Comprehensive analysis of the analytical characteristics and structure of the lateral branches pectin isolated from seeds of wild medlar (Mespilus germanica L.)

E.B. Farzaliyev1,3, G.K. Hafizov2,*, B.J. Jabrailov3

1 Azerbaijan State University of Economics, Baku, Azerbaijan
2 Research Institute of Fruit and Tea Industry of the Ministry Agriculture, Zardabi, Azerbaijan
3 Azerbaijan Technical University, Baku, Azerbaijan

Abstract. The medlar (Mespilus germanica L.) is an important element of the flora of the mountainous part of the Guba district, and its biotechnological potential has been little studied. This study was conducted in order to comprehensively analyze the analytical characteristics of pectin isolated from the seeds of the fruits of this plant. In the mode of low-frequency hydroacoustic cavitation, primary extracts of fresh seeds of fruits of technical maturity were obtained, which were then purified according to a multi-stage scheme using a filtering centrifuge, a diatomite filter, a decanter and ultra-and diafiltration and subjected to low-temperature concentration and spray drying. The analysis of the studied pectin using titrometric, spectrometric and chromatographic conventional methods showed that the pectin of wild medlar seeds is a mixture of linear and branched polymers of α-D-galacturonane and other polysaccharides (mainly high molecular weight). It is pectin with a high degree of esterification (72.4 ± 0.6%) and a high content of the methoxyl component (48±0.25% of acetyl groups in the total mass of galacturonic acids). At the same time, the content of free carboxyl groups in it is quite low and amounted to 4.25 ± 0.09%, which indicates its low complexing ability.

1 Introduction

In Azerbaijan has the richest resources of wild fruit and berry plants, which can be involved in the production of innovative health products in this region as a particularly valuable biologically active raw material [1–2].

Among the wild plants most often used by the population of the republic for therapeutic and preventive purposes, medlar (Mespilus germanica, Rosaceae) stands out, the fruits of which ripen by September.

The phytochemical composition of fruits of various local genotypes of medlar (Mespilus germanica L.) was studied by Turkish authors; it was found that the content of the total amount of phenolic compounds in medlar pulp, depending on its genotype, varies within 41.*
Medlar has become widespread in the Guba region of Azerbaijan, where it is used to prepare bekmes (decoction, thick extract), which is used in the winter-spring period for colds, general physical weakness and other ailments. When studying the chemical composition of the local wild medlar, it turned out that they contain 82.61 ±0.08% water, 1.2 ±0.03% protein, 0.87 ±0.02% fat, 9.42 ±0.05% total sugar and 2.27 ±0.08% pectin [4].

A CO2 extract with the properties of its seed oil compote was obtained from wild medlar [2, 5]. Samples of jam were obtained from medlar using the suвид technology (vacuum cooking at low temperature). Cooking at different temperatures (60 °C, 70 °C or 80 °C) was accompanied by a change in the antioxidant capacity of medlar jam [6]. The AZ I 2016 0061 patent describes the technology for producing assorted marinade from wild and cultivated medlar [7]. The nutritional value of the final product has significantly increased due to simultaneous use in its preparation: 1 - Hard fruits of wild medlar at the technical stage of maturity with a high content of P-active polyphenols for this period (830 mg/100 g of raw mass); 2 - Fresh dog rose hips (Rosa canina), which are a rich source of ascorbic acid and other vitamins [8].

Among the heteropolysaccharides of medlar (Mepilus germanica L.), which are characterized by the presence of two or more types of monomeric units, a special place is given to pectins due to their unique properties. Pectins are compounds belonging to the group of non-digestible carbohydrates [9-10], they are found practically in intercellular tissues, cell walls, leaves, fruits and other organs of plants [11-12].

Due to their specific structure, these compounds can form gels and purify the body of lead ions, other heavy metals and radionuclides [13-14]. The structural features of pectins make it possible to assign them the role of prolongators of many dietary supplements and medicines, blood plasma substitutes, surfactants and even ion exchangers [15-16].

