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PHYSICOCHEMICAL CHARACTERISTICS OF LOCUST BEAN GUM PURIFIED FRACTIONS OBTAINED BY TEMPERATURE FRACTIONATION

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ABSTRACT: *In this study, carob gum (LBG) which is known to be partially soluble at ambient temperature, was fractionated using temperature of solubilisation (at 23°C, 37°C and 80°C). Two approaches were used. In the first approach, three successive fractions were obtained from one LBG sample starting by a solubilisation at 23°C, followed by a solubilisation at 37°C and finally at 80°C on the insoluble residues. In the second approach three different fractions were obtained from three LBG samples, using a simple fractionation at three different temperatures (at 23°C, at 37°C or at 80°C). The purified fractions obtained were investigated using GLC, SEC and Bolhin rheometer instruments, to gain a clearer picture of their chemical and physico-chemical feature. All the results show that the extracted galactomannan fractions, exhibit molecular characteristics (M/G ratio and intrinsic viscosity) and rheological properties which increase with the temperature of solubilisation/extraction. In addition, these fractions were more soluble at their specific temperature of solubilisation, gave more clearly solutions and higher viscosity than the crude gum. These fractions might be applied industrially as refined thickeners to confer desired properties to food and non-food products at a required temperature.*

KEYWORDS: Locust Bean Gum, Galactomannan, Water-Soluble Polymer, Sugar Composition, Macromolecular Data, Rheological Properties

INTRODUCTION

There is a constantly increasing demand for water soluble polysaccharides, also called hydrocolloids, since they offer interesting physico-chemical properties. Starch is the best known reserve polysaccharide in plant seeds; however, other polymers such as galactomannan may be obtained in large amounts. Many gums are extracted from plants. Guar gum (GG), locust bean gum (LBG), Tara gum (TG) and Fenugreek gum (FG) are galactomannans extracted by grinding the endosperm portions of the seeds of the legume plants *Cyamopsis tetragonolobus*, *Ceretonia siliqua* L and *Caesalpinia spinosa*, *Trigonella foenum-graecum*, respectively.

Galactomannans are widely used as additives in the food and non-food industries due to their ability to yield high viscosity at low concentrations. Their solutions are only slightly affected by pH, added ions, and heat processing. Their utilization include ice cream preparation, paper and textile manufactures (as strengthening agents), rheology (flow) control in latex paints and

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uses as thickeners, oil well fracturing and drilling, and gels in blasting agents (Sittikijyothin, Torres, & Gonçalves, 2005). By controlling the rheological properties of the aqueous phase, they confer desired product properties such as stability, texture and controlled release of activities in various pharmaceutical and cosmetic products (Bateja et al., 1991). Being non-digestible, they are considered as dietary fibres in foods (Dea & Morrison, 1975; Dakia, et al., 2017c). They are used as a fat substitute in mayonnaise. They are also used to treat infant with diarrhea and recurrent regurgitation (Wenzl, Schneider, Scheele, Silny, Heimann, & Skopnik, 2003, Dakia, 2011).

Seed galactomannans have a typical chemical structure with a (1–4) linked β-D-mannopyranosyl backbone substituted in varying degrees at O–6 with single unit α-D-galactopyranosyl residues (da Silva & Gonçalves, 1990). They usually vary in their mannose:galactose ratio (M/G), D-galactose repartition along the mannan backbone, molecular weight and molecular weight distribution, depending on their origin and extraction method (Lundi and Hemansson, 1995; Ganter and reicher, 1997; Dakia, Wathelet, & Paquot, 2007; 2010; Gillet, Blecker, Aguedo, Laurent, Paquot & Richel, 2014; Dakia, et al., 2017a).

The locust bean gum (LBG), with an M/G ratio of ~ 4:1 and 20% of galactose content, is slightly soluble in cold water, and heat treatment up to 80°C is required for maximum dissolution and viscosity (García-Ochoa and Cassas, 1992). The guar gum (GG), the other most widely used galactomannan, with a high degree of galactose substitution in the mannan backbone (M/G ratio of ~ 2:1 and 40% of galactose content) is more soluble than LBG at ambient temperature. This variation in solubility is attributed to the extensive hydration of the galactose-rich regions. On the other hand, the intermolecular associations occur between the unsubstituted regions in the mannan backbone and promote high viscosity in LBG compared to GG at high temperature (Dea, Clark, and McCleary, 1986).

It is well established from several studies that the partial solubility of locust bean gum in cold water could be used to fractionate the material (Gaisford, Harding, Mitchell, & Bradley, 1986; da Silva, & Gonçalves, 1990; Pollard & Fischer, 2006; Dakia, Wathelet, & Paquot, 2008). Therefore, it would be beneficial to undertake more careful fractionation experiments, to gain a clearer picture of the entire composition and physicochemical properties of these polysaccharide fractions.

In the present work a crude locust bean gum was fractionated depending on the solubility of the polysaccharides components at different temperatures. The aim is to produce LBG purified fractions with different solubility profile with regards to their dissolution/extraction temperature: at 23°c, at 37° and at 80°C. Before polysaccharide can be optionally exploited by industry, it is necessary to develop rules to predict their structure and functions relationships. So the chemical composition, the structural and the macromolecular characteristics and rheological behaviour of these LBG different fractions, were investigated and compared, using GLC, GPC/SEC and Bolhin rheometer apparatus.

For carob gum flour fractionation, two approaches were used.

The first one aim is to obtain three different soluble fractions, from a same gum sample, using a sequential (or substractive) fractionation. By this approach, one LBG sample was fractionated starting by a solubilisation at 23°C, followed by a solubilisation at 37°C on the 23°C insoluble residue and finally a solubilisation at 80°C on the 37°C insoluble residue. Three sequential (or successive) fractions were obtained, containing galactomannans

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polysaccharides soluble at their specific temperature of dissolution/extraction (at 23°C, at 23°-37°C and at 37°-80°C).

The second approach aim is to obtain also three different soluble fractions, but from three LBG samples, using a simple fractionation. By this approach, three LBG samples were solubilised at three different temperatures (at 23°C, at 37°C or at 80°C). Three integral (or entire) fractions were obtained, containing galactomannans polysaccharides soluble at their specific temperature of dissolution/extraction (23°C and below, 37°C and below, and 80°C and below).

