«Green» synthesis and characterization of galactomannan sulfates obtained using sulfamic acid

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Abstract

In this work, we studied “green” synthesis and characterization of galactomannan sulfates obtained using the sulfamic acid-urea complex by FTIR spectroscopy, X-ray diffraction, scanning electron microscopy, atomic force microscopy, and gel permeation chromatography. It was shown that in the FTIR spectra of sulfated galactomannan there are bands at 1250 cm^{-1}, 805-820 cm^{-1}, which indicate the presence of sulfate groups. By the method of X-ray diffraction it was shown that the initial galactomannan has an amorphous structure, its amorphization increases during sulfation. Using scanning electron microscopy, it was shown that the initial galactomannan consists of particles of various shapes with sizes from 200 to 800 μm, and sulfated galactomannan consists of particles of various shapes with sizes of 50-1000 μm. Thermal analysis showed that the initial galactomannan has endothermic peaks at 254 and 294°C and an exothermic peak at 315°C, and sulfated galactomannan has endothermic peaks at 209 and 275°C and an exothermic peak at 281°C. Using atomic force microscopy, it was shown that a sulfated galactomannan film consists of spherical particles with an average diameter of 200 to 300 nm; according to phase contrast data, it is uniform in composition and has no extraneous impurities. According gel-permeation chromatography initial galactomannan have bimodal particle mass distribution: a high molecular weight fraction with an MM of ~ 1500 kDa and a low molecular weight fraction with an MM of ~ 600 kDa. An increase in the duration of the sulfation process from 120 to 300 min leads to the destruction of the galactomannan polymer chains with a decrease in the molecular weight of the main fraction of the sulfated product from 130 kDa to 110 kDa.

Keywords: galactomannan, sulfated galactomannan, structure, physicochemical study, sulfation.

1. Introduction

Recently, there has been growing interest in the use of biologically active substances of plant origin, which, as a rule, are safer compared to their analogues obtained synthetically or isolated from animal raw materials [1]. Plant biomass is a constantly renewable raw material and a practically inexhaustible resource for the production of biologically active substances [2].

Polysaccharides are high molecular weight polycondensation products of monosaccharides linked to each other by glycosidic bonds and forming linear or branched chains [3,4]. Polysaccharides make up the bulk of the organic matter on Earth [5].

The molecular weights of natural polysaccharides range from several thousand to several million Daltons. The macromolecular nature of these compounds leaves a very significant imprint on their physical and chemical properties [6].

The role of polysaccharides in human nutrition is diverse. They are harmless to the human body and are digested depending on the structure by 69% - 95%. Polysaccharides serve as a source of energy, affect lipid metabolism, play the role of enterosorbents, lower cholesterol, sorb microflora, salts of heavy metals [7,8].

Sulfated polysaccharide derivatives are promising for practical use. They are analogues of heparin, a substance of animal origin with anticoagulant activity [9, 10].

The biological activity of sulfated polysaccharides depends on the method of their preparation, which makes the physicochemical study of their composition and structure relevant [9].

The aim of this work was to study galactomannan sulfates, obtained using the sulfamic acid-urea complex, by FTIR spectroscopy, X-ray diffraction (XRD), atomic force microscopy (AFM), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and gel permeation chromatography (GPC).

2. Experimental

As the source of raw materials are used galactomannan from *Cyamopsis tetraganoloba* (LLC "Mast-sl").

Sulfation of galactomannan (GM) was carried out by the sulfamic acid-urea complex (SAA:U) according to a modified procedure [11, 12]. To do this, the sulfating complex (SC) and galactomannan were triturated to obtain a homogeneous mass. Sulphating complex was obtained by preliminary mixing 7.2 g of sulfamic acid (75 mmol) and 4.5 g of urea (75 mmol). The ratio of galactomannan and sulfating complex was 1:3 (mol/mol) (figure 1). For physico-chemical studies, a sample was taken with a sulfur content of 15.5%.

