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Research of amino acid content of *Cichorium intybus* L. and *Urtica dioica* L. by means of gas-liquid chromatography

Content of amino acids in *Cichorium intybus* L. and *Urtica dioica* L. has been presented. Researches were carried out by means of gas-liquid chromotography. 20 amino acids were revealed in *C. intybus* L. and *U. dioica* L., (mg/100 g), glutamate: 32 < 2568; aspartate: 916 < 1820; alanine: 23 < 1235; proline: 38 < 962; glycine: 21 < 425; leucine: 29 < 42; isoleucine: 24 < 626 and arginine: 36 < 58. Leaves of *U. dioica* L. and stems of *C. intybus*L. contain more glycine than stems of *U. dioica* L. In *C. intybus* L. flowers contain more amino acids than leaves and stems. In *U. dioica* L. leaves contain more amino acids than flowers and stems. In leaves of *U. dioica* L. valine volume was 1.5 times higher than in *C. Intybus* L. Amino acids' content in stems of *C. intybus* L. and *U. dioica* L. were almost identical. Leucine content in leaves and stems was in 1.5–2 times higher in *C. intybus* L. than in *U. dioica* L. The lowest cysteine volume was in leaves and stems of *C. intybus* L. Methionine volume was higher in *U. dioica* L.

Keywords: Cichorium intybus L., *Urtica dioica* L, gas-liquid chromatograph, amino acids, flowers, stems, leaves, BAS.

Introduction

Biologically active substances (BAS) are able to restore pathologically changed organism functions of animals and humans, at the same time they are an admissible source of medicine revealed in various ways. Amino acids are known to be the most important components of biologically active substances. Kazakhstan is rich with both cultivated and important and useful medicinal plants. Area of using the medicines obtained from such plants extends from year to year due to their effectiveness. Identification of plant composition increases quality of selection of raw material for its use in pharmacology. Amino acids hold a specific place in the physiological processes taking place in a human body. At unbalanced nutrition or in case of disease to support the normal level of amino acids the medicines obtained from such medicinal plants are applied. *Cichorium intybus* L. and *Urtica dioica* L. belonging to the *Asteraceae* family are unique among such plants. Researches proved *Cichorium intybus* L. and *Urtica dioica* L. to be the sources of biologically active substances [1].

Totally the genus Urtica includes 50 species, among them one can find only three species in Kazakhstan; and the genus Cichorium includes more than 1000 species, in Kazakhstan one can find only one of them. The most widespread species from these two genera are *Urtica dioica* L. and *Cichorium intybus* L. They are spread across all Kazakhstan and do not choose the soil [2].

Nowadays one of the most used in agriculture, medicine, pharmaceutics, dietology and food industry and effective sources of raw materials are *Cichorium intybus* L. and *Urtica dioica* L. Their various pharmacological, biological and physiological properties are being comprehensively investigated. Nevertheless, studying the amount of biologically active substances and properties in plants with undetermined complete compound, namely in *Cichorium intybus* L. and *Urtica dioica* L., remains very relevant [3, 4] for today.

As it is noticed above amino acids are an important component of biologically active substances [1–3].

Since the molecules of protein compounds which are so important for existence consist of residues of amino acids, their value is great. In the nature there are more than 150 types of amino acids. About 20 of them are monomers of block groups performing very important functions in the structure of protein molecules.

Amino acids are known to take part in the processes of metabolism of an organism. As people and all animals cannot produce amino acids therefore they may get them from the food in ready form. Nowadays the method of biotechnological synthesis is applied (chemistry and microbiology) for producing the amino acids. After that amino acids are added to food of people and animals. Also amino acids are the industrial polyamides which are the products which are always used in production of paints and drugs [2–4].

Under the influence of arginine, one of the major amino acids in a protein molecule functioning in the thyroid gland, which is responsible for exchange of calcium in an organism, improves. Arginine slows down growth of tumors including malignant. It is also applied for increase of functions of purification and excretion of waste of nitric exchange in kidneys [3, 5].

