COMPREHENSIVE BIOTECHNOLOGICAL APPROACH TO PROCESSING OF PEA FLOUR FOR FOOD AND FODDER PURPOSES

Ph.D. Student Denis Kulikov[1]
Ph.D. Ruzaliya Ulanova[2]
Prof. Dr. Valentina Kolpakova[3]
[1, 3] All-Russian Research Institute for Starch Products – Branch of V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Russia
[2] S.N. Vinogradsky Institute of Microbiology, Federal Research Center "Fundamental Foundations of Biotechnology", of Russian Academy of Sciences, Moscow, Russia

ABSTRACT

Investigations were carried out to optimize the growth parameters of the symbiosis of cultures of the yeast *Saccharomyces cerevisiae* 121 and the fungus *Geotrichum candidum* 977 on whey waters formed from pea flour as a secondary product in the production of protein concentrates after precipitation of proteins at the isoelectric point. The whey remaining after protein precipitation is bioconverted at optimal parameters of crop growth (pH of the medium, amount of inoculum, temperature) with the formation of microbial plant concentrate (MPC) for feed purposes. Serum cultures assimilated stachyose, glucose, maltose, arabinose, and other pentoses. The mass fraction of protein in the concentrate was 57.90-61.68 % of DS. The composition of MPC obtained from biomass is balanced in essential amino acids with a speed of 107-226 %. The fatty acid composition is represented by 97 % fatty acids and 3 % - esters, aldehydes, ketones with the properties of fragrances, photo stabilizers, odor fixers, preservatives and other compounds. The ratio of the sum of saturated and unsaturated acids is 1:3, the content of cis-isomers is 91.1 %, trans-isomers are 5.1 %, omega-6 fatty acids are 19.73 %. The quality and safety indicators indicated that it is promising for use in the diet of animals.

Keywords: *pea flour, processing, serum, bioconversion, microbial-plant concentrate, amino acid composition, fatty acid composition*

INTRODUCTION

Modern trends in the development of food production indicate that in the coming years, the deficit of protein in human and animal nutrition will not decrease, and the need for new high-quality (dietary) protein sources will increase. The number of hungry people in the world is growing, at the moment it has reached a third of the entire population of the Earth, part of the population is experiencing a deficiency of complete protein [1]. One of the ways to eliminate the deficit is to obtain protein preparations from plant materials. In this case, secondary products of processing raw materials into proteins or starch can be involved in the scheme of processing by the bioconversion method. The biomass of microorganisms can be

used as part of the diets of farm animals to increase their productivity and for human nutrition as new sources of proteins. Known bioconversion products from various types of by-product sources of the food industry and agriculture. Since ancient times, legumes have been used in the diet of people, including those who, for one reason or another, do not eat meat [2], [3]. Of particular interest is one of the traditional leguminous crops for European countries, including Russia, - peas, the use of which makes it possible to create technologies for protein concentrates, flour, isolates and by-products [4]. Information on the processing of secondary products formed from leguminous crops is still limited, despite the interest in this area. Thus, on the basis of a by-product formed during the extraction of pea protein, using filamentous fungi, a food mycoprotein concentrate was synthesized to "replace" meat. Studies were carried out with strains of fungi: *Aspergillus oryzae*, *Fusarium venenatum*, *Monascus purpureus*, *Rhizopus oryzae* fermentation at 35 ± 2 °C for 48 hours. The protein content in the mushroom biomass reached 43.13-59.74 %. It has been shown that the introduction of this process into production will provide about 680 kg of mushroom biomass with 38 % of additional protein for each 1 ton of by-product [5]. We have also proved the possibility of bioconversion of the secondary product (extract) of processing triticale grain into starch together with pea flour, and obtaining a feed concentrate with a mass fraction in% on DS: protein 55.8–75.1, carbohydrates - 18.9–32.83, fat 3.56–13.56, ash 2.05–8.27 [6]. Cultures of microorganisms actively developing on the substrate were selected, and a symbiotic starter culture from the fungus was compiled from them. *Geotrichum candidum* 977 and yeast *Saccharomyces cerevisiae* 121, providing the growth of biomass in a carbohydrate, nitrogen-containing medium. The serum formed after the isolation of concentrated proteins from the composition with pea flour was benign for culture media of microbiological synthesis with fungus and yeast. We also obtained preliminary positive results with this composition of microorganisms using serum formed during the isolation of proteins from chickpea [7] and pea [8] grains according to the scheme using enzyme preparations without optimization of parameters.

