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Determination Of Amino Acid Concentrations In The "Asshifo" Dietary Supplement Treated With Laser Radiation By The Lc-Ms Method

Ibragim Askarov Rakhmonovich
DSc, Professor, Department of Chemistry, Andijan State University, Andijan, 170100, Uzbekistan

Mirjalol Muminjonov Muqimjon ogli
DSc, Professor, Department of Chemistry, Andijan State University, Andijan, 170100, Uzbekistan

Latipov Khikmatillo Abdulkhamidovich
PhD Student, Department of Chemistry, Andijan State University, Andijan, 170100, Uzbekistan

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Abstract: This article presents the results of determining the amino acid concentrations in the "Asshifo" dietary supplement, developed based on oat (Avena sativa), savory (Satureja hortensis), and meristem tissue of a coniferous plant, treated with laser radiation at different time intervals, using the LC-MS method. The effect of laser radiation on the amino acid content of the dietary supplement was evaluated.

Keywords: Laser technology, exposure time, "Asshifo", LC-MS, Shimadzu LCMS-2020, essential and non-essential amino acids, concentration.

Introduction: It is well known that amino acids are one of the main components determining the biological value of food products. They are products of protein degradation and play an important role in the human diet [1]. Amino acids perform vital biological functions in the human body, including participation in protein biosynthesis [2], serving as an energy source [3], and playing an essential role in the immune system, as well as in the formation and protection of the skin and other organs. In particular, hormones such as insulin, thyroxine, adrenaline, and others are synthesized from amino acids [4]. Glutamine and arginine promote the growth and development of immune cells [5]. Tryptophan is converted into serotonin, while tyrosine is converted into dopamine and norepinephrine, participating in nervous system function and neurotransmitter synthesis [6]. Collagen is synthesized with the participation of glycine, proline, and lysine [7].

Therefore, determining the quantitative composition of amino acids in food products consumed by humans is of great importance for evaluating their nutritional value and therapeutic properties.

The authors developed a natural dietary supplement named "Asshifo", consisting by mass of 59% oat (Avena sativa), 1% savory (Satureja hortensis), and 40% meristem tissue of a coniferous plant – juniper (Juniperus excelsa). This supplement possesses properties such as vasodilation, immune system enhancement, and the treatment and prevention of inflammatory diseases of the stomach, intestines, liver, kidneys, lungs, spleen, and uterus, and has been officially registered by the Ministry of Health of the Republic of Uzbekistan [8, 9].

Although this dietary supplement exhibits the above-mentioned therapeutic properties even without additional processing, treatment with modern technologies, including laser technology (laser irradiation), may further enhance its medicinal properties. Currently, laser technology is successfully applied in many fields of science and technology. In particular, the application of laser radiation in the food industry is expanding [10]. Numerous studies have

investigated the effects of laser radiation on the chemical, nutritional, and physical properties of food products, and most of them have shown that properly applied laser radiation has a positive effect on the chemical components of food products [11–13].

In studies conducted by Smith A., Kumar P., and others, the effects of laser radiation on milk proteins and lipids were investigated. The results demonstrated that laser treatment modifies the structure of milk proteins and lipids, improving their physicochemical properties and bioactivity [11].

Therefore, in this study, laser radiation was applied to the "Asshifo" dietary supplement at different exposure times, and scientific research was conducted to determine the optimal irradiation time by analyzing the amino acid content. The amino acid concentrations in untreated and laser-treated "Asshifo" dietary supplement samples were determined using the LC-MS method. Previously, colorimetric or HPLC methods were mainly used for such purposes; however, their sensitivity and selectivity are lower compared to LC-MS technology. In this study, a Shimadzu LCMS-2020 instrument (Japan) was used, and 20 amino acids were analyzed in SIM mode without derivatization [14, 15].

