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Investigation of the qualitative and quantitative composition of amino acids in the herb of small-flowered galinsoga (*Galinsoga parviflora* L.) using gas chromatography

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Amino acids are important for the human body as initial metabolites of polypeptides and proteins. The grass small-flowered galinsoga (*Galinsoga parviflora* L.) accumulates a large amount of various amino acids, this grass is not poisonous and edible in some countries. The aim of this research is to analyze the qualitative and quantitative composition of amino acids in the herb of small-flowered galinsoga (*Galinsoga parviflora* L.): to select the most suitable extraction conditions of amino acids from the *Galinsoga parviflora* herb; to apply the gas chromatography method for the determination of amino acids.

Keywords: *Galinsoga parviflora*, amino acid, gas chromatography

INTRODUCTION

Amino acids are important for the human body as initial metabolites of polypeptides and proteins. Amino acids also regulate the synthesis of hormones and stimulate immune reactions necessary for gene expression. However, excessive amounts of amino acids promote neurological disorders and increase oxidative stress in the body. Therefore, amino acid homeostasis must be maintained in the body [1]. Small-flowered galinsoga (*Galinsoga parviflora* L.) is an annual dicotyledonous plant that grows up to 60 cm tall and belongs to the Asteraceae (*Asteraceae* L.) family. Galinsoga grows in abandoned places, wastelands and roadsides, but it is also a weed in cultivated fields and flower gardens, it blooms from May to September [2–4]. One plant lives for about 40 days, so during the 7 months of flowering, they mature the seeds even 4–5 times. Small-flowered galinsoga is an invasive plant in North and South America, Europe, Africa, Australia and New Zealand [2, 5–7]. The plant is branched, with a bare or sparsely hairy stem, growing vertically. The leaves are opposite, symmetrical, oval or latent, with a pointed apex and a rounded leaf base, the margins are coarsely toothed.

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The upper and lower surfaces of the leaf are covered with fine, sparse hairs. Young plants have round cotyledons with a slightly concave apex. The seeds are oval or triangular and covered with small hairs. The flowers are small, arranged on long peduncles. The rings consist of two parts, the middle ones of which consist of 8 to 50 rings and the surrounding edges consist of 3–5 rings. The fruits are pointed, angular or flat, dark brown, with one seed [2, 6].

After reviewing the research, it was found that 100 g of dried small-flowered galinsoga grass contains 4 g of protein, 0.5 g of fat, 1.24 g of fiber and 5.29 g of carbohydrates. The raw material accumulates mineral substances, of which 100 g of raw material contains: 162 mg of calcium, 38 mg of phosphorus, 36 mg of sodium, 44 mg of manganese, 3 mg of copper, 14 mg of zinc, 681 mg of magnesium and 27 mg of iron [6, 7].

The herb *Galinsoga parviflora* L. is not poisonous and is therefore edible in some countries. In South America (Mexico, Colombia), the stems and leaves of young plants are eaten raw or dried and used as a spice. In Tanzania, the plant is eaten fresh as a salad [7, 8].

The aim of this research is to analyze the qualitative and quantitative composition of amino acids in the herb of small-flowered galinsoga (*Galinsoga parviflora* L.): to select the most suitable extraction conditions of amino acids from the *galinsoga parviflora* herb and to apply the gas chromatography method for the determination of amino acids.

EXPERIMENTAL

The plant raw material used for the research is the small-flowered galinsoga (*Galinsoga parviflora* L.) grass, collected during flowering in different cities of Lithuania in June 2020 and in June–August 2021. The plant raw material was collected in Vilnius, Kaunas and Merkinė (Table 1).

