

Study of Amino Acid Composition of *Prunus Domestica* Fruits Pectin Complex

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Abstract: Medicines on the basis of herbs are increasingly used for treating of many diseases and provide the following actions laxative, hepatoprotective, membrane stabilizing and antimicrobial. The plant *Prunus domestica* L., family *Rosaceae* is widely cultivated worldwide. The recent studies have shown that fruits have antioxidant, anticancer, antihyperglycemic, anti-hyperlipidemic, antihypertensive, antiosteoporosis, laxative and hepatoprotective activities. As raw material plum of Ukrainian variety 'vengerka' was taken for the research and pectin complex from plum fruits was obtained. The yield of the pectin from the fresh plum fruits was 8,6% of a dry weight. The analysis of plum pectin was performed by HPLC and spectrophotometry methods. 16 amino acids were identified and their contents were determined. It was found that plum pectin contained essential amino acids such as threonine, valine, isoleucine, leucine, phenylalanine, and histidine. The total amount of free and bound amino acids was 33.70 µg/mg, while free amino acids – 1.18 µg/mg. The amount of bound amino acids was 32,52 µg/mg. The content of free amino acids was only 3.50% of the amount of all amino acids found in the pectin complex. L-Aspartic and L-Proline predominated among free amino acids. Among the bound amino acids, L-Aspartic and L-Glutamic acids dominated. The content of the total amount of phenolic compounds calculated with reference to gallic acid in the pectin complex was 0.43%, and the content of hydroxycinnamic acids calculated with reference to chlorogenic acid in this substance was 0.09%.

Keywords: Plum, Fruits, Pectin, Amino Acids, Phenolics, Hydroxycinnamic Acids

1. Introduction

Plums are widely cultivated in many countries of the world. The leaders in the production of plums are China and the USA. Traditionally, plums are also widely cultivated in Ukraine. Plums are used for food in fresh and dried form, as well as for the preparation of juices, jams and compotes. Consumption of plums may help to control obesity, diabetes, and related cardiovascular diseases [1]. At the Ukrainian pharmaceutical market, there is a complex laxative preparation 'Regulax', which includes Senna leaves and fruits, as well as fig and plum fruits. [2]. Several substances from plum fruits and leaves, were obtained and their chemical composition and pharmacological activity were studied at the National

University of Pharmacy. It has been found that these substances contain acidic and neutral polysaccharides [3], flavonoids, hydroxycinnamic acids and other phenolic compounds [4, 5], organic acids. Their microelement composition has been also studied [6]. It has been determined that these substances have the laxative, antioxidant, hepatoprotective and diuretic effects [7-10].

Plum fruits are known to contain pectin substances. Pectin is a carbohydrate polymer consisting of acidic sugars. It is found in various fruits, in the form of protopectin, and is converted into a soluble form when heated with dilute acids, then purified and dried. Pectin, (E-440) is a purified hydrocarbon, in industry it is obtained by extraction of citrus, apple or a beet pulp. It is a gelling agent, stabilizer, thickener, humectant, clarifier, filter aid, and encapsulating agent. Pectin

is used in the production of meat and confectionery products to form and maintain the consistency, texture and shape of food products.

The search and creation of substances that meet the requirements of efficiency, safety, and quality is an important task for Pharmacy. Plum fruits are a promising object for research in this field.

The aim of the present work was to obtain the pectin complex from plum fruits, study its chemical composition, namely the qualitative and quantitative amino acid composition, as well as the content of the total amount of phenolic compounds and hydroxycinnamic acids.

2. Materials and Methods

2.1. Object

The study object was plum (*Prunus domestica*) fresh fruits of 'vengerka' variety harvested in September, in the Kharkiv region of Ukraine, in 2019.

To isolate pectin, 400 g of the plum fruit puree was mixed with 1% citric acid in the ratio of 1:10, stirred for 2 hours at room temperature, centrifuged (the rotation speed – 5000 rpm) for 10 minutes, and the liquid was decanted in a flask and concentrated to 400 ml. After that, 1200 ml of 96% alcohol was added to 400 ml of the concentrated extract, stirred and allowed to stand for 3 hours. A precipitate (pectin complex) was filtered through a paper filter. The pectin thus obtained was washed with 96% ethyl alcohol. The filter with the pectin was dried firstly in air, and then its yield was calculated.

2.2. Determination of the Hydroxycinnamic Acids Content

The quantitative determination of hydroxycinnamic acids was performed by the spectrophotometric method according to State Pharmacopoeia of Ukraine (SPhU) [11].