The published work of the Turkish authors characterizes the pectins of the pulp of the fruits of 10-year-old trees growing on the hillsides in Istanbul [17]. Pectins extracted under optimal conditions (89 °C, 4.83 hours and pH 4.2) were classified as pectins with a high content of methoxyl groups. Analysis of the sugar composition showed that pectin consists mainly of D-galacturonic acid, L-arabinose, L-rhamnose, D-galactose and D-glucose.

Fourier transform infrared spectroscopy, Raman scattering and nuclear magnetic resonance spectra confirmed the molecular structure, revealing the presence of the main chain of D-galacturonic acid. X-rays revealed an amorphous structure. Differential scanning calorimetry showed endothermic (123 °C) and exothermic peaks (192 °C). Thermogravimetric analysis revealed three decomposition regions: 50-225 °C, 225-400 °C and 400-600 °C. Stable and dynamic shift analyses have shown that pectin has pseudoplastic behavior with storage modulus (G') and loss (G") increasing with increasing frequency.

One of our already published works was devoted to the study of the characteristics of the pectins of sea buckthorn fruits (Hippophae rhamnoides L.) growing in Azerbaijan [18]. The main objective of this study was to study in detail the pectins of medlar (Mepilus germanica L.), which is widespread in the wild in some regions of Azerbaijan, including in the Guba region, from where its samples were taken as material for this study.

2 Materials and Methods
2.1 Objects of research

The fruits of wild medlar (M. germanica L.) of the technical stage of maturity, collected in an amount of 10 kg in the mountainous part of the Guba district, located in the northeastern part of the republic, were used as the starting material. Wild medlar fruits were harvested from the same plants twice in mid-October 2021-22.

2.2 Pectin extraction

In this work, a method was used to extract pectin from medlar seeds with specially prepared water with a volumetric electrical resistance of at least 10 m x cm in an environment with a pH of 3.5-3.8 using hydroacoustic cavitation (created in this case by the MT-1500 rotary pulsation apparatus of the Swiss company Kinematica), which is increasingly used in production and research in practice [19].

Extraction was carried out with the following technological parameters: hydromodule 1: (8-10); temperature 65-70°C; extraction time 15-30 minutes.

The resulting extract was first separated under the thrust of a vacuum pump, then purified according to a multistage scheme using a kieselguhr filter and in the mode of tangential filtration through ceramic ISOFLUX membranes (Germany) with a cut-off threshold of 2 kDa.

The purified extract was subjected to low-temperature concentration and spray drying to obtain a high purity pectin powder.

The yield of pectin in the extraction method used was ≥82.7% of its total content in wild medlar seeds.

Modern titrimetric, physico-chemical and statistical research methods were used to evaluate the analytical characteristics, composition, structure and purity of the studied pectin.

2.3 Analysis of the composition of monosaccharides

The monosaccharide composition was determined by high-performance liquid chromatography on a DIKMA Inertsil ODS-3 column (4.6 mm × 150 mm) connected to the Shimadzu HPLC system (LC–20ATvp pump and UV-VIS detector, Shimazu, Tokyo, Japan) after acid hydrolysis of pectin [20].

2.4 Determination of the degree of methylesterification and acetylation

The determination of these characteristics was carried out by the titrometric method [21].

The value of the electrolytic dissociation index (pKa, 25°C, water) was determined by the method of potentiometric titration as a percentage, taking into account the number of titrated carbon groups (C).

2.5 Determination of molecular weight

The molecular weight of pectin substances was determined using an exclusive liquid chromatograph MALS (Wyatt Technology Corp., USA) using ASTRA 6.0 software (Wyatt Technology), molecular standards of Pullulan (Showa Denko k.k. Japan) were used for calibration.

2.6 Identification of pectin
2.7 Determination of the jelly-forming ability of the studied pectin

In short, the strength of the gels was determined using a ridgelimeter, a device with a micrometer, the glass vessels of which have a height of 79.4 mm in the center. The strength of the studied pectin is expressed in the SAG of deflection of unsupported jelly prepared according to the standard method [22].

