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Enrichment of the grains from rye wort after shock-activator-disintegrating processing

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Abstract. In this study**,** the mode of obtaining grains (pellets) from wort prepared from rye processed by the shock-activator-disintegrator (SAD), has been developed. Additionally, the enrichment of the grains by proteins using the strain of yeast producing proteins was carried out. For cultivation of a pure culture of a strain of yeasts producing proteins, grains with a concentration of 43.00 g 100 g^{-1} reducing substances and 62.71 mg g^{-1} of the total amount of amino acid were used. Different concentrations (10%, 20%, 30% and 40% by weight of the grains) of filtrate 24.90% dry matter, 21.00 g 100 g^{-1} reducing substances and 10.82 mg g^{-1} of the total amount of free amino acid was added to the grains. As nitrogen and phosphorus-containing mineral feed, diammonium phosphate was added to the nutrient medium. To obtain a pure culture of *Candida tropicalis, SK-4* yeast strain was isolated. The content of crude protein and the concentration of amino acids were determined. The morphological state of the cells was assessed. The results of this study show that to prepare the nutrient medium for a pure culture of a strain of protein microorganisms-producers and its cultivation, it is necessary to add 30% of the filtrate to the grains, while the proportion of crude protein in the protein-containing additive reaches more than 40%. The resulting protein-enriched product has a balanced amino acid composition, which is of interest as a protein-containing feed additive and its fine particle size distribution allows its use for feeding farm animals.

Key words: shock-activator-disintegrator (SAD), rye filtrate and grains (pellets), proteolytic enzyme complex, *Candida tropicalis*, protein-enriched product.

INTRODUCTION

Shock-disintegrator-activator processing of grain is a promising way of preparing grain raw materials in the process of obtaining the wort. This type of processing allows to carry out deep destruction of starch and to activate own enzymes of grain crops (Alimova, 2018).

By Electron microscopy, it has been shown that during processing there is a deep destruction of the endosperm of the grain. The starchy grains of the endosperm acquire oval-rounded and eye forms, their sizes varying from 25 to 40 microns.

After SDA-processing, protein matrices have a more developed surface. The intermediate protein is separated much easier, freeing more starch granules and partially leaving starch grains in the protein matrix. Moreover, the intermediate protein of the vitreous endosperm is destroyed during grinding together with starch grains strongly attached to it.

According to the electropherograms presented by Sabirov et al. (2018), the flour obtained by grinding on a disintegrator is rich in water-soluble albumin fraction of 17–28 kDa. Herewith, SAD-processing of the grains does not lead to a decrease in the total content of amino acids, the biological value of flour.

From the flour, obtained by processing the grains on the SDA-equipment, the wort is obtained. In order to further enrich the wort and increase its biological value, it is advisable to add proteolytic enzymes (Sabirov et al., 2017b).

Grains or pellets are the residual products obtained in the production of wort. The composition of the grains (pellets) is dominated by cellulose, hemicellulose and indigestible protein. It is the source of nutrients, such as carbohydrates and minerals (Kuznetsov & Ruchai, 2010). However, due to the fact that during the preparation of the wort from the SDA-processed grain, the most complete dissolution of the dry substances of the raw material is achieved. Thus, the energy and biological value of the solid fraction, the pellets, decrease. For this reason, the enrichment of these pellets with nutrients is important. One of such methods is cultivation, on the basis of grains (pellets), of microorganism's strains that produce protein.

The production of feed protein is currently widely used by the yeast of the main *Saccharomycetaceae* family of the genus Candida. They are able to grow on a variety of substrates and give a high biomass yield. Yeast strains, Candida, are commercially introduced, providing a high biomass yield (Azoulay et al., 1980).

Candida is facultative anaerobes. With aeration, the yeast oxidizes sugar in the nutrient medium to water and carbon dioxide (aerobic respiration). The released heat energy is used by yeast for the synthesis of cellular matter and metabolic processes. Under aerobic conditions, much more biomass accumulates in the substrate than during anaerobic respiration. Therefore, cultivation is recommended for continuous aeration of the medium (James et al., 2003; Hosiyev & Plieva, 2014).

The main nutrients for microbial cells are sugars, as a source of energy, and compounds of such macro-elements as nitrogen and phosphorus, which are part of proteins.

