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CHANGES IN AMINO ACIDS CONTENT IN TUBERS OF JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.) CULTIVARS DURING STORAGE

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Abstract. The objective of the three-year study was to determine the influence of post-harvest storage on essential and nonessential amino acids content in tubers of different cultivars of Jerusalem artichoke (*Helianthus tuberosus* L.) – 'Rubik', 'Albik' and 'Sauliai'. The experiment was performed in 2007–2009. The plants were grown in the experimental field of Lithuanian University of Agriculture in Kaunas (from 2011 August – Aleksandras Stulginskis university). The Jerusalem artichoke tubers were stored for 20 weeks at the temperature of 2°C (± 0.5°C) and RH 90–95 % in polypropylene (PP) perforated bags, capacity of 10 kg, in 4 replicates. Amino acids contents were determined immediately after harvest and during storage of the tubers in every 4 weeks by the method of ion-exchange chromatography and then detected photometrically. The data obtained were statistically analyzed with two-factor Anova (STATISTICA software). Standard deviation and the least significant difference at the 95% probability level were calculated with Fisher's LSD test. Data presented in tables are mean values of the three years of the experiment. The results obtained showed that directly after harvest and during the storage the dominating amino acid in tubers of all cultivars was of essential amino acids – arginine, and of nonessential – asparagine, glutamine and alanine. Tubers of cv. 'Sauliai' after 20 week storage accumulated the highest amount of essential amino acids – treonine, valine, isoleucine, leucine, phenylalanine and nonessential – serine, glycine, alanine, tyrosine. Significant correlations between contents of some amino acids in the tubers were found.

Key words: Jerusalem artichoke tubers, cultivars, arginine, functional food

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INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is grown primarily for its edible tubers, which were first cultivated by native Americans before the arrival of the Europeans. Cultivated for centuries, Jerusalem artichoke was at the beginning used as human food, and then also as animal feed [Ben Chekroun et al. 1996]. The plants are easy in field cultivation because are resistant to frosts, dry conditions and poor soils. In Lithuania and Poland this plant is used as an "alternative" vegetable, however so far not very popular. Studies on nutritive value of Jerusalem artichoke tubers showed that they contain some health promoting components. The protein content of Jerusalem artichoke tubers varies from 2 to 3% fw [Bagni et al. 1980, Danilčenko et al. 2009]. There are very little data about amino acid composition of the tubers. Cieślik [2005] and Danilčenko et al. [2009] reported that the tubers contained all essential amino acids in almost ideal proportions for humans. Although high fructans content is the main reason why the tubers can be used as a functional food, also high content of proteins and balanced amino acids composition of the tubers is worth to underline. Proteins play an important role in metabolism of all living organisms. They mechanically support cells and tissues, are components of tissues and act as biologically active compounds (enzymes and hormones). They are essential part of protoplasm and enzymes, participate in cell metabolism, are involved in accumulation and dissimilation of organic substances. They are also compounds of proteins, which consist only of amino acid residues and contain proteid besides protein prosthetic groups. Proteids are found, depending on the prosthetic group, in the form of nucleoproteids in the cell nucleus and in organelles, as well as enzymes throughout the cytoplasm in the mitochondria and microcosms. Until recent years, researchers though that role of specific proteins and amino acids as functional ingredients was restricted to muscle building in humans. Furthermore, protein intakes higher than shown in the Recommended Dietary Allowance (RDA) are often believed as potentially detrimental to renal function and bone mineralization. These concepts have been replaced by a new understanding of the importance of dietary protein for adult health [Ciborowska and Rudnicka 2007].

There is very little data on influence of storage on quality traits of Jerusalem artichoke tubers, including their chemical composition. According to our previous experiments, the tubers can be stored at a temperature close to 0^{o}C and at high humidity level for a period of about 4 months, but significant losses of sugars and dry matter occur [Danilčenko et al. 2008].

The objective of the study was to determine the influence of storage period on changes of essencial and non-essential amino acids content in tubers of different cultivars of Jerusalem artichoke.

