Qualitative indicators of protein concentrates from pea and chickpea flour

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Abstract. A comparative analysis of the qualitative indicators of food and feed protein concentrates (PC) from pea and chickpea flour was carried out. The chickpea PC contains more protein than the pea PC: 83.22±0.35 and 71.78±0.35% on dry matter (DM), respectively, the biological value adjusted for protein digestibility (PDCAAS) in the pea PC (96%) is higher than that in the chickpea PC (76%). The PCs differed in the content of essential amino acids, copper, cobalt, manganese, nickel, the amount of flavonoids and foaming ability. Higher foaming capacity and lower foam stability in the chickpea PC correlated with higher flavonoid content and percent parallel β-structure and anti-parallel 3_{10}-helix proteins. A fodder biomass with a protein content of 61.68-64.10% and a biomass with a cultural liquid with 50.60-53.56% protein on DM were obtained. Biologically valuable concentrates differed in the mass fraction of fat, soluble, insoluble carbohydrates, potassium, magnesium, cobalt, manganese, sodium and the ratio of saturated:unsaturated fatty acids. A correlation was found between the amount of flavonoids, the optical density at D_{590} nm, and the color of preparations (correlation coefficient R=0.895). It is recommended to use the PCs for food purposes, serum concentrates, in feed for various animals.

1 Introduction

In recent years, in the country and abroad, interest in legumes for their use in food and feed products has increased due to the high nutritional value of protein and relatively low cost [1]. Protein concentrates and isolates are used in the production of bakery, pasta, imitation dairy products, curds, yoghurts, meat analogues, baby products, sports nutrition, etc. Therefore, the development of new technologies for concentrated forms of plant protein with high nutritional value and good functional properties is an urgent task [2]. Usually, proteins from plant materials are extracted with alkali solutions, which form new cross-linked covalent bonds, D-isomers of amino acids, etc. These chemical bonds have a negative impact on human and animal health. Safe technologies with enzyme preparations (EP) for obtaining a high yield of biologically valuable pea and chickpea proteins with appropriate functional properties are little known. At the same time, in the production of protein products, liquid by-products are always formed. These are serum waters. Along with the growth of world demand for protein-containing food products and feed from plant raw materials, the volumes of liquid effluents and waste that pollute the environment are also increasing [3]. One of the directions of the scientific and technological development of the world community for the coming years is the rational use of natural resources. Therefore, an important problem is the development of environmentally friendly technologies for the disposal of all types of waste and increasing the level of environmental culture during the deep processing of organic raw materials. Waste is a substrate for the synthesis of biomass of microorganisms [2, 4, 5]. Biomass is a part of the diet of farm animals to increase their productivity and an ingredient in food as a protein, lipid, carbohydrate source or a technical substance [6-8]. For example, a product obtained by fermentation of corn stalks with *Saccharomycetes* or by the consortium of *Saccharomycetes*, *Lactobacillus plantarum* and *Lactobacillus casei* has a positive effect on the animal organism and on the environment [9–11]. *Saccharomyces cerevisiae* yeast in feed recipes for broilers in an amount of 0.8% by weight of the diet also increased the efficiency of its use [12]. The study of the microbiota of fecal samples on days 21 and 42 using polymerase reaction (PMR) revealed a positive effect of supplementation with the yeast *S. cerevisiae* on the microflora of broilers and ruminants, increased fiber digestibility and the population of rumen cellulolytic bacteria *Ruminococcus flavefaciens* [13]. The addition of *S. cerevisiae* and/or *Aspergillus oryzae* biomass to the diet of cattle increased milk yield and fat content [14–16]. Also there are feed additives from coffee sludge [17], from alcohol stillage with wheat bran, which are obtained by growing the yeast *Saccharomyces*

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diastaticus and carotene-forming yeast *Rhodosporidium species* to increase the content of essential amino acids in them [18]. A feed additive with carotenoids and lipids was synthesized with the yeast *Rhodotorula glutinis*, *Rhodotorula mucilaginosa* and *Rhodotorula gracilis* on a medium from wastewater during potato processing together with waste from the production of glycerin [19]. At a sugar cane processing plant, *Aspergillus niger* mushroom biomass with lipids for biodiesel fuel was obtained from wine waste [20]. A microbial-plant concentrate with the yeast *S. cerevisiae* with a mass fraction of protein of 25.2±2.1%, fat - 22.1±3.2%, carbohydrates - 40.8±1.6% was synthesized on the basis of triticale grain extract for feeding pond fish. This concentrate contains potassium, calcium, cobalt, iron, zinc, molybdenum, nickel and other mineral elements.

According to FAO, today the annual protein deficit in the world is estimated at tens of millions of tons and by 2050 will reach 30 million tons [3]. Since ancient times, legumes and their processed products have been used as a source of protein in the diet of people, including those who do not eat meat [21, 22]. Also, little attention is paid to the processes of processing by-products of leguminous crops, which are formed during the production of protein concentrates using bioconversion. For example, it is known that with by-products from the extraction of pea protein, a food vegan protein concentrate is obtained to replace meat. The studies were carried out with 5 strains of filamentous fungi: *Neurospora intermedia*, *A. oryzae*, *Rhizopus oryzae*, *Fusarium venenatum*, *Monascus purpureus*, which were grown at 35±2 °C for 48 h until the protein content in the biomass was 43.13-59.74% on dry matter (DM). For every ton of by-product, about 680 kg of mushroom biomass can be obtained with 38% additional protein [5]. The synthesis of feed concentrates with cultures of *S. cerevisiae* and *Geotrichum candidum* was obtained with a protein mass fraction of 61.68-70.48% from serum, which remains after precipitation of pea protein [6].