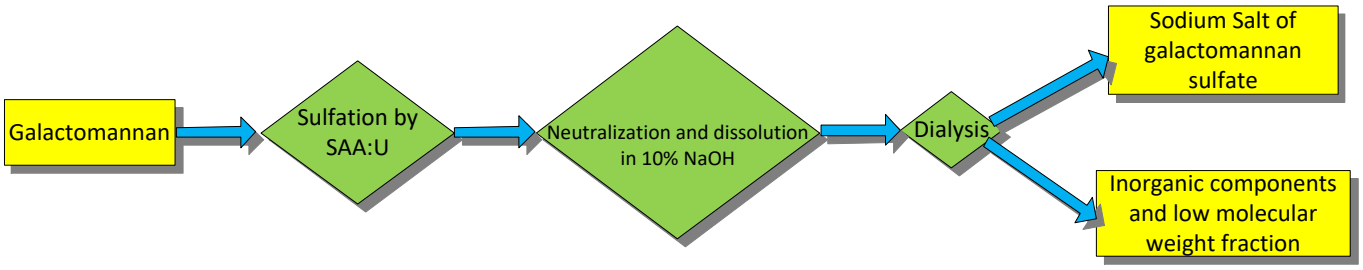


Figure 1. Scheme of experiment of the galactomannan sulfation process with sulfamic acid-urea complex.

At the end of the thermostating process, the melt was cooled to room temperature, the formed solid product was dissolved in 50 ml of water, and the unreacted sulfamic acid was neutralized with a 10% aqueous sodium hydroxide solution to pH 7-8. The resulting solution was evaporated to a volume of 10-15 ml in vacuum of a water-jet pump.

Purification of the sodium salt of sulfated galactomannan (SGM) was carried out by dialysis on cellophane against distilled water. The product was dialyzed for 10 hours, changing the water at intervals of 1-2 hours (fig.1).

The FTIR spectra of initial starch and sulfated starch were recorded using a Shimadzu IR Tracer-100 spectrometer (Japan) within the wavelength range of 400–4000cm^{-1}. The spectral information was analysed using the OPUS program (version 5.0). Solid samples for analysis were prepared in the form of pills in a KBr matrix (2 mg sample/1000 mg KBr).

The X-ray diffraction (XRD) phase analysis was carried out on a DRON-3 X-ray diffractometer using CuKα monochromatized radiation (λ = 0.154 nm), voltage 30 kV, current 25 mA. The scanning step is 0.02 deg; intervals for 1 s per data point. The measurement was carried out in the interval of the Bragg angles 2Θ from 5.00 to 70.00 deg.

The electron microimages were obtained with a TM-1000 HITACHI scanning electron microscope (Japan) with an accelerating voltage of 15 kV and a magnification from 100 to 10000× with a resolution of 30 nm.

Study of the sulfated galactomannan films by AFM in a semicontact mode was carried out using a Solver P47 multimode scanning probe microscope (NT-MDT, Moscow). Scanning was performed at no less than 3–4 points at several sites. Scan speed was 1.5–2.0 Hz, the resolution of the resulting image was 256 × 256 pixels.

Thermogravimetric study and data analysis was performed using the STA 449 F1 Jupiter (NETZSCH, Germany). The thermal degradation of the samples was analyzed in argon in the temperature range from 30 to 600 ° C, the flow rate of the protective and purge gases was 20 and 50 ml / min, respectively. The samples were heated in a dynamic temperature regime (10 deg / min) in corundum crucibles. Processing of the measurement results was performed using the «NETZSCH. Proteus Thermal Analysis.5.1.0» supplied with the instrument.

The average molecular weight (Mw), average molecular mass (Mn) and polydispersity of sulfated galactomannan samples were defined by gel permeation chromatography using an Agilent 1260 Infinity II Multi-Detector GPC/SEC System chromatograph with two detection: by a refractometer (RI) and by a viscometer (VS). The separation was made on two Aquagel-OH columns using the solution 0.2M$NaNO_3$ + 0,01M NaH_2PO_4 in water (pH = 7) as the mobile phase. The column was calibrated using polyethylene glycol standards (Agilent, USA). The flow rate of the eluent was 1 ml / min, the volume of the used sample was 100 μl. Before analysis, the samples were dissolved in the mobile phase (1-5 mg / ml) and filtered through a 0.22 μm PES membrane filter (Agilent). Data collection and data processing were performed using Agilent GPC / SEC MDS software.

