Original scientific paper
UDC 637.1/.3.045

DETERMINATION OF AMINO ACID COMPOSITION AND BIOLOGICAL VALUE IN PROTEIN-BERRY CONCENTRATES

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Abstract

Research of the amino acid composition of proteins, including the content of free and fixed amino acids, and biological value in protein-berry concentrates, obtained by thermo acid coagulation are an actual direction.

Concentrates obtained by thermo acid coagulation of milk proteins with organic acids of berry coagulant and milk-protein concentrates made according to the classical technology were used for researches. The study content of free and fixed amino acids has been realized by ion-exchange chromatography. To evaluate the balance of amino acid composition in concentrates by content of essential amino acids, the amino acid score and biological value have been determined by the calculation method.

Proteins in concentrates have a full amino acid composition and contain all essential amino acids, the content of which from amounts are 41.97% and 43.96%, respectively, for protein-berry and milk-protein concentrates. The total content of amino acids in protein-berry concentrates increased by 20.18% compared to the control sample - through the active coagulating effect of organic acids blackcurrant paste. A quantitative content of 18 free amino acids has been determined in protein-berry and milk-protein concentrates, of which: glutamic acid, histidine and methionine are dominant, and 16 fixed amino acids, where the dominant are: glutamic acid, leucine and proline. Protein-berry concentrates have a differential coefficient of amino acid score below the control sample at 19.73% and the amino acids in them are absorbed more fully.

This confirms the increase in biological value by 2.5 times as compared with the classical concentrate, according to the coefficients of utilitarian, redundancy and rationality of the amino acid composition.

Key words: *Concentrate, Coagulation, Amino acids, Biological value.*

1. Introduction

Protein deficiency in the nutrition of population is characteristic for many countries all over the world. Reducing the amount of protein in the diet has negative affect on health, development of the human body, and reduces its resistance to adverse external influences. The solution to this problem is achieved by optimizing the structure of nutrition by introducing products of high biological value that could satisfy the needs of the human body for nutrients, including proteins, which contain essential and non-essential amino acids. In order to change the situation, it is necessary to attract non-traditional raw materials and change approaches to increase the amount of complete protein.

The biological value of food product is an important indicator of quality along with safety and functionality. These values are depending on the essential amino acids content in the protein. The balance of proteins amino acid composition is relevant to their complete digestibility and its ensuring the necessary level of syntheses process [1]. Therefore, investigating the biological value of products, determine the correspondence of proteins amino acid composition to the formula of "human amino acid needs" [2].

Protein products have a significant place among dairy products. Insufficient amount of dairy raw materials and seasonality of milk manufacturing have effect on volumes of dairy-protein products manufacturing (cottage cheese and dairy products, hard and soft cheeses, etc.) during the year [3]. So far, acidic, rennet, acid-rennet, thermos-calcium, thermos-acid coagulation and ultrafiltrational concentration of milk have been used in the manufacturing technology of these products. Chemical nature of milk proteins coagulation is complicated and not studied in detail up to now. According to some researchers, fundamental changes of protein macrostructure are occurring at denaturational transformations. These changes are related to the weakening of the interaction forces between the protein chains of amino acid residues [4, 5]. The first stage of denaturation consists in change of the polypeptide chains original type. This change is caused by different methods, but it has a common pattern - the gap of the minimal amount of intramolecular ties for globule deploying. All typical manifestations of denaturation are explained by the release of single radicals, which in the native state of the protein are closely grouped among themselves. Secondary effects after denaturation - association of deployed globules and their chemical change leads to the allotment of proteins. Denatured whey proteins in the form of aggregates with irregular shape, surround changed casein particles and probably partially connect with it. With the temperature increasing the degree of whey proteins denaturation increases, and a complex of denatured whey proteins with casein amplifies. The influence of high temperature can't lead to coagulation at the whole milk pH. During acidification whey proteins coagulate as casein [6].

