Ecological approach to the use of secondary products of pea flour and rice grain processing into protein concentrates and phytin

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Abstract. The process of bioconversion of the secondary product of pea flour processing into protein concentrate (serum) into fodder microbial-plant concentrate has been optimized. For this, a composition was selected from the culture of the fungus Geotrichium candidum 977 and the yeast Saccharomyces cerevisiae 121, a mathematical model of concentrate synthesis was developed in the form of an equation that adequately describes the dependence of the biomass yield on technological parameters: pH of the medium, temperature and amount of inoculum. The concentrate from the biomass had a protein mass fraction of 61.68 % of DS, from the biomass with the culture liquid - 57.90 %. Concentrates - biologically complete, the rate of essential amino acids was 107-226 %, out of 30 fatty acids, 97 % were acids that are part of animal fats, vegetable oils or marine organisms. The ratio of saturated and unsaturated acids is 1:3, the content of trans isomers is 5.1 %, omega-6 fatty acids (linoleic) is 19.73 %. The ability of symbiosis between the yeast S. cerevisiae 84/5 and the fungus Trich. cutaneum 656 has been proven. Transform the components of whey remaining after the extraction of phytin from rice bran into protein biomass. The ratio of monocultures by mass fraction is 1:1, pH - 5.0...6.0, duration of growth - 72 hours, digestibility – 90 %. The protein is enriched with methionine, isoleucine, leucine, lysine. The amount of essential acids is 18-21 % higher than in concentrates obtained from individual monocultures. The use of concentrates is advisable to use in the diet of animals.

1. Introduction
The intensive development of the agro-industrial complex is accompanied by an increase in the amount of waste and by-products that pose an ecological threat to the environment [1-3]. A significant danger
is the discharge into the environment of rapidly acidifying liquid effluents generated during the processing of grain raw materials. At the same time, soils, groundwater, and reservoirs are polluted. Reducing such waste can contribute to global food security [4]. At the same time, they represent a valuable but underutilized source of components that can be used as ingredients in the food, feed, chemical or other industries. Modern developments today are directed to the use of by-products of agriculture and food industry, both animal (skin, bones, internal organs) and plant origin (fruit peel, seeds, rice and wheat bran) [5, 6]. The potentialities of extracts, wash water, whey obtained as disposable waste during the processing of agricultural raw materials are great, they can serve as a source of protein or bioactive / functional compounds for food, feed or other types of products (biofertilizers, packaging materials, etc.). Considering the environmental impact and economic losses, by-products from agriculture and the food industry can be used in various innovative processes to obtain various useful products.

There are various approaches to the utilization of by-products of processing plant materials with valuable organic compounds: obtaining feed and food components [7], biofertilizers, gaseous and liquid fuels, additives of biodegradable polymers, chemical products [8, 9], etc. One of the most rational methods is the use by microorganisms of a nutrient medium from liquid products released during extraction and isolation of the target product from plant raw materials (edible crops, straw, stems, leaves, fat-free seed residues, etc.) in the process of protein biomass synthesis. In this case, bacteria, micromycetes, yeasts are used, which assimilate pentose, hexose sugars, starch, nitrogenous compounds of nutrient media [10]. Positive results for obtaining feed, biosurfactants and polymers have been achieved by biotransformation of rapeseed meal (waste from rapeseed oil production) with yeast cultures Yarrowia lipolytica and Aspergillus niger [11, 12]. In the process of obtaining protein feed preparations with the fungus Fusarium sambucinum strains 139, 52, 48, the synthesis of ubiquinone (coenzyme Q10) on hydrolyzate of grape pomace, malt broth, etc. was proved. [13], and with the Pleurotus ostreatus fungus - on the extract, which is a waste from the processing of triticale grain into starch into a full-fledged biomass [14].

