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ISOLATION, CHEMICAL COMPOSITION, AND STRUCTURAL FEATURES OF POLYSACCHARIDES FROM *Lupinus* SPECIES

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Polysaccharides of Lupinus angustifolius *(Bryansk 123 and Krystall varieties) and* Lupinus luteus *(SG-91 variety) are extracted and hydrolyzed. Their chemical composition and structural features are studied. Optimal conditions for extraction, hydrolysis, and purification of the lupine polysaccharides are found. Structural features of the isolated pectins are determined based on ^{13}C NMR and IR spectroscopy.*

Key words: *Lupinus angustifolius, Lupinus luteus*, polysaccharides, structure, hydrolysis.

Plants of the *Lupinus* genus are industrial sources of protein, aminoacids, and other valuble compounds, primarily pectins and uronide-containing hemicelluloses [1-3].

The present work examines results from the isolation of polysaccharides from *Lupinus angustifolius* species (Bryansk-123 and Kristall varieties) and *Lupinus luteus* (SG-91 variety) and studies of their chemical composition and structural features.

We screened the pectin content and extracted hemicelluloses containing acidic sugars, uronic acids. It was found that *Lupinus* species contain, depending on the variety, year, and vegetative stage, from 1.5 to 7% pectins and 3.5-10.5% uronide-containing hemicellulose (per dry weight). It was also found that the Bryansk-123 and Kristall varieties with pectin content up to 7% are promising for industrial production of pectins whereas the SG-91 variety (hemicellulose content up to 11%) is promising for production of uronide-containing hemicellulose.

We obtained various fractions of acidic polysaccharides by carefully choosing the extraction-hydrolysis conditions. These were the water-soluble and acid-soluble pectins and the uronide-containing hemicelluloses. The lupine pectins possess excellent swelling and water-absorption properties, forming a strong gel at 2% concentration.

Table 1 presents the physicochemical properties of the pectins. According to the data, the molecular weight of the pectins as a function of extraction method reaches 100-400 kDa; the degree of esterification, 65-85%. The molecular weight of the hemicelluloses is 1.0-1.5 MDa.

Fractionation of the lupine polysaccharides using ultrafiltration on hollow fibers with a filtration efficiency of 5-100 kDa revealed that pectins of *Lupinus angustifolius*, Bryansk-123 variety, contain mainly a fraction of molecular weight 200-300 kDa (~85%) whereas the fraction from 5 to 15 kDa makes up only 8-10%.

The composition of monosaccharides in the pectin and hemicellulose fractions was studied in detail by HPLC and GLC.

The samples were hydrolyzed using cellulase enzymes (pectofoetidin G20X and celluviridin G20X) and 2 N H_2SO_4. It is noteworthy that the monosaccharide composition varies as a function of extraction method and varietal features of specimens taken from various lupine samples. The pectins obtained by extraction using oxalic acid contain primarily glucose, fructose, and arabinose. The polyuronide hemicellulose obtained by extraction in alkaline medium (pH 9.5-12.0) contains primarily galactose, arabinose, and xylose.

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TABLE 1. Physicochemical Properties of Polysaccharides from *Lupinus angustifolius*, Bryansk 123 Variety

Product	Molecular weight, viscosimetry	Galacturonic acid content, abr. units	Degree of esterification	Number of COOH groups	Yield, mass %
Water-soluble pectin	400000-420000	75.5-85.3	92-93.8	3.43-4.95	5.0-5.5
Acid-soluble pectin	210000-240000	65.5-68.4	91-93.8	4.5-5.4	1.5-2.0
Uronide-containing hemicellulose	1052500-1400000	80.0-82.5	82-83.8	6.75-7.45	8.0-8.5

Content, rel. %	Water-soluble pectin	Acid-soluble pectin	Uronide-containing hemicellulose
Galactose	35.2	-	45.6
Rhamnose	-	3.7	3.9
Glucose	-	35.3	7.6
Xylose	4.2	-	4.7
Fructose	-	24.4	4.8
Arabinose	27.5	36.6	23.2
Saccharose	33.1	-	10.2
Methanol, mass %	0.01	0.05	0.05
Formaldehyde, mass %	0.04	0.14	0.12

Demethoxylation formed methanol and formaldehyde according to GLC of various polysaccharide fractions. The presence of formaldehyde suggests that the polysaccharide chain is partially cleaved at the glucose bonds during the hydrolysis to produce terminal aldehydes. This has been observed previously [4]. The D-glucuronic acid in the hemicellulose occurs primarily as the methyl ester.