3. Results and discussion

3.1 Green synthesis of galactomannan sulfate

Galactomannan sulfation reaction with sulfamic acid-urea complex and the subsequent isolation of galactomannan sulfate was carried out according to the scheme (figure 2).

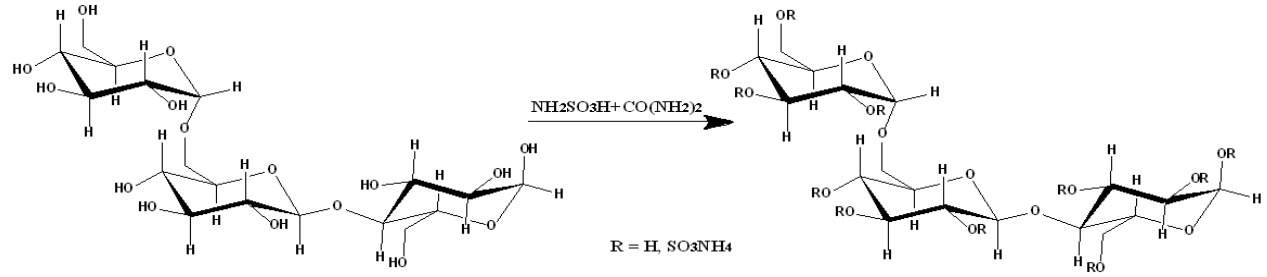


Figure 2. Scheme of galactomannan sulfation reaction.

In the study of galactomannan sulfation by sulfamic acid the time and temperature of the process were varied.

The degree of substitution (DS) was calculated according [13] to the equation 1.

$$DS = \frac{1.62 * S\%}{32 - 1.02 * S\%} \quad (1)$$

S% - sulfur content determination by elemental analysis.

Data on the sulfur content in galactomannan sulfate obtained under these experimental conditions is shown in table 1.

Table 1. Galactomannan sulfation process data by sulfamic acid-urea complex.

№	Temperature, °C	Time, min	DS
1	70	30	0.39
2	70	60	0.61
3	70	120	0.70
4	80	30	1.26
5	80	60	1.67
6	80	120	1.25
7	90	30	0.77
8	90	60	0.56

9	90	120	0.23

It was found that DS of sulfated galactomannan can be controlled by varying the temperature and sulfation process time (table 1). The maximum DS was observed at a process temperature of 80°C and the time of process of 60 minutes. With increasing temperature, the DS decreases to 0.23. The observed regularity is consistent with the data presented in [13, 14], and is probably associated with the destruction of the polysaccharide at high temperature.

3.1 FTIR-analysis

The introduction of the sulfate group into the galactomannan molecule was proved by FTIR spectroscopy (Fig. 3).

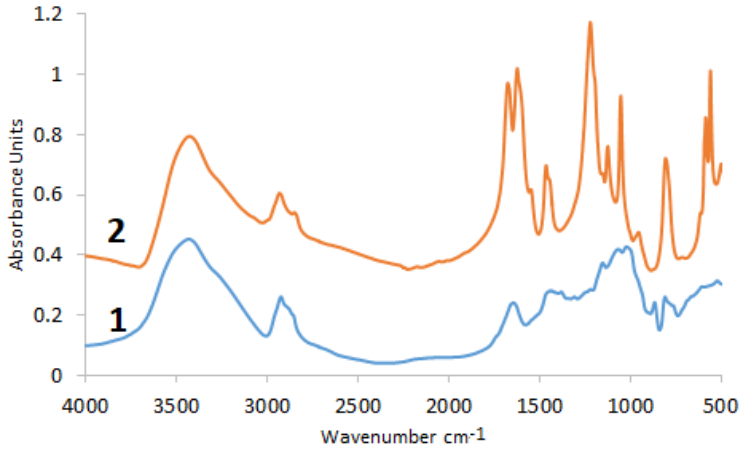


Figure 3. FTIR spectra: 1 - galactomannan, 2 - sodium salt of galactomannan sulfate.

