

## Amino acid composition of homeopathic matrix tinctures of various varieties of Onions onion (*Allium cepa* L.) and Common garlic (*Allium sativum* L.)

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The amino acid composition of garlic (*Allium sativum* L.) and different varieties of onions (*Allium cepa* L.) in homeopathic mother tinctures

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### RESUME

Varietal diversity of *Allium cepa* and *Allium sativum* is not taken into account in the manufacture of medicines (including homeopathy) from the bulbs of onions and garlic. Different varieties of onions with a different chemical composition grow in different countries, but foreign Homeopathic Pharmacopoeias have no reference to the use of specific varieties *Allium cepa* L. and *Allium sativum* L. The present study examines the amino acid composition of homeopathic mother tinctures of different varieties of *Allium cepa* and *Allium sativum* in a comparative perspective.

Comparative HPLC analysis of the amino acid composition of garlic and different varieties of onion homeopathic mother tinctures revealed the presence of a wide spectrum of amino acids; 19 of them were identified. It was shown that all of the analyzed tinctures have similar qualitative amino acids composition (after hydrolysis); quantitative amino acid content varies depending on varieties of onions.

The total content of amino acids after hydrolysis in homeopathic mother tinctures (raw onion materials) ranged from 0.2% (varieties of onion: Holland white, Myachkovski) to 0.5% (Red Baron); in the garlic tincture - 0.6%. The total content of essential amino acids after hydrolysis in onion homeopathic mother tinctures ranged from 0.03% (Holland white, Myachkovski) to 0.08% (Red Baron); in garlic tincture - 0.1%. Regardless of onion varieties, the predominant amino acids in tinctures are: glutamic acid (36–42% of all amino acids), arginine (17–27%) and aspartic acid (7.12%). These amino acids are dominant in the tincture of garlic too. Their content is respectively about 32%, about 31% and about 8%.

keywords: amino acids, HPLC, onion, garlic, *Allium cepa* L., *Allium sativum* L., homeopathic mother tinctures.

### SUMMARY

In the production of medicinal preparations from bulbs of onion and garlic, the varietal diversity of these species is not taken into account. In different countries, different varieties of onions are grown, which have different chemical compositions, but in foreign homeopathic pharmacopoeias there is no indication of the possibility of using raw materials of specific varieties of *Allium cepa* L. and *Allium sativum* L. This study is devoted to the study of the amino acid composition of homeopathic matrix tinctures (HMT) of various *Allium cepa* and *Allium sativum*.

Comparative analysis of the amino acid composition of GMN from raw materials of various varieties of onion and garlic by HPLC made it possible to establish the presence of a wide range of amino acids in them, 19 of which were identified. It was shown that the qualitative composition of amino acids after hydrolysis in all analyzed tinctures is similar, but their quantitative content varies depending on the onion variety. Total content

amino acids after hydrolysis into GMN from raw materials of various varieties of onions range from 0.2% (varieties Dutch white, Myachkovsky) to 0.5% (variety Red Baron); the same indicator for garlic tincture is 0.6%. The total content of essential amino acids after hydrolysis in HMN of various onion varieties ranges from 0.03% (Dutch white, Myachkovsky) to 0.08% (Red Baron variety); in garlic tincture - 0.1%.

It has been established that, regardless of the onion variety, the dominant amino acids in tinctures are: glutamic acid (36–42% of the sum of all amino acids), arginine (17–27%) and aspartic acid (7–12%). The same amino acids are dominant in garlic GMN; their content is respectively about 32%, about 31% and about 8%.

Key words: amino acids, HPLC, onion, garlic seed, *Allium cepa* L., *Allium sativum* L., homeopathic matrix tinctures.

