BIOCONVERSION OF CEREAL SERUM - A SECONDARY PRODUCT FOR PRODUCING PROTEIN CONCENTRATES FROM PEA AND CHICK PEAS

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ABSTRACT

Studies on the bioconversion of whey water formed from chickpea and pea grains in the preparation of protein concentrates have been performed. The serum remaining after precipitation of the main part of the protein was subjected to a symbiotic transformation of *Saccharomyces cerevisiae* 121 and *Geotrichum candidum* 977 yeast cultures with the formation of protein-containing products with a mass fraction of protein (52.27-57.90% of DS) and a complementary amino acid composition. A microbial-plant concentrate was used as an additive in the feeding of Wistar laboratory rats. After 25 days of feeding, there was no negative effect on the physiological parameters and behavior of animals, which indicates the high quality of the protein product and the prospects of its inclusion in the composition of animal feed and diets.

Keywords: *pea flour, chickpea flour, extracts, bioconversion, biomass, concentrates, amino acid composition*

INTRODUCTION

For modern agriculture, new technologies are being developed and sources of fodder and food products are being sought. One of these areas is the involvement of bioconversion of secondary products into food and feed additives in resource-saving flour processing schemes. Microorganisms on certain compositions of nutrient media have a high growth rate and the ability to synthesize a wide range of nutrient compounds: proteins, lipids, carbohydrates, carotenoids, etc. [1]. The biomass of microorganisms is intended for the diets of farm animals and poultry in order to increase their productivity. The basis for the cultivation of feed biomass of microorganisms is often the secondary products of the food industry and agriculture. Thus, a preparation obtained during the fermentation of corn stalks with saccharomycetes or a consortium of saccharomycetes, *L. plantarum* and *L. casei* had a positive and safe effect on the animal organism and the environment [2], [3].

The yeast introduced into the feed of broiler chickens in an amount of 0.8% also increased the efficiency of their use [4]. A study of the microbiota of fecal samples on days 21 and 42, carried out using the polymerase reaction (PR), revealed a positive effect of the additive on the microflora of broiler chickens, and the introduction of *S. cerevisiae* yeast in ruminant feed increased fiber digestibility and increased the population of cellulolytic bacteria *R. flavefaciens* scar [5].

It was also proved that the addition of *S. cerevisiae* and / or *A. oryzae* to the diet of cattle increased milk yield and fat content of milk [6], [7]. Feed additives from coffee sludge [8], from distillery stillage with wheat bran, obtained by cultivation with *S. diastaticus* yeast and carotene-forming yeast *Rh. species* to increase the content of essential amino acids in feed [9]. With yeast *Rh. glutinis, Rh. mucilaginosa* and *Rh. Gracilis* synthesized a feed additive with carotenoids and lipids from a deproteinized wastewater generated during the processing of potatoes and glycerol wastes [10]. Mushroom biomass with *A. Niger* with a yield of 35 g / dm^3 [11] was also obtained from the distillery's waste, and a feed microbial-plant concentrate (FMPC) synthesized with *S. cerevisiae* yeast was extracted from the extract remaining in the production of triticale starch with a mass fraction of protein of 25.2 ± 2.1%, fat - 22.1 ± 3.2%, carbohydrates - 40.8 ± 1.6% [12]. Potassium and calcium prevailed among macroelements, cobalt, iron, zinc, molybdenum, nickel, which are necessary for maintaining the normal development of a living organism, prevailed among microelements.

With secondary products formed during the extraction of pea protein, a food mycoprotein concentrate was obtained to replace meat. The studies were performed with 5 strains of fungi (*A. oryzae, F. venenatum, M. purpureus, N. Intermedia, R. oryzae*), which were grown at 35 ± 2 °C for 48 h with a protein content in biomass of 43.13-59.74% of DS. The process can provide about 680 kg of mushroom biomass with 38% additional protein for every 1 ton of by-product [13]. It is important to note that the processing of secondary products of leguminous crops by bioconversion for other technological schemes has been little studied, so today such studies in this direction are quite relevant.

This work aims to develop a bioconversion process for liquid grain whey, which is formed as a secondary product of the processing of flour from pea and chickpea grains into protein concentrates, by the symbiosis of *S. cerevisiae* yeast and a new strain of *G. candidum* 977.