Approximately 0.1 g (accurate weight) of the pectin obtained was placed into a 100 ml volumetric flask, diluted to the volume with 50% ethyl alcohol and stirred. The extract was filtered through a paper filter (solution A).

1 ml of solution A was placed into a 10 ml volumetric flask, and 2 ml of 0.5 M solution of hydrochloric acid, 2 ml of 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water, 2 ml of dilute sodium hydroxide were gradually added, stirring after each addition, diluted with water to the volume and mixed.

Preparation of the compensation solution. 1 ml of solution A was placed into a 10 ml volumetric flask, 2 ml of 0.5 M hydrochloric acid solution and 2 ml of dilute sodium hydroxide solution were added stirring after each addition, diluted with water to the volume and mixed.

Immediately the optical density of the test solution at a wavelength of 525 nm in a cuvette with a layer thickness of 10 mm was measured on a Hewlett Packard 8453 spectrophotometer against the compensation solution. The content of the total amount of hydroxycinnamic acids calculated with reference to chlorogenic acid, in percent, was determined by the formula:

$$X = \frac{A \cdot 1000}{188 \cdot m} \quad (1)$$

where:

A – is the optical density of the test solution at a wavelength of 525 nm;

m – is the weight of the pectin complex, g.

2.3. Determination of the Phenolic Compounds Content

The quantitative determination of phenolic compounds was performed by the spectrophotometric method [11].

Approximately 0.05 g (accurate weight) of the pectin was dissolved, stirring constantly, in 10 ml of 40% ethyl alcohol. The operation was repeated three times with a new portion of the solvent. The solutions were combined, filtered through a paper filter and transferred in a 50.0 ml volumetric flask, diluting to the volume with the same solvent and stirring (solution A).

1.0 ml of solution A was added to a 25.0 ml volumetric flask, diluted to the volume with 40% ethyl alcohol and mixed. 2.0 ml of the resulting solution was poured into a 25.0 ml volumetric flask and diluted to the volume with the same solvent. The optical density of the solution obtained was measured on a Hewlett Packard 8453 spectrophotometer at a wavelength of 270 nm in a cuvette with a layer thickness of 10 mm against 40% ethyl alcohol.

The content of the total amount of phenolic compounds calculated with reference to gallic acid was determined by the formula:

$$X = \frac{A_1 \times 50 \times 25 \times 25 \times 100}{540 \times m \times 1 \times 2 \times (100 - w)} \quad (2)$$

where:

A – is the optical density of the test solution;

m – is the weight of the sample, g;

540 – is the specific absorption coefficient of gallic acid solution in 40% ethyl alcohol at a wavelength of 270 nm; w – is the loss on drying, %.

2.4. HPLC Determination of the Amino Acid Content

The method is based on the extraction of free amino acids from the plant raw material and acid hydrolysis of plant preparations, followed by the analysis of hydrolysates by high performance liquid chromatography (HPLC) with pre-column derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalic aldehyde (OPA) followed by detection using a fluorescent detector.

The sample preparation and analysis were performed as follows. To determine free amino acids, a portion of the powdered pectin was placed in a vial, 2 ml of an aqueous solution of 0.1 N hydrochloric acid was added, and kept in an ultrasonic bath at 50°C for 3 hours.

Mobile phase: A - 40 mM Na_2HPO_4 pH 7.8; B-ACN: MeOH: water (45:45:10, v/v/v). The separation mode was gradient with a constant flow rate of 1.5 ml/min. The temperature of the column thermostat was 40°C. Precolumn derivatization was performed in an automatic programmable

mode using the FMOc reagent (Agilent 5061-3337) and the OPA reagent (Agilent 5061-3335). Derivatized amino acids were detected using a fluorescent detector [12-14].

To determine the total amount of amino acids, a portion of the pectin was placed in a vial, 2 ml of an aqueous solution of 6N hydrochloric acid was added, and placed in a thermostat at 110°C. The hydrolysis was performed for 24 hours.

0.5 ml of the pectin/hydrolyzate centrifuged was evaporated on a rotary evaporator, washing three times with distilled water to remove hydrochloric acid, re-suspended in 0.5 ml of distilled water and filtered through 0.2 µm regenerated cellulose membrane filters.

Fluorescent derivatives were obtained in the automatic programmable mode before introducing the sample into the chromatographic column.

