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COMPARATIVE ANALYSIS OF THE BIOLOGICAL VALUE OF PROTEIN OF *Chenopodium quinoa* WILLD. AND *Chenopodium album* L. PART I. AMINO ACID COMPOSITION OF THE SEED PROTEIN

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Abstract. The research concerned amino acid content in protein and the yield of amino acids from seeds of *Chenopodium quinoa* and *Chenopodium album*. *Chenopodium quinoa* seed material, cv. Sandowal, came from Germany, and *Chenopodium album* – from the commonly available forms occurring in Poland. Seeds of both *Chenopodium* species were characterized by beneficial amino acid composition, especially by the lysine content. Amino acid proportion profiles in the seeds protein of both studied species showed that their composition was different. Biological value of the protein of *Chenopodium quinoa* measured with the essential amino acid index (EAAI) was higher than the protein value of *Chenopodium album*. However, *Chenopodium quinoa* significantly exceeded *Chenopodium album* with yield of both exogenous and endogenous amino acids as well as with the yield of all amino acids.

Key words: amino acid yield, essential amino acid index, EAAI, limiting amino acid index, pseudocereals

INTRODUCTION

One of the basic components deciding about the nutritive value of plant products is protein. It has a genetically conditioned structure, and it is also characterized by its specific content for every species. Appropriate agricultural techniques allow its content and composition modifications [Wróbel 1993, Barczak 1995, Cwojdziński and Nowak 2000, Tabe et al. 2002], but only to a certain extent. In most species, increase of the rate of nitrogen fertilization results in a decrease of the proportion of exogenous amino acids in protein, and thus in the decrease of the nutritive value [Barczak 1995, Lubowicki et al. 1997]. One of the plants with a high protein content is *Chenopodium quinoa* used as

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a food source for people and animals since the Inca civilization. At that time it was considered as the mother of cereals. Today everyone knows that it is one of the oldest crop plants, included in the group of the so-called 'pseudocereals'. *Chenopodium quinoa* is cultivated and grown in a lot of South American countries, but also in Europe, where it has recently enjoyed a large interest among researchers [Grochowski 2000, Dębski and Gralak 2001, Gęsiński 2008, Martinez et al. 2009, Gonzáles et al. 2010, Vega-Gálvez 2010]. Seeds of this species are distinguished by high nutritive value because of its very good chemical composition, high proportion of vitamins, microelements, fat, including essential unsaturated fatty acids (EFA), mainly linoleic and linolenic acids [Coulter and Lorenz 1990], as well as squalene [Jahaniaval et al. 2000, Ryan et al. 2007]. However, the greatest advantage of this plant is the content and quality of protein.

Chenopodium quinoa is morphologically very similar to *Chenopodium album*, which is a commonly occurring weed. However, from the point of view of agriculture these species differ with several significant traits [Gęsiński 2004]. *Chenopodium album* was used in Poland in hard times of crop failure and hunger, as well as during war. Seeds of this species were also used for baking the so-called 'hunger-bread' and were also eaten by inhabitants of Russia, Denmark, Greece and Northern Italy. Up to this time *Chenopodium album* has been cultivated in Asia [Grochowski 1996].

Research hypothesis assumed that protein in seeds of *Chenopodium quinoa* and *Chenopodium album* may differ in the biological value.

The aim of the research was the study of the composition and yield of amino acids in seed proteins of *Chenopodium quinoa*, the crop plant coming from South America, compared to *Chenopodium album*, commonly occurring in Europe.

