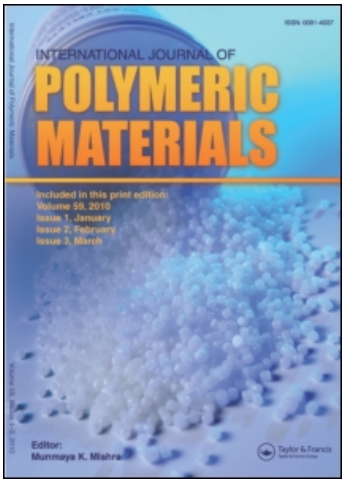

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Galactomannan, a water-soluble heteropolysaccharide, was isolated from the seed of Chinese traditional medicine fenugreek. The polysaccharide was characterized by using both chemical and chromatographic procedures, as well as FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy. The results showed that the polysaccharide consists of D-mannopyranose and D-galactopyranose residues with a molar ratio of 1.2:1.0. The main chain of this galactomannan comprises β-(1,4)-linked D-mannopyranose residues, in which 83.3% of the main chain is substituted at C-6 with a single residue of α-(1,6)-D-galactopyranose. The galactomannan had a molecular weight M_w of 3.23×10^5 g mol^{-1} and an intrinsic water viscosity of 235 mL g^{-1}. Fenugreek gum (seed endosperm) contains 73.6% galactomannan. The viscosity of fenugreek gum at 1% concentration is 286 mPa•s (30°C, 170 s^{-1}). The viscosity of the solutions decreased sharply as the rate of shear increased, but rose with increased concentration, and decreased as the temperature of fully hydrated gum solution was gradually raised from 30 to 90°C. Fenugreek gum can be a highly efficient water-thickening agents and can be used in food industry and drug delivery formulations.

Keywords: fenugreek gum, galactomannan, rheology, structure

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INTRODUCTION

Fenugreek, a leguminous annual plant, is a native of West Africa and now widely cultivated in China, India, Pakistan, Egypt, and Mediterranean countries. Fenugreek seeds have long been known as a Chinese traditional medicine. In recent years fenugreek seeds have been used in Western countries as a medicinal herb and as a flavoring agent to imitate the taste of maple [1]. In north China, the seed powder is mixed with flour to make steamed bread, which is used as a condiment. The seeds contain many nutrients including protein, carbohydrates, fiber, fats, saponins, choline and trigonelline, vitamin, and minerals as well as enzymes. Among the various constituents, fiber and saponin components of the seeds have been shown to have bioactivity. In addition, fenugreek seeds are known to contain steroidal sapogenins [2–4], especially diosgenin (25R-spirost-5-en-3β-ol), which is considered as a basic compound in the hemi-synthesis of steroid drugs such as cortisone and sexual hormones, and thus, they are of great significance to the pharmaceutical industry.

It has been reported that the fenugreek seed contains 26.8% fenugreek galactomannan gum [4]. The effect of the gum on the blood glucose level of the normal and alloxan diabetic rats has been determined after treatment with supplemented diets. Fenugreek gum produces a significant drop in blood glucose both in normal as well as diabetic rats when they are fed with 0.9 and 4.5 g kg^{-1} dose [5–7]. In addition, the fenugreek gum has a highly hypoglycemic effect, compared with fenugreek seeds, by treatment with supplemented diets to the alloxan diabetic rats. There are many investigations on the effect of fenugreek gum on the blood glucose level, but very little work has been done on the structure and rheological properties fenugreek gum. In the present study, the fenugreek gum isolated from the fenugreek seeds was structurally characterized by gas-liquid chromatography (GLC), Fourier transform infrared (FT-IR), and hydrogen and carbon-13 nuclear magnetic resonance (^{1}H, ^{13}C NMR) spectroscopy, and gel permeation chromatography (GPC), and its rheological properties, are also reported.

EXPERIMENTAL

Preparation of Galactomannan

Fenugreek seeds were obtained from Institute of Medicinal Plant, Chinese Academy of Medical Sciences, Beijing, China. The endosperms of the fenugreek seeds were separated by wet milling process

using 20% ethanol. The products were powdered to a 60 mesh and extracted with benzene-ethanol (2:1, v/v) in a Soxhlet for 6 h. The crude polysaccharides were extracted with 100 volumes of water (w/v) under continuous stirring for 6 h at 30°C. The aqueous extraction was separated by centrifugation at 4000 rpm for 15 min. The precipitates were re-suspended in a small volume of water, and the suspension was centrifuged again under the same conditions. The precipitates were decanted, and the clear supernatant liquids were precipitated by addition of 2 volumes of ethanol and overnight incubation at 6°C. The polysaccharides precipitated were separated by centrifugation (4000 rpm, 15 min), washed three times with ethanol under an increasing concentration from 75% to 96%, and then freeze-dried.