In the FTIR spectra of sulfated galactomannan, in contrast to the initial galactomannan, there is an intense band at 1250 cm^{-1}, which refers to asymmetric stretching vibrations υ_{as} (O = S = O). The presence of absorption bands in the region of 805–820 cm^{-1}, which are absent in the FTIR spectrum of the initial galactomannan, indicates the presence of sulfate groups in the sodium salt of sulfated galactomannan (Fig. 3), which is consistent with the data presented in [13, 14].

In addition to sulfate groups, stretching vibrations of C=O groups at 1715 cm^{-1} are observed in the FTIR spectrum. The presence of a carbonyl group also confirms the formation of a carbamate group caused by an adverse reaction between polysaccharides and urea [15]. The formation of esters of polysaccharide acids and carbamates is observed during phosphorylation of starch in the presence of urea [16], as well as using a deep eutectic solvent containing urea [11, 17].

3.2 X-ray diffraction (XRD)

The initial and sulfated galactomannan was analyzed by XRD (Fig. 4).

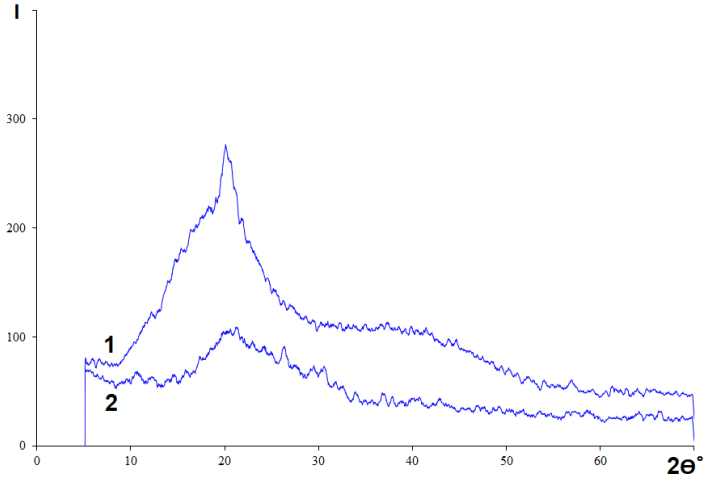
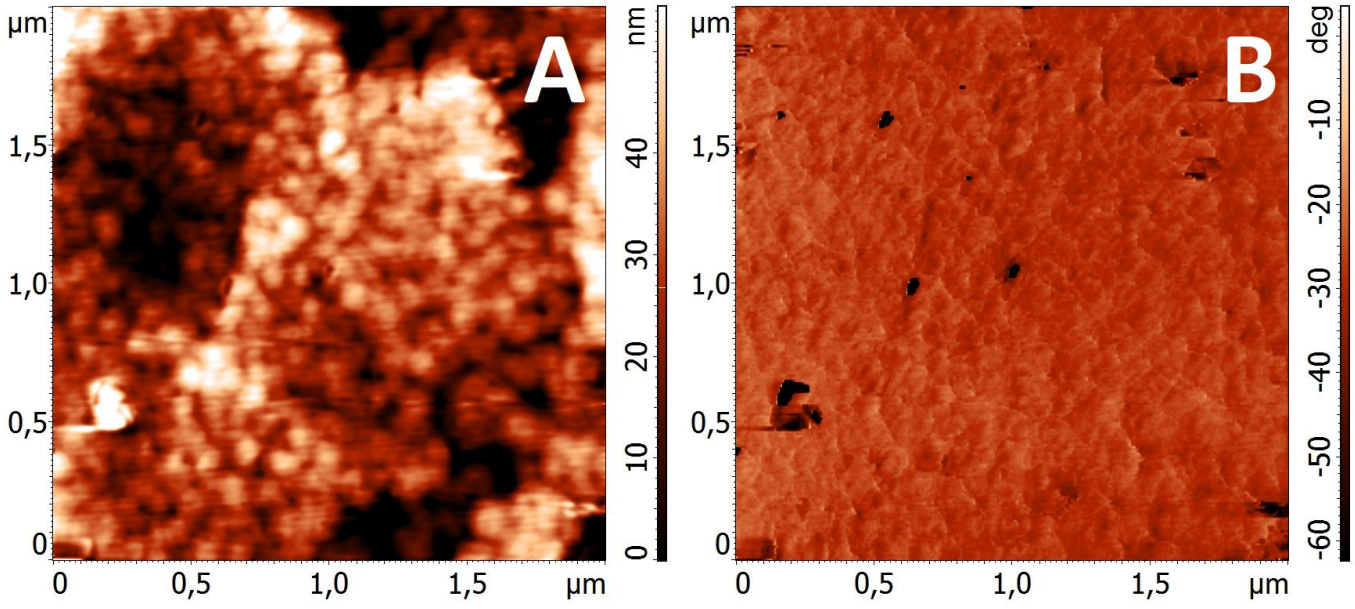