EXPERIMENTAL

Raw Material

A commercial sample of locust bean gum (GRINSTED LBG 047, extra high analytical grade, lot number 3121659 from DANISCO Denmark) was used for fractionation. The composition of this gum flour is, according to product specifications: fibre (max. 83%), water (max. 14%), protein (max. 7.0%), ash (max. 1.2%) and acid-insoluble matter (max. 3.0%), particle size (max. 2% > 150 μm), viscosity (min. 2,800 mPa.s**,** 1% sol. 25°C, Brookfield RVT, spindle no. 3, 20 rpm).

Fractionation and purification procedures

Sequential fractionation

Sequential or successive fractions were obtained as follow: A 10 g sample of crude LBG was dispersed in 500 ml of distilled water, with strong stirring for 60 min at 23°C ± 2°C. The dispersion was centrifuged for 30 min at 2800 ×g at 20°C for 30 min, and the supernatant recovered (LBG F23°C fraction). The pellet (sediment) was washed, resuspended in 500 ml of distilled water, heated to 37°C ± 2°C in a water bath for 45 min, with stirring, centrifuged and the supernatant recovered (LBG sF 23°-37°C sequential fraction). The sediment was washed again and resuspended, with stirring, in 500 ml of distilled water heated to 80°C ± 2°C in a water bath for 30 min, centrifuged and the supernatant recovered (LBG sF37°-80°C sequential fraction). For each fraction, the solubilized galactomannan was precipitated from the crude LBG solution by pouring into two volume excess of ethanol 96%, and allowing the mixture to stand for 1 (or 2) h. The white fibrous precipitate formed were collected and washed twice with ethanol. The precipitates (LBG F23°C, LBG sF23°-37°C and LBG sF37°-80°C) and the insoluble residue were dried at 40°C in an air circulated owen and ground to a fine powder. **Figure 1** shows the entire process.

Integral fractionation

Integral or entire fractions were obtained as follow: A 10 g sample of powdered gum was gradually added to strongly stirred distilled water (500 ml). The dispersion was moderately stirred for 1 h at 23°C ± 2°C. The resulting solution was centrifuged at 2800 ×g at 20°C for 30 min, and the supernatant recovered (LBG F23°C fraction). Other sample was prepared at room temperature, and then heated at 37°C ± 2°C for 45 min in a water bath, with stirring, centrifuged and the supernatant recovered (LBG eF37°C entire fraction). The whole cycle was repeated at 80°C ± 2°C for 30 min and the supernatant recovered (LBG eF80°C entire

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fraction). For each fraction, the solubilized galactomannan was precipitated from the crude LBG solution by pouring into two volume excess of ethanol 96%, and allowing the mixture to stand for 1 h. The polysaccharides precipitated as cord-like filaments were collected and washed twice with ethanol. After drying at 40°C in an air circulated owen, the precipitate was ground to a fine white powder. The corresponding sediments were recovered, dried and ground to a fine powder for further analysis. **Figure 2** shows the entire process.

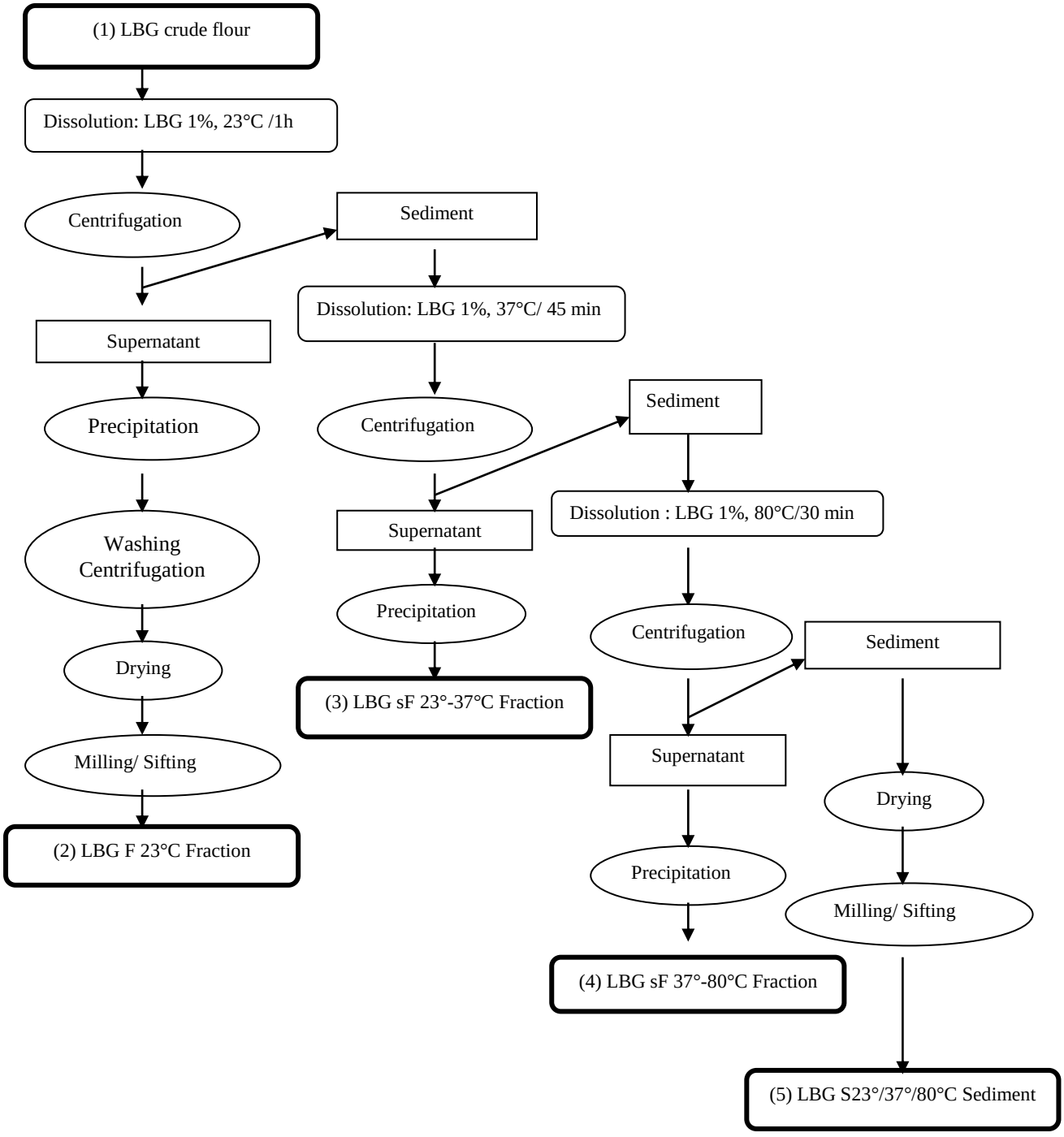

Fig. 1: Process of carob gum flour sequential (subtractive) fractionation by temperature of solubilisation

