Volume 3, Issue 5 May 2025 **ISSN (E):** 2938-3757

RESEARCH ON THE PROCESS OF EXTRACTING PECTIN FROM DOLANA FRUITS

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Abstract
Proper nutrition based on a consistent diet alleviates many digestive diseases and helps prevent serious conditions such as gastritis and stomach ulcers. Proper nutrition also positively affects hormone synthesis, normalizing their balance. As a result, the human body begins to sleep better and can function much better throughout the day. [1].
Nutritional fibers such as pectins, cellulose, hemicellulose, tooth meat, mucilage, and non-carbohydrate compounds like lignin are non-digestible polysaccharides that cannot be digested in the small intestine. Additionally, they include pentosans, chitin, chitosan, and some amino sugars from fungi and crustaceans that are indigestible proteins. [2].
Malnutrition is a risk factor for diseases such as colon cancer, irritable bowel syndrome, constipation syndrome, hypomotor dyskinesia of the large intestine, diverticulosis, appendicitis, diaphragmatic hernia, cholelithiasis, diabetes mellitus, obesity, atherosclerosis, cardiovascular diseases, hyperlipoproteinemia, varicose veins, and thrombosis of the lower limbs. [3].
The purpose of the research: To investigate the processes of obtaining pectin substances from the fruits of the C. songarica and C. turkestanica groups that grow in Uzbekistan.

Keywords: Pectin, oxalic acid, ammonium oxalate, capron membranes, ions, chromatography, aniline-phthalate, pentoses, uronic acids, hexoses.

Introduction

To extract pectin substances, the fruits of C. songarica collected in October 2021 near the village of Arbagi in the Chortoq district of Namangan region, Uzbekistan, and the fruits of C. turkestanica collected on August 30, 2021, at the aforementioned location were used. After being dried in a well-ventilated room for 6 days, the seeds were separated and dried in a drying cabinet at a temperature of 40-45 °C for 4 hours. The moisture content of the raw material was 10-11%.

Method for obtaining pectin substances

Raw material weighing 20 g, crushed (0.5-20 mm), was subjected to extraction three times with a mixture of 0.3% solutions of oxalic acid and ammonium oxalate (1:1) at a temperature of 75-80 °C for 90 minutes while constantly stirring. The obtained extracts were combined, evaporated to 30-40 ml, and precipitated with 96% alcohol in a 1:3 volume ratio. The resulting precipitate was filtered through capron membranes, washed with 96% alcohol, dried in a drying cabinet, and then crushed.

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Determination of monosaccharide composition. The amounts of 100 mg of pectin substances were hydrolyzed with a 2 normal H2SO4 solution at 100 °C for 18-20 hours. After neutralizing the hydrolysate with BaCO3, it was purified from ions using KU-2 cationite (in H+ form), then it was evaporated and subjected to chromatography using n-butanol-pyridine-water (6:4:3) mixture on FN-12 paper in a descending method for 18 hours. After drying the chromatogram, acidic aniline-phthalate was applied, and it was dried again and heated in a drying oven at 110 °C for 2-4 minutes. In the chromatogram, hexoses appeared as brown spots, while pentoses and uronic acids appeared as light pink spots.

Determining the degree of pectin's etherification. A pectin substance weighing 0.15 g was dissolved in 625 ml of water by weakly boiling, and after 2 hours, it was titrated with a NaOH solution of concentration 0.1 N in the presence of phenolphthalein indicator until a light pink color was obtained.

$$k_c = \frac{a}{p} \cdot 0.45\%$$

Here: k_c - the number of free hydroxyl groups;
a – the volume of 0.1 n NaOH solution used for titration, ml (1 ml of NaOH solution corresponds to 0.0045 g of carboxyl groups); r – the amount of pectin taken for titration, g. The identification of etherified carboxyl groups was carried out as follows: to a sample of the obtained pectin substance, 10 ml of 0.1 n NaOH solution was added and hydrolyzed at room temperature for 2 hours, which means that methoxylated carboxyl groups were hydrolyzed under alkaline conditions. To the resulting reaction mixture, 10 ml of 0.1 n HCl solution was added, and its excess was titrated with 0.1 n NaOH solution in the presence of phenolphthalein indicator.

