



## ISOLATION, CHARACTERIZATION, AND MONOSACCHARIDE COMPOSITION OF PECTIC POLYSACCHARIDES FROM FOUR AMARANTH (AMARANTHUS SPP.) VARIETIES CULTIVATED IN UZBEKISTAN

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**Abstract:** In this study, pectic substances were isolated and characterized from four local amaranth varieties (Andijon, Ulug'nor, Uzbekistan, and Marhamat) cultivated in Uzbekistan. Extraction was carried out using an equal mixture of 0.25% oxalic acid and ammonium oxalate solutions at 75 °C in two steps. The monosaccharide composition of the isolated pectins was determined by paper chromatography and gas chromatography, revealing mannose, glucose, galactose, and rhamnose as the major components. The pectin yield ranged from 3.3% to 8.5%. The findings indicate that amaranth pectins represent a promising source of biologically active polysaccharides for food and pharmaceutical industries.

**Keywords:** Amaranth pectins; polysaccharides; monosaccharide composition; extraction; chromatography.

### Introduction

Scientific and industrial interest in pectin substances has increased sharply in recent decades. They are valuable polysaccharides used in the food industry as thickeners, stabilizers, and gelling agents [1]. In the pharmaceutical field, the immunomodulatory, anticarcinogenic, and antithrombotic properties of pectins are being extensively studied [2,3].

Pectin isolated from plants, in its dried form, is a powder ranging from white to grayish-brown, depending on the production source and degree of purification. It is odorless and has a mucilaginous texture when tested on the tongue. Pectin is soluble in water, especially when heated, and it precipitates with alcohol and other organic solvents. Pectin decomposes at temperatures exceeding 100°C. Rapid decomposition occurs in the presence of chloride ions. In addition to its sorption and gelling properties, pectin is one of the most effective gelling agents, which is why it is widely used in the food industry [4].

### Literature review

In addition to traditional sources (apples, citrus), there is a growing interest in new plant resources, including amaranth pectins [5,6]. Although amaranth is well known for its high-protein seeds, extracting pectin from its leaves, stems, and seeds is promising [7]. Therefore, in this study, pectins were isolated from four local amaranth varieties of Uzbekistan, and their monosaccharide composition was analyzed.

The total carbohydrate content of amaranth seeds is slightly lower than that of wheat seeds, with starch being the primary carbohydrate component. Amaranth accounts for 48-69% of the dry grain (depending on the species) and is located in the perisperm cells. It is stored in the form of very small starch granules ranging from 0.8 to 2.5 µm, often of uniform size, in the form of spheres, angles, and polygons. An unusual feature is that they have an almost crystalline structure. The amylose content is very low, ranging from 0.1% to 11.1%. Compared to regular

grain, amaranth starch has a high starch content, and its content is usually low. In the amaranth grain composition, the grain fiber is slightly lower than in wheat and is generally similar; in quinoa and common cereals, it accounts for about 20% of the dry grain. According to other authors, the composition of total blood fibers ranges from 9.8% to 14.5%, depending on the type of soluble and insoluble fibers. Only small amounts of mono- and disaccharides were found in the amaranth seeds. The amount of sucrose is high, but glucose, galactose, fructose, maltose, raffinose, stachiose, and inositol were also found [8].

Shows that the maximum amount of water-soluble polysaccharides in the vegetative part of amaranth varieties was observed in *Amaranthus retroflexus* and amounted to 2%, and the highest amount of pectin was found in *Thlaspi arvensis* and reached 10.7% of the dry mass. The maximum amount of hemicellulose A was found in the vegetative part of *Thlaspi arvensis* and amounted to 9.4%, and hemicellulose B in *Amaranthus retroflexus* reached 23.3%. It is known that hemicelluloses and pectins are part of the cell wall of plants, and their content in tissues is determined by the characteristics of the species, but it can also depend on the climatic conditions of the place of growth.

