AMINO ACID COMPOSITION OF WINTER WHEAT GRAIN PROTEIN DEPENDING ON THE SEED CERTIFICATION CLASS AND ON THE CULTIVAR

Ewa Spychaj-Fabisiak, Bożena Barczak*, Krystian Nowak, Jacek Jagielski

Department of Agricultural Chemistry, University of Technology and Life Sciences, Seminaryjna 5, 85436 Bydgoszcz, Poland, *Corresponding author: e-mail: barczak@utp.edu.pl

ABSTRACT

The present research, which aimed at evaluating the effect of the seed certification class and the cultivar on the amino acid composition of winter wheat grain protein, was based on a two-factor field experiment carried out over 2006-2009. It was set up as a randomised split-plot design in three reps on a farm at Nidowo (the Pomorze Province). The experiment factors included winter wheat cultivars (Bogatka, Zyta, Turnia, Tonacja) and the seed certification class (certified seed of the first (C1), second (C2) and third (C3) seed reproduction). The research showed a relatively high variation in the amino acid composition of grain in the wheat cultivars in respective research years. The greatest fluctuations concerned the content of lysine and isoleucine. The factors, in general, did not have a significant effect on the value of the total sum and sums of exo- and endogenous amino acids in winter wheat grain protein. The cultivar factor significantly determined Chemical Score (CS) values for lysine and methionine (CSlys and CSmeth) as well as Essential Amino Acid Index (EAAI) values. The highest CSlys and CSmeth values were recorded for 'Tonacja' grain protein, while the highest EAAI value – for 'Bogatka' grain. The seed significantly determined the CS value for methionine (CSmeth) and in the interaction with the cultivar factor – also the EAAI value.

Key words: amino acids composition, cultivar, protein, seed certification class, winter wheat.

INTRODUCTION

B iological progress is one of the key factors determining the increase in agriculture efficiency, e.g. by applying certified seed the role of which in plant production is high and in the countries of Western Europe, Nord America and Africa at the end of the 20^{th} century it was considered to account for over 50% (Nalborczyk, 1997; Usman, 2008). In Polish agriculture the importance of biological progress is currently comparable with or lower than that observed in highly developed countries in the 1970 (Wicki, 2007). There is still a low effect of the application of certified seed on the level of yielding, which can be no encouragement for the farmers to apply it. The interest can be getting even lower due to no visible economic effects of its use (Wolski, 1995).

In the applicable literature one can find reports by Piech et al. (1994) and Wolski (1995) who claim that the application of certified seed in cereal production results in an increase in the yield by 0.33 dt⁻ha⁻¹ and 3-4 dt⁻ha⁻¹, respectively. According to Krzymuski (1994), seed degradation can decrease yields by 133% annually. However, there is much literature coverage on an increase in yields as affected by qualifiers (Kwiatkowski, 1997; Wolski, 1987) or reporting on the results which are confusing, making their yieldforming effect depend on weather factors (Dziamba and Rachoń, 1994).

The seed determines not only the cereal grain yield but also its quality. As a result of applying highly certified seed, it is, in general, higher, due to no grain pollution with other species, greater healthiness as well as lower content of grain offal, badlydeveloped grain (Podlaski, 1987). One can assume that that factor also affects the chemical composition of grain, especially the protein content.

Facing little literature coverage on the effect of the seed certification class on the

biological value of grain protein, research has been launched to evaluate the effect of certified seed and its seed certification class on the amino acid composition of grain protein in four winter wheat cultivars.

MATERIAL AND METHODS

The field experiment, being a springboard for research carried out over 2006-2009, was performed on a farm at Nidowo (the Pomorze Province) on very good wheat complex soil, with a slightly acidic reaction (pH_{KCl} 6) and high or very high class of richness in available nutrient forms (145-230-240 mg K 150 mg P. and 118-120 mg Mg \cdot kg⁻¹ of soil). It was a two-factor field experiment set up as a randomized splitplot design in three reps.

