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STUDY OF FREE AMINO ACIDS AND ELEMENTAL COMPOSITION OF COMMON BARLEY GRAINS AND ITS DRY EXTRACT

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The article is devoted studying of free amino acids and elemental composition of common barley grains cultivated in Uzbekistan and its dry extract.

When conducting a comparative analysis of the amino acid composition of barley grains and its dry extract, their identity in qualitative composition was established – 20 amino acids, of which 8 are essential. The presence of all essential amino acids in the studied objects indicates their usefulness and high value as medicinal plant raw materials. It was found that the dry extract by the content of the sum of amino acids (7.71 mg/g) is almost 3 times higher than the grains (2.89 mg/g). But the percentage of the sum of essential amino acids from the total number of amino acids in the dry extract (20.36%) is 1.7 times less than in grains (35.29%).

As a result of the conducted research in common barley grains and dry extract based on them, the presence of 41 mineral elements was found. Toxic heavy metals (As, Pb, Hg, Cd), which are subject to priority control, in the raw materials and dry extract under study are determined within the acceptable values adopted by the World Health Organization and in the State Pharmacopoeia of the Russian Federation XIII edition for medicinal plant raw materials and preparations based on it, which indicates their environmental safety.

The obtained data will be in demand for the chemical characterization of raw materials and dry extract and their subsequent standardization.

Keywords: common barley, dry extract, amino acids, high performance liquid chromatography, macro- and microelements, inductively coupled plasma mass spectrometry, toxic heavy metals.

Introduction

The importance of amino acids for the body is primarily determined by the fact that they are used for the synthesis of proteins, the metabolism of which hold a special place in the metabolic processes between the body and the environment.

Amino acids are directly involved in the biosynthesis of not only proteins, but also other biologically active compounds that regulate the metabolic processes in the body, such as neurotransmitters and hormones -derivatives of amino acids. They contain almost 95% of all nitrogen, therefore, they maintain the nitrogen balance of the body, serve as nitrogen donors in the synthesis of all nitrogen-containing non-protein compounds, including nucleotides, heme, creatine, choline and other substances.

The exclusion of even one essential amino acid from the diet leads to incomplete assimilation of other amino acids and is accompanied by the development of a negative nitrogen balance, depletion, growth arrest and dysfunction of the nervous system [1–3].

In plant raw materials, amino acids not only provide their characteristic biological activity, but also give other biologically active substances an easily digestible form, in addition, they enhance their pharmacological effect [4, 5].

It is known that the medicinal value of many plants is determined by the content of not only biologically active substances, but also macro- and

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microelements, the accumulation of which is due to environmental factors and the nature of plants. According to academician V.I. Vernadsky, all the chemical elements of living and inanimate nature are interconnected and without a sufficient amount of them, the main physiological reactions in the body cannot proceed. This role of macro- and microelements is explained by the fact that they are part of vitamins, respiratory elements, enzymes and coenzymes involved in the regulation of vital processes [6–8].

The main functions of macronutrients are tissue building, maintaining a constant acid-base and osmotic balance. Microelements affect the activity of enzymes and the direction of their action [9]. In addition, they are able to increase the body's resistance to various environmental influences, which helps to fight various diseases.

Individual chemical elements and their complexes with organic compounds can, on the one hand, have a useful pharmacological effect, on the other hand, their increased content is often the cause of toxic effects on the cells of living tissues and the body as a whole. Considering the above, the studying of remedy plants as objects of environmental monitoring, there is recognized as an urgent direction for improving the quality of phytopreparations.

Grain crops have been the main component of human nutrition and have played an important role in the formation of human civilization for thousands of years. They are an excellent source of carbohydrates, an important source of protein, a good source of B vitamins, including folic acid, a good source of many minerals such as iron, magnesium, copper, phosphorus, zinc and others [10].

Common barley (*Hordeum vulgare L.*) is one of the oldest cultivated plants. It is an annual herb from the family of cereals (Poaceae) [11]. Barley is not directly eaten by humans, but mainly turns into cereals (pearl barley and barley groats). The advantage of barley groats is that, unlike pearl barley, it is not subjected to polishing [12] and so the outer part of the endosperm, aleuron, which contains proteins with essential amino acids and vitamins, fiber, omega-3 fatty acids and food minerals, as well as others bioactive compounds are preserved [10, 13].

