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Protein nutritional value of rocket leaves and possibilities of its modification during plant growth

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Abstract: Leafy vegetables are an important source of protein and other nutrients. Vegetable protein is characterized by different and variable amino acid composition. This study determined the protein content and amino acid composition of the leaves of garden rocket (*Eruca sativa* Mill.) as affected by different nitrogen and potassium nutrition regimes. On average, the rocket leaves accumulated 11.7% dry matter, including 16.6% protein. Increased nitrogen rates resulted in a significant decrease in protein content as opposed to increased potassium rates. In the majority of cases, the increasing rates of nitrogen and potassium used in the nutrition of rocket plants significantly modified the amino acid composition of protein, which contained all primary amino acids, both nonessential and essential. In the group of nonessential amino acids, glutamic acid and aspartic acid were predominant. The proportions of all the amino acids determined were substantially lower than in the FAO/WHO reference guidelines. The greatest variation was found in the case of threonine and lysine, while the smallest was for sulfur amino acids. It was shown that the rocket leaves were characterized by a high content of protein with a beneficial amino acid composition, and rocket should be taken into consideration as a diet component. Modifications in the plant nutrition system can be used to increase the amount of rocket protein and to obtain its specific chemical profile.

Key words: *Eruca sativa*, plant protein, amino acids

1. Introduction

Proper nutrition is closely related to the state of human health, both physical and mental. A diverse diet that includes various groups of food products guarantees the supply of the complete range of nutritional and health-promoting substances. Plant products contain on average 1%–2% protein, although the content of this component in some vegetables is much higher (Kunachowicz et al., 2005). Plant proteins, even though they do not contain a sufficient amount of indispensable amino acids, are recommended in Western-type diets as a way to reduce the risk of chronic diseases (Young and Pellett, 1994). Among plant proteins, the protein of leguminous vegetables and nuts is characterized by the highest nutritional value.

The leaves of some plants can be used as a raw material to obtain protein concentrates, in particular those with a high level of protein (about 10% DM) and appropriate amino acid composition. For example, the leaves of alfalfa, spinach, marrow stem kale, mustard, and maize are used for this purpose (Gawęcki, 1998). The garden rocket (*Eruca sativa* Mill.) from the family Brassicaceae, included in the group of green leafy vegetables, is characterized by a rich chemical composition and broad biological activity (Michael et al., 2011). Rocket leaves are considered to be potential nutritional and health-promoting agents (Bukhsh et al., 2007; Tassi and Amaya-Farfan, 2008; Filocamo et al., 2010; Michael et al., 2011; Villatoro-Pulido et al., 2012). Earlier study (Nurzyńska-Wierdak, 2001, 2009) showed that rocket accumulates up to 17% of dry matter in its leaves, including 37.2% of protein, 2.36% of total sugars, 2.00% of monosaccharides, and 243.2 mg 100 g^{-1} of L-ascorbic acid.

The content and chemical profile of some biosubstances can be modified by agronomic factors (Jarosz et al., 2012), among which the level of mineral nutrition is particularly evident (Smatanová et al., 2004; Abou El-Magd et al., 2010; Tunçtürk et al., 2011; Michałojć and Dzida, 2012; Pitura and Michałojć, 2012). An increased rate of nitrogen in growing leaf chicory contributed to an increase in total nitrogen concentration and to a reduction in the levels of methionine, valine, and lysine in the leaves (Custic et al., 2002). In turn, chlorine has a beneficial effect on the nitrogen

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content and protein amino acid composition of marrow stem kale (Michałek, 1992), similarly to phosphorus and potassium on the protein amino acid composition of leaf parsley (Kołota et al., 1994). The protein content of rocket leaves was dependent on the rate and form of nitrogen as well as the potassium fertilization applied (Nurzyńska-Wierdak, 2001, 2009). Different nitrogen and potassium nutrition regimes modified the protein amino acid composition of rocket and kohlrabi leaves (Nurzyńska-Wierdak, 2006), while sulfur application increased the protein content in rocket seed (Singh et al., 1999). An increased rate of nitrogen contributed to an increase in dry matter and free amino acid production in mustard greens plants (Vyas et al., 1995). The results of the above studies show the significant modifications in the plant protein content and amino acid composition as affected by different plant mineral nutrition. Green leafy vegetables are an important component of the daily diet and their consumption in a fresh state ensures complete availability of vitamins. The present study was designed to determine the protein content and amino acid composition of the leaves of garden rocket (*Eruca sativa* Mill.) as affected by different nitrogen and potassium nutrition regimes.