Nitrogen and phosphorus can be added to the nutrient medium in the form of mineral additives, for example, diammonium phosphate. As a source of sugar in the nutrient medium, the wort filtrate can be added. It is noteworthy that the wort prepared from SDA-processed grains, with the addition of proteases, will also contain amino acids and low molecular weight peptides that can be absorbed by the cells of inoculated microorganisms.

For cultivation of a pure culture of a strain of a microorganism of a protein producer, for the purpose of preparing a seed material, a grain mash filtrate diluted with water with a mass fraction of dry substances not more than 10% can be used. It is known (Plieva et al., 2015) that enzymatic hydrolysis of a protein contributes to the dissolution of grain components, enriching the wort with biogenic nitrogen: soluble peptides and free amino acids. This allows intensifying the growth of cells of the strain of

microorganisms cultured on a nutrient substrate from the filtrate and wort grains, by introducing a dose of proteolytic enzymes during its preparation.

Thus, it is possible to create an integrated technology for the processing of grains, including cereals with a high content of non-starch polysaccharides. To obtain grains and protein product, the cultivation of strains of microorganisms producing protein on the basis of grains must be carried out. Those grains can be used to create new functional foods or to intensify fermentation in the alcohol industry (Alimova et al., 2014). It is recommended to add the enriched protein product into the diet of farm animals.

Therefore, the aim of this work is to investigate ways of enrichment of grains obtained after SDA-processing of rye with proteolytic enzymes and protein strain *Candida tropicalis.*

MATERIALS AND METHODS

The object of the study was the rye first-class crop harvested in 2018. The used rye contains 8% moisture content, 53% starch content and trash impurities up to 1%.

The moisture content was determined by using Shimadzu MOC-120H moisture analyser (Sabirov et al., 2017a).

The starch content of the barley was determined by using Polarimeter (PolAAFF55). The determination of the starch content of barley was conducted according to ISO/TC 93- Ewers polarimetric method (ISO/TC 93, 1997).

The rye grains were milled using a DESI-15 shock-activator-disintegrator with a five-row rotor (Disintegrator, Estonia). Then, the milled flour was investigated using a Malvern Mastersizer 2000 laser particle size analyser (Malvern Panalytical Ltd, UK). The average integral particle size of flour was 158.1 μm.

Preparation of rye wort

Milled flour (375 g) was measured and transferred into hand-made mash tuns filled with 1,125 mL of warm water (45 °C). The mixture flour and water was then placed in a water bath a 'LOIP LB-163' (Russia) equipped with temperature regulators and a heating system with the constant stirring. Enzyme preparation was then done by adding an enzyme preparation of the thermostable α-amylase 'AmiloLux-ATS' (0.2 units g^{-1} of starch) for the partial hydrolysis of starch. The hydrolysis of starch was carried out for 1 hour at 60 °C.

After that, an enzyme preparation containing glucoamylase 'GlucoLux-A' enzymes (0.9 units g^{-1} of starch) was added for saccharification. The process of saccharification was carried out for 1.5 hours at 60 °C.

Further, the temperature was decreased to 55 °C, and 'Protosubtilin GZx A-120' enzymes (0.5 units g^{-1} of raw material) were added. The mixture was heated again for 1 hour at 55 °C.

Finally, the enzyme preparation of the acid protease 'Pro100L' (0.3 units g^{-1} of raw material) was added, and the proteolysis process was carried out at 55 °C for 1 h. 'Sibbiopharm Ltd' (Berdsk, Russia) manufactured all used enzymes.

The wort was centrifuged for 60 min at 4,600 rpm. The filtrate was separated from a solid fraction (pellets or grains). The concentration of dry matter (%) of the filtrate was measured similarly to that of Nsengumuremyi et al. (2019). The concentration of dry

matter of filtrate was 24.9%. The solid fraction (grains) was dried at 60 °C to 10% moisture content.