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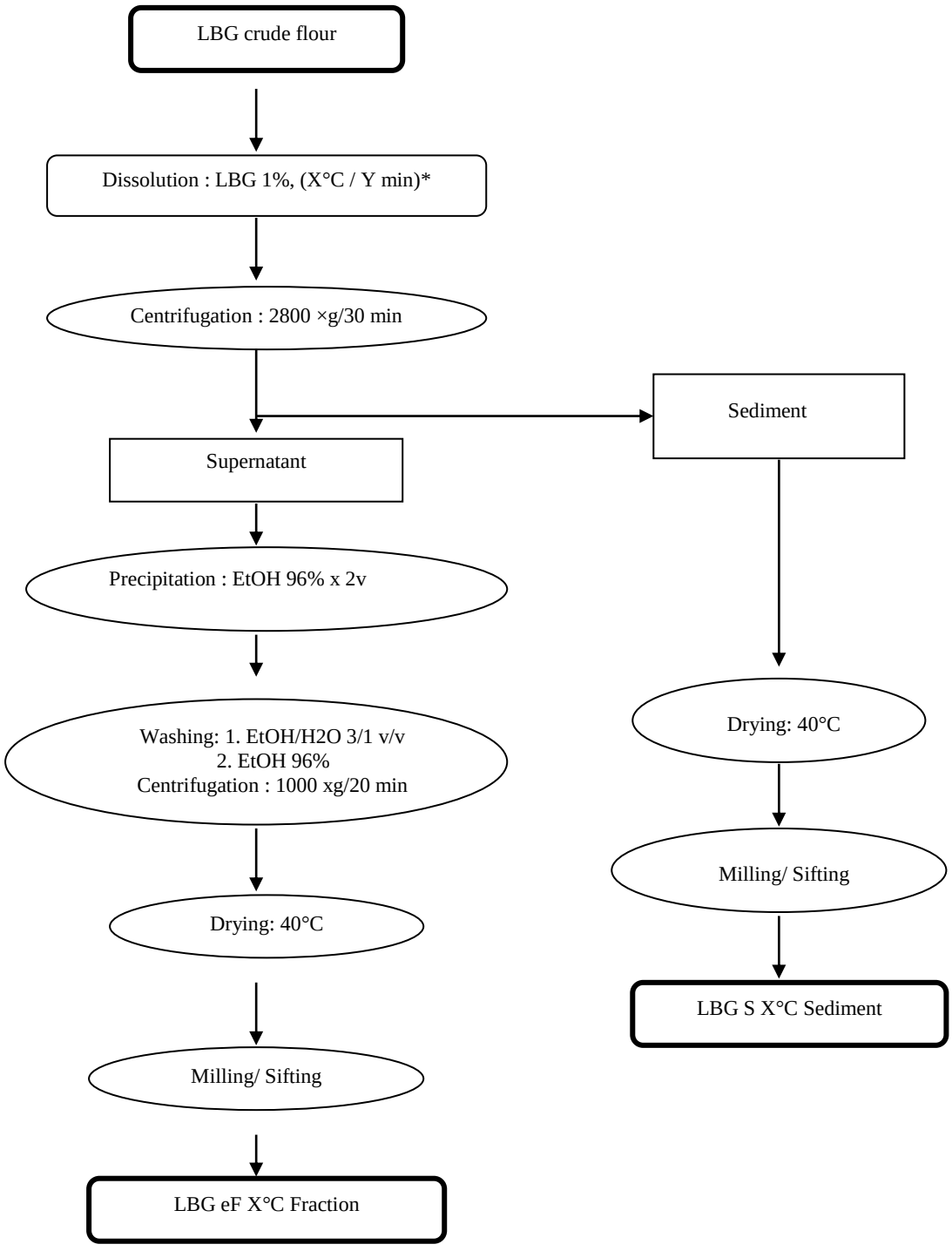

Fig. 2: Process of carob gum flour simple fractionation at different temperature. *(at 23°C/60 min, 37°C/45 min, or 80°C/30 min).

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Chemical analysis

The moisture content of the carob gum was determined gravimetrically after heating the material (500 mg) in an oven at 105°C for 24 h and was taken into consideration in all solution preparations.

The ash content of the carob gum (3 g) was determined gravimetrically after dry mineralization at 600°C for 12 h.

Protein content of the carob gum (150 mg) was determined by the Kjeldahl procedure, after mineralization (with a 1000 KJELTABS MQ tablet and a Digestion System 20, 1015 Digester, Tecator AB, Höganäs, Sweden) and distillation (by a Kjeltec Auto 1030 Analyser, Tecator AB, Höganäs, Sweden) with a conversion factor of 5.87 according to Anderson (1986).

Determination of mannose to galactose ratio by Gas Chromatography (GC)

Individual neutral sugars were quantified by gas–liquid chromatography (GC) after reduction and conversion of acid-released monosaccharides to corresponding alditol acetate derivatives (Albersheim, Nevins, English, and Karr, 1967; Blakeney, Harris, Henry, and Stone, 1983). Hydrolysis of polysaccharides was carried out by 1 M H_2SO_4 sulphuric acid (100°C / 2h). Optimum hydrolysis time is dependent on a balance between the rate of release of hydrolysable polysaccharides and the degradation of monosaccharides that occurs during prolonged treatment under experimental conditions. GC analysis was performed as previously described (Dakia et al., 2008). The hydrolysate was centrifuged (at 2000 rpm, 5 min in a Centrifuge MSE Mistral 4L) and the sugars (0.4 ml of supernatant) were reduced to their corresponding alditols by adding 2 ml of DMSO containing 2% $NaBH_4$. Reduction was performed for 90 min at 40 °C. The excess of sodium borohydride was then destroyed by adding 0.6 ml glacial acetic acid. Acetylation was then performed with acetic anhydride (4 ml, 10 min at room temperature) in the presence of 1-methylimidazole (0.4 ml) as a catalyst. Acetylation was stopped with 10 ml deionized water and the acetylated alditols were partitioned between dichloromethane (4.0 ml) and water. After the phase had separated, the lower one was removed with a pasteur pipette and putted (1 ml) in a septum-cap vial.