Figure 4. X-ray diffraction patterns of samples: 1 - galactomannan, 2 - sodium salt of galactomannan sulfate.

Galactomannan samples have an amorphous structure [18]. Comparison of X-ray diffraction patterns of the samples of galactomannan and the sodium salt of galactomannan sulfate showed (Fig. 4) that further amorphization of the material structure occurs during sulfation. On the X-ray diffraction pattern of the sample of sodium salt of galactomannan sulfate, peak smoothing was observed in the range of angles from 12 to 30˚ 2˚Θ.

3.3 Atomic force microscopy

The synthesized films of sulfated galactomannan were studied by the AFM method (Fig. 5). This method allows one to measure not only the lateral dimensions of nanoscale objects, but also their height with high accuracy up to 0.1 nm [19, 20].



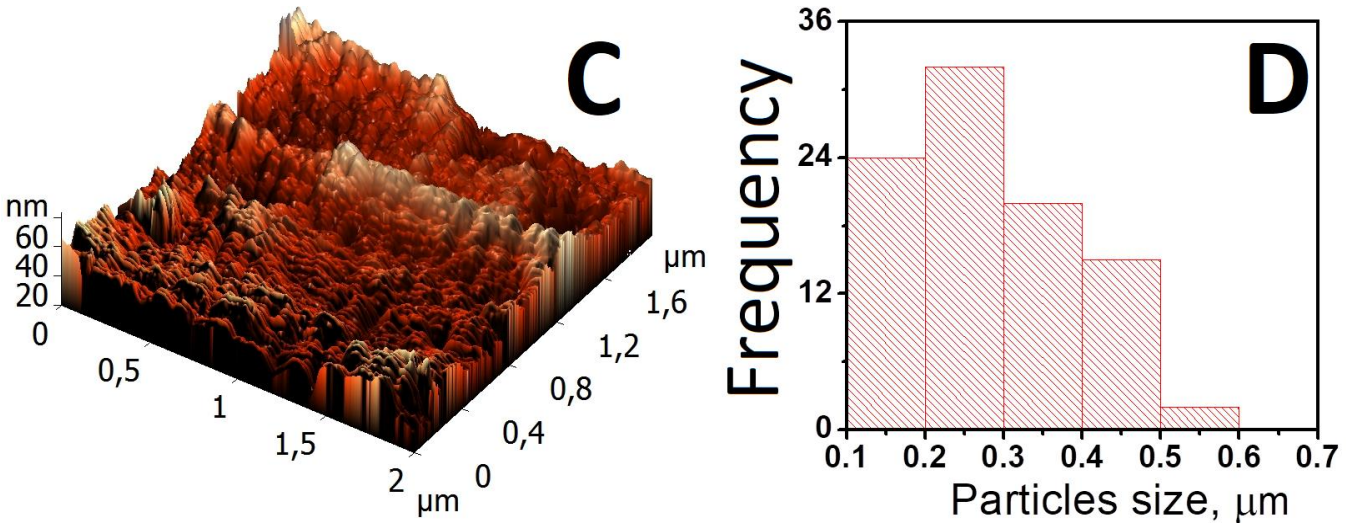


Figure 5. Typical AFM image of the sulfated galactomannan film: (A) relief; (B) phase contrast, (C) 3D surface profile, (D) particle size distribution.

According to AFM data, the surface of a sulfated galactomannan film consists of homogeneous spherical particles with an average diameter of 200 to 300 nm. According to phase-contrast images (Fig. 5, b), the surface of the sulfated galactomannan film is quite homogeneous and does not contain impurities.