The aim of this work was to improve the process of bioconversion of grain whey, formed as a secondary product of the processing of flour from pea grain into protein concentrate, by the symbiosis of the yeast *S. cerevisiae* and the fungus *G. candidum* 977, by optimizing the growth parameters of microorganisms with the subsequent characterization of the feed microbial-plant concentrate.

MATERIALS AND METHODS

The objects used were pea whey made from flour obtained from Yamal grain with 11.6 % moisture and mass fraction, % of DS: protein (Nx6.25) - 25.7; ash - 2.67; fat - 1.46; starch - 51.50; carbohydrates - 18.76. Enzyme preparations from Novozymes A/S (Denmark) were used to isolate protein concentrates and the secondary product of grain whey from flour: Shearzym 500 L, Viscoferm L, Fungamyl 800 L, AMG 300 L 2500, and Distizym Protacid from Erbslon. The yeast *Saccharomyces cerevisiae* 121 from the collection of the Institute of Microbiology named after S.N. Vinogradskiy and a new strain of the fungus *Geotrichum*

candidum 977, the phylogenetic position of which was determined jointly with the State Research Institute of Genetics (Russia) [9].

The amount of protein in the solution was determined by the Lowry method, nitrogenous substances in flour and in MPC - by the Kjeldahl method (GOST 10846-91); moisture - GOST 13586.5-93; ash - GOST 10847-2019; fat - GOST 29033-91, carbohydrates - by the difference between 100 % and the sum of the remaining components. The amino acid composition of MPC was determined on an L-8800 chromatograph (Hitachi, Japan) in the standard mode of analysis of protein hydrolysates with a sulfonated styrene-divinylbenzene copolymer and a stepwise gradient of Na-citrate buffer solution with increasing pH and molarity (GOST 32195-2013). When calculating the rate of essential amino acids, we used the FAO / WHO standard protein scale (2011) [10]. The carbohydrate composition of serum and extracts was investigated on a Shimadzu GCMS 2010 gas chromatograph (Japan), the fatty acid composition of MPC lipids - on a chromatograph with a Simadzu GCMS-QP 2010 Ultra mass detector at 120°C, an injector - 200°C; interface – 205°C, detector – 200 °C on an SLB-IL82 column (30 m, 0.20 mkm, d = 0.25 mm) with a carrier helium at a flow rate of 35.6 cm / s, flow division 1:10. The gradient mode varied from 120°C to 260°C at a rate of 5 °C/min for 2 minutes. Lipids were isolated according to the Folch method, evaporated on a rotary evaporator, dissolved in chloroform, hydrochloric acid methanol (Supelco Methanolic-HCl 0.5 N) was added, sealed in a vial and heated at 90 °C for 1 h. Museum cultures from wort agar were subcultured into a test tube with serum remaining after protein isolation, and cultured for 24 h. Then the culture was subcultured into 300 cm^3 flasks with 50 cm^3 nutrient medium, grown on a shaker at a rotation speed of 150 min^{-1} and a temperature of 27±1°C for 48 hours. Serum with pH 6.0 - 6.5 was used to prepare nutrient media. The serum was sterilized at a pressure of 0.1 MPa, cooled, a suspension of cultures was introduced into the substrate and grown at different temperatures for 24 - 48 h with stirring on a rocking chair at a rate of 150 min-1. The suspension was inactivated at 95 ± 5°C for 10 - 15 min and cooled for 10 - 15 min at a temperature of 22 ± 2°C. The biomass was separated from the culture liquid by centrifugation at 4000 min – 1 for 10 min. The biomass (KMPK-1) and the biomass with the culture liquid (KMPK-2) were dried on a Hochvacuum HVDTG-50 lyophilizer (Germany) in a vacuum at -80 °C.