2-deoxy-D-glucose was employed as internal standard and standards of different carbohydrates (L(+)-rhamnose, D(–)-arabinose, D(+)-xylose, D(+)-mannose, D(+)-glucose and D(+)-galactose from Fluka Chemie (Buchs, Switzerland)) were used.

The analyses were accomplished using a Hewlett–Packard Agilent 6890 series gas chromatograph equipped with a HP1 column (30 m×0.32 mm, film thickness 0.25 µm). Derivatized extracts (1.0 µl) in dichloromethane were injected on-column. Helium was used as the carrier gas with a flow of 1.6 ml/min. The injection temperature was 290 °C and the temperature program was: 1 min at 120 °C, linear increase in 4 min to 220 °C and finally in 35 min to 290 °C and this temperature was then maintained for 4 min. Compounds were detected using a flame ionisation detector at 320 °C.

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Macromolecular characteristics (SEC)

The intrinsic viscosity [η] and the radius of gyration (Rg) of the gums extracted fractions were determined using high performance size exclusion chromatography (SEC). The apparatus used is a HPLC Waters 2690 ALLIANCE equipped with a $TSKGMPW_{XL}$ column (TosoHaas Co. Ltd., Tokyo, Japan) and coupled with refractive index (RI, Model 2410, Waters Corporation, Milford, USA), viscosity (TriSec Dual Detector Model 270, Viscotek, Houston, USA) and right angle laser light-scattering (RALLS, Model 270, Viscotek) detectors. The columns was thermostated at 30 °C, the flow rate was of 0.7 ml/min, the mobile phase was 0.05 M $NaNO_3$ with 0.05% NaN_3 as preservative and the injection volume was of 100 µ1.

Viscosity measurement

Solution preparation

For rheological measurements, all the extracted fractions are solubilised, at 1% on a dry weigh basis, in distilled water under mechanical stirring, at their corresponding dissolution/extraction temperature (23°C/60 min, 37°C/45 min and 80°C/30 min). Note that the entire extracted fractions are fully soluble at their corresponding temperature of dissolution/extraction.

Rotational rheological analysis

Rheological measurements were performed at 23°C using a stress controlled rheometer (Bohlin Instruments Inc., NJ) fitted with a cone and plate geometry (4° cone angle, 40 mm plate diameter, 150 µm gap). The temperature was constant (23 ± 0.2 °C) by circulating water from a constant temperature circulator. On the lower plate, ~2 ml of sample were placed. In this steady state experiments performed shear stress versus shear rate was calculated in the range of 0.08–400 (s^{-1}). The values of viscosity at the weak shear rate could permit to appreciate the consistency in mouth of the product (Morris & Taylor, 1982), while the values of viscosity at the high shear permit to appreciate the viscosity of the product during some hard industrial operations. Data were obtained every 20 s.

Statistical analysis: All analyses reported in this study were carried out in triplicates. Mean value and standard deviation were calculated. Data were assessed by analysis of variance (ANOVA) and Duncan's test was used to evaluate difference between means, with the software Statistica 7.1 (Stat Soft Inc, Tulsa USA Headquarters). Statistical significant difference was stated at $p < 0.05$.

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RESULTS AND DISCUSSION

Characteristics of sequential fractions obtained by increasing fractionation temperature

The results of sequential or successive temperature fractionation are shown in Table 1. The galactomannans obtained by successive fractionation, namely, LBG F 23°C, LBG sF23°-37°C and LBG sF37°-80°C accounted (yields) respectively for 32.62%, 5.69% and 35.42% of dried initial gum (LBG C). Total yield of the three refined fractions accounted for 73.70%. The sediment (insoluble residue from sequential fractionation), namely, LBG S23/37/80°c accounted for 11.10%. The lost part (difference to 100% of initial material was about 15%) eliminated with purification process may be constituted of a part of small molecules of galactomannan, of lipids, proteins and mineral components. It can be observed that LBG gum is a highly unhomogeneous polysaccharide. These observations are in good agreement with the conventional description of carob gum, as being partially soluble at cold water and fully (maximum) soluble in hot water (Hui & Neukom, 1964; Dea et Morrisson, 1975; McCleary, Clark, Dea, & Rees, 1985; Gaisford, Harding, Mitchell, & Bradley, 1986; da Silva & Gonçalves, 1990; Fernandes et al., 1991; Garcia-Ochoa & Casas, 1992; Mannion et al., 1992; Fernandes, 1994; Richardson et al., 1999; Pollard & Fischer, 2006; Dakia, et al., 2008; Gillet et al., 2014, Dakia, et al., 2017b).

According to the protein content (Table 1), it could be calculated that the amounts of proteins recovered in LBG insoluble residue (LBG S23/37/80°C) accounted for ~ 95.5% of the total protein content, showing that protein was drastically reduced in refined fractions. According to some authors (da Silva and Gonçalves, 1990; Dakia et al., 2007, 2008) the protein content (6%) of the crude gum reflects the natural presence of structural proteins and enzymes from the endosperm (gum source), but also a possible contamination with seed germ. On the other hand, the appearance of the insoluble residue indicates the presence of high amount of small pieces of carob seed brown hull (previously present in the crude gum as contaminant). The presence of higher content of minor sugars in the sediment polysaccharide (i.e. 4.26% arabinose, 0.64% xylose and 0.60% rhamnose) may be due to the presence of non-galactomannan polysaccharide such as seed coat polysaccharides (Dakia, et al., 2017b).

The three refined fractions obtained by successive fractionation exhibited M/G ratios which increased (3.11 for LBG F23°C < 3.45 for LBG sF23-37°C < 4.55 for LBG sF37-80°C) with the temperature of solubilisation/extraction. Significant difference ($p=0.05$) was observed in the M/G ratios (structural parameter) between the successive refined fractions. As expected, the solubility of the galactomannans is strongly influenced by the galactose substitution level (M/G ratio). In fact, cold water soluble fraction galactose content (22.45% for LBG F23°C) is relatively higher than the hot water soluble fractions (20.73% for LBG sF23-37°C and 16.88% for LBG sF37-80°C).

