were achieved in 1 ml of 2 M trifluoracetic acid under reflux for 2 h. The hydrolysate was filtered (S4 glass filter) and the residue washed several times with distilled water, the hydrolysate finally being dried in a vacuum oven. If the samples contained insoluble fractions, these were removed from the hydrolysate by centrifugation or filtration. After drying these insolubles (acid resistant portions) were weighed.

The sugar composition was estimated qualitatively. Part of the hydrolysate was diluted to 1% solution and 100–200 μl of it applied to a paper chromatogram (Whatman No: 1). The chromatography was performed in the descending manner using the solvent system: ethyl acetate–pyridine–water, 8:2:1 (v/v) for 20 h. The chromatograms were dried in air and the sugars were detected by spraying with anilinium hydrogen phthalate in acetone followed by heating at 100 °C for 3–5 min.

3. Results and discussion

3.1. Appearance of the locust bean gum samples

The milling process determines the size and the colour of the end product. Increasing the time of the milling process will produce a finer product; however the powder colour may darken due to high temperatures being produced in the process. The colour and the particle size are also an indication of the presence of impurities (Maier et al., 1993; Seaman, 1980).

The test samples showed great differences in particle size and colour. In general, rLBG samples are lighter in colour and smaller in particle size (flour like), in comparison to cLBG samples. Great variation in colour and texture was also found between the seven cLBG samples.

When examined under a microscope it can be seen that rLBG has finer endosperm particle sizes and no observable particulates whereas cLBG varies in endosperm particle sizes and does contain some particulates. The appearance of particulates is as large brown flakes. The success of purification, by alcohol precipitation, is evident by the lack of particulates in ethanol precipitated rLBG samples.

In practice lower grade (cLBG) galactomannan sources are frequently used in canned products. These differ substantially from higher quality (rLBG) samples in composition with high levels of non-galactomannan material being present. The cLBG material used in this study was found to contain a large amount of material (40% of dry weight), termed "particulates", which remained insoluble after heating to 70 °C (Table 1). The variation of particulate

Table 1
Compositional analysis of the samples using oven drying, Kjeldahl, Soxhlet, and GLC

	Compositional distribution of total material (%)					Fractions^{+} (%)		Sugar distribution	
	Moisture	Protein	Fat	Ash	Total* carbohyd.	Sup	Par	% GLC recovery	M/G
rLBG1	11	6.5	0.6	1.0	81	87	0	95 ± 6	3.9 ± 0.2
cLBG1	10	10.3	2.0	2.1	76	80	7	60 ± 6	3.3 ± 0.1
cLBG2	9	11.8	1.8	2.8	75	50	38	51 ± 4	3.3 ± 0.0
cLBG3	9	10.9	1.6	2.6	76	58	27	67 ± 1	3.1 ± 0.1
cLBG4	10	16.8	1.5	3.5	68	70	15	81 ± 3	3.4 ± 0.0
cLBG5	11	13.5	1.3	2.7	73	52	33	71 ± 2	3.3 ± 0.0
cLBG6	11	13.5	1.3	2.7	73	52	33	71 ± 2	3.3 ± 0.0
cLBG7	8	17.1	1.3	3.7	70	50	36	70 ± 2	3.2 ± 0.2

Supernatants recovered by 18,500 × g centrifugation for 15 min, after 70 °C/h solubilisation. (+) Solubilised and non-solubilised fractions after 70 °C/h heat treatment. (Par) particulates, (Sup) supernatant, (*) calculated from total, (±) standard deviation.

content between samples can be observed easily. Notable differences are the clarity of the solutions, varying amount of particulates shown by darker areas and distinct phase separation for some samples.

