COMPARATIVE STUDY OF THE AMINO ACID COMPOSITION OF COMMON PEACH (PERSICA VULGARIS MILL.) LEAVES GROWN IN KARAKALPAKSTAN

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Abstract

For the first time, the amino acid composition of peach leaves harvested in the Republic of Karakalpakstan was studied. Choosing the content's qualitative and quantitative components of amino acids in peach leaves was carried out by high-performance liquid chromatography. 20 proteinogenic amino acids are present, of which eight are necessary (valine, methionine, phenylalanine, threonine, leucine, isoleucine, and tryptophan), 2 partially replaceable (histidine and arginine) and 10 replaceable amino acids (alanine, asparagine, glutamine, aspartic and glutamic acids, proline, glycine, cysteine, tyrosine, serine). The total amino acid content was 29.05 mg/g (2.9%), of which 6.8861 mg/g (0.69%), including histidine, were irreplaceable and 22.16 mg/g (2.22%), including arginine, were replaceable in terms of completely dry raw materials.

As the content of common peach leaves decreases, amino acids are arranged in the following sequence: Cus>Arg>Gly>Asn>Trp>Phe>Thr> Gln>Asp>Tyr>His>Ser>Val>Ala>Glu>IzoIle>Lys> Leu> Pro>Met. The data obtained make it possible to characterize peach leaves as a raw material source of proteinogenic amino acids.

Keywords: amino acids, medicinal plants, common peach leaves, high-performance liquid chromatography.

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Introduction

The pharmacological market is currently expanding rapidly, and there is a trend toward more in-depth research on the chemical properties of medicinal herbs. The main attention is paid to substances of secondary metabolism (phenolic compounds, alkaloids, glycosides, tannins and other biologically active substances) with pronounced pharmacological properties. But no less important role is played by compounds of primary metabolism, which are always present in plant products and herbal remedies, providing the physiological needs of the body and providing a therapeutic effect [1].

One of these important components of the primary metabolism are amino acids. They, as components of proteins, along with carbohydrates, lipids and nucleic acids, are actively involved in all life processes and perform certain functions in the proper functioning of the body and metabolism [2].

Plants can synthesize all currently known amino acids. From various plant sources, more than 300 free amino acids and their metabolic products have been identified, of which only 20 are part of proteins, while the rest can be found in cells and tissues in a free form.

In addition, in plants, amino acids are the starting material for the biosynthesis of several physiologically active compounds: auxins, enzymes, alkaloids, polyphenols, vitamins, etc. [3].

The presence of amino acids in plants and their high biological activity contributes to the effective action of preparations derived from medicinal raw materials on the body, giving them an easily digestible form, while simultaneously potentiating their pharmacological effect [4].

They are included in preparations for the treatment of various diseases of the gastrointestinal tract, and liver, to reduce excitation of the central and peripheral nervous system, improvement of cardiac circulation, etc. [2].

In addition, scientists were able to determine that in stressful situations, plants are able to accumulate a large number of free amino acids that are not bound into peptides and proteins. It is these amino acids that act as a protective mechanism in the presence of unfavourable factors since they are quickly included in the metabolic process as their own [5].

Recently, among medicinal plants, the attention of researchers has been attracted by agricultural and, in particular, food crops, which have a sufficient raw material base.

One such promising medicinal plant rich in proteins, amino acids, polyphenolic compounds and other biologically active substances is the common peach (Persica vulgaris Mill.), which has not only high nutritional qualities but also a wide range of pharmacological effects. Of particular interest are the leaves of the common peach (Persica Vulgaris folia) as a useful and promising type of medicinal plant material.

Peach leaves have traditionally been used in folk medicine due to some pronounced biological activity. Peach leaf extracts exhibit antioxidant, immunomodulating, antitumor, antidiabetic and antibacterial, choleretic, and capillary-strengthening action as an adaptogen, increase the body's resistance to adverse conditions and also slow down prevent premature ageing processes [6].