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Table 1: yields (% on dry matter (DM)), chemical composition (%), sugar composition (%) and macromolecular characteristics of LBG samples from successive fractionation (LBG F23°, LBG sF23-37°, LBG sF37-80° and LBG S23°/37°/80°C sequential fractions)

	Crude LBG	Sequential fraction F23°C and below	Sequential fraction sF23°-37°C	Sequential fraction sF37°-80°C	Sediment (insoluble residue)
Yield (%DM)	100[d]	32.60 ± 2.20[c]	5.70 ± 0.90[a]	35.40 ± 3.30[c]	11.10 ± 1.60[b]
Moisture	9.87 ± 0.42[b]	10.47 ± 0.43[c]	12.33 ± 0.08[d]	9.49 ± 1.01[b]	9.31 ± 0.00a
Protein	6.00 ± 0.02[b]	0.35 ± 0.01a	0.62 ± 0.00[a]	0.73 ± 0.01[a]	36.52 ± 3.38[c]
Rhamnose	0.13 ± 0.04[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.60 ± 0.05[c]
Arabinose	0.79 ± 0.03[b]	0.16 ± 0.01[a]	0.12 ± 0.08[a]	0.09 ± 0.03[a]	4.26 ± 0.23[c]
Xylose	0.21 ± 0.04[c]	0.12 ± 0.00[b]	0.10 ± 0.00[b]	0.04 ± 0.01[a]	0.64 ± 0.02[d]
Mannose (M)	65.79 ± 1.05[b]	69.84 ± 2.09[c]	71.51 ± 3.66[c]	76.85 ± 0.13[d]	30.19 ± 1.20[a]
Glucose	2.01 ± 0.01[c]	0.91 ± 0.03[b]	0.90 ± 0.03[b]	0.97 ± 0.05[b]	0.77 ± 0.00[a]
Galactose (G)	17.55 ± 0.31[a]	22.45 ± 0.81[b]	20.73 ± 3.79[b]	16.88 ± 0.04[a]	22.72 ± 0.27[b]
GM=M+G	83.34±2.36[b]	92.72±2.90[c]	92.24±2.45[c]	93.73±0.17[c]	52.91±1.47[a]
M/G ratio	3.74 ± 0.01[d]	3.11 ± 0.32[b]	3.45 ± 0.04[c]	4.55 ± 0.06[e]	1.33 ± 0.05[a]
	(tD80° C)	*(tD23° C)*	*(tD37° C)*	*(tD80° C)*	-
[η] (dl/g)	13.30 ± 0.25[c]	9.66 ± 2.01[a]	11.23 ± 1.05[b]	13.59 ± 1.05[c]	-
Rg (nm)	79.52 ± 1.07[d]	59.64 ± 1.82[a]	69.84 ± 1.05[b]	76.64 ± 1.05[c]	-

Values given are mean ± standard deviation of triplicate determination. Means with different letters within the same row denote significant differences among LBG fractions (p=0.05).

tD: Dissolution (or preparation) temperature

GM=M+G: Total galactomannan as the sum the Mannose and Galactose contents

Gum extracted fractions presented ~93% of total galactomannan (GM) content while the crude LBG C presented ~83% galactomannan content. This means that purified fractions correspond to relatively pure polysaccharides.

Table 1 displays also some macromolecular characteristics, of the successive fractions, obtained by size exclusion chromatography (SEC) with multidetection. The intrinsic viscosity and radius of gyration (Rg) (which are a measure of hydrodynamic volume and chain extension) values, increase with the temperature of solubilization/extraction: [η]: 9.66 < 11.23 < 13.59 dl/g; Rg: 59.64 < 69.84 < 76.64 nm for LBG F23°C, LBG sF23-37°C and LBG sF37-80°C, respectively. Significant difference (p=0.05) was observed in the different macromolecular characteristics ([η], Rg) studied between the successive refined fractions.

Lower values for LBG F23°C, suggest a great presence of small molecules in LBG F23°C and may also explain its relatively high solubility in cold water, in addition of its relatively high galactose content. Therefore, it could be noticed that at low temperature, lower molecular weight molecules and molecule with high galactose content were dissolved, whereas higher molecular weight components with less degree of galactose substitution were dissolved at high temperature.

Note that the three galactomannan sequential fractions obtained (without sediment) are more pure, totally soluble at their corresponding dissolution/extraction temperature and the

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resulting solutions are clearer and more stable than those obtained with the crude gum (LBG C). In addition, the unacceptable flavours of the crude gum are also removed.

The effect of shear rate on dynamic viscosity of the three sequential fractions solutions, prepared at their corresponding temperature of extraction, are shown in Figure 3. These results show that the viscosity of these sequential fractions solutions were shear rate dependent and in all cases the behaviour was shear-thinning (or pseudoplastic). The shear-thinning behaviour of LBG sequential fractions may be regarded as arising from modifications in macromolecular organization in the solution as the shear rate changes. The disruption of entanglements by the imposed shear made that molecules align in the direction of flow and the dynamic viscosity decreases (Fig. 3) with increasing shear rate (Mao & chen, 2005; Sittikijyothin *et al*., 2005; Dakia *et al*., 2008).

