PROTEIN COMPOSITIONS ON THE BASE OF PLANT BASED PROTEINS WITH BALANCED AMINO ACID COMPOSITION

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ABSTRACT

The data presents the theoretical and practical bases of creating new two-component protein compositions from various types of grain raw materials with using the transglutaminase enzyme in order to apply them in the production of food products to increase the biological value and improve quality indicators. On the basis of Monte-Carlo calculation method, has been developed program that allows automatic selection of qualitative and quantitative selection of protein components from various sources of vegetable protein for balancing composites according to their amino acid composition. The program provides calculations for compositions containing from 2 to 8 proteins with different origins. In accordance with this program, have been developed composites based on dry wheat gluten (DWG), which is known to have a low biological value, with the addition of amaranth, pea, potato and oat protein. An experimental way for the biocomposites of the composition of the DWG-pea protein, DWG-potato protein, potato protein-oat protein at certain ratios of dry preparations determined the optimal parameters of the reaction: water ratio, exposure time, the concentration of the enzyme preparation.

Composites are enriched with all three limiting amino acids: lysine, threonine, sulfur-containing amino acids. For each of the essential amino acids, amino acid score was 100% and higher. Further studies on the functional properties of composites, the physicochemical properties of the proteins that make up them, and structural features will make it possible to determine the uses in the manufacture of food products based on their ability to bind fat, water, form foam, and so on.

Keywords: *protein compositions, plant based preparations, amino acid score, biological value*

INTRODUCTION

The increase in the population of the planet allows experts today to predict the progressive shortage of protein foods. The shortage of protein on the planet is estimated at 10-25 million tons per year. Approximately half of the world's population suffers from a lack of protein. The lack of food protein is not only an economic but also a social problem of the modern world [1]. Biotechnological processes with the usage of microorganisms have not yet had any success in obtaining new alternative sources of this mandatory and valuable component of food. This dramatically increases the role of natural proteins, enhances the importance of high-tech technological processes in their production and use in the form of new forms. Plant based diet containing a complete protein in the required amount might be created on the base of usage of protein preparations obtained from protein-containing sources with different chemical compositions and biological values. The most common and affordable cereal protein is dry wheat gluten (DWG). Most of the cereals are deficient in lysine, one of the most important from the essential amino acids in human nutrition, while legumes and solanaceae contain this amino acid in sufficient quantity. On the other hand, cereal proteins can supplement legume and solanaceous proteins with the deficient amino acid methionine [2]. Along with soy proteins, with appropriate functional properties, pea and potato proteins also can be successfully used for enrichment and enhancing of the biological value of food products [3]. With the usage of enzyme preparation transglutaminase for the biosynthesis of composite protein products with the increased biological value from the technological point of view, it may be important to vary the content of free amino groups in the used plant preparations. Adding proteins with a high content of free amino groups, in particular lysine, to proteins with a low amount of them will increase the reactivity of the latter [4] and form modules with a given composition and functional properties.

The aim of this work is the formation of multi-protein polymers based on biologically inferior DWG with the selection of compositions formula on the base of amino acid composition data for the intake of complete protein into the human body and the expansion of the use of this protein product in food production.

MATERIALS AND METHODS

The main materials used were samples of DWG (BioRosva, Kaluga region, Russia) and protein concentrates: potato (Roquette, France), oat (Tate & Lyle, Sweden), pea (Roquette, France). The chemical composition of protein concentrates is shown in Table 1. The enzyme preparation used was the enzyme preparation (EP) of the ‘classical’ transglutaminase (TG) (Novozymes, Denmark).

Table 1. The chemical composition of protein products

Protein product	Humidity, %	Protein, %	Fat, %	Insoluble fibers, %	Carbohydrates, %
DWG	3,6	83.7	1,4	0,2	11,1
Protein concentrates:					
Pea	10,0	84.0	5,0	1,0	0
Potato	8,0	78,0	5,0	0,5	8.5
Oat	6,0	56,3	3,0	1.5	33.2

Conclusion about the reaction, with the transglutaminase enzyme, between proteins with different chemical nature were based on the amount of released amine nitrogen. Amine nitrogen was determined by formol titration. To do this, 50 cm^3 of distilled water was added to 10 g of the fermented DWG mixture with protein concentrates taken at certain ratios, then the mixture was dispersed for 4-5 minutes at 500 min^{-1}. The mixture were centrifuged at 5500 min^{-1} for 20 minutes. The supernatant was decanted, 5 cm^3 from it was transferred into a glass beaker, mixed with 20 cm^3 of distilled water after has been measured the pH. The pH meter electrodes were loaded into the test suspension. Neutralization of free carboxyl groups was performed with 0.05 N NaOH solution. Alkali was added while stirring, following the readings of the potentiometer. When the pH of the solution reached 7, was added 0.5 cm^3 of the formula mixture with phenolphthalein 50 cm^3 of 40% formalin + 2 cm^3 of 1% alcohol solution of phenolphthalein). The mixture was titrated with 0.05N NaOH solution to pH 9.1-9.5, which corresponded to the bright red staining of the sample. All reagents were chemically pure.