Medium preparation and growth of starter culture

The medium used to grow starter culture was prepared by the same procedure as the preparation of rye wort described above with modification. The same dose of amylolytic enzymes was added while different doses of fungal and bacterial proteolytic enzymes were applied: (a) 0.25 units PS (bacterial protease) g^{-1} of raw material + 0.15 units PS (fungal protease) g^{-1} of raw materials; (b) 0.5 units PS (bacterial protease) g^{-1} of raw material + 0.30 units PS (fungal protease) g^{-1} of raw materials; (c) 1 unit PS (bacterial protease) g^{-1} of raw material + 0.6 units PS (fungal protease) g^{-1} of raw materials. The hydrolyzate was centrifuged for 60 min at 4,600 rpm. The liquid fraction (filtrate) was separated from the solid fraction (grains). The filtrate was diluted to 6 ± 0.1% of dry matter and was used in the preparation of nutrient medium.

As an additional source of mineral elements, diammonium phosphate with a calculation of 0.4% nitrogen and 0.06% per 1 g of the grain was added to the nutrient medium.

The prepared nutrient medium was sterilized at 1 atm., 121 °C for 30 min. The medium was cooled down to 35 °C.

The inoculation of strains of *Candida tropicalis* was carried out in sterile conditions in the laminar cabinet. The growth of strains of *Candida tropicalis* was carried out under aerobic conditions in incubator-shaker (ES-20) at 35 °C at 220 rpm.

Enrichment of biomass with proteins

4 samples were prepared for cultivation. 4 conical flasks were prepared and in each conical flask, 50 g of dried rye solid sediment (grains) were dissolved in 450 mL of water. Then, 1.5 g of carbamide (urea) was added to each sample and acidified with orthophosphoric acid to pH 5.5. As a source of carbohydrate for yeasts, different concentrations of the filtrate were added to every sample. In the1st sample, 10% (5 mL of filtrate) was added; 20% (10 mL of filtrate) was added to the 2nd sample; 30% (15 mL of filtrate) was added to the 3rd sample and 40% (20 mL of filtrate) was added to the 4th sample. All samples were sterilized in an autoclave (Tuttnauer 2540MK, USA) at 1 atm. for 30 min. The pH of all samples was adjusted to 6 ± 0.1 by adding a 10% solution of orthophosphoric acid. A strain of the yeast *Candida* CK-4, derived by ecological selection, was taken as the protein-producing microorganisms. Cultivation was carried out under conditions of continuous aeration of the medium with an air flow rate of 100 m^3 h^{-1} per medium volume of 1 m^3. Aeration was performed using a Rocker 420 laboratory compressor. After 2 days, the biomass was centrifuged. The supernatant was discarded and the precipitate was dried to 9 ± 0.3% moisture content.

In the process of cultivation, the control was carried out by counting the cells of the microorganism strain.

Determination of parameters

Alpha-amino nitrogen was determined by the Ninhydrin colourimetric method (Lie, 1973).

The mass fraction of glucose and maltose in the filtrate were determined after the method of oxidation of the aldose group of sugars with iodine (Barakova & Tishin, 2010).

The content of crude protein was determined by the Kjeldahl method on an automated Vadopest installation. The installation includes a burning unit, a distiller and a titrator. The absolute error of the device is not more than 0.5% (Ibatullin et al., 2015).

The haemocytometer was used to count cells of yeasts. The total; budding and dead cell count were determined (Thomson et al., 2015).

The concentration of amino acids was determined using the 'KNAUER' amino acid analyzer: the calculation of aminogram was carried out by comparing the areas of the standard and the sample (Kondratenko et al., 2015).

Data analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made in at least triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

For the growth of microbial culture cells, carbohydrates are used as an energy source. Therefore, the nitrogen-containing substances are needed. In the process of preparing the wort from milled flour, proteins are hydrolysed into peptides and free amino acids. Depending on the complex of proteolytic enzymes activity and applied dose, it is possible to produce wort with various degrees of protein hydrolysis into low molecular weight compounds, which can be estimated by the content of α-amino nitrogen. According to the study of Amelyakina et al. (2012), the data show that the most complete hydrolysis of proteins can be achieved by the combined use of bacterial and fungal proteases. Therefore, it is important to determine the dose of this proteolytic complex that can be added in the preparation of wort. The latter is used as the basis of the nutrient medium for the growth of microorganisms, which will contribute to the maximum growth of cells. Therefore, three samples of filtrate were prepared using different doses of fungal and bacterial proteolytic enzymes.

The concentration of α-amino nitrogen, glucose content, maltose content and the osmolality of filtrate were recorded in Table 1. This filtrate has been used in the preparation of the liquid nutrient medium, in which the protein-producing yeasts were grown.