Amino acids, necessary for human, enter our bodies together with food. Valine, leucine, isoleucine, methionine, threonine, phenylalanine, lysine, arginine, histidine and tryptophane are the most necessary among them. Availability of these amino acids as a part of our food increases nutritional value of any product. It is known that about twenty amino acids take part in protein biosynthesis [4–7].

Amino acids hold a specific place in a human body. For example, glutamine was one of the first the synthesized amino acids. The Japanese scientist Kinosita in nature found a special bacterium *Micrococcus glutamicus* and extracted from it this acid. This bacterium can be used for obtaining glutamic acid from nearly 50 % of glucose added to the medium. Nowadays about one hundred thousands of tons of this acid per a year are manufactured in Japan and the USA at special plants. Glyutamic acid in food industry is added to products, improving their quality. In medicine glutamic acid is applied in treatment of diseases of a nervous system.

One of the important amino acids in a molecule of protein is the lysine. 2.1 kg of synthesized lysine being added will cause animals' weight increase for 13.6 % in average, and costs for protein will decrease by 20–25 %. This amino acid is considered to be the most valuable and necessary additive in nutrition ration [5].

Applying lysine in bread baking increases its nutrition value. The lysine in a large amount is also produced by *Enterobacter aerogenes*, *Proteus spp.*, *Bacillus subtilis* and *Torula utilis*.

Under the ultraviolet influence *Micrococcus glutamicus* can synthesize lysine. Nowadays lysine production by using this bacterium is promoted to the industrial level. As the result about ten thousand tons of lysine per a year is produced in Japan, the USA and in other countries [3]. There are two leading companies in the world market of L-lysine production such as the Japanese Ajinomoto Co and the American Archer Daniels & Midlands (ADM), which control 33 % of the world production. Other large players in the market are Degussa-Huels (Germany), BASF (Germany), Kyowa Hokko (Japan) and Cheil Jedang Corporation (South Korea) [8].

In our research it was revealed that such plants as *Cichorium intybus* L. and *Urtica dioica* L., in turn, are the sources of amino acids. In this connection the aim of our research is to determine the volume of amino acids in *Cichorium intybus* L. and *Urtica dioica* L. The amount of amino acids in the samples of *Cichorium intybus* L. and *Urtica dioica* L. was determined by means of gas-liquid chromotography using the device Carlo-Erba-4200 (Italy-USA).

Experimental

Samples of *Cichorium intybus* L. and *Urtica dioica* L. collected in September-October 2016–2017 in the Medeo mountains of Almaty area were the objects of our research.

1 g of raw material was hydrolyzed at 105 ºC within 24 hours in 6N HCl. The obtained hydrolyzate was evaporated in a drying rotor by 3 times, before drying at a temperature of 40–50 ºC and atmospheric pressure in 1 atmosphere. The residue formed was dissolved in 5 ml of sulfosalicylic acid. In 5 minutes the centrifuged liquid of ionomixed pitch was passed through a pipe by means of a mesh of Dauks 50, N-8, 200–400 with velocity of 1 drop per a second. After that pitch was rinsed in 1–2 ml of water and 2 ml of 0.5N acetic acid; then pitch was rinsed with water till obtaining the neutral medium. For an elution of amino acids a sample was passed through a pipe of 3 ml of 6N NH_4OH with velocity of 2 drops per a second. Eluate was gathered together with water into a flask with a round bottom. After that the substance from a flask was evaporated in a rotor-evaporator under the pressure of 1 atmosphere at 45–50 ºC, till drying. Into the same flask there was added 1 drop of freshly prepared solution of 1.5 % $SnCl_2$, 2,2-dimetoxypropane, HCl and 1–2 ml of dense propanol; then it was heated at 110 ºC temperature. This temperature was maintained during 20 minutes, and then the eluate was again evaporated in a rotor-evaporator.

At the following stage 1 ml of freshly prepared acetylated reagent was added to a flask, heated during 1.5–2 minutes at temperature of 60 ºC. This reagent was evaporated till drying, then a sample was again evaporated in a rotor evaporator; 2 ml of ethyl acetate and 1 ml of saturated NaCl solution were poured into the flask. The flask was mixed slowly, at this moment the 2 layers were formed; for gas chromatographic analysis the top layer was taken. Experiment was carried out by the method of gas chromatographic analysis using «Carlo-Erba-4200» device (Italy-USA) [9–11]. The data obtained during the research are presented in Table 1.