There are many studies on the chemical makeup of peach fruits, but few on the chemical composition of the plant's other parts, such the leaves. The high content of proteins in common peach leaves makes it one of the important sources of amino acids and makes it possible to use it to compensate for the deficiency in the diet since it contains both essential and non-essential amino acids [7,8].

In Uzbekistan, in particular, the Republic of Karakalpakstan, the common peach (Persica Vulgaris Mill.) is distributed throughout the region.

It should be noted that the species composition of amino acids in peaches grown in Karakalpakstan has not been previously determined. It is appropriate to note that an acutely unfavourable ecological environment remains in the region, associated with the consequences of the drying up of the Aral Sea and other polluting factors. These factors dictate the need for thorough chemical research on individual classes of substances of each raw material, in order to ensure food safety and eliminate the toxic effects of negative environmental factors on the raw materials of medicinal plants. In a previous

article, we studied the macro- and microelement composition of Karakalpakstani peach leaves [9]. In this report, we describe the findings of a high-performance liquid chromatography investigation on the amino acid composition of peach leaves (HPLC).

The goal of this study was to compare the amino acid composition of peach leaves grown in stressful, ecologically unfavorable conditions in Karakalpakstan.

Materials and methods

For work, we used raw materials harvested in the Kungrad region of the Republic of Karakalpakstan (RK) in 2021. After collection, medicinal raw materials were sorted and damaged and spoiled leaves were separated. Dried in a ventilated room at a temperature of 15-20 ℃.

High-performance liquid chromatography was used to measure the qualitative and quantitative levels of amino acids in peach leaves (HPLC). The method used for chromatographic analysis was gradient elution.

By allowing proteins and peptides from an aqueous extract of the samples to settle in centrifuge cups, free amino acids were isolated. This was accomplished by mixing 1 ml of the test sample with 1 ml of 20% TCA (tetrachloroacetic acid) (precise volume). Centrifugation at 8000 rpm for 15 minutes separated the precipitate after 10 minutes. 0.1 ml of the supernatant was separated, and then freeze-dried. The Kohan approach was used for the precolumn modification of amino acids [10].

Phenylthiocarbamyl derivatives (PTC) of amino acids were obtained by reaction with phenylthioisocyanate according to the method [10]. HPLC was used to carry out additional amino acid derivative identification. HPLC setup: 75x4.6 mm Discovery HS C18 column, Agilent Technologies 1200 optoelectronic device with DAD detector. B: CH_3CN; Solution A: 0.14 M CH_3COONa + 0.05% TEA pH 6.4. 1.2 ml/min flow rate and 269 nm absorption. Gradient %B/min: 1-6%/0–2.5 min; 6-30%/2–40 min; 30–60 min; 60–60 min; 60–0 min.

Results and discussion

In the studied sample of raw materials, 20proteinogenicamino acids, of which 8 are essential amino acids (valine, methionine, phenylalanine, threonine, leucine, isoleucine, lysine, tryptophan), 2 are partially replaceable (histidine and arginine) and 10 are non-essential amino acids (alanine, asparagine, glutamine, aspartic and glutamine acids, proline, glycine, cysteine, tyrosine, serine) (Table 1 and Fig. 1-2).