For medicinal purposes of common barley has long been used in folk medicine. Malt extract is used for bronchitis and for feeding young children. It is drunk in case of metabolic disorders, expressed in the appearance of skin rashes, furuncles [14].

Despite the noted circumstances and the richest raw material base, the grain of common barley due to the lack of sufficient scientific justification did not found for their use. In this regard, a comprehensive study of common barley grains with the aim of introducing them into medical practice is highly relevant.

This research is aimed at studying of free amino acids and elemental composition of common barley grains and its dry extract, based on them for the chemical characterization of raw materials required for subsequent standardization.

Experimental

The object of the study was the mature grains of common barley cultivated in the Tashkent region. The analysis was carried out on average samples of raw materials selected in accordance with the instructions that meet the requirements of the General Pharmacopoeia Monograph "Sampling of medicinal plant materials and herbal medicinal preparations" [15]. Common barley grains were identified at the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan by Dr. Duschanova G.M. [16].

Dry extract from barley grains was obtained by hot fractional maceration. Purified water was used as the solvent.The solvent was completely removed at a gentle temperature mode. The finished product is a dry friable powder of light gray color, spicy taste, readily soluble in water-alcohol mixtures, soluble in water when it is heated.

HPLC analysis of PTC-derivatives of amino acids. From the averaged crushed homogeneous sample of barley grains and dry extract for analysis, an accurate sample was weighed into test tubes with an error of no more than 0.1%. Next, free amino acids were isolated.

Precipitation of proteins and peptides from the aqueous extract (1 : 10) of the samples was carried out in centrifuge beakers. To do this, 1 ml (exact volume) of 20% trichloroacetic acid was added to the test samples (1 : 1). After 10 minutes the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. After separating 0.1 ml of the supernatant fluid was lyophilically dried.

Phenylthiocarbamyl derivatives (PTC) of amino acids were obtained by reaction with phenylthioisocyanate according to the method of Steven A., Cohen Daviel [17].

Identification of PTC-amino acids is carried out on an Agilent Technologies 1200 chromatograph using a Discovery HS C^{18} column, 75×4.6mm with a Diode-array detector. Solution A: a mixture of 0.14 M sodium acetate and 0.05% triethylamine, pH 6.4. Solution B: acetonitrile. The flow rate is 1.2 ml/min; absorption is 269 nm. Gradient %B/min: 1–6%/0–2.5 min; 6–30%/2.51–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min.

Qualitative analysis and quantitative calculation of the concentration of the studied free amino acids was carried out by comparing the retention time and peak areas of the standard and studied PTC-derivatives of amino acids.

The determination of the elemental composition was carried out by the inductively coupled plasma method on an NEXION-2000 ICP-MS mass spectrometer (Perkin Elmer, Inc., USA) – a method for studying a substance based on determining the mass-to-charge ratio of ions formed during the ionization of sample components of interest, one of the most powerful methods of qualitative identification of substances that can also be quantified. In the ICP-MS method, argon plasma also serves as a source of inflame ions, but each chemical element of the periodic table has a unique set of stable isotopes, which allows one to accurately identify the presence of this element in the sample by mass spectrometry [18].

A sample of raw materials was prepared according to the method specified in PND F 16.1:2.3:3.11-98, "Quantitative chemical analysis of soils. Methods for measuring the content of metals in solid objects by the method of spectrometry with inductively coupled plasma" [19]. A weighed portion of the analyzed sample weighing 0.5 g was placed in teflon autoclaves and there were added 10 ml of concentrated nitric acid. Then the autoclaves were closed and placed in a Berghoff microwave decomposition device with MWS-3 software, observing all safety precautions. Determined the decomposition program, based on the type of test substance, indicated the degree of decomposition and the number of autoclaves (up to 12 pieces).

Following heating mode was applied for the sample: raising the temperature to 210 °C for 25 min, holding for 10 min at a temperature of 210 °C, and cooling to a temperature of 45 °C. The cooled autoclave was gently shaken to stir the contents and the lid was slightly opened to balance the pressure (a qualitatively decomposed sample after distilling off nitrogen oxides should be a colorless or yellowish transparent solution, without undissolved particles on the bottom and on the walls of the fluoroplastic insert). Then the solution cooled to room temperature was quantitatively transferred into a volumetric flask with a capacity of 50 or 100 ml, depending on the expected content of the element in the sample, the walls of the insert were washed in small portions with 0.5% nitric acid, brought to the mark and thoroughly mixed. A "blank sample" was prepared in parallel with a batch of analyzed samples, performing all the above operations. To measure the mass concentrations of elements in solutions, the analyzed sample solutions were fed into the spray chamber of the mass spectrometer using a peristaltic pump, and in an argon flow (gas, purity 99.995%), the resulting aerosol entered the burner, in which the atoms were ionized. After receiving the data, the true quantitative content of the substance in the test sample was automatically calculated by the device in mg/kg or µg/g with error limits – RSD in %.