The experimental data were processed in the TableCurve 2D 5.1, TableCurve 3D 4.0, Mathematica 10.3, and Statistica 10 programs. The confidence interval of the arithmetic means was calculated according to the significance level p = 0.05.

RESULTS AND DISCUSSION

The extraction of proteins from the pea suspension was carried out by a biotechnological method using hydrolytic enzyme preparations (EPs) of various actions (cellulases, xylanases, amylases, proteases) in stages. The scheme and parameters of protein extraction for each stage are presented in [8]. The hydromodule 1:15 was used, the EP concentration was 1.5 %/g of protein, the fermentation time was 4 hours, the reaction temperature was 55 ± 1°C, and the stirring speed was 200 min^{-1}. After precipitation of the protein at the isoelectric point and centrifugation of the suspension, serum was formed, which was subjected to

bioconversion to synthesize feed protein preparations. The mass fraction of dry substances (DM) in pea whey averaged 3.5±0.5 %, nitrogenous substances (Nx6.25) - 28.35 ± 0.8 %, true proteins - 11.06±0.23 %, in% of DS. Table 1 shows that in the process of protein extraction from flour with amylases, cytases and hemicellulases, the amount of high molecular weight carbohydrates in the dissolved part after the 2nd stage decreased by 2 %, tri-, tetra-disaccharides - almost 2 times, and the amount of glucose, on the contrary, - increased by 36 %, fructose, galactose, xylose - 3 times.

Table 1. *The content of carbohydrates by stages of protein extraction,% of the total content in flour*

Product	HMWC*	Stachyose Raffinose	Sucrose, maltose	Glucose	Fructose, galactose, xylose	Arabinose
Extract Stage 1	23.43	23.93	0+31.81	10.11	8.40	2.31
Extract Stage 2	21.12	11.95	6.70+12.33	20.48	24.79	2.64
Extract Stage 1	14.77	20.27	8.99+ 10.91	13.89	28.39	2.78
Serum	32.01	26.38	0+14.98	9.66	12.06	4.90

Note: HMWC – High molecular weight compounds*

At the third stage of extraction, under the influence of proteases, the share of HMWC decreased by 37 %, disaccharides - by 38 %, but the amount of monosaccharides (fructose galactose, xylose) increased 3.4 times. Thus, the nutrient medium for the synthesis of substances by microorganisms has been enriched with assimilable low molecular weight carbohydrates.

To determine the optimal conditions for increasing the productivity of the yeast *S. cerevisiae* and the fungus *G. candidum* 977, we studied the effect of the substrate pH, temperature, and the amount of inoculum on biomass synthesis for 3 days. For this, the matrix of the experiment was compiled (Table 2), the results of which were processed in the Statistica 12.5 program.

Table 2. *Matrix for planning the experiment of growth of cultures on serum*

No.	pH	Temperature, °C	Seed amount, %	Mass fraction of biomass, g/dm^3
1	5	20	3	0.611
2	5	25	2	0.816
3	5	30	1	0.757
4	5	35	4	0.570
5	6	20	4	0.776
6	6	25	3	0.774
7	6	30	2	0.711
8	6	35	1	0.573
9	7	20	1	0.791
10	7	25	4	0.811
11	7	30	3	0.708
12	7	35	2	0.413
13	8	20	2	0.616
14	8	25	1	0.751
15	8	30	4	0.553
16	8	35	3	0.313

Table 3 shows the values of the regression coefficients and the level of significance p. The equation for the dependence of the mass fraction of biomass md, g/dm^3 on influencing factors was as follows:

$$md = -2.94 + 0.544 \cdot pH - 0.0356 \cdot pH^2 + 0.181 \cdot t - 0.003 \cdot t^2$$
$$-0.147 \cdot cm + 0.0276 \cdot cm^2 - 0.00447 \cdot pH \cdot t$$

All coefficients of the equation are significant ($p \leq 0.05$) (Table 3). An adequate description of the data was indicated by the results of the experiment, the data of the calculation by the equation, their absolute error (Table 4) and the correlation graph $R = 0.9644$ (Figure 1).