MATERIAL AND METHODS

Material for analysis of the amino acid composition and quality of seed protein of *Chenopodium quinoa* cv. Sandowal and *Chenopodium album* came from the field experiment conducted at the Experimental Station of Cultivar Testing in Chrząstowo (53°09' N; 17°35' E) in the years 2006-2008. The field experiment was carried out as a single-factor experiment in the randomized subblock design, in four replications, on the soil of the very good rye complex. Row spacing on plots of the area 11.2 m^2 was 40 cm. Sowing density of 9 $kg \cdot ha^{-1}$ of seeds was applied, as well as fertilization of 60 $kg \cdot ha^{-1}$ nitrogen, 21 $kg \cdot ha^{-1}$ phosphorus and 60 $kg \cdot ha^{-1}$ potassium. The following seed yields were harvested: *Chenopodium quinoa* cv. Sandowal – 1.95 $t \cdot ha^{-1}$, *Chenopodium album* – 1.48 $t \cdot ha^{-1}$. Samples for analysis were collected from each plot.

Chenopodium quinoa cv. Sandowal was characterized by the following agricultural-functional properties (commercial information): length of the growing season – 135 days, plant height – 140 cm, weight of 1000 seeds – 2.7 g, cultivar resistant to cold, health – good, susceptibility to seed shedding – low, fertility – good, protein content in seeds – very high, protein content in the green matter – very high, requirements for farming standard – average to high, required soil fertility – average.

Seed material of *Chenopodium album* came from the forms commonly occurring in Poland, and *Chenopodium quinoa* from Germany.

In the studied seed samples of *Chenopodium quinoa* and *Chenopodium album* the total nitrogen content was determined with the use of Kjeldahl method, and next the total protein was calculated using the conversion factor 6.25.

Amino acid content was determined after acid hydrolysis [Rakowska et al. 1978] with high-pressure liquid chromatography HPLC [Lindroth et al. 1979]. Separation of amino acids was done with HPLC analyzer (Knauer GmbH, Germany) equipped with an automatic feed pump, column 250 × 4 mm with Hipersil packing ODS 5 μm and a fluorescent detector. Separation of amino acids was done using buffer solutions: 12.5 mM sodium phosphate with pH 6.5 with 10% methanol as well as methanol with addition of 3% tetrahydrofuran. Amino acid detection was conducted with fluorescence photometer (measurement range: Ex 340, Em 455 nm), after preliminary aldehydation of amino acids with ortho-phthaldialdehyde (OPA) and mercaptoethanol. Amino acid content was calculated from the plot area of peaks compared with standard peaks using software Eurochrom 2000 for Windows (Knauer GmbH, Germany). Standard peaks were obtained from standardized measurements of solutions of proteinous amino acids. Analyses from each sample were conducted in two replications.

On the basis of amino acid composition, the total of exogenous and endogenous amino acids was calculated. For seed protein of the analyzed species of *Chenopodium* the following parameters were calculated: essential amino acid index (EAAI) as an n root (number of amino acids included in the calculation) of the ratio product of amino acid content in protein of the studied sample and in the chicken egg protein according to Oser [1951] – and the limiting amino acid index expressed as a percentage (Chemical Store – CS).

Statistical evaluation of obtained results was conducted in two stages. First, comparison of formation of amino acid content in the studied protein of both species was done with multivariable method, profile analysis.

For profile comparison, Cohen's profile similarity coefficient r_c was used which was calculated from the formula:

$$r_C = \frac{\sum_{i=1}^{n} A_i B_i + nm^2 - m\left(\sum_{i=1}^{n} A_i + \sum_{i=1}^{n} B_l\right)}{\sqrt{\left(\sum_{i=1}^{n} A_i^2 + nm^2 - 2m\sum_{i=1}^{n} A_i\right)\left(\sum_{i=1}^{n} B_i^2 + nm^2 - 2m\sum_{i=1}^{n} B_i\right)}}$$

where:

A_i, B_i – unitarized values of traits included in the compared profiles A and B,
n – number of traits in the profile,
m – midpoint of the ranking scale.

This coefficient value was measured in the range from (–1.0) to (1.0). With $r_c < 0$, profile similarity was evaluated as negative, with $r_c > 0$, the similarity was positive, whereas with $r_c = 0$, lack of profile similarity was found. The closer were the values of r_c to boundary values (–1) or (+1), the stronger was the evaluated similarity.

