Available online at www.sciencedirect.com

ScienceDirect

Food Chemistry 101 (2007) 1508–1515

Food Chemistry

www.elsevier.com/locate/foodchem

The analysis of crude and purified locust bean gum: A comparison of samples from different carob tree populations in Tunisia

N. Bouzouita [a], A. Khaldi [b], S. Zgoulli [c], L. Chebil [a], R. Chekki [a], M.M. Chaabouni [a,*], P. Thonart [c]

[a] Ecole Supérieure des Industries Alimentaires, 58, Avenue Alain Savary, 1003 Tunis, Tunisia
[b] Institut National des Recherches en Génie Rural, Eaux et Forêts (INGREF) BP 10, Ariana 2080, Tunisia
[c] Centre Wallon de Biologie Industrielle Université de Liège Sart Tilman, B 40, 4000 Liège, Belgium

Received 12 September 2005; accepted 16 March 2006

Abstract

The crude and purified locust bean gum (LBG) from seven areas of the north and centre of Tunisia (Bouarada, Bargou, Kessra, Haffouz, Borj Toumi, Ben Arous and INRGREF) were analyzed for moisture, ash, protein, acid-insoluble matter and mannose/galactose ratio. The purified samples exhibited higher mannose/galactose ratios and lower amounts of ash, protein and acid-insoluble matter than the crude gum. The purified LBG from different regions had 3.43–6.99% moisture, 0.87–2.06% ash, 0.61–2.46% protein, 0.00–1.20% acid-insoluble matter and 3.55–4.32 mannose/galactose ratios. Statistical analysis revealed that purification significantly affected ($P < 0.05$) moisture, ash, protein, insoluble matter contents and mannose/galactose ratios of the crude LBG and purified LBG for all samples from different areas. The rheological properties of the different carob gum samples were determined, the best rheological properties are those of spontaneous carob trees of Bargou, Bouarada and Kessra areas. The climatic and geographic origin of carob and the cultivation mode influence the chemical and rheological properties. The purification of crude galactomannan samples by precipitation with isopropanol gave a clear and more stable solution, due to the elimination of impurities and endogenous enzymes.

Keywords: Carob gum; *Ceratonia siliqua*; Locust bean gum; Mediterranean vegetation; Galactomannan; Purification

1. Introduction

The carob tree has been included in a national list of priority forest genetic resources for conservation and management in Tunisia, a country bordering the southern Mediterranean shores. Carob is the fruit of an evergreen, *Ceratonia siliqua*, which grows widely in the mediterranean region and belongs to the genus leguminosae. The seeds of carob, which represent about 10% of the weight of the fruit, are composed principally of galactomannans (Egli, 1969; Karawya, wassel, Baghdadi, & Ammar, 1980; McCleary & Matheson, 1976). The chemical composition of carob has been studied (Avallone, Plessi, Baraldi, & Monzani, 1997; Calixto & Canellas, 1982; Yousif & Alghzawi, 2000). Carob gum is the ground endosperm of the seeds of fruit; owing to its remarkable water-binding properties, it is widely used to improve the texture of food (Glicksman, 1969; Imeson, 1997; Meyer, Rosa, Hischenhuber, & Meyer, 2001). The gum-aqueous solutions have a high viscosity, even at low concentrations; for this reason, the gum is used as a substitute for pectins, agar and other mucilagenous substances. It is used as a thickener, microorganism growth medium, food stabiliser and other applications in the textile, food, cosmetic and pharmaceutical industries (Calixto & Canellas, 1982). However, there are some problems with the food application of these gums which mainly arise from the high variability in the quality of several brands in the market. The purification of these

* Corresponding author. Tel.: +216 71 770 399; fax: +216 71 771 192.
E-mail address: chaabouni.medmoncef@iresa.agrinet.tn (M.M. Chaabouni).