Table 3. *Regression coefficients and significance level p*

	Regr. Coefficients; Var.:md; R-sqr=,9644; Adj: 93325 (Spreadsheet1) 3 factors, 1 Blocks, 16 Runs; MS Residual=,0014517 DV: md					
Factor	Regression Coeff.	Std.Err.	T (8)	p	-95, % Cnf.Limt	+95, % Cnf.Limt
Mean/Interc.	-2,93662	0,593210	-4,95040	0,001120	-4,30457	-1,56868
(1) pH(L)	0,54434	0,133016	4,09228	0,003475	0,23760	0,85107
pH(Q)	-0,03563	0,009525	-3,74006	0,005705	-0,05759	-0,01366
(2)t(L)	0,18057	0,023871	7,56456	0,000065	0,12553	0,23562
t(Q)	-0,00304	0,000381	-7,99191	0,000044	-0,00392	-0,00217
(3)cm(L)	-0,14700	0,054163	-2,71404	0,026492	-0,27190	-0,02210
cm(Q)	0,02756	0,010471	2,63238	0,030067	0,00342	0,05171
1L by 2L	-0,00447	0,001739	-2,57322	0,032962	-0,00849	-0,00046

Table 4. *Experimental (1), calculated (2) data and absolute error*

No.	1	2	Absolute error	No.	1	2	Absolute error
1	0.611	0.647450	-0.036450	9	0.791	0.775625	0.015375
2	0.816	0.762500	0.053500	10	0.811	0.809175	0.001825
3	0.757	0.780425	-0.023425	11	11	0.708	0.672100
4	0.570	0.554225	0.015775	12	12	0.413	0.437900
5	0.776	0.756350	0.019650	13	0.616	0.631775	-0.015775
6	0.774	0.793900	-0.019900	14	0.751	0.734825	0.016175
7	0.711	0.734325	-0.023325	15	0.553	0.593750	-0.040750
8	0.573	0.577625	-0.004625	16	0.313	0.282050	0.030950

The equation made it possible to determine the dependence of the mass fraction of biomass *md* on the influencing factors and to determine their values for its maximum yield. Figure 2 shows, as an example, the regularity of the change in biomass from the pH value and the temperature of the environment C^0 with the amount of seed $cm = 2\%$.

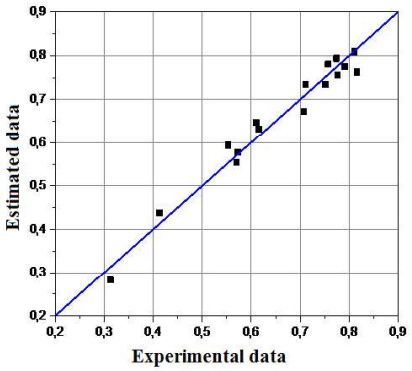

Fig. 1*. Correlation graph*

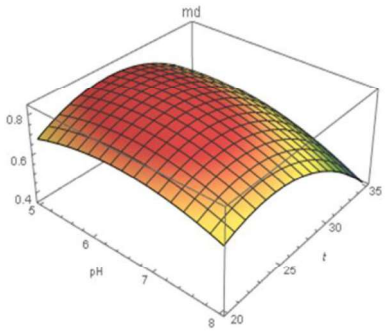

Fig. 2. *Dependence of the mass fraction of biomass on pH and temperature*

From the equation in the Mathematica 12.1 program, the values of the factors for the maximum biomass yield (0.88 g/dm3) are determined: pH of the medium - 6.03, *C*=25.7°, seed quantity *cm*=4%. At lower pH values (4.5-5.0) or higher (7.5-8.0), the growth of microorganisms slowed down. Cultivation of symbiosis of cultures had a positive effect on the accumulation of biomass and protein; for the symbiosis of cultures, the amount of protein in the biomass was 61.68% of DS (Table 5), while from biomass with the culture liquid - 57.90%.

