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Chemical characterization of carbohydrate fractions and free amino acids in fruits of wild pear (*Pyrus pyraster* L.)

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ABSTRACT

This study aimed to characterize the carbohydrate fractions and free amino acid composition of fruits of wild pear (Pyrus pyraster L.) growing in the Jizzakh region of Uzbekistan. Particular attention was given to both low-molecular-weight sugars and structural polysaccharides, as well as their contribution to the overall chemical profile of the fruit. Carbohydrate fractions were obtained using stepwise extraction, including alcohol-soluble sugars, water-soluble polysaccharides, pectic substances, and hemicelluloses. Monosaccharide composition was determined after acid hydrolysis, while sugars were analyzed by HPLC with refractive index detection. Free amino acids were quantified by HPLC after derivatization. The results showed that the fruits contain typical mono- and disaccharides, including fructose, glucose, and sucrose. The yields of water-soluble polysaccharides, pectic substances, and hemicelluloses ranged from 1.9% to 2.8%. The isolated polysaccharides exhibited a heteropolysaccharide structure with the presence of neutral sugars and uronic acids. The degree of esterification of pectic substances was 15.6%, indicating their low-esterified nature. The total content of free amino acids reached 12.857 mg/g (dry weight), with alanine, valine, and leucine being the predominant components. The obtained data indicate that wild pear fruits represent a source of structurally diverse polysaccharides and free amino acids. These findings may be useful for further evaluation of their nutritional properties and potential application in food systems.

Contribution/Originality: This study provides new data on the composition of carbohydrate fractions and free amino acids in fruits of wild pear (Pyrus pyraster L.), a species that remains poorly characterized compared to cultivated varieties. The work combines fractionation of structural polysaccharides with amino acid profiling, allowing a more complete description of the fruit's chemical composition.

1. INTRODUCTION

Wild edible fruits represent a valuable but still insufficiently studied source of nutrients and biologically active compounds. Interest in such plant materials has increased due to their potential use in functional food systems and their relevance for sustainable use of natural plant resources (González-Zamorano, Cámara, Morales, & Cámara, 2025). Investigation of wild species also makes it possible to complement existing data obtained mainly for cultivated fruit crops.

Recent studies have shown that wild fruit raw materials may serve as valuable sources of natural polysaccharides and other biologically active compounds with potential application in functional food systems (Kocira et al., 2021; Schepetkin & Quinn, 2006).

Fruits are complex biological systems in which several classes of compounds determine both nutritional value and technological properties. Among these, carbohydrates play a central role. Low-molecular-weight sugars such as glucose, fructose, and sucrose contribute to sweetness and energy value, whereas structural polysaccharides of the cell wall influence texture, water-binding capacity, and behavior during processing (Delmer, Dixon, Keegstra, & Mohnen, 2024; Shi, Li, Grierson, & Chen, 2023).

Plant cell wall polysaccharides, including pectic substances and hemicelluloses, are of particular interest due to their functional properties. These compounds are capable of forming gels and viscous systems, interacting with other components of food matrices, and contributing to product stability (Benalaya, Alves, Lopes, & Silva, 2024; Freitas, Coimbra, Souza, & Sousa, 2021; Xiang, Yang, Li, Lin, & Kai, 2024). Their functional characteristics depend on structural features such as monosaccharide composition and degree of esterification, which makes their detailed study important for both fundamental and applied research (Arumuganainar et al., 2025; Dang et al., 2025).

Particular attention has recently been paid to fruit polysaccharides because their structural characteristics are closely related to antioxidant, immunomodulatory, and technological properties (Jin, 2012; Xie et al., 2022).

In addition to carbohydrates, free amino acids represent an important component of fruit chemical composition. They participate in the formation of taste and aroma, reflect metabolic processes, and may serve as indicators of plant physiological status (Łozowicka, Kaczyński, & Iwaniuk, 2021; Zhang et al., 2022). The amino acid profile of fruits varies depending on species, environmental conditions, and stage of maturity, which makes it a useful parameter for comparative characterization of plant materials (Sun et al., 2022).

