

Barbara SAWICKA¹ and Stanisław KALEMBASA²

**FLUCTUATION OF PROTEIN NITROGEN LEVEL
IN TUBERS OF *Helianthus tuberosus* L.
CAUSED BY VARYING LEVELS
OF NITROGEN FERTILISATION**

**FLUKTUACJA AZOTU BIAŁKOWEGO
W BULWACH *Helianthus tuberosus* L.
W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO**

Abstract: The effect of nitrogen fertilisation against potassium and phosphorus fertilisation on the total and true protein content in tubers of *Helianthus tuberosus* L. was examined. Two cultivars of the test plant (Albik and Rubik) were cultivated for 3 years in a field experiment on slightly acidic soil with a granulometric composition of loamy sand. The experimental objects were: 1) control – no fertilisation; 2) PK fertilisation at $\text{kg} \cdot \text{ha}^{-1}$; 52.4 – P and 149.4 – K and (against PK fertilisation) four levels of nitrogen fertilisation: 50, 100, 150 and $200 \text{ kg} \cdot \text{ha}^{-1}$. The total protein in tubers was calculated by multiplying the nitrogen content determined by the Kjeldahl method by 6.25, while the true protein content was determined by the Bernstein method. The protein content in tubers of the Jerusalem artichoke cultivars under study was significantly affected by their genetic features. The Rubik cultivar was found to contain significantly more of both forms of protein than the Albik cultivar. Irrespective of the cultivar, phosphorus and potassium fertilisation increased the total protein content compared with the control. The application of nitrogen at a dose as low as $50 \text{ kg} \cdot \text{ha}^{-1}$ increased the content of both forms of protein.

Keywords: Jerusalem artichoke, cultivar, total protein content, protein proper, share of proper protein in total protein

Introduction

Proteins are nutrients which determine the metabolism in live organisms. They are chemical compounds formed by the polycondensation of 18–20 amino acids. These are macromolecular biopolymers (with molecular weights ranging from approx. 10,000 to

¹ Department of Plant Production Technology and Commodity, University of Life Sciences in Lublin, ul. Akademicka 15, 20–950 Lublin, Poland, email: barbara.sawicka@up.lublin.pl

² Department of Soil Science and Agricultural Chemistry, Siedlce University of Natural Sciences and Humanities, ul. B. Prusa 14, 08–110 Siedlce, Poland, email: kalembasa@uph.edu.pl

several million Daltons) and biological polycondensates, made up of amino acid residues connected by peptide bonds $-CONH-$. Proteins occur in all live organisms and in viruses. Protein synthesis is mediated by special cell organelles called ribosomes. The number of amino acid residues in a single polypeptide chain is larger than 100 and the entire molecule can contain many polypeptide chains [5, 8, 21]. Due to their multiple functions (enzymatic catalysis, transport, storage, buffers, construction material, immune functions, regulators, etc.) proteins are very important. Crop plants, including Jerusalem artichoke, are a good source of proteins. Large doses of nutrients in the form of fertilisers, mainly nitrogen, phosphorus and potassium, increase the productivity of overground parts and tubers of *Helianthus tuberosus* L. [17, 19]. However, such large doses of those nutrients are suspected of reducing protein content and to increase nitrate accumulation in plants. The aim of the study was to examine the effect of nitrogen fertilisation at different doses used against potassium and phosphorus fertilisation on the total and true protein content in tubers of *Helianthus tuberosus* L.

Materials and methods

A field experiment was conducted at the Field Experimental Station in Parczew in 2003–2005, on slightly acidic soil with a granulometric composition of loamy sand. The experiment was conducted by the randomised blocks method in triplicate and the following factors were analysed:

- primary factors – cultivars (2 cultivars: Albik and Rubik);
- secondary factors – nitrogen doses (5 nitrogen doses, $\text{kg} \cdot \text{ha}^{-1}$), ie 0, 50, 100, 150 and 200 against a constant level of potassium and phosphorus fertilisation ($52.4 \text{ kgP} \cdot \text{ha}^{-1}$ and $149.4 \text{ kgK} \cdot \text{ha}^{-1}$). The mineral fertilisation was applied against manure fertilisation at $25 \text{ Mg} \cdot \text{ha}^{-1}$.