2. Materials and methods

2.1. Plant material

During the period of 2010–2012, a plant growth experiment was conducted in a detached heated greenhouse, situated in the north-south direction and belonging to the Department of Vegetable Crops and Medicinal Plants of the University of Life Sciences in Lublin. Garden rocket seeds, acquired from the seed production company PNOS Ożarów Mazowiecki, were sown individually in 2-dm^3 pots. Deacidified sphagnum peat with a pH of 6.5 was used as the growing medium. The experiment was set up as a completely randomized design in 14 replicates. One pot in which 3 plants were grown was one replicate. Seeds were sown on approximately 20 March, having first been dressed in Funaben T (5 g kg^{-1} seed). The study investigated the effects of different levels of nitrogen and potassium nutrition of rocket plants on biomass accumulation and chemical composition simultaneously with different levels of calcium, sulfur, and chlorine, resulting from the varying quantities of accompanying ions. The experimental design included 2 rates (g dm^{-3} medium) of nitrogen applied as $Ca(NO_3)_2$, 0.3 N (and 0.37 Ca) and 0.6 N (and 0.74 Ca), as well as 3 rates of potassium applied as K_2SO_4 [0.3 K (and 0.34 S), 0.6 K (and 0.47 S), 0.9 K (and 0.6 S)] and as KCl [0.3 K (and 0.27 Cl), 0.6 K (and 0.54 Cl), 0.9 K (and 0.81 Cl)]. In addition, the following nutrients were applied: 0.4 P and 0.2 Mg, as well as micronutrients (mg dm^{-3} medium): 8.0 Fe, 13.3 Cu, 5.1 Mn, 1.6 B, 3.7 Mo, and 0.74 Zn. Phosphorus was applied in the form of granulated triple superphosphate (20% P); magnesium as $MgSO_4.H_2O$; iron in the form of chelate; copper, manganese, and zinc as sulfates; boron as boric acid; and molybdenum as ammonium molybdate. Sulfur was supplied with the application of potassium and magnesium (K_2SO_4 and $MgSO_4.H_2O$) as well as in a small amount as sulfates of Mn, Cu, and Zn (micronutrients); calcium as the ion accompanying nitrogen; and chlorine as the ion accompanying potassium. The reason for the application of potassium in the form of K_2SO_4 and KCl was the assumption that an increasing amount of sulfur (K_2SO_4) would be supplied with the uptake of a significant amount of chlorine by garden rocket plants, as found in an earlier study (Nurzyńska-Wierdak, 2006), without a negative effect on their yield. Nitrogen doses were divided into 3 equal parts and applied as follows: 1 day before seeding, 30 days after seeding, and 10 days before harvest. The nutrient solution was applied to the roots. The other nutrients were applied only once before seeding without differentiating between their amounts and forms. The plants were watered 1–2 times a day with 250 mL of water as necessary. All plants received the same amount of water. Leaf rosettes were harvested on 10 May, i.e. 48–54 days after seeding. After harvest, the percentage of dry matter in fresh leaves was determined; the plant material was dried at a temperature of 70 °C and the contents of total nitrogen and raw protein, as well as the protein amino acid composition, were determined according to the Kjeldahl method.

2.2. Determination of the total nitrogen and protein content

The analyzed sample was mineralized in concentrated sulfuric acid with the addition of selenium as a catalyst, hydrogen peroxide as an oxidant, and potassium sulfate to elevate the acid boiling point (380–410 °C). NaOH was added to the cooled solution at a rate of 30%, and the ammonia produced was distilled into a saturated boric acid solution with the addition of an indicator dye (methyl red + bromocresol green). The boric acid-ammonia solution was determined by titration with standard 0.01 M hydrochloric acid. The total nitrogen content was calculated using the following formula:

$$\% \mathrm{N} = \frac{(M_1 - M_2) \times n \times 0.014}{m} \times 100$$

where M_1 is the volume in cm^3 of 0.1 N NaOH consumed for titration of the control sample, M_2 is the volume in cm^3 of 0.2 N NaOH consumed during titration of the material sample, n is the titer of the NaOH solution, 0.014 is the milligram equivalent of nitrogen, 100 is the conversion to percentage, and m is the the sample weight in grams corresponding to 50 cm^3 of the solution taken for distillation. The obtained total nitrogen content was then converted to protein content using a conversion factor of 6.25.