The distance between the lower surface of the micrometer screw and the upper surface of the glass plate (on which the jelly is placed) must correspond to the height of the jelly on it (79.4 mm). The micrometer screw is calibrated in such a way that one rotation is equal to one scale division - 0.8 mm. After exactly 1 minute, the height of the unsupported jelly is measured, lowering the micrometer until its sharp end touches the upper surface of the jelly. The loss of height by a jelly without support is called deflection, and one division of the scale is equal to a deflection of 1%. The device is calibrated according to the well-known standard pectin with strength of 100 SAG, which gives a deflection of about 20%. Examining standard jellies with different pectin content, a graph is constructed that should cover the deflection range from 10 to 35%. This graph is used to determine the degree of the studied pectin.

2.6 Processing of primary data

The data were analyzed using basic descriptive tools such as the mean and standard deviation of a set of repeated measurements. All tests are checked for compliance with the level of statistical significance equal to 0.05 or 5%. Verification of compliance with the significance level of 5% was carried out using the SPSS (Statistical Package for the Social Sciences) software.

3 Results

Analytical characteristics, such as the degree of esterification and others, determine the functionality of pectin. Pectins with a high degree of esterification and a low value of free carboxyl groups show, as a rule, a high jelly-forming ability. As is known, with a degree of esterification of 40% or less, pectins do not dissolve in water and have a high complexing ability, that is, such pectins can only be effective as functional ingredients capable of binding and removing heavy metals and other toxins from the body.

The analytical characteristics of the studied pectin are summarized in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esterified carbonyl groups, %</td>
<td>17.15</td>
</tr>
<tr>
<td>Total phenols, mg GAE/g</td>
<td>1.12 ± 0.05</td>
</tr>
</tbody>
</table>

Table 1. Analytical characteristics of wild medlar seed pectin
Free carboxyl groups, %  
4,25 ± 0,09

Uronid component, %  
65,50 ± 0,5

Acetyl groups, %  
1,15 ± 0,06

The degree of esterification (DE), %  
72,4 ± 0,6

Poly-GalA, %  
72,5 ± 3,5

The methoxyl component, % of the methoxyl groups in the total mass of Poly-GalA  
48 ± 0,25

pH (25°C)  
0,3% solution  
1,2% solution  
3,5 ± 0,06  
3,8 ± 0,07

pKa (25°C, water), %  
4,75 ± 0,10

From this table it can be seen that the pectin of wild medlar with a high degree of esterification is 72.4 ± 0,6%.

Summing up the data of many published works, it can be argued that the degree of esterification of pectin from different batches of lemon peel varies from 69.5 to 75.1%, apple pectin – from 60.9 to 73.6%.

In the studied pectin, the proportion of polygalacturonic acid (Poly-GalA) accounted for 72.5 ± 3,5%.

The chemically active methoxyl component also has a strong effect on the formation of jelly. Pectins with high values of methoxyl groups form more durable jellies than pectins with their low values.

Methoxyl component (percentage of acetyl groups in the total mass of galacturonic acids)  
The studied pectin sample was 48 ± 0,25. In samples of pectins with a high gelatinous ability, obtained fruits and berries of other wild plants, this indicator is low and varies from 7,23...13.64 % [23].

As can be seen from Table 1, the studied pectin is characterized by a relatively low content of carboxyl groups at the level of 4.25 ± 0,09%; this indicates its low complexing ability. In the pectin samples of some wild fruits and berries, the value of this indicator was even lower – 1–3% (viburnum) to 3.9% (dogwood) [23].

Acetyl groups associated with hydroxyl groups of pectin substances significantly worsen their jelly-forming properties.

As can be seen from Table 1, the studied pectin is characterized by a relatively low content of carboxyl groups at the level of 4.25 ± 0,09%; this indicates its low complexing ability. In the pectin samples of some wild fruits and berries, the value of this indicator was even lower – 1–3% (viburnum) to 3.9% (dogwood) [23].

Fine purification of extracts formed during hydroacoustic treatment of wild medlar seeds made it possible to obtain a dry pectin product of milky-beige color, in which the value of the electrolytic dissociation index (pKa), which reflects the strength of acids, was equal to 4,75 ± 0,10 (Table 1).

The studied samples of dry pectin were achromatic (with a slight presence of a gray component in the composition of the colored paint) of color, without absorption in the range of 400-700 nm.