3.2. Gross composition of seed gum samples

The gross compositions of the gums are given in Table 1. This table of compositional analysis compares the composition of a typical rLBG preparation with cLBG materials from seven different sources. The values for the rLBG compare favourably with expected values (Maier et al., 1993; Gaisford, Harding, Mitchell, & Bradley, 1986). The major difference for cLBG samples is the high protein contents that were recorded. The expected protein content is about 5–6% for the good quality samples; however, cLBG samples exceeded this level, averaging 13.4% with a maximum of 17%. GLC studies were undertaken in order to establish the composition of samples with respect to sugar distribution. The recoveries were diverse, especially for cLBG samples; however, the results were still within the range of reported M/G values (Fox, 1992; Gaisford et al., 1986; McCleary, Clark, Dea, & Rees, 1985). Later studies attempted to increase GLC recovery by increasing the acid concentration and the digestion time. However this had limited success.

3.3. Determination of sugars

Galactomannans are linear polysaccharides based on a backbone of β(1–4)-linked L-mannose residues. Single α-D-galactose residues are linked to the chain by C-1 via a glycosidic bond to C-6 of mannose. The degree of galactose substitution varies from one botanical source to the next as well as between molecular species of one gum.

The functionality of seed gums depends on their sugar composition and it is believed that substitution levels are responsible for differences in behaviour between the gums (Sand, 1982). Determination of the sugar profile of different galactomannan samples available to this study was undertaken in order to quantify differences in the degree of galactose substitution.

It is generally reported that the galactose content of guar is greater than that of LBG and Tara gum. The average mannose to galactose ratio (M/G) in LBG is in the region of 4.0 and Tara 3.0 compared to GG ratios reported between 1.7 and 2.0 (Dea, 1979, 1981; Cairns, Morris, Miles, & Brownsey, 1986; Fernandes, Goncalves, & Doublier, 1991). This greater degree of substitution in guar enhances solubility as the presence of side-chains interferes with the formation of stable crystalline regions and promotes water penetration (Nussinovitch, 1997). Different M/G ratios within gum species have also been reported. Fernandes et al. (1991) have reported M/G ratios for two different industrial LBG gum samples as; 3.6 and 4.0 with 3.5, 3.8, and 4.0 for their laboratory LBG samples.

During this analysis of the sugar distribution two major issues arose; the sugars detected and the levels of recovery. It had been assumed that the only sugar components in these gum samples would be galactose and mannose. However these supplied gum samples often contained up to 7% glucose and 3% xylose (Tables 3 and 4). A third large and unexpected sugar peak also appeared occasionally on the trace (Fig. 1). Careful checks were made using standard sugars and it would appear that the unknown sugar was L-arabinose. The levels of arabinose could be very high. In some gum samples over 45% (as high as 51%) of the sugars would seem to be arabinose (Table 4). Therefore a review was made of arabinose presence in seed gums reported in previous research.

3.4. Presence of arabinose in seeds and seed gums

Arabinose levels, occurring as a free sugar, are normally low (Shiba, Yamada, Hara, & Okada, 1993; Cleemput, Roels, Vandort, Grobet, & Delcour, 1993). This sugar normally occurs in a side chain where xylose (arabinoxylan) or galactose (arabinogalactan) forms the backbone. Structures are shown in Figs. 2 and 3.

Arabinoxylan isolated from rice endosperm cell wall, was composed of the linear backbone of an α-1,4-linked β-xylose residue highly substituted at the c3 position with α-L-arabinofuranose (Yui, Imada, Shibuya, & Ogawa, 1995). In wheat bran polysaccharide the main constituent

Table 2
Sugar distribution of cLBG native samples and cLBG6 heat treated samples obtained using paper chromatographic technique

Native	Sugar distribution
cLBG1	***
cLBG2	**
cLBG3	**
cLBG4	**
cLBG5	**
cLBG6	**
cLBG7	**
Heat treated cLBG6[a]	
S 25 °C/h	***
S 70 °C/h	man + gal: 80%, xyl: 15%, ara, glc: <5%
S 90 °C/h	***
S 90 °C/2.5 h	***
S 121 °C/h	**
P 25 °C/h	man + Gal: 70%, ara: 25%
P 70 °C/h	*
P 90 °C	*
P 90 °C/2.5 h	*
P 121 °C/h	man + gal: 50%, ara: 30%, glc: 15%, xyl: 5%