Scientific results on the use of secondary products of processing grain of peas and rice are also known. Trends in the development of production of agricultural products indicate that the deficit of protein in animal feeding and human nutrition in the future will not decrease, and the need for new high-quality sources of proteins will increase. The number of hungry people in the world now reaches one third of the entire population of the Earth, and part of the population is deficient in complete protein [15]. One of the ways to eliminate the deficit is to obtain additional protein preparations from plant raw materials to animal sources. At the same time, secondary products of processing of raw materials in the starch industry, both for proteins and starch, can be involved in the scheme of processing by the method of bioconversion to obtain biomass of microorganisms used in the composition of animal feed to increase their productivity, and in human diets - in the form of new dietary sources proteins. Since ancient times, legumes have been used in the diet of people, including those who, for one reason or another, do not eat meat [16, 17]. 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 (starch, dietary fiber) [18]. Information on the processing of secondary products from leguminous crops is still limited, despite the significant interest in this area. So, for example, on the basis of a by-product formed during the extraction of pea protein, a food mycoprotein concentrate is synthesized with filamentous fungi to "replace" meat. The studies were carried out with strains of fungi: Aspergillus oryzae, Fusarium venenatum, Monascus purpureus, Rhizopus oryzae by fermentation at 35±2 °C for 48 hours. The protein content in the mushroom biomass reached 43.13-59.74%. The introduction of this process into production can provide about 680 kg of mushroom biomass with 38% of additional protein for each 1 ton of by-product [19]. The possibility of bioconversion of pea flour together with an extract (a by-product of the processing of triticale grain into starch) has been proven to obtain a feed concentrate from them with a mass fraction in % of DS: protein 55.8-75.1 with an improved amino acid composition; carbohydrates - 18.9-32.83; fat 3.56-13.56; ash 2.05-8.27 [20]. For this, the optimal cultures of microorganisms developing on the substrate were
determined, and a symbiotic starter culture was made from the fungus Geotrichum candidum 977 and the yeast Saccharomyces cerevisiae 121, which provides active growth of biomass on a carbohydrate-nitrogen-containing medium. The serum formed after the isolation of concentrated proteins from the pea flour extract composition without additional ingredients was a good-quality nutrient medium for biomass synthesis. Preliminary positive results with this composition of microorganisms were obtained when using serum formed during the isolation of proteins with hydrolytic enzymes, both separately from chickpea grains [21] and pea grains [22], but the authors did not fully determine the optimal parameters of fodder synthesis biomass.

The share of by-products of rice processing, including bran, accounts for up to 25-50% of the mass of crushed grain [23]. Such products are typically disposed of using few or no environmentally friendly disposal methods. However, innovative methods of processing rice bran are also visible, including technologies for biotransformation of raw materials to increase added value after grain harvest. Thus, the authors of [24] obtained a biomass of unicellular microorganisms with three types of cultures: Aspergillus oryzae, Trichoderma viride, and Aspergillus niger by solid-phase fermentation from defatted rice bran formed during grinding of grain into flour. The biomass contained 39.2 - 44.0% protein and 5.3 - 7.2% nucleic acids. From rice grain on the basis of its waste from starch production by microbial bioconversion with lactic acid cultures Lactobacillus plantarum, Lactobacillus casei, and Lactobacillus fermentum, food additives were obtained [25], and lactic acid was synthesized using Bacillus coagulans [26]. Bioconversion of the steep waters of rice grain together with other wastes from wine production made it possible to achieve a protein content in the biomass of 68.5±1.0% using a symbiotic culture from Candida utilis and Geochichum candidum, made in a 1:1 ratio. According to the authors of [27], the use of mixed cultures of yeast and fungi is especially promising for the production of microbial protein from this type of raw material. The sediment formed as a waste from the production of wine from rice grains in China, for example, reaches 1 million tons per year and is a particularly dangerous pollutant of the environment. The processing of such cheap waste made it possible to obtain a feed additive with a protein mass fraction of 48.0% and with the yeast Saccharomyces cerevisiae [28].