**1-jadval. *Amaranthus retroflexus*, *Agastache rugosa* va *Thlaspi arvensis* vegetativ amount of polysaccharides in the mass, % (n=4)**

Polisaxaridlar	<i>Amaranthus retroflexus</i>	<i>Agastache rugosa</i>	<i>Thlaspi arvensis</i>
VPIS (water-soluble polysaccharides)	2,0 ± 0,2	1,4 ± 0,1	1,6 ± 0,2
Pektin	9,3 ± 0,7	8,7 ± 0,7	10,7 ± 1,1
Gemitsellyuloza A	4,4 ± 0,4	8,8 ± 1,8	9,4 ± 1,2
Gemitsellyuloza B	23,8 ± 2,2	15,1 ± 1,2	11,4 ± 1,3
ΣPIS (umumiy polisaxaridlar)	39,4 ± 3,4	33,8 ± 2,9	32,9 ± 3,7

**2-jadval. *Amaranthus retroflexus*, *Agastache rugosa* va *Thlaspi arvensis* qualitative and quantitative composition of pectins**

Plant	Relative total amount, %							MED, %
	Ara	Gal	Rha	Man	Xyl	Glc	GalA	
<i>Amaranthus retroflexus</i>	13,3	26,6	3,9	10,9	1,8	15,7	27,7	29,5
<i>Agastache rugosa</i>	23,9	24,2	3,1	6,6	tr.	8,4	33,8	31,5
<i>Thlaspi arvensis</i>	16,2	22,2	14,9	3,4	1,7	6,8	34,9	38,1

Note: tr. - a small amount (i.e., a very small amount).

According to the research results of the authors [10], the sugar content in amaranth plants varies widely depending on the species of the sample: in the leaves from 3.0 to 13.25%, in the stem from 3.0 to 24.25%, and in the flowering organs (baskets) from 2.0 to 15.0% (in dry matter). According to the data, the highest sugar content was found in the stem - 13.93%, in the leaves - 10.69%, and in the flowers - 7.26%. When comparing amaranth stems with main fodder crops, it was found that they are not inferior to most of them in terms of sugar content.

It was previously established that the qualitative monosaccharide composition of the isolated pectin substances, when measured using thin-layer chromatography and high-performance liquid chromatography methods, was distributed as follows: galacturonic acid - 67%, rhamnose - 4.1%, arabinose - 6.6%, glucose - 8.3%, galactose - 7.7%, fructose - 4.1%, xylose - 2.1% [11].

Subsequent studies [12] have determined the molecular weight of pectins isolated from amaranth seeds, which ranges from 20,000 to 25,000. Additionally, the qualitative monosaccharide composition was determined using thin-layer and high-performance liquid chromatography methods: galacturonic acid - 67%, rhamnose - 4.1%, arabinose - 6.6%, glucose - 8.3%, galactose - 7.7%, fructose - 4.1%, and xylose - 2.1%.

Based on the monosaccharide composition, specifically the ratio of galacturonic acid and rhamnose (16:1), it is necessary to classify the pectin isolated from amaranth as type II rhamnogalacturonan (RG-II).

It has also been proven that pectin substances are highly esterified compounds: the degree of esterification is 65–70% when determined by the titrometric method and infrared spectroscopy.

### Research methods

Natural carbohydrates and their fractions were isolated according to methods cited in the literature. In this process, alcohol-soluble carbohydrates (ASC), water-soluble carbohydrates (WSC), pectin substances (PS), and hemicelluloses were isolated, and their quantities were determined (see Table 3).

As is evident from the table, the oilseed cake contains a higher amount of water-soluble carbohydrates, which are a white, amorphous, water-soluble powder. To determine the monosaccharide composition of the carbohydrates, samples were hydrolyzed in a 2 n H<sub>2</sub>SO<sub>4</sub> solution at 100°C using the complete acid hydrolysis method. The hydrolysis time was 8 hours for WSC and 24 hours for PS and hemicelluloses. The presence of the monosaccharide glucose was identified in the hydrolysates of all polysaccharide samples using paper chromatography. It was also found that the WSC hydrolysates contain very small amounts of xylose and arabinose [13].

**Isolation of pectin substances (PS).** 100 g of crushed material from four different amaranth varieties was extracted twice. A mixture of equal volumes of 0.25% oxalic acid (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) and ammonium oxalate ((NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) solutions was used as the extraction solvent. The process was conducted at a temperature of 75°C.

The extraction was performed at hydromodule ratios (raw material to solvent) of 1:4 and 1:3. The resulting extract was separated by filtration, then dialyzed against running water, subsequently concentrated by evaporation in an evaporator, and the pectin substance was isolated by precipitation using three volumes of alcohol (ethanol, C<sub>2</sub>H<sub>5</sub>OH).