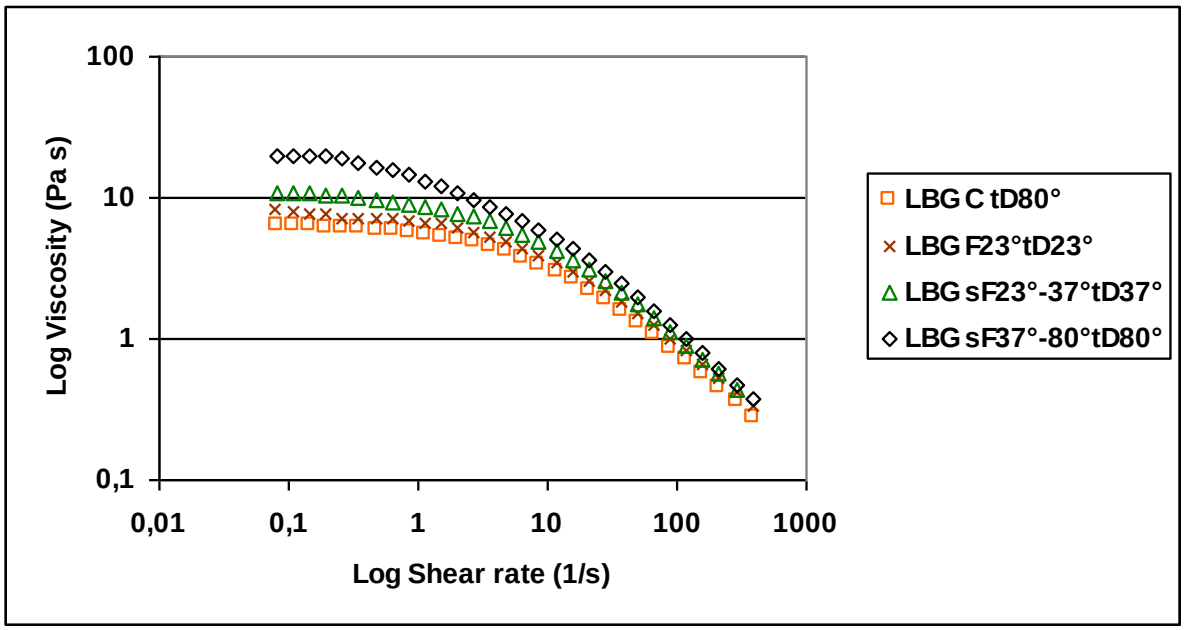

Fig.3. Plot of apparent viscosity behaviour *vs.* Shear rate for sequential purified fractions (LBG sF) and crude (LBG C) of Locust bean gum measured at 23°C, 1% concentration. tD= Dissolution temperature.

It can be observed also in figure 3, throughout the shear rate range, that viscosity values of LBG sequential fractions, at similar concentration (1%), increased with increasing temperature fractionation and M/G ratio: LBG F23°C (M/G=3.11) < LBG sF23-37°C (M/G=3.45) < LBG sF37-80°C (M/G=4.55). This observation suggest that galactomannan with less content of galactose show evidence of intermolecular associations in solutions; with the strength of the interaction increasing with decreasing galactose quantity. It should be inferred that the high proportion of unsubstituted mannan chain segments, are prone to forming intermolecular association that give rise to viscosity.

The crude gum (LBG C) had a lesser viscosity than the purified gum fractions throughout the shear rate range (Fig. 3). This difference may reflect difference in the total galactomannan

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content (~93% for LBG extracted fractions and ~83% for crude LBG) or gum purity.

Characteristic of LBG entire fractions obtained by fractionation at different temperature

Following the simple fractionation at different temperature described previously in figure 2, three different entire fractions were obtained. LBG F23°C was obtained by dissolution/extraction at 23°C and contains galactomannan polysaccharides which are soluble at 23°C and below. LBG eF37°C contains galactomannan polysaccharides which are soluble at 37°C and below, and LBG eF80°C contains galactomannan polysaccharides which are soluble at 80°C and below.

The extraction yields showed that the crude LBG contains 32.86% of LBG F23°C, 43.76% of LBG eF37°C and 75.16% of LBG eF80°C (Table 2). The sediments (aqueous insoluble matter) corresponding to each refined fractions accounted for 54.69% for LBG S23°C, 43.76% for LBG S37°C and 14.66% for LBG S80°C. Our extraction yields at 23°C (LBG F23°C) were similar, and were in accordance to those reported by Gainsford (1986) and da Silva et Gonçalves (1990) whom obtained ~38% yield at 20-25°C for carob gum.

Concerning the monosaccharide composition (Table 2), the three soluble entire fractions obtained by a simple fractionation at 23°C, at 37°C or at 80°C, exhibited M/G ratios which increased (3.11 for LBG F23°C < 3.39 for LBG eF37°C < 3.99 for LBG eF80°C). Significant difference (p=0.05) was observed in the M/G ratios between the entire refined fractions. It can be assumed that, the solubility at low temperature increases with increasing galactose content (22.45% for LBG F23°C > 19.87% for LBG eF37°C > 17.05% for LBG eF80°C). Note that the solubility is attributed to the extensive hydration of the galactose-rich regions. Therefore, LBG M/G ratio can differ depending on the temperature of fractionation/extraction process, in addition of gum origin and crude gum extraction method. Total galactomannan (GM) content is higher in entire fraction (~91% of GM) than in the crude LBG C (~83% of GM) meaning that the purified fractions are more pure polysaccharides.

According to macromolecular data, the intrinsic viscosity and the radius of gyration (Rg) (a type of molecular size) values, increase with the temperature of solubilisation/extraction ([η]: 9.66 < 11.00 < 11.78 dl/g; Rg: 59.64 < 65.64 < 67.92 nm, for LBG eF23°C, LBG eF37°C and LBG eF80°C, respectively). Significant difference (p=0.05) was observed in the different macromolecular characteristics ([η], Rg) between the entire refined fractions. This suggests that hot water soluble fractions contain great amount of hight molecular weight components.

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Table 2: Yields (%DM), chemical composition (%), and sugar composition (%), macromolecular of LBG samples from integral fractions (LBG F23° / S23 °C, LBG eF37 °/ S37 °C, LBG eF80° / S80 °C entire fractions)