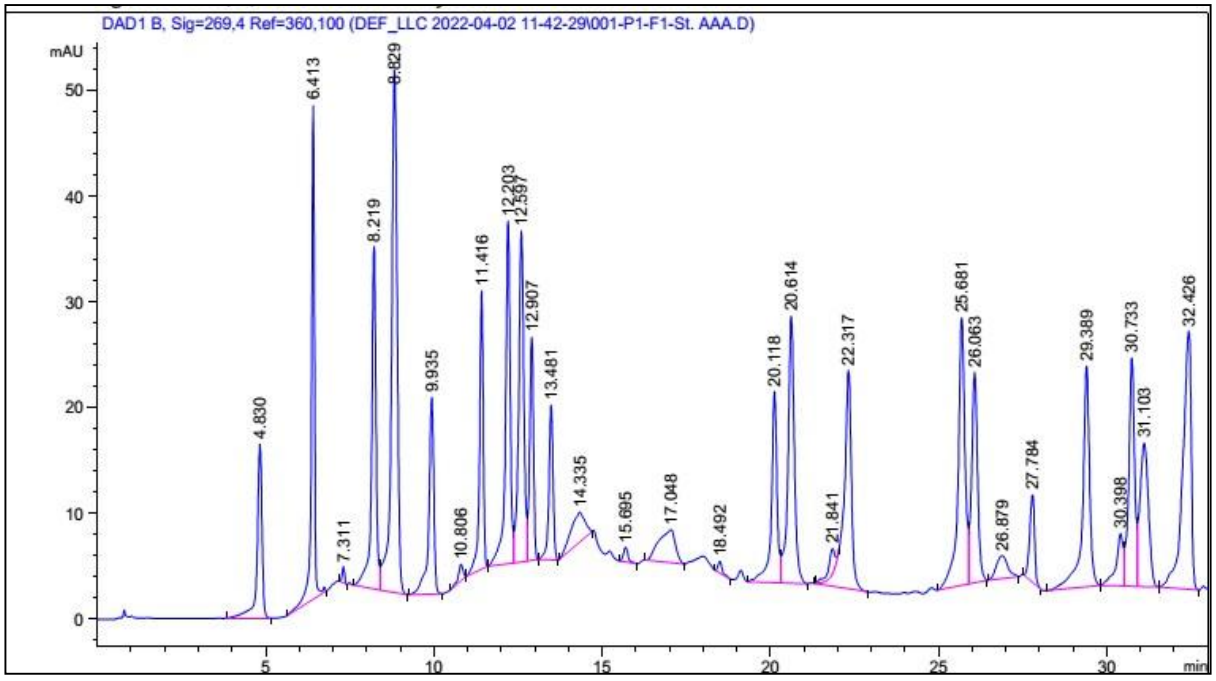


Fig. 1. Chromatogram of a standard sample of amino acids

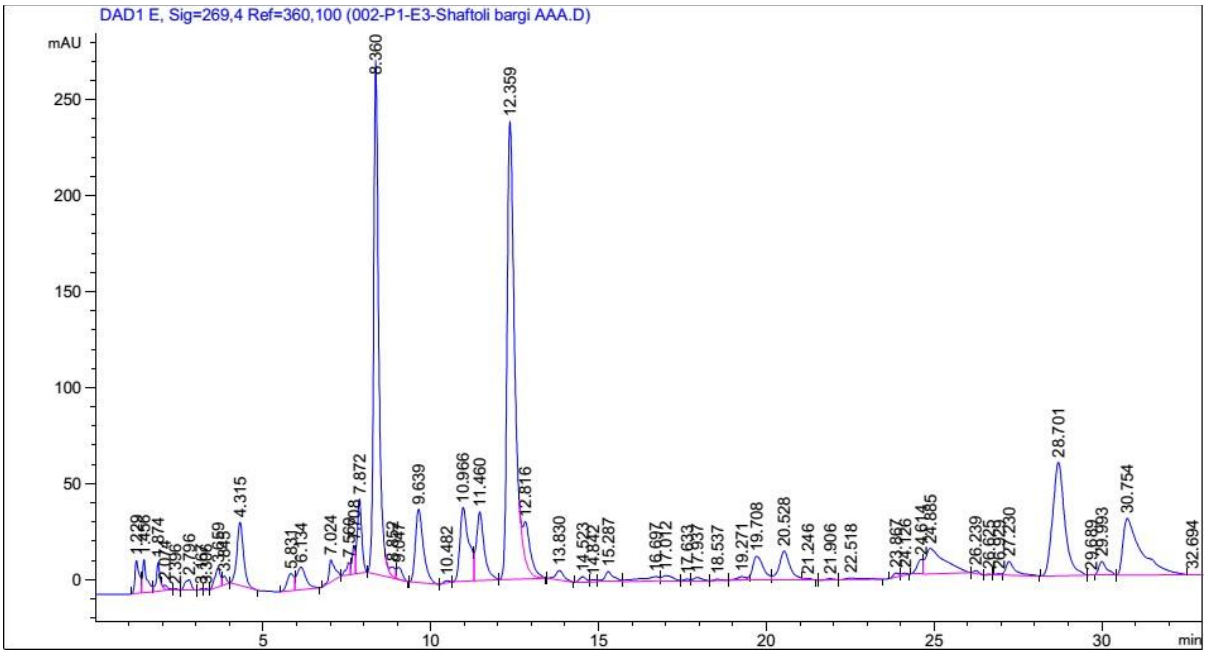
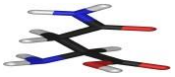
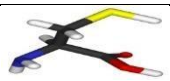
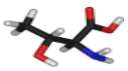
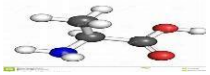
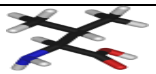
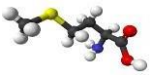
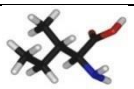
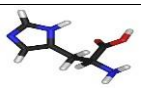


