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Rheological Properties and Sugar Composition of Locust Bean Gum from Different Carob Varieties (*Ceratonia siliqua* L.)

VALERIA RIZZO, FILIPPO TOMASELLI, ALESSANDRA GENTILE, STEFANO LA MALFA, AND EMANUELE MACCARONE*

Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA), Università degli Studi di Catania, Via S. Sofia 98, 95123 Catania, Italy

The seeds of the main Italian carob varieties, Latinissima and Tantillo, and those of two selected accessions of Latinissima were evaluated in terms of yield, rheological properties, and sugar composition of the endosperm (LBG). The separation of the seed components in Latinissima and its seedlings yielded meanly 52.2% gum, 17.4% germ, and 30.5% tegument, whereas Tantillo furnished a lower gum yield (38.5%) and a higher yield of tegument (45.8%). The viscosity of 1% LBG aqueous solutions was measured at different shear rates (3−60 rpm), pH values (3.0−6.0), and temperatures (10−60 °C). The best results were shown by Latinissima, whereas Tantillo provided always the poorest thickening capacity. The content of free simple sugars and sucrose in the raw flours, the total monosaccharide residues after acidic hydrolysis, the mannose/galactose ratio, and the distribution of polysaccharides by size exclusion chromatography accounted for the observed viscosities. The seeds of Latinissima showed the highest technological potential.

KEYWORDS: ***Ceratonia siliqua* L.; locust bean gum; mannose/galactose ratio; molecular weight of galactomannans; rheological properties; sugar composition**

INTRODUCTION

Carob is a typical tree of the semiarid environments in the Mediterranean area. It produces edible pods used as a fodder for breeding cattle; it has also a long history of application as a source of health products (*1*). Improvement of the living standard in the past decades caused a reduction of production and a gradual disappearance of carob trees in southern Italy and Sicily. Cultivation is concentrated in the Ragusa district (Sicily), where 70% of the Italian carob fruits are produced (*2*). Nowadays, carob is exploited prevalently for the industrial transformation of the seeds, for obtaining a flour called locust bean gum (LBG), used as thickening agent in food preparations because of its ability to form viscous solutions and to stabilize emulsions and dispersion (*3*). The European Codex classifies LBG as a fully accepted food additive for human use (E 410).

The carob fruit contains about 90% pulp, rich in sucrose, glucose, cellulose, and tannins, and 10% seeds (*1*). The seed endosperm consists prevalently of galactomannan, the polysaccharide responsible for thickening properties, which is able to give synergic interaction when used in combination with other charged polysaccharides (*4*−*7*).

Galactomannans are neutral energy reserve polysaccharides made of a linear chain 1→4 linked β-D-mannopyranosyl units, with α-D-galactopyranosyl residues 1→6 joined as irregularly spaced side chain (*3*). The galactose distribution in the mannose linear chain controls the rheological properties of LBG; in particular, a higher mannose/galactose ratio (M/G) leads to higher thickening ability (*8*) and influences solubility (*9*), mechanism, and temperature of gel formation (*7*). Galactomannans exist as not greatly soluble "random coils"; for this reason high temperature and vigorous agitation are required for their complete dissolution in water, to achieve the best water-binding capacity. Carboxylated, hydroxylated, and phosphate derivatives of galactomannans have been prepared to increase solubility (*4*).

Recent studies increased the knowledge of the other carob constituents. Pinitol (*O*-methyl inositol) was identified in the pulp and used as a marker to ascertain adulteration of cacao by carob pulp flour (*10*). Composition of the lipid, protein, and phenol fractions was studied in the seed germ (*11*). Volatile components of aroma were separated and identified, and methylpropanoic and hexanoic acids were recognized as the most important contributors to the carob flavor (*12*). Various *C*-glycosyl- and *O*-glycosyl-apigenines were identified in the seeds (*13*). The level of condensed tannins and gallotannins was determined in pulp and seeds (*14*). Roasting effects of pulp and seeds on the composition of the aromatic fraction were investigated (*15*, *16*). Utilization of the seed oil to prepare alkyd resins was also reported (*17*).

In previous papers we reported the distribution of fatty acids and phytosterols in the carob seed oil (*18*) and evaluated the

* Author to whom correspondence should be addressed (fax +39 095 7141960; e-mail emacca@unict.it).