MATERIAL AND METHODS

The experiment was performed in 2007–2009. Jerusalem artichoke tubers of cultivars 'Rubik', 'Albik' (late-yielding ones), and 'Sauliai' (medium-early yielding one) were used in the experiment. The plants were grown in the experimental field of

Lithuanian University of Agriculture in Kaunas (54°54' N 23°56' E), in the soil of the following characteristics: neutral, medium humus, limnoglacial loam on moraine loam, calcareous deeper gleyic luvisol (*Calearie Luvisol*). The soil was medium phosphorus rich and medium potassium rich (tab. 1).

Table 1. Characteristics of soil conditions in the field used in the experiment

pH	Humus content (%)	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})
6.6	1.76	126	121

The tubers were planted out at the end of April, and plants harvested at the beginning of November. After harvest the tubers of marketable quality (sound, not injured) were stored for 20 weeks, from the beginning of November till the end of April of the next year in the cold chamber with controlled temperature of 2°C (±0.5°C) and RH 90–95%. The tubers were kept in polypropylene (PP) perforated bags, capacity of 10 kg, in 4 replicates.

Amino acids contents were determined immediately after harvest and during storage of the tubers in every 4 weeks. Amino acids were separated by the method of ion-exchange chromatography and then detected photometrically. Light absorbance was measured at wavelength 570 nm with an automatic analyzer of amino acids, Mikrotechna AAA 339, using a glass column filled with ionite Ostion LGANB. Hydrolysis of the samples was performed in the presence of 6 M HCl at 105°C for 24 h. The concentrations of the nonessential amino acids: asparagine, serine, glutamine, proline, glycine, alanine, tyrosine and the essential amino acids: treonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine and arginine were determined [Technical regulation of amino acids 2003].

The data obtained were statistically analyzed with two-factor Anova (STATISTICA software). Standard deviation and the least significant difference at the 95% probability level were calculated with Fisher's LSD test. Data presented in tables are mean values of the three years of the experiment.

RESULTS AND DISCUSSION

Long-term storage of Jerusalem artichoke tubers is perhaps the main problem associated with their commercial production. Storage has been found to result in deterioration of quantity and quality of carbohydrates [Dorrell 1977, Danilčenko et al. 2008]. In our study content of amino acids in the tubers was relatively high and varied from 40.1 to 59.4 mg kg^{-1} dw, depending on the cultivar and the storage term. Maximum total amino acid content was determined in all investigated artichoke cv. tubers storage 8 weeks (fig. 1). The largest increasing of total amino acids amount was in the cv. 'Albik' tubers and the smallest – in 'Sauliai' cv. tubers. The differences between the har-

vesting day until 8 week were respectively 8.31 and 6.34 mg kg^{-1} dw. Some authors argue that increasing amounts of amino acids in Jerusalem artichoke tubers depending on their germination [Cieślik 2005]. Other proposed that during storage, the amino acids change were influenced by various other factors including the composition of proteins and free amino acids variations [Brierley et al. 1996]. In our research at the end of storage the total amino acid amount of the tubers increased, because the tubers starts awake and germinate. This increase of total amino acids content from harvest to 20-weeks was significant in tubers of 'Sauliai' cv. from 43.76 to 52.84 mg kg^{-1} dw. In other cultivars of Jerusalem artichoke tuber the amino acids amount change had been insignificant.