The protein amino acid composition was determined with an AAA 400 amino acid analyzer (INGOS, Czech Republic) by the external reference method. The plant material was acid-hydrolyzed without oxidation (Davies and Thomas, 1973) and was additionally subjected to oxidative hydrolysis to determine sulfur amino acids (Moore, 1963). Cysteine was oxidized to cysteic acid and methionine to methionine sulfone using formic acid (Schramm et al., 1954). The prepared hydrolysate sample was injected into the column of the amino acid analyzer where the amino acids were separated. The individual amino acids (AAs) were derivatized in a reactor to colored AA-ninhydrin complexes. Identification of derivatized AAs was performed using photometric detectors. The content of the so-called conditionally essential AAs, cysteine and tyrosine, was determined. In the body, they can only be produced from essential AAs: tyrosine from phenylalanine, and cysteine from methionine. The protein AA content (without tryptophan) was evaluated in relation to the pattern of requirements for indispensable AAs in adult humans (FAO/WHO, 1991). The chemical quality of the protein was characterized using the Chemical Score (CS) and the Essential Amino Acids (EAA) Index, following the FAO/WHO standard (1999). The CS was determined as a ratio of the content of the limiting essential AA in the test protein (a_0) to the content of this AA in the reference protein (a_{0w}) (FAO/WHO, 1991):

$$CS = \frac{a_0}{a_{0w}} \times 100$$

The EAA score was determined as the geometric mean of the ratio of the content of all essential AAs as well as of histidine and arginine in the test protein ($a_1 - a_{10}$) to the content of these AAs in the reference protein ($a_{1w} - a_{10w}$) (FAO/WHO, 1991):

$$EAA = \frac{a_1}{a_{1w}} \times 100 \cdots \frac{a_{10}}{a_{10w}} \times 100$$

3. Results and discussion

On average, the rocket leaves accumulated 11.7% dry matter, including 16.6% protein. Plants fed with potassium chloride accumulated significantly more protein and less dry matter than those supplied with potassium sulfate (Table 1). The form of potassium differentiates the level of

Table 1. Protein and endogenic amino acid (mg g^{-1}) content. Values followed by the same letter in a row are not significantly different.

K source (A)	N dose (B)	K dose (C)	DW	Protein	Asp	Ser	Glu	Pro	Gly	Ala
	g dm^{-3}		%	% DW						
K_2SO_4	0.3	0.3	9.36	28.01	13.71	4.81	14.15	10.92	7.90	9.87
		0.6	9.44	31.39	13.29	5.05	15.56	12.78	8.07	9.75
		0.9	8.45	25.30	12.42	3.97	13.21	12.71	6.99	7.99
	0.6	0.3	7.56	24.44	13.15	4.40	14.42	13.40	7.67	8.92
		0.6	12.10	21.75	12.81	4.45	13.06	11.80	7.29	8.45
		0.9	7.85	26.25	13.57	4.46	14.36	14.47	7.69	8.94
Mean (A)			13.33a	26.19b	13.16b	4.52b	14.13b	12.68b	7.60b	8.99b
KCl	0.3	0.3	9.78	24.16	17.47	7.69	19.70	17.11	10.01	11.53
		0.6	9.17	29.68	23.08	11.10	26.16	21.20	11.89	15.23
		0.9	10.16	28.20	19.41	10.06	21.78	19.86	10.37	13.17
	0.6	0.3	8.82	30.60	14.23	4.59	14.50	15.38	7.90	8.96
		0.6	7.95	28.45	12.50	4.65	14.99	16.85	7.23	8.51
		0.9	8.20	27.73	13.10	4.84	15.60	18.83	7.64	8.53
Mean (A)			9.01b	28.14a	16.63a	7.16a	18.79a	18.21a	9.17a	10.99a
Mean (B)	0.3		9.39a	27.79a	16.56a	7.11a	18.43a	15.76a	9.21a	8.94a
	0.6		8.75b	26.54b	13.23b	4.57b	14.49b	15.12a	7.57b	8.72a
Mean (C)	0.3		8.88b	26.80b	14.64a	5.37b	15.69b	14.20c	8.37a	9.82a
	0.6		9.67a	27.82a	15.42a	6.31a	17.44a	15.66b	8.62a	10.49a
	0.9		8.67b	26.87b	14.63a	5.83ab	16.24b	16.49a	8.17a	9.66a