From all existing ways of proteins allotment only the thermos-calcium, thermos-acid coagulation and ultrafiltrational concentration of milk are providing comprehensive selection of proteins that allows receiving dairy-protein product with increased biological value. Expansion of protein products manufacturing made by intensive technologies is actual. In particular thermos-acid method of milk processing, which is based on the simultaneous coagulation of milk casein and whey proteins under the influence of acid and high temperature deserves attention. The degree of proteins application in this method is up to 95 - 97%, while about 90% at acid coagulation, and about 85% at rennet [7 - 9].

Whey milk proteins have a high biological value, which is due to the content of essential amino acids such as: lysine, tryptophan, methionine, and branched chain amino acids - valine, leucine and isoleucine. As a result, the full utilization of whey proteins is actual [10].

It is common knowledge that protein amino acids are in free and bound states. In the first case, the amino acid molecule is not bound by chemical bonds with other structural elements, which promotes faster fixation and instant absorption into the blood. In the second case, the molecules are covalently connected to each other in the composition of peptides and proteins. These compounds are important organic compounds involved in the formation of substances in secondary biosynthesis. According to the literature [11], after proteopepsis, proteins form a "free amino acids fund" which can be spent on the formation of new body tissues or on energy. The more of them in the product, the easier they are absorbed by the body. Moreover, free amino acids may experience further metabolic transformations to other low molecular weight compounds that have protective properties [12]. However, no studies can be found on the qualitative and quantitative composition of bound and free forms of protein amino acids in concentrates obtained by milk thermos-acid coagulation with organic acids of berry coagulant.

The aim of this work is determination of the amino acid composition and biological value of protein-berry concentrates, compared with milk-protein concentrate. Also, the content of essential amino acids has been investigated, and evaluation of their balance has been conducted in relation to the reference protein by calculating the amino-acid score.

2. Materials and Methods

Object of research was protein-berry concentrate (PBC), obtained by thermos-acid coagulation of milk proteins, using as coagulant - homogenized blackcurrant paste in the amount of 7% with an organic acid content of 0.21%, and an active acidity of 2.8. This amount of berry coagulant changes the active acidity in the mixture to provide a balanced isoelectric state of milk proteins in the entire volume at pH 4.6 - 4.7 and leads to their active coagulation. Thermal conditioning of the milk mixture and proteins coagulation have been realized according to the classical technology with optimization of schedule of thermo-acid coagulation (t = 75 ± 2 ^{0}C in duration of 2 ± 1 min.) based on the results of previous studies [13]. The thermo-acid coagulation allows more complete use of milk proteins and especially whey proteins - the most biologically complete with the contents of essential amino acids.

Determination of the amino acid composition in PBC, obtained by thermo-acid coagulation of milk proteins with organic acids of berry raw materials, was performed by ion-exchange chromatography [14]. Meaning of the method is hydrolysis of the sample to the amino acids and following their identification by method of high-performance liquid chromatography on the amino acid analyzer LC 3000 of the company "Eppendorf-Biotronic" (Germany). Sample was hydrolyzed with a solution of 6N hydrochloric acid, at a temperature of 120 ± 2 ^{0}C for 24 hours. This method allows to determine with accuracy to ± 5 - 10% the presence of up to 18 amino acids with a minimum level of their content in solution (0.500 ± 0.006) µmol/mL. Quantitative evaluation of the content of individual amino acids has been realized by comparing the peak areas on the aminograms calculated using the

Winpeak integration systems of the company "Eppendorf-Biotronic" (Germany) to a similar level, with peak areas obtained by analyzing a standard mixture of amino acids containing 2.5 μmol each amino acids in 1 cm^3 of standard solution.

The amino acid content in the protein-berry concentrate was compared with their content in the control sample - the milk-protein concentrate (MPC) obtained according to the classical technology (t = 90 ± 2 ^0C during 5 min.), using an acid whey with titrated acidity 160 - 180 ^0T as a coagulant, in the amount of 10% from the milk mass.

Amino acid score was determined in order to assess the balance of the amino acid composition of protein-berry concentrates by content of essential amino acids. It shows the percentage content of each amino acid in the investigated protein, relatively to its content in the ideal protein [2]. Then it was compared with the amino acid score of the ideal protein recommended by FAO/WHO. Amino acid fast has been calculated by the formula:

$$C_j = \frac{A_j}{A_{ej}} \cdot 100$$

Where: A_j - mass fraction of the j-th acid EAA in the PBC, g/100 g of protein; A_{ej} - the mass fraction of the j-th acid of EAA, in standard, the proportion of the physiologically necessary standard for a certain consumer group, g/100 g of protein.