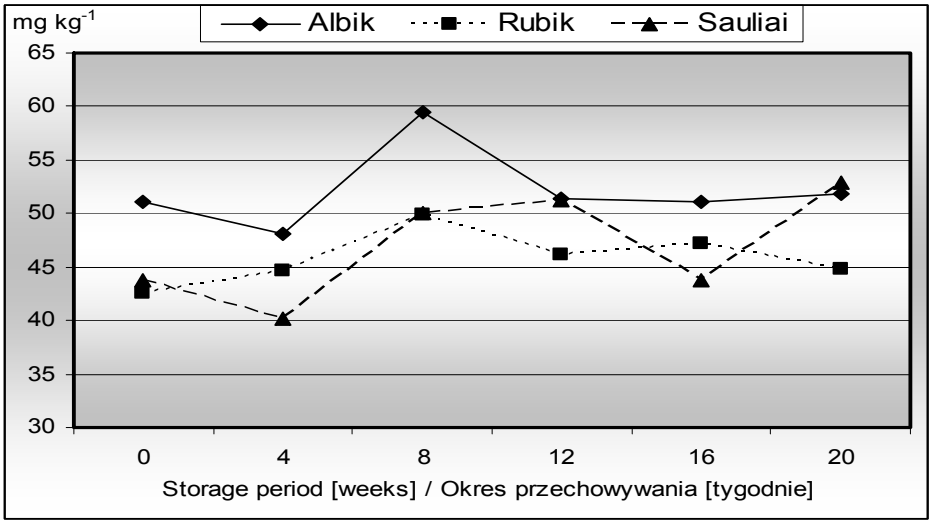

Fig. 1. Total amount of amino acids in Jerusalem artichoke tubers during storage

Essential amino acids made up 55% of all amino acids found in the tubers. Regardless of cultivar and storage period, the dominating amino acid of essential ones was arginine and nonessential – asparagine, glutamine and alanine. The lowest content in the tubers showed tyrosine and methionine. Tyrosine is not desirable in the tubers due to the fact that it negatively affects color of raw tubers. At harvest time tubers of 'Albik' accumulated the highest amount of glutamine (9.18 mg kg^{-1} dw), glycine (2.31 mg kg^{-1} dw), leucine (2.77 mg kg^{-1} dw) (tab. 2, 3). Jerusalem artichoke tubers contained similar amount of amino acids as the fodder beet, but lower than potato tubers [Kaldy et al. 1980].

Tuber dormancy is a period of slow metabolism, which ends, from a physiological point of view, when the utilization of spare substances begins (mid/end of February). Arginine and glutamine continue to be accumulated throughout dormancy [Bagni et al. 1980, Serafini-Fracassini and Mossetti 1985]. Leucine participates in numerous metabolic processes, its obvious role being as an indispensable amino acid for new proteins synthesis. Leucine is also a critical regulator of translation initiation of protein synthesis. In human body it acts as the nitrogen donor for muscle production of alanine and

Table 2. Contents of essential amino acids during storage of Jerusalem artichoke tubers (means of 2007–2009)

