OBTAINING PROTEIN CONCENTRATES FROM TRITICALE EXTRACT AND PEA FLOUR WITH APPLICATION OF ENZYMES

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ABSTRACT

The purpose of this work is to obtain protein concentrates (composites) for food purposes with improved amino acid composition, in relation to the raw materials. The raw material for obtaining these products was the secondary product of grain processing of triticale for starch (extract, soaking water) and whole ground pea flour. With the use of hydrolytic enzyme preparations (cellulase, xylanase, amylase, protease), food protein concentrates were obtained from a mixture of triticale extract and pea flour at a ratio of 1:3 to 1:5 protein fractions with a protein content of 80.40 % and 75.44 % on dry substance. Amino acid score by lysine in mentioned protein products was more than 100 %, threonine – 96-98 %, methionine+cystine – 60-62 %. All chemical indicators and functional and technological properties of the obtained protein concentrates corresponded to indicators of commercial samples of dry wheat gluten. Has been proved the feasibility of bioconversion of whey waters remaining after separation of the protein composite from TE and pea flour, for growing fodder biomass with the fungus *Geotrichum candidum 977* in combination with *Saccharomyces cerevisiae*. Triticale extract was a benign raw material resource for food protein concentrates in a complementary mixture with pea flour, having chemical composition and functional properties that meet the requirements of these types of products.

Keywords: *triticale extract, pea flour, protein concentrates, enzyme preparations.*

INTRODUCTION

The world food and processing industry annually uses various types of raw materials for effective use of which requires the introduction of advanced processing technologies, which allow production, along with traditional products, food and feed protein preparations with desired properties. Despite the fact that in recent years the structure of nutrition in the world has improved, the consumption

of protein-containing foods by the population does not meet the standards [1]. The growth in the output of the starch industry is accompanied by an increase in the yield of by-products. The basic raw material for starch production is grain, during the processing of which secondary products are formed, often infected with various types of microorganisms, and representing a danger to the environment of the enterprise [2]. A promising way to eliminate the negative impact of soaking water (extracts) on the environment is their biocatalytic or biosynthetic modification with the production of food. Since cereals contain proteins that are unbalanced in amino acid composition, it is also advisable to solve the problem of improving the quality of commercial preparations through the creation of protein composites of enhanced biological value. One of such ways is the development of technological solutions using physicochemical and biotechnological methods for the joint processing of grain and leguminous raw materials. In this work, triticale extract (TE) was studied, which is advisable to mix with pea flour, since pea protein has a high content of essential amino acids and increased solubility [3]. Therefore, in order to eliminate the deficiency of protein-containing preparations from plant raw materials, methods and technological solutions for protein extraction are developed using modern physicochemical and biotechnological methods of processing grain, leguminous plants and other types of crops. Such methods include processes using enzyme preparations (EP), which exclude the destruction of the structure and composition of protein fractions of grain and leguminous raw materials, as opposed to the use of acids and alkalis [4].

The aim of the work is to obtain protein concentrates for food and feed purposes from the secondary product of processing triticale grain (extract) for starch and pea flour and characterizing their chemical composition and functional and technological properties.

MATERIALS AND METHODS

Triticale extract was obtained at the All-Russian Research Institute for Starch Products from Legion, Bard, and Consul grain sorts provided by the Don-Zonal Agricultural Research Institute and ungraded grain from the Mglinsky starch plant (Bryansk region, Russia). The chemical composition of grain, % of dry substance (DS): starch 62.8±0.5, protein (Nx5.7) 10.1±0.4, lipids 1.50±0.05, ash 1.72±0.20, reducing sugars 10.0±0.3. TE had chemical composition, % on DS: mass fraction of DS 11.00±1.05, protein (Nx5.7) 20.40±2.10, pH of the medium 5.10±0.1.

To develop a biocatalytic process for obtaining food and feed preparations, triticale grains were soaked for 48 hours in 0.4 % sodium metabisulfite solution, soaking water was removed, the grain was crushed, cellulolytic EP was added to the mass, centrifuged, and the centrifugal was mixed with soaking water. The pulp (fiber) was separated from the grain mass on the sieves, washed and used for feeding purposes. The starch-protein suspension was separated by centrifugation into starch A and starch B. Starch B was mixed with soaking water, hydrolyzed with α-amylase, and a triticale extract was obtained.