There were considered the following factors:

Factor I – winter wheat cultivars (n=4): Bogatka, Zyta, Turnia, Tonacja.

Factor II – the seed (seed certification class) (n=3):

Certified seed of the first reproduction (C1),

Seed of the second reproduction -(C2)

Seed of the third reproduction -(C3)

The experimental plots were 15 m^2 each.

Nitrogen, phosphorus and potassium doses were determined based on the soil richness in those nutrients as well as on nutritional requirements of winter wheat and were applied as follows: 200 kg N·ha⁻¹; 30.8 kg P·ha⁻¹ (70 kg P₂O₅·ha⁻¹) and 100 kg K·ha⁻¹ (120 kg K₂O·ha⁻¹) in a form of ammonium nitrate, ammonium phosphate and potassium salt, respectively. Winter wheat grain was sown at the rate of 450 kernels per m² in the first decade of October in each research year. Rape constituted the forecrop for wheat.

In the representative wheat grain samples there was determined the amino acid composition of protein after hydrolysis as exposed to hydrochloric acid at the concentration of 6 mol·dm⁻³ with the High Performance Liquid Chromatography (HPLC). The amino acid detection was made using the fluorescent photometer following their aldehydation with *o*-ftaldialdehyde. With the amino acid composition of grain protein defined, there were calculated the values of the following indices:

- EAAI (Essential Amino Acid Index):

EAAI= $(c_1/c_{01} \times c_2/c_{02} \times \ldots \times c_n/c_{0n})^{1/n}$, where:

 $c_1, c_2, ..., c_n$ – the content of successive essential amino acids in protein,

 $c_{01}, c_{02}, ..., c_{0n}$ – the content of successive essential amino acids in the reference protein assumed as chicken egg protein.

- CS (Chemical Score):

$$CS = (c_i/c_{0i}) \times 100\%,$$

where:

 c_i – the content of successive essential amino acids in protein;

 c_{0i} – the content of successive essential amino acids in the reference protein assumed as chicken egg protein.

The results were statistically verified with the analysis of variance following the reference compliant with the experimental design and using the Tukey test to evaluate the significance of differences. To define the compounds and relationships between the parameters, the results were exposed to the analysis of simple correlation and equations of linear regression were calculated.

RESULTS AND DISCUSSION

An essential component of protein properties characteristics is its amino acid composition, which is its main structural characteristic, irrespective of the kind, origin and physiological function (Shewry and Halford, 2002). Determining the amino acid composition of cereal grain protein and the proportions between respective amino acids facilitates the evaluation of its potential nutritive value, especially creating the possibility of applying synthetic amino acids to enhance the nutritive value of foodstuffs and animal feed mixtures produced from grain.

The present research demonstrated a relatively high variation in the amino acid composition of wheat grain in respective research years (Figure 1, Table 1). The lowest total sum of amino acids, irrespective of the factors studied, was reported for wheat grain

EWA SPYCHAJ-FABISIAK ET AL.: AMINO ACID COMPOSITION OF WINTER WHEAT GRAIN PROTEIN DEPENDING ON THE SEED CERTIFICATION CLASS AND ON THE CULTIVAR

in 2009 (75.9 g·16 N⁻¹), while the highest – in 2007 (83.9 g·16 N⁻¹). Of all the amino acids determined, average contents of methionine, aspartic acid, glutamic acid and serine in

respective years were relatively stable, whereas relatively high fluctuations concerned the content of lysine and isoleucine, followed by histidine, alanine and leucine (Figure 1).



Figure 1. Grain protein amino acid composition in the wheat cultivars researched over 2007-2009

The variation in the amino acid composition of winter wheat grain protein in successive research years determined the differences in the sums of exo- and endogenous amino acids (Table 1).

