MATHEMATICAL MODELS FOR THE SYNTHESIS OF PLANT-BASED COMPOSITIONS WITH IMPROVED AMINO ACID COMPOSITION

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ABSTRACT

The aim of the work was to optimize the process of obtaining multicomponent protein compositions with high biological value and higher functional properties than the original vegetable protein products. Was realized studies to obtain biocomposites on the base of pea protein-oat protein and pea protein-rice protein. Developed composites were enriched with all limited amino acids. For each of the essential amino acids, the amino acid score was 100% and higher. Protein products used in these compositions are not in major allergen list, which allows to use these compositions in allergen-free products and specialized nutrition. To determine biosynthesis parameters for compositions from pea protein and various protein concentrates with the use of transglutaminase enzyme, was studied effect of concentration and exposition time on the amount of amino nitrogen released during the reaction. Decreasing of amino nitrogen in the medium indicated the occurrence of a protein synthesis reaction with the formation of new covalent bonds. Were determined optimal parameters of reaction: the hydromodule, the exposure time, the concentration of EP of the preparation, were obtained mathematical models. Studies on the functional properties of composites, the physicochemical properties of the proteins that make up their composition, 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, gels, and etc.

Keywords: *plant-based preparations, transglutaminase, protein compositions, 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]. With the help of 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 composition and biological value. Most of the cereals are deficient in lysine, one of the most important from the essential amino acids in human nutrition, while legumes contain this amino acid in sufficient quantity. On the other hand, cereal proteins can supplement legume proteins with the deficient amino acid methionine [2]. Along with soy proteins, with appropriate functional properties, pea, rice and oat 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 increased biological value from a 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 an optimization of parameters of biosynthesis multi-protein compositions, created on the base of pea protein with the selection of their composition on the basis of the quantitative content of amino acids in them, and above all, essential, so that the human body received full proteins, and this protein product could be more widely used in the manufacture of food products.

MATERIALS AND METHODS

The main materials used were samples of pea (Roquette, France), rice (Beneo, Belgium) and oat (Tate & Lyle, Sweden),. 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, %
Pea	10,0	84,0	5,0	1,0	0
Rice	12,0	79,0	5,0	3,2	6,0
Oat	6,0	56,0	3,0	2,0	18,0

Conclusion about the reaction, with the transglutaminase enzyme, between proteins with different chemical nature was 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 was 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 3,6 cm^3 of phosphate buffer solution with predetermined pH, mixed vigorously, and was added 1 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.

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, in accordance with a given hydromodule, mixed vigorously, and was added 1 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, data for the reference protein based on the recommendations of the FAO WHO (2011) [5]. For pea and oat concentrates, the optimum ratio of proteins in the composition was 1: 1 (table 2), for pea and rice concentrates - 1: 0.6 (table 2). These ratios provided the optimal amino acid fastness and were economically feasible.

Table 2. *An amino acid score of proteins from compositions of the protein concentrates,%*

Indicators	Protein compositions			Protein concentrate	
	PEA	OAT	RICE	PEC/OC	PEC/RC
Mass fraction of proteins, %	84.0	56,3	79.5	70.2	81.8
Amino acids	Score, %				
Valine	125	65	137	132	157
Leucine	134	62	124	135	158
Isoleucine	156	62	113	149	170
Threonine	152	56	176	142	196
Lysine	148	33	147	117	141
Methionine + cysteine	151	103	136	181	177
Phenylalanine + tyrosine	47	82	147	101	105

Note: PEC – pea concentrate; OC – oat concentrate; RC-rice concentrate

The data showed that rice concentrate had the highest amino acid scores, oat protein had the lowest values, pea concentrate contained insufficient amounts of sulfur-containing amino acids, which does not contradict the literature data. Amino acid composition of protein compositions with pea concentrate, in comparison with individual samples, was significantly improved due to rice and oat concentrates. This increase is especially valuable in all two-component composites for lysine, threonine, phenylalanine and rytosine, sulfur-containing amino acids, the deficiency of which is noted in most grain crops [6]. The most balanced in terms of amino acids was the PEC/RC composite composition, the most unbalanced (in sulfur-containing amino acids) - the PEC/OC composition composite.

To optimize the parameters of the biosynthesis of compositions from protein concentrates using TG was studied the effect of concentration of EP, exposure time and hydromodule on the amount of amino nitrogen released during the reaction with the enzyme.