Table 1

The volume of amino acids in the samples of *Cichorium intybus* L. and *Urtica dioica* L., mg/100 g

Amino acids	*Cichorium intybus* L. leaves	*Cichorium intybus* L. stem	*Cichorium intybus* L. flower	*Urtica dioica* L. leaves	*Urtica dioica* L. stem	*Urtica dioica* L. root
Threonine* (Thr)	198	182	220	750	384	19
Serine (Ser)	248	234	266	206	68	30
Glycine (Gly)	265	225	300	425	228	21
Alanine (Ala)	714	610	915	1235	754	23
Valine* (Val)	224	202	235	558	325	24
Methionine(Met)	83	73	98	82	30	-
Isoleucine* (Ile)	368	352	372	626	406	24
Leucine* (Leu)	342	315	364	317	214	29
Aspartate (Asp)	1208	1118	1278	1820	916	–
Glutamat (Glu)	2456	2340	2568	348	194	32
Cystine (Cys)	36	26	42	4	2	–
Lysine* (Lys)	224	196	260	342	156	25
Arginine(Arg)	328	305	355	585	478	36
Ornithine(Orn)	2	1	3	376	190	14
Tyrosine (Tyr)	284	270	303	4	2	–
Phenylalanine* (Phe)	255	248	271	2996	2486	–
Tryptophane (Trp)	65	55	88	156	78	–
Proline (Pro)	452	435	476	962	722	38
Histidine (His)	209	192	228	492	322	–
Oxyproline (Oxn)	2	1	3	524	318	32

Note. * — non mixed amino acids.

Results and Discussion

The composition of *Cichorium intybus* L. and *Urtica dioica* L. was revealed to be rich with amino acids. 20 types of amino acids were found in *Cichorium intybus* L. and *Urtica dioica* L. Among them (mg/100 g) glutamate: 32 < 2568, aspartate 916 < 1820, alanine: 23 < 1235, proline: 38 < 962, glycine: 21 < 425, leucine: 29 < 425, isoleucine: 24 < 626 and arginine: 36 < 58. According to results of our research, in comparison with *Cichorium intybus* L. leaves of *Urtica dioica* L. contain more glycine; and in turn stems of *Cichorium intybus* L. contain more glycine than stems of *Urtica dioica* L.

In *Cichorium intybus* L. flowers contain more amino acids than leaves and stems. In *Urtica dioica* L. leaves contain much more amino acids than flowers and stems, twice. In leaves of *Urtica dioica* L. valine amino acid volume was 1.5 times higher than in *Cichorium intybus* L. Content of amino acids in stalks of plants of *Cichorium intybus* L. and *Urtica dioica* L. were almost identical. In leaves and stem of *Cichorium intybus* L. leucine content in 1.5–2 times more than in *Urtica dioica* L. The volume of cysteine was least of all in leaves and a stalk of Cichorium intybus L. Methionine volume in *Urtica dioica* L. was higher than in *Cichorium intybus* L.

Results of our research have something in common with literary sources about structure of the herbs, which underwent an experiment, namely they are rich source of such amino acids as glutamate, aspartate, alanine, proline, arginine, leucine, serine, isoleucine, tyrosine, lysine and glycine [12, 13]. The plants studied by us are widely used in traditional medicine at treatment of many diseases; also they have homeopathic properties. They have property to keep amino acids in the normal state. Also they can be applied not only in the medical purposes, but also as technical raw materials [14].

Amino acids are considered to be a unit of structural proteins. Also proteins participate in such phenomena as digestion, irritation, division, reproduction, the movement; and they are a source of life of live organisms. Protein acts as antibodies protecting a human body from microbes and pathogenic agents [13, 15]. In recent years great attention is paid to production of amino acids necessary for protein synthesis. Before they were made by hydrolysis from valuable raw materials with a large amount of protein, and it demanded big expenses. For the last ten years the new microbiological method of obtaining amino acids has been developed [14].