0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2006.03.056

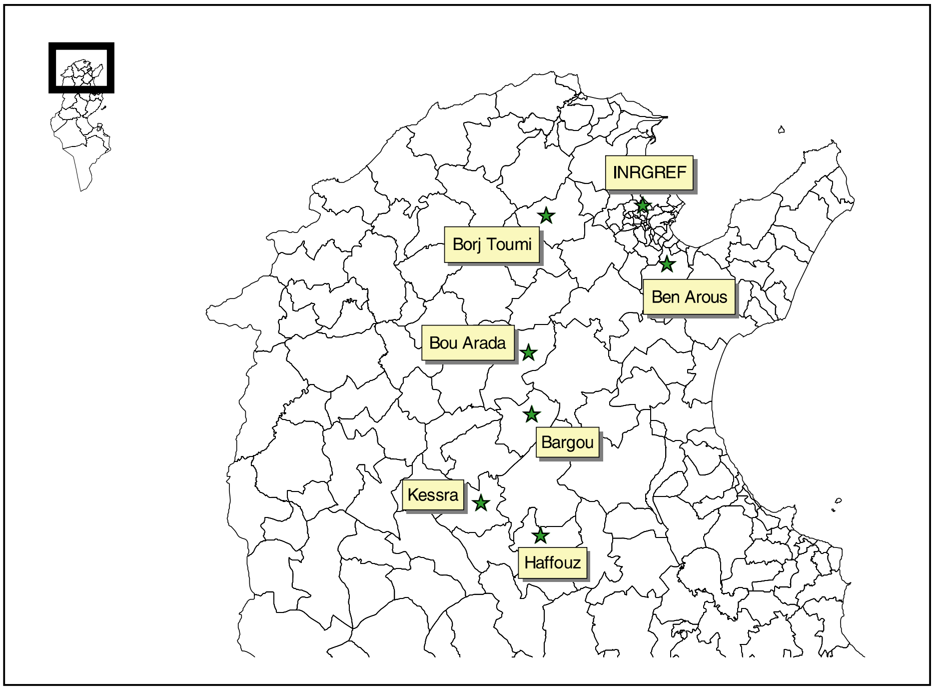

Fig. 1. The geographical distribution of carob sites.

polysaccharides improves this situation; unacceptable flavours of the crude gums are removed and the purified gums give clear and more stable solutions due to the elimination of impurities and endogenous enzymes (Dea & Morrison, 1975). There are several methods for the purification of crude galactomannan samples. Precipitation with ethanol was largely used (Anderson, 1949; Doublier, 1975; McCleary & Matheson, 1974). Purification by methanol (Rafique & Smith, 1950) and by copper (McCleary, Matheson, & Small, 1976) or barium complexes (Kapoor, 1972) has also been used. Purification by precipitation with isopropanol, though sparsely used in the laboratory, is the method of choice in the industrial processes (Dea & Morrison, 1975). In the present work, different samples, from seven areas of Tunisia (Fig. 1), of locust bean gum were purified by precipitation with isopropanol. The chemical analysis: moisture, ash, protein, acid-insoluble matter, mannose/galactose ratio and rheological behaviour of the purified and crude samples were compared.

2. Materials and methods

2.1. Selection and preparation of samples

Samples of carob were collected during August–September, in 2001, from different areas of the centre of Tunisia, where they grow naturally: Bou Arada, Bargou, Kessra, Haffouz and areas of the north of this country, where carob is cultivated: Borj Toumi, Ben Arous, INRGREF.

Samples were stored at 18 °C and analyzed within 5 months of collection. The seeds were removed and ground to particles of $\leqslant$1 mm; the locust bean gum obtained, as a mucilage from the seed endosperm of the carob was kept in polyethylene bags in a deep freezer until used.

2.2. Measurement of carob pods

Every site was characterized through the following morphological parameters. Lg: the pods length, Wdp: the pods width, Whtp: the pods weight, Nsd: the number of seeds per pod, Wsds: the weight of seeds of per pod (in grammes). We also calculated, by direct measurement, the ratio *R*, seed weight/pod weight, an important parameter for this characterization. Length was measured using a measuring tape, width by using a vernier calliper, and weight was taken using a top-loading balance.

2.3. Preparation of solutions

The required amount of the powdered gum was gradually added to strongly stirred distilled water. The precipitation is initiated at 1.3 g 100 ml^{-1} concentrated aqueous solution. The dispersion was moderately stirred for 1 h, at room temperature, and then heated at 80 °C in a water bath for 30 min, with continuous stirring. The resulting solution was allowed to cool, centrifuged for 1 h at 21,875*g* (20 °C) and the supernatant, corresponding to crude LBG solution, separated for use in the purification process.

2.4. Purification of the gum

The solubilized galactomannan was precipitated from crude LBG solution by pouring into a two volume excess of isopropanol, and allowing the mixture to stand for 30 min. The white fibrous precipitate formed was collected by filtration with screen (53 μm), and washed twice with isopropanol and with acetone. After drying under vacuum overnight at 30 °C, the precipitate was ground to a fine powder.