In the rice bran fractions, especially in the aleurone layer, there is a bioactive compound - phytic acid [29], known as inositol or hexaphosphate (IP6). If IP6 is in the form of a salt, it is called phytin (calcium-magnesium salt of inositol phosphoric acid). Phytin, in addition to limiting the bioavailability of minerals (Zn, Fe, Ca and / or Mg) [30], is a strong antioxidant in vivo, suppresses lipid peroxidation. Phytin inhibits the formation of free radicals [31, 32], deactivates xanthine oxidase, thereby preventing the formation of ADP-iron-oxygen complexes [33], and stimulates hematopoiesis, growth and development of bone tissue. It inhibits the crystallization of calcium oxalate salts in urine, prevents the development of kidney stones, and lowers glucose levels in vivo [34]. An imbalance of myo-inositol is observed in mental illness, its use is effective for the treatment of depression, anxiety, compulsive disorders, for the prevention of cognitive impairment in aging and neurodegenerative diseases [35]. The principle of obtaining phytin as a pharmacological additive is to extract it with dilute organic, mineral acids, salts and / or using enzymes. Then it is precipitated with urea, nitric acid salts of calcium, magnesium, etc. [36, 37], washed with water and dried. When phytin is extracted from rice bran, wash water is released in an amount of 6-7 tons per day. Rinse water can be reused for phytin extraction, which reduces drinking and waste water volume by 56%, increasing yield and shortening the process. In addition, rice bran is a rich source of proteins, fats, carbohydrates, vitamins, minerals, and dietary fiber. In terms of chemical and functional properties, rice protein is superior to other proteins (soy flakes, peanuts, sorghum, beans) [38, 39], therefore they are raw materials for various schemes for the isolation of protein concentrate, hydrolysates, active peptides [5]. However, it is not possible to utilize the liquid secondary products of rice bran processing in the production of phytin and protein substances using completely known methods, but at the same time, one of the most promising methods should be considered the processing of liquid waste by bioconversion with a reasonable selection of microorganisms to modify the composition of the medium used.
In general, by-products of grain processing, formed in the starch and phytochemical industries, do not require complex and expensive preparation as substrates for microbial conversion, which is important for the economic performance of enterprises.

The purpose of this work is to optimize the parameters of serum bioconversion (waste product of concentrate from pea flour obtained by precipitation of proteins at the isoelectric point) and to determine the rational parameters of serum bioconversion (waste of phytin production from rice bran), with the subsequent characterization of quality indicators of the obtained feed microbial and plant concentrates.

2. Materials and methods

The object used was whey obtained from pea flour, ground from Yamal grain and after proteins were removed from it. Chemical composition of flour: 11.6 % moisture; mass fraction, % of DS (dry matter): protein (Nx6.25) - 25.7; ash - 2.67; fat - 1.46; starch - 51.50; carbohydrates - 18.76. Peas were grown in the Altai Territory in 2018 with a yield of 18-20 c / ha. To isolate protein concentrates from flour, we used enzyme preparations from Novozymes A / S (Denmark): Shearzym 500 L, Viscoferm L, Fungamyl 800 L, AMG 300 L 2500, and Distizym Protacid from Erbslon [22]. After a three-stage isolation of proteins, an extract was obtained, from which protein substances were precipitated at the isoelectric point (pH 4.2±0.05). The remaining serum with pH 6.0–6.5 was used to obtain a feed microbial-plant concentrate (FMPC) by bioconversion with the yeast Saccharomyces cerevisiae 121 and a new strain of the fungus Geotrichum candidum 977, which we have deduced [40]. The mass fraction of DS in pea whey was 3.5±0.5 %, nitrogenous substances (Nx6.25) - 28.35±0.8 %, true proteins - 11.06±0.23 %, in % of DS.

The molecular weights of pea flour proteins, the extract isolated by EP, and serum were determined using denaturing gel electrophoresis (SDS-PAAG). Protein samples in an amount of about 50 μg were mixed with buffer in a 1:1 ratio. To prepare the buffer, a weighed portion of 30 g of urea, 2 g of sodium dodecyl sulfate, 10 mg of bromophenol blue was placed in a glass, 12.5 cm³ of Tris-HCl buffer (pH 6.8) was added. 5 cm³ of β-mercaptoethanol was added to the solution and the volume of the solution was brought to 100 cm³.