Table 1. **Small-flowered galinsoga (*Galinsoga parviflora* L.) herb samples**

Place of raw material collection	Time of collection	Sample abbreviation
Kaunas	08.2020	K (1)
Kaunas	06.2021	K (2)
Kaunas	07.2021	K (3)
Kaunas	08.2021	K (4)
Vilnius	08.2020	V (1)
Vilnius	06.2021	V (2)
Vilnius	07.2021	V (3)
Vilnius	08.2021	V (4)
Merkinė	08.2020	M (1)
Merkinė	06.2021	M (2)
Merkinė	07.2021	M (3)
Merkinė	08.2021	M (4)

The following reagents and standards were used: 96.3% (v/v) ethanol from AB Stumbras (Kaunas, Lithuania); acetonitrile (Buchs, Switzerland) and formic acid from Sigma-Aldrich Chemie (Germany); methanol (99.9%) (Sigma-Aldrich, Germany); N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) (>97%) (Sigma-Aldrich, Germany); 6N hydrochloric acid (Sigma-Aldrich, Germany); the mixture of amino acid standards, 2.5 μmol/ml of each amino acid (16 different amino acids: alanine, glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, glutamic acid, lysine, histidine, tyrosine and cysteine) from Sigma-Aldrich, Germany. Ultrapure water was purified with a Millipore water cleaning system (Bedford, USA).

Preparation of a test solution for the determination of free amino acids

1 g of crushed small-flowered galinsoga grass is weighed on an analytical balance (to the nearest 0.0001). The weighed herb is poured into 10 ml glass measuring flasks. The flasks are filled with 70% methanol to the 10 ml exact amount. The flasks are transferred to an ultrasonic bath and kept for 10 min. The samples are transferred to a centrifuge and the material is centrifuged for 5 min in a speed of 8000 rotations per min. After the centrifugation, 100 μl of the liquid phase is transferred into dark glass chromatographic vials.

Preparation of a test solution for the determination of amino acids after hydrolysis

1 g of crushed small-flowered galinsoga grass is weighed on an analytical balance (to the nearest

0.0001). The weighed herb is poured into 10 ml glass measuring flasks and is dissolved in 10 ml of a 6 N hydrochloric acid solution. The samples are sealed tightly and transferred to a glycerol bath, where they are heated for 5 h at 110°C. The samples after hydrolysis are transferred to test tubes and placed in a centrifuge. The material is centrifuged for 5 min in a speed of 8000 rotations per min. 100 µl of the liquid phase is transferred into dark glass chromatographic vials.

Amino acids derivatization

It is not possible to determine amino acids directly by the gas chromatography method, because the temperature inside the apparatus is high, which breaks down thermolabile amino acids, also amino acids are not volatile. To prevent amino acids from breaking down and to analyze by gas chromatography, derivatization is required. Due to the derivatization amino acids become more flexible and thermostable. Amino acid derivatives improve substance recognition [4].

Sample preparation: 100 µl of the prepared test solution is dried under a stream of nitrogen to a dry residue. The precipitate is poured into 100 µl of acetonitrile and 100 µl of a MTBSTFA derivatizer. The resulting solution is heated in a glycerol bath at 100°C for 2.5 h.

Preparation of a standard solution: 100 µl of an amino acid standard mixture is taken and dried under a stream of nitrogen until dry sediments. The precipitate is then poured into 100 µl acetonitrile and a 100 µl MTBSTFA derivatizer. The received solution is heated in a glycerol bath at 100°C for 2.5 h.

GC analysis of amino acids

A chromatograph (Shimadzu GC-2010 PLUS, Shimadzu Corporation, Japan) with a flame ionization detector (FID) was used. The gas chromatographic separation was carried out using a 30 m × 0.25 mm, 0.25 µm capillary column, filled with a liquid stationary phase (5% diphenyl and 95% polysiloxane). Ultra-high purity helium was used as a carrier gas at a column flow rate of 1.18 mL/min. The general elution flow rate was 27.8 mL/min. The temperature of the injector was 260°C. The pressure was 258.7 kPa. The injection volume was 1 µL, with a split ratio of 1:20. The temperature of the column changed from 70 to 290°C in 10 min and from 290 to 320°C in 20 min, holding for 5 min. The research time was 41 min [16].

Determination of amino acid quantity

For the quantitative analysis, calibration curves were used, which show a direct proportionality, for the analyte concentration, in terms of peak area. These calibration curves are constructed using the standard solutions of the known concentration. In the graphs, the concentration is marked on the abscissa axis, and the peak area on the ordinate axis. The calibration curves for glycine and L-valine are shown in Fig. 1.

Amino acid concentrations were calculated using formulas. The formulas and parameters of calibration curves are given in Table 2.