2.5. Chemical analysis

Moisture and ash contents were determined according to Food Chemical Codex (Food & Nutrition Board-National Research Council, 1981). Total nitrogen was determined by the Kjeldahl modified method; a conversion factor of 6.25 was used to obtain the protein content (Anderson, 1986).

2.6. Measurement of mannose–galactose ratios

2.6.1. Preparation of solution

Twenty milliliters of H_2SO_4 was added to 400 mg of carob bean gum. After boiling, the mixture was cooled and treated with add $BaCO_3$ to pH 7, then filtered and evaporated (30 °C and 50 °C) to obtain crystalline residue (or syrupy) which was dissolved in 2 ml of deionised water and analysed by HPLC.

2.7. Determination mannose/galactose ratio

To optimise the hydrolysis conditions, we have undertaken an experimental methodology (the complete factorial plan with two levels) to test three parameters: the acid concentration, the time and the temperature of hydrolysis. The optimum conditions for carob gum hydrolysis obtained were temperature 100 °C, 1 M H_2SO_4 concentration and hydrolysis time 2 h. Cationic-exchange liquid chromatography analyses were performed on a Agilent HP 1100 HPLC with waters differential refraction detector, equipped with a Pb^{2+} column at 80 °C.

2.8. Viscosity measurements

The rheological study was carried out on the crude and purified LBG solutions, using an Ares Advanced Rheometric Expansion System from Rheometric Scientific. This rheometer is a coaxial cylindrical system witch works under both dynamic and static regimes. The samples were sheared in the gap between the fixed inner cylinder ($\Phi = 32$ mm, $h = 33$ mm) and the rotated mobile outer cylinder ($\Phi = 34$ mm).

2.9. Statistical analysis

The obtained data were analyzed statistically using a statistical analysis system STATGRAPHICS® version 5.0 (2001). Values of all parameters were presented as means ± standard deviation. Analysis of variance (ANOVA) was used in statistical analysis of chemical properties. We consider statistically significant P values less than 0.05.

3. Results and discussion

3.1. Measurements of carob pods

Results of carob pod measurements are shown in Fig. 2. The longest pods were measured in the sites of INRGREF, Ben Arous and Borj Toumi, with, respectively, of 150.6, 140.3 and 116.8 mm lengths (average). The shortest were Haffouz and Kessra with, respectively, 89.3 and 95.9 mm length averages (a). The widest pods were at the same time, the longest with 7.6 mm for the Ben Arous's site and 7.3 mm for INRGREF and Borj Toumi sites. The sites with the lowest widths were Kessra and Haffouz with, respectively, 5.0 and 5.5 mm averages (b). The weight of pods follows the same tendency and the heaviest pods were in the sites of INRGREF and Ben Arous with, respectively, 20.9 and 16.8 g averages per pod (c). The sites with the lowest pod weight are those of Haffouz and Kessra with, respectively, 6.7 and 7.1 g averages per pod. The number of seeds per pod varied between 6.4 for the Haffouz's site and 12 for the INRGREF's site (d). The weight of seeds per pod oscillates, on average, between 1.0 g per pod in the Haffouz site and 2.4 g in the INRGREF site (e). The calculated ratio, seed weight/pod weight, showed that the highest percentages were obtained in the sites of Kessra, Bou Arada and Bargou (spontaneous ecotypes) with 18%, then Haffouz with 16.8%. The three other sites were below 13% (f). These results show that it is comparatively difficult to choose origin on the basis of the dimensions of pods. Data obtained from other studies showed that the carob pods ranged from 100 to 200 mm long, 20 to 50 mm wide, 9 to 10 mm thick and 20 to 40 g in weight (Blenford, 1979). Length was 123.4 mm, width 19.5 mm, thickness 9.3 mm and weight 31.8 g/pod in one study (Yousif & Alghzawi, 2000). The results obtained in this study, however, are in agreement with the literature. It is well established that the physical measurements of the whole carob pods indirectly indicate the quality of those pods.