Fig.2. Chromatogram of amino acids from common peach leaves

In terms of completely dry raw materials, the total amino acid content was 29.0459 mg/g (2.9%), of which 6.8861 mg/g (0.689%) included histidine and 22.16 mg/g (76.29%) included arginine were irreplaceable.

Table 1. The content of amino acids in the leaves of common peach (RK)

No.	Name amino acids (AA)	Molar mass, g/mole	AA content			Structural formula
			mg/g	%	Share of AA from the total content, %	
1	Aspartic acid Asp	133.1	0.909494	0.091	3.131	
2	Glutamic acid Glu	147.1	0.230207	0.023	0.7925	
3	Serene Ser	105	0.641163	0.064	2.207	
4	Glycine Gly	75	3.001708	0.300	10.334	
5	Asparagine Asn	132.1	2.97329	0.297	10.236	
6	Glutamine Gln	146.1	1.209437	0.121	4.1639	

7	Cysteine Cys	12	8.06079	0.806	27.752	
8	Threonine Thr	119.1	1.283847	0.128	4.42	
9	argenin Arg	174.2	3.910585	0.391	13.463	
10	Alanine Ala	89	0.254017	0.025	0.8745	
11	Proline Pro	115.1	0.177353	0.018	0.6106	
12	Tyrosine Tyr	181.1	0.791823	0.079	2.726	
13	Valine Val	117.1	0.45842	0.046	1.5783	
14	Methionine Met	149.2	0.06461	0.006	0.2224	
15	Isoleucine Ile	131.1	0.209765	0.021	0.7221	
16	Leucine Leu	131.1	0.181899	0.018	0.6263	
17	Histidine His	155.1	0.735453	0.073	2.532	
18	tryptophan Trp	204.2	2.011445	0.201	6.925	
19	Phenylalanine Phe	165.1	1.747872	0.175	6.0176	
20	Lysine HCl Lys	146.1	0.192783	0.019	0.6637	

The amino acids found in peach leaves, as their content decreases, can be arranged in the following order:

Cus>Arg>Gly>Asn>Trp>Phe>Thr> Gln>Asp>Tyr>His>Ser>Val>Ala>Glu> Ile>Lys> Leu>Pro>Met.