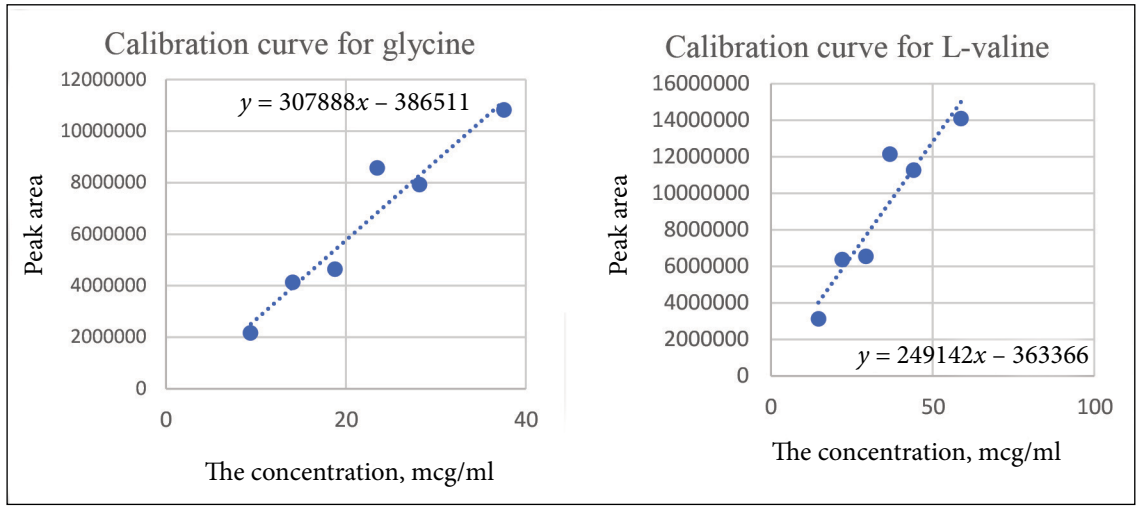

Fig. 1. Calibration curves for glycine and L-valine

Table 2. **Parameters of calibration curves**

Amino acid	Function equation	Coefficient of determination
L-Alanine	$f(x) = 281766.732385*x–151475.614966$	$r^2 = 0.930087$
Glycine	$f(x) = 307888.469976*x–386511.363171$	$r^2 = 0.924776$
L-Valine	$f(x) = 249141.957926*x+363365.493606$	$r^2 = 0.886084$
L-Leucine	$f(x) = 287041.492244*x–201473.326389$	$r^2 = 0.927946$
Izoleucine	$f(x) = 233454.537256*x+544450.385570$	$r^2 = 0.896224$
L-Proline	$f(x) = 288232.903171*x+1720910.693438$	$r^2 = 0.865114$
L-Methionine	$f(x) = 191580.533564*x–317706.645069$	$r^2 = 0.924414$
L-Serine	$f(x) = 324303.113917*x+1971749.329881$	$r^2 = 0.886962$
L-Trethionine	$f(x) = 254206.553589*x+2531324.218197$	$r^2 = 0.902862$
L-Phenylalanine	$f(x) = 211654.925651*x+421367.953259$	$r^2 = 0.888505$
L-Aspartic acid	$f(x) = 273314.973170*x+2701137.416486$	$r^2 = 0.881785$
L-Glutamic acid	$f(x) = 218542.759110*x–352240.735532$	$r^2 = 0.933645$
L-Lysine	$f(x) = 236839.828705*x–3007313.281102$	$r^2 = 0.939495$
L-Histidine	$f(x) = 290134.615620*x–2836462.193286$	$r^2 = 0.916866$
L-Tyrosine	$f(x) = 218746.004720*x+4267862.926887$	$r^2 = 0.813934$

The coefficient of determination (r^2) is close to 1, which means that these calibration curves can determine the concentrations of amino acids quite accurately.

Statistical analysis

The research data were analyzed and systematized in the Microsoft Office 365 Excel version (Microsoft, USA), and SPSS 28.0 (IBM, USA) was used for the data analysis. All results were presented as a concentration of different amino acids in mcg/g. The total amino acid content was calculated by summing all concentrations. Differences between the groups were determined using the Kruskal–Wallis test. This research method is suitable for comparing more than two groups of the data that are not distributed according to the normal distribution (standard deviation). Statistically significant differences between the groups are determined when the p value is lower than the α value (0.05).