3.4 Scanning electron microscopy

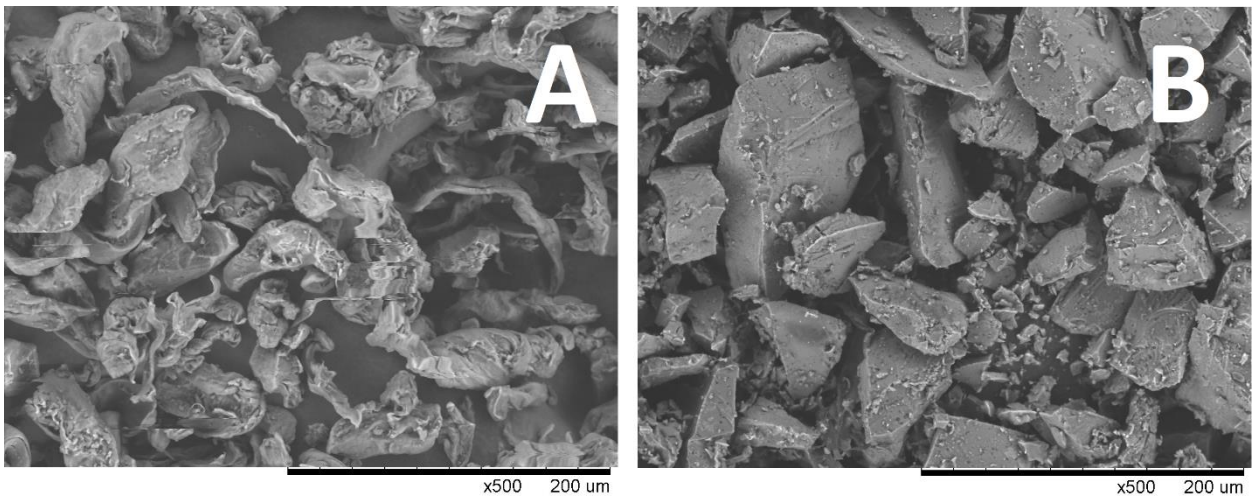


Figure 6. SEM images of samples of (A) the initial galactomannan and (B) sulfated galactomannan.

According to scanning electron microscopy, the sample of the initial galactomannan consists of uneven asymmetric particles with average sizes from 200 to 800 μm (figure 6. A). After sulfation, the samples have a morphology slightly different from the morphology of the initial galactomannan (figure 6. B). Sodium salt of sulfated galactomannan consists of particles of various shapes and sizes from 50-1000 μm.

3.5 Thermal analysis

The DSC analysis (Fig. 7) shows the thermal behavior of the starting and sulfated galactomannans. For the initial galactomannan, endothermic peaks were detected at 254 and 294 °C, and an exothermic peak was detected at 315 °C. Sulfated galactomannan showed endothermic peaks at 209 and 275 °C and an exothermic peak at 281 °C.

The calorimetric profile of GM and SGM showed endothermic and exothermic peaks. The first endothermic peak corresponds to the early removal of adsorbed water, while the second endothermic peak corresponds to the onset of thermal decomposition of organic matter (Fig. 7) [18, 21, 22]. The wide peak of GM decomposition at 254 ° C can be associated with the cleavage of galactose and mannose from the main chain of GM. The same SGM decomposition peak shown at 209 ° C. showed a smaller width than the original galactomannan. GM sulfation resulted in a decrease in the decomposition temperature of the exothermic peak.

