

Amino Acid Composition and Biological Value of Protein of New Pea Morphotypes

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Abstract

Background/Objectives: The research goal is to investigate the amino acid composition and biological protein value of seeds of new morphotypes of peas. **Methods/Statistical analysis:** The crude protein content is determined by decomposition of organic matter by sulfuric acid over catalyst. The crude protein content was calculated according to the amount of nitrogen release using the coefficient 6.25. Amino acid composition of protein was studied by capillary electrophoresis. Protein biological value was calculated based on the ideal protein according to FAO/WHO requirements for the control. Investigations were carried out in triplicate. Statistical data processing was performed using the Microsoft Excel 2010 statistical package determining the error of the mean and standard deviation at a significance point of 95%.

Findings: The study of protein complex of new varieties and forms of peas revealed the identity of the amino acid composition of the protein in different morphotypes. The biological value of pea seed proteins is limited by methionine, an essential amino acid, and to a lesser extent by valine. The wrinkle-seeded types prevail over the smooth-seeded types by the amount of essential amino acids. Proteins of wrinkle-seeded Amior variety and Amich-99-1132 breeding line were characterized by the largest biological value in terms of the sum of essential amino acids. For the first time the research conducted in terms of amino acid composition of protein in pea varieties with modified leaf architectonics demonstrated that they are more useful compared to the parent varieties. Sources of methionine (B-Agrimut, Agritek Mutant), phenylalanine (Ras-type) and tryptophane (Multik) were identified among varieties with non-traditional leaf architectonics. The obtained data confirm the feasibility and efficiency of breeding work in improving the habitus of pea plants, as well as the possibility of their further use as the initial material to improve the quality of seeds, and for food purposes. **Applications/Improvements:** To expand the resource base of the processing industry it is recommended to include modern varieties and breeding lines of peas, differing with high content of protein and some amino acids, in breeding programs for improving the quality of seeds.

Keywords: Amino Acid, Composition, Morphotype, Pea, Protein

1. Introduction

Today the problem of food availability and elimination of the protein deficit in the world is becoming increasingly important, not only because of the global economic crisis and the rise in prices for many types of food and energy sources, but also because of the continued population growth and urbanization, as well as the allocation

of some agricultural raw materials for the biofuel production.

According to FAO data, the mankind is facing the task of increasing food production by 70%. As estimated by the experts, about 20% of the increasing food needs can be satisfied through the involvement of more land in the crop rotation, about 10% – by improving productivity. The remaining 70% should be satisfied by means

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of more efficient processing technologies and the use of agricultural raw materials^{1,2}.

The most promising and valuable crops – suppliers of low-cost and complete vegetable protein are grain legumes, including, peas.

The main types of protein preparations that can be obtained from pea grains are defatted flour (56–59% of protein), concentrated pea protein (65–72% of protein) and isolate (at least 90% of protein)³. According to the MR 2.3.1.2432-08, in terms of amino acid composition and assimilation legume protein isolates and concentrates are close to proteins of animal origin.

2. Literature Review

According to the data obtained by a number of authors, albumins, globulins and glutenins are the main fractions of the pea proteins. The basic part of protein complex (up to 80–90 %) consists of reserve proteins globulins⁴. Separate groupings of salt-soluble proteins contain unequal amount of essential amino acid limiting nutritional value – methionine, tryptophane and cysteine. Their low content is characteristic of vicilines and viciline-related proteins. They are present in large amounts in legumines and legumine-related proteins⁵. Albumins are characterized by high content of sulphur-containing amino acids, lysine, threonine, as well as aspartic and glutamic acids⁶.

Therefore, the forms with increased content of water-soluble proteins and legumine-related proteins in the composition of globulins are of the greatest value as sources of deficient amino acids⁷.

The genotype has a significant impact both on the content, and on the composition of pea proteins⁸.

Currently, in addition to the 'traditional' leafy and leafless pea morphotypes forms Russian breeders created a number of promising forms with modified leaf architecture. Thus, chameleon variety obtained by Zelenov⁹, which differs in a tiered heterophylly, became a basis for the creation of Spartak varieties, exceeding the area-specific standard variety both in seed production, and in the content of protein¹⁰. Pea varieties with dissected leaves (Ras-type)¹¹ and with multiple or double imparipinnate leaves¹² are of considerable interest, though they have not become widespread in agricultural practice. A fundamentally new trend is pea selection for increased amylose content in the seed starch, which resulted in the creation of the first high-amylose Amior variety in Russia¹³.

The purpose of this research was to study amino acid composition and determine biological value of seed protein of new pea morphotypes.

3. Methodology

Material for study included the seeds of green pea varieties and breeding lines grown in All-Russia Scientific Research Institute of Grain Legumes and Cereal Crops, Orel, Russia:

- Smooth-seeded: leafy (Orlovchanin variety), leafless (Batrak, Multik varieties), chameleon (Spartak variety), with multiple imparipinnate leaves (Agritek Mutant, Pap-485/4 breeding lines), with dissected leaves (Ras-type breeding line), with double imparipinnate leaves (B-Agrimut breeding line);
- Wrinkle-seeded: leafless (Amior variety), chameleon (Amich-99-1132).

