Structure and properties of pectin substances of wild sea buckthorn (Hippophae rhamnoides L.) growing in Azerbaijan

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Abstract. The use of sea buckthorn fruits in medicine, cosmetology and as a source of ingredients that improve the quality of food has already been studied and continues to be studied. One of these ingredients can serve as pectin substances of sea buckthorn, isolated from it in its pure form. Considering the relevance of this direction, the main task of this work was the study and identification of pectin substances isolated from the fruits of wild sea buckthorn growing in Azerbaijan. Using cavitation-membrane technology, samples of pectin substances from sea buckthorn pomace were obtained under optimal technological conditions, after which the physicochemical characteristics were studied and their identification was carried out using physicochemical methods - elementary analysis, IR and NMR spectroscopy. Data have been obtained indicating that sea buckthorn pectin is a low-esterified pectin, which consists of a mixture of linear and highly branched polymers of α-D-galacturonane and other polysaccharides, whose macromolecules include galacturonic acid residues and neutral sugars. The high complexing ability of the obtained pectin in relation to lead ions has been established, which makes it possible to recommend it as an active ingredient for therapeutic and prophylactic products. The data obtained allow us to get a more complete picture of the biotechnological potential of local sea buckthorn as a source of pectin substances.

1 Introduction

Azerbaijan's vegetable raw materials are widely represented by food, vitamin and medicinal plants, from which hydrophilic extracts and beverages containing a wide range of nutrients and biologically active substances can be obtained [1-2].

In the list of wild plants growing on the territory of Azerbaijan, buckthorn buckthorn (Hippophae rhamnoides L.) stands out for abundant fruiting. Here, the potential volume of harvesting the fruits of this plant is 3,000 tons.

Sea buckthorn fruits are an excellent raw material for processing into lipophilic extracts and other products with high antioxidant, antibacterial, anti-carcinogenic and anti-radiation activity [3-4].

In recent years, interest in this plant has been growing everywhere as a source of biologically active substances capable of ensuring the normal functioning of the human

body, increasing its resistance to oncological and viral diseases, stress, negative environmental influences and prolonging life [5-6].

Among the many natural components of sea buckthorn fruits, plant polysaccharides, in particular, pectin substances, are of particular interest.

Pectin substances (pectins) include protopectin, pectin polysaccharides and associated arabinans, galactans and arabinogalactans, which, as a rule, have a complex chemical structure and structure [7-8].

Pectin substances are a multifaceted family of complex plant polysaccharides that form a functionally important part of primary cell walls together with cellulose and hemicellulose. They ensure the strength of plant cells, plant resistance to drought and low temperatures, provide water-salt exchange, are characterized by a high gel-forming ability and play an important role in human nutrition as components of "dietary fibers" with complexing ability [9].

Changes in the composition and structure of pectin substances were revealed depending on their origin by types of raw materials, places of their cultivation and methods of manufacture [10]. This makes it necessary to conduct systematic studies aimed at clarifying the structure, properties and direction of use of pectin substances from various sources, and in particular from local wild sea buckthorn, which was the main task of this work.

2 Materials and Methods

2.1 Objects of research

The object of the study was the fruits of sea buckthorn harvested in the period September-November 2019 in the Babek administrative region of Azerbaijan, with a content of 10.32% (per absolutely dry mass) pectin substances in the form of a water-soluble form (hydropectin) and protopectin fraction.

2.2 Isolation of pectin substances

Modern trends in the development of technology of pectin substances provide not only economic aspects, but also environmental aspects related to both reducing the release of harmful chemicals into the environment and creating favorable working conditions. Another important aspect of technological improvement of the processes of obtaining pectin substances is the intensification of individual resource-determining stages.

One of the effective technological solutions is the use of the method of hydroacoustic treatment of pectin-containing raw materials in rotary cavitation extractors, since cavitation treatment of an aqueous extractant changes its physico-chemical properties, increases the pH of water, contributing to its activation, as a result of such treatment, water temporarily becomes an active solvent with acidic properties without the introduction of chemical reagents [11].