Recently, the industry has been particularly interested in chickpea - a legume crop, which makes it possible to create technologies for protein concentrates (PC), isolates and a number of other useful products [2, 3]. This crop is resistant to hot climates and is a promising addition to soybeans and peas in conditions of recurring drought and global warming [1, 23–25]. The gross harvest of chickpeas in Russia in 2019 amounted to 506.3 thousand tons, but it decreased by 18.4% compared to the record harvest of chickpeas in 2018 (620.4 thousand tons). Since 2014, the chickpea harvest has been on an upward trend, increasing by 15.0%. In recent years, chickpea sown areas have increased by 355.2 thousand hectares (71.6%), over 5 years – by 178.1 thousand hectares, which amounted to 26.5%. These data indicate the stability of the raw material base of chickpeas and peas in Russia, which is promising to be used for the production of protein preparations of various compositions and purposes [2]. The urgency of the problem of providing the population with protein from this type of crop dictates the development of various technologies for protein preparations, therefore, the creation of methods for the utilization of liquid secondary products for future introduction into production is in demand. The purpose of this work is a comparative description of the qualitative indicators and properties of protein concentrates obtained by a biotechnological method for food purposes, and feed concentrates synthesized with the help of microorganisms from serum remaining after the extraction of the bulk of the dietary protein from pea and chickpea flour.

2 Materials and methods

As objects, we used pea flour from grain of the Yamal variety grown in the Altai Territory and chickpea flour from grain of the Volzhanin variety grown in the Volgograd region in 2019-2020. The chemical composition of pea flour in % in dry matter in mass fraction of protein (N×6.25) – 24.30±1.40, ash – 2.87±0.20, fat – 1.58±0.12, carbohydrates – 73.02±3.66. The chemical composition of chickpea flour in % in dry matter in mass fraction of protein (N×6.25) – 24.54±0.23, ash – 2.91±0.02, fat – 4.89±0.31, carbohydrates – 67.66±0.56. The amount of total nitrogenous substances in flour and in concentrates was determined by the Kjeldahl method, the mass fraction of moisture, ash and fat according to Russian Standards (GOST 10846-91, GOST 13586.5-93, GOST 10847-2019 and GOST 29033-91). Carbohydrates were determined by the difference between 100% and the sum of other components.

For isolation from PC and the secondary product (serum), we used enzyme preparations (EP) from Novozymes (Denmark): Shearzym 500 L from *Aspergillus aculeatus* with a xylanase activity of 500 U/g and optimal conditions of action 65–75 °C, pH 4.5–5.5. As a source of cellulase, carboxymethyl cellulase and β-glucanase activity, Viscoferm L was used, produced by *Trichoderma* and *Aspergillus* strains with a cytolytic activity of 600 U/g of raw material and an optimum of action at a temperature of 50–60 °C and pH 4.8–5.8. As a source of α-amylase, we used Fungamyl 800 L from the mold *A. oryzae*, which cleaves α-1,4 glucosidic bonds to form maltodextrins and maltose (50-60 °C, pH 5.0-6.5). The amyloglucosidase-containing EP was AMG 300 L 2500, isolated from the fungus *A. niger*, which cleaves both α-1,4 and α-1,6 glucosidic bonds in starch, dextrins and oligosaccharides to form glucose. The optimum action lay in the region of 55-60 °C, pH - 4.5-5.5. Alcalase 2.4 L EP from *Bacillus licheniformis* with a protease activity of 2.4 units/g was used as a source of proteases. We determined the enzymatic activity of the preparations according to Russian Standards (GOST P54330-2011, GOST R 53974-2010 and GOST R 55302-2012). All reagents were chemically pure. Ultrasound treatment of the protein suspension was performed on a Soniprep 150 ME device (MseLtd., UK).

To obtain feed microbial-plant concentrates (FMPC) from the collection of the Institute of Microbiology named after S.N. Vinogradsky (Moscow) we used the yeast *S. cerevisiae* 121 and the fungus *G. candidum* 977,

the phylogenetic position of which was determined at the Research Institute of Genetics (Russia). Museum cultures from agar wort were subcultured into a test tube with pea or chickpea serum, which was pre-sterilized at a pressure of 0.1 MPa and cooled. Microorganisms were grown for 24 h, after which they were subcultured into 300 cm^3 flasks with 50 cm^3 of serum (pH 6.0-6.5). Cultivation was carried out on a shaker at a flask rotation speed of 150 min^{-1} and a temperature of 27±1 °C for 24–48 h. The suspension was inactivated at a temperature of 95±5 °C for 10-15 min and cooled to a temperature of 22±2 °C. The biomass was separated by centrifugation at 4000 min^{-1} for 10 min, dried separately or together with the culture liquid in a Hochvacuum HVDTG-50 lyophilizer (Germany) in a vacuum at –80 °C, and feed microbial-plant concentrates (FMPC-1 and FMPC-2) were obtained [26].

The amount of protein in solutions was determined by the Lowry method, soluble and insoluble carbohydrates in concentrates according to the method described in book [4]. The method is based on the enzymatic hydrolysis of starch and protein compounds with amylase, protease and amyloglucosidase to mono-, di-, oligosaccharides and peptides. For the hydrolysis of proteins and carbohydrates, α-amylase Fungamyl 800 L, protease Alcalase 2.4 L, and amyloglucosidase AMG from Novozymes (Denmark) were used. Soluble dietary fiber was precipitated with 4 volumes of 95% (v/v) ethanol for 2 hours at 4 °C, washed 2 times with 95% ethanol. The amount of dried mass was determined by the gravimetric method, the mass fraction of carbohydrates was expressed as a percentage (g/100 g) [27]. The digestibility of feed concentrates was determined according to Russian GOST 24230-80.