Before starting the analysis, conversion of the data values was done for all traits separately into the same range scale. Obtained results were in the 9-point scale, in which trait value within the studied group was: 1 – very low, 2-4 – low, 5-6 – average, 7-8 – high, 9 – very high [Brzeziński 2002].

At the second stage, variation development of particular amino acids of both *Chenopodium* species was compared with the use of t-Student test. It concerned the amino acid content in the studied protein and in the seeds. The amino acid yield was also calculated. Analysis results are presented in tables.

While preparing both parts of this paper, computer program EXCEL was used as well as the statistical package STATISTICA.

RESULTS

It was found that seeds of *Chenopodium quinoa* cv. Sandowal and *Chenopodium album* are characterized by a large content of total protein (176 $g \cdot kg^{-1}$ and 142 $g \cdot kg^{-1}$ respectively). Comparative analysis of protein profiles of *Chenopodium quinoa* and *Chenopodium album*, based on the amino acid proportion (Fig. 1), proved lack of similarity on the basis of the calculated Cohen's profile similarity index r_c (coefficient value 0.147).

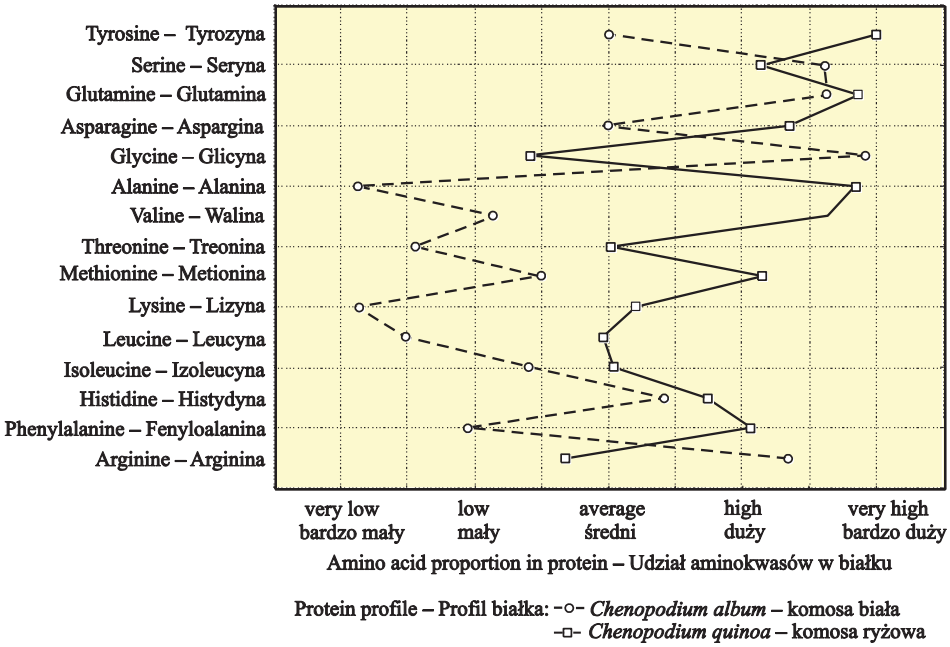

Fig. 1. Profiles of amino acid proportion in the seed protein of *Chenopodium quinoa* and *Chenopodium album*

Rys. 1. Profile udziału aminokwasów w białku nasion komosy ryżowej i komosy białej

Analysis of the amino acid composition proved lack of significant differences between analyzed species in the content of three exogenous amino acids: phenylalanine, histidine and isoleucine. Part of other exogenous amino acids differed significantly in favour of *Chenopodium quinoa*, with the exception of arginine, which was higher in the protein of *Chenopodium album* seeds (Table 1). As a result, the total of exogenous amino acids was slightly, though significantly, higher in *Chenopodium quinoa*.