Additionally, on the basis of the well-known formulas [15, 16], the following indicators of the balance of amino acid composition and biological value were calculated:

• Differential coefficient of amino acid score (DCAS) - characterizes the average value of the excess amino acid score of essential amino acids compared to the score of limiting amino acid:

$$DCAS = \frac{\sum \Delta DAS}{n},$$

Where: DAS - the difference in amino acid score for each EAA compared to the AS of a limiting amino acid%; n - the number of amino acids.

• Biological value of the protein concentrate has been calculated by the formula, %:

$$BV = 100 - DCAS$$

• Utilitarian coefficient (U) - characterizes the balance of protein essential amino acids in relation to the standard and the closer to one, the more balanced concentrate protein:

$$U = C_{min} \frac{\sum_{j=1}^{8} A_{ej}}{\sum_{j=1}^{8} A_j},$$

Where: A_{ej} - mass fraction of the j-th acid EAA in the standard, mg/g of protein; A_j - mass fraction of the j-th acid EAA in the product protein, mg/g of protein; C_{min} - score of the first limited acid EAA, units.

• Redundancy coefficient (σ_{red}) – shows the mass fraction of EAA in 100 g protein concentrate, which is not fully used by the body – for compensation of energy consumption and biosynthesis of nonessential amino acids:

$$\sigma_{red} = \frac{\sum_{j=1}^{k}(A_j - C_{min} A_{ej})}{C_{min}}$$

• Rationality coefficient of amino acid composition of the product (Rrat) - characterizes the balance of EAA in relation to the standard:

$$R_{rat} = \frac{\sum_{i=1}^{n} A_j \, U_j}{\sum_{i=1}^{n} A_j}$$

Where: U_j - utilitarian coefficient j-th acid EAA.

The study of the biological value of protein-berry concentrates will allow to justify their use in the production of multi-functional food products.

3. Results and Discussion

Analysis of the amino acid composition in the protein-berry (PBC) and milk-protein (MPC) concentrates has shown that the protein fractions contain a full set of amino acids, including essential, which confirms their high biological value. Aminograms of the free and bound amino acids content in the PBC and MPC are shown in Figure 1.

Based on the aminogram shown in Figure 1, the free and bound amino acids content, including essential (EAA) and non-essential (NAA), in the protein-berry and milk-protein concentrates has been calculated. The results are presented in Table 1.

According to the results (Table 1), the total amino acids content in PBC increased by 20.18% compared with the control sample, as a result of the action of organic blackcurrant paste. Probably the concentrates production by thermo-acid coagulation of milk proteins with this type of berry raw material increases their biological value. The maximum amount of glutamic acid in all samples has been recorded. 18 free amino acids, among which histidine and methionine predominate, and 16 bound amino acids (leucine and proline prevail) have been identified in concentrates. All essential amino acids (EAA) have been identified in the PBC and MPC. Their importance is due to the fact that they are not synthesized by the body, and the deficiency affects the protein regeneration. The content of the above amino acids from the total amount is 41.97% and 43.96%, respectively.

In addition, glutamic acid in a bound state accumulates in large quantities in protein-berry concentrates at the level of 3.007/100 g, due to its high content in black currant (34.7 g/100 g, of which 33.2 g/100 g in a bound state [17]). The above mentioned amino acid