$$К_э = \frac{B}{p} \cdot 0.45\%$$

B - the volume of 0.1 n NaOH solution spent on the second titration. The total amount of carboxyl groups. Ко = Кс+Кэ.
Broadcasting level (СЭ) It is calculated according to the following formula:

$$СЭ = \frac{К_э}{К_о} \cdot 100\ \%$$

Determining the relative viscosity (ηrelative) of pectin substances. 100 mg was taken from each sample and dissolved in 10 ml of water. The relative viscosity of the pectin substances was measured at a temperature of +20-21°C using an Oswald viscometer with a capillary diameter of 0.73 mm. The outflow time of water from the capillary is 30 seconds. The outflow times of pectin solutions from the capillary are: C. songarica 61 sec for pectin; relative viscosity ηнисб=2,03; C. turkestanica ηнисб=6,56.
Determining the molecular weights of pectins extracted from two types of chokeberry. Gel chromatography analysis was conducted using an Agilent 1260 Infinity high-performance liquid chromatograph with a refractometric detector. The flow rate of the eluent was 0.8 ml/min, and the concentration of the sample in the injector was 3 mg/ml. The volume of the sample in the injector was 20 µl. The analysis was performed using the Agilent Chemstation program. A cylindrical stainless steel chromatographic column was used. (25 x 0,8 см) TSK GM PWXL (Toya Soda, Japan) filled with sorbents.

The IR spectra of the samples from the Perkin Elmer model 2000. ИК- Obtained on KBr plates in a Fourier spectrometer.

Despite the availability of sufficient local raw material reserves and opportunities for obtaining pectin substances in our republic, currently Uzbekistan is importing pectin substances from foreign countries. Taking this into account, we studied the pectin substances of two types of wild plants, namely C. songarica and C. turkestanica fruits. It was found that the pectin content in the fruits of C. songarica and C. turkestanica is 9.58% and 11.25%, respectively.

The monosaccharide compositions determined by the results of acid hydrolysis of the pectin samples are shown in Table 21.

Table 21. Two types of Crataegus plants, the amount of pectin substances and monosaccharide composition.

Plant	Amount, %*	Monosaccharide composition					
		Gal	Glu	Ara	Xyl	Rha	UAc
Crataegus turcistanica	11,25	+	+	++	+	Жуда оз	++
Crataegus songarica	9,58	+	+	++	+	Жуда оз	++

The amount of pectin is taken relative to the initial mass of raw materials, Gal - galactose, Glu - glucose, Ara - arabinose, Xyl - xylose, Rha - rhamnose, UAc - uronic acids.

Table 21 According to the provided data, the pectin compound monosaccharide compositions of C. songarica and C. turkestanica fruits do not differ from each other. They differ in the amount of monosaccharides, but in both analyzed pectin molecules, uronic acids and arabinose are the main components, which is characteristic of both biopolymers.

The pectin substances extracted from two types of willow plants, when mixed with iodine solution, produce a blue color, which proves the presence of starch-like glucans in their composition.

Table 22. The titrimetric indices of pectins from the fruits of C. songarica and C. turkestanica.

Pectin source	Кс, %	Кэ,%	Ко,%	СЭ, %
Crataegus turkestanica	20,9	34,79	55,69	62,4
Crataegus songarica	22,5	36,59	59,09	61,92

In order to determine the degree of etherification of the isolated pectin substances, the titrimetric indices of the pectin substances were determined.

The titrimetric analysis data indicates that both pectin substances studied Кс, Кэ, Ко ва СЭ the values do not differ significantly from each other (Table 22). The high amount of carboxyl groups

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produced by the pectin substances of the studied types of hawthorn is characteristic, meaning they are highly esterified.

The relative viscosity of pectin substances measured by Ostwald viscometer (ηнисб) C. songarica for ripening its fruit ηотн= 2,03 ва C. turkestanica for ripening its fruit ηотн= 6,56 the equality of values has been determined.

In the IR spectra of the extracted pectin substances, absorption peaks characteristic of carboxypolysaccharides were observed. Among them, first and foremost, 3498 and 3466 см-1 it has been noted that there are high intensity and broad absorption bands in the hydroxyl group regions. The values are 2913, 2924 $см^{-1}$ and the low-intensity winning lines indicate the presence of –CH groups in the composition of pectin substances. The valence vibrations of the carbonyl groups (C=O) are at the frequencies of 1748 and 1739 $см^{-1}$ It appears in its fields. Pectin substances in plants occur in the form of sodium, potassium, or calcium salts. This is characteristic of the absorption peaks at 1622 and in their IR spectra of ionized carboxyl (COO-) groups. 1440 $см^{-1}$ (*C. songarica* пектини) and 1630, 1454 $см^{-1}$ (*C. turkestanica* пектини) it leads to being manifested in its fields.