Results and discussion

Chromatograms of a mixture of amino acid standards, barley grains and dry extract are shown in figures (1–3) of the electronic application.When conducting a comparative analysis of the amino acid composition of barley grain and its dry extract, it was found that they are identical in terms of their qualitative composition – 20 amino acids, of which 8 are essential (Table 1). The presence of all essential amino acids in the studied objects indicates their usefulness and high value as medicinal plant raw materials. The predominant amino acid in grains is methionine (0.56 mg/g) and arginine (0.47 mg/g). Their percentage of the total amounted to 19.38% and 16.26%, respectively. And the composition of the dry extract contains large amounts of amino acids glutamic acid (1.23 mg/g) and arginine (1.27 mg/g). Together they made up almost 32.7% of the total amount of amino acids. It was found that the dry extract by the content of the sum of amino acids (7.71 mg/g) is almost 3 times higher than the grains (2.89 mg/g). But the percentage of the sum of essential amino acids from the total number of amino acids in the dry extract (20.36%) is 1.7 times less than in grains (35.29%).

The composition of the detected elements and their quantitative content are shown in table 2. As can be seen from the table, there were found 41 elements were found in the studied raw material and its dry extract. The composition of the mineral elements of the dry extract is completely identical to the initial raw material, which indicates the correctness and rationality of the technology of its production.

Table 1. Amino acid composition of barley grains and its dry extract

N	Amino acids	Grains (mg/g)	% from the total quantity	Dry extract (mg/g)	% from the total quantity
1	Aspartic acid	0.09	3.11	0.51	6.61
2	Glutamic acid	0.12	4.15	1.23	15.95
3	Serine	0.04	1.38	0.19	2.46
4	Glycine	0.04	1.38	0.47	6.10
5	Asparagine	0.04	1.38	0.51	6.61
6	Glutamine	0.26	9.00	0.41	5.32
7	Cysteine	0.18	6.23	0.67	8.69
8	Threonine*	0.07	2.42	0.19	2.46
9	Arginine	0.47	16.26	1.27	16.47
10	Alanine	0.20	6.92	0.40	5.19
11	Proline	0.09	3.11	0.19	2.46
12	Tyrosine	0.21	7.27	0.18	2.33
13	Valine*	0.05	1.73	0.28	3.63
14	Methionine*	0.56	19.38	0.12	1.56
15	Isoleucine*	0.05	1.73	0.09	1.17
16	Leucine*	0.05	1.73	0.09	1.17
17	Histidine	0.13	4.50	0.11	1.43
18	Tryptophan*	0.07	2.42	0.15	1.95
19	Phenylalanine*	0.14	4.84	0.58	7.52
20	Lysine HCl*	0.03	1.04	0.07	0.91
The sum of essential amino acids		1.02	35.29	1.57	20.36
Total		2.89	100.00	7.71	100.00

*Essential amino acids

Table 2. Elemental composition of common barley and its dry extract

N	Element	Raw materials (mg/kg)	Dry extract (mg/l)	N	Element	Raw materials (mg/kg)	Raw materials (mg/kg)
1	Li	0.154	0.679	22	Ge	0.002	0.007
2	Be	0.004	0.012	23	As	0.018	0.065
3	B	5.611	18.478	24	Se	0.258	0.226
4	Na	145.030	786.439	25	Rb	1.599	1.925
5	Mg	1464.587	2615.622	26	Sr	0.802	6.726
6	Al	85.570	246.653	27	Zr	0.141	0.116
7	Si	560.159	2159.590	28	Nb	0.003	0.006
8	P	4365.949	5093.183	29	Mo	0.848	0.821
9	S	702.983	1234.237	30	Ag	0.006	0.012
10	K	4992.366	13712.903	31	Cd	0.010	0.011
11	Ca	1754.766	4639.406	32	Sn	0.257	0.432
12	Ti	1.522	4.952	33	Sb	0.003	0.006
13	V	0.091	0.342	34	Ba	2.191	2.250
14	Cr	1.868	3.495	35	W	0.014	0.013
15	Mn	19.924	114.189	36	Re	0.000	0.002
16	Fe	73.804	231.171	37	Hg	0.007	0.002
17	Co	0.034	0.117	38	Tl	0.001	0.002
18	Ni	0.444	1.339	39	Pb	0.613	0.894
19	Cu	3.189	3.201	40	Bi	0.001	0.002
20	Zn	19.600	20.353	41	U	0.015	0.133
21	Ga	0.150	0.177				