The purity of pectin can be judged by the uronide component, which is contained in the studied pectin sample in an amount of 65.50 ± 0,5%. In samples of pectins with a high gelatinous ability, obtained fruits and berries of other wild plants, this indicator varied within the range of 60.35...97.58% [23].

In the studied pectin sample, the nitrogen content was low (at the level of 0.23 ±0.01), as well as polyphenols prone to oxidation (1.12 ± 0.05 mg GAE/g).
The high content of the methoxyl component (48±0.25%) determines the high molecular weight (55–95 kDa) and the jelly-forming ability (145 ±5 o SAG) of the studied pectin (Table 2).

Table 2. The content of ballast substances in the pectin of wild medlar seeds accompanying this type of pectin, its molecular weight and gelling ability

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>82.61±0.08</td>
</tr>
<tr>
<td>Total sugar, %</td>
<td>9.42 ±0.05</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.33 ± 0.01</td>
</tr>
<tr>
<td>Acid-insoluble ash, %</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>Fat, %</td>
<td>0.87±0.02</td>
</tr>
<tr>
<td>Nitrogen, %</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Total phenols, mg GAE/g</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td>Average molecular mass (kDa)</td>
<td>55–95</td>
</tr>
<tr>
<td>Gelling power, о SAG</td>
<td>145 ±5</td>
</tr>
</tbody>
</table>

Elemental analysis of dry wild medlar pectin showed that it is within the following parameters: C–28%, H–42%, O–24%, which corresponds to the gross formula C14H21O12, i.e., it is really pure pectin.

Table 3 presents data on the qualitative composition of monosaccharides of the studied pectin sample.

From this table, it can be seen that five different neutral sugars were found in it: rhamnose (L), arabinose (Ara), galactose (Gal), glucose (Glc) and mannose (Man).

Table 3. Monosaccharide composition of pectin

<table>
<thead>
<tr>
<th>Uronic acid (w/w,%)</th>
<th>Neutral sugar composition (w/w,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin of wild medlar seeds</td>
<td>Rha 65.5 Ara 5.2 Xyl 6.6 Man 0.0 Glc 18.3</td>
</tr>
</tbody>
</table>

Two types of polysaccharide sites were observed in pectin isolated from seeds of wild medlar fruits: a smooth site (homogalacturonan) and a hairy site (rhamnogalacturonan).

The data obtained indicate that the most complex section of wild medlar seed pectin consists of alternating residues of galacturonic acid and L-rhamnose and branches consisting of chains of D-galactose and L-arabinose attached to L-rhamnose. The presence of rhamnose, galactose and arabinose indicates that this pectin contains the rhamnogalacturanane I (RG-I) (2024)
IR spectra of pectin substances carry information about their composition and structure, the purity of the preparations, the absolute and relative number of functional groups, and others.

**Fig. 1.** IR spectrum of pectin isolated from seeds of wild medlar fruits.

The IR spectrum of the studied pectin (Fig. 1) showed typical peaks for a number of specific groups. Intense wide asymmetric peaks with maxima at 3200 - 3600 cm$^{-1}$ correspond to fluctuations of OH groups in the molecule of this pectin. The region with a maximum of approximately 2926 cm$^{-1}$ contains peaks corresponding to fluctuations of various groups containing C-H bonds. Areas with maxima of 1500 - 2000 cm$^{-1}$ correspond to fluctuations of C=O groups. Peaks typical for ester and carboxylic acid C=O were observed at 1730 - 1760 cm$^{-1}$ (COO-R) and 1600 - 1630 cm$^{-1}$ (COO-), respectively. The last two peaks were at maximum intensity, which is associated with the degree of methylation of the studied pectin.

The area with maxima between 1200 and 800 cm$^{-1}$ is called the fingerprint area, and the intensity of the individual bands in this area is unique for each polysaccharide. At the same time, the absorption bands at 1145, 1104 and 1020 cm$^{-1}$ are characteristic of pectin polymers, and the bands at 1077 and 1050 cm$^{-1}$ correspond to fluctuations of neutral glycans based on arabinose and galactose; characteristic peaks of 1146, 1095, 1074, 1050 and 1015 cm$^{-1}$ were present in this spectral region.