Due to the fact that pectin substances are usually esterified with methanol, there are 1365 and in their IR spectra 1370 $см^{-1}$ there will be winning lines in their fields.

In addition, in pectin substances CH_3- the winning lines of characteristic ether gods of groups (C-O-S) 1244 $см^{-1}$ (C. songarica fruits) will be shown. Of the spectrum 1144, 1103, 1013, 9511$см^{-1}$ (*C. songarica пектини*); 1093, 1074, 1020, 945 $см^{-1}$ (*C. turkestanica* пектини) the winning lines in its fields reflect the oscillations in the following segments of the pyrazole cycles: C-H, C-O, CH2, and so on.

Of the spectrum 827 $см^{-1}$ characteristic achievement lines in the field between the residues of D-galacturonic acid in the biopolymer chain α- indicates the presence of glycoside binding. The IR spectra at 632 and 637 $см^{-1}$ the winning lines in its branches indicate the presence of β-glycosidic bonds among the neutral monosaccharide residues in the pectin composition.

Thus, the analysis of the pectin substances of the fruits of C. songarica and C. turkestanica showed that the main chain of the studied biopolymers is linked by α – 1→4 glycosidic bonds and belongs to methylated carboxyl polysaccharides.

The molecular weights of the samples were determined using the calibrated universal gel chromatography method.

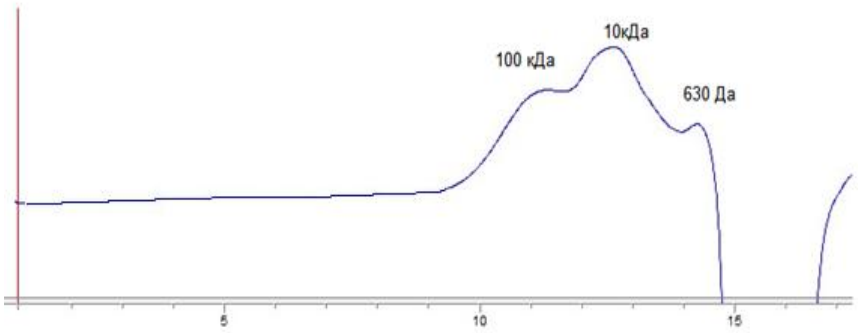

Figure 1. Chromatogram of pectin from C. songarica plant Pectin substance isolated from the fruit of C. songarica consists of three components, the molecular masses of which are shown in Figure 1. The total molecular mass of this pectin is 110,630 Da.

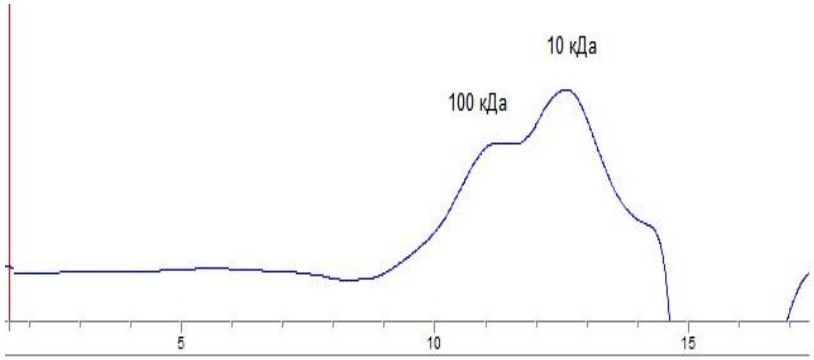

Figure 2. C. turcestanica chromatogram of the plant extract

The pectin extracted from C. turcestanica consists of two components (Figure 2), where the molecular mass of the first component is 100,000 Da, and that of the second component is 10,000 Da.

Conclusion

The fruits of hawthorn belonging to the species C. songarica and C. turkestanica, which grow in Uzbekistan, are rich in pectin compounds, and according to their physical-chemical characteristics, they can be used in the food industry and medicine. The pectin content in the fruits of C. songarica is 9.58%, while in C. turkestanica it is 11.25%. The monosaccharide compositions of the pectin compounds in the fruits of both studied hawthorn species are almost identical. The total molecular weight of pectin extracted from C. songarica fruits is 110,630, and from C. turkestanica fruits is 110,000 Daltons. It has been determined that the extracted pectin compounds are highly esterified.

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