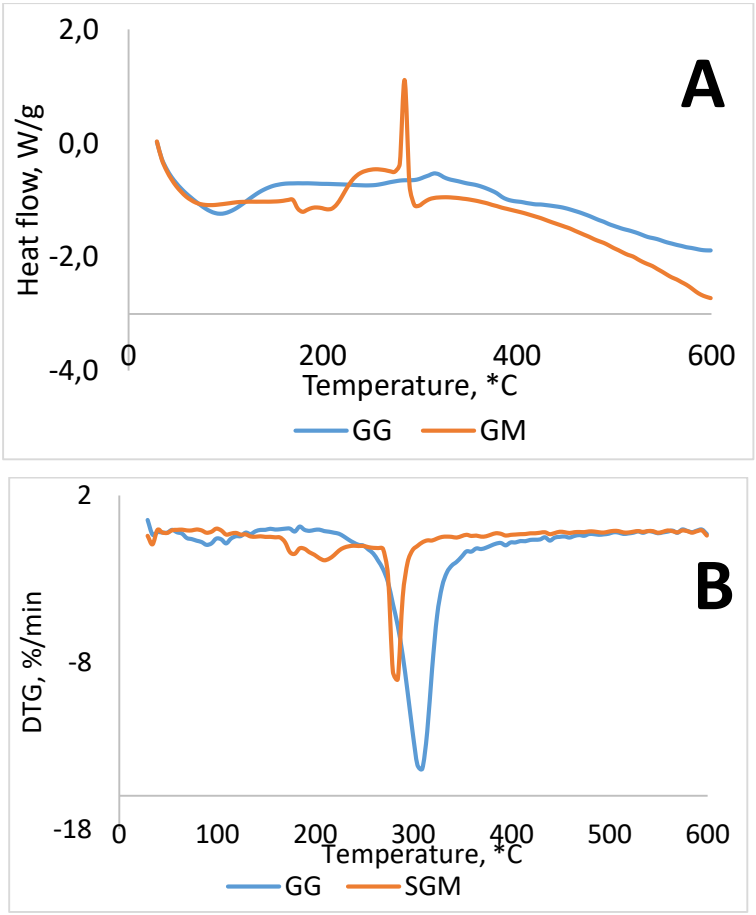


Fig. 7. DSC (A), DTG (B) analysis of samples of the initial galactomannan and sulfated galactomannan.

Thermogravimetric analysis of the initial and sulfated galactomannans is shown in Fig. 8. The values of the temperature of the broad peak of decomposition of GM and SGM, obtained from the DSC

curve, were confirmed by TGA thermograms. The thermal stability of the polymer is an important property that can make the material suitable for use in the food and pharmaceutical industries [18]. TGA residual mass profiles demonstrated the stability of sulfated galactomannan over a smaller temperature range compared to the original galactomannan.

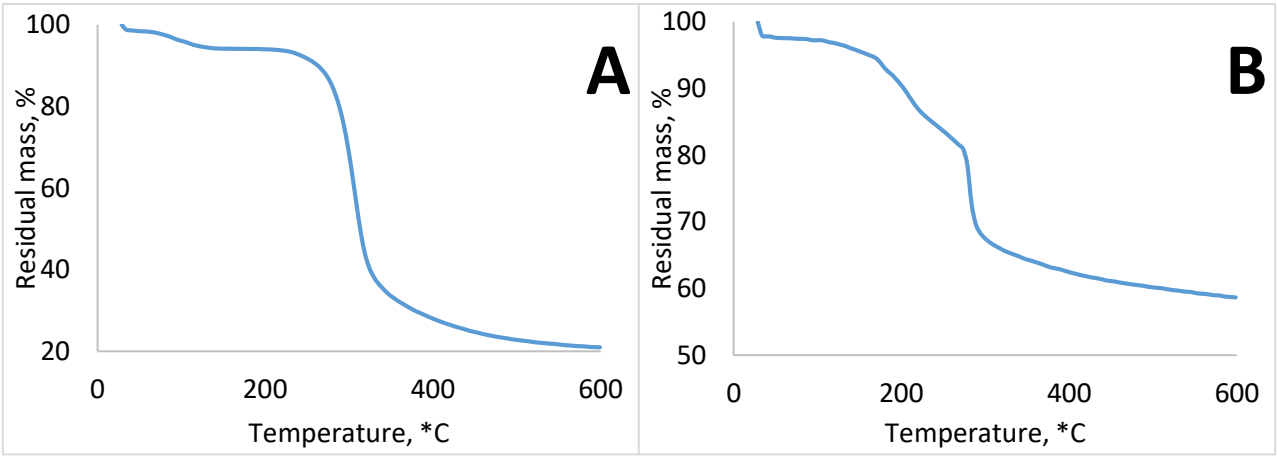


Figure 8. TGA analysis of samples of (A) the initial galactomannan and (B) sulfated galactomannan.