In a rotary-cavitation type extractor, under optimal conditions, the processes of grinding of pectin-containing raw materials (the area of the solid phase increases by 60-75 times), hydrolysis of the protopectin (water-insoluble) fraction of pectin substances and the actual extraction (diffusion) of pectin substances into the aqueous phase occur simultaneously.

Another important technological technique that allows preserving the nativity of pectin biomolecules is the use of membrane processes to purify them from ballast substances and concentrate them, since these processes take place at ambient temperature and without phase transitions.

To obtain pectin substances, freshly obtained extracts of wild sea buckthorn fruits were used, which were crushed to a size of 2-3 mm.

The extraction process of crushed pomace was carried out at the following parameters: hydromodule 5:1 – (deionized water: pomace); cavitation index 0.6; temperature - 65 ° C, process time - 20 min.

At the output, a white-beige (achromatic) powder was obtained, colorless both in dissolved form and in gels and emulsions, without absorption in the wavelength range with a length of 400-700 nm, with parameters L=90-92, a = (-3.7) – (-1), b = (+2)-(+15).

2.3 Identification and testing of pectin substances

To identify the isolated pectin substances, elemental microanalysis, IR spectra on the Impact 410 “Nicolet” brand infrared Fourier spectrometer in the range of wave numbers 400-4000 cm-1 in KBr tablets with a spectral resolution of 2 cm-1, NMR spectra of 1 H and 13 C on the Bruker Avantes device were used. Optical rotation was determined on a Perkin-Elmer 141 device in water at a temperature of 20 ° C.

The resulting pectin from wild sea buckthorn was tested for physico-chemical characteristics in accordance with the US Pharmacopoeia form "Pectin 9000 -69-5 FT-FSIJK254368”.

The sorption capacity of pectin substances in relation to lead ions was determined by complexometric titration (reverse titration technique) [8].

The results of experimental studies were evaluated using Microsoft Office Excel 2013 and Statistica 8.0 for Windows application packages.

3 Results and Discussion

Microanalysis of isolated pectin substances from wild sea buckthorn pomace showed the following chemical parameters: C-28%, H-42%, O- 24%, which corresponds to the gross formula C14 H21 O12, i.e. it is really pure pectin.

Table 1 shows the physico-chemical characteristics of sea buckthorn pectin.

Table 1. Physical and chemical characteristics of sea buckthorn pectin.

Indicators	quantity %
Free carboxyl groups	21.80
Esterified carbonyl groups	7.15
Esterification degree	28.80
Uronid component	74.45
Acetyl groups	1.12
Methoxyl groups	8.14
Molecular mass, Da	$42.0 \cdot 10^3$
pH 1% solution	3,25
Jelly fortress, kPa	70.68

Table 1 shows that according to the degree of esterification, the resulting pectin belongs to the group of low-esterified pectins. Due to the sufficiently high content of free carboxyl groups (21.80%), it should have a high complexing ability.

This was confirmed during further study of the complexing ability of sea buckthorn pectin in relation to Pb^{2+} ions. It amounted to 284.5 mg Pb/g, which can serve as a basis for creating functional products based on it with a high antidote potential in relation to heavy metals and radionucleides.

To identify the obtained pectin substances, IR-Fourier spectra and NMR spectra of ^{1}H and ^{13}C were taken (Figure 1). Figure 2 shows the results of their mathematical processing and interpretation.