The resulting precipitate was processed according to the method described above. The yield of pectin substances (PS) is presented in Table 4.

**Complete acid hydrolysis of pectin substances (PS).** 100 mg of the isolated pectin substances were hydrolyzed in 3 ml of 1 N H<sub>2</sub>SO<sub>4</sub> solution at 100°C. The hydrolysis process continued for 24 hours. After the specified time, the hydrolysate was transferred to a beaker and neutralized with barium carbonate. The resulting precipitate was filtered off, and the filtrate was deionized using the KU-2 cation-exchange resin, then evaporated to a small volume

(0.5 ml) and chromatographed on FN-18 paper. A butanol-1-pyridine-water (6:4:3) system was used as the solvent, with known monosaccharides serving as control samples [14]. The resulting precipitate was filtered, and the filtrate was deionized using the KU-2 cation exchanger, then dried by evaporation to a small volume (0.5 ml), and chromatographed on FN-18 paper.

The obtained chromatograms were dried, then treated with acidic aniline phthalate and heated in a drying oven at 110°C for 1–2 minutes. As a result, mannose, glucose, galactose, and rhamnose were identified in the monosaccharide composition of the pectin substances. See Table 4.

**Results**

**Table 3. Carbohydrate complex of the aerial parts of amaranth samples, % by mass**

Sort	Sample Natural	carbohydrates	SPEU	SEU	PM	GMS	Kletchatka
Ulugnor	Oil cake	69,6	3,5	51,5	4,1	3,3	7,2
	Shrot	64,3	3,1	43,4	7,9	2,7	7,2
	Leaf, shoot	56,8	3,3	15,3	3,4	7,6	27,2
	Bouquet	33,4	1,2	16,4	5,1	7,5	3,2
Marhamat	Oil cake	67,9	3,5	50,5	4,1	2,8	7,0
	Shrot	62,4	3,0	42,2	7,8	2,4	7,0
	Leaf, shoot	56,7	2,8	15,8	3,6	7,5	27,0
	Bouquet	32,9	1,1	16,4	5,0	7,4	3,0
Uzbekistan-M	Oil cake	63,7	3,8	51,8	4,8	3,3	7,2
	Shrot	61,5	3,0	41,5	7,5	2,5	7,2
	Leaf, shoot	59,1	3,4	16,8	3,6	7,5	27,8
	Bouquet	33,7	1,5	16,4	5,1	7,4	3,3

PM and GMS hydrolysates traces of glucouric acids, arabinoz, xylose, and galactose were also detected.

**Table 4. PM yield and their monosaccharide composition**

PM	Unum, %	Monosaxarid qoldiqlarining nisbati						UA, BX
		Gal	Glc	Ara	Man	Xyl	Rha	
<b>Andijan</b>								
PM	8.5	3,0	5,0	-	5,5	-	1,0	+
<b>Ulugnor</b>								
PM	7.6	5,2	4,8	-	6,0	-	1,0	+
<b>Uzbekistan</b>								
PM	3,7	3,0	2,8	-	5,0	-	1,0	+
<b>Marhamat</b>								

PM	3,3	2,8	-	3,5	5,2	3,0	1,0	+
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**Discussion**

The results showed that the pectin yield varies from 3.3% (Marhamat) to 8.5% (Andijan). These values are close to the literature data for citrus and apple pectins [15,17].

The presence of mannos, glucose, galactose, and rhamnos in the monosaccharide was confirmed. This is confirmed by other researchers (Maran [18]; This is consistent with the results recorded by Martău [16]. In terms of color, the pectins isolated from the Uzbekistan and Markhamat varieties were light cream, while the pectins obtained from the Andijan and Ulugnor varieties were light brown. This difference is explained by the content of phenolic substances.

Such a composition of amaranth pectins makes them a promising raw material for the food and pharmaceutical industries.