[a] S, soluble; P, particulates (insoluble).
* man + gal: ⩽50%, ara ~45%.
** man + gal: ~90%, ara >8%.
*** Purest: man + gal: ~95%.

is arabinoxylan (AX), and in grains other than oats and barley the major hemicellulosic polysaccharides in the soluble fractions are mainly AXs (Shiba et al., 1993; Marlett, 1993). Experimentation on rye bran AX dispersions has indicated that there are two main AX types, water-insoluble (WISX) and water soluble (WSX), which together account for over 60% of cell wall polysaccharide (Ebringerova & Hromadkova, 1992).

The literature review did not bring to light much research concerning arabinogalactans in terms of structural characteristics and their rheological properties. Arabinogalactans consisting of β-D-(1 → 3) galactan backbone and four kinds of arabinogalactan side chain, including α-L-(1 → 3,5) arabinan side chains, have been reported in the roots of the medicinal herb *Angelica acutiloba* (Kiyohara, Zhang, & Yamada, 1997).

Arabinose polysaccharides, when dissolved, can impart high viscosities; intrinsic viscosities of 80–310 cm^3/g have been reported for rye bran AX (Ebringerova, Hromadkova, & Berth, 1994).

A major literature search did not highlight any reports of high arabinose contents in locust bean gum samples. The normal LBG of commerce is usually a white powder with the typical composition, galactomannan 88%,

Table 3
Analysis of sugar distribution of the native, 70 °C/h solubilised (supernatants) and ethanol precipitated LBG samples using GLC

Samples	Man	Gal	Arab (%)	Xyl	Glc	GLC recovery	M/G
rLBG1 (NP)	78	20	1	0	1	95 ± 6	3.9 ± 0.2
rLBG1 (sup)	78	22	0	0	0	77 ± 27	3.6 ± 0.3
rLBG1 (EP)	79	22	0	0	0	94 ± 5	3.7 ± 0.5
*						89 ± 13	3.7 ± 0.3
cLBG1 (NP)	65	20	10	1	5	61 ± 6	3.3 ± 0.1
cLBG1 (sup)	75	24	1	0	0	67 ± 27	3.2 ± 0.4
cLBG1 (EP)	75	22	4	0	0	78 ± 4	3.3 ± 0.2
*						69 ± 12	3.3 ± 0.2
cLBG2 (NP)	61	18	13	3	4	51 ± 4	3.3 ±0.0
cLBG2 (sup)	78	22	0	0	0	52 ± 31	3.6 ± 0.4
*						51 ± 18	3.4 ± 0.2
cLBG3 (NP)	63	21	10	2	4	70 ± 1	3.1 ± 0.1
cLBG3 (sup)	77	23	0	0	0	55 ± 33	3.3 ± 0.0
*						61 ± 17	3.2 ± 0.1
cLBG4 (NP)	53	16	27	1	4	81 ± 27	3.4 ± 0.0
cLBG4 (sup)	78	22	0	0	0	67 ± 35	3.6 ± 0.3
cLBG4 (EP)	76	25				95	3.1
*						81 ± 31	3.3 ± 0.1
cLBG5 (NP)	52	16	25	2	6	71 ± 21	3.3 ± 0.0
cLBG5 (sup)	77	21	1	0	1	74 ± 24	3.5 ± 0.3
cLBG5 (EP)	79	21	0	0	0	71 ± 14	3.7 ± 0.4
*						72 ± 20	3.5 ± 0.2
cLBG6 (NP)	50	16	27	3	5	69 ± 19	3.2 ± 0.2
cLBG6 (sup)	75	22	3	0	0	51 ± 47	3.5 ± 0.9
*						60 ± 33	3.3 ± 0.5
rLBG2 (NP)	78	20	0	0	2	93 ± 9	3.9 ± 0.3
rLBG2 (sup)	78	22	0	0	0	73 ± 49	3.5 ± 0.1
*						83 ± 29	3.7 ± 0.2

Results are given in total weight percentage (%).
(NP) native powder; (sup) supernatant; (EP) ethanol precipitated; (±) standard deviation: for replicates of (NP) see Table 1, (*) average of total.