The essential amino acids cysteine, arginine, glycine and asparagine are dominant, while tryptophan, phenylalanine and threonine predominate among the essential ones. Of the monoaminodicarboxylic acids, which play an important role in the functioning and development of the nervous system, the content of aspartic acid is almost 4 times higher than glutamic acid. The lowest content in the general series falls on methionine and proline.

With a total content of proteinogenic amino acids29.04596 mg/g, the content of essential amino acids from the total content of AA is only 6.89 mg/g (23.71%) (Table 2). The sequence of arrangement of irreplaceable AAs as their quantitative decrease is arranged in the following order:

Trp > Phe > Thr > His > Val > Ile > Lys > Leu > Met.

Non-essential amino acids account for the remaining 22.16 mg/g (76.29%) of the total

amino acid content. At the same time, a series of decreasing content among replaceable AA is presented as follows:

Cys> Arg> Gly> Asn> Gln> Asp>Tyr> Ser> Ala> Glu> Pro.

For a qualitative and quantitative assessment of the amino acid composition of common peach leaves growing in ecologically unfavourable conditions of the Republic of Karakalpakstan (RK), we conducted a comparative analysis with the results of the amino acid composition of the extract of common peach leaves according to the Ukrainian author [11] and the leaves of the common peach subspecies Nectarine (Prunus persica var. nucipersica), harvested in two districts of the Fergana region of the Republic of Uzbekistan according to the authors [12].

The analysis of these data revealed significant differences due to different climatic conditions of plant growth. So, for example, in the extract of peach leaves by Ukrainian authors [11], the total amount of amino acids is lower and such important amino acids as methionine and tyrosine are absent (Table 3).

Similarly in the Ferghana samples, the data show some quantitative differences and the absence of the partially nonessential amino acid histidine.

Table 2 presents the quantitative content of essential and non-essential amino acids in peach leaves growing in the Republic of Karakalpakstan and two accessions of peach subspecies common nectarine (Prunus persica var. nucipersica), harvested in Kuva(sample 1) and Altaryk (sample 2) districts of the Fergana region of Uzbekistan.

Table 2. The quantitative content of AA in the leaves of common peach from the Republic of Karakalpakstan and the Fergana region of the Republic of Uzbekistan, mg/g

Interchangeable AKs		Sample RK	Sample 1, Fergana	Sample 2, Fergana
1	Aspartic acid	0.909494	0.736573	0.836573
2	Glutamic acid	0.230207	0.703812	0.603912
3	Serine	0.641163	0.105741	0.103741
4	Glycine	3.001708	0.261532	0.160032
5	Asparagine	2.97329	0.262671	0.362622
6	Glutamine	1.209437	1.650929	1.850139
7	Cysteine	8.06079	0.884615	0.984712
8	Tyrosine	0.791823	0.822542	1.022532
9	Arginine	3.910585	0.603679	0.513679
10	Alanine	0.254017	3.361894	2.361874
11	Proline	0.177353	4.400817	3.900817
Sum		22.1599	13.7948	12.7007
Essential AKs				
12	Threonine	1.283847	2.867409	3.067309
13	Valine	0.45842	1.191397	1.192396
14	Methionine	0.06461	1.364142	1.368182
15	Isoleucine	0.209765	1.108212	1.908912
16	Leucine	0.181899	0.742721	1.542791
17	Histidine	0.735453	0	0
18	Tryptophan	2.011445	0.775985	0.978984

19	Phenylalanine	1.747872	0.713934	1.913994
20	Lysine Hcl	0.192783	0.06688	0.18680
Sum		6.8861	8.8307	12.1593
General		29.046	22.62549	24.86

As can be seen from Table 2, the amino acids of the Karakalpak sample of common peach leaves are superior to the Fergana samples in terms of essential and total amino acid content, but inferior in the content of essential amino acids.

In table 3, we also presented a series of amino acid sequences in descending order of their quantitative content.