	Crude LBG	Entire fraction F 23°C	Sediment S 23°C	Entire fraction eF 37°C	Sediment S 37°C	Entire fraction eF 80°C	Sediment S 80°C
Yield (%DM)	100^{f}	32.86 ± 1.10^{b}	54.69 ± 0.63^{d}	43.76 ± 1.31^{c}	45.83 ± 2.25^{c}	75.16 ± 2.44^{e}	14.66 ± 1.42^{a}
Moisture	9.87 ± 0.42^{c}	10.26 ± 1.81^{d}	8.49 ± 0.13^{a}	10.81± 0.85^{d}	9.75 ± 0.15^{b}	9.93 ± 0.18^{c}	11.29 ± 0.00^{e}
Ash	1.03 ± 0.18^{e}	0.16 ± 0.01^{b}	0.32 ± 0.08^{c}	0.21 ± 0.02^{c}	0.30 ± 0.06^{c}	0.14 ± 0.01^{a}	0.60 ± 0.28^{d}
Protein	6.00 ± 0.02^{b}	0.56 ± 0.00^{a}	8.73 ± 0.06^{c}	0.51 ± 0.01^{a}	10.82 ± 0.02^{b}	0.82 ± 0.00^{a}	30.58 ± 2.48^{e}
Rhamnose	0.13 ± 0.04^{d}	0.00 ± 0.00^{a}	0.15 ± 0.02^{d}	0.05 ± 0.02^{c}	0.16 ± 0.04^{d}	0.00 ± 0.00^{a}	0.02 ± 0.00^{b}
Arabinose	0.79 ± 0.03^{b}	0.16 ± 0.01^{a}	0.97 ± 0.08^{c}	0.17 ± 0.01^{a}	1.20 ± 0.03^{d}	0.15 ± 0.03^{a}	3.33 ± 0.01^{e}
Xylose	0.21 ± 0.04^{b}	0.12 ± 0.01^{a}	0.17 ± 0.03^{b}	0.10 ± 0.01^{a}	0.18 ± 0.08^{b}	0.08 ± 0.00^{a}	0.04 ± 0.02^{a}
Mannose	65.79 ± 1.05^{c}	69.84 ± 1.12^{d}	66.36 ± 2.50^{c}	69.42 ± 2.01^{d}	61.43 ± 1.26^{b}	73.78 ± 5.23^{e}	37.33 ± 3.43^{a}
Glucose	2.01 ± 0.01^{d}	0.91 ± 0.02^{c}	0.95 ± 0.09^{c}	0.73 ± 0.05^{b}	0.88 ± 0.02^{c}	0.68 ± 0.02^{b}	0.19 ± 0.01^{a}
Galactose	17.55 ± 0.31^{b}	22.45 ± 2.20^{d}	15.20 ± 1.40^{a}	19.87 ± 2.01^{c}	14.10 ± 1.44^{a}	17.05 ± 1.50^{b}	12.79 ± 0.99^{a}
GM (M+G)	83.34 ± 1.36^{d}	92.72 ± 3.32^{f}	81.20 ± 3.90^{c}	89.29 ± 4.02^{e}	75.53 ± 2.60^{b}	90.83 ± 6.73^{e}	50.12 ± 4.52^{a}
M/G ratio	3.74 ± 0.01^{d}	3.11 ± 0.68^{b}	4.39 ± 0.71^{f}	3.39 ± 0.02^{c}	4.35 ± 0.00^{f}	3.99 ± 0.05^{e}	2.92 ± 0.01^{a}
	(tD80° C)	*(tD23° C)*	*(tD80° C)*	*(tD37° C)*	*(tD80° C)*	*(tD80° C)*	*(tD100° C)*
[η] (dl/g)	13.30 ± 1.25^{e}	9.66 ± 1.42^{a}	13.02 ± 0.80^{d}	11.00 ± 1.14^{b}	14.44 ± 0.78^{f}	11.78 ± 1.06^{c}	15.21 ± 1.07^{g}
Rg (nm)	79.52 ± 1.07^{f}	59.64 ± 1.02^{a}	70.15 ± 0.91^{d}	65.64 ± 0.81^{b}	72.26 ± 1.24^{e}	67.92 ± 1.02^{c}	78.20 ± 4.23^{f}

Values given are mean ± standard deviation of triplicate determination. Means with different letters within the same row denote significant differences among LBG fractions (p=0.05).

tD: Dissolution (or preparation) temperature

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The effect of shear rate on dynamic viscosity of the three integral fractions solutions, prepared at their corresponding temperature of extraction, are shown in Figure 4. These results show that the viscosity of these entire fractions solutions were shear rate dependent and in all cases the behaviour was shear-thinning.

The rheological values show that viscosity of LBG entire fractions seems to evolve (LBG F23°C < LBG eF37°C < LBG eF80°C) with the temperature of dissolution/extraction. These observations confirm that galactomannan with high intrinsic viscosity and high M/G ratio (LBG F23°C (9.66 dl/g and 3.11) < LBG eF37°C (11.00 dl/g and 3.39) < LBG eF80°C (11.78 dl/g and 3.99)) are prone to forming intermolecular associations that give rise to viscosity.

In addition, the entire fractions are more viscous than the crude gum (LBG C) throughout the shear rate range (Fig. 3.), due to the difference in purity in term of total galactomannan content (additional material being solubilised), as described in Table 2: ~90.5% for integral fractions and ~83% in LBG C.

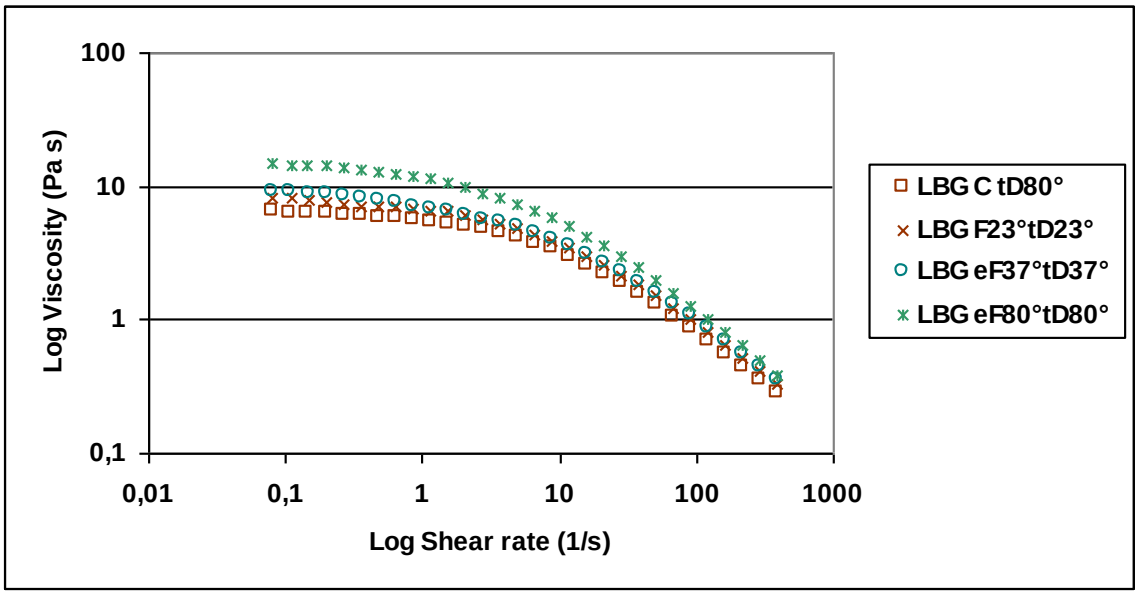

Fig.4. Plot of apparent viscosity behaviour *vs.* Shear rate for entire purified fractions (LBG eF) and crude (LBG C) of Locust bean gum measured at 25°C, 1% concentration. tD= Dissolution temperature.

Comparison of LBG entire and sequential fractions rheological properties

All the purified (extracted) fractions are more viscous (Figure 5) than the crude gum. This may reflect the difference in the total GM content (Table 3) or in the purity of galactomannan polysaccharides. This observation is in disagreement with the observation of da Silva and Gonçalvez (1990) that found that the crude gum had a higher viscosity than the purified gum. This could be due to the method used to obtain the purified gum solution. In the present study, LBG refined fractions are redissolved at the same temperature than the fractionation

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temperature, in order to avoid macromolecular depolymerization (Doublier, 1975) which may occur in their method.