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morphological traits of the fruits of the most widespread Italian genotypes, Latinissima and Tantillo, and of some seedlings of Latinissima (*19*). The present work originated from the interest of the food industry in LBG, which is produced using the seeds of all available carob varieties. However, if the heterogeneity of varieties is not relevant for using the pod flour as a source of sugar and flavor in confectionery, it could be important for using the seeds, because the higher is the yield and quality of LBG, the higher is the value of the seeds. Moreover, considering that past selections of carob tree were made to favor the "pulp" character and reduce the "seed" one (*20*, *21*), it is useful to know which carob variety today present in the territory is more suitable for yield and rheological properties of LBG, with the aim of selecting new genotypes able to satisfy the market requirement. The goal of this study is the knowledge of the technological potential of the seeds of Latinissima and Tantillo, evaluating the results in terms of structure−property relationships.

MATERIALS AND METHODS

Seed Characterization. Four samples of carob seeds were investigated: one of the Tantillo hermaphrodite variety (T), one of the Latinissima female variety (L), and two seedlings of Latinissima belonging to our department collection, designated LM and LS. The moisture of the seeds was determined by drying in an oven at 70 °C for 15 h to constant weight. The volume and weight of seeds were determined by filling a graduated cylinder to 100 mL; seeds were weighed and computed. The separation of endosperm (germ and gum) from the external tegument was performed by hand, after the seed treatment in boiling water for 45 min and storage in the same water for 12 h at room temperature. After hulling, germ, gum, and tegument were dried in an oven at 70 °C for 15 h to constant weight, to refer yield of each component to 100 g of dry seeds. All of the measurements were carried out in triplicate runs.

Flour Preparation and Viscosity Measurements. Dry gum was ground with a cutmill (model MF10, IKA, Staufen, Germany) with a grid of decreasing diameter and sieved using test sieves (model BS 410) to obtain a fine flour of 106 μm (140 mesh). Standard aqueous solutions for viscosity measurements were prepared as follows: LBG (1 g) was added to 100 mL of citrate/citric buffers (pH 3, 4, 5, 6); the suspension was heated to 85 °C under stirring at 500 rpm for 5 min and then at 1000 rpm for another 25 min in the thermostated bath. After cooling, the viscosity values (cps) were measured at constant temperature (10−60 °C) with a rotational viscometer (model Visco Star R, Selecta, Milan, Italy), using different spindles (R2−R3) and shear rates (3−60 rpm).

LBG Sugar Composition. Monosaccharide composition was determined after acid hydrolysis of polysaccharides using trifluoroacetic acid (TFA) and transformation of the monosaccharide residues in volatile trimethylsilyl-oxime derivatives by treatment with hydroxylamine and hexamethyldisilazane (HMDS), as previously described (*22*, *23*). Advantages of this method are the relatively short reaction time, the simple sample handling, and the inexpensive reagents. The popular methodology of sugar transformation in volatile alditol acetates (*24*) was not used because fructose forms both glucitol and mannitol acetates epimers. Mannitol acetate is also formed from mannose, thus causing an overestimation of the actual content of mannose, which is misleading in the determination of the mannose/galactose ratio.

Separation, identification, and quantification of monosaccharides and sucrose were performed by GC-FID by comparison with a mixture of standard sugars (galactose, rhamnose, glucose, arabinose, mannose, xylose, fructose, and sucrose). The analysis of the standard mixture was performed as follows: in a vial, 10 mg of each sugar was added to 2 mL of Stox reagent (25 mg/L hydroxylamine and 6 mg/L phenyl-β-D-glucopyranoside as GC internal standard in pyridine as a solvent). The solution was heated for 30 min at 75 °C, and then 1 mL of HMDS and 0.1 mL of TFA were added. The reaction mixture was mixed for 30 s and stored for 30 min. After addition of isooctane to a known volume, the solution was ready for GC analysis. An HP instrument (model 5890) was used under the following conditions: column, HP 5% MS (L 30 m, i.d. 0.25 mm, film 0.25 μm); oven temperature, initial isotherm, 50 °C for 1 min, gradient, 10 °C/min to 120 °C, 2 °C/min up to 200 °C, and 10 °C/min to 280 °C, final isotherm, 280 °C for 10 min; helium as gas carrier at a linear velocity of 9 cm/s; injector, 270 °C; detector, 300 °C.