3.2. Purification and chemical analysis

Fig. 3 shows the extraction and purification processes used in this work to obtain the purified galactomannans and efficiency varies from 26.66% to 33.16% (Table 2). The mean value of purification yield is 30.14%. The sample from the INRGREF area presented the highest yield 33.16%, and the measurements of carob pods showed that the pods of this sample were the longest, the widest, the heaviest and had the highest number of seeds per pod and the highest weight of seeds per pod. In the literature, the yields were higher: 77% (Lopes da Silva & Gonçalves, 1990) and 46% (Andrade, Azero, Luciano, & Gonçalves, 1999). Moisture, ash, protein and acid-insoluble matter contents of the crude locust bean gum and purified locust

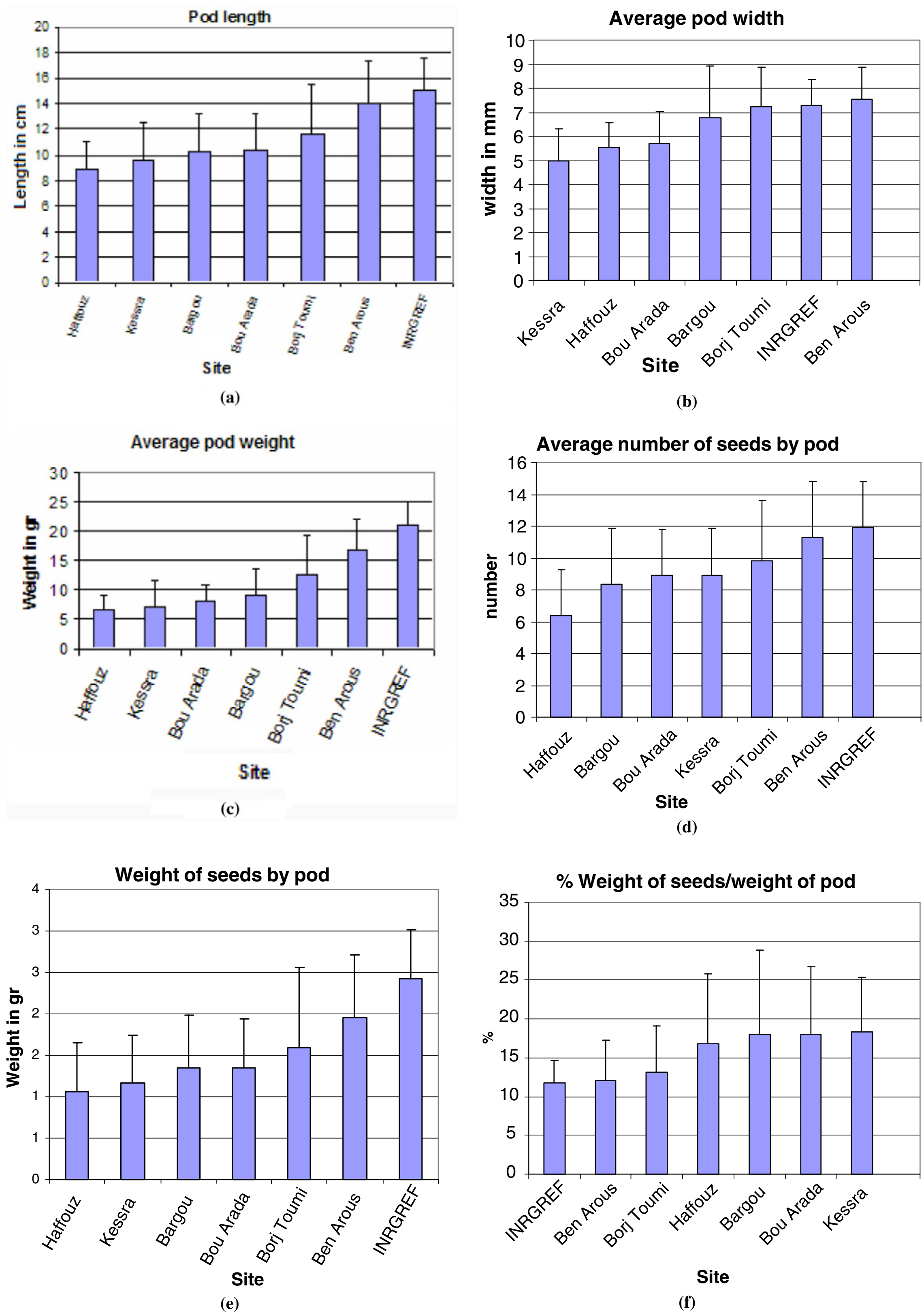

Fig. 2. Representation of the measured and calculated parameters of the site's morphological characteristics: (a) pod length, (b) pod width, (c) pod weight, (d) number of seeds by pod, (e) seed weight by pod, (f) yield in % seed weight/pod weight.

bean gum are shown in Table 1. There are significant differences in the contents of ash and protein between the analyzed crude LBG and purified LBG. The crude LBG of the INRGREF area contained lower amounts of ash (3.73% vs the mean value 3.96%) and more protein (4.12% vs the mean value 3.39%). The acid-insoluble