Crude protein content was determined under GOST P 51417-99 by mineralizing organic substances with sulfuric acid in the presence of a catalyst to form ammonium sulfate; by destructing ammonium sulfate with alkali followed by liberation of ammonia; ammonia stripping with steam into a sulfuric acid solution with subsequent titration. Crude protein content was calculated based on the amount of nitrogen, using a coefficient of 6.25. Amino acid composition of protein was studied by capillary electrophoresis using "Kapel" CE system. Biological value of proteins was calculated based on amino acid scores using FAO/WHO scale¹⁴.

Statistical data processing was performed using the Microsoft Excel 2010 statistical package determining the error of the mean and mean root square deviation at a significance point of 95%.

4. Results and Discussion

Nutritional value of pea proteins is determined by the amino acid content therein. It was found that in pea varieties with different types of seeds proteins are identical in composition and contain all the essential and nonessential amino acids (Table 1). Nonessential amino acids make 63.47–64.06%, the largest share among these was taken by the aspartic and glutamic acids. Arginine content ranged from 8.88 (Temp) to 9.41 % (Spartak).

Sum of essential amino acids in the proteins of the investigated pea varieties made on average 36.30 %, ranging from

35.94 (Spartak) to 36.53 % (Amich-99-1132). It was also revealed that in terms of the total number of essential amino acid the wrinkle-seeded forms surpass the smooth-seeded ones on average by 0.36%. Protein usefulness and digestibility depends primarily on the content of the most deficient amino acids – methionine, tryptophane and lysine. It was established that in the proteins of the investigated varieties the share of methionine totaled to 1.06-1.10 %; tryptophane – 1.12-1.14 %; lysine – 7.06–7.36 %. Cumulative content of isoleucine and leucine reached 12.86 (Spartak) – 13.46 % (Amich-99-1132) in the studied proteins.

Table 1. Amino acid composition of pea seed proteins

Amino acid	Content, % to the total protein			
	Temp	Spartak	Amior	Amich-99-1132
Aspartic + glutamic acid	32.77	32.68	32.22	31.90
Threonine	4.06	4.00	4.12	4.10
Serine	4.26	4.36	4.31	4.60
Proline	4.36	4.22	4.50	4.44
Glycine	4.18	4.13	4.25	4.13
Alanine	3.96	4.02	3.92	3.92
Valine	4.94	4.75	4.78	4.73
Methionine	1.08	1.06	1.10	1.09
Isoleucine+ leucine	13.18	12.86	13.12	13.46
Tyrosine	2.65	2.62	2.73	2.62
Phenylalanine	4.86	4.77	4.88	4.74
Histidine	2.64	2.62	2.65	2.64
Lysine	7.06	7.36	7.29	7.28
Arginine	8.88	9.41	9.00	9.22
Tryptophane	1.12	1.14	1.13	1.13
Sum of essential amino acids	36.30	35.94	36.42	36.53

The research demonstrated that proteins of smooth-seeded and wrinkle-seeded pea varieties are balanced with regard to most essential amino acid (Table 2).

Chemical score as to lysine made 128.4-133.8 %. Pea proteins were provided with phenylalanine and tyrosine on average by 24.5 % higher than with standard protein. Pea proteins also exceeded 'FAO protein' by the content of such amino acids as threonine, isoleucine and leucine.

Chemical score as to tryptophane in the proteins of all studied varieties also exceeded 100 %. Methionine is an essential amino acid limiting protein usefulness of the studied varieties and breeding lines, its score being 48.2-50.0 % (calculated with reference to coefficient 2.2 for methionine). Also proteins of the studied pea varieties were insufficiently balanced with regard to valine. Proteins of wrinkle-seeded Amior variety and Amich-99-1132 breeding line were characterized by the largest biological value in terms of the sum of essential amino acids.

Also research was conducted in terms of amino acid composition and biological value of seed proteins in pea varieties with modified leaf architectonics compared to the conventional varieties.

In the studied seeds of pea morphotypes crude protein content ranged from 21.40 to 26.55 % (Table 3). Majority of leafy mutants differed in higher value of this parameter compared to area-specific standard variety Orlovchanin, excluding breeding line Ras-type with dissected leaves.

A number of forms with modified leaf architectonics had higher protein content than the initial ones. Thus, in the seeds of B-Agrimut with double imparipinnate leaves the crude protein content was 24.80 %, whereas the initial form Agritek Mutant (with multiple imparipinnate leaves) showed 22.50 %, which is apparently due to the increased leaf surface and, consequently, higher level of biosynthetic processes.