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) belong to the Alliaceae family and are the most popular food plants used in herbal medicine and homeopathy worldwide [2]. There are numerous data on the chemical composition [3] and the experience of using these two representatives of the genus *Allium* in traditional and folk medicine [2]. Onion bulbs contain essential oil, a wide range of flavonoid compounds, amino acids, macro- and microelements, vitamins A, B<sub>one</sub>, V<sub>2</sub>, V<sub>5</sub>, V<sub>6</sub>, V<sub>9</sub>, C, D, E, H, PP, U and other biologically active substances (BAS). Bulbs of garlic also contain essential oil, which is a mixture of sulfides, disulfides and trisulfides of various structures, vitamins, proteins and other biologically active substances [3].

In domestic medical practice, the use of onion bulbs and preparations from it - Allylchep, Allylglycer, garlic bulbs and preparations based on it - garlic tincture and a thick extract, which is part of the Allohol preparation, is allowed [1]. When harvesting medicinal plant raw materials and producing preparations from it, the varietal diversity of *Allium cepa* and *Allium sativum* is not taken into account, including in homeopathy. Despite the fact that different varieties of onions with different chemical compositions are grown in different countries, foreign homeopathic pharmacopeias [6–9] also do not indicate the possibility of using raw materials of specific varieties of onions and garlic.

The purpose of this study is to study the amino acid composition of homeopathic matrix tinctures of various varieties of *Allium cepa* and *Allium sativum* in a comparative aspect.

#### Materials and methods

The objects of the study were homeopathic matrix tinctures from onions garlic and onion (varieties Stuttgarten Risen, Central Asian, Dutch White, Red Baron, Myachkovsky, Strigunovsky, widely cultivated in Russia).

Preparation of tinctures. Homeopathic matrix tinctures of onion was prepared from fresh bulbs using 86% (by mass) (90% by volume) ethanol. The onions were chopped and left in a closed jar at 20°C for 18 hours. Next, the raw material was poured with 86% (by mass) ethanol, vigorously shaken and left for 10 days at a temperature of 20°C, periodically shaken. After 10 days, the tincture was filtered. All tinctures were stored in a place protected from light, at a temperature not exceeding 20°C. Matrix tincture

garlic was made by maceration with 86% (by weight) ethanol. Fresh onions were thoroughly crushed and left in a closed vessel for 18 hours. Then 1.4 parts of 86% (by mass) ethanol were poured into the raw material and macerated for 10 days, shaking from time to time. Filtered and stored in a place protected from light. The method of preparation of tinctures is explained by the peculiarities of raw materials: fresh

vegetable raw materials of onion contain essential oil and more than 70% of squeezed juice; raw garlic also contains a large amount of essential oil, less than 70% juice and has a moisture content of more than 60%.