Modern chromatographic methods are widely used for the characterization of biologically active compounds and the evaluation of functional properties of plant-derived polysaccharides and related metabolites (Xu et al., 2016; Zhang & Li, 2018).

Most available studies on fruits of the genus *Pyrus* are focused on cultivated varieties and report significant variation in carbohydrate composition depending on cultivar and growing conditions (Peng et al., 2022; Su et al., 2022; Zheng et al., 2022). However, information on the chemical composition of wild species remains limited. In particular, there is a lack of detailed data on the polysaccharide complex of wild pear (*Pyrus pyraster* L.), as well as on its free amino acid profile.

In addition, available studies usually consider carbohydrate components and amino acid composition separately. Such an approach does not fully reflect the complexity of fruit chemical composition, since these groups of compounds are metabolically interconnected and together influence both nutritional and functional properties of plant materials. Therefore, the absence of integrated studies combining analysis of polysaccharide fractions and free amino acid profiles in wild fruit species represents a noticeable gap.

Therefore, a combined analysis of carbohydrate fractions and free amino acids in wild pear fruits appears justified. Such an approach allows a more complete characterization of the chemical composition of the plant material and provides a basis for evaluating its potential use.

The aim of the present study was to characterize the carbohydrate fractions, including alcohol-soluble sugars and structural polysaccharides, and the free amino acid composition of fruits of wild pear (*Pyrus pyraster* L.) growing in the Jizzakh region of Uzbekistan, using chromatographic methods.

The paper is structured as follows. The Materials and Methods section describes sample preparation, extraction procedures, and analytical techniques. The Results and Discussion section presents the obtained data on carbohydrate fractions and amino acid composition and compares them with available literature. The final section summarizes the main findings of the study.

2. MATERIALS AND METHODS

All experiments were carried out in triplicate to ensure reproducibility of the analytical procedures. The procedures were organized to ensure reproducibility of extraction and analytical measurements. Carbohydrates and free amino acids were determined using standard chromatographic techniques.

2.1. Plant Material

The object of the study was the fruits of the wild pear (*Pyrus pyraster* L., family Rosaceae). The fruits were collected under natural conditions in the Bakhmal district of the Jizzakh region, Uzbekistan. Only fully ripened fruits without visible mechanical damage, decay, or microbiological contamination were selected for analysis.

After collection, the plant material was cleaned of surface impurities and foreign particles. Sample preparation included air-drying the fruits at room temperature under conditions excluding direct sunlight exposure. The dried fruits were ground to a fine powder to obtain a homogeneous material, ensuring reproducibility of extraction and analytical procedures.

The prepared plant material was stored in airtight containers in a dry, light-protected environment until extraction. Analytical samples consisted of accurately weighed portions of air-dried powdered material. All quantitative results for carbohydrates and amino acids were expressed on a dry weight basis.

2.2. Preparation of the Crude Extract from Pyrus Pyraster Fruits

For the preparation of the crude extract, 100 g of air-dried powdered plant material was extracted with a hydro-organic solvent. A 70% acetone solution prepared with distilled water was used as the extractant. The raw material-to-solvent ratio was 1:14 (w/v).

Extraction was performed at 45°C in a water bath. The extraction mixture was maintained for 48 hours with periodic stirring to ensure efficient recovery of polar and moderately polar compounds without thermal degradation.

After extraction, acetone was removed under reduced pressure using a rotary evaporator until complete solvent removal. The resulting aqueous extract was used for preparing samples for carbohydrate and amino acid analyses.

2.3. Sample Preparation for Carbohydrate Analysis

A portion of the aqueous extract obtained after acetone removal was used for carbohydrate analysis. To remove co-extracted lipophilic and moderately polar compounds that could interfere with chromatographic determination, the aqueous phase was subjected to sequential liquid–liquid extraction with organic solvents.

Initially, 500 mL of ethyl acetate was added to the aqueous extract. The mixture was stirred on a magnetic stirrer, transferred to a separatory funnel, and allowed to stand until complete phase separation occurred. The ethyl acetate phase was then removed. The extraction of the aqueous phase was repeated with 350 mL of ethyl acetate. This step is primarily aimed at removing phenolic and other weakly polar components.