Table 1. Amino acid content and yield in seeds of *Chenopodium quinoa* cv. Sandowal and *Chenopodium album*
Tabela 1. Zawartość i plon aminokwasów w nasionach komosy ryżowej odmiany Sandowal i komosy białej

Amino acid Aminokwas	Content in protein – Zawartość w białku g·16 g^{-1} N			Content in seeds – Zawartość w nasionach g·kg^{-1}			Yield – Plon kg·ha^{-1}		
	Chenopodium quinoa komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$	*Chenopodium quinoa* komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$	*Chenopodium quinoa* komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$
Arginine – Arginina	10.6	12.1	1.12	18.66	17.18	1.45	36.4	25.4	3.21
Phenylalanine – Fenyloalanina	3.50	3.45	ns – ni	6.16	4.90	0.95	12.0	7.3	1.66
Histidine – Histydyna	3.80	3.72	ns – ni	6.69	5.28	0.87	13.0	7.8	1.52
Isoleucine – Izoleucyna	2.39	2.35	ns – ni	4.21	3.34	0.69	8.2	4.9	1.21
Leucine – Leucyna	5.63	5.34	0.23	9.91	7.58	0.38	19.3	11.2	0.66
Lysine – Lizyna	6.98	5.68	0.52	12.28	8.07	0.85	24.0	11.9	1.49
Methionine – Metionina	1.75	1.60	0.14	3.08	2.27	0.23	6.0	3.4	0.40
Threonine – Treonina	4.01	3.77	0.21	7.06	5.35	0.34	13.8	7.9	0.60
Valine – Walina	2.83	2.62	0.18	4.98	3.72	0.30	9.7	5.5	0.52
Σ of exogenous amino acids Σ aminokwasów egzogennych	41.5	40.6	0.84	73.0	57.7	1.38	142	85	2.41
Alanine – Alanina	3.16	2.36	0.35	5.56	3.35	0.57	10.8	5.0	1.00
Glycine – Glicyna	5.88	7.30	0.46	10.35	10.37	ns – ni	20.2	15.3	1.32
Aspartic acid Kwas asparaginowy	4.28	4.18	ns – ni	7.53	5.94	1.18	14.7	8.8	2.07
Glutamic acid Kwas glutaminowy	9.45	9.55	ns – ni	16.63	13.56	1.62	32.4	20.1	2.84
Serine – Seryna	4.34	4.55	ns – ni	7.64	6.46	0.43	14.9	9.6	0.75
Tyrosine – Tyrozyna	4.15	3.70	0.26	7.30	5.25	0.79	14.2	7.8	0.75
Σ of endogenous amino acids Σ aminokwasów endogennych	31.3	31.6	ns – ni	55.0	44.9	2.45	107	67	4.29
Σ tested – Σ badanych	72.8	72.2	ns – ni	128.0	102.6	3.46	249	152	6.06

ns – ni – non-significant differences – różnice nieistotne

However, protein of the studied species did not differ in the total of endogenous amino acids as well as in the total of all amino acids. Among analyzed endogenous amino acids, the content of aspartic acid and glutamic acid as well as serine in protein did not differ in both *Chenopodium* species. *Chenopodium quinoa* was characterized by higher content of alanine and tyrosine, however glycine was higher in protein of *Chenopodium album*. The content of all amino acids, with the exception of glycine, was significantly higher in *Chenopodium quinoa*. This species was also characterized by the higher total of exogenous and endogenous amino acids as well as of all studied amino acids.

Higher yields of amino acids from *Chenopodium quinoa* were obtained than from *Chenopodium album*. The yield of all amino acids from *Chenopodium quinoa* (apart from arginine and glycine) were higher by 60% than amino acid yields from *Chenopodium album*, and the yields of lysine and alanine themselves were even by 100% higher (Table 1). Comparison of seed protein of *Chenopodium quinoa* and *Chenopodium album* was done also on the basis of the essentials amino acid index (EAAI). *Chenopodium quinoa* was distinguished by its higher value (Table 2). On the basis of analysis of the value of limiting amino acid index (CS) it was found that isoleucine is the first, and valine the second amino acid reducing nutritive value of the seed protein of both *Chenopodium quinoa* and *Chenopodium album*.