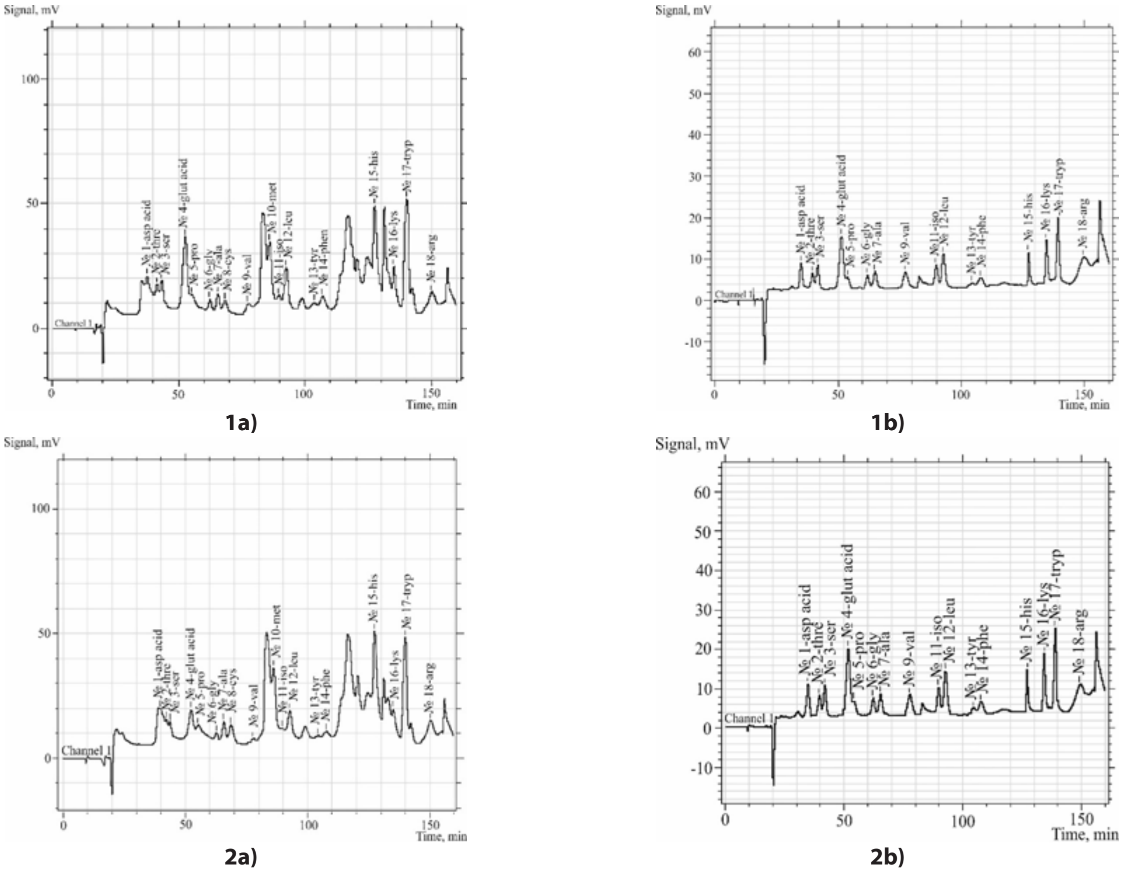

Figure. 1. Aminograms of free (a) and bound (b) amino acids content in protein-berry (1) and milk-protein (2) concentrates
1 - Aspartic acid, 2 - Threonine, 3 - Serine, 4 - Glutamic acid, 5 - Proline, 6 - Glycine, 7 - Alanine, 8 - Cysteine, 9 - Valine, 10 - Methionine, 11 - Isoleucine, 12 - Leucine, 13 - Tyrosine, 14 - Phenylalanine, 15 - Histidine, 16 - Lysine, 17 - Tryptophan, 18 - Arginine

Table 1. The content of free and bound amino acids in protein-berry and milk-protein concentrates (n = 3, p ≤ 0.05)