Amino acid identification was performed by comparing retention times with a mixture of amino acid standards (Agilent 5061-3334). The content of bound amino acids was determined by subtracting the content of free amino acids from their total content.

The content of free/total amino acids was calculated by the formula:

$$X = C \times V / M \quad (3)$$

where:

C – is the concentration according to the chromatographic system (µg/ml);

V – is the volume of the solvent for extraction (free)/volume of the solvent for hydrolysis (total), ml;

M – is the weight of the pectin complex, mg.

3. Results and Discussion

The pectin substance obtained from the plum fruit is a powder of a dark reddish brown color, with a sweet and sour taste, readily soluble in water, insoluble in ethyl and methyl

alcohol, chloroform, ether. The pectin yield from the fresh plum fruits was 8.6% (dry weight).

The content of the total amount of phenolic compounds calculated with reference to gallic acid in the pectin complex was 0.43%.

The content of hydroxycinnamic acids calculated with reference to chlorogenic acid in this substance was 0.09%, determined by the SPhU method.

The results of identification of free and bound amino acids in the pectin complex are shown in Table 1 and Figures 1 and 2.

In the pectin complex obtained from plum fruits, 16 amino acids were found. Among them the content of L-Aspartic acid was the highest. The plum pectin contained essential amino acids, such as threonine, valine, isoleucine, leucine, phenylalanine, and histidine. The total amount of free and bound amino acids was 33.70 µg/mg, while the amount of free amino acids was 1.18 µg/mg. The amount of bound amino acids was 32.52 µg/mg. The content of free amino acids was only 3.50% of the amount of all amino acids found in the pectin complex. L-Aspartic and L-Proline acids predominated among free amino acids. Among the bound amino acids, L-Aspartic and L-Glutamic acids dominated.

In our previous research the composition of free amino acids in extracts obtained from cherry, peach, and apricot leaves was studied [15]. Among the free amino acids found the extracts contained L-Proline in the greatest or significant amount from 23.3% in apricot leaf extract to 58.5% in peach leaf extract [15]. Comparing the composition of amino acids in the pectin complex obtained from plum fruits with these results it can be seen that L-Proline was also contained in a significant amount, slightly inferior only to L-Aspartic acid. The L-Proline content was 20.25% of the total amount found in free amino acids.

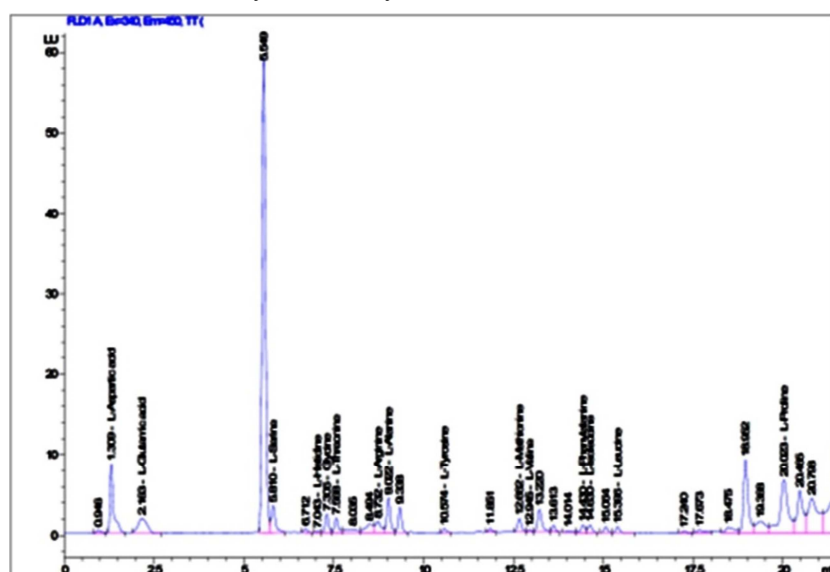


Figure 1. The chromatogram of studying free amino acid in the plum pectin.