dry matter and some minerals in crop plants (Michałojć, 1998; Nurzyńska-Wierdak, 2006, 2009), whereas the sulfate form promotes dry matter accumulation, which is confirmed by the results of the present study. The increased nitrogen rates resulted in a significant decrease in protein content as opposed to the increased potassium rates. An opposite relationship was found in earlier studies (Nurzyńska-Wierdak, 2006; Akanbi et al., 2007; Petek et al., 2012). On the other hand, rocket grown during the autumn season accumulated protein in a similar amount, regardless of increasing nitrogen rate (Nurzyńska-Wierdak, 2009). It should be noted that plant foods derive more than 12% of their calorific value from protein (Aberoumand, 2009); thus, it is an important building and energetic block of vegetables. Experimental results show that rocket contained more protein than related species and other species of *Brassica* vegetables (Januškevičius et al., 2012; Ng et al., 2012; Saeed et al., 2012).

The AA composition of protein determines its nutritional value. The tested rocket protein contained all primary AAs, both nonessential and essential (Tables 1 and 2). In the group of nonessential AAs, glutamic acid (Glu) and aspartic acid (Asp) were predominant. Their average contents were 16.46 and 14.90 mg g^{-1}, respectively. Among the essential, conditionally essential, and nonessential AAs determined, the content of leucine (Leu) and lysine (Lys) was clearly distinguishable, averaging 13.83 and 10.85 mg g^{-1}, respectively. The presence of sulfur AAs (Met + Cys: 7.7 mg g^{-1}), which generally reduce the value of plant protein, should also be noted. The proportions of all AAs determined were much lower than in the reference protein guidelines (FAO/WHO, 1991) (Table 3). The greatest variation was found in the case of threonine and lysine, while the lowest variation was found in sulfur amino acids.

CS values showed that the first limiting AA biological value of rocket leaf protein was Thr, followed by Lys and then Leu. The EAA value was 22.6%. The rocket protein, similarly to other plant proteins, is not a complete protein, since it does not contain a sufficient amount of essential AAs. Nevertheless, due to its content and composition, rocket leaves can be taken into consideration as valuable dietary supplements. The protein content and its AA composition is genetically controlled (Ceyhan et al., 2014). However, the proportions of individual AAs in protein can change under the influence of agronomic and climatic factors (Bell et al., 2000; Smatanová et al., 2004). The AA composition of rocket protein was significantly affected by

Table 2. Exogenic and relatively exogenic amino acid composition of rocket protein (mg g^{-1}). Values followed by the same letter in a row are not significantly different.

K source (A)	N dose (B)	K dose (C)	Thr	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
	g dm^{-3}												
K_2SO_4	0.3	0.3	3.44	4.30	8.71	3.19	6.49	13.38	5.19	8.30	3.90	10.03	8.99
		0.6	3.77	3.60	8.71	3.83	6.59	13.51	5.12	8.35	3.80	10.34	9.34
		0.9	2.55	2.91	7.81	3.24	5.86	11.94	3.92	7.42	3.53	9.08	7.88
	0.6	0.3	3.00	2.78	8.41	2.87	6.38	12.91	5.29	7.93	3.89	9.87	8.71
		0.6	3.20	3.87	7.70	3.66	5.72	12.13	4.39	7.13	3.29	8.91	8.18
		0.9	2.86	2.05	8.21	2.93	6.22	12.86	3.63	7.28	3.49	9.34	8.62
Mean (A)			2.60b	3.25b	8.26b	3.29b	6.21b	12.79b	4.59b	7.74b	3.65b	9.60b	8.62b
KCl	0.3	0.3	7.38	5.05	10.86	3.63	8.00	16.14	8.06	10.18	5.31	13.14	11.51
		0.6	11.65	5.45	13.00	4.21	9.51	18.74	11.43	12.42	6.56	15.82	14.30
		0.9	10.34	5.31	11.32	3.77	8.28	16.58	10.42	10.66	5.81	13.52	12.71
	0.6	0.3	3.21	4.75	8.57	4.35	6.36	13.26	4.15	7.76	3.67	10.10	8.88
		0.6	3.44	4.21	8.09	3.89	5.96	11.90	4.40	7.37	3.75	9.76	7.98
		0.9	3.52	4.78	8.41	3.67	6.31	12.57	4.59	7.82	3.95	10.30	8.71
Mean (A)			6.59a	4.93a	10.04a	3.92a	7.40a	14.87a	7.18a	9.37a	4.84a	12.11a	10.68a
Mean (B)	0.3		6.52a	4.44a	10.07a	3.65a	7.46a	15.05a	7.36a	9.56a	4.82a	11.99a	10.79a
	0.6		3.21b	3.74a	8.23b	3.56a	6.16a	12.61b	4.41b	7.55b	3.67a	9.71b	8.51b
Mean (C)	0.3		4.26b	4.22a	9.14a	3.51a	6.81a	13.92a	5.67a	8.54a	4.19a	10.79a	9.52a
	0.6		5.52a	4.28a	4.28b	3.90a	6.95a	14.07a	6.34a	8.82a	4.35a	11.21a	9.93a
	0.9		4.82ab	3.76a	8.94a	3.40a	6.67a	13.49a	5.64a	8.30a	4.20a	10.56a	9.48a