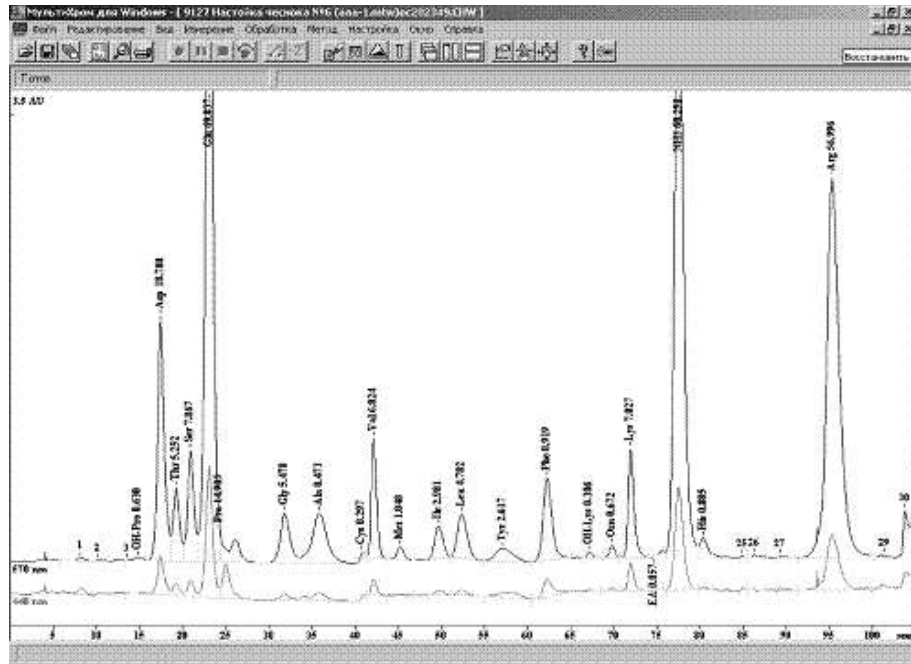
The study of the amino acid composition was carried out on the basis of the Research Institute of Physicochemical biology them. A.N. Belozersky Moscow State University. M.V. Lomonosov by HPLC method on a Hitachi amino acid analyzer (Japan), model 835 with a Hitachi Custom Ion-Exchange column 1.5 m x 2.6 mm filled with Resin-2619 cationic resin. The instrument was calibrated using a standard mixture of amino acids containing 3 nmol of each amino acid. Amino acids were eluted using a stepwise gradient of four buffer solutions: 0.2 M Na<sup>+</sup>- citrate buffer pH 3.3 containing 13% ethanol; 0.2 n. Na<sup>+</sup>- citrate buffer pH 3.3 containing 2% ethanol; 0.2 N, Na<sup>+</sup>-citrate buffer pH 4.3; 1.2 n. Na<sup>+</sup>-citrate buffer pH 4.9. Amino acids were detected at 570 nm, with the exception of proline, which is detected at 440 nm. The detection system is a solution of ninhydrin (pH 5.5) in the presence of a reducing agent (tin chloride or titanium). The flow rate of the buffer solution is 0.225 ml/min., the ninhydrin reagent is 0.3 ml/min. The column temperature is 53-C, maintained constant throughout the analysis.

Sample preparation technique. The study of the total number of amino acids carried out after acid hydrolysis. Sample preparation was carried out according to the standard procedure [10]: 100 µl of the matrix tincture was evaporated to dryness in a Labconco vacuum concentrator; for hydrolysis, 50 µl of the supernatant was taken and hydrolyzed under vacuum with a mixture of concentrated hydrochloric acid and trifluoroacetic acid (2:1) with the addition of 0.001% β-mercaptoethanol, in an amount of 300 µl; the ampoules were sealed and hydrolysis was carried out at 155-C for 1 hour. After hydrolysis, the tubes were cooled and opened. The contents were quantitatively transferred into plastic tubes and evaporated to dryness in a Labconco vacuum concentrator. The dry residue was dissolved in 300 µl of 0.1N hydrochloric acid. To 30 µl of the resulting solution was added 240 µl of water. For analysis in an amino acid analyzer, 145 µl of the resulting solution was taken.