The polysaccharides isolated were further purified as follows: a viscous solution (0.5% w/v) was prepared under continuous stirring for 6 h at 30°C and precipitated with the Fehling reagent. The Cu^{2+} complex was centrifuged and de-complexed with 2 M acetic acid under continuous stirring for 12 h. The corresponding polysaccharide was recovered from the centrifuged solution by precipitation with ethanol, washed successively with ethanol, acetone, and ether, and finally freeze-dried.

Monosaccharide Composition of Galactomannan

The polysaccharides were first treated with 72% sulfuric acid for 30 min under stirring at room temperature, and then further hydrolyzed at 100°C for 2 h with 3% aqueous sulfuric acid. The resulting aldoses were reduced to alditols with sodium borohydride in dimethylsulfoxide (DMSO). Acetylation was effected with pyridine-acetic anhydride (1:1 v/v, 1 h, 100°C). The dried alditol acetates were dissolved in dichloromethane and injected (1 μL) into a GLC capillary column DB-225. The column was operated isothermally at 220°C, using hydrogen as a carrier gas at a flow rate of 0.8 mL min^{-1}. Identification of the peaks was based on comparison of the retention times of the compounds sought and commercial monosaccharides. Quantitative measurements were performed through calculating the areas of the corresponding peaks using a graphical procedure.

The polysaccharides were methylated in the usual way by the Haworth procedure, followed by treatments with the Purdie method until FT-IR $-$OH absorption at 3600–3400 cm^{-1} was negligible. The product was then subjected to hydrolysis and analysis of the methylated fragments by GC–MS.

Analyses of Galactomannan by GPC, FT-IR, and NMR Spectroscopy

The measurements of the average molecular weight and intrinsic viscosity of the polysaccharides were performed on a Waters/GPC2000 at 25°C with a concentration of 1 g L^{-1} galactomannan solution after filtration through 0.2 μm Millipore filters. FT-IR spectra of samples embedded in KBr were recorded on a TENSOR 27 instrument. NMR spectrum of the sample dissolved in D_2O (10 mg mL^{-1} for ^1H; 100 mg mL^{-1} for ^{13}C) was recorded on a Bruker DRX 300 with a 65° pulse and a repetition time of 0.8 s.

Determination of Rheological Properties of Fenugreek Gum

Endosperm was separated from the seed hull and embryo by taking advantage of the difference in hardness of the various seed components. The endosperm was then ground to powder form and marketed as fenugreek gum. The moisture content of the fenugreek gum was determined by drying the sample in an oven at 103°C for 12 h. The ash content was measured by heating the dried sample in a muffle furnace at 500°C for 16 h and then at 900°C for 1 h. The protein was determined by the Kjeldahl method. The galactomannan content and the insoluble substance were determined, respectively, using the purification method of the polysaccharides as described earlier.

Gum solutions were prepared by dispersing gum samples from 0.3% to 1.5% (wt) at 30°C with mechanical stirring in distilled water for 3 h. The resulting solution was translucent in nature. The viscosity of different concentrations of the samples was measured at 30°C with a ZNN-D6 viscometer at different shearing rates. The viscosity of 1% (wt) solution of the samples was measured at 30°C with a NDJ-79 viscometer at different temperatures. The viscosity of 1% (wt) solution of the samples was also measured at 30°C with a ZNN-D6 viscometer (shearing rate 170 s^{-1}) at different hydration times.

RESULTS AND DISCUSSION

Characterization of the Polysaccharide

Upon complete hydrolysis, the purified galactomannan showed the presence of D-galactose and D-mannose. Their ratios, determined by GLC and ^1H NMR, are reported in Table 1. The mannose/galactose ratios, calculated from the relative intensities of ^1H NMR signals, were in good agreement with those obtained by chemical analysis by

TABLE 1 Physicochemical Property of Fenugreek Galactomannan

Mannose/galactose ratio		Weight average molecular weight	Number average molecular weight	Intrinsic Viscosity
GLC	^{1}H-NMR	M_w	M_n	mL/g
1.23	1.18	5.01×10^5	3.23×10^5	235

GLC. The GPC revealed that the fenugreek galactomannan had a weight-average molecular weight (M_w) of 3.23×10^5 g mol^{-1} with a polydispersity of 1.6 and an intrinsic water viscosity of 235 mL g^{-1} (Table 1) in water.