2. MATERIALS AND METHODS

2.1. Preparation of Standard Solutions

Individual standard solutions with a concentration of 500 µg/mL were prepared for each amino acid. For this purpose, 50 mg of each amino acid was weighed and dissolved in 100 mL of water, 0.1 N HCl, or 0.1 N NaOH. Some amino acids (tyrosine, tryptophan, phenylalanine) were dissolved using an ultrasonic bath. The solutions were filtered through a 0.22 µm PVDF filter and stored at 4°C.

2.2. Laser Treatment of the "Asshifo" Dietary Supplement

The "Asshifo" dietary supplement was ground and passed through a 1 mm sieve. The powdered sample, spread in a layer 0.5 cm thick, was treated with laser radiation at a frequency of 1000 Hz for 5, 10, and 15 minutes. The laser radiation source was a "Vityaz" quantum therapy device positioned 5 cm above the sample.

2.3. Preparation of "Asshifo" Dietary Supplement Samples

Samples treated with laser radiation at different time intervals, as well as untreated samples, were prepared as follows. To defat the sample, 25 mL of hexane was added to 1 g of the sample and mixed for 1 minute using a vortex mixer. The mixture was then centrifuged at 7000 rpm for 10 minutes using an Eppendorf 540 centrifuge. The supernatant was discarded, and the residue was dried. Subsequently, 25 mL of 0.1 N HCl was added, and ultrasonic extraction was carried out for 60 minutes. After centrifugation (7000 rpm, 10 minutes), the supernatant was filtered through a 0.22 µm filter and transferred into LC-MS vials.

2.4. LC-MS Analysis Conditions

- Instrument: Shimadzu LCMS-2020
- Ionization mode: ESI positive, SIM mode
- Column: Shim-pack GIST C18 (150 × 4.6 mm, 5 µm)
- Mobile phase:
  - A: Water + 0.1% formic acid
  - B: Acetonitrile + 0.1% formic acid
- Gradient program:
  - 0 min (98% A)
  - 4 min (95% A)
  - 12–16 min (80% A)
  - 18–20 min (50% A)
  - 20.1 min (98% A)
- Flow rate: 0.1 mL/min
- Injection volume: 5 µL
- DL temperature: 250°C
- Block temperature: 400°C
- Interface voltage: 4.5 kV

2.5. Calibration

Calibration solutions were prepared for each amino acid at concentrations of 0.2, 0.5, 1, 2, and 10 µg/mL. Linear calibration curves with correlation coefficients $R^2 \geq 0.995$ were obtained.

3. RESULTS AND DISCUSSION

Based on the obtained chromatograms, the m/z values and retention times of 20 amino acids were determined. A linear calibration curve was constructed for each amino acid. The repeatability (RSD%) values did not exceed 3%. The results of the analysis of the "Asshifo" dietary supplement samples are presented in the table below.

Table 1.

Results of amino acid analysis in extracts of "Asshifo" dietary supplement samples treated with laser radiation.