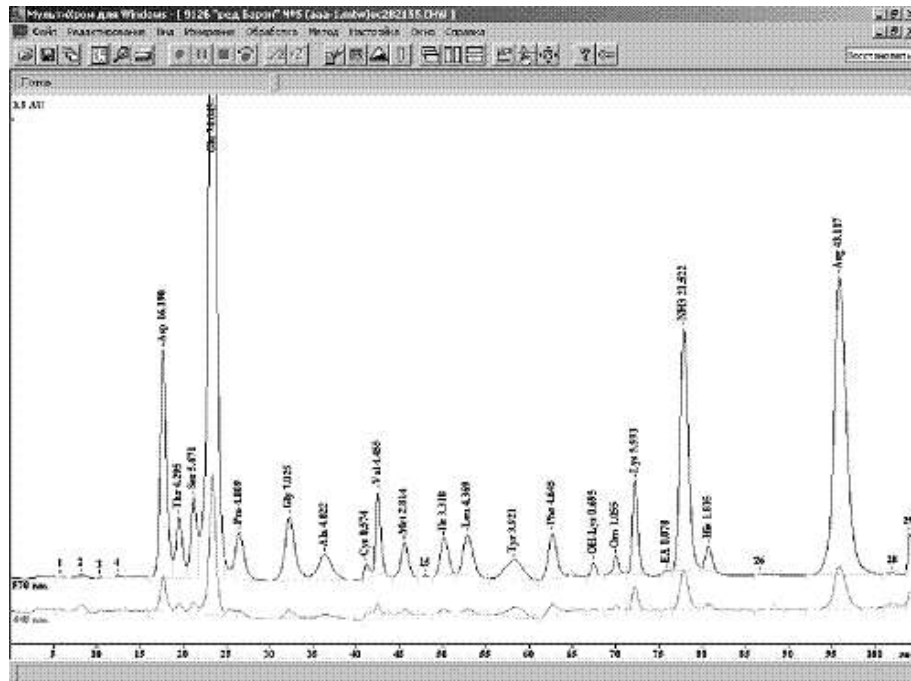
The qualitative composition of amino acids was determined by retention times. V A standard calibration mixture of amino acids was used as an internal standard.

Quantitative calculation of the results of amino acid analysis was carried out automatically, resulting in data on the content of each amino acid in the hydrolyzate. The amount of each identified amino acid was determined in nmol in an aliquot taken for analysis, then the quantitative content of amino acids in milligrams per 1 ml of the matrix tincture was calculated; the percentage of each amino acid and the amount of amino acids, as well as the relative

the percentage of each amino acid from the sum of amino acids. Figures 1 and 2 show typical chromatograms of the amino acid composition of matrix tinctures of onion and garlic.



Rice. 1. Chromatogram of a matrix homeopathic tincture of garlic: Amino acid Hitachi Analyzer Model 835, Hitachi Custom Jon-Exchange 1.5 m x 2.6 mm column packed with Resin-2619 cationic resin. Detection of amino acids at 570 nm (proline at 440 nm). The detection system was a solution of ninhydrin (pH 5.5), column temperature 53-C.



Rice. 2. Typical chromatogram of an onion bulb matrix homeopathic tincture Bulb (Red Baron): Hitachi Model 835 Amino Acid Analyzer, Hitachi Custom Jon-Exchange 1.5 m x 2.6 mm column packed with Resin-2619 cationic resin. Detection of amino acids at 570 nm (proline at 440 nm). Detecting system - solution ninhydrin (pH 5.5), column temperature 53-C

The results of the quantitative determination of amino acids (in%) in tinctures matrix onions and garlic are summarized by us in table 1.

Table 1

The content of amino acids after hydrolysis in various samples of matrix tinctures  
homeopathic onions and garlic, %

Amino acid	one	2	3	4	5	6
asp	0.026	0.018	0.025	0.030	0.040	0.046
Thr	0.004	0.003	0.005	0.007	0.009	0.012
Ser	0.005	0.003	0.005	0.004	0.011	0.015
Glu	0.084	0.089	0.130	0.129	0.203	0.188
Pro	0.002	0	0.006	0.006	0.010	0.032
gly	0.006	0.007	0.008	0.011	0.010	0.008
Ala	0.008	0.007	0.009	0.008	0.007	0.014
Cys	0.001	0.001	0.002	0.002	0.003	0.001
Val	0.004	0.003	0.006	0.004	0.010	0.015
Met	0.004	0.003	0.003	0.004	0.008	0.003
lie	0.002	0.003	0.005	0.004	0.008	0.007
Leu	0.005	0.005	0.010	0.007	0.011	0.012
Tyr	0.004	0.006	0.009	0.011	0.013	0.009
Phe	0.008	0.008	0.009	0.012	0.014	0.027
OH Lys	0.002	0.002	0.005	0.003	0.002	0.001
Orn	0.002	0.001	0.002	0.002	0.003	0.002
Lys	0.006	0.013	0.015	0.012	0.015	0.019
His	0.002	0.003	0.003	0.003	0.005	0.002
Arg	0.044	0.038	0.086	0.097	0.139	0.184
Total content AK	0.218	0.213	0.343	0.357	0.521	0.597
Total content irreplaceable AKs	0.033	0.031	0.053	0.050	0.075	0.095

Note to table 1.