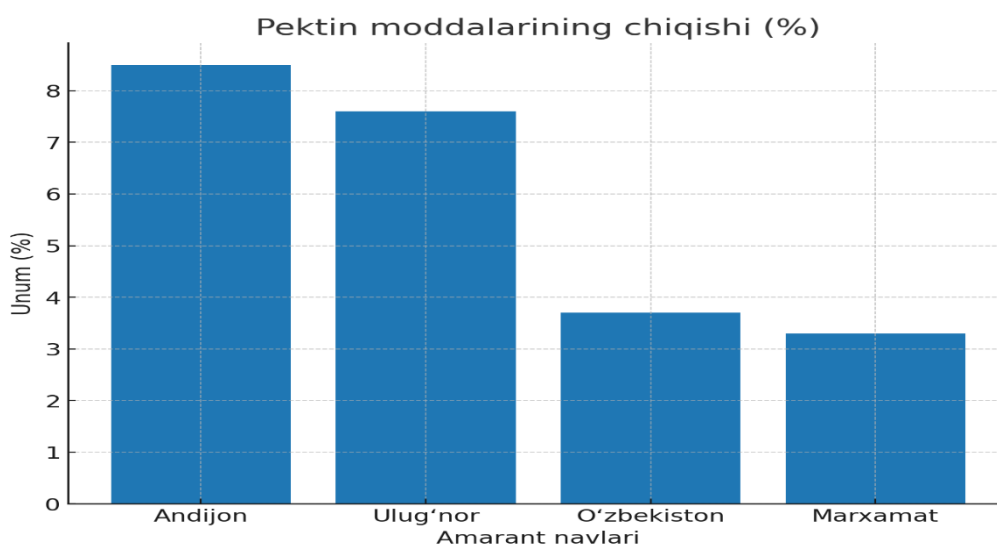
**Conclusion:**

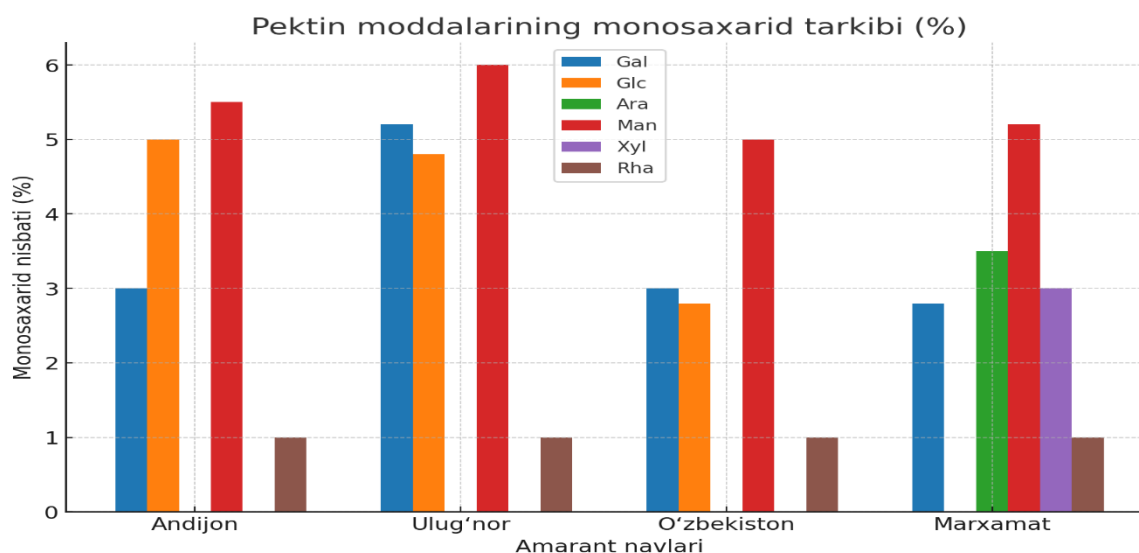
The yield of pectins isolated from local amaranth varieties ranges from 3.3 to 8.5%. Monosaccharides primarily consist of mannoza, glucose, galactose, and rhamnose. Amaranth pectins possess properties similar to citrus and apple pectins and are promising as biologically active additives.

Thus, the carbohydrate complex found in the aerial parts of amaranth is characterized by quantitative and qualitative indicators, and the predominance of water-soluble glucan-type carbohydrates in the complex is shown.

**Appendix**

- **Graph 1:** Pectin release (%)
- **Graph 2:** Monosaccharide composition of pectin substances (%)





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