Samples and solutions of marker proteins in the amount of 102 μg / 30 μL were heated for 5 min at 95-100 °C. The homogenizates were centrifuged at 20,000 min⁻¹ for 20 minutes in a centrifuge (Eppendorf, Germany). To prepare a 15 % separating gel, 4.5 cm³ of AB solution was mixed (a weighed portion of 29.6 g of acrylamide was dissolved in a small amount of water, 0.4 g of bisacrylamide was added, the volume was adjusted to 100 cm³), 2.5 cm³ of Tris-HCl buffer, pH 8.8, 3 cm³ of water, 20 μL TEMED, 100 μL ammonium persulfate (PSA). The solution was shaken and poured between slides using an automatic pipette. To prepare the concentrating gel, 1 cm³ of AB solution, 1 cm³ of Tris-HCl buffer, pH 6.8, 3 cm³ of water, 15 μL of TEMED, 37.5 μL of PSA were mixed, the contents were shaken and poured into a device with electrophoresis plates. A comb was placed between the glasses and wells for protein samples were formed. Electrophoresis was carried out at a voltage of 50-60 V to enter the samples into the gel, then at 120 V. The finished electrophoregrams were stained with Coomassie G-250 brilliant blue and images were obtained using a Bio-5000 plus scanner (Serva, Germany).

Museum cultures from wort agar were subcultured into a test tube with serum and cultured for 24 hours. The culture was subcultured into flasks with a capacity of 300 cm³ with 50 cm³ of nutrient medium and grown on a shaker for 48 hours at a rotation speed of 150 min⁻¹ and a temperature of 27±1 °C. To prepare nutrient media, the initial serum was sterilized at a pressure of 0.1 MPa and cooled to 20 °C. Then the culture suspension was introduced into the substrate. The culture was grown at different temperatures for 24-48 h with stirring on a shaker 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 (FMPC-1) and the biomass with the culture liquid (FMPC-2) were dried on a Hochvacuum HVDTG-50 lyophilizer (Germany) in a vacuum at -80 °C.

To isolate phytin, we used rice bran with a chemical composition, in %: fats - 19.75, proteins - 13.05, carbohydrates - 50.49, water - 6.83, ash - 9.88 and a particle size of 250–300 μm. The culture medium
was obtained by removing undissolved particles from the serum waters remaining after precipitation of phytin, adjusting the pH, followed by pouring into flasks, and sterilizing at 0.5 atm. 20 minutes. The nutrient substrate was inoculated with a microbial suspension: 3 % for one day - for yeast and bacteria, and two days - for microscopic fungi. Yeast and microscopic fungi for subsequent inoculation were grown on wort agar, bacteria on meat-peptone agar. Inoculants were subcultured into flasks with serum. Yeast and fungi were incubated at 28-30 °C, bacteria of the genus Bacillus - at 30 °C, Flavobacterium - at 28 °C. The amount of protein in solutions was determined by the Lowry method, total nitrogenous substances in flour, feed concentrates, whey - by the Kjeldahl method [41], moisture [42], ash [43], fat [44], carbohydrates - by the difference between 100 and the sum of the remaining components.

The amino acid composition of FMPC was determined on a Hitachi L-8800 chromatograph (Japan) in the standard mode of analysis of protein hydrolysates with a sulfonated styrene-divinylbenzene copolymer and a stepwise gradient of a Na-citrate buffer solution with increasing pH and molarity [45]. When calculating the amino acid score, we used the FAO / WHO standard protein scale (2011) [46]. The carbohydrate composition of serum and extracts was investigated on a Shimadzu GCMS 2010 gas chromatograph (Japan), the fatty acid composition of lipids in concentrates - on a chromatograph with a Shimadzu GCMS-QP 2010 Ultra mass detector at 120 °C. Injector temperature - 200 °C; interface - 205 °C, detector - 200 °C on an SLB-IL82 column (30 m, 0.20 mm, d = 0.25 mm) with a helium carrier 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 from FMPC were isolated by the Folch method, evaporated on a rotary evaporator, dissolved in chloroform, hydrochloric acid methanol (SupelcoMethanolic-HCl 0.5 N) was added, sealed in a vial, and heated for 1 h at 90 °C. The digestibility of whey protein from phytin was determined according to GOST 24230-80 [47], the essence of the method was to determine the degree of digestibility (dissolution) of dry matter using the enzymes pepsin and celloviridin. The amount of mineral substances were determined by the method described in the manual [48], ammonium nitrate - according to GOST 13496.19-2015 [49], inositol phosphates - according to the methods described in [50]. The experimental data were processed in the programs TableCurve 2D 5.1, TableCurve 3D 4.0, Mathematica 10.3, and Statistica 10. The confidence interval of the arithmetic mean was calculated according to the significance level p = 0.05.