Table 2. Value of the essential amino acid index (EAAI) and limiting amino acid index (CS) of seed protein of *Chenopodium quinoa* and *Chenopodium album*

Tabela 2. Wartości wskaźnika aminokwasów egzogennych (EAAI) i wskaźnika aminokwasów ograniczających (CS) białka nasion komosy ryżowej i białej

Index – Wskaźnik	*Chenopodium quinoa* Komosa ryżowa	*Chenopodium album* Komosa biała
EAAI ($LSD_{0.05}$ – $NIR_{0,05}$ 2,11)	72.8	69.5
CS I – isoleucine – izoleucyna	35	34
CS II – valine – walina	38	35

DISCUSSION

One of the most important arguments convincing us to cultivate *Chenopodium quinoa* in Poland is the rich chemical composition of its seeds [Jacobsen 2000, Pulvento et al. 2010]. The content of nutrients is higher than in the seeds of many cultivated now plant species [Wahli 1990, Grochowski 2000, Vega-Gálvez et al. 2010]. *Chenopodium quinoa* is a good source of minerals and vitamins [Gorinstein et al. 2002]. Lack of gluten gives the possibility to use *Chenopodium quinoa* in the gluten-free diet in patients with celiac disease [Jacobsen 2003, Pulvento et al. 2010]. Interesting also seems to be the fact of squalene presence in the seeds [Jahaniaval et al. 2000, Ryan et al. 2007], thanks to which *Chenopodium quinoa* may constitute an alternative source of this component. High nutritional qualities of *Chenopodium quinoa* seeds, and of *Chenopodium album* seeds, also result from the large protein content of well-balanced composition. Balzotti et al. [2008] state that it reaches the level of 126-137 $g \cdot kg^{-1}$. It is confirmed by Aufhammer [2000], while Ahamed et al. [1998] give the content of 190 $g \cdot kg^{-1}$. It is higher than that of cereals: rice, corn and millet [Charalampopoulos et al. 2002], as well as wheat, barley and oat [Wahli 1990, Grochowski 1996]. In own research, the protein content in *Chenopodium quinoa* cv. Sandowal was found to be 176 $g \cdot kg^{-1}$ and in *Chenopodium*

album 142 g·kg^{-1}. Amino acid composition of seed protein of *Chenopodium quinoa* exceeds FAO recommendations concerning protein consumption [Ruas et al. 1999]. Particularly large content of lysine and amino acids containing sulfur (cystine and methionine) was found. These amino acids occur most often in small amounts in grain of cereals and legume plants. It is confirmed by own research results.

It was found that protein of *Chenopodium quinoa* is characterized by the high level of polymorphism, similarly to protein of soybean, buckwheat and amaranth [Drzewiecki et al. 2003]. These species are phylogenetically distant, however similarities of their soluble fraction of protein and in the amino acid composition were confirmed. Thus, these plants may be substituted by each other, as well as they can constitute supplementation with nutrients for the commonly consumed cereals [Drzewiecki et al. 2003].

Seed protein of *Chenopodium quinoa* and *Chenopodium album* was characterized by similar and large proportion of exogenous amino acids (app. 57%), while with other species cultivated in Poland these values were much lower. Protein of winter barley contained maximum 40.6% of exogenous amino acids [Barczak et al. 1994], and protein of spring barley, rape and winter wheat below 50%, only in potato this proportion was 51% [Cwojdziński and Nowak 2000]. Essential amino acid index for winter barley was 54, while for *Chenopodium album* 70, and for *Chenopodium quinoa* it exceeded 70. From among plants cultivated in Poland, *Chenopodium quinoa* and *Chenopodium album* were only equaled by bean with regard to this index value [Nowak et al. 2007], as well as by potato cultivated on manure. Spring barley, rape and winter wheat were characterized by its lower value [Cwojdziński and Nowak 2000]. Biological value of rye grain protein, evaluated on the basis of the above indexes, was also lower than of both analyzed *Chenopodium* species [Nowak and Barczak 1996].