Amine nitrogen (in mg /%) (N) was calculated by the formula: N = A * 0.7 * 100 / V, where: A -is the amount of cm^3 of 0.05N NaOH, followed by titration; V- is the amount of solution for titration; 0.7- is the amount of nitrogen in g, corresponding to 1 cm^3 of 0.05 N NaOH solution.

For the preparation of two-component fermented compositions, weighed protein products at their specific ratios were mixed on a stirrer at a speed of 500 min^{-1}. Samples of transglutaminase EP were placed in a microbiological test tube with a cap, was added 10.5 cm^3 of distilled water , mixed vigorously, and was added 3 g of a mixture of protein products. The tubes were placed in a thermostat, shaken at 170 min^{-1} and a temperature of 50 ° C, and the proteins were reacted at different flow times and concentrations.

RESULTS AND DISCUSSION

With the help of the program developed by us on the basis of the Monte Carlo calculation method, were compiled protein compositions with an improved amino acid profile. In time of calculating was used amino acid composition data of protein products for the proposed mixtures. For DWG and pea concentrate, the optimum ratio of proteins in the composition was 1: 1.5 (table 2), for DWG and potato

concentrate - 1: 2.0 (table 3), for potato and oat - 1: 1.5 (table 3). These ratios provided the optimal amino acid fastness and were economically feasible due to the low cost of DWG compared to other protein concentrates.

Table 2. Amino acid score of proteins from compositions of the DWG and protein concentrates,%

Indicators		Protein concentrate			Protein compositions		
	DWG	PEA	POTATO	OAT	DWG /PEC	DWG /POC	POC/ OC
Mass fraction of proteins, %	83.0	84.0	78.0	56.3	84.5	80.5	67.2
Amino acids	Score, %						
Valine	72	100	110	52	105	119	121
Leucine	95	117	87	54	129	113	105
Isoleucine	85	117	115	47	124	129	122
Threonine	65	95	117	35	98	122	115
Lysine	27	129	112	29	101	100	107
Methionine + cysteine	40	31	97	54	42	94	113
Phenylalanine + tyrosine	147	153	153	77	180	189	173

Note: PEC – pea concentrate; POC – potato concentrate; OC – oat concentrate

The data shows that potato concentrate had the highest amino acid values of all acids, oat concentrate and DWG had the lowest values, and insufficient amounts of sulfur-containing amino acids are present in pea concentrate, which does not contradict the literature data. Amino acid composition of protein compositions with DWG and oat concentrate, compared with individual samples, was significantly improved due to potato and pea concentrates. This increase is especially valuable in all two-component composites for lysine (101-107%), threonine (98-122), and sulfur-containing amino acids (42-113), the deficiency of which is noted in most grain crops [5]. Composites of the POC / OS and DWG / POC composition were the most balanced, the most unbalanced (for sulfur-containing amino acids) was the DWG / PEC composition composite. For a complete optimization of the amino acid level of such a composite, potato concentrate should be added to its composition.

To determine the parameters of the biosynthesis of compositions from DWG and protein concentrates, was studied the effect of the concentration of EP and the exposure time on the amount of amino nitrogen released during the reaction. Considering that in reactions involving transglutaminase, the transfer of amino groups - NH_2 occurs between molecules with the formation of new covalent bonds, the amount of amino nitrogen in the systems under study decreases, so the number of unreacted amino groups can be judged on the course of the synthesis process between different types of proteins. Studies were performed at two concentrations of EP: 0.0015 and 0.0024 g / g of protein and the duration of the reaction was 5, 10, 15, 30, 90, and 120 min. The amount of amine nitrogen in the original protein preparations, which were taken as control samples, is shown in Figure 1. It can be seen that the smallest amount of free amino groups contained DWG, the highest - potato concentrate (more than 3 times as compared to DWG), the other two concentrates occupied an intermediate position on this indicator between them. Therefore, greater reactivity could be expected from potato and pea concentrate

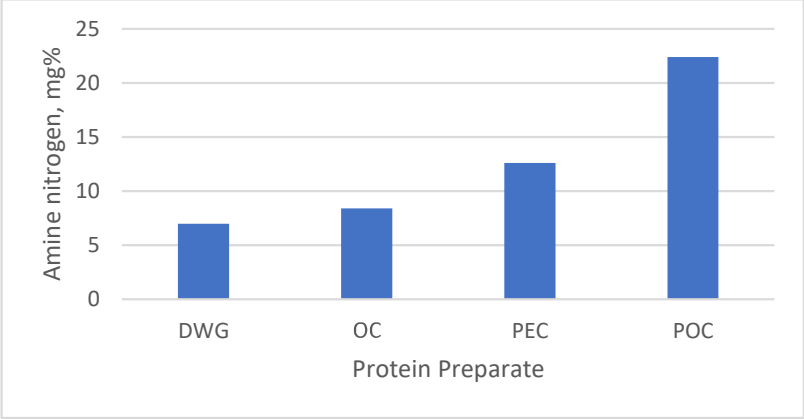


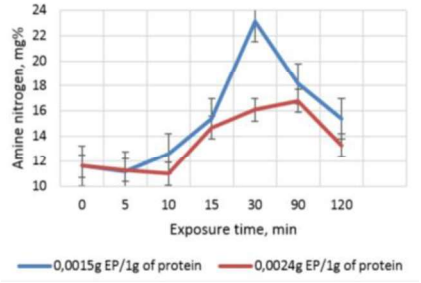
Figure 1. Amount of amine nitrogen in protein products