RESULTS AND DISCUSSION

Amino acids determination by gas chromatography

Amino acids are thermolabile, polar and non-volatile, so it is impossible to study them directly by the gas chromatography-mass spectrometry method. Due to the properties of these amino acids, it was decided to perform derivatization. The derivatizer used in the study is N-tert-butyldimethylsilyl-N-methyl-trifluoroacetamide (MTBSTFA). During the reaction, amino acids attach the -silyl functional group and t-BDMS derivatives are obtained (Fig. 2).

During the study, 15 amino acids were identified: 1 – alanine (retention time 15.227 s), 2 – glycine (15.51 s), 3 – valine (16.74 s), 4 – leucine (17.241 s), 5 – isoleucine (17.621 s), 6 – proline (18.054 s), 7 – methionine (20.25 s), 8 – serine (20.478 s), 9 – threonine (20.81 s), 10 – phenylalanine (21.501 s), 11 – aspartic acid (22.103 s), 12 – glutamic acid (23.186 s), 13 – lysine (24.145 s), 14 – histidine (25.936 s) and 15 – tyrosine (26.317 s).

The samples were analyzed by gas chromatography to determine the amino acids present in the small-flowered galinsoga grass. The results are presented in Table 3.

15 hydrolyzed amino acids were found in the small-flowered galinsoga herb. L-Cysteine was not detected in any sample. L-Lysine was not detected only in samples K-4 and V-1; L-Histidine was not detected in K-3, V-1, V-4 and M-1 samples.

The data of the study were compared with the studies conducted at the Lithuanian University of Health Sciences, during which the composition of amino acids was determined in the raw material of fruiting bodies of edible mushrooms [13] and in bee products [14]. The amino acid isolation methods used in these studies were identical to those used in this study. 30 hydrolyzed amino