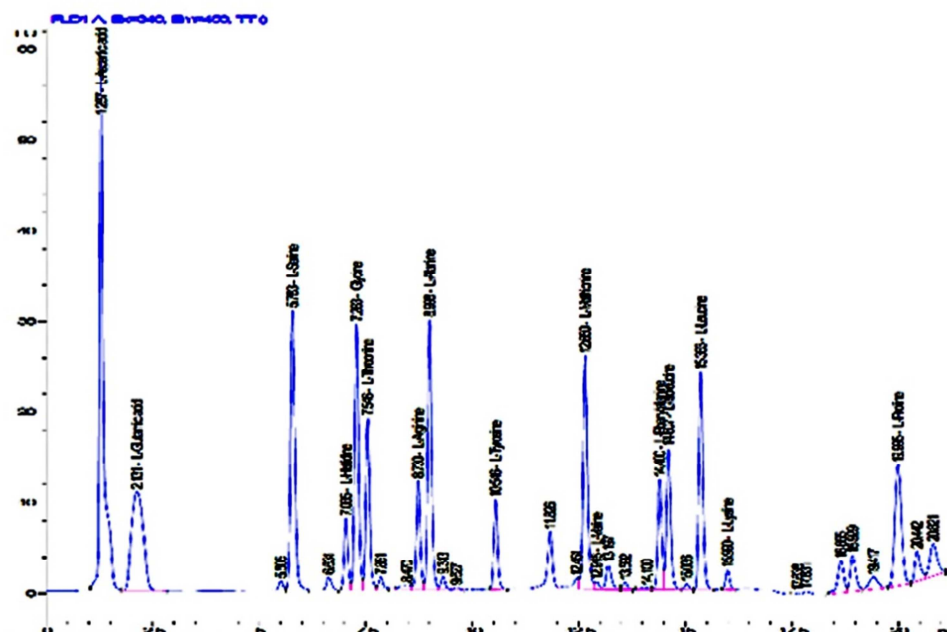


Figure 2. The chromatogram of studying the total amount of amino acids in the plum pectin after hydrolysis.

Table 1. The results of amino acid identification in the plum pectin.

Amino acid	Free amino acids		Total amino acids		Bound amino acids amount, µg/mg
	retention time	amount, µg/mg	retention time	amount, µg/mg	
L-Aspartic	1,309	0,282	1,287	7,021	6,738
L-Glutamic	2,163	0,196	2,131	4,728	4,533
L-Serine	5,81	0,087	5,783	2,636	2,549
L-Histidine	7,043	0,010	7,035	1,976	1,967
Glycine	7,306	0,034	7,283	1,626	1,592
L-Threonine	7,568	0,046	7,546	1,672	1,625
L-Arginine	8,732	0,069	8,73	1,372	1,304
L-Alanine	9,022	0,081	8,998	1,923	1,843
L-Tyrosine	10,574	0,017	10,546	1,249	1,232
L-Valine	12,662	0,041	12,65	2,192	2,151
L-Methionine	12,945	0,006	12,916	0,065	0,059
L-Phenylalanine	14,43	0,032	14,4	1,363	1,331
L-Isoleucine	14,63	0,023	14,607	1,331	1,307
L-Leucine	15,396	0,016	15,363	2,059	2,043
L-Lysine	16,055	0,000	15,99	1,251	1,251
L-Proline	20,023	0,239	19,995	1,232	0,993

The pectin complex obtained from plum fruits has a reddish brown color, probably due to the presence of such pigments as flavonoids and anthocyanins [16]. These substances belong to phenolic compounds. Their content in the pectin complex has been found to be rather significant – 0.43%. These substances possess such types of biological activity as the anti-inflammatory, vasoconstrictor, diuretic activity, and have the antioxidant effect. The current research has shown its multi-spectrum pharmacological benefits for the treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia [17-19]. Among the phenolic compounds of plum, hydroxycinnamic acids were found by the methods of TLC, HPLC, paper chromatography [4, 20]. Their content in the pectin complex obtained was 0.09%, which was 20.93% of the total amount of all phenolic compounds determined by spectrophotometry. Hydroxycinnamic acids, namely chlorogenic acid,

neochlorogenic acid, and ferulic acid, have the laxative, membrane-stabilizing and anti-inflammatory effect.

4. Conclusion

Plum are widely cultivated in the world and have been traditionally used in food for centuries and, accordingly, are not toxic. Many studies have been carried out by scientists around the world to study the chemical composition of plum fruits.

Several substances from plum fruits that have various types of the pharmacological action and are not inferior to the known natural and synthetic substances concerning the laxative and hepatoprotective action have been obtained at our lab. The present research continues to search in this direction. It has been found that the pectin complex obtained contains amino acids, their composition has been studied, the content

of the total amount of phenolic compounds and hydroxycinnamic acids in the pectin complex has been determined. The data obtained show the prospects for further study of the antioxidant activity and pharmacological effects of the pectin complex obtained for creating new pharmacologically active agents based on it.

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