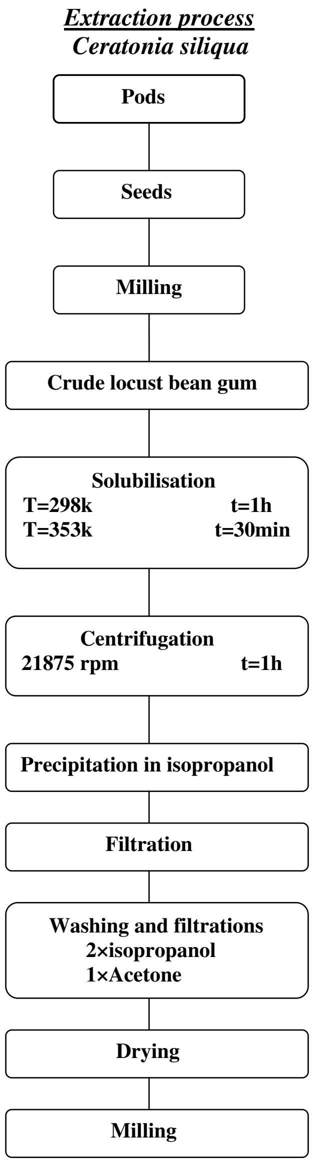

Fig. 3. Extraction and purification process of galactomannans.

matter value decreased from 6.05% to 0.00%. The purification of this sample, eliminated practically all fat and fibre; the ash and protein contents were also drastically reduced. The statistical analysis (Tables 3 and 4) revealed that purification significantly affected ($P < 0.05$) moisture, ash,

Table 2
Yields of purification and sugar compositions of the samples (crude LBG and purified LBG)

Sample	Yield (% total gum, dry weight basis)	Mannose:galactose ratio	
		Crude LBG	Purified LBG
Kessra	28.21 ± 1.20	3.03 ± 0.18	3.77 ± 0.24
Ben Arous	30.42 ± 1.72	3.47 ± 0.13	3.96 ± 0.18
Haffouz	29.01 ± 2.80	3.10 ± 0.23	3.91 ± 0.19
INRGREF	33.16 ± 1.66	3.36 ± 0.22	4.06 ± 0.12
Bouarada	30.47 ± 1.82	3.29 ± 0.11	3.78 ± 0.11
Borj Toumi	26.66 ± 1.89	3.34 ± 0.15	4.32 ± 0.20
Bargou	33.06 ± 2.45	3.05 ± 0.20	3.55 ± 0.14

The values shown are the means of three determinations ± standard deviation.

protein and insoluble matter contents of the crude LBG and purified LBG for all samples from different areas. Significant differences ($P < 0.05$) were also noticed between crude LBG and purified spontaneous samples and cultivated samples. On the other hand, the origin or the factors spontaneous and cultivated types did not show any significant differences (Tables 3 and 4). The mannose/galactose ratios were not the same for all samples (Table 2) the purified samples exhibited higher M/G ratios than did the crude gum. The contents of galactomannan for the 7 samples were deduced from a calibration curve obtained from five synthetic mixtures of β-D-galactose and β-D-mannose in known proportions. The M/G ratios for the sample of INRGREF was 3.36 vs the mean value 3.23 for the crude LBG; this value increased to 4.06 vs the mean value of 3.91 for the purified LBG. As seen in Table 2, the purification process improved the yield of galactomannan and gave good resolution in chromatograms. Also isomer forms were reduced in chromatograms of the non purified gum. The M/G ratio varied from 3 to 3.5 for crude gums and from 3.8 to 4.3 for purified ones, this was due to the elimination of insoluble residue by a successful purification process. Our results were similar to those reported by Lopes da Silva and Gonçalves (1990). Tables 3 and 4 show that significant differences ($P < 0.05$) also existed between mannose/galactose ratios of crude LBG and purified LBG.