We investigated the IR spectra of the fractions at various stages of separation. The IR spectra of lupine pectins differ substantially from those of other known pectins. Three groups of bands are observed in the region 400-2000 cm^{-1}. The first is due to stretching vibrations of carbonyls in various environments. There are very strong ν_{as} (COO^-) and strong ν_s (COO^-) bands of ionized carboxylate in the regions 1600 and 1400 cm^{-1}, respectively. These are characteristic of pectin salts that contain a large number of amides. The second group of bands appears in the range 1200-1500 cm^{-1} and is due to δ(C–H) deformations of methyl groups, pyranose rings, and the COH fragment. The strongest groups of bands is located at 1000-1200 cm^{-1} and corresponds to stretching vibrations of C–C and C–O bonds of pyranose rings. The nature of the distribution of the strong bands in the second and third groups has implications for the pectin structure of lupine.

We recorded ^{13}C NMR spectra of purified pectin (Bryansk-123 variety) before gel chromatography and of the pectin fraction (>15 kDa) obtained by ultrafiltration. It was found that the main part of the purified pectin that was not fractionated was polygalacturonic acid containing both free (or ionized) and esterified carboxylates. A series of bands with chemical shifts δ_C 179.14, 179.11, 179.06, 178.97, 178.95, 171.80-172.54, 169.79-168.44, and 168.18-168.03 ppm (broad signals) correspond to these groups.

The abundant signals for carbonyls indicates that the pectin structure is complicated and nonuniform, with extensive branching. Both α-1,4-and β-1,4-bonded monomers are present. This is consistent with the very complicated spectrum for the acetal C: δ_C 102.0-104.2, 97.46-98.06, 96.82-96.88 ppm (a series of doublets with two types of coupling constants $^1J_{HC}$ 168-171 and 158-162 Hz). The presence of a large number of signals in this region indicates also that significant quantities of neutral sugars are present (mainly rhamnose and galactose). Other nuclei of the C ring of the galacturonic chain and neutral sugars resonant in the range δ_C 64-80 ppm, forming a complicated splitting pattern. The methoxyl C appears characteristically at high field with δ_C 54.5, 50.14-50.29, and 50.48 ppm as a quartet with spin—spin coupling constants $^1J_{HC}$ 142-143 Hz.

A series of signals with δ_C 78-82 (C^4 of the galacturonic acid of pectin), 62-68 (rhamnose), and 19-20 ppm (rhamnose

CH_3) is noteworthy. The degree of methoxylation of the carboxylic groups can be judged from the ratio of the integrated intensities of the peaks for the carboxylate C atoms with δ_C 160-175 ppm and the methoxyl C atoms with δ_C 50-60 ppm. According to the spectra, the degree of esterification is >80%.

The data suggest that pectin from *Lupinus* is a mixture of linear and highly branched polymers, predominantly with high molecular weights, of α-D-galacturonan and other polysaccharides. The macromolecules contain galacturonic acid and neutral sugars: galactose, rhamnose, glucose, and fructose.

Thus, the stepwise method developed by us can produce lupine pectins and hemicellulose in a single production cycle. The observed unusual properties of lupine pectins are due primarily to their structural features, which should be studied in more detail.