Similarly, upon complete hydrolysis, the methylated polysaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-galatose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-mannose in the molar proportions of 1.00:0.22:1.02 as determined by GC-MS. Tetra-*O*-methyl-D-mannose, tri-*O*-methyl-D-galactose, and di-*O*-methyl-D-galactose were not identified from the hydrolysates of the methylated galactomannan. The proportions of the isolated methyl sugars are consistent with the mannose to galactose ratios of the polysaccharides determined by GLC and ^{1}H NMR.

The absorption curve of the fenugreek galactomannan in the FT-IR range of the spectrum was identical to the guar curve between 500 and 4000 cm^{-1} (Figure 1). In the range of 700–1000 cm^{-1}, weak absorption bands at 813, 872, and 890 cm^{-1} are due to the CH oscillations of (β-mannopyranose residues. The absence of the protein absorption band amide II (approximately at 1560 cm^{-1}) in the spectrum indicated

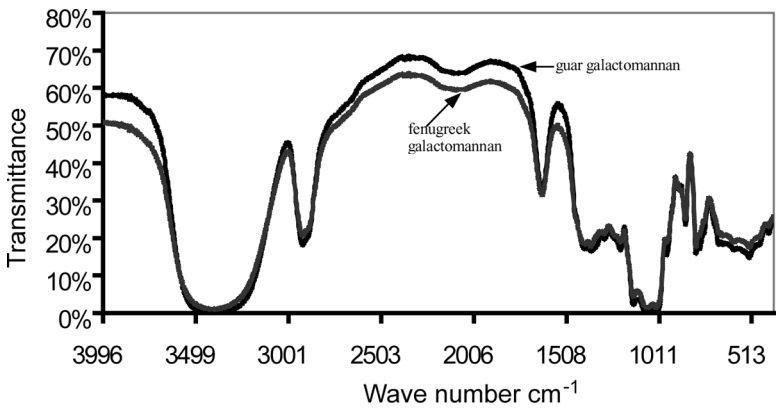

FIGURE 1 FT-IR spectra of fenugreek and guar galactomananns.

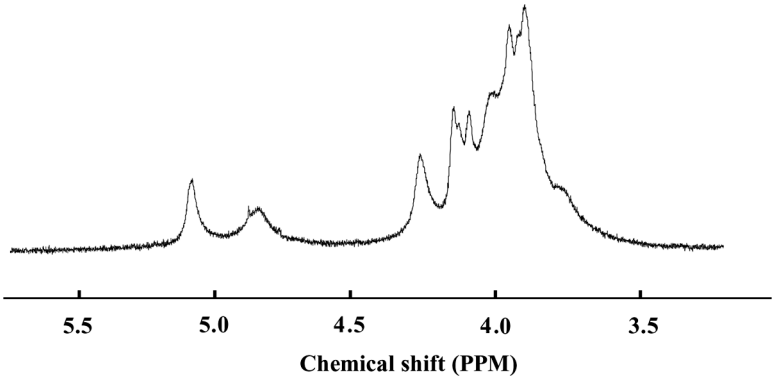

FIGURE 2 H NMR spectrum of fenugreek galactomanann.

that the galactomannan preparation isolated is free of the protein contaminants.

As shown in Figure 2, the resonance of the anomeric protons in the ^{1}H NMR spectrum are well separated, and their identification is evident from the known monomeric composition of polysaccharide (Gal:Man = 1.00:1.18) [8]. Measurement of the ratios of the corresponding peak-areas gave results for monomeric composition (Gal: Man ratio), which were in good agreement with those obtained by chemical analysis. Obviously, the spectrum gives a signal at 4.8 ppm