Amino acids	Cultivar	Content (mg kg $^{-1}$)					
		at harvest	storage period (weeks)				
			4	8	12	16	20
Treonine	'Albik'	2.05 ±0.39a	1.87 ±0.09a	2.16 ±0.06a	2.00 ±0.09a	1.98 ±0.14a	1.94 ±0.18a
	'Rubik'	1.94 ±0.10a	2.07 ±0.10a	2.28 ±0.10a	2.12 ±0.07a	2.10 ±0.02a	2.02 ±0.06a
	'Sauliai'	1.96 ±0.23a	1.81 ±0.34a	2.19 ±0.39a	2.20 ±0.31a	1.94 ±0.26a	2.81 ±0.72b
Valine	'Albik'	2.15 ±0.25a	1.97 ±0.06a	2.24 ±0.19a	2.03 ±0.13a	1.91 ±0.03a	1.86 ±0.07a
	'Rubik'	2.09 ±0.21a	2.20 ±0.19a	2.33 ±0.03a	2.15 ±0.02a	2.12 ±0.09a	2.00 ±0.07a
	'Sauliai'	2.09 ±0.28a	1.90 ±0.28a	2.20 ±0.40a	2.18 ±0.27a	1.89 ±0.19a	2.39 ±0.24b
Metionine	'Albik'	0.56 ±0.39a	0.38 ±0.13a	0.53 ±0.03a	0.50 ±0.16a	0.45 ±0.03a	0.32 ±0.05a
	'Rubik'	0.60 ±0.34a	0.61 ±0.05a	0.59 ±0.08a	0.55 ±0.11a	0.42 ±0.05a	0.37 ±0.08a
	'Sauliai'	0.55 ±0.25a	0.45 ±0.14a	0.50 ±0.02a	0.45 ±0.23a	0.23 ±0.06a	0.45 ±0.11a
Isoleucine	'Albik'	2.51 ±0.68a	2.30 ±0.46a	2.67 ±0.36a	2.38 ±0.28a	2.30 ±0.05a	2.25 ±0.07a
	'Rubik'	2.35 ±0.63a	2.51 ±0.63a	2.78 ±0.22a	2.62 ±0.28a	2.62 ±0.30a	2.46 ±0.03a
	'Sauliai'	2.23 ±0.71a	2.18 ±0.78a	2.53 ±0.54a	2.67 ±0.61a	2.24 ±0.38a	2.70 ±0.01b
Leucine	'Albik'	2.77 ±0.22b	2.44 ±0.10a	2.73 ±0.19a	2.52 ±0.12a	2.43 ±0.05a	2.43 ±0.17a
	'Rubik'	2.44 ±0.25a	2.73 ±0.21a	2.91 ±0.14a	2.74 ±0.04a	2.73 ±0.08a	2.58 ±0.01a
	'Sauliai'	2.54 ±0.41a	2.34 ±0.34a	2.61 ±0.37a	2.63 ±0.37a	2.42 ±0.30a	2.98 ±0.33b
Phenylalanine	'Albik'	2.02 ±0.05a	1.91 ±0.18a	2.21 ±0.07a	1.99 ±0.04a	1.94 ±0.18a	1.87 ±0.23a
	'Rubik'	1.83 ±0.11a	1.96 ±0.05a	2.20 ±0.26a	2.00 ±0.10a	1.96 ±0.03a	1.88 ±0.10a
	'Sauliai'	1.81 ±0.22a	1.71 ±0.30a	2.12 ±0.32a	2.04 ±0.31a	1.83 ±0.21a	2.23 ±0.22b
Lysine	'Albik'	2.82 ±0.17a	2.54 ±0.10a	2.49 ±0.61a	2.64 ±0.30a	2.54 ±0.05a	2.62 ±0.20a
	'Rubik'	2.57 ±0.20a	2.51 ±0.08a	2.89 ±0.31a	2.76 ±0.13a	2.68 ±0.10a	2.56 ±0.13a
	'Sauliai'	2.44 ±0.14a	2.34 ±0.22a	2.68 ±0.17a	2.78 ±0.61a	2.45 ±0.39a	2.81 ±0.38a
Hystidine	'Albik'	1.59 ±0.19a	1.57 ±0.23a	1.79 ±0.19a	1.72 ±0.21a	1.53 ±0.12a	1.48 ±0.22a
	'Rubik'	1.35 ±0.05a	1.47 ±0.13a	1.71 ±0.35a	1.46 ±0.03a	1.68 ±0.03a	1.54 ±0.10a
	'Sauliai'	1.37 ±0.09a	1.41 ±0.04a	1.76 ±0.24a	1.67 ±0.36a	1.43 ±0.04a	1.62 ±0.15a
Arginine	'Albik'	12.18 ±3.02a	12.89 ±2.41a	16.90 ±2.63a	11.78 ±0.72a	12.82 ±2.57a	14.46 ±3.99a
	'Rubik'	8.58 ±0.08a	7.41 ±1.74a	9.03 ±2.46a	8.23 ±0.04a	7.98 ±0.66a	8.30 ±1.46a
	'Sauliai'	7.53 ±1.02a	6.65 ±2.01a	8.76 ±1.39a	8.47 ±4.04a	7.09 ±2.51a	8.21 ±0.83a

Means followed by the same letter are not significantly different at $P \leq 0.05$

Table 3. Contents of nonessential amino acids during storage of Jerusalem artichoke tubers (means of 2007–2009)