For the production of protein two-component composites, along with TE, commercial pea flour with chemical composition was used, in % on dry substance:

moisture 8.9, protein 22.9, ash 3.10, fat 1.70, starch 52.7, other carbohydrates 19.60. Enzyme preparations (EP) were obtained from Novozymes: Shearzym 500 L with xylanase activity, Viscoferm L with basic cytolytic activity, Fungamyl 800 L and AMG 300 L with amylase and glucoamylase activity, Distizym Protacid from Erbslon with proteolytic activity.

RESULTS AND DISCUSSION

Pre-calculated for the first three limiting amino acids of triticale grains (lysine, threonine, methionine + cystine), taking into account their mass fraction in 100 g of the product, mass fraction of protein in the product (in %) and the "reference" scale of the FAO / WHO (1973), obtained amino acid scores in %. Table 1 presents the results for the proteins of the initial grain and 2-component composites of triticale and peas, compiled at certain ratios of the mass fraction of proteins from various sources. It is confirmed that the triticale grain is poor in lysine, threonine, sulfur-containing amino acids, and peas only in methionine + cystine.

Table 1 – Amino acid score of grain proteins and composites

Cultures	Amino acid (%)			
	Lysine	Threonine	Methionine + Cystine	
Triticale	58	76	85	
Pea	137	102	54	
Composites		Amino acid (%)		
	The ratio of the mass fraction of protein	Lysine	Threonine	Methionine + Cystine
Triticale - Pea	1:1	98	89	70
Triticale - Pea	1:3	117	96	65
Triticale - Pea	1:5	122	98	60

When balancing triticale protein with pea protein at ratios of 1:1 ÷ 1:5, sulfur-containing amino acids did not exceed 70 %. However, threonine fasting was close to 100 %, lysine over 100 % (except for the 1:1 ratio). Therefore, to balance the amount of lysine and threonine in the composition with a necessary component, it was advisable to use pea flour protein.

Initially, a separate process for the isolation of proteins from pea flour was carried out according to a scheme with technological regimes developed by us earlier for triticale grains [2] (Figure 1). At the same time, we studied the effect of the particle size of pea flour on the protein yield in the solution after the raw material was treated with enzyme preparations. Initially, pea flour was poured with tap water containing cellulolytic and amylolytic EP at a concentration of 50 and 2 units/1 g DS, respectively, to a water ratio of 1:19. The treatment was carried out for 3 hours at 50 °C and pH 5.0 with stirring, after which the pH of the suspension was adjusted to 4.3, solutions of EP of xylanase and glucoamylase action were added, based on an activity of 50 and 2 units/g DS, respectively. The treatment was carried out for 3 hours at 55 °C with stirring. The solution was separated from the insoluble

residue, and a solution of proteolytic EP was added to the residue at the rate of 0.4 units/g DS to a hydronic module 1:12 at pH 3.0 and a temperature of 50 °C. The treatment was carried out for 1 hour with stirring. The extract was centrifuged, all the protein solutions were combined, concentrated to a content of 16 % of DS at 50 °C, and a solution of EP transglutaminase was added at a concentration of 14 units/g of DS and pH 5.5.