The carbohydrate composition of serum and culture liquid was studied on a gas chromatograph model GCMS-QP 2010 (Japan, Shimadzu Corporation) with a ReproGel Na column (9 μm, 8x300 mm). The amino acid composition of proteins was determined on a Hitachi model L-8800 chromatograph (Japan) with a cation exchanger (sulfonated copolymer of styrene with divinylbenzene) and a stepwise gradient of sodium citrate buffer solutions with increasing pH and molarity. The data were processed in the on-line system "MultiChrom 1.52" (Russia). A ninhydrin reagent was used to detect eluted amino acids. The instrument was calibrated with standard amino acid mixtures after dilution. The reproducibility in terms of release time was 0.3%, in terms of peak area – 1%, the lower limit of sensitivity – 3 pmol (signal-to-noise ratio = 2 for Asp). For acid hydrolysis, 3–5 mg of a sample was placed in a molybdenum glass ampoule and 0.3 cm^3 of a hydrolyzing mixture (concentrated hydrochloric and trifluoroacetic acids in a ratio of 2:1 with 0.1% β-mercaptoethanol) was added. The sample was frozen in liquid nitrogen, evacuated, and melted in a glass ampoule. Hydrolysis was carried out at 155°C for 1 hour, after which the ampoule was opened, the contents were transferred into a plastic tube (Eppendorf, Germany) and the hydrolyzing mixture was removed on a CentriVap Concentrator LABCONCO (US). 0.1 N HCl was added to the dry residue of the hydrolyzate, vigorously mixed in a closed plastic tube, and centrifuged for 5 min at 8000 min^{-1} in a Microfuge 22R centrifuge (Beckman-Coulter, USA). When calculating the amino acid score of the concentrates, the FAO/WHO reference protein scale was used [28]. Lipids were isolated from FMPC by the Folch method. After the lipids were evaporated in a rotary evaporator, chloroform, methanol hydrochloride (SupelcoMethanolic-HCl 0.5 N) were added to them, and the mixture was heated for 1 h at 90°C. The fatty acid composition of lipids was studied on a chromatograph with a Simadzu GCMS-QP 2010 Ultra mass detector at a temperature of 120°C, an injector at 200°C; interface – 205°C, detector – 200°C on an SLB-IL82 column (30 m, 0.20 μm, d = 0.25 mm) with a gel carrier at a flow rate of 35.6 cm/sec and its division 1:10. The gradient mode was changed from 120°C to 260°C at a rate of 5 °C/min for 2 minutes.

The content of lead and cadmium in the concentrates was determined according to GOST 30178-96, the remaining macro- and microelements were determined after dry ashing by the atomic absorption method on a Hitachi instrument (model Z 5300) in an air-acetylene flame with Zeeman correction. The functional properties of PC were studied according to the methods described in the works [29], flavonoids - by the spectrophotometric method with light absorption at 276 nm [30].

The molecular weights of flour proteins, extracts isolated under the action of enzyme preparations (EP), and serum were determined using gel electrophoresis under denaturing conditions (SDS-PAGE). Protein samples in an amount of about 50 μg were mixed with buffer in a ratio of 1:1. To prepare the buffer, 60 cm^3 of glycerol and 1 mg of bromophenol blue were poured into a beaker, and the pH was adjusted to 6.8 with concentrated HCL. 5 cm^3 of ß-mercaptoethanol was added to the solution, and the volume of the solution was adjusted to 100 cm^3. Samples and solutions of proteins markers in the amount of 102 μg/30 μl were heated for 2 min at 95–100°C. To prepare a 15% separating gel, 4.5 cm^3 of an AB solution were mixed (a sample of 29.6 g of acrylamide was dissolved in a small amount of water, 0.4 g of bisacrylamide was added and the volume was adjusted to 100 cm^3), 2.5 cm^3 of Tris-HCl buffer with pH 8.8, 3 cm^3 of water, 20 μl of TEMED and 160 μl ammonium persulfate (PSA). The solution was shaken and poured between glasses. To prepare a concentrating gel, 1 cm^3 of AB solution, 1 cm^3 of Tris-HCl buffer, pH 6.8, 3 cm^3 of water, 20 μl of TEMED, and 160 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 for the entry of samples into the gel, then at 120 V to separate the components. Ready electrophoregrams were stained with Coomassie bright blue and scanned. The experimental data were processed using TableCurve 2D 5.1, TableCurve 3D 4.0, Mathematica 10.3, and Statistica 10 programs. The confidence interval of the

arithmetic mean was calculated using the significance level $p = 0.05$.

3 Results and discussion

3.1 Protein concentrates for food purposes

The extraction of proteins from pea and chickpea flour was carried out with hydrolytic EPs: cellulase, xylanase, amylase and protease at 3 stages according to the scheme shown in Figure 1. To determine the optimal parameters of protein extraction, an experiment planning matrix was compiled from 25 experiments on the dependence of protein solubility (R) on the EP concentration (sv), hydromodule (gl), and extraction duration (t). After solving the tasks, the proteins were transferred into the solution at optimal values of the parameters: for pea flour: sv – 1.50%, t – 4.2 h, g1 – 15; for chickpea flour: sv – 1.81%, t – 5 h, $g1$ – 25 at a mixing speed of both suspensions of 200 min^{-1}. Protein solubility was increased by 23±1% to 84±1% with a 3-minute ultrasonic treatment (UST) of the protein extract and a wave amplitude of 10 μm.