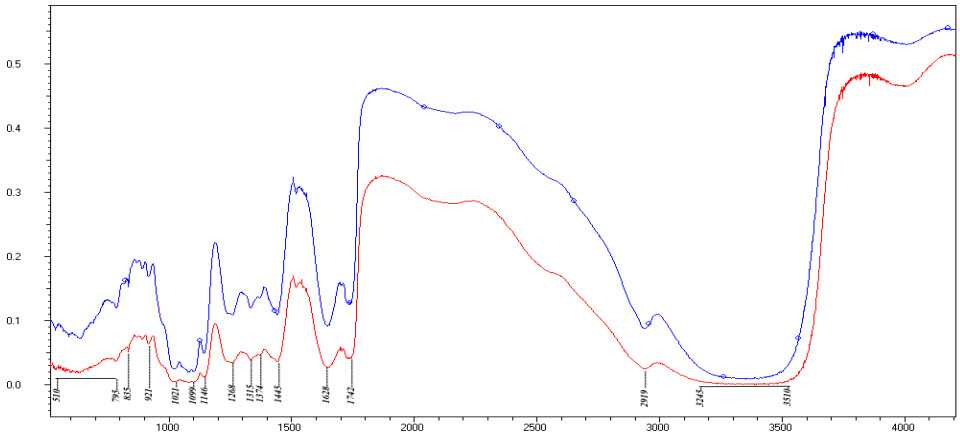

Fig. 1. IR spectra of sea buckthorn pectin samples.

Upon a detailed examination of the ir spectra, it can be concluded that pectin contains a large amount of galacturonic acid (intense absorption bands in the region of 1010-1150 cm^{-1}). The band in the region of 1374 cm^{-1} is caused by deformation fluctuations of the C-H groups of the pyranose ring. absorption bands are observed in the area of 1610-1740 cm-1, indicating the presence of free carboxyl groups. The available oscillation bands of the CH_3 groups indicate partial esterification of carboxyls.

The assignment of bands in the experimental IR absorption spectra of sea buckthorn pectin is presented in Table 2 based on a comparison of absorption bands with data from the NIST spectral database (ASTM).

Table 2. Assignment of bands in IR absorption spectra.

Band, cm^{-1}	Preferred types of oscillations
3245-3510	□(OH)$_C$, □(H_2O)
2919	□(CH)
1742	□(C=O)$_E$
1628	
1445	□$_{as}$(CH_3)□
1374	□$_s$(CH)□
1315	□(CH)□
1268	
1146	□(C–O–C)
1099	□,□ (C–OH)$_C$, □(C–C, C–O)□
1021	□(C–C, C–O)$_K$
921	□(OH)$_C$
835	□(CH_3)$_E$
510-795	Pulsating vibrations of pyranose rings

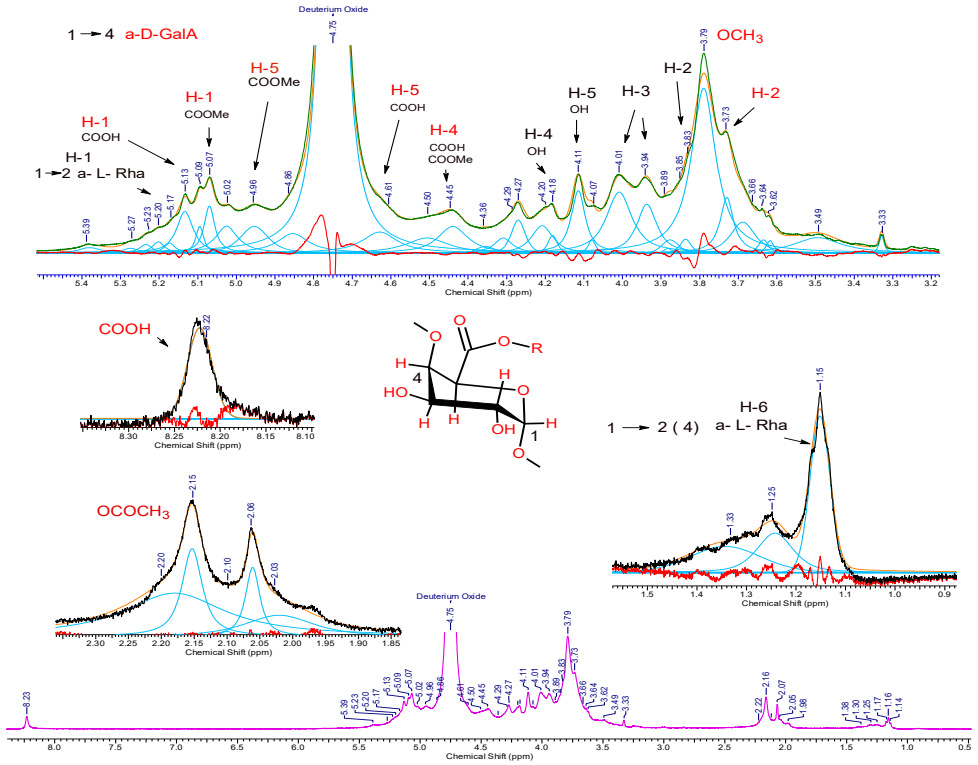

Fig. 2. The mathematical processing and interpretation.