One of the amino acids of seed protein of *Chenopodium quinoa* and *Chenopodium album* which shows much higher content than in the cereal grain is lysine. In protein of both winter barley and winter wheat, it constitutes an amino acid which reduces the biological value of proteins [Barczak and Nowak 1995, Nowak and Majcherczak 2002]. This amino acid content in the protein of *Chenopodium quinoa* and *Chenopodium album* seeds exceeds not only all cereals but also amaranth, and *Chenopodium quinoa* even exceeds soybean [Ahamed et al. 1998]. Both analyzed species of *Chenopodium* are also characterized by the higher content of this amino acid than the protein standard determined by FAO/WHO. Higher biological value of the seed protein of *Chenopodium quinoa* and *Chenopodium album* compared to cereals, but also to amaranth, results from the content of such exogenous amino acids as arginine, phenylalanine, histidine, leucine, and threonine, except lysine [Ahamed et al. 1998, Aufhammer 2000]. Comparing the amino acid yield of the studied species with cereals it appears that these relations are different. *Chenopodium album* in the case of most amino acids reaches lower yields, except arginine and histidine, which are higher by app. 9 and 2 kg·ha^{-1}. However, *Chenopodium quinoa* is characterized by higher amino acid yields compared with cereals. In oat grain, only valine and phenylalanine from exogenous amino acids have higher yields, and from endogenous amino acids aspartic acid and glutamic acid [Barczak et al. 2006, Majcherczak et al. 2006]. Therefore, exogenous amino acid yield from *Chenopodium quinoa* is by 40 kg·ha^{-1} higher than from oat, similarly to the yield of all amino acids (by app. 15 kg·ha^{-1}). These facts prove not only the high biological value of the protein of both *Chenopodium* species, but also its high concentration in the yield. In relation to cereals, *Chenopodium quinoa* is also characterized by much higher concentration of exogenous amino acids, especially lysine.

CONCLUSIONS

1. Seeds of the analyzed *Chenopodium* species are characterized by the beneficial amino acid composition with regard to the exogenous amino acid content, especially lysine.

2. Profiles of the amino acid proportion in the seed protein of both studied species indicate that their composition differs. Measured with the essential amino acid index (EAAI), biological value of seed protein of *Chenopodium quinoa* is higher than of *Chenopodium album* protein.

3. *Chenopodium quinoa* significantly exceeds *Chenopodium album* with the yield of both exogenous and endogenous amino acids, as well as with the yield of all amino acids.

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ANALIZA PORÓWNAWCZA WARTOŚCI BIOLOGICZNEJ BIAŁKA KOMOSY RYŻOWEJ (*Chenopodium quinoa* WILLD.) I KOMOSY BIAŁEJ (*Chenopodium album* L.) CZ. I. SKŁAD AMINOKWASOWY BIAŁKA NASION

Streszczenie. Badania dotyczyły zawartości aminokwasów w białku i plonu aminokwasów nasion komosy ryżowej i komosy białej. Nasiona komosy ryżowej odmiany Sandowal pochodziły z Niemiec, a komosy białej z powszechnie występujących w Polsce form. Nasiona obu gatunków komosy odznaczały się korzystnym składem aminokwasowym, szczególnie zawartością lizyny. Profile udziału aminokwasów w białku nasion obu gatunków pokazały, że ich skład się różnił. Mierzona wskaźnikiem aminokwasów egzogennych (EAAI) wartość biologiczna białka nasion komosy ryżowej była wyższa niż białka komosy białej. Komosa ryżowa znacząco przewyższała jednak komosę białą plonem zarówno aminokwasów egzogennych, jak i endogennych oraz plonem wszystkich aminokwasów.

Słowa kluczowe: plony aminokwasów, pseudozboża, wskaźnik aminokwasu ograniczającego, wskaźnik aminokwasów egzogennych (EAAI)

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