MATERIALS AND METHODS

As objects, pea serum was used from flour obtained from the grain of the Yamal variety with 11.6 % moisture and mass fraction, % of DS: protein (Nx6.25) – 25.7; ashes – 2.67; fat – 1.46; starch – 51.50; carbohydrates – 18.76 and chickpea whey from flour, ground from Volzhanin grains with 9.1% moisture and mass fraction, % of DS: protein (Nx6.25) – 23.40; ashes – 2.91; fat – 4.89; starch – 43.82; carbohydrates – 24.98. Peas were grown in the Altai Territory in 2017, chickpeas – in 2018 in the Volgograd Region with a yield of 18-20 kg/ha.

To isolate protein concentrates and a by-product of cereal whey from flour, enzyme preparations from Novozymes A / S (Denmark) were used: Shearzym 500

L, Viscoferm L, Fungamyl 800 L, AMG 300 L 2500 and Distizym Protacid from Erbslon. To obtain FMPC, we used cultures of the fungus Geotrichum candidum 977 and yeast Saccharomyces cerevisiae 121 from the collection of S.N Vinogradsky Institute of Microbiology. The phylogenetic position of the new strain *G. candidum* 977 was determined in conjunction with the Federal State Budget Scientific Research Institute of Genetics (Russia).

RESULTS AND DISCUSSION

Proteins were extracted from a suspension of pea and chickpea flour separately by a biotechnological method with the stepwise addition of hydrolytic enzyme preparations (EP) of various actions (cellulases, xylanases, amylases, proteases). The scheme and parameters of protein extraction for each stage are presented in [14]: hydromodule 1:15, EP concentration 1.5% / g protein, fermentation time 4 hours, reaction temperature 55 ± 1 °C, stirring speed 200 min^{-1}. After protein precipitation and centrifugation of the suspension, formed whey, which was subjected to bioconversion for processing flour into food and feed protein preparations. The use of liquid whey without any processing in the composition of the feed is difficult, due to the impossibility of long-term storage and the complexity of transportation, so we further selected the conditions for the preparation of the nutrient medium and microorganisms to assimilate its components and obtain dry feed additives. Whey contained digestible nitrogenous substances, mono- and oligosaccharides (Table 1).

Table 1 – The average chemical composition of pea and chickpea serum

DS, %	Nitrogen Substances, % of DS (Nx6,25)	Serum carbohydrates, mg / 100 g of product				
		Fructose	Glucose	Maltose	Maltotriosis	HMWC*
1.85±0.35	13.94±5.32	144.3±0.2	185.7±1.2	270.6±23	51.1±1.2	398.2±0.43

** Note: HMWC – high molecular weight compounds*

For this substrate, microorganisms were selected from yeast of the genera *Pichia, Rhodotorula, Hansenula, Saccharomyces* and micromycetes used in cheese making (*Geotrichum, Penicillium*). Representatives of the genera *Pichia* and *Saccharomyces* grew with the highest speed and activity on both types of serum, while *Rhodotorula* and *Hansenula* developed poorly. For further studies, *S. cerevisiae* yeast and *G. candidum* 977 micromycete, which we deposited at the Federal State Budget Scientific Research Institute of Genetics (registration number VKPM Y-300), were selected. The *G. sandidum* 977 culture was able to grow in a wide pH range of the nutrient medium and regulate the acidity of the medium with alkalization of the substrate to alkaline values *G. candidum* 977 was used in our experiments in a consortium with *S. cerevisiae* at a ratio of 1:1. Pea and chickpea whey had a positive effect on the morphology of cells, both of individual monocultures, and their consortium (Figure 1). To determine the growth conditions of microorganisms, we studied the effect of substrate pH, temperature, and the amount of seed on biomass formation for 2 days.

Figure 1 – Cells of monocultures and their consortium: 1 – S. cerevisiae; 2 – G. candidum 977;

3 – S. cerevisiae + G. candidum 977

The most effective for the synthesis of biomass was a pH value of 6.0-6.5. At lower pH values (4.5-5.0) and higher (7.5-8.0), the growth of microorganisms slowed down (Figure 2). An effective amount of seed was a dose of 3 % (Figure 3), a growth temperature of 26-28 °C.