Table 5. *Chemical composition of MPC from biomass on serum*

Moisture, %	Mass fraction, % of DS			
	Protein (Nx6.25)	Ash	Lipids	Carbohydrates
6.81±0.4	61.68±0.47	8.60±0.03	8.31±0.36	21.41±0.55

In the process of synthesis, stachyose, maltose, arabinose were completely absorbed from serum, more than half - glucose and almost all other pentoses (Table 6). The assimilation of stachyose by these yeasts corresponded to the literature data. On the other hand, in the MPC, the number of HMWC has doubled, the nature of which has to be deciphered.

Table 6. *Carbohydrate composition of pea serum (1) and MPC (2),% of the total*

Product	HMWC	Stachyose	Raffinose	Sucrose, maltose	Glucose	Fructose, galactose, xylose	Arabinose
1	32.01	26.38	0	0+14.98	9.66	12.06	4.90
2	68.83	0	26.21	0	3.87	1.09	0

The amino acid composition of MPC from biomass and from biomass with culture liquid is mostly represented by glutamic, aspartic acids, glycine, alanine, lysine (Figure 3).

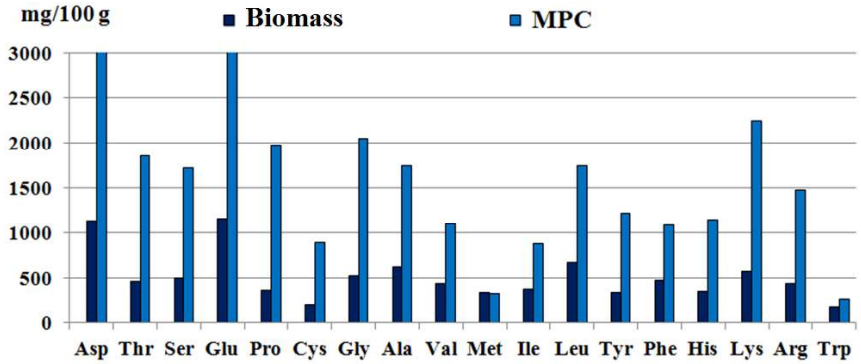

Fig. 3. *Amino acid composition of biomass and MPC*

The amino acid rate of biomass of cultures for all essential acids was 107-226%, for MPC with a culture liquid it was high for histidine, lysine, threonine, sulfur-containing amino acids (81-128%), and for valine, leucine, isoleucine it was not higher 48%. The fatty acid composition (FAC) of MPC is represented by 30 components, among which 97 % are fatty acids that are part of animal fats, vegetable oils, marine organisms, 3 % are esters, aldehydes, ketones with the properties of aromatizing essential oils, metabolites of the human body, photo stabilizer, odor fixatives, preservatives and other compounds. The ratio of the sum of saturated (23.51 %) and unsaturated fatty acids (71.67 %) is 1:3, the content of cis isomers is 91.1 %, trans isomers - 5.1 %, omega-6 fatty acids (linoleic) -

19,73 %. MPC did not have a negative effect on the vital parameters of experimental rats [11], which indicated its safety and prospects for use.

CONCLUSION

The optimization of the process of biotransformation of the chemical composition of the secondary product of pea flour processing into food protein concentrate (serum) into a microbial-plant concentrate by a symbiosis of cultures of the fungus *G. candidum* 977 and yeast *S. cerevisiae* 121 has been carried out. adequately describing the dependence of the crop biomass yield on technological parameters: pH of the medium, temperature and amount of seed. The microbial-plant concentrate from the biomass of cultures with a protein mass fraction of 57.90 and 61.68 % of DS was biologically valuable (the rate of essential amino acids was 107-226 %), had a high biological efficiency of lipids: out of 30 types of fatty acids, 97 % were acids included in composition of animal fats, vegetable oils and marine organisms. The ratio of saturated (23.51 %) and unsaturated fatty acids (71.67 %) was 1:3, the content of trans isomers was 5.1%, and omega-6 fatty acids (linoleic) were 19.73 %. The use of the concentrate is promising for animal diets.

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