The purified aqueous fraction was subsequently used for carbohydrate determination. Prior to chromatographic analysis, samples were filtered and adjusted to the required concentration when necessary.

2.4. Sample Preparation for Free Amino Acid Analysis

For the determination of free amino acids, the aqueous fraction of the crude extract was used after removal of low- and medium-polar components. For additional purification, the aqueous solution was extracted with *n*-butanol to remove interfering compounds.

To the aqueous phase, 500 mL of *n*-butanol was added, and the mixture was stirred on a magnetic stirrer for 1–2 hours. The mixture was then transferred to a separatory funnel and allowed to stand until two clearly defined layers formed. Considering the lower density of *n*-butanol ($\rho = 0.81$ g/cm^3), the upper organic layer was separated. The extraction was repeated using 350 mL of *n*-butanol. After fractionation, the purified aqueous phase containing free amino acids was used for further analysis.

To remove proteins and high-molecular-weight impurities, 1 mL of the sample solution was mixed with 1 mL of 20% trichloroacetic acid, allowed to stand for 10 minutes, and centrifuged at 8000 rpm for 15 minutes. The supernatant was collected and lyophilized.

The dry residue was dissolved in a triethylamine–acetonitrile–water mixture (1:7:1) and evaporated to dryness; the procedure was repeated twice to neutralize residual acid. The samples were derivatized with phenylisothiocyanate according to the established procedure (Cohen & Strydom, 1988).

Chromatographic separation of phenylthiocarbamyl derivatives of amino acids was performed using high-performance liquid chromatography on an Agilent Technologies 1200 system equipped with a diode-array detector. Separation was carried out on a Discovery HS C18 column (75 × 4.6 mm). The mobile phase consisted of solvent A (0.14 M sodium acetate containing 0.05% triethylamine, pH 6.4) and solvent B (acetonitrile). The flow rate was 1.2 mL/min, and detection was performed at 269 nm. Identification and quantification of amino acids were conducted using reference standards.

2.5. Determination of Carbohydrate Composition

2.5.1. Isolation of Alcohol-Soluble Sugars

For analysis of low-molecular-weight sugars, the plant material was preliminarily treated to remove pigments and non-carbohydrate components. One hundred grams of air-dried powdered material were twice treated with a boiling methanol–chloroform mixture (1:1), filtered, and dried. The dried material was then extracted twice with boiling ethanol (82 °C) at a hydromodule of 1:6 for 1 hour. The ethanol extracts were combined, concentrated, and used for identification of alcohol-soluble sugars by paper chromatography (Kozłowicz & Chizhov, 1967).

2.5.2. Isolation of Water-Soluble Polysaccharides (WSP)

The residue remaining after ethanol extraction was extracted twice with cold water at room temperature for 3 hours (hydromodule 1:4). The aqueous extracts were filtered, concentrated to a small volume, and precipitated with a threefold volume of ethanol. The precipitate was collected by centrifugation (5000 rpm, 10 minutes), washed with ethanol, and dried. The yield of WSP was 1.9 g.

2.5.3. Isolation of Pectic Substances (PS)

The residue after WSP extraction was extracted twice with an equal mixture of 0.3% oxalic acid and 0.3% ammonium oxalate at 70°C (hydromodule 1:4 and 1:3). The extract was filtered, dialyzed against running water, concentrated, and precipitated with a threefold volume of ethanol. The precipitate was collected and processed similarly to the WSP fraction. The yield of PS was 2.1 g (based on air-dried raw material).

2.5.4. Isolation of Hemicelluloses (HC)

The residue after PS extraction was treated twice with 5% KOH solution at room temperature for 1.5–2 hours (hydromodule 1:3). The extracts were filtered, neutralized, dialyzed against running water, concentrated, and precipitated with a threefold volume of ethanol. The yield of hemicelluloses was 2.8 g.