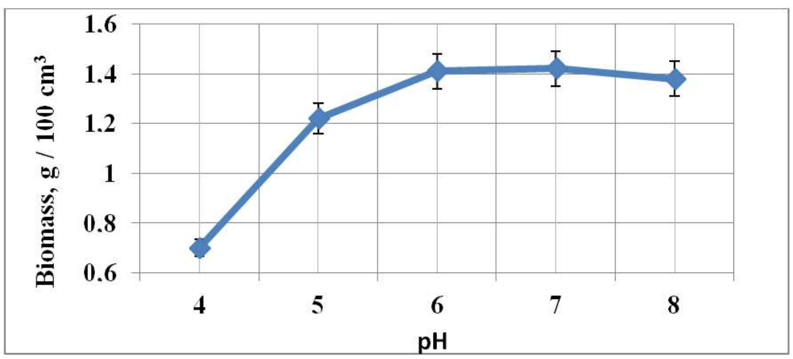

Figure 2 – Effect of pH on the amount of biomass G. candidum 977 + S. Cerevisiae 121

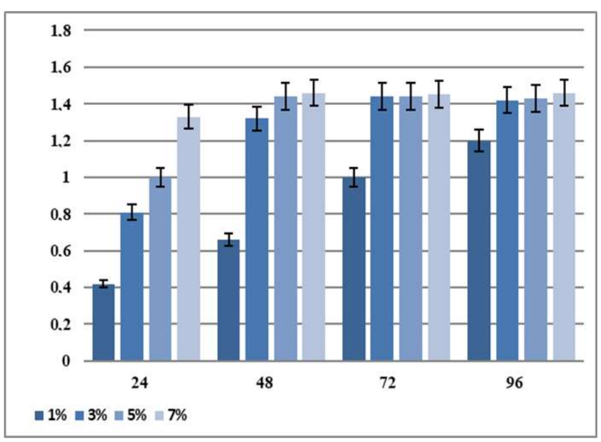

Figure 3 – Effect of seed dose on the amount of biomass (pH 6-7)

Co-cultivation of yeast and micromycete positively influenced the accumulation of biomass and the formation of protein, the mass fraction of which was 57.90 ± 0.1 % of DS for pea whey and 52.27 ± 0.72 % of DS for chickpea whey, while in some cultures the amount of protein reached only 16.39...50.23 % of DS. The least amount of protein was contained in biomass with *G. candidum* 977 fungus (Table 2).

Table 2 – Mass fraction of protein in biomass with microorganisms, %

Biomass with culture	Dry substance (DS), %	Mass fraction of protein, % of DS (Nx6,25)
G. candidum 977	17.49±0.21	16.39±0.31
S. cerevisiae	19.21±0.34	50.23±0.42
G. candidum 977 + *S. cerevisiae*	18.89±0.41	57.90±0.51

The culture fluid and biomass were dried and FMPC-2 was obtained, and after centrifugation of the total suspension, washing the precipitate with water and drying it, the FMPC-1 biomass preparation was obtained. According to the organoleptic characteristics, the concentrates were crumbly powders: FMPC-1 light cream color, FMPC-2 cream color, odorless. A typical chemical and amino acid composition of FMPC-2 obtained from pea whey biomass with *S. cerevisiae* 121 and *G. candidum* 977 is shown in table 3.

Table 3 – Chemical and amino acid composition of FMPC-2 from biomass crops

Moisture, %	Mass fraction, % of DS			
	Protein (Nx6.25)	Ash	Lipids	Carbohydrates
26.8±0.5	61.68±0.47	8.60±0.03	8.31±0.36	21.41±0.55

Amino acid composition, mg / g of product								
Val	His	Ile	Leu	Lys	Met+Cys	Thr	Trp	Phe+Tyr
10.98	11.45	8.79	17.44	22.40	12.23	18.46	2.59	23.07

The safe properties of dry pea concentrate FMPC-2, obtained from a microbial suspension of pea whey with a consortium of cultures of *G. candidum* 977 and *S. cerevisiae* 121, were studied when feeding rats. The introduction of FMPC-2 in the feed did not change the color, smell and its uniformity. The effect of the concentrate on the appetite, tolerance and growth of animals, studied over 25 days, showed that the degree of eating, estimated by the remainder of the feed, is the same in both the control and experimental groups. There was no difference between the experimental and control rats for appetite, no digestive disorders, inhibition of behavior, increase or decrease in motor activity. The response to external stimuli (transfer of animals from the cells, fixation of the animal during weighing) was also the same (Table 4).