Phosphorus was applied as a granulated superphosphate 19 % (P_2O_5), potassium – as potassium salt 60 % (K_2O), while nitrogen was applied as urea 46 %. The nitrogen

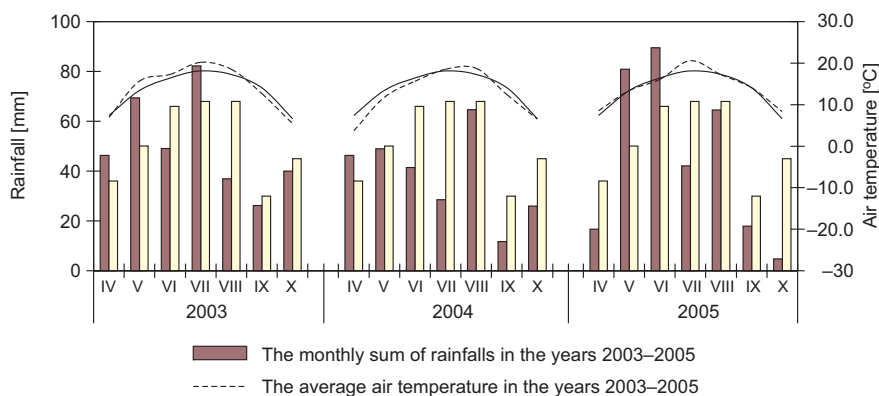


Fig. 1. Rainfalls and air temperature in vegetation periods of *Helianthus tuberosus* acc. to meteorological station of COBORU in Uhnin (years 2003–2005)

doses below and equal to $100 \text{ kg} \cdot \text{ha}^{-1}$ were applied on a one-off basis before tubers were planted; those above $100 \text{ kgN} \cdot \text{ha}^{-1}$ were applied twice: before tuber planting and after the plant sprouting, at phase 14 according to the BBCH scale. Tubers were planted in spring (on 26–27 April), spaced every 40 cm, with the rows spaced every 62.5 cm. The surface area of each plot was equal to 20 m^2 . All the agricultural procedures were performed in accordance with the requirements for the crop. The weather conditions in the years of the experiment varied. The year 2003 was average in terms of the rainfall level, while 2004 was dryish (Fig. 1) and 2005 was extremely dry. 50 tubers of different sizes were taken for laboratory analyses during the autumn (mid-October) harvest. The following were determined in the fresh matter of tubers: dry matter by drying, total protein – by converting the nitrogen content ($\times 6.25$) determined by the Kjeldahl method and true protein – by the Bernstein method [1]. The significance of the sources of variance was tested with the Fisher-Snedecor test and the significance of differences between average values was analysed by Tukey's test.

Experiment results

The genetic features of the cultivars under study proved to significantly affect the total and true protein in tubers of *Helianthus tuberosus*. The Rubik cultivar accumulated significantly more total and true protein in its tubers than the Albik cultivar. The feature variance was low, with the total protein content being more stable in the Rubik cultivar, while the true protein content was more stable in the Albik cultivar (Table 1).

Table 1

Effect of cultivars on the content of total and proper protein in the tubers of *Helianthus tuberosus* [$\text{g} \cdot \text{kg}^{-1}$ d.m.]

Specification	Cultivar				LSD _{0.05}
	Albik		Rubik		
	mean	C _v *	mean	C _v	
Total protein	111.36	10.44	121.72	8.18	3.84
Proper protein	58.38	7.66	63.23	11.04	2.06

* Variability coefficient; d.m. = dry matter.

Mineral fertilisation significantly modified the average total protein content and true protein content in both cultivars of *Helianthus tuberosus* tubers (Table 2, Fig. 2).

Phosphorus and potassium fertilisation increased the content of total protein in tubers as compared with the control (no fertilisation), while the content of true protein did not change significantly (Table 2). Nitrogen fertilisation had a decisive effect on protein content. A significant increase in the total and true protein content was observed in tubers harvested on plots fertilised with $50 \text{ kgN} \cdot \text{ha}^{-1}$, against constant phosphorus and potassium fertilisation. Larger doses of nitrogen did not cause the content of both protein forms to increase so clearly. The regression analysis showed the existence of

Table 2

Effect of mineral fertilisation and years on the content of total and proper protein in the tubers of *Helianthus tuberosus* [g · kg⁻¹ d.m.]