3. Results and discussion

The extraction of proteins from the suspension of pea flour was carried out by a biotechnological method using hydrolytic enzyme preparations (EP): cellulase, xylanase, amylase, protease at 3 stages with the optimal parameters described in [22]. The hydromodule 1:15 was used, the concentration of EP was 1.5 % / g of protein, the duration of fermentation was 4 hours, the reaction temperature was 55±1 °C, and the stirring rate of the suspension was 200 min⁻¹. After precipitation of the protein at the isoelectric point, the serum was subjected to bioconversion to synthesize FMPC. 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 soluble part after the 1st and 2nd stages decreased by 2 %, tri-, tetrasaccharides decreased almost 2 times, and the amount glucose, on the contrary, increased by 36 %, fructose, galactose, xylose - 3 times.

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

| Product   | HMWC a | Raffinose + stachyose | Sucrose + maltose | Glucose | Fructose, galactose, xylose | Arabinose |
|-----------|--------|-----------------------|-------------------|---------|--------------------------|-----------|
| Extract   | 23.43  | 23.93                 | 0+31.81           | 10.11   | 8.40                     | 2.31      |
| Stage 1   |        |                       |                   |         |                          |           |
| Extract   | 21.12  | 11.95                 | 6.70+12.33        | 20.48   | 24.79                    | 2.64      |
| Stage 2   |        |                       |                   |         |                          |           |
| Extract   | 14.77  | 20.27                 | 8.99+10.91        | 13.89   | 28.39                    | 2.78      |
| Stage 3   |        |                       |                   |         |                          |           |
| Serum     | 32.01  | 26.38                 | 0+14.98           | 9.66    | 12.06                    | 4.90      |

a - HMWC – High molecular weight compounds
At the third stage of extraction, under the influence of proteases, the proportion of HMWC decreased by 37%, disaccharides by 38%, but the amount of monosaccharides (fructose, galactose, xylose) increased 3.4 times. Electrophoresis of serum proteins remaining after precipitation of the bulk of proteins in PAGE showed that of 28.35±0.8%, true proteins accounted for 11.35% (figure 1). The patterns of protein bands without mercaptoethanol differed from the samples with it, which indicated that mercaptoethanol broke S-S bonds in proteins and reduced them to –SH. Pea flour proteins contained up to 21 components with a molecular weight (MW) of 12 to > 250 kDa. After breaking S-S bonds, 26 components were found in pea proteins. The proteins most pronounced in the pea sample without mercaptoethanol had a MW of 70, 50, and 35 kDa, while in the sample with mercaptoethanol these components had a lower MW, the bands were shifted slightly lower.

![Figure 1. Molecular weight of pea proteins and taps, kDa. Samples without mercaptoethanol: 1 – flour, 3 – extract, 5 – markers, 6 – serum. Samples with mercaptoethanol: 2 - flour, 4 - extract, 7 – serum.](image)

The extract obtained for the isolation of proteins contained almost all the components of the native protein, both before and after the rupture of S-S bonds. In the serum obtained after the removal of the bulk of the proteins, the bulk of the proteins is represented by single-stranded components with MW 16-17 and 19-20 kDa.

Thus, the nutrient medium for bioconversion of serum in the biotechnological process has been enriched with assimilable low molecular weight carbohydrates and proteins.