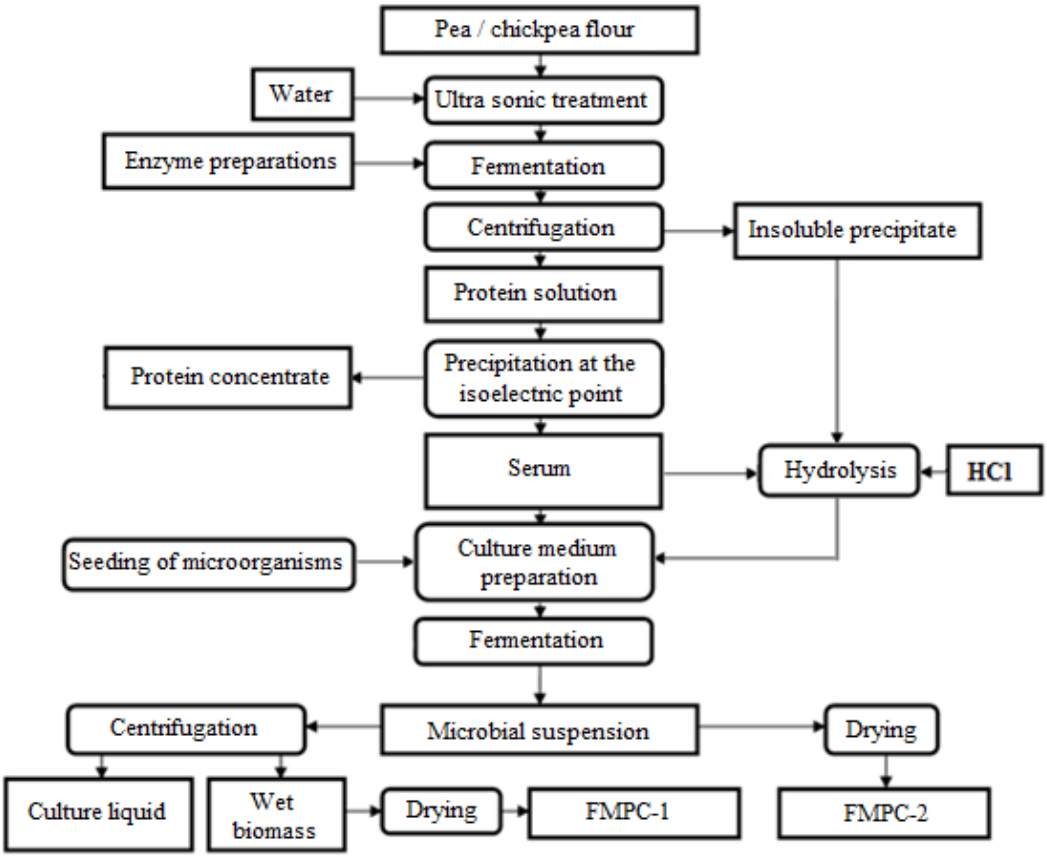


Fig. 1. Technological scheme for processing pea and chickpea flour into food and feed concentrates.

Proteins from the solution were precipitated with 10% HCL at the isoelectric point (pH 4.2), followed by centrifugation of the suspension at 5500 min^{-1} and separation of the precipitate from the serum. The precipitate was washed twice with water, freeze-dried, and PCs were obtained with the chemical composition presented in Table 1. The yield of proteins was 65–70% of their content in the raw material. Both concentrates have protein amino acid scores of 100% or more, with the exception of the valine score of the chickpea PC.

Table 1. Chemical composition of pea (PPC) and chickpea (CPC) protein concentrates.

Moisture, %	**Mass fraction, % on DM**							
	Protein (Nx6.25)	**Lipids**	**Ash**	**Alimentary fiber**				
				Soluble	**Insoluble**			
	PPC							
3.86±0.20	71.78±0.35	4.47±0.27	1.80±0.27	9.67±0.76	7.57±0.26			
	CPC							
9.76±0.11	83.22±0.35	2.23±0.31	1.11±0.38	8.01±0.70	5.81±0.48			
Amino acid score, %								
Val	His	Ile	Leu	Lys	Met+Cys	Thr	Trp	Phe+Tyr

PPC								
110	144	166	144	145	109	160	212	183
CPC								
86	120	120	111	100	100	136	184	195

The amount of essential amino acids in the PPC did not differ much from the amount of such acids in the CPC and was equal to 256.21 and 247.9 mg/100 g, respectively (Figure 2). Adjusted for protein digestibility (PDCAAS) (88%) the biological value of the PPC is 96%, and that of the CPC is 76%.

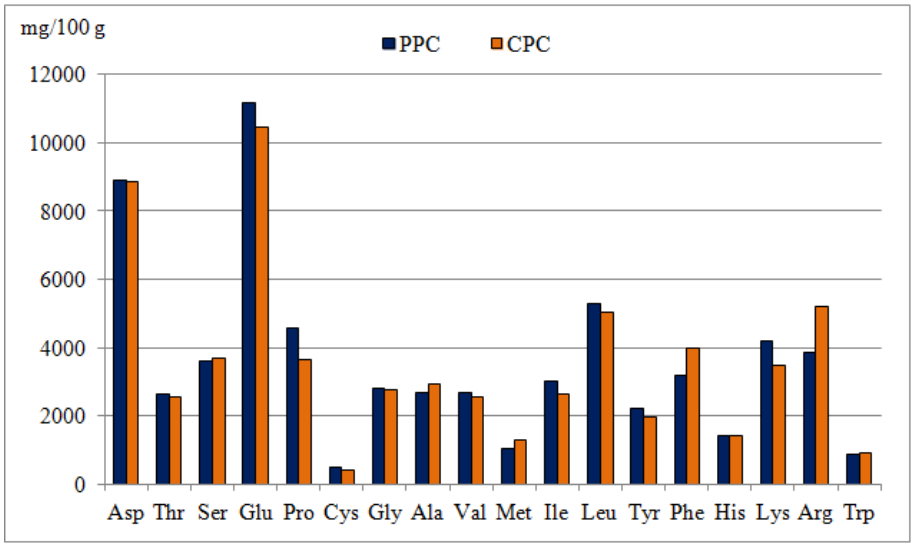


Fig. 2. Amino acid score of PPC and CPC.

The composition of macro- and microelements of PCs was characterized in Table 2. In the CPC, the mass fraction of iron was 2.1 times lower, and zinc was 1.4 times less as compared to the PPC, while copper, cobalt, manganese and nickel, on the contrary, were 1.2-4.8 times more than that in the PPC.

Table 2. The content of macro- and microelements in grains and protein concentrates.

No	Macro- and microelements	Pea		Chickpea	
		Grain	Protein concentrate	Grain	Protein concentrate
1	Sodium, mg/100g	33.0±2.5	103±7.0	4.93±0.49	109±7.0
2	Potassium, mg/100g	872±10.0	259±14.0	795.4±95.4	263±15.0
3	Calcium, mg/100g	115.2±1.5	219±14.0	96.5±9.7	207±18.0
4	Magnesium, mg/100g	106.5±2.0	10.3±0.7	122.9±1.23	10.7±0.6
5	Iron, mg/100g	6.80±1.3	114±8.0	5.16±1.33	53.9±4.0
6	Zinc, mg/100g	3.18±0.31	3.10±0.25	2.54±0.25	2.20±0.20
7	Copper, mg/100g	0.74±0.06	0.36±0.02	0.66±0.06	1.73±0.05
8	Manganese, mg/100g	1.70±0.08	0.51±0.04	2.91±0.29	0.92±0.07
9	Cobalt, mcg/100g	13.0±1.0	92.0±2.0	9.0±1.30	109.0±3.0
10	Nickel, mcg/100g	241.6±11.0	190±16.0	356±36.0	340±25.0
11	Cadmium, mg/kg	0.026±0.006	0.086±0.005	0.030±0.007	0.071±0.003
12	Chromium, mg/kg	0.007±0.002	≤0.005±0.001	0.008±0.001	≤0.005±0.002
13	Molybdenum, mg/kg	0.074±0.001	≤0.040±0.001	0.050±0.001	≤0.040±0.001

Table 3. Functional properties and amount of flavonoids in PCs.