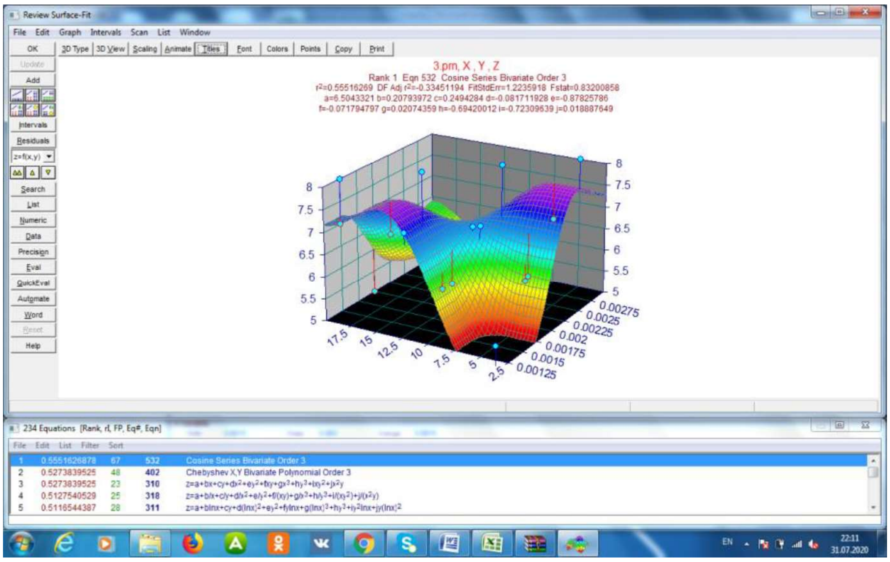
Considering that in transglutaminase reactions, the transfer of amino groups -NH_2 between molecules occurs with the formation of new covalent bonds, the amount of amino nitrogen in the studied systems decreased, therefore, by the number of unreacted amino groups, it was possible to judge the course of the synthesis process between different types of proteins.

To obtain results on the influence of various factors on the content of amino nitrogen for the composition of pea protein-oat protein, methods of mathematical planning of the experiment were used, with pH = 6.8–7.0 (const), temperature = 50 ° C (const). The exposure time (X3) was changed in the range of 5 ÷ 20 min, the hydronic module (X1) - in the range of 1: 5 to 1: 8, the concentration of EP (X2) - from 0.0015 to 0.003 U / g protein (table 3).

Table 3. *The effect of the concentration of EP, the exposure time and hydromodule on the amount of amine nitrogen in the process of synthesis of the composition of the pea concentrate-oat concentrate*

№	Concentration of EP, U/g for DS	Exposure time, min	Hydromodule	Amine nitrogen, mg%
1	0,0015	5	1:5	7,98
2	0,0015	10	1:6	7,28
3	0,0015	15	1:7	6,16
4	0,0015	20	1:8	7,98
5	0,002	5	1:6	9,38
6	0,002	10	1:7	7,56
7	0,002	15	1:8	6,86
8	0,002	20	1:5	8,4
9	0,0025	5	1:7	6,44
10	0,0025	10	1:8	7,84
11	0,0025	15	1:5	7,14
12	0,0025	20	1:6	8,4
13	0,003	5	1:8	7,7
14	0,003	10	1:5	7,42
15	0,003	15	1:6	7,7
16	0,003	20	1:7	7,7

According to the experiment data, using the TableCurve 3D program, were constructed response surfaces for amino nitrogen (Fig. 2). Data processing in programs Matematika and Table Curve 3D



$$Y = 7.4865 + 149X_1 + 0.0335X_2 - 0.1145X_3$$

where x –concentration of EP, g/g of protein; y-time of exposition, min; z- back hydromodule