2.5.5. Determination of Monosaccharide Composition

The monosaccharide composition of WSP, PS, and HC fractions was determined after complete acid hydrolysis. One hundred milligrams of isolated polysaccharide were hydrolyzed with 3 mL of 1 N H_2SO_4 at 100 °C. WSP was hydrolyzed for 8 hours, whereas PS and HC were hydrolyzed for 24 hours. The hydrolysates were neutralized with barium carbonate, filtered, deionized using KU-2 cation-exchange resin, concentrated to 0.5 mL, and analyzed by paper chromatography in the *n*-butanol–pyridine–water (6:4:3) system using standard monosaccharides. Chromatograms were developed with acidic aniline phthalate, followed by heating.

2.5.6. HPLC Analysis of Sugars

Quantitative/semi-quantitative determination of sugars was performed by HPLC with a refractive index detector (RID) using a Shim-pack GIST NH2 column (250 × 4.6 mm). The column temperature was maintained at 40 °C. The flow rate was 1.0 mL/min. The mobile phase consisted of water/acetonitrile (25:75). The injection volume was 60 µL.

2.5.7. Determination of Degree of Esterification of Pectins

Free carboxyl groups in pectic substances were determined by titration. A 0.25 g portion of PS was mixed with 25 mL of water, gently heated, stirred, and allowed to stand for 2 h. The solution was titrated with 0.1 M NaOH in the presence of phenolphthalein until a stable pale pink color appeared (Arasimovich, 1984).

3. RESULTS AND DISCUSSION

3.1. Carbohydrate Composition of Pyrus pyraster Fruits

3.1.1. Alcohol-Soluble Sugars

Fructose, glucose, and sucrose were identified in the fruits of Pyrus pyraster L., which corresponds to the typical carbohydrate profile of species of the genus Pyrus. According to literature data, fructose usually predominates in pear fruits and largely determines their sweetness, while sucrose content varies depending on cultivar and stage of ripeness. The results obtained in the present study are consistent with these general patterns. Table 1 presents the relative monosaccharide composition of liquid fractions obtained from 40% and 70% ethanol extracts of Pyrus pyraster L. fruits.

Table 1. Relative monosaccharide composition of the liquid fractions obtained from 40% and 70% ethanol extracts of *Pyrus pyraster* L. fruits.

No.	Extract	Volume (mL)	Monosaccharide residue ratio		
			Fru	Glc	Suc
1	40% ethanol extract	110	2.5	1.5	1.0
2	70% ethanol extract	115	6.0	3.0	1.0

Note: Values are presented in relative units and represent the ratio of chromatographic peak areas obtained by HPLC; the data do not correspond to absolute concentrations.

3.2. Polysaccharide Complex of Pyrus Pyraster Fruits

A polysaccharide complex composed of water-soluble polysaccharides (WSP), pectic substances (PS), and hemicelluloses (HC) was isolated from the fruits of Pyrus pyraster L. The yields of these fractions were 1.9%, 2.1%, and 2.8%, respectively, calculated on an air-dried raw material basis. The predominance of hemicelluloses and pectic substances aligns with the structural organization of cell walls in pome fruits and indicates a significant contribution

of structural carbohydrates to the fruit matrix. Comparison with published data on cultivated pear varieties (Pyrus communis L.) indicates that the content of pectic substances and hemicelluloses in Pyrus pyraster is of a comparable level. At the same time, the relatively lower proportion of water-soluble polysaccharides may reflect differences related to the wild-growing nature of the species, environmental conditions, and stage of fruit development. Table 2 shows the yields of isolated polysaccharide fractions and their monosaccharide composition in fruits of Pyrus pyraster L.

Table 2. Yields of isolated polysaccharide fractions and their monosaccharide composition (dry weight basis) in fruits of *Pyrus pyraster* L.

Polysaccharide fraction	Yield (%)	Monosaccharide residue ratio (HPLC analysis)					UA, qualitative test	$\eta_{intrinsic}$
		Rha	Ara	Xyl	Glc	Gal		
WSP	1.9	-	5.2	1.0	4.9	2.9	+	10.1
Pectins	2.1	-	8.8	1.0	2.7	2.0	+	1.9
Hemicelluloses	2.8	-	3.7	1.4	1.2	1.0	+	1.3

3.3. Monosaccharide Composition of Polysaccharide Fractions

It was established that all isolated polysaccharide fractions possess a heteropolysaccharide nature and are characterized by different ratios of neutral sugars and uronic acids.