Sample of PCs	WAC, g/g	FC, %	FS, %	OAC, g/g	EA, %	ES, %	PS, %	Flavonoids, mg/g of protein
PPC cream	2.79±0.04	42±1	32±1	2.24±0.01	52±2	47±9	11.60±0.25	2.78±0.24
PPC brown	2.44±0.03	91±1	10±1	2.25±0.03	56±3	51±3	13.10±0.15	15.05±0.71
CPC light yellow	2.82±0.05	85±0	12±0	2.26±0.03	55±1	52±1	12.20±0.50	14.06±0.41

Note: WAC – water absorption capacity; FC - foaming capacity; FS - foaming stability; OAC - oil absorption capacity; EA - emulsifying activity; ES - emulsifying stability; PS – protein solubility.

The values of functional properties (Table 3) are at the level of the values of the functional properties of cereal PCs obtained from wheat, rice, barley, rye, etc.

Table 4 presents data on the elements of the secondary structure of proteins of light powder of the PPC and the CPC, obtained on the basis of circular dichroism (CD) spectra. The measurements were carried out in a solution of 0.05 M acetic acid at 20°C and protein concentrations from 0.10 to 0.16 mg/cm^3.

Table 4. Elements of the secondary structure of proteins in the PPC and CPC, % of the sum of structures.

PPC cream		**CPC light yellow**	
α – helix **-7.2±0.3**	Regular - 3.0±0.1	α – helix – **0.1±0.005**	Regular – 0.1±0.0
	Distorted - 4.2±0.2		Distorted – 0.00
Antiparallel 3_{10}-helix - **26.9±0.5**	Left-twisted - 0.8±0.1	Antiparallel 3_{10}-helix – **39.2±0.3**	Left-twisted - 4.7±0.1
	Relaxed - 12.6±0.4		Relaxed - 17.6±0.4
	Right-twisted - 13.4±0.5		Right-twisted – 16.9±0.5
Parallel β - structure **- 7.1±0.3**		Parallel β - structure - **3.0±0.2**	
Turn β – curves – 14.5±0.6		Turn β – curves – 14.8±0.7	
Others - 44.3±0.7		Others - 42.9±0.6	

The CPC proteins differed from the PPC proteins by the 7 time-less regular and irregular (distorted) α-helix and 2 time-less parallel β-structure. However, the CPC contains 1.26-6 times more proteins with an antiparallel 3_{10}-helix: left-twisted, right-twisted and relaxed. Turn β-curves and other types of the secondary structure are present in equal amounts. Consequently, the high FC, but the low value of FS in the PCs are due to the parallel β-structure, all types of proteins of the antiparallel 3_{10}-helix and flavonoids.

3.2 Feed microbial-plant concentrates from pea and chickpea serum

On serum after precipitation of pea and chickpea proteins from extracts that were isolated using hydrolytic EP, feed protein concentrates were synthesized with a consortium of yeast *S. cerevisiae* 121 and micromycete *G. candidum* 977 at a weight ratio of 1:1. Plans for the experiment were drawn up in the form of Latin squares, in which the function was the mass fraction of biomass (g/dm^3), factors - pH of the substrate (*pH*), temperature (*t*) and the amount of seed (*cm*) during the growth of microorganisms for 4 days. Through the listing of the solution, the coefficients and optimal values of the factors influencing the maximum yield of the mass fraction of biomass *md*, g/dm^3 were determined. The equations for the dependence of the biomass yield (*md*) on the influencing factors for chickpea serum had the form (1) for pea serum (2):

$$md = 3.798\ (0.44 + 0.00585cm^2)\ e^{-332624e\text{-}t}\ (0.3402 + 5.5403 / pH^2) \quad (1)$$

$$md = -2.94 + 0.544\ pH - 0.0356\ pH^2 + 0.181\ t - 0.003\ t^2 - 0.147\ cm + 0.0276\ cm^2 - 0.00447\ pH\ t. \quad (2)$$

Note: md is the mass fraction of biomass, cm is the amount of seed, t is temperature, pH is the pH of the medium.

The correlation coefficients for equation 1 and equation 2 were significant ($p \leq 0.05$), they were equal to R=0.9644 and R=0.9869, respectively, which indicated that they adequately described the experimental data. From the equations, the optimal values of the factors providing the maximum biomass yield *md* = 0.82 g/dm^3 for pea serum were pH (*pH*) – 6.03, temperature (*t*) – 25.7°C, the amount of seed (*cm*) – 2–3%; for chickpea serum: *md* = 0.88 g/dm^3 with pH (*pH*) – 5.0, temperature (*t*) – 16.96℃, the amount of seed (*cm*) is 4%.

In the process of biomass synthesis from both types of serum, microorganisms completely assimilated glucose, xylose, arabinose, galactose, fructose, in total their number decreased more than 10 times. Raffinose, stachyose and maltose were practically not assimilated, the appearance of sucrose in the culture liquid was possibly due to the hydrolysis of the alpha 1 → 6-glycosidic bond between the residues of galactose and sucrose in raffinose. The amount of high molecular compounds (HMC) in the liquid increased due to the quantitative redistribution of fractions (Table 5).

Table 5. Carbohydrate composition of serum and culture liquid during biomass growth.

Days of growth of the biomass	**Carbohydrate composition of serum and culture liquid, % of total amount**						
	HMC	**Raffinose, stachyose**	**Sucrose**	**Maltose**	**Glucose**	**Xylose, galactose**	**Arabinose, fructose**
	Serum						
	29.75±0.43	17.65±1.2	0.0	4.75±2.3	2.29±1.2	39.07±1.6	6.49±0.2
	Cultural liquid during biomass growth						
1	57.56±0.10	26.00±0.81	4.73	8.21±0.07	0.0	0.0	3.51±0.41

2	53.78±0.09	33.30±0.70	0.20	8.07±0.06	0.0	4.86±0.13
3	55.88±0.08	28.28±1.20	3.05	7.79±0.08	0.0	5.02±0.05
4	58.47±1.10	27.04±0.92	0.25	10.05±0.10	0.0	4.45±0.33

Efficient accumulation of biomass was also due to the presence of relatively low molecular weight nitrogenous components compared to the native flour. The molecular weight (MW) of the components was determined by electrophoresis in SDS-PAGE with and without mercaptoethanol to break disulfide bonds in proteins. If the flour contains multichain components with MM from 15 to >250 kDa, which decomposed into single-chain polypeptides with MM (from 10 to 150 kDa) under the action of mercaptoethanol, then the serum contains components with a lower MM: from 10 to 25 kDa (Figure 3).