Conclusions

For the first time the volume of amino acids in *Cichorium intybus* L. and *Urtica dioica* L. growing in the Medeo Mountains in Kazakhstan was revealed. In total 20 amino acids were revealed, among them the most amount was found in flowers of *Cichorium intybus* L. and in leaves of *Urtica dioica* L.

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Cichorium intybus L. және *Urtica dioica* L. өсімдіктерінің құрамындағы аминқышқылдарының мөлшерін газсұйықтық хроматография әдісімен зерттеу

Мақалада *Cichorium intybus* L. және *Urtica dioica* L. өсімдіктерінің құрамындағы аминқышқылдарының мөлшерін анықтау нәтижелері көрсетілген. Зерттеу жұмысы газсұйықтық хроматография әдісімен «Carlo-Erba-4200» (Италия–США) газсұйықтық хроматографында жүргізілді. Зерттеу нәтижесі бойынша *Cichorium intybus* L. және *Urtica dioica* L. өсімдіктерінде 20 аминқышқылдары табылды (мг/100 г), олардың мөлшері: глютамат: 32 < 2568; аспаратат: 916 < 1820; аланин: 23 < 1235; пролин: 38 < 962; глицин: 21 < 425; лейцин: 29 < 42; изолейцин: 24 < 626 және аргинин: 36 < 58 аралығында.

U. dioica L. жапырағында және *C. intybus* L. сабағында глициннің мөлшері көп, *U. dioica* L. сабағына қарағанда *C. intybus* L. гүлінде аминқышқылдарының мөлшері жоғары, жапырағы мен сабағына қарағанда. *U. dioica* L., жапырағында сабағы мен тамырына қарағанда, аминқышқылдарының мөлшері көп. *U. dioica* L. жапырағында валиннің мөлшері 1,5 есе жоғары, *C. intybus* L. қарағанда, *C. intybus* L. және *U. dioica* L. өсімдіктерінің сабағында аминқышқылдарының мөлшері шамалас мәнге ие. *C. intybus*. жапырағы мен сабағында, *U. dioica* L. өсімдігіне қарағанда, лейциннің мөлшері 1,5–2 есе жоғары. Ал цистеиннің мөлшері *C. intybus* L. жапырағы мен сабағында ең аз мөлшерді көрсетті. Сондай-ақ метионинның мөлшері, *C. intybus* L. қарағанда, *U. dioica* L. жоғары мөлшерде табылды.

Кілт сөздер: Cichorium intybus L., *Urtica dioica* L, газсұйықтық хроматография, аминқышқылдары, гүлі, сабағы, жапырағы, ББЗ.

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Исследование объема аминокислот в составе *Cichorium intybus* L. и *Urtica dioica* L. с помощью газожидкостной хроматографии

В статье представлены результаты определения объема аминокислот у *Cichorium intybus* L. и *Urtica dioica* L. Исследования проводились методом газожидкостной хроматографии на приборе «Carlo-Erba-4200» (Италия–США). 20 аминокислот были обнаружены у *C. Intybus* L. и *U. Dioica* L. (мг/100 г), глютамат: 32 < 2568; аспартат: 916 < 1820; аланин: 23 < 1235; пролин: 38 < 962; глицин: 21 < 425; лейцин: 29 < 42; изолейцин: 24 < 626 и аргинин: 36 < 58. Листья *U. dioica* L. и стебли *C. intybus* L. содержат больше глицина, чем стебли *U. dioica* L. У *C. intybus* L. цветки содержат больше аминокислот, чем листья и стебли. В листьях *U. dioica* L. содержится больше аминокислот, чем в цветах и стеблях. В листьях *U. dioica* L. объем валина был в 1,5 раза выше, чем в *C. intybus* L. Содержание аминокислот в стеблях *C. intybus* L. и *U. dioica* L. было практически одинаковым. Листья и стебли *C. intybus*. L. содержат в 1,5–2 раза больше лейцина, чем в *U. dioica* L. Объем цистеина был меньше всего в листьях и стеблях *C. intybus* L. Объем метионина в *U. dioica* L. был выше чем, у *C. intybus* L.

Ключевые слова: Cichorium intybus L., Urtica dioica L., газожидкостная хроматография, аминокислоты, цветы, стебли, листья, БАВ.

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