3.3. Rheological characteristics

The flow curves of the different crude LBG samples presented in Fig. 4 show that all the solutions had a

Table 1
Compositions of seven different carob samples

Sample	Moisture %		Ash %		Protein%		Acid-insoluble matter%	
	Crude LBG	Purifed LBG	Crude LBG	Purifed LBG	Crude LBG	Purifed LBG	Crude LBG	Purifed LBG
Kessra	6.08 ± 0.01	5.96 ± 0.01	3.87 ± 0.01	1.01 ± 0.01	4.52 ± 0.01	0.92 ± 0.01	8.20 ± 0.02	1.20 ± 0.01
Ben Arous	7.09 ± 0.02	5.62 ± 0.01	3.93 ± 0.01	2.06 ± 0.01	3.00 ± 0.01	2.46 ± 0.01	8.46 ± 0.01	0.52 ± 0.01
Haffouz	5.82 ± 0.01	4.39 ± 0.01	3.87 ± 0.01	1.44 ± 0.03	2.65 ± 0.03	1.16 ± 0.01	7.95 ± 0.01	0.00 ± 0.00
INRGREF	8.43 ± 0.01	3.43 ± 0.01	3.73 ± 0.01	0.87 ± 0.02	4.12 ± 0.01	0.61 ± 0.01	6.05 ± 0.02	0.00 ± 0.00
Bouarada	5.93 ± 0.02	5.01 ± 0.02	4.48 ± 0.02	1.20 ± 0.01	5.06 ± 0.02	0.82 ± 0.02	6.32 ± 0.01	0.03 ± 0.01
Borj Toumi	6.73 ± 0.01	6.09 ± 0.02	4.10 ± 0.21	1.90 ± 0.02	2.57 ± 0.01	1.28 ± 0.01	8.60 ± 0.02	0.50 ± 0.03
Bargou	9.3 ± 0.02	6.99 ± 0.01	3.78 ± 0.08	1.18 ± 0.01	1.82 ± 0.02	0.72 ± 0.01	5.38 ± 0.01	0.20 ± 0.02

Comparison between crude locust bean gum and purified locust bean gum. The values shown are the means of three determinations ± standard deviation.

Table 3
Analysis of variance of chemical properties according to the origin[a] (areas) for crude and purified locust bean gum[b]

	Source of variation	Degree of freedom	Sum of squares	Mean square	*F* value	P
Moisture	A: origin[a]	6	11.376	1.896	1.460	0.329
	B: LBG[b]	1	10.098	10.098	7.76	0.031
	Error	6	7.808	1.301		
	Total	13	29.282			
Ash	A: origin[a]	6	1.134	0.189	1.14	0.438
	B: LBG[b]	1	24.420	24.420	147.26	0.000
	Error	6	0.994	0.165		
	Total	13	26.549			
Protein	A: origin[a]	6	4.248	0.708	0.650	0.694
	B: LBG[b]	1	17.763	17.763	16.25	0.006
	Error	6	6.557	1.093		
	Total	13	28.57			
Acid-insoluble matter	A: origin[a]	6	7.778	1.296	2.030	0.205
	B: LBG[b]	1	168.087	168.087	262.61	0.000
	Error	6	3.84	0.640		
	Total	13	179.706			
Mannose: galactose ratio	A: origin[a]	6	0.430	0.071	4.010	0.057
	B: LBG[b]	1	1.584	1.548	88.380	0.0001
	Error	6	0.107	0.017		
	Total	13	2.123			

[a] Areas.
[b] Crude and purified locust bean gum.

Table 4
Analysis of variance of chemical properties according to the origin[a] (spontaneous and cultivated) for crude and purified locust bean gum[b]

	Source of variation	Degree of freedom	Sum of squares	Mean square	F value	P
Moisture	A: origin[a]	1	1.213	1.213	0.740	0.407
	B: LBG[b]	1	10.098	10.098	6.180	0.030
	Error	11	17.970	1.633		
	Total	13	29.282			
Ash	A: origin[a]	1	0.025	0.025	0.130	0.723
	B: LBG[b]	1	24.420	24.42	127.660	0.000
	Error	11	2.104	0.191		
	Total	13	26.549			
Protein	A: origin[a]	1	0.021	0.021	0.02	0.885
	B: LBG[b]	1	17.763	17.763	18.12	0.001
	Error	11	10.784	0.98		
	Total	13	28.569			
Acid-insoluble matter	A: origin[a]	1	0.709	0.709	0.720	0.415
	B: LBG[b]	1	168.087	168.087	169.50	0.000
	Error	11	10.908	0.991		
	Total	13	179.706			
Mannose: galactose ratio	A: origin[a]	1	0.265	0.265	10.710	0.007
	B: LBG[b]	1	1.584	1.584	63.87	0.000
	Error	11	0.272	0.024		
	Total	13	2.123			

[a] Spontaneous and cultivated.
[b] Crude and purified locust bean gum.