Table 3. Exogenic and relatively exogenic amino acid content in rocket protein in comparison to pattern (mg g^{-1}).

Amino acid (AA)	AA content*	CS (%)	Pattern		
			FAO/WHO (1991)	Millward (1999)	Young et al. (1994)
Lys	10.9	18.8	58	31	50
Leu	13.8	20.9	66	44	65
Ile	6.8	24.3	28	30	35
Thr	4.9	14.4	34	26	25
Val	9.2	26.3	35	23	35
Met + Cys	7.7	30.8	25	27	25
Tyr + Phe	14.5	23.0	63	33	65
His	4.3	22.6	19	-	-
Arg	9.7	-	-	-	-
∑ AA	81.8		328	214	300
EAA (%)	22.6				
LAA**	Thr + Lys + Leu				

*: Mean values irrespective of mineral nutrition; **: limiting amino acid.

the mineral nutrition used (Tables 1 and 2). The content of all AAs determined was significantly higher in the protein of plants fed with potassium chloride compared to those fed with potassium sulfate. In the majority of cases, the increasing rates of nitrogen and potassium, applied in the nutrition of rocket plants, significantly modified the proportions of AAs in the protein. The lower nitrogen rate (0.3 g N dm^{-3}) proved to be more beneficial for the accumulation of Asp, threonine (Thr), serine (Ser), Glu, glycine (Gly), valine (Val), Leu, tyrosine (Tyr), phenylalanine (Phe), Lys, and arginine (Arg). In the case of the other AAs, no relationship was found between their proportion in the protein and the amount of nitrogen applied. The increased rate of potassium modified the level of AAs in the rocket protein to a lesser extent. The higher rate of potassium significantly increased the content of Thr, Ser, and proline (Pro); in turn, the concentration of Val was significantly lower in the protein of plants fed with a medium dose of potassium. The proportions of the other AAs were not modified by the different rates of potassium. As reported by Smatanová et al. (2004), cysteine and methionine determined in the fresh matter of spinach and pepper positively responded to sulfur fertilization and to the increased rate of nitrogen in particular. The absence of such relationships in the protein AAs of rocket can be explained by the specific nutritional requirements of this plant, which also accumulates other substances containing nitrogen and sulfur. It should be noted that there is a small number of scientific papers discussing similar issues that are important from the point of view of nutrition. The protein of Brassicaceae plants, which has a beneficial AA composition and is accumulated in large amounts in their edible parts, should be taken into consideration as a diet component. Modifications in plant nutrition systems as well as other factors leading to increased nutritional value of plant raw material should be taken into account in rational plant production.

It can be concluded that rocket leaves are characterized by a high content of protein with a beneficial AA composition and should be taken into consideration as a diet component. Rocket protein should be considered incomplete, although at the same time it contains all primary AAs. The results presented in this study show that it is possible to modify the protein content and chemical composition of rocket leaves by applying an appropriate system of plant mineral nutrition. The application of potassium chloride significantly increased the content of protein and all protein AAs. The increase in nitrogen rate, on the other hand, caused a decrease in the concentration of protein and in the following protein amino acids: Asp, Thr, Ser, Glu, Gly, Val, Leu, Tyr, Phe, Lys, and Arg. The higher rates of potassium contributed to an increase in the content of Thr, Ser, Glu, and Pro in the rocket protein.

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