Amino acid name: Asp - aspartic acid, Thr - threonine, Ser - series, Glu - glutamic acid, Ala - alanine, Cys - cysteine, Val - valine, Met - methionine, Not - isoleucine, Leu - leucine, Tyr - tyrosine, Phe - phenylalanine, OH-Lys - OH-lysine, Orn - ornithine, Lys - lysine, His - histidine, Arg - ar-ginine.

Matrix tinctures: 1 - Dutch white onion; 2 - onions of the Myachkovsky variety; 3 - onion of the Stuttgarten Risen variety; 4 - onion of the Central Asian variety; 5 - red baron onion: b - garlic sowing.

#### The discussion of the results

As can be seen from the data in Table 1, the qualitative amino acid composition of tinctures from bulbs of all the studied varieties of onions and garlic is similar and is represented by 19 identified amino acids. The quantitative content of amino acids in onion tinctures varies considerably depending on the variety of the producing plant. The highest total amount of amino acids was found in the onion tincture of the Red Baron variety (up to 0.52%). In the tincture of the white variety of onion - Dutch white, the amount of amino acids is almost 2 times less than in the tincture of the Red Baron variety and is only 0.22%. The total content of amino acids in the Myachkovsky, Central Asian and Stuttgarten Risen varieties is almost the same. Tincture of garlic contains the maximum amount of amino acids compared to onion tinctures - up to 0.60%.

The maximum amount of essential amino acids (lysine, threonine, valine,

isoleucine, leucine, phenylalanine, methionine) was found in garlic tincture - up to 0.10% and Red Baron onion tincture - 0.08%.

The relative content of each of the amino acids identified after hydrolysis (in % of the sum of amino acids) in onion and garlic tinctures is given in Table. 2.

From the data in Table. Table 2 shows that the dominant amino acids in all the studied onion tinctures are: glutamic acid (36–42% of the sum of identified amino acids), arginine (17–27% of the sum of all amino acids) and aspartic acid (7–12% of the sum of amino acids in depending on the type of onion). Garlic tincture is dominated by: arginine (about 31% of the total amino acids), glutamic acid (about 32%), aspartic acid (about 8%), proline (about 5%), phenylalanine (about 4.5%).

The biological role of the identified amino acids is well studied. Some of them are drugs approved for medical use in the Russian Federation (glutamic acid, glycine, methionine, histidine) [1, 4]. Standard solutions of amino acids for parenteral nutrition of patients (Aminosol, Aminoplasmal, etc.) are widely used in order to maintain body homeostasis, eliminate negative nitrogen balance, and disorders of protein and water-electrolyte metabolism [5].

table 2

The content of individual amino acids after hydrolysis in tinctures of matrix homeopathic garlic and various varieties of onions (% of the sum of amino acids)