Total free and bound monosaccharides in the flour were determined according to the following procedure: LBG flour (50 mg) and TFA (1 mL) were heated in a vial at 100 °C for 60 min; the solution was dried in rotavapor at 45 °C, and 2 mL of Stox reagent was added to the dry residue. This solution was dried in an oven at 75 °C for 30 min, and hence the same procedure described for the standard sugar analysis was followed. The free monosaccharides and sucrose were determined in LM and T samples by an analogous procedure, but without using the first step of acid hydrolysis by TFA.

Free and bound sugar determinations were performed in duplicate runs and analyzed twice by GC. Deviation from the mean value does not exceed ±8%.

The reaction between monosaccharides and hydroxylamine yielded two oxime stable stereoisomers because of the hindered rotation around the newly formed carbon−nitrogen double bond. Both isomers remained after silylation of OH groups; therefore, two peaks for each sugar appeared in the gas chromatogram: the former, at lower retention time due to the *anti* isomer, and the latter to the *syn* one (**Figure 1**). The two peaks of xylose, rhamnose, and fructose were well separated, and quantitation of each sugar was performed by summing up the corresponding peak areas. Glucose, mannose, and galactose showed also two peaks, but the second was accidentally isochronous for the three sugars, as it was ascertained by preliminary experiments analyzing various standard sugar mixtures. The relative contribution of each of three *syn* stereoisomers to the total area of the second peak was calculated with a series of calibrated determinations. Sucrose showed only one peak due to the silylated derivative, because it cannot form oxime being acetal in structure.

Size Exclusion Chromatography. The size exclusion chromatography apparatus consisted of a Waters 515 HPLC pump connected to a prefilter, a guard column, two TSK-Gel PW_{XL} G4000 and G5000 columns, and a Waters 410 differential refractive index detector. The eluent was water purified by a Simplicity system from Millipore. The columns were calibrated with pullulan molecular weight standards (Shodex) and ethylene glycol (0.1% w/v). Samples to be analyzed were dissolved (0.1% w/v) and filtered through 0.45 μm disposable syringe filters, and 100 μL was injected. The flow rate was 1 mL/min. The signal from the detector was sampled by a DAC interface and recorded and elaborated with the GPC for Windows software from ChemWare.

RESULTS AND DISCUSSION

Table 1 reports the weight and number of seeds in 100 mL; it reports also the yield of germ, gum, and tegument referred to 100 g of dry seeds. The weight ranges from 86.3 g for T to 90.1 g for LS. The number of seeds varies from 414 for L to 600 for T. The seeds of L are bigger and heavier than those of the other samples, in particular, the mean weight of a single seed changes from 0.14 g for T to 0.21 g for L. The yield of germ does not show relevant variations, whereas yield in gum changes from 38.5% for T to 53.0% for LM. Such a difference is reflected in the yield of tegument, which is maximum in T (45.8%) and minimum in L (29.0%). The best variety for the gum yield is Latinissima, whereas Tantillo is the worst. Other advantages of Latinissima are the seed yield (*19*), the big size of the seeds, which makes the hulling process easier, and the minor amount of tegument that is a byproduct.

Barbagallo et al. (*23*) studied the seeds of 16 Sicilian carob cultivars to evaluate morphological characteristics and percentage of endosperm. Our results range within the reported mean