Table 3. A series of amino acids in descending order of quantitative content in the leaves of common peaches of various samples

Several amino acids in common peach leaves growing in Karakalpakstan	
Common AK range	Cus>Arg>Gly>Asn>Trp>Phe>Thr>Gln>Asp>Tyr>His>Ser>Val>Ala>Glu>Ile> Lys>Leu>Pro>Met
NAC	Trp> Phe>Thr>His>Val>Ile>Lys>Leu>Met
ZAK	Cys>Arg>Gly>Asn>Gln>Asp>Tyr>Ser>Ala>Glu>Pro
Several amino acids in the extract of peach leaves of Ukraine	
	Pro> Pro>Asn>Ala>Arg>Phe>Asp>Glu>Val>Ile>Ser>Leu>Thr>Gly>Leu>Gln>His
NAC	Phe>Val>Ile>Trp>Leu>Thr>Lys>His
ZAK	Pro>Asn>Ala>Arg>Asp>Glu>Ser>Gly>Gln> Cys
Several amino acids from peach leaves Kuvinsky district of the Fergana region, sample 1	
Common AK range	Pro>Ala>Thr>Gln>Met>Val>Ile>Cys>Tyr> Trp>Leu>Asp>Phe>Glu>Arg>Asn> Gly>Ser>Lys
NAC	Thr>Met>Val>Ile>Trp>Leu>Phe>Lys
ZAK	Pro>Ala>Gln>Cys>Tyr>Asp>Glu>Arg>Asn>Gly> Ser
Several amino acids from peach leavesAltaryk district of Fergana region, sample 2	
Common AK range	Pro> Thr>Ala> Phe> Ile>Glu> Leu>Met>Val>Tyr>Cys>Trp>Asp> Glu>Arg>Asn>Lys>Gly>Ser
NAC	Thr>Phe>Ile>Leu>Met>Val>Trp>Lys
ZAK	Pro>Ala>Glu>Tyr>Cys> Asp>Glu>Arg>Asn> Gly>Ser

Note: AA - amino acids; NAA - essential amino acids, NAA - non-essential amino acids.

Analyzing the data on amino acids of peach leaves from Karakalpakstan, it can be seen that the content of tryptophan (2.011445 mg/g), phenylalanine (1.748 mg/g) and threonine (1.284 mg/g) significantly predominates over other essential amino acids. Phenylalanine is a coded, essential amino acid for humans and animals, especially necessary for the functioning of the thyroid gland and adrenal glands in a child. The norm is required for children at 90 mg/kg of body weight per day [13,14].

With a lack of tryptophan, severe muscle atrophy develops, and growth slows down.

According to [8], tryptophan is normally required per day for a child's body 22 mg/kg of body weight.

Comparison with the data of Ferghana samples shows that the content of tryptophan in samples 1 and 2 is almost 3 and 2 times less, respectively, and phenylalanine in sample 1 is 2.4 times less, and in sample 2 it is higher compared to Karakalpakstan.

It is appropriate to note here that in the body phenylalanine is used only in the synthesis of proteins. The entire unused supply of amino acids is converted into tyrosine [13].

In the case of threonine, significant differences are also observed. The content of threonine in Ferghana samples is 2-3 times higher than its content in Karakalpakstan peach leaves.

Threonine performs several important biological functions, participates in the construction of muscle protein and maintains the desired protein balance in the body, improves the condition of the cardiovascular system, liver and immune system, and also serves as an additional source of energy. From threonine in the body, other amino acids are synthesized - glycine and serine, which are necessary for building muscle tissue, collagen and elastin. Synthesis of immune proteins and many enzymes of the digestive system is impossible without threonine [15].

The least content is methionine (0.06461 mg/g), which is crucial for biosynthetic methylation. In the human body, mention is also required to ensure that the liver detoxifies, that the neurological system is operating, and that it participates actively in the metabolism of fats and phospholipids.

As noted earlier, there is no mention in the peach leaf extract of the Ukrainian sample, and the content of methionine in the Fergana samples is significantly higher (1.364-1.368 mg/g) compared to Karakalpakstan.