Name amino acids	Amino acid content in % of the sum of amino acids after hydrolysis					
	one	2	3	4	5	6
asp	11.87	8.45	7.29	8.43	7.70	7.73
Thr	1.82	1.41	1.47	1.97	1.73	1.94
Ser	2.28	1.41	1.47	1.12	2.11	2.57
Glu	38.35	41.78	37.90	36.24	38.96	31.53
Pro	0.91	-	1.75	1.69	1.92	5.32
gly	2.74	3.29	2.33	3.09	1.92	1.28
Ala	3.65	3.29	2.62	2.25	1.34	2.34
Cys	0.45	0.45	0.58	0.56	0.56	0.22
Val	1.84	1.41	1.76	1.12	1.92	2.48
Met	1.84	1.41	0.87	1.12	1.54	0.48
lie	0.92	1.41	1.46	1.12	1.54	1.21
Leu	2.28	2.35	2.91	1.97	2.11	1.95
Tyr	1.83	2.81	2.62	3.09	2.50	1.47
Phe	3.65	3.76	2.62	3.37	2.69	4.57
OH Lys	0.91	0.94	1.46	0.84	0.38	0.15
Orn	0.91	0.47	0.58	0.56	0.56	0.34
Lys	2.74	6.10	4.37	3.37	2.88	3.19
His	0.92	1.41	0.87	0.84	0.96	0.39
Arg	20.09	17.84	25.07	27.25	26.68	30.82
Total	100.00	100.00	100.00	100.00	100.00	100.00

Note to table. 2

Matrix tinctures: 1 - Dutch white onion; 2 - onions of the Myachkovsky variety; 3 - onion of the Stuttgarten Risen variety; 4 - onion of the Central Asian variety; 5 - onion of the Red Baron variety; b - garlic sowing.

Glutamic acid is involved in protein and carbon metabolism, stimulates

energy processes, promotes the synthesis of norepinephrine and ATP [4]. Glutamic acid is classified as a neurotransmitter amino acid that stimulates the transmission of excitation in the CNS synapses [4].

In medical practice, glutamic acid is most often used in the treatment of CNS diseases - epilepsy, psychosis, depression, etc. In pediatrics, it is used for mental retardation, cerebral palsy, and Down's disease [4].

Aspartic acid plays an important role in protein metabolism by stimulating protein synthesis. It has a pronounced antihypoxic effect. Increases oxygen consumption by the affected areas of the myocardium, improves collateral circulation of the heart. A specific property of aspartic acid is its ability to carry K ions+and Mg<sup>2+</sup>into the intracellular space. Like glutamic acid, aspartic acid is a neurotransmitter substance [4].

Arginine is synthesized in the human body in insufficient quantities and, by some experts, is classified as a conditionally essential amino acid, tk. with a decrease in its amount, the processes of protein synthesis are significantly inhibited [5]. Arginine exhibits a protective effect in hyperoxia, hypothermia, liver damage, promotes the conversion of ammonia into urea, and has an immunostimulating effect. Arginine also stimulates the secretion of insulin and growth hormone [5].

Aspartic acid, glycine, arginine have a hypolipidemic effect and normalize lipoprotein metabolism. The positive effect of methionine, leucine, glutamic and aspartic acids on the cardiovascular system is known [4, 5].

The high content of these amino acids in the matrix tinctures of *Allium cepa* and *Allium sativum* can presumably determine the corresponding types of action of onion and garlic, as well as preparations from them, including allopathic ones.

#### CONCLUSIONS

1. Comparative analysis of the amino acid composition of homeopathic matrix tinctures from raw materials of various varieties of onions and garlic by HPLC allowed us to establish the presence in them of a wide range of amino acids, 19 of which were identified. The qualitative composition of amino acids after hydrolysis in all analyzed tinctures is similar, and their quantitative content varies depending on the onion variety.

2. The total content of amino acids after hydrolysis in homeopathic tinctures matrix from raw materials of various varieties of onions ranges from 0.2% (varieties Dutch white, Myachkovsky) to 0.5% (variety Red Baron); the same indicator for garlic tincture is 0.6%.

3. Total content of essential amino acids after hydrolysis in tinctures homeopathic matrix different varieties of onions range from 0.03% (Dutch white, Myachkovsky) to 0.08% (Red Baron variety); in garlic tincture - 0.1%.

4. It has been shown that, regardless of the onion variety, the dominant amino acids in tinctures are: glutamic acid (36–42% of the sum of all amino acids), arginine (17–27%) and aspartic acid (7–12%). These same amino acids are dominant in garlic tincture; their content is respectively about 32%, about 31% and about 8%.

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