shear-thinning behavior (the apparent viscosity decreases when the shear rate increases). The apparent viscosity at 10 s^{-1} varies from 0.76 to 1.92 Pa s; the Newtonian plateau varied from 1.11 to 3.64 Pa s and the critical shear rate from 1.07 to 2.69 s^{-1}. The best rheological properties were those of the INRGREF sample (the highest apparent viscosity 1.92 Pa s, the highest Newtonian viscosity 3.64 Pa s and the lowest critical shear rate 1.07 s^{-1}). This is in agreement with the fact that this sample had the highest galactomannan concentration (482 mg/g). The rheological properties of the different purified LBG samples have also been determined. The results are illustrated as flow curves and are summarized in Fig. 5. These curves show that all the studied purified LBG solutions have a shear-thinning behaviour that is significantly better than what is shown by the crude LBG solutions. The apparent viscosity at

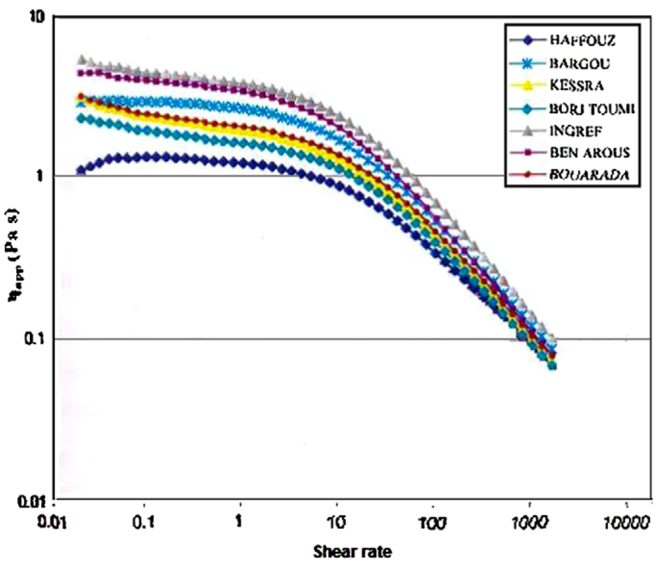

Fig. 4. Flow curves of crude LBG samples (concentration 2% m/m at 23 °C).

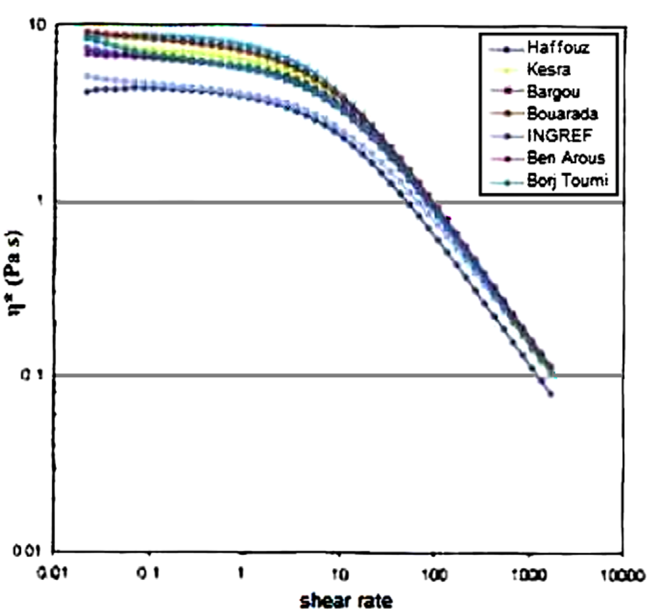

Fig. 5. Flow curves of purified LBG samples (concentration 2% at 23 °C).

10 s^{-1} varied from 1.85 to 3.14 Pa s, the Newtonian viscosity varies from 3.87 to 8.02 Pa s and the critical shear rate from 0.67 to 1.07 s^{-1}. The best rheological properties were those of Bargou, Bouarada and Kessra samples. They had the highest apparent viscosities, respectively, 3.14, 3.01 and 2.84 Pa s, the highest Newtonian viscosities 8.02, 7.33 and 6.57, and the lowest critical shear rate, 0.67 for the three samples. These results could be explained by the fact that these samples had low proteins, respectively, 0.72%, 0.82%, 0.92%, and high galactomannan amounts (983, 980 and 952 mg/g). The INRGREF sample has lower protein than Bargou, Bouarada and Kessra samples (0.61%) but a lower apparent viscosity (2.79 Pa s). The rheological properties of locust bean gum do not depend only upon protein, but the important factor is the galactomannan amount and the mean branching degree. In solution, the smoothest galactomannan macromolecules are more probably aggregated than isolated, and a relationships between aggregate formation and degree of branching was established. The botanical origin also influences these properties.