**Fig. 2.** 13C NMR spectrum of wild medlar pectin.

The analysis of NMR 13C spectra showed that the chemical nature of the studied pectin is determined by the presence of carboxylic (δ=103.03 - 103.64 ppm), methoxylic (δ=57.40 ppm) and methylene groups (ppm - millionth fraction).

Carbon atoms located in position 1 of the pyranose fragment give a signal in the strong field region at 63.78, 72.50 and 77.65 ppm. The second and third carbon atoms are characterized by the manifestation of a signal at 81.01, 67.47 and 76.92 ppm.
Fig. 2. NMR 13C spectrum of pectin isolated from seeds of wild medlar fruits. The carbon in position 4, involved in the connection of pyranose fragments by an oxygen bridge, resonated at 44.05 and 40.44 ppm. The chemical shift of the fifth carbon atom of the galactopyranosiluronic link, influenced by carboxyl and methoxyl groups, was equal to 63.80 and 74.19 ppm.

Fig. 3. The estimated structure of pectin isolated from the seeds of wild medlar fruits. In the proton spectrum of the pectin sample in the region of a strong field (δ=0.98 – 1.02, δ=1.43 – 1.77 ppm), a CH- group signal was observed at position 4. Protons of the carbon atom of position 1 of the pyranose cycle resonate at 3.51 ppm and chemical shift s at 3.36 and 3.92 ppm belong to carbon protons in positions 2 and 3 of the galactopyranosiluron fragment. The carbon proton directly bound to the carboxyl group resonates in the region of a weak field (δ=4.67 – 5.07 ppm). For the methoxyl and carboxyl groups, signals were characteristic at 3.60 and 3.86 ppm, respectively.

An objective criterion for the biochemical classification of pectin substances is the nature of their connection in a plant cell. Regarding the nature of the bonds of pectin substances in plants, hypotheses have been expressed based on the reactivity of functional groups of pectins — primarily hydrogen bonds formed by carboxyl and hydroxyl groups.

Analysis of data on 1H spectra of wild medlar seed pectin showed a distinct manifestation of doubling of the signal group, which speaks in favor of the predominance of two different sites in the polymer molecule of pectin.

Based on the IR and NMR data obtained, the structure of oligosaccharide fractions is unambiguously α-1,4-D-glucans. The abundance of carbonyl group signals indicates a very complex and heterogeneous structure of the pectin molecule, which has a rather branched structure.
4 Discussion

The analytical characteristics of pectin substances make it possible to predict the physicochemical properties of pectins and their use. Esterification of galacturonic acid with methanol (methylesterification) and/or acetic acid (acetylation) is important for revealing the structure of pectin. The degree of methoxylation (degree of esterification) is the ratio of the number of methoxyl groups to all acid residues in a molecule. Pectins are divided into high- and low-methoxylated pectins with methoxylation degrees above and below 50%, respectively [24]. The degree of methoxylation affects the resistance to hydrolysis, solubility, gelatinization and other physicochemical properties of the jelly. According to GOST (State Standard of Russia) 29186-91 [25], pectins with a degree of esterification of more than 50% belong to highly esterified and are divided into types. Type A includes pectins with a degree of esterification of at least 70%.

Table 1 shows that the studied pectin samples have a degree of esterification of 72.4 ± 0.6%. Consequently, they, having such a high degree of esterification, are able to form gels. The content of acetyl groups in pectins from different sources can vary from hundredths of a percent to 2.5%. The presence of a large number of acetyl groups affects the decrease in the jelly-forming ability of pectin, which is the main indicator for pectins used in the food industry. Therefore, the permissible limits of the content of acetyl groups for jelly-forming pectin have been established - no more than 1%.