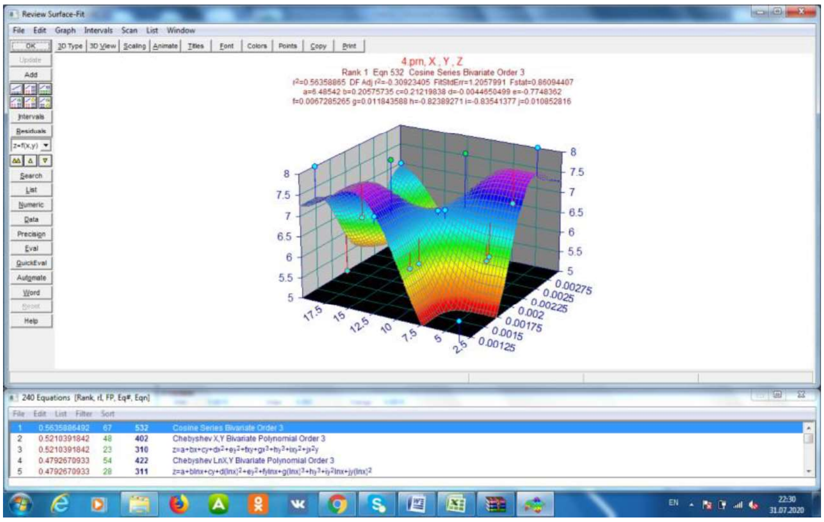
Fig. 2. *The dependence of the amount of amine nitrogen on the reaction parameters for the composition of pea concentrate-oat concentrate*

To obtain the results on the influence of various factors on the content of amino nitrogen for the composition of pea protein concentrate-rice protein, were used methods of mathematical planning of the experiment. Taking into account the data obtained during the protein concentrate-rice protein experiment, the most effective reaction parameters were selected: pH - 6.8–7.0 (const), temperature 50°C (const). The exposure time (X3) was changed in the range of 5 ÷ 20 min, the hydromodule (X1) - in the range of 1: 5 to 1: 8, the concentration of OP (X2) - from 0.0015 to 0.003 U / g protein (table 4).

Table 4. *The effect of the concentration of EP, the exposure time and hydromodule on the amount of amine nitrogen in the process of synthesis of the composition of the pea concentrate-rice concentrate*

№	Concentration of EP, U/g for DS	Exposure time, min	Hydromodule	Amine nitrogen, mg%
1	0,0015	5	1:5	7,28
2	0,0015	10	1:6	7,14
3	0,0015	15	1:7	7,14
4	0,0015	20	1:8	6,72
5	0,002	5	1:6	7,28
6	0,002	10	1:7	7,28
7	0,002	15	1:8	7,14
8	0,002	20	1:5	7,84
9	0,0025	5	1:7	7
10	0,0025	10	1:8	7,42
11	0,0025	15	1:5	7,98
12	0,0025	20	1:6	7,98
13	0,003	5	1:8	7,28
14	0,003	10	1:5	8,4
15	0,003	15	1:6	8,26
16	0,003	20	1:7	7,84

According to the experiment data, using the TableCurve 3D program, were constructed response surfaces for amino nitrogen (Fig. 3). Data processing in programs Matematika and Table Curve 3D



$$Y = 7.5775 + 567X_1 + 0.0245X_2 - 0.2555X_3$$

where x –concentration of EP, g/g of protein; y-time of exposition, min; z- back hydromodule

Fig. 3. *The dependence of the amount of amine nitrogen on the reaction parameters for the composition of the pea concentrate-rice concentrate*

For the composition of pea concentrate-oat concentrate, the patterns of change in the amount of amine nitrogen in the course of the reaction were similar to the patterns characteristic of the composition of pea concentrate-rice concentrate. The minimum amount of amino nitrogen after reaction with TG in the composition of pea concentrate-oat concentrate was observed in the reaction medium at a concentration of 0.0015 g / g of protein, exposure time of 15 minutes and a water ratio of 1: 7. The minimum amount of amino nitrogen after reaction with TG in the composition of pea concentrate-rice concentrate was observed in the reaction medium at a concentration of 0.0015 g / g of protein, exposure time of 20 minutes and a water ratio of 1: 8.

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 [7], [8], [9]. 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], [10], [11] and isolated studies are known to produce compositions from vegetable proteins [12].

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 (peas, rice, 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: pea concentrate-oat concentrate, pea concentrate-rice 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 of pea concentrate-oat concentrate, the minimum amount of amino nitrogen after reaction with TG in a two-component product was observed in the reaction medium at a concentration of 0.0015 g / g of protein, exposure time 20 minutes and a water ratio of 1: 7; for the composition of pea concentrate-rice concentrate, the minimum amount of amino nitrogen was released in reaction with TG at a concentration of 0.0015 g / g of protein, exposure time of 20 minutes and a water ratio of 1: 8. These data indicated a high intensity of the reaction of the 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 possess and in which food products they can be used.

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