It was found that in the proton spectrum of the sample in the region of a strong field (δ =0.97-1.47; δ = 1.47-1.73 m.d.), the manifestation of signals of CH groups located in position 4 is observed. Protons of the carbon atom of position 1 of the pyranose cycle resonate at 3.94-3.38 m.d., and chemical shifts 3.48-3.59 m.d. refer to carbon protons in positions 2 and 3 of the galactopyranosiluron fragment. The methoxyl and carboxyl groups are characterized by the manifestation of signals at 3.67 and 3.91 m.d, respectively. Analysis of the NMR spectrum on 13C nuclei showed the presence of carboxyl (δ =103.03 -103.64 m.d.), methoxyl (= 57.40 m.d.) and methine groups in the structure. Carbon atoms located at position 1 of the pyranose fragment give a signal in the strong field region at 63.78, 72.50 and 77.65 m.d. For 2 and 3 carbon atoms, the manifestation is characteristic at 77.33, 81.01 and 76.92 m.d. Carbon of position 4, participating in the connection of pyranose fragments by an oxygen bridge, resonates at 44.05, 40.44 m.d.

Figure 2 shows the NMR spectra of sea buckthorn pectin, as well as the data of their mathematical processing and interpretation.

It can be said that the NMR spectra clearly show a doubling of the signal group, which speaks in favor of the predominance of two different sections of the polymer molecule of pectin.

Based on this, the structure of oligosaccharide fractions is unambiguously α -1,4-D-glucans.

Mathematical processing of NMR spectroscopy data makes it possible to make assumptions about the structural features of pectin (Figure 3).

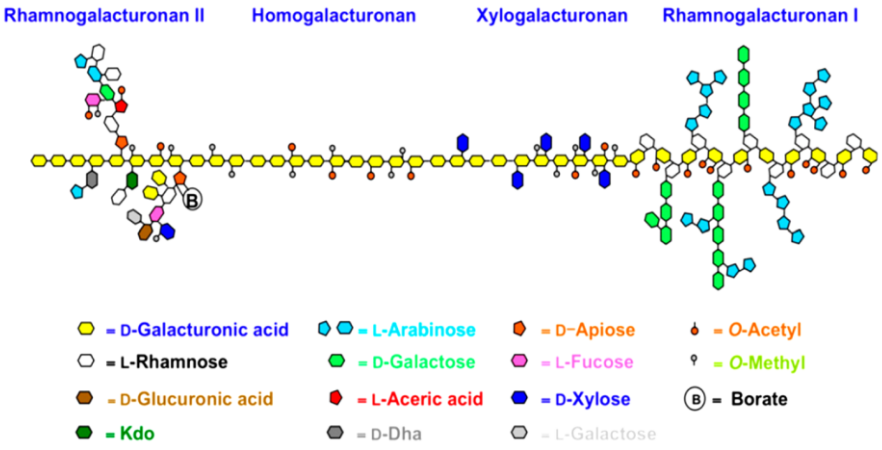

Fig. 3. Prospective structure of sea buckthorn pectin.

The abundance of carbonyl group signals indicates a very complex and heterogeneous structure of pectin, which has a rather branched structure. The presence of a large number of signals in the acetal carbon region also indicates the presence of a significant amount of neutral sugars, mainly rhamnose and galactose.

4 Conclusions

The data obtained indicate that pectin from wild sea buckthorn fruits mainly consists of a mixture of linear high-molecular polymers α-D-galacturonane and other polysaccharides, whose macromolecules include galacturonic acid residues and neutral sugars; this is a low-esterified pectin with a high antidote potential with respect to heavy metals and radionucleides.

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