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates in 1-butanol—acetic acid—water (8:3:2). Spots of monosaccharides were detected visually using acidic anyline phthalate. The content of sugars was determined quantitatively using HPLC. The analyses were carried out on a Likvokhrom 2010 (Hungary) apparatus. Sugars were identified using an RIDK 102 refractometer. The eluent was CH_3CN—H_2O (85:15) with a 3×300 column and Separon^{TM} SGX NH_2 stationary phase. The elution rate was 1 ml/min at 90 bar with a sample volume of 20 μl. Elution curves were constructed using an RIDK 102 (Czech Republic) differential refractometer. Solutions were lyophilized or evaporated under vacuum. The optical rotation was determined on a Perkin—Elmer 141 instrument in water at 20 °C.

Gas chromatography of the pectin hydrolysates was performed on a Khrom-4 (Laboratorni pristroje, Czech Republic) chromatograph equipped with glass columns and a flame-ionization detector under conditions previously described [5].

IR spectra were recorded on an IRS-113 (Bruker) IR-Fourier spectrometer at 1 cm^{-1} resolution in the range 400-4000 cm^{-1}. Samples of polysaccharides were ground and mixed with optically pure dry KBr powder and then pressed in a special die at 10 t/cm^2.

We recorded ^{13}C NMR spectra on an MSL-400 (Bruker) instrument at 100.6 MHz in D_2O at 60-80 °C. We used DMSO as an internal standard. The polysaccharide concentration was 0.5%.

Ultrafiltration was carried out on an automated ultrafiltration apparatus AUF-0.6 with hollow fibers. The filtration limits were 5, 15, 50, and 100 kDa. The working pressure during filtration was 0.8-1.2 mPa. The filtration area was 200 cm^2. The filtration rate was 1-10 l/h depending on the filtering module. The molecular weight was determined using an Oswald viscometer of diameter 0.56 mm. The molecular weight was determined as before [6]. The principal functional characteristics were determined by the usual methods [7].

Isolation of Polysaccharides from *Lupinus* Species. We used *Lupinus angustifolius* species (Bryansk-123 and Kristall) and *Lupinus luteus* (SG-91), which are widely cultivated and grown in the Bryansk district. Polysaccharides from *Lupinus* species were isolated by stepwise extraction of the plant material with hot water (70-75 °C) for 1 h, by 0.25-0.5% aqueous oxalic acid for 1-2 h at 70-75 °C at a 1:10 ratio, and by aqueous KOH at pH 10.45-12.45 for 30 min at 40-45 °C. The resulting extracts were cooled to room temperature, concentrated, and precipitated with ethanol.

The dry ground green biomass of *Lupinus angustifolius* (Bryansk-123, 100 g) was extracted with hot water (1 l, twice, 70-75 °C, 1 h). The combined aqueous extracts were concentrated and precipitated with two portions of 70% aqueous ethanol acidified with HCl to pH 2.9-3.5. The resulting precipitate was suspended in water and lyophilized. The yield of water-soluble pectins was 5.0-5.5 g.

The remaining biomass (*Lupinus angustifolius*, Bryansk-123) was extracted with 0.25-0.5% aqueous oxalic acid (1:10 by weight, 70-75 °C, 1 h). The extract was concentrated, precipitated with alcohol, and lyophilized. The yield of acid-soluble pectins was 1.5-2.0 g. The biomass of this plant material was then extracted with aqueous KOH at pH 10.45-12.45 (1:10 by weight, 40-50 °C, 20-30 min). The extract was neutralized to pH 3.5-4.0, passed over KU-1, concentrated, precipitated with alcohol, and lyophilized. The yield of uronide-containing hemicellulose was 8.0-9.5 g.

Enzymatic Hydrolysis. Polysaccharides (100 mg) were suspended in water (10 ml) containing 0.7% celloviridin G20X (cellulolytic activity 0.1 ME/mg) and 0.2% pectofoetidin G20X with cellulolytic activity 150 E/mg (per dry polysaccharide weight) and incubated at 37 °C for 3 days. The reaction mixture was centrifuged or filtered. The monosaccharide composition of the resulting samples was determined by HPLC, paper chromatography, and TLC.

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