Difference between the five fractions may reflect difference in polysaccharide homogeneity according to extraction process (polysaccharide from sequential fraction are more homogenous than polysaccharide from entire fractions), in addition of molecular properties.

Sequential fractions (LBG sF23-37°C and LBG sF37-80°C) may be more pure and homogene than the entire fractions (LBG eF37°C and LBG eF80°C) and the LBG F23°C. In general, there is not evident difference in viscosity measurement between the extracted fractions. However, it seems that apparent viscosity of LBG extracted samples evolve slightly with the M/G ratio (3.11 < 3.39 < 3.45 < 3.99 < 4.55) and the intrinsic viscosity (9.66 < 11.00 < 11.23 < 11.78 < 13.59), combining probably with the total GM content.

Note that intrinsic viscosity related to the hydrodynamic volume occupied by the macromolecule in solution is directly related to apparent viscosity. The higher is the intrinsic viscosity the higher is the viscosity measurement. In addition, the interchain associations related to the M/G ratio give rise to the viscosity.

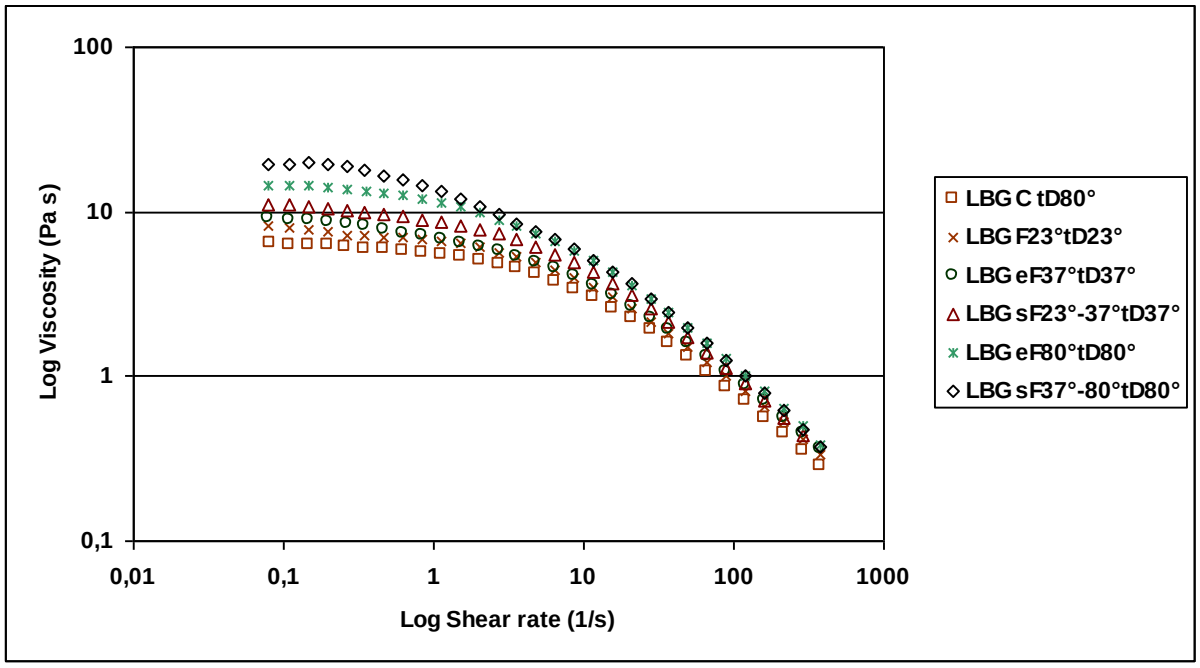

Fig.5. Viscosity curves rheograms of all extracted LBG samples and crude gum (LBG C) measured at 25°C, 1% concentration. tD= Dissolution temperature.

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Table 3: Molecular properties of all analyzed LBG samples (LBG C, LBG F23°, LBG sF23°-37°C, LBG sF37°-80°C, LBG eF37°, LBG eF80°C)

	Crude LBG	Fraction F 23°C	Entire fraction eF 37°C	Sequential fraction sF23°-37°C	Entire fraction eF 80°C	Sequential fraction sF37°-80°C
GM (M+G)	83.34±1.36^{a}	92.72±2.90^{c}	89.29±4.02^{b}	92.24±7.45^{b}	90.83±6.73^{b}	93.73±0.17^{c}
M/G ratio	3.74 ± 0.01^{c}	3.11 ± 0.50^{a}	3.39 ± 0.02^{b}	3.45 ± 0.04^{b}	3.99 ± 0.05^{d}	4.55 ± 0.06^{e}
[η] (dl/g)	13.30±0.25^{d}	9.66 ± 2.01^{a}	11.00±1.14^{b}	11.23 ± 1.05^{b}	11.78±1.06^{c}	13.59 ± 1.05^{e}
Rg (nm)	79.52±1.07^{e}	59.64 ± 1.82^{a}	65.64±0.81^{b}	69.84 ± 1.05^{c}	67.92±1.02^{c}	76.64 ± 1.05^{d}

Values given are mean ± standard deviation of triplicate determination. Means with different letters within the same row denote significant differences among LBG fractions (p=0.05).

In general, the rheological behavior of each fraction could be considered to be itself of interest in some applications. These refined fractions could be used in human food, pharmaceutical and cosmetic industries (Dakia, 2011), while the insoluble residues recovered as by-products during LBG C thermodynamic partitioning, although contaminated by impurities, could be fractionated again or directly use in animal feed industries.

CONCLUSIONS

This study shows clearly that the galactomannan polysaccharides in carob gum flour can be fractionated according to temperature of solubilisation. The galactomannan soluble fractions obtained are more pure, fully soluble at their corresponding temperature of solubilisation than the crude gum.

The degree of galactose substitution (M/G ratio), the intrinsic viscosity and the total galactomannan content in galactomannans, determine in most cases their physicochemical properties. Galactomannan with higher M/G ratio and higher intrinsic viscosity cause higher apparent viscosity. These parameters can differ in galactomannans according to the extraction process in term of temperature of dissolution/extraction, in addition of the carob seeds source.

These extracted fractions may be industrially applied as refined thickeners because these polymers have the advantage of being full or more soluble in water at a desired temperature than the commercial gum flours.

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