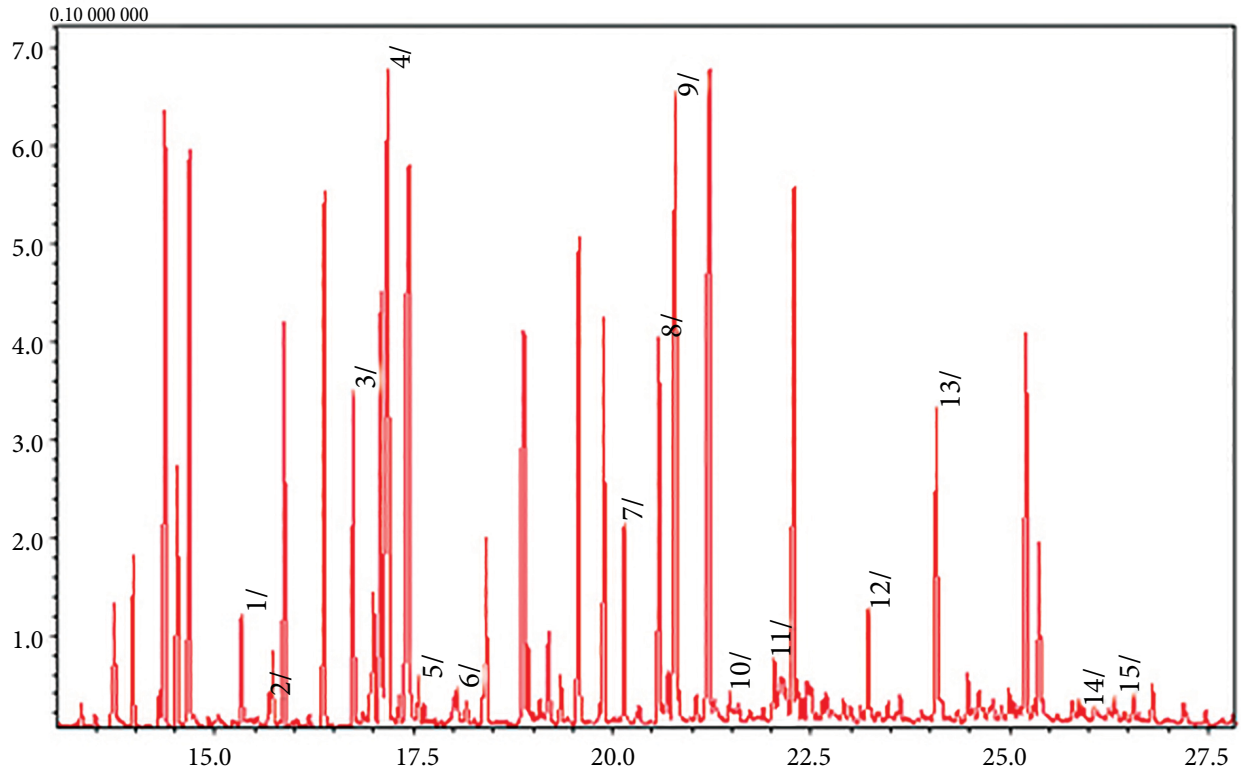

Fig. 2. The chromatogram of amino acids after hydrolysis

Table 3. **Amino acids detected in galinsoga samples after hydrolysis**

Amino acid	K (1)	K (2)	K (3)	K (4)	V (1)	V (2)	V (3)	V (4)	M (1)	M (2)	M (3)	M (4)
L-Alanine	+	+	+	+	+	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+	+	+	+	+	+
L-Valine	+	+	+	+	+	+	+	+	+	+	+	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+	+
Izoleucine	+	+	+	+	+	+	+	+	+	+	+	+
L-Proline	+	+	+	+	+	+	+	+	+	+	+	+
LMethionine	+	+	+	+	+	+	+	+	+	+	+	+
L-Serine	+	+	+	+	+	+	+	+	+	+	+	+
L-Trethionine	+	+	+	+	+	+	+	+	+	+	+	+
L-Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+
L-Aspartic acid	+	+	+	+	+	+	+	+	+	+	+	+
L-Glutamic acid	+	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	–	–	+	+	+	+	+	+	+
L-Histidine	+	+	+	–	–	+	+	–	–	+	+	+
L-Tyrosine	+	+	+	+	+	+	+	+	+	+	+	+
L-Cysteine	–	–	–	–	–	–	–	–	–	–	–	–

acids were detected by comparison, and it was observed that the same variety of amino acids is detected in the raw material of the small-flowered galinsoga compared to bee products. In both bee products and galinsoga raw material, cysteine was not detected in any sample, and lysine and histidine were not detected in some samples. After comparing the amino acid diversification of galinsoga grass with the amino acid analysis of fruiting bodies of edible mushrooms, it was observed that

no histidine was detected in any of the mushroom samples, while histidine was detected in many samples of galinsoga raw material [13, 14].

Determination of free amino acids in galinsoga raw

Free amino acids in the samples were identified. Many different amino acids were detected, but less than after the hydrolysis. The data are presented in Table 4.

According to the data, it was noticed that the samples do not contain free amino acids: L-Methionine, L-Histidine and L-Lysine. L-Cysteine was also not detected in the samples after hydrolysis.

11 free amino acids were identified in the bearded iris raw material [15]. In comparison with the free amino acids identified in this study, it was observed that 11 of the 12 detected amino acids are the same.

Evaluation of the total amino acid content of small-flowered galinsoga grass

The total amino acid content was determined in the methanolic extracts of small-flowered galinsoga grass. 12 samples were prepared for the study: 4 of them were collected in Kaunas, 4 in Vilnius and 4 in Merkinė. During the study, the total amount of amino acids after hydrolysis and free amino acids were determined. The results are presented in Fig. 3.

The data presented in the diagram show that the lowest amount of hydrolyzed amino acids was established in the raw materials collected in Vilnius in August 2021 – 2026.8 μg/g, and the highest amount was found in the raw materials collected in Vilnius in June 2021 – 13457.1 μg/g. Comparing the tested samples and after performing the statistical analysis, it was determined that there is a statistically significant variation in the amount of amino acids between different samples ($p < 0.01$). It was observed that the maximum amount of amino acids was determined in the raw materials collected in Kaunas in June 2021 – 11082.1 μg/g, in Vilnius in June – 13457.1 μg/g and in Merkinė in June – 9725.7 μg/g.