3.6 Gel Permeation Chromatography

According to the molecular mass distribution of galactomannan (Table 2), the initial sample is a low molecular weight type GM with an MW ~660 kDa, are in agreement with the results reported in the literature [23]. At the same time, GM has a bimodal particle mass distribution: a high molecular weight fraction with an MM of ~ 1500 kDa and a low molecular weight fraction with an MM of ~ 600 kDa. The degree of polydispersity in this case was 2.55.

Table 2. Number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity of galactomannan samples.

Sample	M_n(kDa)	M_w(kDa)	PD
GM	258,0	659,3	2,55
SGM 120/80	69,9	192,5	2,75
SGM 300/80	57,8	276,8	4,78

After the sulfation process was carried out for 120 minutes and 80 ° C, the destruction of the GM chains and the redistribution of molecular masses in the sample probably occur - the low molecular weight peak of the fraction with MM ~ 600 kDa disappears and a new peak appears corresponding to the reaction product with MM ~ 130 kDa. The proportion of the high molecular weight fraction in the sample also decreases markedly (Fig. 9).

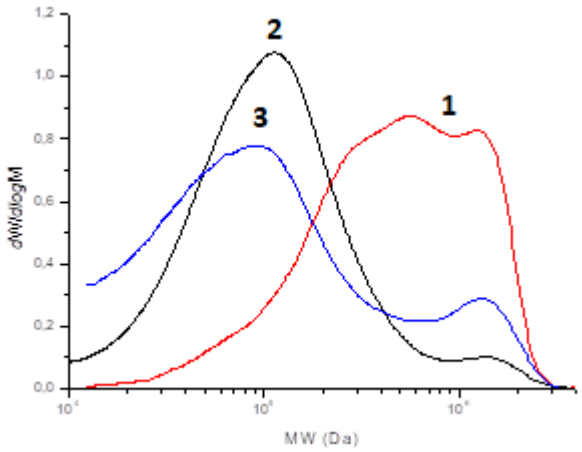


Figure 9. Molecular weight distribution of galactomannan (1) and sulfated galactomannan obtained at 80°C, 120 min. (2) and 300 min. (3).

An increase in the duration of the sulfation process to 300 min leads to further destruction of the GM polymer chains with a decrease in the molecular mass of the main fraction of the sulfated product to ~ 110 kDa; at the same time, a slight decrease in the total fraction of the low molecular weight fraction in the product is observed. Simultaneously, the proportion of the high molecular weight fraction, on the contrary, slightly increases, probably due to the greater resistance of the molecules to destruction and an increase in the degree of sulfation of GM. Redistribution of molecular masses leads to an increase in polydispersity from 2.75 to 4.78.

Conclusion

For the first time, galactomannan sulfates obtained by fusion with a sulfamic acid-urea complex were studied using FTIR spectroscopy, X-ray diffraction, scanning electron microscopy, atomic force microscopy, and gel permeation chromatography.

The presence of sulfate groups in the molecule of sulfated galactomannan was proved by FTIR spectroscopy. The FTIR spectra of sulfated galactomannan contain bands at 1250 cm^{-1}, 805-820 cm^{-1}, which indicate the presence of sulfate groups.

Using scanning electron microscopy, it was shown that the initial galactomannan consists of particles of various shapes with a size of 200 to 800 μm, and sulfated galactomannan consists of particles of various shapes with a size of 50-1000 μm.

Thermal analysis showed that the initial galactomannan has endothermic peaks at 254 and 294°C and an exothermic peak at 315°C, and sulfated galactomannan has endothermic peaks at 209 and 275°C and exothermic peak at 281°C.

Using atomic force microscopy, it was shown that a sulfated galactomannan film consists of spherical particles with an average diameter of 200 to 300 nm; according to phase contrast data, it is uniform in composition and has no extraneous impurities.

In gel-permeation chromatogram initial galactomannan have bimodal particle mass distribution: a high molecular weight fraction with an MM of ~ 1500 kDa and a low molecular weight fraction with an MM of ~ 600 kDa. An increase in the duration of the sulfation process from 120 to 300 min leads to the destruction of the galactomannan polymer chains with a decrease in the molecular weight of the main fraction of the sulfated product from 130 kDa to 110 kDa.

Acknowledgments

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

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