Table 4 – The effect of additives with FMPC-2 on the characteristics of behavior and microbiological parameters of experimental and control rats

Indicators	Control group	Experienced group
Animal condition (motility, appetite)	Animals are mobile, appetite is good	Animals are mobile, appetite is good
Degree of Eating and Tolerance	Animals actively ate food, normal manifestation of thirst	Animals did not refuse food, actively ate food

Behavior (motor activity, reaction to external irritations)	Disorders of behavior and oppression were not observed	Disorders of behavior and oppression were not observed
The condition of the skin of the coat and eyes	The condition of the skin of the coat and eyes	The condition of the skin of the coat and eyes
Feces characterization	Spindle-shaped with a dark gray tint, about 10 mm long, characteristic for this group of animals	Spindle-shaped, with a dark gray tint, about 10 mm long, the first 2 days are more humid and shiny than in the control
Microflora, CFU / g (on day 25)		
General bacterial contamination	1,5 x 10^8	1,6 x 10^8
Escherichia coli bacteria	1,3 x 10^6	1,1 x 10^6
Lactic acid bacteria	1,1 x 10^5	1,2 x 10^5

Studies of the microflora of fecal samples, performed at the beginning and at the end of feeding the rats for 25 days, showed no changes in the number and composition of the microbiocenosis of the experimental and control animals (Table 4). The total bacterial contamination, the number of bacteria of the group of Escherichia coli (Escherichia, Citrobacter, Enterobacter, Serratia) and lactic acid bacteria in the feces of both groups of animals was identical. The conclusion is made about the benigness of FMPC-2 and the prospects of its inclusion in the composition of animal feed and diets. On the base of the data obtained, was developed a concept for the processing of whey obtained from the isolation of protein concentrates from pea and chickpea flour with the formation of FMPC (Figure 4), which requires further testing under experimental conditions.

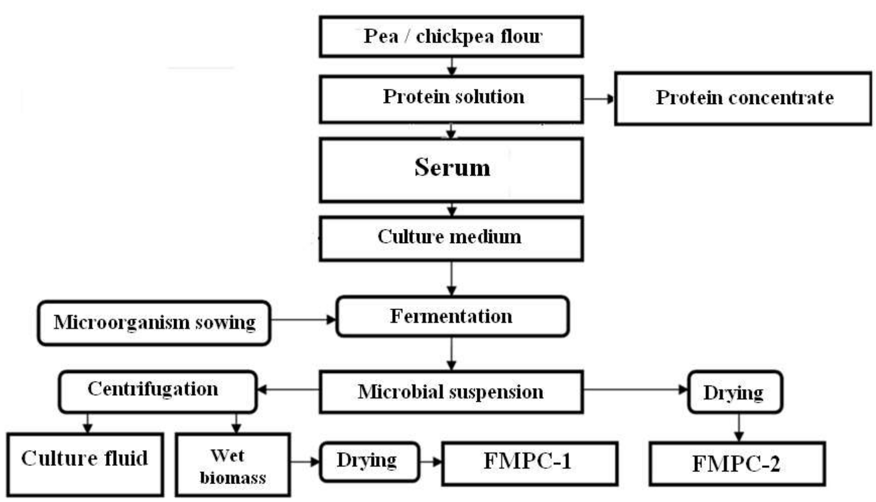

Figure 4 – Scheme for the production of FMPC by microbial transformation of serum

CONCLUSION

The possibility of processing the whey formed during the isolation of protein from a suspension of pea and chickpea flour by bioconversion with a symbiosis of cultures of the fungus *G. candidum* 977 and the yeast *S. cerevisiae* 121 into FMPC was established. A composition from cultures of microorganisms is proposed for the synthesis of FMPC from grain serum of peas and chickpeas. FMPC obtained from pea whey had a protein mass fraction of 57.90% of DS, from chickpea whey – 52.27% of DS. The resulting protein concentrate did not adversely affect the behavior and performance of experimental rats, which indicated its safety and prospects for animal diets. Therefore, for whey obtained as a secondary product in the processing of one triticale grain [12], and in the processing of triticale serum together with pea flour into starch and protein concentrates [15], the symbiosis of these types of microorganisms is also effective for the bioconversion of one pea or chickpea whey with a sufficiently high mass fraction of protein in the FMPC.

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