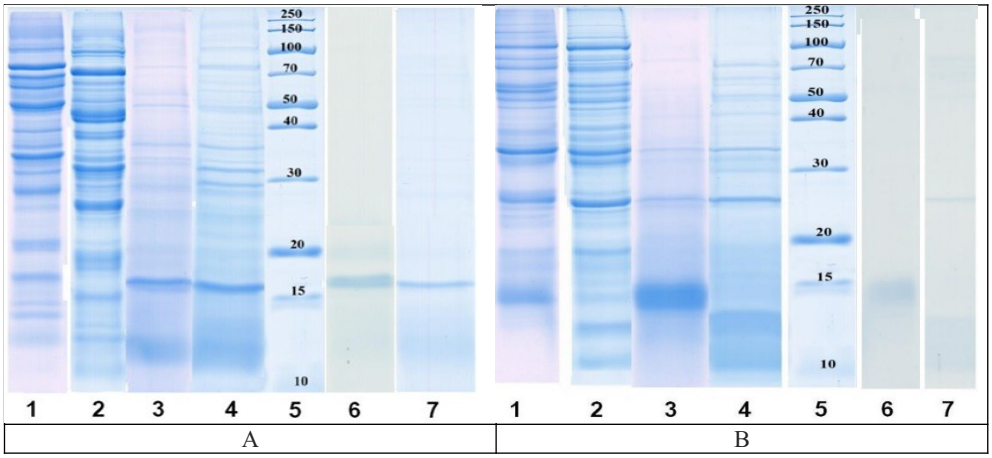


Fig. 3. Protein components in PAAG from: A – pea; B - chickpea; without mercaptoethanol: 1 – flour, 3 – extract of the 1st stage, 5 – markers, 6 – serum; with mercaptoethanol: 2 – flour, 4 – extract of the 1st stage, 7 – serum.

Carbohydrate and protein compositions of the nutrient medium already on the second day of growth (24 hours) of microorganisms on serum ensured the formation of a consortium of yeast and micromycetes, which positively affects the cell morphology (Figure 4 - D) and the chemical composition of the finished concentrates (Table 6).

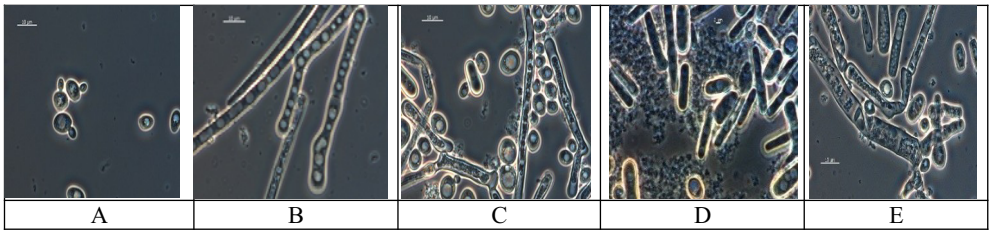


Fig. 4. Cells of monocultures and their consortium: pea serum: A - *S. cerevisiae* 121, B - *G. candidum* 977, C - consortium (48 hours of growth); chickpea serum: D - consortium (24 hours of growth), E - consortium (48 hours of growth)

Table 6. Chemical composition of microbial-plant concentrates from biomass with culture liquid (FMPC-1) and from biomass (FMPC-2).

Product	**Moisture, %**	**Mass fraction, % on DM**			**Carbohydrates**	
		Protein (Nx6.25)	**Ash**	**Lipids**	**soluble**	**insoluble**
FMPC from chickpea serum						
FMPC-1	7.20±0.26	50.60±0.58	12.27±0.05	2.65±0.01	15.22±0.64	20.31±0.33
FMPC-2	9.10±0.50	64.10±0.88	8.93±0.04	9.57±0.42	8.80±0.72	8.60±0.27
FMPC from pea serum						
FMPC-1	6.81±0.40	53.56±0.37	8.60±0.03	2.04±0.19	10.51±0.55	20.48±0.35
FMPC-2	6.81±0.41	61.68±0.47	8.60±0.04	8.31±0.36	7.13±0.55	14.27±0.44

The mass fraction of protein and lipids in the FMPC-2 from biomass is greater than the mass fraction of these compounds in FMPC-1 from biomass with culture liquid in both cultures by 15-27% and is 3.6-4.1 times, respectively. The sum of soluble and insoluble carbohydrates, on the contrary, is 1.4–2.0 times less. Differences in the chemical composition were also revealed for FMPC obtained on different serum. Differences in the mass fraction of protein amounted to 5-8%, in both types of chickpea FMPCs there are 15-29% more lipids than that in pea concentrates and 20-45% more soluble carbohydrates. The mass fraction of insoluble carbohydrates is almost the same in pea and chickpea FMPC-1, while in the FMPC-2 obtained from pea biomass, their amount is 66.5% that is more than that in the FMPC-2 from chickpea serum. A higher content of insoluble carbohydrates in the pea FMPC-2 (by 66%) compared to the chickpea FMPC-2 corresponded to a slightly lower value of their *in vitro* digestibility with EP: 84.41±0.32% and 88.46±1.30%, respectively. The differences are probably due to the characteristics of the chemical composition of the grain of the cultures, because the technology of serum bioconversion is the same.

Proteins of FMPCs contain 18 amino acids: glutamic, aspartic acids, glycine, proline, lysine, etc. (Figure 5).

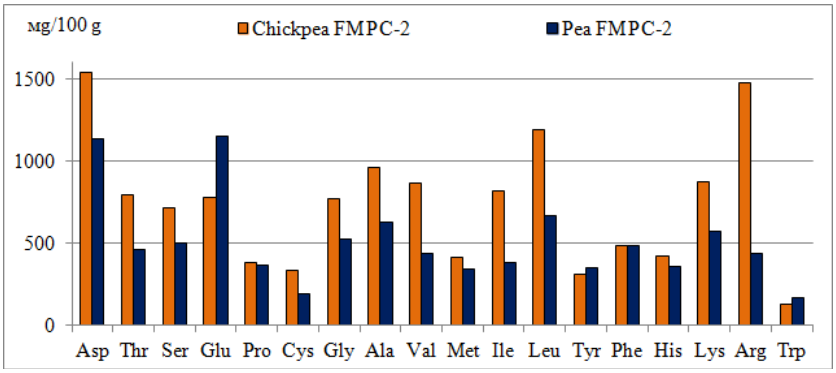


Fig. 5. Amino acid composition of feed microbial-plant concentrates from biomass (FMPC-2).