Table 4
Replicated sugar composition analysis of native cLBG4 and cLBG6 samples obtained using GLC technique

Samples	Total recovery (%)	M/G	Man (%)	Gal (%)	Arab (%)	Xyl (%)	Glc (%)
cLBG4	62	3.4	68	20	6	1	4
cLBG4	100	3.4	38	11	47	1	3
cLBG6	54	3.3	63	19	8	2	7
cLBG6	57	3.2	62	19	10	2	7
cLBG6	57	3.3	64	19	9	0	7
cLBG6	93	3.3	38	11	46	1	4
cLBG6	96	3.2	34	11	51	1	4

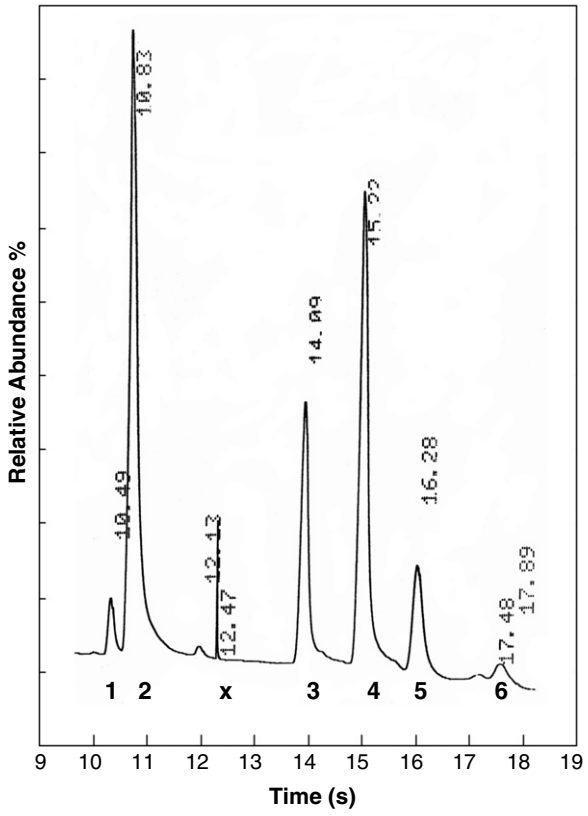

Fig. 1. GLC chromatogram of cLBG4. The peak assignments were: (**1**) D-arabinose, (**2**) L-arabinose, (**3**) allose (added as internal standard), (**4**) mannose, (**5**) galactose, (**6**) glucose, (**x**) unidentified.

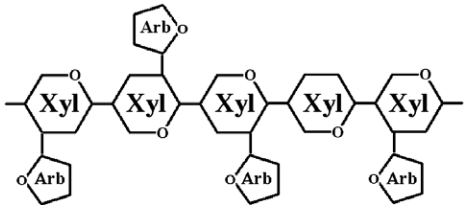

Fig. 2. Structure of Arabinoxylan (Girhammar et al., 1986).

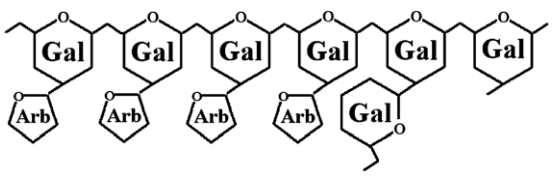

Fig. 3. Structure of Arabinogalactan (Girhammar et al., 1986).

pentosan 3–4%, protein 5–6%, cellulose 1–4%, and ash 1% (Glicksman, 1969).