Irrespective of the factors studied, the highest mean values of those sums were recorded in 2007 (34.3 and 49.6 g·16g N⁻¹, respectively). The lowest sum of exogenous amino acids was recorded for wheat grain protein from 2009 (on average – 30.0 g·16g N⁻¹), and endogenous — from 2008 (45.7 g·16g N⁻¹). The quantitative proportions between the sums and respective research years were as follows: 0.69:1 (2007), 0.67:1 (2008), 0.65:1 (2009).

The differentiation in the results in respective research years comes from the fact that the real effect on the seed yield size and quality is a result of the interaction between the cultivar potential and the production technology applied with natural growing conditions, which are determined, e.g. by humidity and temperature factors which in our country's moderate climate show a high variation.

Table 1. Value of the sums and indices of grain protein amino acid composition in winter wheat cultivars in respective research years

Sum		N			
Sum	2007	2008	2009	Mean	
Total sum of amino acids	83.9	76.3	75.9	78.7	
Sum of egzogenous amino acids	34,3	30,7	30,0	31,7	
Sum of endogenous amino acids	49.6	45.7	45.9	47.0	
CS _{meth}	30.0	31.5	29.6	30.3	
CS _{lys}	35.4	37.3	27.6	33.4	
EAAI	60.0	54.1	52.8	55.7	

One shall stress that the share of sums of the content of exogenous amino acids in the total amount of amino acids in wheat grain protein in successive years was relatively stable and accounted for 40.9%, 40.2% and 39.5%. respectively. Manv authors (Majcherczak et al., 2003; Petkov et al., 2006; Woźniak and Staniszewski, 2007) point to a high variation in the cereal grain protein amino acid composition in respective research years and to an important role of weather conditions in determining it. In the present research the hydrothermal conditions which would be most favourable to the accumulation of protein and the content of amino acids in winter wheat grain occurred in 2007, the year

which recorded much higher rainfall than the multi-year mean. Some authors (Barczak, 1999; Żurek et al., 1991) stress that the importance of the amount of rainfall and its distribution as well as temperature in determining the amount and quality of plant protein is greater than the cultivar effect, which coincides with the present research. investigated The factors differentiated significantly neither the total sum of amino acids nor the sums of exogenous and endogenous amino acids in the protein of the wheat cultivars researched (Table 2). For the total sum and for the sum of endogenous amino acids only their significant interaction was shown.

Table 2. Value of the sums and indices of grain protein amino acid composition of the winter wheat cultivars studied depending on the experiment factors (three-year means)

Cultivar	Seed certification class	Total sum of amino acids	Sum of exogenous amino acids	Sum of endogenous amino acids	CS _{lys}	CS _{meth}	EAAI
Bogatka	C1	80.2	32.4	47.8	33.2	32.7	57.3
	C2	78.7	32.2	46.4	33.1	31.9	56.9
	C3	78.6	31.9	46.8	33.4	30.9	56.2
Mean		79.2	32.2	47.0	33.2	31.9	56.7
Zyta	C1	77.7	31.1	46.6	32.3	28.7	54.1
	C2	78.2	30.7	47.5	31.1	28.5	53.7
	C3	78.0	31.8	46.2	32.9	30.2	55.6
Mean		78.0	31.2	46.8	32.1	29.1	54.5
Turnia	C1	79.2	31.8	47.4	33.0	29.6	55.9
	C2	80.1	32.0	48.1	33.5	27.5	55.9
	C3	79.7	31.6	48.1	31.8	28.2	55.0
Mean		79.7	31.8	47.9	32.8	28.4	55.6
Tonacja	C1	77.1	31.3	45.7	34.8	33.8	55.7
	C2	77.5	31.0	46.5	35.9	29.7	54.8
	C3	79.4	32.1	47.3	36.0	32.4	56.9
Mean		78.0	31.5	46.5	35.6	32.0	55.8
Mean	C1	78.5	31.6	46.9	33.3	31.2	55.7
	C2	78.6	31.5	47.1	33.4	29.4	55.3
	C3	78.9	31.8	47.1	33.5	30.4	55.9
Mean		78,7	31.7	47.0	33.4	30.3	55.7
$LSD^{1}_{(\alpha=0.05)}$ for:	I factor ²	ns ⁴	ns	ns	2.83	2.73	1.72
	II factor ³	ns	ns	ns	ns	1.52	ns
	I x II	3.32	ns	2.16	ns	ns	2.63

¹LSD-Least Significant Difference; ²factor I – cultivar; ³factor II – seed cultivar class; ⁴ns-difference not significant.