The data showed the content of elements K, P, Ca, Mg in the composition of grains in relatively large quantities (more than 1000 mg/kg), S, Si, Na in concentrations from 100 mg/kg to 1000 mg/kg and AI, Fe, Mn, Zn in concentrations from 10 mg/kg to 100 mg/kg, B, Cu, Ba, Cr, Rb, Ti in concentrations from 1 mg/kg to 10 mg/kg, the remaining elements in very small concentrations.

In the dry extract of barley grains, the elements are contained in concentrations in the following order: from 10000 mg/kg to 15000 mg/kg, the content of 1 element (K) was noted, from 1000 to 10000 mg/kg – 5 elements (P, Ca, Mg, Si, S), from 100 to 1000 mg/kg – 4 elements (Na, Al, Fe, Mn,), from 10 to 100 mg/kg – 2 elements (Zn, B), from 10 to 1 mg/kg – 7 elements (Sr, Ti, Cr, Cu, Ba, Rb, Ni) and below 1 mg/kg – 22 elements (Pb, Mo, Li, Sn, V, Se, U, Co, Ga, Zr, As, W, Ag, Be, Cd, Ge, Nb, Sb, Re, Hg, TI, Bi).

As the quantitative content, mg/kg, decreases, the mineral elements found in the studied raw material (1) and dry extract (2) can be arranged in the following sequence:

1) K>P>Ca>Mg>S>Si>Na>AI>Fe>Mn>Zn>B>Cu>Ba>Cr>Rb>Ti>Mo>Sr>Pb>Ni>Se>Sn>Li>Ga>Zr>V>Co>As>U>W>Cd>Hg>Ag>Be>Sb=Nb>Ge>Re=Tl=Bi

2) K>P>Ca>Mg>Si>S>Na>AI>Fe>Mn>Zn>B>Sr>Ti>Cr>Cu>Ba>Rb>Ni>Pb>Mo>Li>Sn>V>Se>U>Co=Ga>Zr>As>W>Ag=Be>Cd>Ge>Nb>Sb>Re=Hg=Tl=Bi

There were found in the studied raw material and dry extract potassium, calcium, magnesium, phosphorus, zinc, copper, manganese, etc., there have a positive effect on the vital activity of the body, to a certain extent, contribute to an increase in the pharmacological value of this remedy plant material due to its combination with its main biologically active substances.

The concentration of heavy metals in the studied raw materials and dry extract practically corresponds to the concentrations of uncontaminated areas.

Toxic heavy metals (As, Pb, Hg, Cd), which are subject to priority control, in the raw materials and dry extract under study are determined within the acceptable values adopted by the World Health Organization and in the State Pharmacopoeia of the Russian Federation XIII edition for medicinal plant raw materials and preparations based on it, which indicates their environmental safety [20, 21].

Conclusions

There have been studied for the first time, the elemental composition of common barley grains cultivated in Uzbekistan and a dry extract based on them.

When conducting a comparative analysis of the amino acid composition of barley grains and its dry extract, their identity in qualitative composition was established – 20 amino acids, of which 8 are essential. The presence of all essential amino acids in the studied objects indicates their usefulness and high value as medicinal plant raw materials. It was found that the dry extract by the content of the sum of amino acids (7.71 mg/g) is almost 3 times higher than the grains (2.89 mg/g). But the percentage of the sum of essential amino acids from the total number of amino acids in the dry extract (20.36%) is 1.7 times less than in grains (35.29%).

As a result, the presence of 41 mineral elements was found. It has been shown that common barley grains and their dry extract are sources of vital elements, and they can be considered as a means of correcting the imbalance of these elements in the body.

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