The study of the effect of the duration of the enzymatic reaction with transglutaminase at two different concentrations of EP revealed that the composition of DWG-pea concentrate had the minimum amount of amino nitrogen in the medium at an AF concentration of 0.0024 g / g protein and an exposure time of 10 minutes (Fig. 2a). At a concentration of 0.0015 g / g of protein at 10 minutes of reaction, the amount of amine nitrogen in the reaction medium was contained. Over 10 minutes of the reaction, and especially at 30 minutes of its course, there was a sharp jump in the number of free groups for both values of the EP concentration. This was probably due to competitive inhibition of the transfer of amino groups by accumulating reaction products or substrate inhibition of the active center of the enzyme.

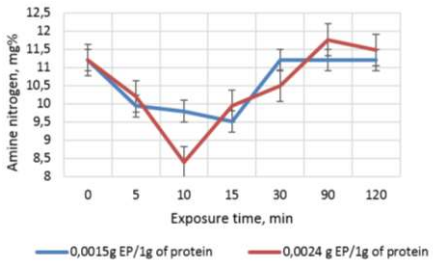
For the composition of the DWG with potato concentrate, the patterns of change in the amount of amino nitrogen in the course of the reaction were similar to the patterns characteristic of the composition of the DWG-pea concentrate. The minimum amount of amino nitrogen after reaction with TG was also observed in the reaction medium at a concentration of 0.0024 g / g of protein and an exposure

time of 10 minutes (Fig. 2b). The absolute value of the nitrogen remaining after the transfer of the amine groups was lower by 10% by 30%, and by 30 minutes by 3.7 times lower than that of the composite with pea concentrate, which indicated a greater reactivity of the proteins of the potato concentrate than pea concentrate, possibly due to their greater number in the original concentrates and before the reaction (table 2).

For the composition of potato concentrate-oat concentrate, the minimum amount of amino nitrogen after reaction with TG remained at an EP concentration of 0.0015 g / 1g of protein and an exposure time of 15 minutes (Fig. 2c), with significant differences in the amount of nitrogen with a concentration of 0.0024 g / g protein was not observed.



A



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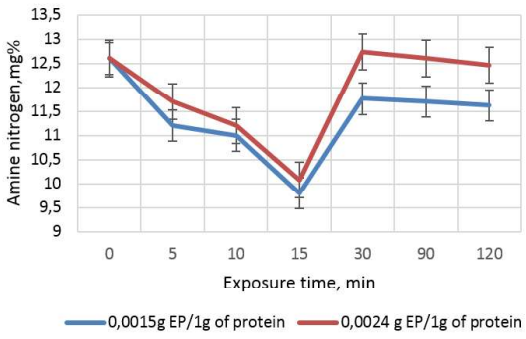

C

Figure 2. Amount of amine nitrogen in protein products: A - DWG-pea concentrate; B - DWG-potato concentrate; C - Potato concentrate - oat concentrate

CONCLUSION

Due to its unique properties, TG is widely used in the meat and dairy industry for the aggregation of protein molecules in the production of restructured products from raw materials of various qualities [6], [7], [8]. The enzyme is safe, produced by inexpensive sources of biosynthesis, which makes its use wide. Significantly less

TG is used in the manufacture of baked goods (bread, biscuits) [4], [9], [10] and isolated studies are known to produce compositions from vegetable proteins [11].

With the help of a program developed on the basis of the Monte-Carlo calculation method, were compiled protein concentrate compositions with improved amino acid composition. Taking into account the mass fraction of protein and the amino acid composition of the concentrates, their ratios and amino acid levels are determined for protein-protein composites obtained from various types of plant materials (wheat, peas, potatoes, oats). Composites are enriched with lysine, threonine, sulfur-containing amino acids in relation to cereals and leguminous crops. Using biotechnological reactions with the participation of the enzyme class transferase (transglutaminase) obtained biocomposites composition: DWG-pea concentrate, DWG-potato concentrate, potato concentrate-oat concentrate. Experimentally using the method of formol titration according to the amount of amino nitrogen remaining in the reaction medium, the reaction parameters were determined: the duration of its flow and the concentration of the enzyme preparation. For the composition DWG-pea concentrate and DWG-potato concentrate, the least amount of amino nitrogen after reaction with TG remained in the medium at an EP concentration of 0.0024 g / g protein and a reaction time of 10 minutes; for the composition, a potato concentrate-oat concentrate — at an EP concentration of 0.0015 g / g protein and exposure time 15 minutes. These data indicated a high intensity of the reaction of synthesis of new forms of proteins. Compositions of concentrates with potato protein did not contain deficient essential amino acids, soon approached the reference protein as much as possible, or it was higher. Further studies will show what functional and technological properties created protein composites will have and in which food products they can be used.

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