Fertilisation [kg · ha ⁻¹]	Total protein				Proper protein			
	Years			Mean	Years			Mean
	2003	2004	2005		2003	2004	2005	
0	85.50	110.40	90.48	95.46	42.38	64.92	53.65	53.65
P ₄₄ K ₁₂₅	95.75	129.78	86.74	104.09	41.20	68.07	54.63	54.63
N ₅₀ P ₄₄ K ₁₂₅	120.23	159.27	104.02	127.84	50.43	76.08	63.26	63.26
N ₁₀₀ P ₄₄ K ₁₂₅	116.18	137.30	87.37	113.61	51.27	71.92	61.59	61.59
N ₁₅₀ P ₄₄ K ₁₂₅	136.23	161.60	106.19	134.68	57.97	67.98	62.98	62.98
N ₂₀₀ P ₄₄ K ₁₂₅	132.95	139.40	98.35	123.57	73.27	64.17	68.72	68.72
LSD _{0,05}	34.56			11.52	18.52			6.17
Mean	114.47	139.63	95.53	116.54	52.75	68.86	60.80	60.80
LSD _{0,05}	5.76				3.09			

a third order curvilinear relationship between the content of total and true protein and mineral fertilisation, with R² equal to: 0.87 and 0.74, respectively (Fig. 2).

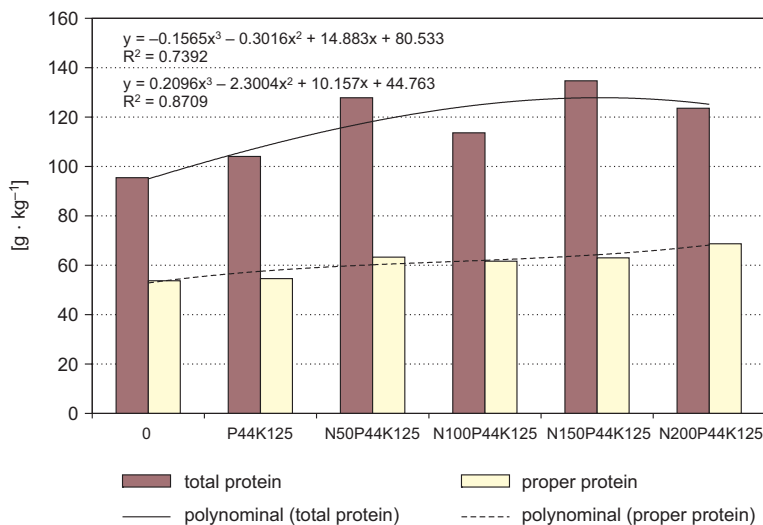


Fig. 2. Effect of mineral fertilization on total protein and proper content in tubers of *Helianthus tuberosus* (Mean 2003–2005)

The plant cultivars under study reacted differently to mineral fertilisation in regard to the accumulation of both protein forms. The phosphorus and potassium fertilisation did not affect the total and true protein content as compared with the control, whereas nitrogen fertilisation did. The total protein content in tubers of the Albik cultivar was found to increase on the plot fertilised with 150 kgN · ha⁻¹, while that in tubers of the

Rubik cultivar – on the plot fertilised with $50 \text{ kgN} \cdot \text{ha}^{-1}$ (Fig. 3). As compared with the control plot, the true protein content in tubers of the Albik cultivar was found to increase significantly on the plot fertilised with $50 \text{ kgN} \cdot \text{ha}^{-1}$, while that in tubers of the Rubik cultivar increased significantly on the plot fertilised with $200 \text{ kgN} \cdot \text{ha}^{-1}$ (Fig. 4).