To determine the optimal conditions for increasing the productivity of the yeast S. cerevisiae and the fungus G. candidum 977, the effect of the substrate pH, temperature and the amount of inoculum on the synthesis of biomass was studied 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.

| №  | pH of medium | Temperature, °C | Seed amount, % | Mass fraction of biomass, g/dm³ |
|----|-------------|----------------|----------------|---------------------------------|
| 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                           |
the pH value and the temperature of the medium

\[
md = -2.94 + 0.544 \cdot pH - 0.0356 \cdot pH^2 + 0.181 \cdot t - 0.003 \cdot t^2
\]

\[-0.147 \cdot cm + 0.0276 \cdot cm^2 - 0.00447 \cdot pH \cdot t\]

It can be seen that all the coefficients of the equation are significant \((p \leq 0.05)\) (table 3). The experimental data and calculation by the equation, their absolute error (table 4), as well as the type of the correlation graph \(R = 0.9644\) (figure 2) indicated an adequate description of the results.

Table 3. Regression coefficients and significance level \(p\).

| Factor      | Regr. Coeff. | Std.Err. | T (8) | p     | -95, % Cnf.Limit | +95, % Cnf.Limit |
|-------------|--------------|----------|-------|-------|------------------|------------------|
| 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 |

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 to ensure the maximum yield. Figure 3 shows, as an example, the regularity of the change in biomass from the pH value and the temperature of the medium \(t\), °C with the amount of inoculum \(cm = 2\) %.

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

| № | 1     | 2     | Absolute error | № | 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 | 0.708 | 0.672100 |
| 4 | 0.570 | 0.554225 | 0.015775 | 12 | 12.0 | 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 |
From the obtained equation in the Mathematica 12.1 program, the values of the factors for the maximum biomass yield (0.88 g / dm$^3$) were determined: pH of the medium - 6.03, $t = 25.7 \, ^\circ\text{C}$, the amount of seed $cm = 4 \,$%. At lower pH values (4.5–5.0) and temperatures (20–24.5 °C) or higher: pH 7.5–8.0, temperature - 26–35 °C, the growth of microorganisms slowed down. The cultivation of symbiosis of cultures had a positive effect not only on the accumulation of biomass, but also on the amount of protein. For symbiosis of cultures, the amount of protein in the biomass (FMPC-1) was 61.68 % of DS (table 5), in the biomass with the culture liquid (FMPC-2) - 57.90 %.

Table 5. Chemical composition of FMPC-1 from biomass obtained on pea serum.

| Moisture, % | Mass fraction, % of DS |
|-------------|------------------------|
| 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 from pea whey, microorganisms completely assimilated stachyose, maltose, arabinose, more than half - glucose and almost all other pentoses (table 6). The assimilation of stachyose by these yeast species corresponded to the literature data. On the other hand, the FMPC has doubled the number of HMWCs, the nature of which has yet to be deciphered.

The amino acid composition of FMPC-1 from biomass and FMPC-2 prepared from biomass together with the culture liquid is mostly represented by glutamic, aspartic acids, glycine, alanine, lysine (figure 4).

Table 6. Chromatographic composition of pea whey and FMPC carbohydrates, % of the total content.

| Product | HMWC | Stachyose | Raffinose | Sucrose, maltose | Glucose | Fructose galactose xyllose | Arabinose |
|---------|------|-----------|-----------|------------------|---------|---------------------------|-----------|
| Serum   | 32.01±0.2 | 26.38±1.1 | 0 | 14.98±1.3 | 9.66±0.7 | 12.06±0.8 | 4.90±0.4 |
| FMPC    | 68.83±0.8 | 0 | 26.21±0.6 | 0 | 3.87±0.2 | 1.09±0.4 | 0 |

The amino acid rate of FMPC-1 for all essential acids was 107-226 %, for FMPC-2 it was high for histidine, lysine, threonine, sulfur-containing amino acids (81-128 %). The fatty acid composition of FMPC-1 is represented by 30 components (table 7), 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 fragrances, essential oils, photostabilizers, odor fixatives, preservatives and other compounds.
The ratio of the sum of saturated (23.51%) and unsaturated fatty acids (71.67%) - 1:3, the content of cis isomers - 91.1%, trans isomers - 5.1%, omega-6 fatty acids (linoleic) - 19.73%. The FMPC did not have a negative effect on the vital parameters of experimental rats [11], which indicated its safety and prospects for use. Determination of the chemical composition of whey waters formed during the production of phytin from rice bran showed that they contain the main sources of nutrition necessary for the development of microorganisms, and there are no compounds undesirable for a living organism in their composition (figure 5).