The name of amino acid		Concentration of amino acids in concentrates, g/100 g			
		Milk-protein		Protein-berry	
Essential amino acids:		Free	Bound	Free	Bound
1	**Valine**	0.055 ± 0.002	1.012 ± 0,030	0.184 ± 0.006	1.05 ± 0.032
2	**Isoleucine**	0.041 ± 0.002	0.939 ± 0,037	0.187 ± 0.007	0.840 ± 0.034
3	**Leucine**	0.635 ± 0.0191	1.732 ± 0,0519	0.164 ± 0.005	1.507 ± 0.045
4	**Lysine**	0.536 ± 0.0268	1.422 ± 0,071	0.922 ± 0.046	1.246 ± 0.062
5	**Methionine**	2.092 ± 0.0418	-	2.145 ± 0.043	-
6	**Threonine**	0.069 ± 0.0021	0.761 ± 0,023	0.362 ± 0.011	0.624 ± 0.019
7	**Tryptophan**	0.604 ± 0.006	0.532 ± 0,005	0.889 ± 0.0089	0.468 ± 0.005
8	**Phenylalanine**	0.193 ± 0.0078	0.766 ± 0,031	0.603 ± 0.024	0.878 ± 0.035
Amount		**4.23** ± 0.132	**7.16** ± 0,223	**6.46** ± 0.201	**6.61** ± 0.206
Nonessential amino acids:					
9	Alanine	0.305 ± 0.006	0.569 ± 0,011	0.301 ± 0.006	0.499 ± 0.010
10	Arginine	0.506 ± 0.015	0.727 ± 0,022	0.646 ± 0.019	0.759 ± 0.023
11	Aspartic acid	0.077 ± 0.002	1.287 ± 0,026	0.360 ± 0.007	1.132 ± 0.023
12	Histidine	2.909 ± 0.116	1.054 ± 0,042	2.362 ± 0.094	0.905 ± 0.036
13	Glycine	0.068 ± 0.003	0.333 ± 0,013	0.143 ± 0.006	0.314 ± 0.013
14	Glutamic acid	0.966 ± 0.019	0.868 ± 0,017	2.843 ± 0.057	3.007 ± 0.060
15	Proline	1.390 ± 0.070	1.464 ± 0,073	1.397 ± 0.070	1.365 ± 0.068
16	Serine	0.107 ± 0.001	0.868 ± 0,009	0.430 ± 0.004	0.747 ± 0.007
17	Tyrosine	0.049 ± 0.001	0.367 ± 0,007	0.259 ± 0.005	0.209 ± 0.004
18	Cysteine	0.603 ± 0.012	-	0.389 ± 0.008	
Amount		**6.98** ± 0.188	**7.54** ± 0,204	**9.13** ± 0.247	**8,94** ± 0.241
Total amount of amino acids		**11.21** ± 0.326	**14.70** ± 0,428	**15.59** ± 0.454	**15,55** ± 0.453

plays an important role in nitrogen metabolism, supports respiration of brain cells and performs the function of a neurotransmitter in the human body [18, 19]. Leucine, which activates the endocrine and immune systems, prevails in the milk-protein concentrate (1.732 g / 100 g). Methionine and cysteine were not detected in all samples. In the free state, histidine which is involved in the formation of proteins and affects metabolic reactions, is present in large quantities (2.362 and 2.909 g/100 g, respectively) in the concentrates.

Non-essential amino acids (NAA) are performing important functions in the body: glucose synthesis (alanine), take part in enzymatic processes (arginine), improve the skin structure (proline), and some of them (cysteine, tyrosine, glutamic acid) play a physiological role, no less than essential amino acids [20]. Glutamic acid, proline and histidine are dominant in concentrates, among essential amino acids that perform the functions of precursors in the protein synthesis and other biologically active compounds. Glutamic acid supports the respiration of brain cells, plays an important role in the inactivation of ammonia, which is released as a result of the protein metabolism and, together with glucose, is an energetic material and source of nitrogen [21, 22]. According to the results, the content of NAA from the amount of total amino acids in the PBC and MPC is 58.03% and 56.04%, respectively.

It is important that the protein-berry concentrate contains 50.06% of amino acids in the free state (Figure 1a), including the content of essential amino acids is 41.44%. The resulting patterns indicate that the protein-berry concentrate has an easier digested form than the classical concentrate obtained by thermo-acid coagulation of milk proteins by acid whey. Analyzing the data presented in the Table 1, we can say that there is an increase in the amount of EAA in the protein-berry concentrate by 14.75% and NAA by 24.45% in the following amino acids: threonine by 0.156 g%, lysine by 021 g%, tryptophan by 0.221 g%, and phenylalanine by 0.525 g% compared with the control sample.

The level of biological value depends on protein quality, and primarily, the content and quantity of all essential amino acids. Their balance is determined by appropriate comparisons with the physiological norms that have been proposed by the international FAO/WHO expert committee in the form of the amino acid scale of an ideal protein. The low content of a certain limiting essential amino acid indicates an imbalance in the product and reduces the level of biological value [23].