Study of amino acid composition of protein of various pea morphotypes demonstrated that nonessential amino

Table 2. Biological value of pea seed proteins

Variety/breeding line	Chemical score of amino acid, %						
	threonine	valine	methionine	isoleucine + leucine	phenylalanine + tyrosine	lysine	tryptophane
Temp	101.5	98.8	49.0	119.8	125.2	128.4	112.0
Spartak	100.0	95.0	48.2	116.9	123.2	133.8	114.0
Amior	103.0	95.6	50.0	119.3	126.8	132.5	113.0
Amich-99-1132	102.5	94.6	49.5	122.4	122.7	132.4	113.0

Table 3. Crude protein content and amino acid in the protein of pea variety seeds

Variety/breeding line	Crude protein content, %	Content in total protein, mg/100 g		Content of essential amino acids, % to the of amino acids sum
		of amino acids sum	of essential amino acid	
Orlovchanin – standard	22.05±0.10	21644.0	6975.0	33.22
Spartak	26.55±0.12	26208.0	8873.0	33.86
Batrak	22.35±0.08	21946.0	7429.0	33.85
Multik	25.20±0.11	25156.0	8445.0	33.57
Pap-485/4	23.05±0.04	23036.0	7882.0	34.22
Ras-type	21.40±0.08	17810.0	6198.0	34.80
Agritek Mutant	22.50±0.06	21992.0	6773.0	30.80
B-Agrimut	24.80±0.10	24626.0	8345.0	33.89

Table 4. Biological value of pea seed proteins

Variety/breeding line	Chemical score of amino acid, %							
	threonine	valine	methionine + cystine	isoleucine	leucine	phenylalanine + tyrosine	lysine	tryptophane
Orlovchanin – ct.	89.5	73.6	59.7	98.0	97.8	124.5	127.4	114.0
Spartak	103.8	76.6	48.6	90.5	108.6	122.7	147.3	94.0
Batrak	99.0	80.0	34.0	94.5	103.1	122.5	145.8	115.0
Multik	101.0	83.6	48.8	88.0	102.1	128.8	144.2	112.0
Pap-485/4	102.0	80.0	49.1	96.5	104.8	121.2	151.4	110.0
Ras-type	103.0	88.0	44.6	92.2	106.7	158.0	144.0	108.0
Agritek Mutant	89.0	72.0	68.0	98.75	87.7	107.7	131.6	108.0
B-Agrimut	103.8	80.2	55.7	95.2	105.6	117.7	142.9	110.0

acids make 66.11–69.20 % of the sum of all amino acids. Moreover, aspartic and glutamic acids account on average for 14.01 and 19.36%, respectively, of their total amount. Spartak and Multik varieties and B-Agrimut breeding line stand out with regard to the composition of the amino acid complex and of essential amino acid (mg/100 g of protein). Amount of essential amino acids made 33.53 % on average in protein of the studied pea forms, ranging from 30.80 (Agritek Mutant) to 34.80 % (Ras-type).

The largest percentage of the total content of essential amino acids – making on average 7.80% - accounted for lysine. The highest content of this amino acid is typical of seed proteins of Spartak variety (8.10 %) and Pap-485/4 breeding line (8.33 %). The forms with increased content of methionine – Agritek Mutant (1.43 %) and B-Agrimut (1.25 %) breeding lines are of the greatest breeding and practical value. The highest content of tryptophane was revealed in the protein of the following varieties: Orlovchanin (1.14 %), Batrak (1.15 %), Multik

(1.12 %). Leafy mutants showed the percentage of this amino acid at the level of 1.08–1.10 %. Also the varieties were distinguished which had increased content of threonine (Ras-type, B-Agrimut, Spartak), valine (Ras-type), leucine (Ras-type, Spartak), isoleucine (Agritek Mutant, Orlovchanin), phenylalanine (Ras-type).

The study of biological value of proteins in the leafy pea mutants in comparison with the standard variety (FAO/WHO protein) showed that their usefulness was limited to the greatest extent by such amino acids as methionine and cystine (Table 4), having cumulative amino acid score 34.0–68.0 %.

Agritek Mutant protein was most provided with these amino acids, exceeding both the initial forms and area-specific standard variety Orlovchanin. Protein of Pap-485/4 proved to be more valuable in biological respect than the protein of the parent variety Batrak, surpassing it by the content of practically all essential amino acids, excluding the sum of tyrosine and phenylalanine

and tryptophane. Protein of B-Agrimut with double imparipinnate leaves also had higher scores of a number of essential amino acids compared to the initial form Agritek Mutant (with multiple imparipinnate leaves).

5. Conclusion

Research of the protein complex of pea varieties and forms showed identical amino acid composition of protein in various morphotypes. Methionine and to less extent – valine is limiting biological value of pea seed proteins. Wrinkle-seeded forms slightly surpass the smooth-seeded ones with regard to the sum of essential amino acids. Protein of breeding lines with modified leaf architectonics differs in greater protein usefulness as compared to the parent forms. The sources of methionine (B-Agrimut, Agritek Mutant), phenylalanine (Ras-type), and tryptophane (Multik) were distinguished among varieties with unconventional leaf structure.

The obtained data suggest the feasibility and efficiency of breeding work in improving the habitus of pea plants, as well as the possibility of using new forms of peas in an initial material to improve the quality of seeds, and for food purposes.

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