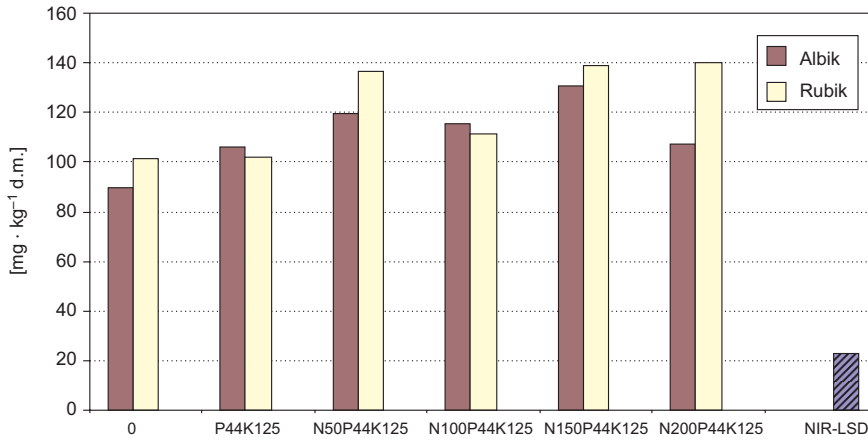


Fig. 3. Effect of cultivars and mineral fertilization on the content of total protein in the tubers of *Helianthus tuberosus* (mean 2003–2005)

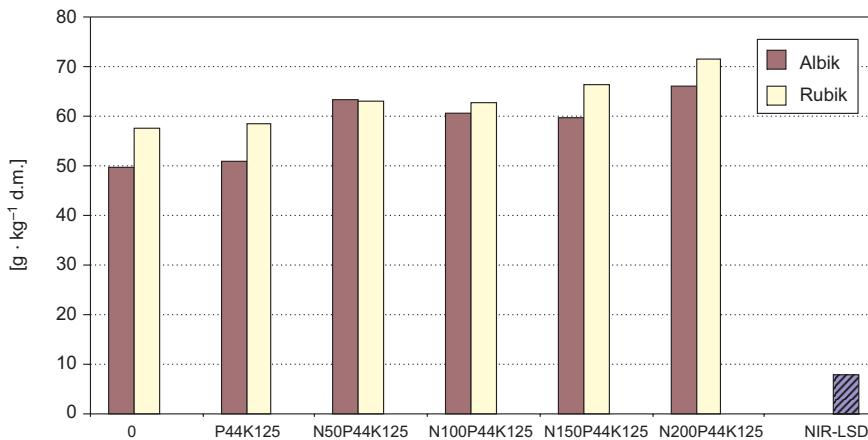


Fig. 4. Effect of variety and mineral fertilization on the content of protein proper in the tubers of *Helianthus tuberosus* (mean 2003–2005)

The effect of mineral fertilisation, compared with the control, depended on the weather conditions during the years of the study (Table 2). In the years 2003–2004, the total protein content increase was found to be caused by fertilisation with 50 kg of $\text{N} \cdot \text{ha}^{-1}$, against phosphorus and potassium fertilisation. In 2005, with a drought lasting from July to the end of October (during the period of tuberisation and tuber growth), no significant changes in the total protein content were found to be caused by mineral

fertilisation (Table 2). True protein content on plots fertilised with $200 \text{ kgN} \cdot \text{ha}^{-1}$ was found to increase only in 2003, when the air temperature was higher than the multi-year average and the total rainfall was close to the multi-year average. Phosphorus and potassium fertilisation alone did not significantly affect the value of the feature (Table 2).

The cultivar effect was observed together with the year of study (Table 3). The Rubik cultivar accumulated significantly more total protein in tubers than the Albik cultivar in 2003–2004, whereas no cultivar-related differences were observed in 2005. No year-related differences for the cultivars were recorded in the true protein content.

Table 3

Effect of cultivars and years on the content of selected elements in dry mass of aboveground parts of Jerusalem artichoke [$\text{g} \cdot \text{kg}^{-1}$ d.m.] (mean for 2003–2005)

Years	Total protein		Proper protein	
	Cultivars			
	Albik	Rubik	Albik	Rubik
2003	108.60	120.30	50.22	55.29
2004	133.00	146.20	66.54	71.27
2005	92.40	98.60	58.38	63.23
LSD _{0.05}	11.52		n*	

* not significant at $\alpha \leq 0.05$.