The absolute values of the amino acid composition in PBC do not allow sufficiently objectively to estimate the biological value of concentrates. Indicators of the balance of amino acid composition were additionally calculated, which characterize not only the qualitative and quantitative difference of protein from the ideal, but also take into account the excessive content of essential amino acids and their ratio to non-essential ones.

The indicator of amino acid score and balancing definition of protein amino acid composition in protein-berry and milk-protein concentrates are shown in Tables 2 and 3.

Table 2. Amino acid score of protein-berry and milk-protein concentrates

The name of amino acid (EAA)	Milk-protein concentrate			Protein-berry concentrate		
	EAA, mg/100 g of protein	Weight fraction EAA (A), in 100 g of protein	Amino acid score (AS), %	EAA, mg/100 g of protein	Weight fraction EAA (A), in 100 g of protein	Amino acid score (AS), %
Valine	1,067	4.12	82.37	1,234	3.96	79.27
Isoleucine	980	3.78	94.57	1,027	3.3	82.46
Leucine	2367	9.14	130.53	2,671	8.58	122.55
Lysine	1958	7.56	137.42	2,168	6.96	126.6
Methionine + cysteine	2695	10.4	297.23	2,145	6.89	196.83
Threonine	830	3.2	**80.1**	986	3.17	**79.17**
Tryptophan	1,136	4.39	438.51	1,357	4.36	435.83
Phenylalanine+ tyrosine	1,375	5.31	88.46	1,481	4.76	79.28
Amount AA, mg/100 g of protein	25,906			31,136		
Total weight fraction EAA, mg %/100 g of protein		47.9			41.97	
Difference amount of amino acid score (Δ DAS), %			708.41			568.63

Table 3. Balancing definition of **protein amino acid composition in protein-berry and milk-protein concentrates**

Concentrates indicators	Milk-protein	Protein-berry
Differential coefficient of amino acid score, DCAS	88.551	71.079
Biological value, BV	11.45	28.92
Utilitarian coefficient EAA (U)	0.602	0.679
Redundancy coefficient EAA (σ_{red})	56.198	49.418
Rationality coefficient of amino acid composition (Rrat)	0.752	0.858

The score of each amino acid gives a general idea about biological value of the concentrate. The data in Table 2 indicate that threonine is the first limiting amino acid of MPC and PBC and the protein using on anabolic needs of the body is limited to its contents at the level of 80.10% and 79.17%, respectively. Excessive content of other essential substances is used to compensate energy consumption and biosynthesis of non-essential amino acids. To assess the degree of protein utilization, the differential coefficient of amino acid score (DCAS) has been calculated. According to the data (Table 3), the protein-berry concentrate has this indicator below the control by 19.73%, while the amino acids are absorbed more fully. Biological value increase of the PBC has confirmed at 28.92%, which is 2.5 times higher than the indicated indicator for the MPC.

For the numerical characterization of the balance of all EAA proteins in PBC and MPC, the utilitarian coefficient (U), the redundancy coefficient (σnad) and the rationality coefficient (Rrats) have been calculated relative to the standard (Table 3). The utilitarian coefficient for the PBC grows by 12.79%, and the rationality coefficient decreases by 6.78% compared with the control sample. This results indicate a better balance of EAA and effective utilization of PBC proteins. Moreover, the rationality coefficient of amino acid composition of the protein-berry concentrate is 0.858, which is 14.1% higher than in the concentrate obtained by the classical technology.

4. Conclusions

- The amino acid composition of concentrates has been determined by the method of high-performance liquid chromatography and it has been found that proteins of the protein-berry concentrate contain all essential amino acids and its biological value is 2.5 times higher compared with concentrates obtained by thermo-acid coagulation by acid whey.

- Obtained results confirm the use efficiency of berry coagulant for thermo-acid coagulation of milk proteins. Protein-berry concentrate has a 13% higher balance of amino acids and accessibility by the human body compared with the milk-protein concentrate.

- Research results should be used in the technology of cheese products, and the proposed method of protein coagulation can function in production systems.

5. References

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