The water-soluble polysaccharide (WSP) fraction was dominated by arabinose, galactose, glucose, and xylose, and also contained uronic acids, indicating its pronounced polarity. Pectic substances were characterized by elevated levels of arabinose and galactose, along with the presence of glucose and galacturonic acid. Hemicellulose fractions showed a predominance of xylose and glucose, with comparatively lower contents of uronic acids.

3.4. Physico-Chemical Characteristics of Pectin Substances

The study of the physicochemical properties of pectin substances isolated from the fruits of wild pear (*Pyrus pyraster* L.) showed that the obtained pectins were light-brown powdery substances, well soluble in water with the formation of highly viscous solutions. The good solubility of pectins indicates their high hydrophilicity and ability to form structured colloidal systems. According to titrimetric analysis, the isolated pectin substances belong to low-esterified pectins. The degree of esterification (DE) was 15.6%, indicating the predominance of free carboxyl groups in the polysaccharide structure. Low-esterified pectins are capable of forming gels in the presence of calcium ions, which expands their potential practical applications in the food and pharmaceutical industries (Cohen & Strydom, 1988). Analysis of titrimetric parameters showed that the content of free carboxyl groups (Cs) was 4.5 %, the content of esterified carboxyl groups (Ce) was 24.3 %, and the total carboxyl group content (Co) was 28.8 %. The obtained results confirm that the studied pectins belong to the low-esterified type of polysaccharides. Figure 1 illustrates the physicochemical characteristics of pectin substances isolated from the fruits of Pyrus pyraster L.

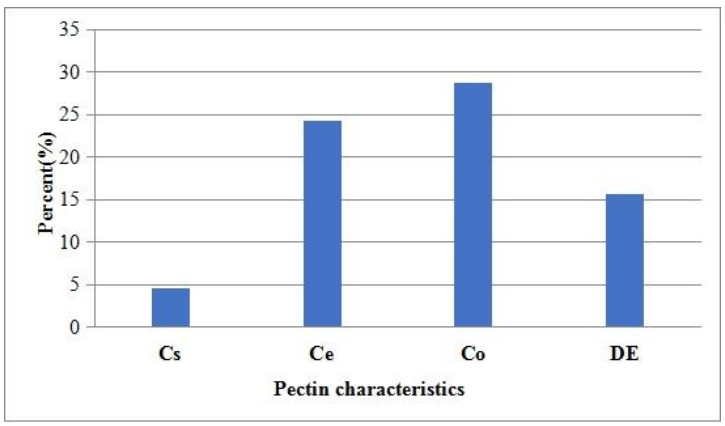

Figure 1. Physico-Chemical Characteristics of Pectin Substances.

The low degree of pectin esterification may increase its biological activity, including sorption properties and the ability to bind toxic compounds, which is of interest for developing functional foods and biologically active supplements, including for preventing metabolic disorders.

3.5. Amino Acid Composition of Pyrus Pyraster L. Fruits

The analysis of free amino acids in fruits of Pyrus pyraster L. demonstrated the presence of both essential and non-essential amino acids, with a total content of 12.857 mg/g (dry weight). The profile was characterized by a predominance of branched-chain amino acids, particularly alanine, valine, and leucine.

The predominance of these amino acids may be related to specific features of nitrogen metabolism in fruit tissue and can influence both nutritional value and sensory properties. In particular, branched-chain amino acids are known to contribute to the formation of aroma-related compounds, which may be relevant for characterizing wild fruit species. Figure 2 demonstrates the quantitative profile of free amino acids in the fruits of Pyrus pyraster L.