Figure 4. Amino acid composition of FMPC from biomass and biomass with culture liquid.

Table 7. Fatty acid composition of FMPC from pea serum.

| №  | Fatty acid composition                          | Mass fraction, % | №  | Fatty acid composition                          | Mass fraction, % |
|----|------------------------------------------------|------------------|----|------------------------------------------------|------------------|
| 1  | Decanoic acid (Capric acid) $C_{10:0}$          | 0.10             | 16 | 7-Hexadecenoic acid (Hypoheic acid) $C_{16:1(7)}$| 0.56             |
| 2  | Undecanoic acid $C_{11:0}$                      | 0.05             | 17 | Trans-9-Hexadecenoic Acid (Palmitoleic acid) $C_{16:1(9)}$ | 3.65             |
| 3  | (R) - 3.4 Methyleneimmonium acetamide $C_{11}H_{13}NO_{2}$ | 0.17             | 18 | Hexadecanoic acid (Palmitic acid) $C_{16:0}$ | 15.03             |
| 4  | Dodecanoic Acid (Lauric Acid) $C_{12:0}$        | 0.28             | 19 | 10-heptadecanoic acid $C_{17:1(10)}$          | 0.63             |
| 5  | Nonanedioic acid (Azelaic acid) $C_{9}H_{16}O_{4}$ | 0.09             | 20 | Heptadecanoic acid (Margaric acid) $C_{17:0}$ | 0.52             |
| 6  | Lauric aldehyde $C_{12}H_{25}O$                 | 0.05             | 21 | Octadecadiene - 9Z, 12Z - oic acid Linoleic acid $C_{18:2(9,12)}$ | 19.73             |
| 7  | 1- Nonadecene $C_{19:1(9)}$                    | 0.81             | 22 | 9-Octadecenoic acid Oleinovaya $C_{18:1(9)}$ | 40.43             |
| 8  | 10- Methylundecanoic acid $C_{13}H_{26}O_{2}$   | 0.05             | 23 | 6- octadecenoic acid (petroselinic acid) $C_{18:1(6)}$ | 4.31             |
| 9  | Diphenol ketone ($C_{6}H_{5})_{2}CO$            | 0.08             | 24 | Octadecanoic acid (Stearic acid) $C_{18:0}$ | 7.10             |
| 10 | 3-phenyl-2-butyl propenoic acid ester $C_{13}H_{16}O_{2}$ | 0.09             | 25 | 7- Hexadecenoic acid (Hypoheic acid) $C_{16:1(7)}$ | 0.56             |
| No. | Compound                                                      | Formula                  | Concentration | Description                                      |
|-----|--------------------------------------------------------------|--------------------------|---------------|-------------------------------------------------|
| 9   | 9-Tetradecenovaya kislota                                    | C₁₄:₁(9)                 | 0.25          | Trans-9-Hexadecenoic Acid (Palmitoleic acid) C₁₆:₁(9) |
| 10  | Myristolovaya kislota                                        | C₁₄:₀                    | 1.36          | Hexadecanoic acid (Palmitic acid) C₁₆:₀          |
| 11  | Tetradecanoic Acid (Myristic Acid) C₁₄:₀                    | 0.45                     |               | 10-heptadecenoic acid C₁₇:₁(10)                 |
| 12  | Pentadecanoic acid C₁₅:₀                                     | 0.41                     |               | Heptadecanoic acid (Margaric acid) C₁₇:₀        |
| 13  | n-Hexyl benzoic acid ester C₁₃H₁₈O₂                            | 0.31                     |               | Octadecadiene - 9Z, 12Z- oic acid (Linoleic acid) C₁₈:₂₀:₁₁,₁₂ |
| 14  | Benzoic acid heptyl ester C₁₄H₂₀O₂                            | 0.41                     |               |                                                  |