The assumption of the chemical method of evaluating the nutritive value of protein is to compare the amino acid composition of protein with the reference protein composition which should, in theory, fully meet the protein requirements of a given organism. Bearing than in mind, there were calculated the values of indices considering the content of exogenous amino acids, including CS (Chemical Score) (Mitchell and Block, 1946) which expresses the ratio of the content of amino acid in the protein to the content of a given amino acid in chicken egg protein, considered to be the reference protein, with an optimal nutritive value. The CS value analysis showed that in the grain of the wheat cultivars studied the role of the first deficit amino acid, limiting the nutritive value of protein, was played by methionine (mean value of $CS_{meth} - 30.3\%$) (Table 2). The second amino acid limiting protein biosynthesis was lysine (33.4%).

According to some authors (Shewry and Halford, 2002), for cereal grain the first amino acid remaining as minimum is lysine. However there is much literature coverage pointing to such position of methionine (Majcherczak, et al., 2003; Spychaj-Fabisiak, et al., 2009). The values of the index for methionine and lysine varied significantly thanks to the cultivar factor. For Bogatka and Tonacja grain protein there were shown significantly higher CS_{met} values than for the other cultivars. Tonacja also demonstrated the highest CS_{lvs} value. The seed certification class was only essential for the value of CS_{meth}. The difference between CS_{meth} for wheat grain from certified seed sowing and the value of that index for grain from C3 was, on average, 2.6%.

The second, next to CS, criterion of nutritive value of protein is EAAI (Essential Amino Acid Index) (Oser, 1951), defined as, expressed as a percentage, geometric mean from the product of the ratios of the content of respective exogenous amino acids in the protein researched to their content in reference protein. The index makes providing more complete characteristics of the nutritive value of protein, as compared with CS, possible since its value depends on the share of all the amino acids. Some authors exogenous (Barczak, 1999), however, point to the fact that as for EAAI, the surpluses of some amino acids can compensate for a deficit of others, and in protein biosynthesis, and thus in animal and human nutrition, no exogenous amino acid can be missing. A lack or a partial deficit of any of them in nutrition limits the use of other amino acids and disturbs protein biosynthesis. In the present research the highest EAAI value was demonstrated for

wheat grain protein from 2007 (on average – 60.0%), the lowest – from 2009 (52.8%) (Table 1). As shown from the analysis of variance, the index varied significantly due to the cultivar factor – the difference between extreme, as far as the index value is concerned, cultivars, Bogatka and Zyta, was only 4.03%.

CONCLUSIONS

A relatively high variation in the amino acid composition of protein in grain of the wheat cultivars in respective research years was demonstrated. The greatest fluctuations concerned the content of lysine and isoleucine.

The factors (the cultivar and the seed class) did not significantly influence the value of the total sum and sums of exo- and endogenous amino acids in winter wheat grain protein.

The cultivar factor significantly affected the values of CS for lysine and methionine $(CS_{lys} \text{ and } CS_{meth})$ and EAAI. The highest CS_{lys} and CS_{meth} values were reported for Tonacja grain protein, while the highest EAAI value – for Bogatka grain. The seed class significantly determined the CS value for methionine (CS_{meth}) and in the interaction with the cultivar factor – also the EAAI value.