The proportion of true protein in the total protein, as an important factor in the evaluation of the protein value, depended on mineral fertilisation, cultivar and year of study, and was co-affected by those factors. Mineral fertilisation modified the value of the feature significantly. The proportion of true protein in total protein was found to decrease significantly on plots fertilised with $150 \text{ kgN} \cdot \text{ha}^{-1}$ as compared to the control. An analysis of regression showed the existence of the 5th order polynomial relationship between the proportion of true protein in total protein and fertilisation, with the determination coefficient $R^2 = 1.0$ (Fig. 5).

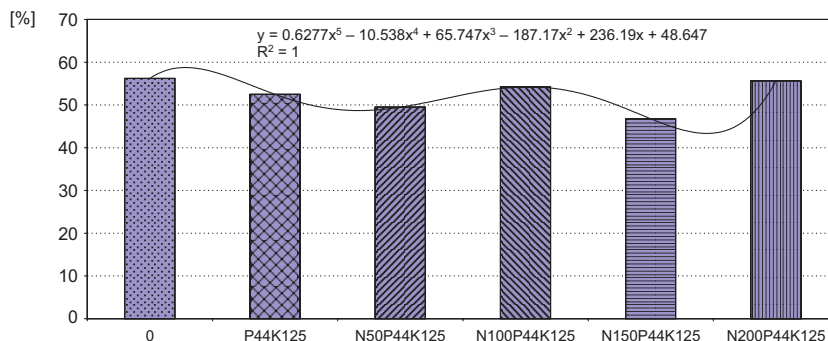


Fig. 5. Effect of mineral fertilization on the share of proper protein in total protein

The value of the feature also depended on the cultivar reaction on mineral fertilisation (Table 3). The highest proportion of true protein in total protein for the Rubik cultivar was found in the control plot; for the Albik cultivar it was on plots fertilised with 200 kg of N · ha⁻¹. The relationship for the Albik cultivar can be described by the 2nd order parabolic regression equation; for the Rubik cultivar – by the 4th order polynomial regression equation. The determination coefficients for those equations were equal to: R² = 0.81 and 0.99, respectively (Fig. 6). The highest proportion of true protein in total protein in 2003, with sufficient rainfall level, was caused by fertilisation at 200 kgN · ha⁻¹; in dry 2005 – by fertilisation with 100 kgN · ha⁻¹, and in extremely dry 2004 – on the control plot (Fig. 7).

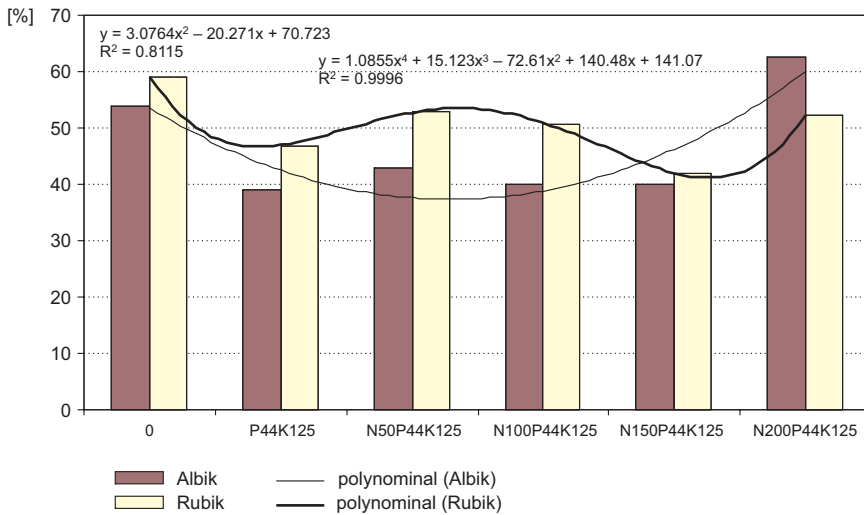


Fig. 6. Effect of mineral fertilization and variety on the proportion of proper protein to total protein