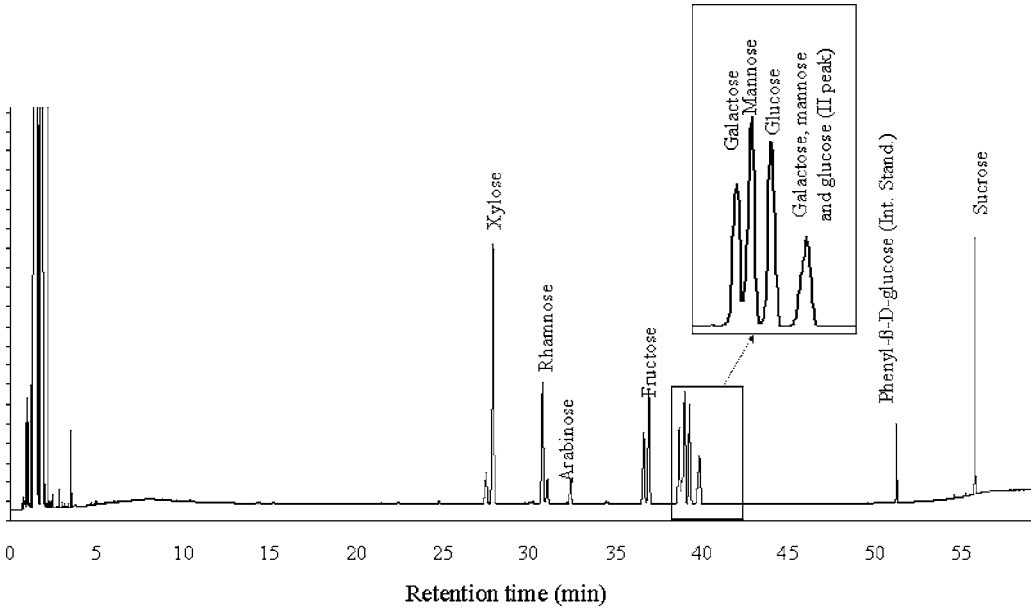

Figure 1. Gas chromatogram of standard sugars after derivation with hydroxylamine and hexamethyldisilazane.

Table 1. General Characteristics of Carob Seeds of Different Genotypes[a]

sample	L	LM	LS	T
wt of 100 mL of seeds (g)	89.6 (0.4)	88.0 (0.5)	90.1 (1.1)	86.3 (0.7)
seed no. in 100 mL	419 (18)	444 (7)	437 (7)	600 (8)
seed moisture (%)	3.7 (0.6)	5.8 (0.4)	6.7 (0.7)	4.4 (0.6)
yield of germ on dry seeds (%)	18.2 (0.3)	17.9 (0.4)	16.1 (0.6)	15.6 (1.9)
yield of gum on dry seeds (%)	52.8 (1.0)	53.0 (0.2)	50.8 (0.7)	38.5 (1.5)
yield of tegument on dry seeds (%)	29.0 (0.7)	29.2 (0.2)	33.2 (1.3)	45.8 (3.4)

[a] Mean value of triplicate runs. Standard deviation in parentheses.

Table 2. Total Content of Monosaccharides and Sucrose (Grams per 100) of Raw LBG of Different Genotypes after Acidic Hydrolysis[a]

sample	L	LM	LS	T
xylose	13.9	15.8	15.4	18.5
rhamnose	1.9	2.1	2.8	1.8
fructose	4.6	4.6	5.5	5.8
galactose	9.9	10.0	10.3	7.1
mannose	40.0	40.4	38.2	20.8
glucose	6.8	6.5	7.5	7.5
sucrose	1.9	1.9	1.8	1.1
total sugars	79.0	81.3	81.5	62.6
fructose, glucose, and sucrose	13.3	13.0	14.8	14.4
M/G	4.0	4.0	3.7	2.9

[a] Mean values from duplicate experiments and duplicate GC determinations. Deviation does not exceed ±8%.

values; in particular; the cv. Latinissima, which can be assimilated to Gibiliana (synonym), is similar with regard to the seed weight and gum yield, but it furnished a major percentage of tegument.

Garcia Ochoa and Casas observed that apparent viscosity of LBG aqueous solution increased with increasing solubilization temperature, and this behavior was related to difference in the molecular weight of polysaccharides dissolving at various temperature, which produced a different mannose/galactose ratio in the solution, from 2.56 (25 °C) to 3.57 (80 °C). Therefore, we dissolved all LBG samples at 85 °C to perform a homogeneous comparison of viscosity measurements and M/G ratio.

Figure 2 shows the viscosity values (cps) of 1% LBG solutions (w/v) measured at 20 °C and pH 5 according to shear rate. LM furnishes the highest viscosity and T the lowest. **Figure 3** shows the variation of viscosity at 20 °C when the pH is changed from 3.0 to 6.0, using a shear rate of 20 rpm. LM induces the highest viscosity at all pH values, whereas T gives systematically lower viscosity values. In all cases a maximum value is observed at pH 5. As galactomannans are neutral polysaccharides this behavior can be ascribed to the presence of charged contaminants (proteins), which can modify the ionic strength of the solutions depending on pH value.

Figure 4 shows the temperature effect on the viscosity of solutions at pH 5; differences among the samples are enhanced at 10 °C and decrease with increasing temperature, as expected. The solutions of T flour look in all aspects the less viscous.