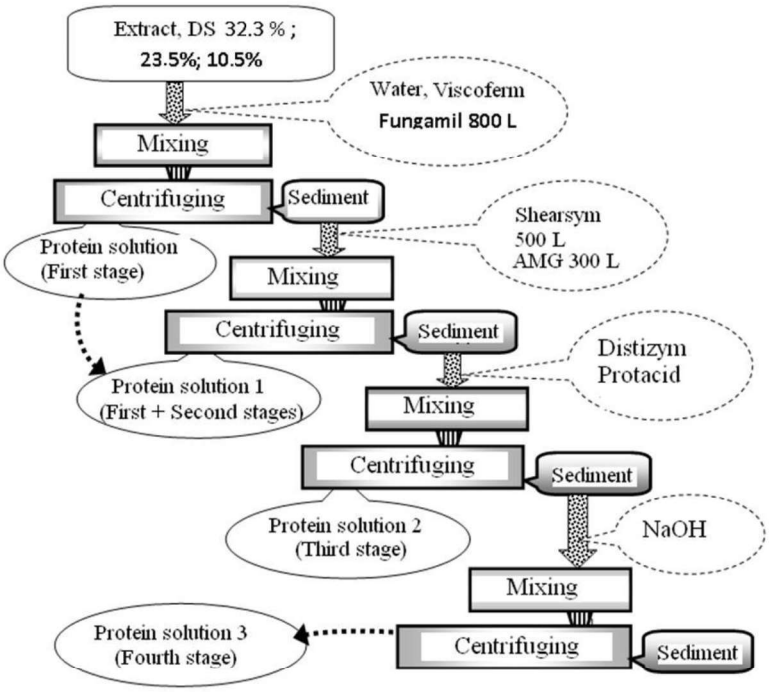

Figure 1 – Preparation of protein solutions from triticale extract

The protein suspension was kept for 30 minutes at 50 °C, after which the pH of the solution was adjusted to 3.0, centrifuged for 15 minutes at 5000 rpm. The precipitation was neutralized with a 5 % NaOH solution to pH 6.2, washed with water and dried in a lyophilic method.

From table 2 it is seen that the maximum yield of protein from pea flour is greater, the smaller the size of its particles. The total protein yield after all stages of extraction with a particle size of 110.4 micrometers was 93.15±3.01 % against 68.12±1.0 % with a size of 237.7 micrometers. Pea flour with a particle size of 237.7 micrometers and in mixtures with TE also provided relatively low protein solubility (63.51±1.6 % of the total amount in the raw material).

Table 2 – The effect of the particle size of pea flour on the protein yield (% of the total in raw materials)

Solutions and EP	DS (%)	Weighted average particle size (micrometers)	
		237.7	110.4
Solution after first stage (cellulase, amylase)	1.00±0.03	21.46±1.02	25.9±1.01
Solution after second stage (xylanase, glucoamylase)	0.53±0.02		7.00±0.09
Solution after third stage (protease)	0.70±0.05	36.2±0.08	16.5±0.06
Solution after fourth stage (alkali)	1.20±0.04	11.46±1.04	43.75±3.02
Total		**68.12**±1.02	**93.15**±1.01
Sediment after fourth stage	19.7±0.08	30.10±1.07	8.12±1.08
Total	-	98.22±1.10	101.27±2.02

For pea flour with a particle size of 110.4 micrometers, the feasibility of carrying out the third stage of processing with proteolytic EP Distizym Protazyd was separately studied, taking into account that the molecular masses of pea proteins are ten times smaller than those of cereal crops, therefore further hydrolytic cleavage of polypeptides could be undesirable. However, the results showed that in the presence of proteases, the transition of the protein into the solution is higher than without them by 16.5 % (Table 3); therefore, it was not advisable to exclude them from the scheme for obtaining a concentrate from peas at this stage.

Table 3 – The effect of proteases on the yield of protein from pea flour in solution (% of total in the solution)

Solutions and EP	DS (%)	Without protease	With protease
Solution after first stage (cellulase, amylase)	1.00±0,03	23.60±1.05	25.9±1.01
Solution after second stage (xylanase, glucoamylase)	0.53±0.02	8.70±0.10	7.00±0.09
Solution after third stage (protease)	0.70±0.05	-	16.5±0.06
Solution after fourth stage (alkali)	1.20±0.04	63.80±1.04	43.75±3.02
Total		**96.10**±1.02	**93.15**±1.01
Sediment after fourth stage	19.7±0.08	2.10±1.07	8.12±1.08
Total	-	98.20±1.10	101.27±2.02

As a result without the use of alkali, the total protein of triticale and pea flour in an amount of about 50 % of the initial raw material was transferred into the solution using EP.

The results of the composition and functional-technological properties of protein concentrates are shown in Table 4. It was established that protein preparations had a high mass fraction of protein (75-80 %) the remaining indicators of composites also correspond to the group "Concentrates".