Behind methionine, the lowest content falls on leucine, a monoaminomonocarboxylic acid involved in metabolic processes, especially influencing the formation of new proteins more than any other amino acid. Quantitatively, isoleucine and lysine are close to the content of leucine. If we make a comparison here with the Ferghana samples, then the content of leucine is 4 and 8 times lower in the Karakalpak sample, similarly, for isoleucine, approximately 5-9 times excess remains in the Fergana samples. Lysine in sample 1 is approximately three times lower, but in sample 2 it is close to its content in the Karakalpak sample. Lysine is an important amino acid that is required for tissue growth and repair, the manufacture of antibodies, hormones, enzymes, and albumins, and the maintenance of the body's nitrogen balance.

Partially essential amino acids include arginine and histidine. Histidine is found in significant amounts in haemoglobin and is also part of carnosine and anserine. This is why a shortage of histidine causes haemoglobin levels to drop. One of the body's histidine reserves is hemoglobin, and when histidine levels are low, hemoglobin is more easily destroyed, releasing histidine into the bloodstream [15].

In turn, the absence of histidine slows down the synthesis of haemoglobin and leads to the development of anaemia due to the fact that the protein part of haemoglobin requires a sufficiently large amount of histidine since heme is bound to the globin component through histidine residues in the polypeptide chains of the haemoglobin molecule [13,14].

Histidine is present in our case at 0.7355 mg/g, and it was not found in the Fergana samples.

Arginine is a diaminomonocarboxylic acid, the lack of which disrupts insulin synthesis and lipid metabolism in the liver. The content of arginine (3.91 mg/g) in peach leaves from Karakalpakstan significantly prevails over other samples. As can be seen from Table 2, in the Ferghana samples, the quantitative content of arginine is 0.6 mg/g and 0.5 mg/g in samples 1 and 2, respectively, which is 6-8 times lower.

The content of replaceable AA to the total content of proteinogenic amino acids is 76.29%. The series in descending order of the content of replaceable AA is presented as follows:

Cys> Arg> Gly> Asn> Gln> Asp>Tyr> Ser> Ala> Glu> Pro.

The lowest content (0.177353 mg/g) falls on proline, a heterocyclic amino acid that is responsible for the synthesis of collagen in the human body and promotes cartilage healing and joint elasticity. In the case of Fergana samples (4.4 mg/g and 3.9 mg/g) and even in the extract of common peach leaves from

Ukraine, the content of proline quantitatively significantly predominates over other amino acids.

According to the literature data, an increase in the content of proline and its derivatives in plant organisms is observed under water, salt, and temperature stresses. It is believed that proline increases the resistance of plants to moisture deficiency, contributing to an increase in the osmotic pressure of the cell [5]. From this point of view, it was logically correct to assume the maximum content of proline in the Karakalpak sample, as being under strong negative stress, but in fact, we have the opposite picture. Most likely, the influence of other factors of growing conditions that were not taken into account prevailed here.

Somewhat superior to proline is glutamic acid (0.230207 mg/g). Glutamic acid (aspartic acid and N-methyl-D-aspartate) is a neurotransmitter excitatory amino acid. Glutamic acid (α-aminoglutaric) is one of the most important amino acids in plant and animal proteins. Being a direct participant in many metabolic processes, glutamic acid affects amino acid, protein, carbohydrate, lipid metabolism, and the distribution of potassium and sodium in the body.

As noted above, the content of glutamic acid (0.23 mg/g) is almost 4 times lower than that of aspartic acid (0.91 mg/g). In the Fergana samples, both of these amino acids are quantitatively close to each other and vary in the range of 0.6-0.8 mg/g.