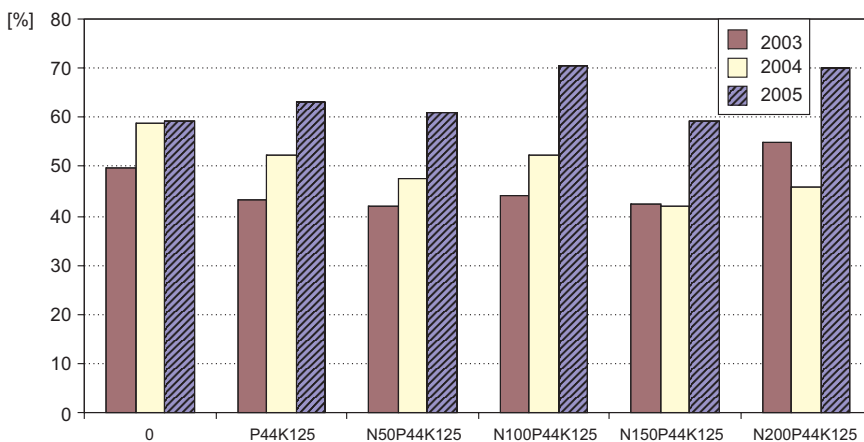


Fig. 7. Effect of mineral fertilization and years on the share of proper protein in total protein

Discussion

Proteins of tubers of *Helianthus tuberosus* stand out among plant proteins in terms of their biological value. They contain exogenous amino acids, including methionine and tryptophan [5]. The average content of total protein in dry matter of tubers of *Helianthus tuberosus* ranged from 78.38 to 170.80 g · kg⁻¹, and that of true protein from 42.20 to 72.90 g · kg⁻¹. According to Mikos-Bielak et al [13], the content of total protein in dry matter ranged from 87.1 to 134.3 g · kg⁻¹, and that of true protein from 43.2 to 57.0 g · kg⁻¹. The findings of a study by Florkiewicz et al [7] showed tubers of the species to contain 65–86 g · kg⁻¹ of total protein. Those values are higher than the tabular data on the potato and root vegetables [5, 6]. A wider range of variance for total and true protein content in tubers is a result of the effect of biotic and abiotic factors, such as: cultivar-related factors, habitat conditions, agrotechnical factors and harvest time [3, 8, 9, 11].

Nitrogen fertilisation had a significant effect on total and true protein content in tubers of *Helianthus tuberosus*. The dose of 50 kg of N · ha⁻¹ has proven to be a safe one, which does not reduce the protein content. These findings have been corroborated by a study conducted by Berghofer and Reiter [2]. An earlier study conducted by Sawicka [17, 19] showed that 100 kgN · ha⁻¹ is such a dose in regard to tuber quality. In the opinion of Mikos-Bielak et al [13], nitrogen fertilisation increases the protein content in a plant and, in consequence, changes its amino acid profile. Changes of proportions between exogenous amino acids, especially lysine and methionine, seem to be of special importance as their deficit, according to many authors [5, 14–16], decreases the nutritional value of the proteins of *Helianthus tuberosus*.

The proportion of true protein in total protein in dry matter of *Helianthus tuberosus* tubers was equal to 46.8–56.2 % – depending on mineral fertilisation, 46.4–50.7 % – depending on the cultivar and 46.1–63.8 % – depending on the year of the experiment. True protein accounts for approx. 60 % of all the nitrogen-containing substances found in tubers of *Helianthus tuberosus*, with amides, peptones, ammonia, nitrates, nitrites accounting for the other 40 % [5, 13, 14, 20]. According to Praznik et al [15] and Florkiewicz et al [7], tubers harvested in autumn contain more protein than those harvested in spring. A different tendency was shown in a study conducted by Ververde et al [21] and Berghofer and Reiter [2], which probably resulted from differences in vegetation and cultivation conditions of various cultivars.

The cultivar-related properties modified the content of total and true protein. The results are similar to the findings of other studies [3, 4, 13, 17–19]. According to Ververde et al [21], the cultivar with reddish pigmentation of the peel contain more proteins than those with white peel. According to Sawicka [18, 19], the differences in protein content are caused by the phenotypic variation of the cultivars of Jerusalem artichoke, which is the combined effect of genetic and environmental variation. Jasiewicz et al [10] suggest that stressful conditions (prolonged drought or excessive rainfall) could disrupt chloroplast membrane function, which directly reduces productivity of the PS II system, which is the most sensitive indicator of the action of different

stressful factors. The diversity of the conditions in which the species grows modifies internal regulation processes within the entire plant and its shoots.