When comparing the results with the study conducted (2020), which examined bee products, it was observed that the amounts of hydrolyzed amino acids are similar [14]. During the research, the highest amount of hydrolyzed amino acids was determined in the bee bread collected in the Biržai District, which was 10845.18 μg/g, and in the small-flowered galinsoga grass grown in 2021. In Vilnius in June, the amount of hydrolyzed amino acids was determined to be 13457.1 μg/g. This is a 20% higher quantity. And when comparing the lowest

Table 4. **Free amino acids detected in galinsoga grass samples**

Amino acid	K (1)	K (2)	K (3)	K (4)	V (1)	V (2)	V (3)	V (4)	M (1)	M (2)	M (3)	M (4)
L-Alanine	+	+	–	+	+	+	+	+	+	+	+	+
Glycine	–	+	–	–	–	+	+	–	+	+	–	+
L-Valine	+	+	+	+	+	+	+	+	+	+	+	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+	+
Izoleucine	+	+	+	+	+	+	+	+	+	+	+	+
L-Proline	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine	–	–	–	–	–	–	–	–	–	–	–	–
L-Serine	+	–	–	+	–	+	+	+	+	+	+	+
L-Trethionine	+	+	–	+	+	+	+	+	+	+	+	+
L-Phenylalanine	+	+	–	+	+	+	+	+	+	+	+	+
L-Aspartic acid	+	+	–	+	+	+	+	+	+	+	–	+
L-Glutamic acid	–	+	–	–	+	+	+	+	–	+	–	+
L-Lysine	–	–	–	–	–	–	–	–	–	–	–	–
L-Histidine	–	–	–	–	–	–	–	–	–	–	–	–
L-Tyrosine	+	+	–	+	–	+	+	+	+	+	–	+
L-Cysteine	–	–	–	–	–	–	–	–	–	–	–	–