The content of polygalactomannans and the M/G ratio are the main factors in determining the thickening properties of LBG: a higher M/G value indicates a major linearity of the polymer (low number of galactose side chains) and a greater "effective volume" of the macromolecules (*3*). To correlate the observed viscosity with the above parameters, total free and bound monosaccharide residues were identified and quantified after acidic hydrolysis of the raw flours (**Table 2**).

Mannose is the predominant sugar, with a level of ∼40% in the samples of L, LM, and LS and ∼20% in T. The content of galactose is ∼10% in L, LM, and LS, whereas a minor amount is found in T (7.1%). Xylose varies from 18.5% in T to 13.9% in L. Glucose, fructose, rhamnose, sucrose, and traces of arabinose are also present. The total sugars content is ∼80%, except for T (62.7%). The M/G ratio is near 4 for L, LM, and LS and <3 for T. The low quantity of mannose in T flour, together with the low M/G ratio, accounts for the reduced thickening capacity. It is reasonable to argue that mannose and galactose are the products of acid hydrolysis of polygalactomannans, whereas glucose and fructose come from hydrolysis of sucrose. As far as the foreseen 1:1 ratio for these sugars is concerned, it can be hypothesized that the lower concentration of fructose relative to glucose could be ascribed to faster degradation of the former during the acidic hydrolysis treatment (*25*).

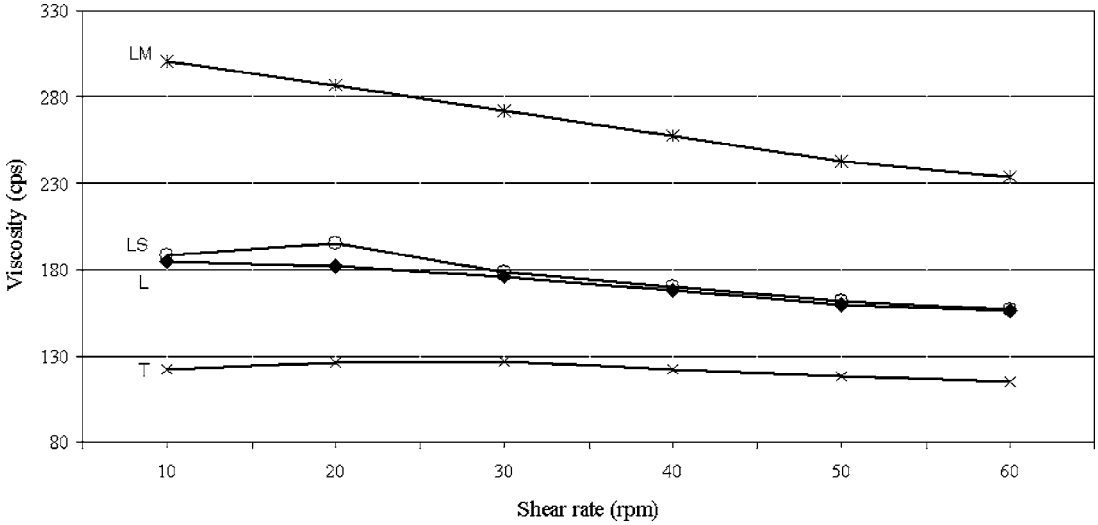

Figure 2. Viscosity values (cps) of 1% LBG aqueous solutions at pH 5 and 20 °C versus shear rate (spindle R2).