The soil and weather conditions caused the greatest modifications of protein accumulation in tubers. Prośba-Białczyk [16] found tubers of plants cultivated on heavy soil to contain less protein than those cultivated on medium-heavy soil. The variance of weather-related factors in the habitat (rainfall and temperature) can affect the tuber yield by modifying the rate of plant growth and development and can also affect their chemical composition [3, 18, 19, 21–23].

Conclusions

1. The cultivar-related properties of Jerusalem artichoke proved to significantly affect the total and true protein in tubers of the plant. The Rubik cultivar accumulated significantly more total and true protein than did the Albik cultivar. Elucidating the phenotypic variance of the cultivars of *Helianthus tuberosus* and identifying, separately, genetic and environmental variance, would enable choosing a cultivar with the highest stability of the desired feature for cultivation.

2. Irrespective of the cultivar, phosphorus and potassium fertilisation increased the total protein content in tubers of *Helianthus tuberosus* as compared with the control. The true protein content was not significantly changed by phosphorus and potassium fertilisation alone.

3. Total and true protein accumulation was changed differently in both cultivars by increasing doses of nitrogen. The total protein content in tubers of the Rubik cultivar was found to increase significantly on the plot fertilised with $50 \text{ kgN} \cdot \text{ha}^{-1}$, while that in tubers of the Albik cultivar increased on the plot fertilised with $150 \text{ kgN} \cdot \text{ha}^{-1}$. The true protein content in tubers of the Rubik cultivar was found to increase on the plot fertilised with $200 \text{ kgN} \cdot \text{ha}^{-1}$, while that in tubers of the Albik cultivar increased on the plot fertilised with $50 \text{ kgN} \cdot \text{ha}^{-1}$.

4. The dose of $50 \text{ kgN} \cdot \text{ha}^{-1}$ proved to be an environmentally safe dose of nitrogen fertilisation of Jerusalem artichoke. The proportion of true protein in total protein was found to decrease significantly on plots fertilised with $150 \text{ kgN} \cdot \text{ha}^{-1}$ against phosphorus and potassium fertilisation.

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**FLUKTUACJA AZOTU BIAŁKOWEGO W BULWACH *Helianthus tuberosus* L.
W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO**

¹ Katedra Technologii Produkcji Roślinnej i Towaroznawstwa
Uniwersytet Przyrodniczy w Lublinie

² Katedra Gleboznawstwa i Chemii Rolniczej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach

Abstrakt: Badano wpływ nawożenia azotem, na tle nawożenia fosforowo-potasowego, na zawartość białka ogólnego i właściwego w bulwach *Helianthus tuberosus*. W 3-letnim doświadczeniu polowym, na glebie lekko kwaśnej, o składzie granulometrycznym piasku gliniastego mocnego, oceniano dwie odmiany słonecznika bulwiastego (Albik i Rubik). Obiektami doświadczenia były: 1) kontrola bez nawożenia, 2) cztery poziomy nawożenia azotem: 50, 100, 150 i 200 kg · ha⁻¹ na tle stałego nawożenia fosforowo-potasowego (52,4 kgP · ha⁻¹ i 149,4 kgK · ha⁻¹) i pełnej dawki obornika (25 Mg · ha⁻¹). Całkowitą zawartość białka w bulwach określano, mnożąc zawartość azotu ogólnego oznaczonego metodą Kjeldahla przez 6,25, a zawartość białka właściwego oceniano metodą Bernsteina. Zawartość białka w bulwach *Helianthus tuberosus* zależała istotnie od cech genetycznych badanych odmian. Stwierdzono, iż odmiana Rubik zawierała znacznie więcej obu form białka niż odmiana Albik. Nawożenie fosforowo-potasowe zwiększało poziom białka ogólnego, w stosunku do obiektu kontrolnego, w bulwach *Helianthus tuberosus*, niezależnie od odmiany. Stosowanie azotu w dawce 50 kgN · ha⁻¹ powodowało istotny wzrost zawartości obu form białka.

Słowa kluczowe: słonecznik bulwiasty, odmiany, zawartość białka ogólnego i właściwego, udział białka właściwego w białku ogółem