The score for all essential amino acids of the FMPC-2 from biomass on both types of serum is above 100% (Table 7), which indicates a high biological value of these concentrates.

Table 7. Amino acid score of feed microbial-plant concentrates from biomass (FMPC-2).

FMPC-2 from:	Score of essential amino acids, %								
	Val	His	Ile	Leu	Lys	Met+ Cys	Thr	Trp	Phe+Tyr
Pea serum	107	219	124	107	116	226	179	247	197
Chickpea serum	151	188	197	136	127	225	221	137	135

Table 8. Fatty acid composition of FMPC from pea and chickpea serum.

No	Fatty acid composition	Mass fraction, %		No	Fatty acid composition	Mass fraction, %	
		Pea serum	Chickpea serum			Pea serum	Chickpea serum
1	Decanoic acid Capric acid $C_{10:0}$	0.10	-	17	7-hexadecenoic acid Hypogeic acid $C_{16:1(7)}$	0.56	-
2	Undecanoic acid $C_{11:0}$	0.05	-	18	Hexadecanic acid Palmitic acid $C_{16:0}$	15.03	20.09
3	(R)-3,4-methyledimoxymethamphetamine $C_{11}H_{15}NO_2$	0.17	-	19	Trans-9-hexadecenoic acid Palmitoleic acid $C_{16:1(9)}$	3.65	8.26
4	Dodecanoic acid Lauric acid $C_{12:0}$	0.28	-	20	10-heptadecenoic acid $C_{17:1(10)}$	0.63	-
5	Nonandioic acid Azelaic acid $C_9H_{16}O_4$	0.09	-	21	Heptadecanoic acid Margaric acid $C_{17:0}$	0.52	-
6	Lauric aldehyde $C_{12}H_{24}O$	0.05	-	22	Octadecadiene- 9Z,12Z acid Linoleic acid $C_{18:2(9,12)}$	19.73	-
7	1-nonadecene $C_{19:1(9)}$	0.81	-	23	9-octadecenoic acid Oleic acid $C_{18:1(9)}$	40.43	16.56
8	10-methyldodecanoic acid $C_{13}H_{26}O_2$	0.05	-	24	6-octadecenoic acid Petroselic acid $C_{18:1(6)}$	4.31	1.01
9	Diphenolketone $(C_6H_5)_2CO$	0.08	-	25	Octadecanoic acid Stearic acid $C_{18:0}$	7.10	1.82

10	3-phenyl-2-butyl ester propenoic acid $C_{13}H_{16}O_2$	-	-	26	7-hexadecenoic acid Hypogeic acid $C_{16:1(7)}$	0.56	-
11	9-tetradecenoic acid $C_{14:1(9)}$ Myristolic acid	0.25	-	27	Trans-9-hexadecenoic acid $C_{16:1(9)}$ Palmitoleic acid	3.65	-
12	Tetradecanoic acid Myristic acid $C_{14:0}$	1.36	1.35	28	Hexadecanic acid Palmitic acid $C_{16:0}$	-	-
13	Pentadecanoic acid $C_{15:0}$	0.45	2.14	29	10-heptadecenoic acid $C_{17:1(10)}$	0.63	-
14	n-Hexyl ester of benzoic acid $C_{13}H_{18}O_2$	0.41	-	30	Heptadecanoic acid Margaric acid $C_{17:0}$	0.52	-
15	Heptyl ester of benzoic acid $C_{14}H_{20}O_2$	0.31	-	31	Octadecadiene-9Z,12Z acid Linoleic acid $C_{18:2(9,12)}$	19.73	41.26
16	1,4-benzenedicarboxylic acid diethyl ester	-	4.08	32	Nonadecanol-1 $C_{19}H_{39}OH$	-	3.42

However, for the FMPC-2 from chickpea serum, the score for threonine, lysine, leucine, isoleucine, and valine is higher than that for FMPC-2 from pea serum and less for aromatic amino acids and tryptophan. The score for sulfur-containing amino acids is the same.

The fatty acid composition of FMPC-1 from chickpea serum is represented by 10 components, from pea serum - 30 compounds (Table 8), among which the first concentrate has: 92.5% fatty acids of vegetable oils and animal fats, 7.50% esters and alcohols with the properties of flavors, essential oils and metabolites, in the second concentrate - 97.0% and 3.0%, respectively.

In the chickpea concentrate, the ratio of the sum of saturated (25.40%) and unsaturated fatty acids (67.09%) is 1:2.6, the content of omega-6 fatty acids (linoleic acid) is 41.26%. In the pea concentrate, respectively, the ratio is 1:3 (23.5% / 71.67%) and omega-6 fatty acids are 19.73%. The content of cis-isomers in the concentrate is 91.1%, trans-isomers is 5.1%. Thus, in terms of the composition and type of fatty acids, both FMPC-1s are close to edible oils and fats, while chickpea FMPC-1 contained 4.5% less as compared to these acids and 21.5% more omega-6 fatty acids (linoleic). The total amount of unsaturated fatty acids in pea FMPC is higher than that in chickpea FMPC (the ratio of saturated:unsaturated fatty acids is 1:3 and 1:2.6).

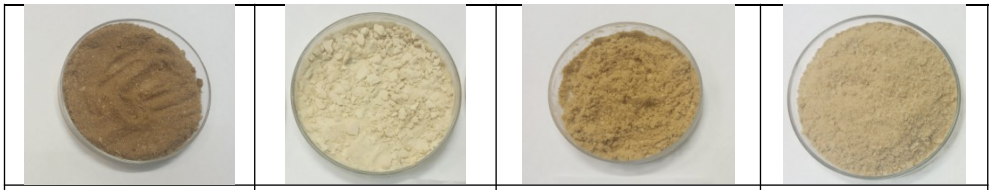
The mineral composition of FMPC-1 is represented by 14 macro- and microelements (Table 9). Chickpea FMPC-1 contains 1.8 times more potassium, magnesium, and cobalt, 10 times more manganese, and 2 times more sodium than pea FMPC-1 does. The amount of calcium, iron, and zinc is almost the same in both preparations.