4. Conclusion

The carob pods of every site were characterised by the following parameters: the percentage seed weight/pod weight, and in first range were the three sites, where the carob trees are spontaneous: Bouarada, Kessra and Bargou. The purification of LBG by precipitation with isopropanol causes drastically reduced ash and protein contents. The mean value of yield of purification is 30.38%, this purification gives a clear and more stable solution, due to the elimination of impurities and endogenous enzymes. If the purification procedure is repeated, an almost pure galactomannan sample is obtained. The rheological behaviours of the purified and crude samples were compared; the flow curves of the different samples showed that all the studied purified LBG solutions had a shear-thinning behaviour that is significantly altered from what is shown by the crude LBG solution. The three sites, where the carob tree is spontaneous, Bouarada, Kessra and Bargou, corresponding to the centre of Tunisia, have the best rheological properties. This result will contribute to safeguarding these trees, which are often degraded and about to disappear. Indeed, the importance of these spontaneous ecotypes as concerns economical value, since the buyers of pods, to extract seeds, will use this parameter and will search for the best possible yield.

Acknowledgements

The authors express their gratitude to DRI (Region Wallonne) and technical staff of INRGREF, without whose help this study would not have been possible. We thank Abdelfatteh El Omri for statistical analysis.

References

Anderson, D. M. W. (1986). *Food Additives and Contaminants, 3*, 231–234.

Anderson, E. (1949). *Industrial and Engineering Chemistry, 41*, 2887–2890.

Andrade, C. T., Azero, E. G., Luciano, L., & Gonçalves, M. P. (1999). Solution properties of the galactomannans extracted from the seeds of *Caesalpinia pulcherrima* and *Cassia javanica*: comparison with locust bean gum. *International Journal of Biological Macromolecules, 26*, 181–185.

Avallone, R., Plessi, M., Baraldi, M., & Monzani, A. (1997). Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. *Journal of Food Composition and Analysis, 10*, 166–172.

Blenford, D. E. (1979). Toasted carob powder. *Confectionery Manufacture and Marketing, 16*, 15–19.

Calixto, F. S., & Canellas, J. (1982). Components of nutritional interest in carob pods *Ceratonia siliqua*. *Journal of the Science of Food Agriculture, 33*, 1319–1323.

Dea, I. C. M., & Morrison, A. (1975). *Advances in Carbohydrate Chemistry and Biochemistry, 31*, 241–312.

Doublier, J. L. (1975). Propriétés rhéologiques et caractéristiques macromoléculaires des solutions aqueuses de galactomannanes, PhD thesis, Université Paris VI, France.

Egli, W. (1969). Origin,characteristics, and possible applications of plant hydrocolloids, particularly galactomannan. *Schweizerusche Zeitschrift fuer Obst-und Weinbau, 105*, 470–477.

Food and Nutrition Board-National Research Council. (1981). *Food chemicals codex*. Washington, DC: National Academy Press.

Glicksman, M. (1969). *Gum technology in the food industry*. New York: Academic Press.

Imeson, A. (Ed.). (1997). *Thickening and gelling agents for food* (2nd ed.). New York: Culinary and Hospitality Industry Publication Services.

Kapoor, V. P. (1972). *Phytochemistry, 11*, 1129–1132.

Karawya, M. S., wassel, G. M., Baghdadi, H. H., & Ammar, N. M. (1980). Mucilagenous contents of certain Egyptian plants. *Plant. Med. Phytother., 38*, 73–78.

Lopes da Silva, J. A., & Gonçalves, M. P. (1990). *Food Hydrocolloid, 4*, 277–287.

McCleary, B. V., & Matheson, N. K. (1976). Galactomannan utilization in germinating legume seeds. *Phytochemistry, 15*, 43–47.

McCleary, B. V., & Matheson, N. K. (1974). *Phytochemistry, 13*, 1747–1757.

McCleary, B. V., Matheson, N. K., & Small, D. M. (1976). *Phytochemistry, 15*, 1111–1117.

Meyer, K., Rosa, C., Hischenhuber, C., & Meyer, R. (2001). *Journal of AOAC International, 84*, 89.

Rafique, C. M., & Smith, F. (1950). *Journal of American Chemical Society, 72*, 4634–4637.

Yousif, A. K., & Alghzawi, H. M. (2000). Processing and characterization of carob powder. *Food Chemistry, 69*, 283–287.