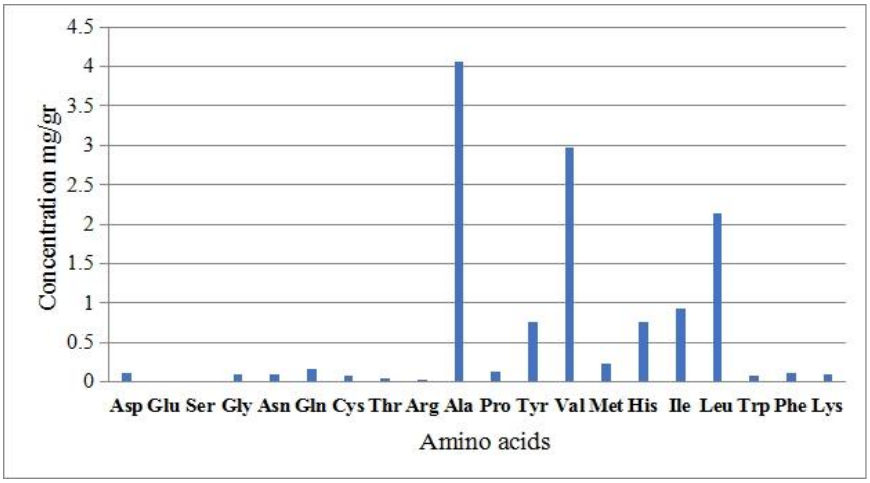

Figure 2. Quantitative profile of free amino acids in fruits of *Pyrus pyraster* L.

The highest concentrations were observed for alanine (4.055 mg/g), valine (2.968 mg/g), and leucine (2.135 mg/g). These amino acids play important roles in energy metabolism, maintenance of protein synthesis, and overall metabolic activity. Moderate levels were detected for isoleucine (0.929 mg/g), tyrosine (0.760 mg/g), histidine (0.766 mg/g), methionine (0.224 mg/g), proline (0.122 mg/g), glycine (0.097 mg/g), asparagine (0.097 mg/g), lysine (0.095 mg/g), tryptophan (0.085 mg/g), and cysteine (0.070 mg/g). The presence of these amino acids may contribute to the overall antioxidant potential of the raw material and support metabolic regulation processes (Lobo, Patil, Phatak, & Chandra, 2010). Particular interest in the context of functional food development is associated with amino acids involved in carbohydrate metabolism regulation (Newsholme et al., 2011). Proline and glycine may participate in cellular protective mechanisms under oxidative stress conditions, which frequently accompany metabolic disturbances such as impaired glucose metabolism. Additionally, sulfur-containing and aromatic amino acids contribute to the maintenance of cellular redox balance (Newsholme et al., 2011). Relatively low levels of certain amino acids, such as arginine (0.026 mg/g), threonine (0.044 mg/g), and phenylalanine (0.116 mg/g), may be associated with specific features of amino acid biosynthesis in plant tissues at the stage of fruit maturity.

Taken together, these results indicate that the fruits of wild pear may be considered a promising source of biologically active compounds. Fruits of wild pear may be considered a promising plant source of biologically active compounds with potential metabolic-regulatory relevance. The diversified amino acid profile may contribute to nutritional value and support physiological metabolic processes. However, further pharmacological and clinical studies are required to more precisely evaluate any potential hypoglycemic or metabolic effects.

4. CONCLUSION

The present study provides a detailed characterization of carbohydrate fractions and free amino acid composition in fruits of wild pear (Pyrus pyraster L.) collected in the Jizzakh region of Uzbekistan. The results demonstrate that the fruits contain both low-molecular-weight sugars and structurally diverse polysaccharides, including water-soluble polysaccharides, pectic substances, and hemicelluloses. The predominance of hemicelluloses and pectic substances indicates an important contribution of structural carbohydrates to the fruit's composition, while the low degree of esterification of pectins suggests their potential applicability in food systems requiring gel formation under mild conditions. The identified amino acid profile, characterized by a relatively high content of alanine, valine, and leucine, reflects specific features of fruit metabolism and may influence both nutritional value and sensory properties.

At the same time, the study has several limitations. The analysis was performed on samples collected from a single geographical region and at one stage of fruit maturity, which may affect the generalization of the results. Additionally, the work focused on compositional analysis without a detailed investigation of biological activity.

Further studies should include a broader geographical sampling, evaluation of seasonal variability, and investigation of the functional and biological properties of the identified compounds. Such data would enable a more comprehensive assessment of the potential of wild pear fruits as a source of biologically active substances.

Taken together, the obtained results expand current knowledge of the chemical composition of *Pyrus pyraster* fruits and provide a basis for their further study and potential use in food and related applications.

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