Table 2 shows that the content of acetyl groups in the pectin of wild medlar seeds is 1.15 ± 0.06%, which is more than 1%, but significantly less than 2.5%. Comparison with pectins isolated from other sources showed that the pectins forming persistent gels have a higher degree of esterification than the pectin of wild medlar fruit seeds studied by us. For example, the degree of esterification of citrus pectin can reach 76% and even 85.7% [26-27]. The degree of esterification of bee pectin varied in the range of 61.27-80.42%, the content of acetyl groups - in the range of 2.00-2.74% [28]. From the official website of the Chinese company Focus Technology Co. Ltd. we learned that commercial apple pectin contains >65% galacturonic acids, >5% ash, >1% nitrogen, it should be with a degree of esterification of 65-68% and with a gelling capacity of 150 ± 5 oSAG (access: https://huaxuan.en.made-in-china.com/product/QKgJyOmAvxfj/China-Supply-Favorable-Fruit-Pectin-Price.html, 2023).

The pectin we studied contained 72.5 ± 3.5% galacturonic acid, 1.33 ± 0.01% ash, 0.23 ± 0.01% nitrogen that is the content of galacturonic acids in it is significantly higher, and the content of ash and nitrogen is lower than in commercial apple pectin. This may partly be due to the finer purification of the studied pectin sample from ballast substances, which was carried out by us. In the studied pectin sample, the content of the methoxyl component is high - 48 ± 0.25%.

The taste and smell of the studied pectin sample were determined by organoleptic methods. It was a milky-white beige pectin with a slightly astringent sweet and sour taste and a slightly pronounced characteristic odor.

The determination of the uronide component is based on measuring the optical density of the colored products of the interaction of pectin hydrolysate with carbazole at a wavelength (2024)
The indicator is the pectin content in terms of galacturonic acid and characterizes the degree of purity of the pectin preparation. In the studied pectin, the value of this indicator was 65.50 ± 0.5%.

The gel-forming ability is one of the most important indicators of pectins used in the food industry, determines the stability of the pectin gel. Therefore, the finished product will have a more stable shape. Structure, consistency, which is ultimately beneficial not only for confectionery manufacturers, but also trade organizations and consumers.

To determine this indicator, a sample of pectin concentrate was taken from the calculation of the content of pectin substances in it 4,625 g, jelly was prepared from it. When suspended, less than 300 ml, it is topped up with distilled water, with a larger volume boiled to the same value. Then the pH of the mixture is adjusted to 3.1 with buffer salts. With further boiling of the solution with 417 g of sugar, a buffer solution (25 ml) is additionally introduced. The mass of jelly is brought to 600 g. The cooking time is no more than 12 minutes, the dry matter content in the jelly is 70-71% according to the refractometer, the pH of the 50% solution is 3.05-3.15. The resulting mass is poured into two vessels so that its level is 12.7 mm below the upper edge of the paper rim, and left covered for gelatinization at a temperature of 15.5-18.5 °C for 18 hours. Finally, after removing the rim, part of the jelly is removed, cutting it off at the level of the edge of the vessel, and the rest is placed on a glass plate of a regeliometer and used for strength tests.

From the table, it can be seen that the strength of the standard jelly made of pectin from wild medlar seeds was 189.0 ± 5.0, which corresponds to ≈18.0% deflection of the jelly without any support under pressure exerted on it by a micrometer. For comparison, commercial citrus pectin with a degree of esterification of 70% makes it possible to obtain standard gels with a strength of 150 OSA USA [29].

The more acetyl (−COOH3) and carboxyl (−COOH) groups there are in pectin, the higher its complexing ability and the lower its jelly-forming ability. The studied pectin contained 1.5% acetyl groups (in jelly-forming pectin there should be no more than 1%) and 4.25% carboxyl groups, which somewhat affected the decrease in its jelly-forming ability. Therefore, standard gels prepared using the studied pectin, although they did not have a clear cubic shape, still had a clear contour; this is consistent with the data of the Turkish authors [17].

Consequently, in terms of its rheological characteristics, this type of pectin is closer to jelly-forming pectins than to complexing pectins.

5 Conclusions

Thus, gels based on seeds of wild medlar fruits had a viscoelastic structure, more elastic than viscous.

Accordingly, the pectin of seeds, which are biowaste for the production of jam and (or) extract from wild medlar fruits, should be considered as a potential functional ingredient of thick liquid gels and cream gels, contributing to their thickening.

Understanding the functional properties of this type of pectin is necessary for the development of many innovative applications.

References