Amino acids	Cultivar	Content (mg kg $^{-1}$)					
		at harvest	storage period (weeks)				
			4	8	12	16	20
Asparagine	'Albik'	4.3 ±0.39a	4.27 ±0.56a	6.46 ±1.77a	5.32 ±0.26a	5.72 ±1.07a	5.81 ±1.70a
	'Rubik'	4.05 ±0.16a	4.36 ±0.08a	5.60 ±1.42a	5.20 ±0.36a	5.46 ±0.29a	5.48 ±0.86a
	'Sauliai'	4.07 ±0.19a	4.10 ±0.55a	5.13 ±0.79a	5.57 ±0.97a	4.90 ±0.46a	6.23 ±1.01a
Serine	'Albik'	1.8 ±0.45a	1.85 ±0.02a	2.41 ±0.63a	1.91 ±0.25a	1.93 ±0.06a	1.85 ±0.04a
	'Rubik'	1.98 ±0.18a	2.18 ±0.18a	2.36 ±0.29a	2.00 ±0.10a	2.08 ±0.11a	1.88 ±0.08a
	'Sauliai'	2.00 ±0.12a	1.86 ±0.32a	2.33 ±0.72a	2.19 ±0.09a	1.86 ±0.29a	2.62 ±0.16b
Glutamine	'Albik'	9.18 ±2.20b	6.60 ±1.50a	8.03 ±0.98a	8.43 ±1.57a	7.77 ±1.60a	7.18 ±1.04a
	'Rubik'	6.43 ±0.70a	7.66 ±1.74a	7.86 ±1.51a	7.79 ±0.44a	8.83 ±1.51a	7.60 ±1.40a
	'Sauliai'	7.65 ±0.77a	5.86 ±0.69a	9.02 ±0.65a	9.39 ±1.54a	7.59 ±0.06a	7.97 ±0.43a
Proline	'Albik'	1.14 ±0.32a	1.08 ±0.15a	1.32 ±0.82a	1.30 ±0.48a	1.08 ±0.53a	1.00 ±0.39a
	'Rubik'	0.94 ±0.40a	1.02 ±0.46a	0.99 ±0.43a	1.07 ±0.36a	0.95 ±0.29a	0.89 ±0.31a
	'Sauliai'	1.05 ±0.48a	0.91 ±0.45a	1.05 ±0.58a	1.20 ±0.62a	1.04 ±0.55a	1.12 ±0.33a
Glycine	'Albik'	2.31 ±0.03b	1.97 ±0.21a	2.28 ±0.20a	2.13 ±0.12a	1.97 ±0.02a	1.83 ±0.34a
	'Rubik'	2.23 ±0.10a	2.45 ±0.15a	2.54 ±0.05a	2.26 ±0.03a	2.30 ±0.12a	2.15 ±0.10a
	'Sauliai'	2.27 ±0.22a	2.18 ±0.19a	2.42 ±0.51a	2.61 ±0.38a	2.14 ±0.24a	2.74 ±0.32b
Alanine	'Albik'	3.38 ±0.66a	3.47 ±1.13a	4.00 ±0.13a	3.66 ±0.72a	3.73 ±0.37a	3.71 ±0.40a
	'Rubik'	2.23 ±0.10a	2.45 ±0.15a	2.54 ±0.05a	2.26 ±0.03a	2.30 ±0.12a	2.15 ±0.10a
	'Sauliai'	3.24 ±0.55a	3.45 ±0.49a	3.65 ±0.05a	4.03 ±0.25a	3.67 ±0.09a	4.57 ±0.40b
Tyrosine	'Albik'	0.93 ±0.03a	1.00 ±0.12a	1.18 ±0.10a	1.01 ±0.05a	1.00 ±0.07a	0.96 ±0.12a
	'Rubik'	0.94 ±0.03a	1.05 ±0.05a	1.24 ±0.18a	1.01 ±0.05a	1.05 ±0.01a	1.0 ±0.062a
	'Sauliai'	0.96 ±0.03a	0.95 ±0.07a	1.19 ±0.27a	1.12 ±0.12a	1.00 ±0.09a	1.39 ±0.44b

Means followed by the same letter are not significantly different at $P \leq 0.05$

glutamine. Some authors indicate that protein metabolism in the tubers during storage increases and also amount of amino acids increases, especially when dormancy period is completed. The process is related to building structural elements of soluble amines and amides. After the dormancy period, with activation of plant physiological processes in tissues, one amino acid can change into another. For example, from the glycine serine is formed, and the latter is converted into cysteine. Glutamine is formed from ornithine