No	Amino acid	Amino acid concentration in sample extracts, µg/mL			
		Laser irradiation:			
		Untreated	Treated for 5 minutes	**Treated for 10 minutes**	Treated for 15 minutes
1	L-Alanine*	1,851±0,176	1,957±0,008	1,874±0,053	1,927±0,034
2	L-Arginine*	4,558±0,367	5,603±0,074	4,670±0,108	4,901±0,170
3	L-Asparagine*	2,935±0,202	5,422±0,324	2,047±0,064	3,607±0,280
4	L-Aspartic Acid*	2,591±0,169	6,284±0,086	1,419±0,064	2,476±0,232
5	L-cysteine*	2,596±0,325	2,639±0,033	2,659±0,043	2,761±0,059
6	L-Glutamic Acid*	1,441±0,022	1,491±0,010	1,459±0,010	1,447±0,027
7	L-Glutamine*	8,112±0,698	8,165±0,087	7,038±0,093	8,123±0,058
8	Glycine*	1,694±0,075	1,669±0,013	1,681±0,083	1,456±0,112
9	L-Proline*	5,005±0,204	5,466±0,016	5,557±0,051	5,359±0,107
10	L-Serine*	2,859±0,095	2,976±0,028	2,980±0,051	1,519±0,007
11	L-Tyrosine*	0,956±0,023	1,038±0,015	0,851±0,017	0,917±0,022
12	L-Histidine**	3,008±0,258	3,241±0,035	2,850±0,045	3,090±0,034
13	L-Isoleucine**	0,890±0,075	1,084±0,064	0,761±0,029	0,878±0,029
14	L-Leucine**	0,693±0,047	0,813±0,012	0,776±0,007	0,689±0,004
15	L-Lysine**	8,024±0,627	8,182±0,042	7,033±0,151	8,087±0,035
16	L-Methionine**	0,146±0,024	0,142±0,010	0,062±0,003	0,150±0,028
17	L-Phenylalanine**	1,103±0,130	1,171±0,011	0,896±0,010	1,015±0,013
18	L-Threonine**	1,925±0,649	1,847±0,321	1,379±0,161	2,548±0,049
19	L-Tryptophan**	0,947±0,159	0,971±0,005	0,750±0,018	1,003±0,005
20	L-Valine**	11,042±0,449	11,571±0,009	10,968±0,149	10,502±0,126

Note: * – non-essential amino acids; ** – essential amino acids.

As can be seen from Table 1 and Figure 1, the content of amino acids in the "Asshifo" dietary supplement without laser treatment decreases in the following order from valine to methionine: Val > Gln > Lys > Pro > Arg > His > Asn > Ser > Cys > Asp > Thr > Ala > Gly > Glu > Phe > Tyr > Trp > Ile > Leu > Met. In addition, the data presented in Table 1 indicate that, apart from valine, the concentrations of amino acids such as glutamine (Gln), lysine (Lys), proline (Pro), and arginine (Arg) are relatively high in the dietary supplement. These amino acids are biologically important natural compounds and confer various therapeutic properties to the "Asshifo" dietary supplement. As can be seen from Table 1 above, when determining the amino acid concentrations in samples of the "Asshifo" dietary supplement that were not treated with laser radiation and those treated for 5, 10, and 15 minutes, the sample treated with laser radiation at a frequency of 1000 Hz for 5 minutes (blue column) exhibited the highest concentrations of almost all non-essential amino acids compared to the other samples. In particular, a sharp increase in the concentrations of Asp, Arg, and Asn amino acids is clearly observed in the samples treated with laser radiation for 5 minutes.

In the samples treated for 10 and 15 minutes, these indicators initially decreased and then increased again, which can also be seen in the diagram shown in Figure 2. The concentration of some non-essential amino acids (for example, Cys) was higher in the sample treated for 15 minutes compared to the others. However, the concentrations of all other amino acids showed lower values compared to those in the sample treated with laser radiation for 5 minutes.

4. CONCLUSION

In conclusion, it can be stated that when plant-based dietary supplements are treated with laser radiation, the extractability of amino acids into the solvent is relatively enhanced. The results obtained indicate that treating the “Asshifo” dietary supplement with laser radiation for 5 minutes leads to an increase in the concentrations of almost all essential and non-essential amino acids, particularly Asp, Arg, Asn, Val, His, and Ile.

Thus, it has been scientifically proven that the optimal exposure time for increasing the amino acid content of the “Asshifo” dietary supplement using laser radiation at a frequency of 1000 Hz is 5 minutes. As a result, the laser-treated “Asshifo” dietary supplement can exhibit higher biological activity compared to the untreated sample, due to the increased extractable concentrations of biologically important amino acids such as Asp, Arg, Asn, Val, His, and Ile that are essential for the human body.

Overall, it can be confidently stated that laser radiation treatment has a positive effect on the chemical composition and therapeutic properties of the dietary supplement.

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