Table 9. The content of macro- and microelements in FMPC-1 from chickpea and pea serum.

No	**Macro- and microelements**	**FMPC-1 from:**	
		chickpea serum	**pea serum**
1	Sodium, mg/100g	2460±172	1163±81
2	Potassium, mg/100g	3377±200	1844±100
3	Calcium, mg/100g	2010±156	2000±120
4	Magnesium, mg/100g	222±10	121±8
5	Iron, mg/100g	5.1±0.35	6.3±0.46
6	Zinc, mg/100g	11.4±0.90	14.0±1.2
7	Copper, mg/100g	1.50±0.04	1.12±0,04
8	Manganese, mg/100g	12.0±0.56	1,56±0,08
9	Cobalt, mcg/100g	107±3.00	57±2.00
10	Nickel, mcg/100g	210±15	440±36
11	Plumbum, mg/kg	≤0,001	≤0,001
12	Cadmium, mg/kg	0.151±0.007	0.171±0.009
13	Chromium, mg/kg	≤0.005	≤0.005
14	Molybdenum, mg/kg	≤0.04	≤0.04

The results must be taken into account when formulating feed and supplement recipes for different groups of animals.

One of the BA samples obtained from pea flour (Sample A) has a dark brown color (Figure 6).



A B C D

Fig. 6. Appearance of PCs from: A - pea flour-1; B - pea flour-2; C - chickpea flour; D - FMPC from chickpea flour.

The color may be related to the reaction of melanoidin formation between the carbonyl groups of reducing sugars and the amino groups of proteins and amino acids, the formation of melanins with the participation of the amino acid tyrosine and enzyme tyrosinase, and the oxidation of −OH groups of flavonoids. The first of two reasons has not received experimental confirmation, while the relationship between the amount of flavonoids in samples and color shades has been established. The mass fraction of flavonoids in flours and PCs, expressed in mg/g of protein, correlated with the color shades of concentrates, while the amount of % of them on DM and in mg/g of the product did not reflect these features (Table 8).

Table 10. Mass fraction of flavonoids in flours and protein concentrates.

Product	The color of product	Optical density, D_{590} nm	Mass fraction of protein, % on DM	Mass fraction of flavonoids		
				% on DM	mg/g of product	mg/g of protein
Pea flour and concentrates						
Flour-1	Yellow	0.390	20.38	1.14	12.70	56.00
PC-1	Dark brown	0.080	71.78	1.08	11.22	15.05
FMPC-1	Light brown	0.100	42.50	1.11	12.23	39.14
Flour-2	Light yellow	0.080	20.20	0.02	1.79	9.89
PC-2	Light yellow	0.040	72.07	0.02	1.88	2.78
FMPC-2	Light yellow	0.040	61.68	1.12	12.37	2.85
Chickpea flour and concentrates						
Flour-3	Yellow	0.380	19.27	1.11	12.24	54.49
PC-3	Dark yellow	0.080	83.22	1.17	12.84	14.06
FMPC-3	Dark yellow	0.085	47.15	1.11	12.28	26.68

The less flavonoids in the flour, the less flavonoids in the composition of PC. Light yellow PC-2, obtained from flour-2 with a 5.6 time-lower amount of flavonoids, compared to flour-1, also contains less flavonoids than dark brown PC-1 does (5.4 times). The same dependence is typical of FMPC. Light yellow FMPC-2 contains 1.7 times less flavonoids than light brown FMPC-1 does. The high content of flavonoids in chickpea flour is also accompanied by a large amount of them in the finished PC-3 with a dark yellow color. The value of the optical density of aqueous solutions of all the studied products, measured at D_{590} nm, highly correlates with the mass fraction of total flavonoids, expressed in mg/g of protein (R = 0.895).

4 Conclusions

A comparative analysis of the qualitative indicators of food and feed PC from leguminous crops obtained with hydrolytic EP and ultrasonic processing of raw materials with the achievement of the solubility of pea and chickpea proteins up to 84±1% and their yield of 65-70% was carried out. Chickpea PC contains more protein than pea PC does: 83.22±0.35 and 71.78±0.35% on DM, but the biological value index, adjusted for protein digestibility (PDCAAS) (88%), was higher in pea PC than that in chickpea PC: 96% and 76%, respectively. The amount of essential amino acids is higher in pea PC (256.21 mg/100 g) as compared to chickpea PC (247.9 mg/100 g). PCs differ in the content of copper, cobalt, manganese, nickel, the amount of flavonoids, foaming ability and secondary structure elements. The higher content of flavonoids and more parallel β-structure and anti-parallel 3_{10}-helices in chickpea PC corresponds to a higher foam ability and lower foam stability compared to pea PC. Twisted β-curves and other types of the secondary structure do not affect the values of the functional properties of PCs.

No differences were found in the assimilation of carbohydrates by the yeast *S. cerevisiae* 121 and the micromycete *G. candidum* 977 from different types of serum. From carbohydrates and proteins with MM from 10 to 25 kDa, on the second day of growth, a consortium of microorganisms is formed with a mass fraction of protein of 50.60–53.56% on DM in biomass together with the cultural liquid and 61.68–64.10 in one biomass. Differences were found in feed PC from pea and chickpea serum biomass in the mass fraction of fat, soluble and insoluble carbohydrates. The amino acid score of all essential amino acids in concentrates from both types of serum is above 100%. The amount of unsaturated fatty acids in pea FMPC-1 is higher than that in chickpea FMPC-1 (the ratio of saturated: unsaturated fatty acids is 1:3 and 1:2.6), but chickpea FMPC-1 contained 21.5% more omega-6 fatty acids (linoleic) and more potassium, magnesium, cobalt, manganese, sodium. For all types of concentrates, a high correlation was found between the mass fraction of flavonoids in raw materials, concentrates and the color of dry preparations. The correlation coefficient R between the optical density of aqueous solutions measured at D_{590} nm and the mass fraction of flavonoids (in mg/g of protein) is 0.895. PCs of both types are recommended to be used for food purposes, FMPCs should be in animal feed, taking into account the characteristics of their chemical composition and quality indicators.

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