REFERENCES

- Barczak, B., 2009. Role of nitrogen fertilisation in developing the biological value of winter barley grain protein. Roczniki Nauk Rolniczych, A, 114: 205-218.
- Dziamba, S., Rachoń, L., 1994. Effect of successive reproductions of the sowing material on triticale yielding. Folia Universitatis Agriculture Stetinensis, Agriculture, 162: 41-44.
- Krzymuski, J., 1994. Optimization of the frequency of the exchange of cultivars and seeds of cereals. Part 4, Seed degeneration. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 189: 141-149.
- Kwiatkowski, J., 1997. Degeneration of winter triticale in the seed reproduction cycle. Folia Universitatis Agriculture Stetinensis, Agricultura, 175: 225-228.
- Majcherczak, E., Cwojdzińsk, i W., Nowak, K., 2003. Effect of increasing nitrogen fertilisation on the amino acid composition of winter barley protein. Acta Scientiarum Polonorum, Agricultura, 2(2): 11-18.

- McDonald, M. B., *Seed certification in the United States*. http://seedbiology.osu.edu/HCS630_files/April%2010 /Seed%20Certification%20USA,%20text.pdf
- Mitchell, H. H., Block, R. J., 1946. *The corelation of the amino acid composition of proteins wit their nutrive value*. Nutrient Review (F) 16: 249-259.
- Nalborczyk, E., 1997. *Biological progress and the development of agriculture at the end of the 20th century and the beginning of the 21st century. Agricola, 33, SGGW, Warsow.*
- Oser, B. L., 1951. *Metod for integrating essential amino acid content in the nutritional evaluation of protein*. Journal American Dietetic Association, 27: 396-411.
- Petkov, K., Bobko, K., Biel, W. Jaskowska I., 2006. Evaluation of winter triticale grain based on the chemical and amino acid composition as well as the protein quality. Folia Universitatis Agriculture Stetinensis, Agricultura, 247(100): 141-144.
- Piech, M., Stankowski, S., Maciorowski, R., Mikulski, W., 1994. *Effect of the fraction on winter triticale yielding.* Folia Universitatis Agriculture Stetinensis, Agricultura, 162: 197-199.
- Podlaski, S., 1997. Some factors affecting the degeneration of generatively reproduced cultivars. Self-pollinating plants. Postępy Nauk Rolniczych, 5: 43-55.
- Shewry, P., Halford, N., 2002. *Cereal seed storage proteins: structures, properties and role in grain utilization.* Journal of Experimental Botany, 53: 947-958.

- Spychaj-Fabisiak, E., Ralcewicz, M., Knapowski, T., Kozera, W., Barczak, B., Nowak, K., Majcherczak, E., Murawska, B., Janowiak, J., 2009. Effect of foliar fertilization with nitrogen and magnesium on chemical composition, biological value and bacing quality of spring wheat grain. Monography, UTP, Bydgoszcz, Poland: 359-374.
- Usman, I., 2008. Recommended seed production practices. Proc. Training Workshop "Production of Legume and Cereal Seeds". 24.01-10.02. 2008, International Institute of Tropical Agriculture, Kano, Nigeria: 11-15.
- Wicki, L., 2007. Effect of biological progress on the yielding and economics of winter cereals production. Roczniki Nauk Rolniczych, G, 94(1): 74-85.
- Wolski, T., 1987. Evaluation and triticale production prospects in Poland. New Agriculture, 6: 1-4
- Wolski, T., 1995. Present and future in small breeding seed production in Poland. Fragmenta Agronomica, 2(45): 52-55.
- Woźniak, A., Staniszewski, M., 2007. Effect of weather conditions on the technological grain quality of Opatka cv. spring wheat and Korweta cv. winter wheat. Acta Agrophisica, 9(2): 525-540
- Żurek, J., Płoszyński, M., Dietrych-Szóstak, D., 1991. Chemical composition and nutritive value of new triticale cultivars. Pamiętnik. Puławski, 98: 55-65.