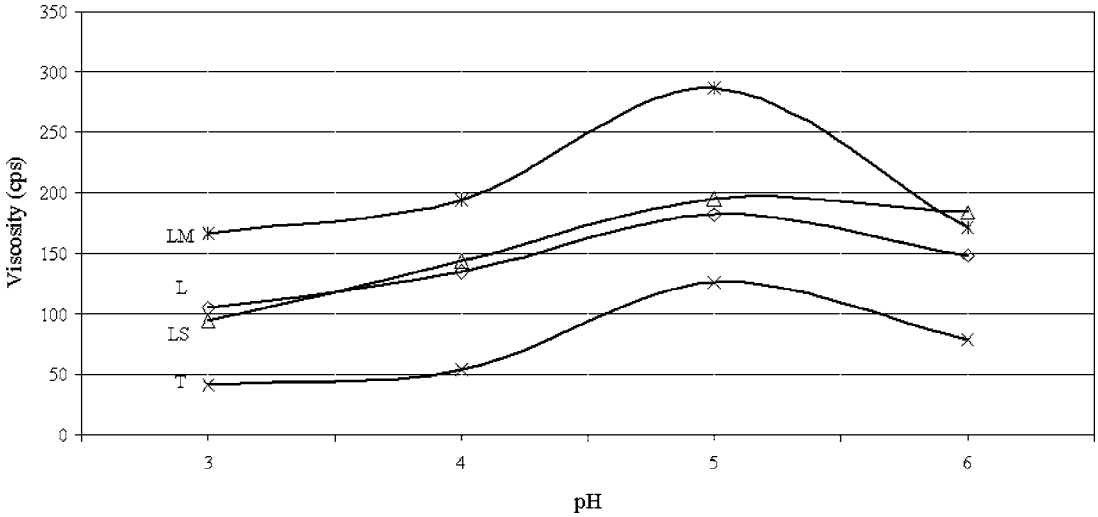

Figure 3. Viscosity values (cps) of 1% LBG aqueous solutions at 20 °C versus pH value (spindle R2 at 20 rpm).

Table 3. Content of Free and Bound Monosaccharides (Grams per 100) of LM and T Raw LBG[a]

	LM		T	
sugar	free	bound[b]	free	bound[b]
xylose		15.8	0.9	17.6
rhamnose	2.7		1.4	
fructose	1.1		1.7	
galactose	0.5	9.5		7.1
mannose		40.4	0.8	20.0
glucose	1.6		3.0	
sucrose	11.5		15.8	
total sugars	17.4	65.7	23.6	44.7
fructose, glucose, and sucrose	14.2		20.5	

[a] Mean values from duplicate experiments and duplicate GC determinations. Deviation does not exceed ±8%. [b] Calculated by difference between total (**Table 2**) and free sugar contents.

These results are roughly similar with those of the literature (*9*) relative to mannose, galactose, and glucose; in fact, the reported percentages are 45.5, 14, and 5.1%, respectively (M/G 3.3), but remarkable differences are observed for other sugars: a much higher level of arabinose (33.9%) and a lower content of xylose (1.7%), whereas rhamnose, fructose, and sucrose were not reported.

Table 3 reports the content of free monosaccharides in the unhydrolyzed LBG. The results confirm the above hypothesis. In fact, the raw flours contain ~20% of total free sugars (17.4% for LM and 23.6% for T) represented prevalently by sucrose and by small amounts of rhamnose, glucose, and fructose. These sugars do not give any contribution to the thickening properties, whereas mannose, galactose, and xylose affect the rheological properties, being constituents of the polysaccharide structures. In particular, xylose is the monomer unit of pentosans, the presence of which in LBG has been recognized for a long time (*26*). The total amount of fructose, glucose, and sucrose is slightly higher in the unhydrolyzed flours than in the hydrolyzed ones because of partial thermal degradation in the acidic medium (*25*).

The polysaccharide distribution of LM and T raw flours and of a sample of a refined commercial LBG was analyzed by gel permeation chromatography (GPC). The distribution of the molecular weights (MW) was calibrated using solutions of known MW standard pullulans. **Figure 5** shows the superimposed chromatograms of LM and refined flours, whereas **Table 4** reports other informative chromatographic data. Two peaks occur in the gel permeation chromatograms: the first, at lower retention volume, due to polysaccharides (mean MW = 2.7×10^6) and the second corresponding to a mean MW of 520. The ratio between the peak areas of the commercial refined flour is