Tyrosine- serves as a biosynthetic precursor of catecholamines, in particular, thyroid hormones - thyroxine and triiodothyronine, and is an iodinated component of the specific thyroid protein thyroglobulin. Tyrosine supplements assist manage anxiety and depression that cannot be treated with medicine, according to clinical studies [1]. The content of tyrosine (0.79 mg/g) in peach leaves from Karakalpakstan is close to its content in sample 1 (0.82 mg/g) and inferior to sample 2 (1.02 mg/g) of the Fergana leaves of common peach (peach subspecies Nectarine). In contrast to tyrosine, serine (0.64 mg/g) significantly exceeds its content of 0.1 mg/g in both samples 1 and 2 of the Fergana nectarine.

As can be seen from Table 2, the gap in the alanine content of the compared samples is very large. Alanine is one of the predominant amino acids in nectarine leaves, yielding quantitatively only to proline in sample 1 (3.36 mg/g), and in the case of sample 2 (2.36 mg/g) and threonine, while in Karakalpak leaves the content alanine corresponds to only 0.254 mg/g.

As for the sequenceCys> Gly> Asp> Gln>, it should be noted that the amino acids cysteine (8.07 mg/g), glycine (3.0 mg/g) and asparagine (2.97 mg/g) significantly prevailed in peach leaves from Karakalpakstan (Table 2), only in the case of glutamine (1.21 mg/g) is there a lower value compared to samples 1 (1.65 mg/g) and 2 (1.85 mg/g).

Cysteine, an amino acid that contains sulfur, needs special consideration. Both cysteine and a dipeptide-cystine complex, which consists of two cysteine molecules covalently linked to one another by a disulfide bridge, can be found in the protein's composition. Because of this characteristic, cysteine plays a crucial role in maintaining the protein molecule's structural stability. The amino acid cysteine is essential for the production of insulin and immunoglobulins (antibodies). It is important for detoxification processes. Cysteine is an extremely important amino acid due to the fact that it is the only source of organic sulfur for body cells. As a result of metabolic reactions, this sulfur passes into the composition of other sulfur-containing substances - phosphoadenosine phosphosulfuric acid (PAPSA), coenzyme A, and glutathione [15].

Glycine is found more frequently in proteins, especially in comparison to other amino acids. In the biosynthesis of porphyrin and purine bases, it functions as a precursor. It is known that glycine, along with gamma-aminobutyric acid (GABA), belongs to inhibitory neurotransmitters. Today, indications for the use of glycine are considered stressful conditions, psychoemotional tension, increased excitability, neurosis, vascular-vegetative dystonia, the consequences of traumatic brain injury, encephalopathy, sleep disorders.

As a raw material for the creation of aspartic acid, asparagine plays a significant role in the body. The most prevalent free amino acid in the human body, glutamine, is broken down in practically all tissues. Glutamine serves as an interorgan nitrogen transporter in the body.

This substance regulates the balance of alkalis and acids, promotes the production of new cells, and prevents early ageing. With a lack of glutamine, tissues are destroyed, and the body begins to use muscle tissue as a source of glutamine [16].

Conclusion

So a study of the amino acid composition of peach leaves growing in stressful, environmentally unfavourable conditions of Karakalpakstan showed that the medicinal raw material contains all 20 proteinogenic amino acids.

The essential amino acids cysteine, arginine, glycine and asparagine are dominant, while tryptophan, phenylalanine and threonine predominate among the essential ones.

With a total content of proteinogenic amino acids of 29.04596 mg/g, the content of essential amino acids from the total content of AA is only 6.89 mg/g (23.71%), and the share of non-essential amino acids from the total content of amino acids accounts for the remaining 22.16 mg/g (76.29%).

Comparative analysis of the quantitative content of amino acids of the test sample with the literature data obtained for the extract of peach leaves and its subspecies Nectarine, growing on the territory of Ukraine and the Fergana region of the Republic of Uzbekistan, respectively, shows differences in both the content and composition of the amino acids of the samples depending on the place of growth.

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