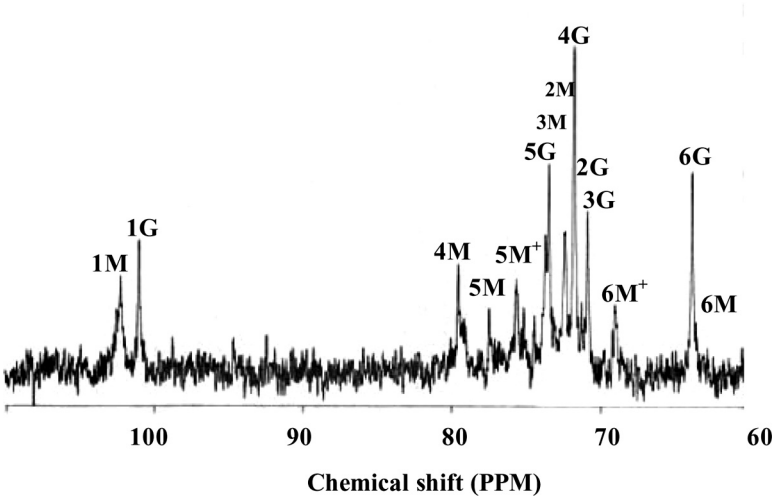

FIGURE 3 ^{13}C NMR spectrum of fenugreek galactomanann.

TABLE 2 Position of the Signals of ^{13}C NMR Spectrum of Fenugreek Galactomannan

Type of unit	C-l	C-2	C-3	C-4	C-5	C-6
α-D-Galactopyranosyl	101.1	72.1[a]	71.1	72.3	74.2	63.2
β-D-mannopyranosyl (unbranched)	102.8	72.2	73.6 79.0[b]	78.7[a]	77.8	63.2
β-D-mannopyranosyl (branched at O-6)	102.8	72.2	73.6 79.4[b]	79.0[a]	75.9	69.1

[a]As the preceding D-mannosyl units is unbranched.
[b]As the preceding D-mannosyl units is branched.

($Ji_{1,2} \sim 1.0$ Hz) of the anomeric proton (H-1) of the D-mannopyranosyl units, which, accordingly, have the β-D configuration. Another signal at 5.0 ppm. ($J_{1,2} \sim 3.2$ Hz) is assigned to H-1 of the galactopyranosyl units, which, therefore, have the expected α-D configuration.

In the ^{13}C NMR spectrum (Figure 3), the separated lines are in accord with those reported for guar gum. Table 2 lists the chemical shifts of the fenugreek galactomannan. As can be seen, three types of structural units can be clearly differentiated and identified. These units are (i) the α-D-galactopyranosyl nonreducing end-units, (ii) the otherwise un-substituted (1 → 4)-linked β-D-mannopyranosyl units of the mannan backbone, and [iii] the *O*-6-substituted (1 → 4)-linked β-D-mannopyranosyl units of the mannan backbone [9]. The fenugreek galactomannan molecule has therefore a "comb" structure, that is, a backbone of 1,4-β-D-mannan with a substitution degree of C-6-carbon atoms by a single α-D-galactopyranosyl residue equal to 0.83. Such a structure is typical of majority of leguminous galactomannans.

Characterization of the Fenugreek Gum

Table 3 gives the chemical composition of the fenugreek gum and guar gum. In comparison, the water insolubles of fenugreek gum (8.5%)

TABLE 3 Chemical Composition and Physicochemical Property of Fenugreek and Guar Gum Powder

	Moisture %	Ash %	Protein %	Water insoluble %	Galactomannan %	Viscosity* mPa•s
Fenugreek gum	10.2	0.5	5.5	8.5	73.6	286
Guar gum	9.6	0.7	4.5	23.1	74.6	313

*Determined on a ZNN-D6 viscometer at 170 s^{-1} shearing rate and 30°C.

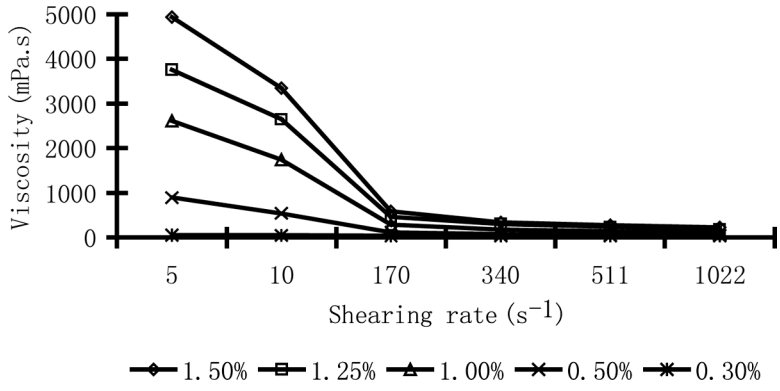

FIGURE 4 Effect of the concentration and shear rate on the viscosity of fenugreek gum (measured on a ZNN-D6 viscometer after 3 h hydration at 30°C).

were much lower than those of guar gum (23.1%), the clarity of fenugreek solutions was therefore higher than that of the guar solutions. Interestingly, the two gums contained approximately the same levels of galactomannans (73.6, 74.6%).