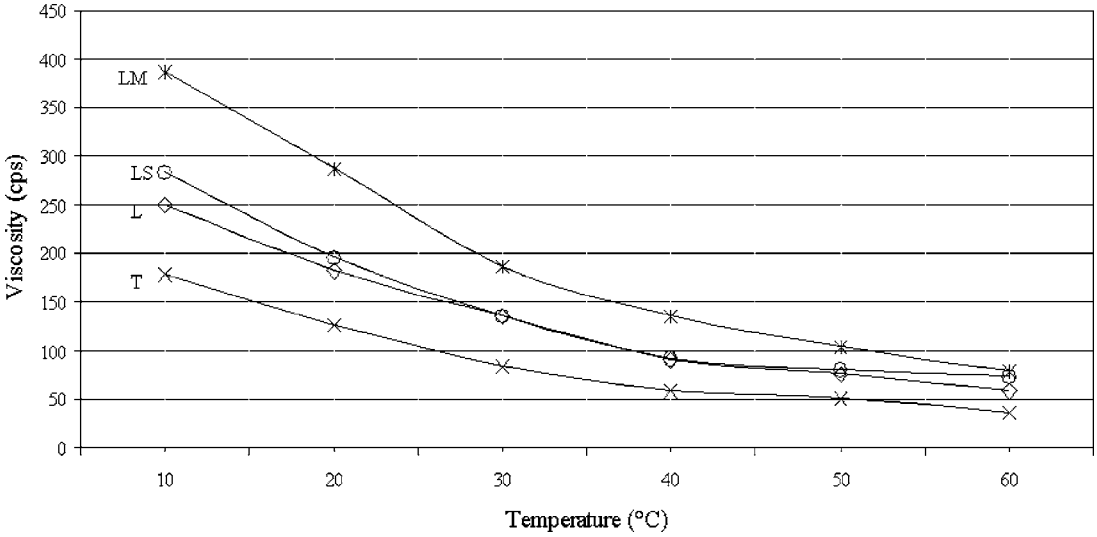

Figure 4. Viscosity values (cps) of 1% LBG aqueous solutions at pH 5 versus temperature (spindle R2 at 20 rpm).

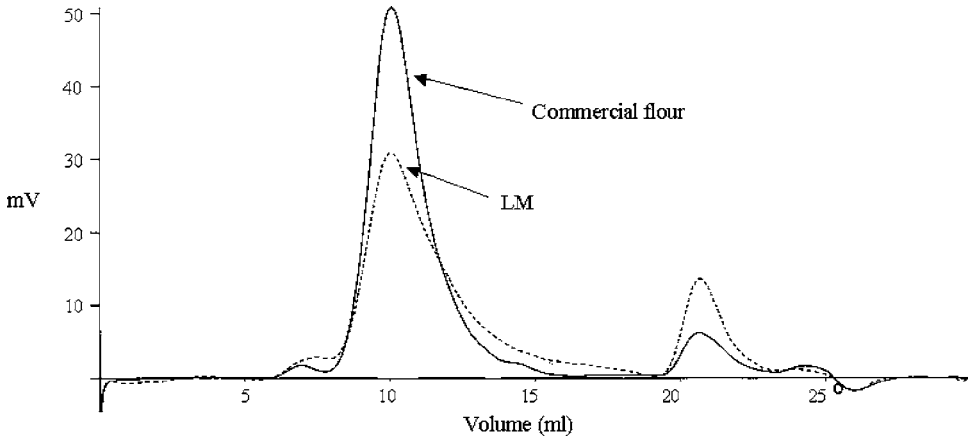

Figure 5. Gel permeation chromatogram of a commercial refined LBG and LM raw flour.

Table 4. Comparison of GPC Data between the Raw Flour (LM and T) and a Refined Commercial Flour

	LM		T		commercial flour	
	GPC peak I	GPC peak II	GPC peak I	GPC peak II	GPC peak I	GPC peak II
retention volume (mL)	10.1	20.6	9.9	20.7	10.1	20.6
molecular weight	2.6×10^6	508	2.7×10^6	552	3.0×10^6	510
area (%)	80.8	18.2	75.4	17.2	89.3	8.7
peak area sum (%)	99.0		92.6		98.0	
peak area ratio	4.4		4.4		10.3	

higher than the raw flours, because the low molecular weight components have been largely removed by the flour refinement.

In conclusion, Latinissima was the most suitable variety for LBG production, owing to the yield and size of the seeds and the yield and quality of the flour. The best thickening properties are measured for the LM seedling, according to the higher value of M/G ratio and the lower content of sucrose. Although the mean molecular weights of polysaccharides are almost the same in all samples, the level of polysaccharides is higher in Latinissima. The seeds of Tantillo are less suitable with regard to all considered aspects. It is relevant to emphasize that Latinissima produces only female flowers and requires pollen of other genotypes to bear fruits; for this reason, farmers seeking new carob plants prefer the hermaphrodite Tantillo variety, which is worse with regard to technological potential. Divulgence of the present results might contribute to the reversal of such a trend.

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