In this study the levels of xylose detected by GLC for the cLBG samples are low with respect to the arabinose levels (Table 4). The low levels of xylose would indicate that typical arabinoxylans were not present in the samples. If an arabinoxylan was present the xylose would be expected to be present in higher amounts than the arabinose (typically values is a ratio of 0.5–1.0% arb/xyl). This was also confirmed by the enzymic work using xylanase. The viscosity of cLBG6 (2%) showed no marked drop in viscosity when xylanase was added. Hence, it could be reasoned that arabinoxylases were not present in any significant amount that could contribute to the viscosity.

Therefore it would seem possible that an arabinogalactan is present. Although, the amount of galactose is not great, the M/G ratio recorded in the samples is lower than expected. For example an average of 3.2 found for cLBG3, compared to a typical value of 4 quoted in literature (Fox, 1992).

However the presence of a polysaccharide containing arabinose is still not clear. There may be a fraction unaccounted for as the GLC method does not detect charged sugars. The missing fraction within the analysis might be the material linking the pentoses. For example high levels of glucuronic acid and uronic acid have been reported in association with other sugars in gum arabic, tragacanth and ghatti (Aspinall, 1970).

3.5. Inconsistency of recovery levels

Another feature of the GLC sugar studies was the marked differences in recovery for the samples. Gross analysis of the samples indicated that some had high levels of protein, up to 17% (Table 1). However, at least 70% of the product should be carbohydrate. For some samples the sugar analysis indicated recoveries as low as 30%. The recovery values were not reproducible and for the same sample 35–100% recovery has been recorded. For example, Table 4 shows the inconsistency in recovery and arabinose levels for cLBG4 and cLBG6. It is notable, in the same table, that a high arabinose level was only detected when the recovery levels were high.

In an attempt to increase the recovery rate the process was altered by increasing H_2SO_4 concentration and the time for digestion. Typically all the recovery values

Table 5
Sugar distribution of the soluble material of LBGs at 70 °C and 121 °C

Solubles	Total recovery (%)	M/G	Man (%)	Gal (%)	Arab (%)	Xyl (%)	Glc (%)
cLBG1 *	83 ± 3	3.3 ± 0.5	75	23	2	0	0
cLBG1 **	89	3.0	75	25	0	0	0
cLBG2 *	74	3.8	79	21	0	0	0
cLBG3 *	78	3.3	77	23	0	0	0
cLBG4 *	87 ± 8	3.6 ± 0.4	78	22	0	0	0
cLBG6 *	83 ± 8	3.8 ± 0.2	65	17	1	0	1
cLBG6 **	87 ± 14	3.3 ± 0.3	49	13	19	0	1
cLBG7 *	85	2.9	70	24	5	0	0
rLBG1 *	100 ± 4	3.8 ± 0.2	78	21	1	0	0
rLBG1 **	96	3.3	77	23	0		0
rLBG2 *	100	3.6	78	22	0	0	0
rLBG2 **	100	3.4	77	23	0	0	0

The results given as averages with the standard deviation of duplicates (±).
* 70 °C Treatment is for 1 h.
** 121 °C Treatment is for 30 min.

increased with this alteration. However, it must be noted that this may cause changes to some sugars present. To try and use the data to compare samples, the decision has been made not to include data when recoveries are less than 50% for the cLBG samples and 70% for the purified samples rLBG.

When the samples are solubilised (70 °C/h) or heat-treated (121 °C/30 min) the recovery of gas liquid chromatography (GLC) increases significantly (Table 5), thus indicating that it is the insoluble fraction that determines the recovery levels of the samples. Arabinose content of the soluble fractions was low, with the exception of cLBG6 when heat-treated at 121 °C for half an hour. It is possible that high temperature treatment forces some insoluble material, such as arabinogalactans, to become soluble.