Fenugreek gum is an edible carbohydrate polymer, which is useful as a thickening agent for water and as a reagent for adsorption and hydrogen bonding with mineral and other carbohydrate surfaces. Solutions of fenugreek gum are non-Newtonian and classed as

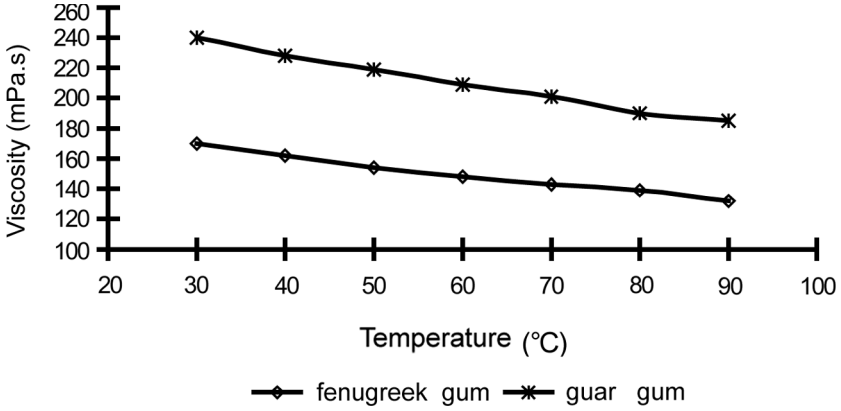

FIGURE 5 Effect of temperature on the viscosity of fenugreek and guar gums (measured on a NDJ-79 viscometer after 3 h hydration).

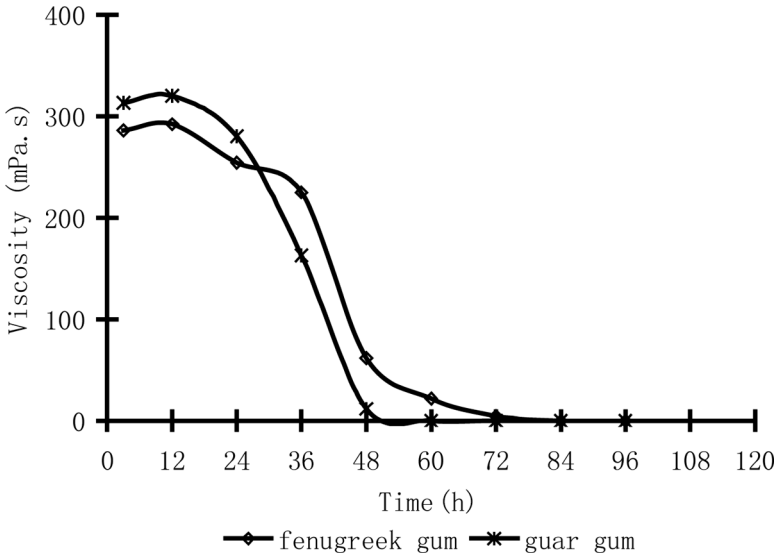

FIGURE 6 Viscosity development as the function of time for fenugreek and guar gum (measured on a ZNN-D6 viscometer at 30°C, shearing rate 170 s^{-1}).

pseudo-plastic (Figure 4). The viscosity of the solutions decreased sharply as the rate of shear was increased and then approached a minimum limiting value that is dependent on the concentration of the solutions. In general, the viscosity of solution is independent of time and prior shear testing. It makes no difference whether the final rate of shear is approached from low shear rate to high shear rate or vice versa. This implied that the shear rates are not high enough to degrade the molecular structure. As illustrated in Figure 5, when the temperature of the fully hydrated gum solutions is gradually raised from 30 to 90°C, the viscosity decreases. In addition, the rheological properties of fenugreek and guar gums were also investigated at various hydration times. As shown in Figure 6, when the hydration time is less than 12 h, the viscosities raise with time. In contrast, after 12 h, both of the gums begin to be degraded, and their viscosities decreased. In the food industry, solutions of gum serve as a food source for common microorganisms. Where it is necessary to hold solutions for a length of time, common food preservatives, such as benzoic or sorbic acid, may be added to inhibit bacterial growth. Fenugreek gum can be a highly efficient water-thickening agent used in food industry and drug delivery formulations.

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