Table 4 – The chemical composition of protein concentrates (PC) at different ratios of TE protein and pea flour

The ratio of protein TE and pea flour in the composition of PC	Moisture (%)	Mass fraction (% on DS)			
		Protein Nx6.25	Lipids	Ash	Carbo-hydrates
1:3	2.90±0.04	80.40±1.03	1.97±0.04	3.53±0.06	14.10±1.0
1:5	4.80±0.03	75.44±0.09	4.94±0.20	2.93±0.3	16.73±0.7
	Functional properties				
	Solubility (%)		WBC (%)	FBC (%)	FEC (%)
1:3	0.39±0.05		230±2	131±0.5	45.0±1.0
1:5	0.13±0.03		263±1	131±0.0	45,0±0.0

Note: WBC – water-binding capacity, FBC - fat binding capacity, FEC - fat emulsifying capacity

All functional properties, as well as chemical indicators of protein concentrates, are similar to those of commercial samples of dry wheat gluten. Significant differences in performance, depending on the different ratios in their composition of proteins (triticale : pea - 1:3 and 1:5), were not found.

In order to biosynthetic modification of the serum released after precipitation of proteins isolated by EP from TE and pea flour, a symbiotic composition of *Saccharomyces cerevisiae* yeast and yeast-like fungus *Geotrichum candidum 977* was selected. In the process of growing symbiotic cultures on serum, yeast absorbed low molecular weight carbohydrates, amino acids formed after hydrolysis of starch, fiber, hemicelluloses, and proteins at the 1st and 2nd stages of protein isolation (Table 5).

Table 5 – The carbohydrate composition of the extract after the treatment of EP for growing symbiotic cultures (% of total)

Stage	Carbohydrates					
	High molecular weight compounds	Malto tetrose	Maltotrios, raffinose	Sucrose, maltose	Glucose	Xylose, galactose
Before EP processing suspension of triticale : peas						
	38.06±0.09	0	4.96±0.10	41.97±1.12	13.27±0.08	1.74±0.4
After EP processing suspension of triticale : peas						
1	60.0±0.34	0	24.87±0.31	5.64±0.21	9.01±0.46	Traces
2	9.95±1.29	1.95±0.04	2.61±0.01	52.51±0.58	25.06±0.90	8.19±0.2

At the 1st stage of protein extraction after-treatment of the grain suspension with amylases and cellulases, the number of high-molecular compounds (HMC), probably released from the connection with protein triosis, sharply increased, but the relative amount of disaccharides and glucose decreased. In the second stage, the effect of xylanase and glucoamylase reduced the number of HMCs by almost 4 times, the number of trioses increased almost 2 times and the amount of glucose increased 2 times, the number of disaccharides increased almost 10 times compared to the 1st stage, and almost 5 times - xylose and galactose. Consequently, the nutrient medium based on enzymatic hydrolysis was enriched with simple carbohydrates, which was favorable for the growth of microorganisms, biomass growth and protein synthesis (Figure 2). If whey was used as a nutrient medium formed at a ratio of protein triticale: pea 1:3, then its mass fraction in biomass was 55.8±0.40 %, if 1:5, then 75.10±0.05 %, which was quite a high figure.

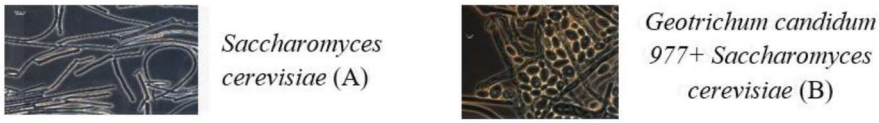

Figure 2 – Yeast cells (A) and yeast-like fungus (B) colonies

CONCLUSION

Thus, the biocatalytic process of obtaining food protein composites from TE and pea flour with a complementary amino acid composition (lysine score is over 100 %, threonine score 96-98 %, a score of sulfur-containing amino acids 60-62 %) is carried out at protein ratios of 1:3 and 1:5 using hydrolytic EP (cytase, xylanase, amylase, protease). The data of chemical composition are similar to the values of standard indicators of dry wheat gluten; the mass fraction of protein in the final production was 75-80 % on DS. Functional and technological properties of protein

products are characteristic of concentrates derived from other grains (rye, barley, amaranth, rice). The feasibility of bioconversion of whey waters remaining after separation of the protein composite from TE and pea flour for growing fodder biomass with the fungus *Geotrichum candidum 977* in combination with *Saccharomyces cerevisiae* has been proved.

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