Particulates of cLBG4 and cLBG6, recovered from centrifugation after samples were solubilised, contained significantly high arabinose levels, as high as 64% of total carbohydrate (Table 6). Heat treatments of 100 and 121 °C have improved the GLC recoveries a great deal. Surprisingly recovery was also good at ambient temperature, but as expected poor at 70 °C with cLBG6 recovery as low as 36%.

Ethanol precipitated samples did not show any trace of arabinose at all with the exception of cLBG6 (Table 7). Even for cLBG1 the amount of arabinose is very low at 4% thus allowing us to assume that these samples consist mainly of galactomannan. The recoveries of GLC were also reasonable, ranging from 71 to 94%.

The recovery of GLC dropped considerably to 53%, as expected, for the husk from Turkish carob seed. In contrast GLC recovery of the locust bean gum (endosperm) from this seed was high being 85% with a high M/G ratio of 4.0 (Table 8).

Table 6
Sugar distribution of particulates (insoluble fraction) of locust bean gum recovered by centrifugation (except*) before being subjected to GLC

	Total recovery (%)	M/G	Man (%)	Gal (%)	Arab (%)	Xyl (%)	Glc (%)
cLBG6 (25 °C)	89	4.4	38	9	52	2	–
cLBG6 (70 °C)	36 ± 1.8	6.4 ± 4.2	43	9	41	6	1
cLBG6 (100 °C)	94	2.9	23	8	64	3	2
cLBG6 *	42	3.3	41	12	39	7	2
cLBG6 (120 °C)	95	3.0	23	8	64	3	2
Average	71 ± 0.9	4.0 ± 2.2					
cLBG4 (70 °C)	55	28.1	68	2	30	–	–
Husk (native)**	53	3.1	54	18	17	4	7

* Particulate recovered by filtration (cheese cloth) and washed with 100 °C distilled water.
** Turkish origin LBG husk obtained from whole seed, (–) not detected, (±) standard deviation, (+) out of four readings.

Table 7
Sugar distribution of the ethanol precipitated LBG samples

	Total recovery (%)	M/G	Man (%)	Gal (%)	Arab (%)	Xyl (%)	Glc (%)
rLBG1	94 ± 4.6	3.7 ± 0.5	79	22	–	–	–
cLBG1	78 ± 4.2	3.3 ± 0.2	75	22	4	–	–
cLBG6	71 ± 14	3.7 ± 0.4	79	21	–	–	–

(–) Not detected, (±) standard deviation of duplicates.

Table 8
Sugar distribution of whole carob fruit obtained from Turkey; containing endosperm (LBG), husk and the fruit

	Total recovery (%)	M/G	Man (%)	Gal (%)	Arab (%)	Xyl (%)	Glc (%)	Unknown (%)
LBG	85	4.1	78	19	1	0	1	–
Husk	53	3.1	54	18	17	4	7	–
Fruit*	51	5.2	7	1	3	6	74	9

(*) Excluding seeds, (–) not detected.

Paper Chromatography analysis also showed that the presence of arabinose ranged between 10 and 45% (Table 2). This technique only determines the sugars on a qualitative basis compared to GLC (which is also quantitative). Although there is a wide range in the data obtained, the presence of high arabinose content is clear. For example sugar composition of cLBG6 in duplicate gave two different profiles; mannose and galactose total was about 70% followed by 25% arabinose with less than 2% glucose and xylose, alternatively mannose and galactose were about 90% with 8% arabinose and the rest is again estimated as glucose and xylose. Another finding of this technique was that insolubles are non-solubilised forms of lignin, proteins and some condensed material from sugars.