The selection of microorganisms was carried out at a pH of 5.0 for yeast and microscopic fungi, and 7.0 for bacteria. In biological production, it is important to select high-tech producers who actively synthesize the target product. In biological production, the selection of producers, actively synthesizing the target product. The choice of protein biomass producers was carried out among bacillus bacteria, flavobacterium, yeast Candida, Saccharomyces, Torulopsis, Rhodotorula. Hansenula Pichia Trichosporon and Mycelial Mushrooms Penicillium Fusarium Aspergillus Rhizopus, highlighted by their various regions of Central Asia. The selection of yeast and microscopic mushrooms was performed at pH 5.0, bacteria - at pH 7.0. It has been established that the yeast 18-21% better absorbed the substrate than micromycetes and bacteria, 65% of yeast, 44% of micromycetes and 47% of bacteria assimilated serum with varying degrees of activity (figure 6).

Further work was carried out with yeast, as it assimilated the substrate better than bacteria and fungi. The sequence of obtaining phytin from rice bran and protein preparation is shown in figure 7.

Using the most productive yeast *S. cerevisiae* 84/5 and *Trich. cutaneum* 656 in a 1:1 ratio, a symbiotic starter was compiled, which differs from monocultures by an increased protein content in the biomass (figure 8).
The supply of nutrients to the cell depends on the concentration of hydrogen ions, which affects the yield of yeast. It was found that the pH of the substrate 3.0 and 8.0 inhibited the formation of biomass and protein, and pH 5.0...6.0 was optimal for the growth of the yeast association (figure 9). The highest yield of monocultures was achieved by 96 hours of growth, yeast association - by 72 hours (figure 10).

The biomass of the symbiotic culture contained more than the biomass of the monocultures, methionine, isoleucine, leucine, lysine, and total essential amino acids (figure 11).
The biomass protein of Trich. cutaneum 656 contained an increased amount of glutamic acid, and the protein of the symbiotic culture - methionine, isoleucine, leucine, lysine, and the sum of amino acids (figures 12, 13).

The quality of feed preparations is determined not only by the amount of protein and the composition of amino acids, but also by its assimilation by the animal organism. Protein assimilation is characterized by its ability to undergo hydrolysis to amino acids and peptides by gastrointestinal enzymes. The study of the attackability of the protein of the preparation obtained on serum found that 90% of the proteins are hydrolyzed by proteolytic enzymes (pepsin) (GOST 24230-80). A pilot batch of the drug has been successfully tested at the poultry farm of JSC "Uzbekistan" (Tashkent region) as a protein feed additive to compound feed. Thus, the ability of biological agents to transform serum components into protein biomass and to obtain qualitatively new products with high practical potential has been proven.

4. Conclusions
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 symbiosis of cultures of the fungus G. candidum 977 and yeast S. cerevisiae 121 has been carried out. adequately describing the dependence of the yield of biomass of crops 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 ability of symbiosis between the yeast S. cerevisiae 84/5 and the fungus Trich. cutaneum 656 has been proven to transform the components of serum remaining after the extraction of phytin from rice bran into protein biomass and obtain a product with high practical potential. The ratio of monocultures by mass fraction (1:1), pH - 5.0...6.0, duration of growth - 72 hours, digestibility – 90 % has been established. The biological value of protein in terms of the amount of the sum of essential acids and their individual representatives (methionine, isoleucine, leucine, lysine, etc.) is high. The use of concentrates from secondary products of pea and rice bran processing for protein preparations and phytin is promising for animal diets.

Acknowledgments
This work was carried out under the grant of the Russian Science Foundation 21-16 00025 SSI NIIMMP. Grant sponsors were not directly involved in the development, analysis, or writing of this article.

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