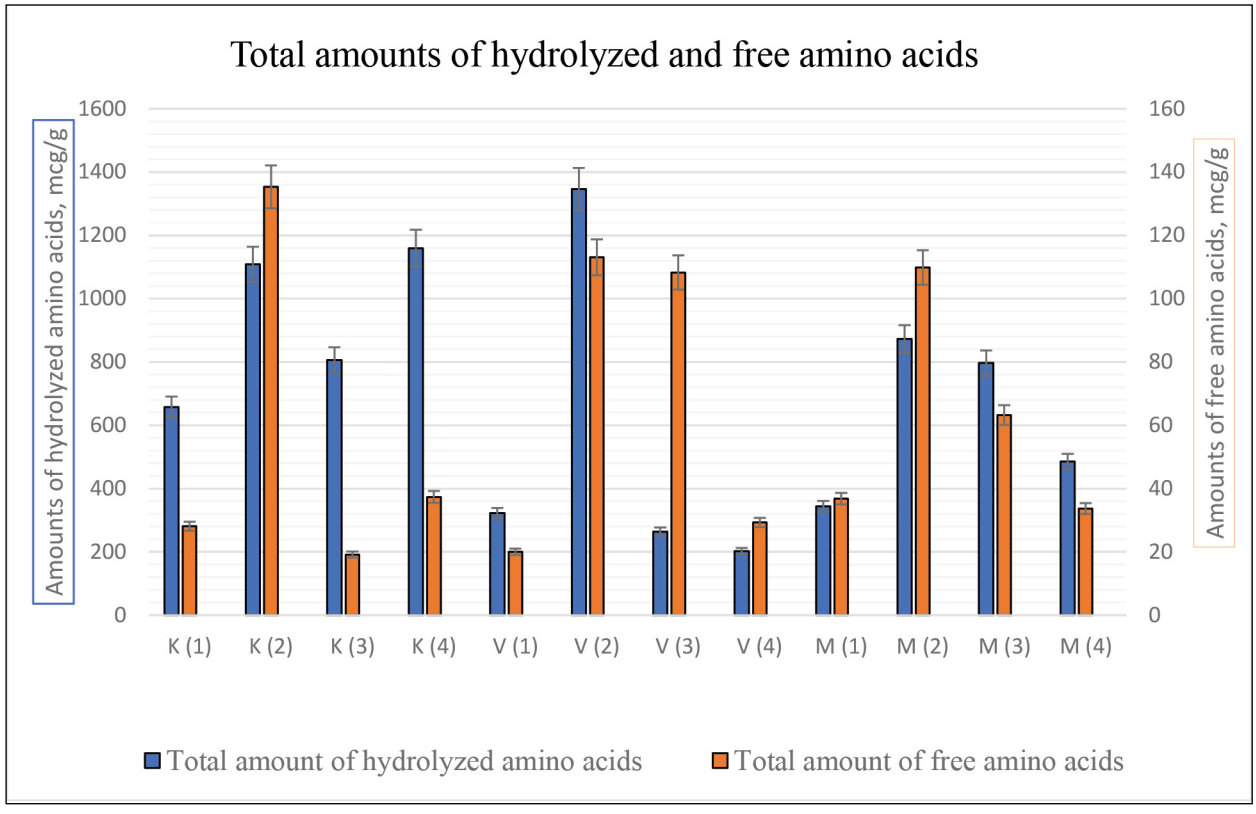

Fig. 3. Total amounts of the hydrolyzed and free amino acids determined in small-flowered galinsoga grass samples

determined total amounts of hydrolyzed amino acids, it was observed that in August 2021, only 2026.8 μg/g was detected, and the lowest amount detected in bee products was 8578.04 μg/g, which is 4.2 times higher than in galinsoga grass.

According to the research data, it was observed that the amounts are much lower than the analyzed samples after hydrolysis. The minimum amount of free amino acids was determined in the raw materials collected in Kaunas in July 2021 – 191.3 μg/g, and the highest amount was determined in the raw materials collected in Kaunas in June 2021 – 1353.5 μg/g. Comparing all samples, a statistically significant ($p < 0.01$) difference in the amount of free amino acids was determined. According to the data, it was observed that the amounts of amino acids differ statistically significantly between different locations and the period when the sample was collected. The maximum amounts of free amino acids are determined in the raw materials collected in June 2021 – 1353.5 μg/g in Kaunas, 1130.0 μg/g in Vilnius and 1098.2 μg/g in Merkinė. The minimum quantities of free amino acids were determined in the raw materials collected in Kaunas in June 2021 – 191.3 μg/g, in Vilnius in August 2021 – 200.14 μg/g and in Merkinė in August 2021 – 336.68 μg/g.

CONCLUSIONS

Derivatization was used to analyze amino acids by gas chromatography. After analyzing the data and applying it in practice, it was observed that the most suitable derivatizer is MTBSTFA. After the research, 15 hydrolyzed amino acids were found in the small-flowered galinsoga – alanine, glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, glutamic acid, lysine, histidine and tyrosine. 12 free amino acids were detected. Free histidine, lysine and methionine were not detected. Comparing the amounts of small-flowered galinsoga amino acids collected in different places, it was observed that the highest amounts of hydrolyzed amino acids were found in Kaunas – the total amount of hydrolyzed amino acids of all samples was 37313.43 μg/g. The lowest amount was detected in the raw materials collected in Vilnius – 21351.98 μg/g. Comparing the amounts of free amino acids in the raw materials of galinsoga collected in different places, it was observed that the highest amount of free

amino acids of all samples was found in the raw materials collected in Vilnius – 2706.65 μg/g, and the lowest amount was found in the samples from Kaunas – 2199.39 μg/g.

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SMULKIAŽIEDĖS GALINSOGOS (*GALINSOGA PARVIFLORA* L.) AMINORŪGŠČIŲ KOKYBINĖS IR KIEKYBINĖS SUDĖTIES TYRIMAS DUJŲ CHROMATOGRAFIJOS METODU

S a n t r a u k a

Aminorūgštys yra svarbios žmogaus organizmui, nes dalyvauja polipeptidų ir baltymų apykaitoje. Smulkiažiedė galinsoga (*Galinsoga parviflora* L.) sukaupia daug įvairių aminorūgščių, ši žolė nenuodinga ir kai kuriose šalyse vartojama maistui. Šio tyrimo tikslas – ištirti kokybinę ir kiekybinę aminorūgščių sudėtį smulkiažiedės galinsogos (*Galinsoga parviflora* L.) žolėje: parinkti tinkamiausias aminorūgščių ekstrahavimo iš smulkiažiedės galinsogos žolės sąlygas, taikyti dujų chromatografijos metodą aminorūgštims nustatyti.