4. Conclusion

The values obtained for M/G ratio of crude and refined LBG samples range from 3.1 to 3.9. This is in agreement with the literature (Gaisford et al., 1986; McCleary et al., 1985) for refined samples (~3.7), but lower for crude samples (Fernandes et al., 1991). The range is an indication of the differences between the species as well as the variation found within *Ceratonia siliqua* (LBG) from different sources. In reality, the limitation of separation of the testa (husk) from endosperm allows impurities in the commercially sold gum and obviously this has a direct impact on sugar composition. Research has shown that purifying LBG by the filtration process favors inter and intramolecular associations. Systematic differences were observed for the intrinsic viscosity and Huggins' constant values determined for samples of the same galactomannan purified by different purification procedures (Azero & Andrade, 2002).

The levels of arabinose are shown to be higher in cLBG samples. Sugar analysis by paper chromatography suggests that the particulates contain high levels of arabinose (Table 2). GLC analysis of whole cLBG samples showed significant levels of arabinose while supernatants of the same samples contain little or none, confirming that arabinose is largely found in the insoluble fraction (Table 3). GLC studies on the particulate fraction of cLBG6 further confirm the high levels of arabinose (Table 6).

A major literature search has not highlighted any report of high arabinose contents in LBG samples. However the relevance of high levels of arabinose containing material is reported when occurring in seed and cereal preparations. Its presence often changes water holding capacity of the system (Ebringerova et al., 1994). The arabinose is not known to be a component of seed gums; however it may be from a co-extracted arabinan, arabinogalactan or even a coextracted xylan (Dea & Morrison, 1975). The levels of xylose detected by GLC for the cLBG samples are low with respect to the arabinose level. As the xylose would normally be present in higher amounts than the arabinose (typically values for an arabinoxylan are ratios of 0.5–1.0% ara/xyl) it would seem possible that arabinoglactan is present. In plant tissues, arabinogalactans are implicated in such diverse functions as cell–cell adhesion, nutrition of growing pollen tubes, response to microbial infections, and also as markers of identity expressed in the terminal sequences of saccharide chains (Tharanathan, 2002).

The mannose to galactose ratio recorded in the refined samples is typical of that expected for LBG i.e., 3.9 found for both rLBG samples, compared to an average value of 4.0 (Fox, 1992). However, values for the lower quality cLBG samples were lower than expected, being as low as 3.1 for cLBG3 and a maximum of 3.4 for cLBG4.

The only explanation for the low values of M/G ratio for the cLBG samples, in comparison to rLBG samples, is that the galactose contents of the former are high. If this is the case it is possible that arabinose is attached to the galactose units thus forming an arabinogalactan. A GLC analysis of the sugar content in the non particulate fraction (supernatant) of cLBG6 (70 °C/h preparation) showed that this contained less than 2% arabinose expressed as a proportion of the total sugar, the remaining being mannose and galactose. However, high levels of arabinose were also recorded, up to 71%, for those supernatants obtained at 25 °C, 65 and 62% at 100 and 121 °C preparations respectively. The GLC recovery from the particulate fraction is low (Table 6). However, taken together these results suggest that in these samples the particulate phase probably contains a glycoprotein with a high arabinose content and the supernatant is primarily galactomannan with a mannose to galactose ratio of approximately 3.3. NMR studies on the crude LBG would help to establish the source of the arabinose, to date such studies have concentrated on pure forms of LBG (Chaubey & Kapoor, 2001; Vieira & Gil, 2005). In addition, in order to ascertain whether the source is glycoprotein or arabinogalatomannan, detailed research into structure and at the same time determining the amino acid content is a necessary area of future studies.

The major objective of this work was to estimate the galactomannan, from the mannose to galactose ratio, as

this often has a great bearing on the properties of the material. However the functionality of the sample is also significantly influenced by the non-galactomannan fraction (Kök et al., 1999). The high levels of arabinose do indicate that other polysaccharides could be present in the materials. These arabinose containing polysaccharides could also contain galactose or mannose in their structure. If this was the case the M/G ratio as predicted from the sugar analysis would not be accurate to the M/G ratio of the galactomannans.

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