and aspartic acid from arginine and threonine. Cysteine is formed of methionine [Dougall 1966]. In our experiment, tubers of 'Sauliai' cv. accumulated the highest amounts of amino acids after 20-week storage, such as treonine, valine, isoleucine, leucine, phenilalanine, serine, glicine, alanine and tyrosine. The amounts of nonessential amino acids serine, glicine, alanine and tyrosine also significantly increased (tab. 2, 3). This could be explained in this way that at the end of dormancy period all amino acids are used for synthesis of proteins in competition with amino acids synthesized from glucose [Dougall 1966]. During the whole storage period contents of asparagine, glutamine, proline, methionine, lysine, histidine and arginine were quite stable (tab. 2, 3). Most plants during amino acids metabolism process and catalyzing enzyme aminotransferase involved in glutamine and asparagine acids. During this process, the amino group of the amino acid glutamine is transferred to keto acid. Therefore consisted the keto acid, and from it – the amino acid asparagine. So it is thus possible to explain the basis for their content of tubers during storage. Proline biosynthesis is activated and its catabolism repressed during dehydration, whereas rehydration triggers the opposite regulation [Strizhov et al. 1997, Kiyosue et al. 1996, Verbruggen 1996, Deuschle et al. 2001, Xue et al. 2009]. Proline was shown to protect Complex II of the mitochondrial electron transport chain during salt stress and therefore stabilized mitochondrial respiration [Hamilton and Heckathorn 2001]. The recently discovered P5C-proline cycle can deliver electrons to mitochondrial electron transport without producing glutamate and, under certain conditions, can generate more ROS in the mitochondria [Miller 2009]. Proline catabolism is, therefore, an important regulator of cellular ROS balance and can influence numerous additional regulatory pathways. Although proline is usually considered to be a metabolite with protective functions, several reports show that, under certain conditions, exogenous proline can be deleterious to plants and can inhibit growth and cell division [Maggio et al. 2002]. Essential amino acid methionine, containing sulfur, synthesized from homocysteine which in turn – is formed from the *reciprocity* reaction between cysteine and homoserine.

Table 4. Correlation matrix between contents of chosen amino amino acids in tubers

	Tyrosine	Glycine	Phenilalanine	Izoleucine	Histidine	Leucine
Treonine	0.891**	0.765**	0.822**	–	–	0.824**
Valine	0.705**	0.851**	0.769**	0.821**	–	0.917**
Phenilalanine	0.801**	0.682*	–	0.551*	0.704**	0.828**
Leucine	0.721**	0.782**	0.828**	0.757**	–	–

* correlation significant at $P \leq 0.05$, ** correlation significant at $P \leq 0.01$

Relationship between amounts of some amino acids in the tubers during storage was also examined. Strong positive relationship was found between treonine and tyrosine, glicine, phenilalanine and leucine; as well as between valine and glycine, phenilalanine, leucine and izoleucine, also between leucine and glycine, tyrosine, as well as between phenilalanine and izoleucine (tab. 4).

CONCLUSIONS

1. At harvest and during storage period the dominating essential amino acids in tubers of all Jerusalem artichoke cvs. was arginine and from nonessential amino acids asparagine, glutamine and alanine

2. After 20-week storage, tubers of 'Sauliai' cv. accumulated the highest amount of essential amino acids, such as treonine, valine, isoleucine, leucine, phenylalanine and nonessential – serine, glycine, alanine, tyrosine.

3. Tubers of 'Sauliai' cv. are especially rich in amino acids and these tubers could be successfully used as a functional food.

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ZMIANY ZAWARTOŚCI AMINOKWASÓW W BULWACH ODMIAN TOPINAMURU (*Helianthus tuberosus* L.) PODCZAS PRZECHOWYWANIA

Streszczenie. Celem trzyletnich badań było określenie wpływu przechowywania pozbiorczego na zawartość egzogennych i endogennych aminokwasów w bulwach topinamburu (*Helianthus tuberosus* L.) trzech odmian uprawnych: ‘Rubik’, ‘Albik’ i ‘Sauliai’. Bulwy przechowywano przez okres do 20 tygodni w temperaturze 2 C (± 0,5 C) oraz warunkach wilgotności względnej (90–95%). Wyniki wskazują, że bezpośrednio po zbiorze oraz podczas przechowywania dominującym aminokwasem spośród amainokwasów egzogennych u wszystkich odmian była arginina, a spośród aminokwasów endogennych asparagina, glutamina i alanina. Bulwy odmiany ‘Sauliai’ po 20 tygodniach przechowywania akumulowały najwięcej egzogennych aminokwasów – treoniny, waliny, izoleucyny, leucyny, fenyloalaniny i endogennych – seryny, glicyny, alaniny i tyrozyny. Stwierdzono silne korelacje między zawartościami niektórych aminokwasów w bulwach.

Słowa kluczowe: topinambur, odmiany, arginina, żywność funkcjonalna

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