Table 1. Qualitative parameters of the filtrate used in the preparation of the liquid nutrient medium

Samples	α-amino nitrogen, mg L^{-1}	Glucose content g 100 mL^{-1}	Maltose content G 100 mL^{-1}	Osmolality, mmol L^{-1}
Sample A*	402.7 ± 0.3	10.87 ± 0.06	20.66 ± 0.07	1,104.66 ± 0.06
Sample B**	441.7 ± 0.2	11.03 ± 0.04	20.95 ± 0.05	1,172.66 ± 0.02
Sample C***	503.9 ± 0.2	11.06 ± 0.03	21.02 ± 0.06	1,265.33 ± 0.04

*Bacterial protease (0.25 unit g^{-1} of raw material) + fungal protease (0.15 unit g^{-1} of raw materials); **Bacterial protease (0.50 unit g^{-1} of raw material) + fungal protease (0.30 unit g^{-1} of raw materials); ***Bacterial protease (1 unit g^{-1} of raw material) + fungal protease (0.60 unit g^{-1} of raw materials).

Statistically at the 0.05 significance level, the concentrations of α-amino nitrogen are significantly different. By increasing the dose of bacterial and fungal protease, the concentration of α-amino nitrogen increases. On the other side, the increment of bacterial of fungal protease has not affect the concentration of glucose and maltose. Statistically, at the 0.05 significance level, glucose or maltose contents of 3 samples are NOT significantly different. Regarding the osmolarity, stastically the means are NOT significantly different at 0.05 level.

At the next stage, the filtrate was used to prepare the nutrient medium for a pure culture of *Candida tropicalis*. The concentration of dry matter of filtrate was 24.90 ± 0.02%. Therefore, to dilute pure cultures of microorganism strains of protein producers, the filtrate was diluted with water to a 6 ± 0.1% of dry matter. Data on the growth of microbial cells after 24 hours are presented in Fig. 1.

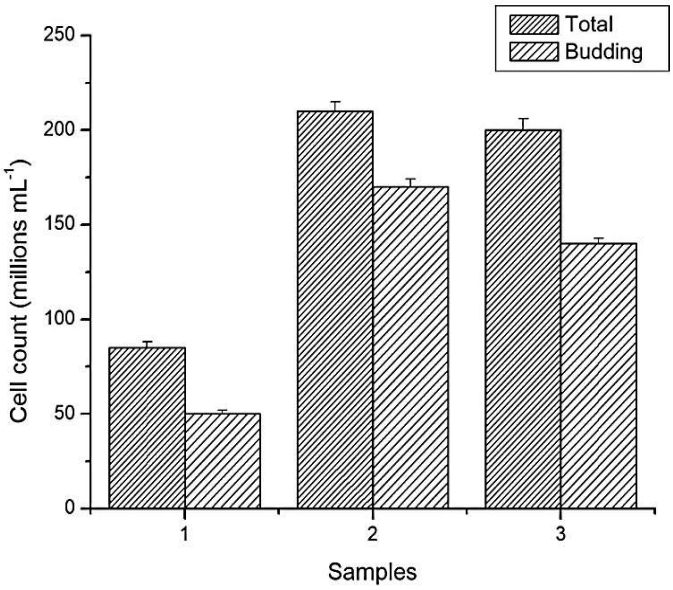

Figure 1. The growth of cells of microorganism-producing protein strains in the process of cultivation of pure culture after 24 hours (millions mL^{-1}).

The figure above represents how the cells of yeasts strains that produce protein have grown in a nutrient medium. The media are based on the filtrate of wort obtained by adding different doses of bacterial and fungal proteases. Sample 1; 2 and 3 represent cell count of protein-producing strains on a nutrient medium based on the filtrate of wort with a dose of Bacterial protease (0.25 unit g^{-1} of raw material) + fungal protease (0.15 unit g^{-1} of raw materials); bacterial protease (0.50 unit g^{-1} of raw material) + fungal protease (0.30 unit g^{-1} of raw materials) and bacterial protease (1 unit g^{-1} of raw material) + fungal protease (0.60 unit g^{-1} of raw materials) respectively.

In all three samples of the pure culture of strains of microorganisms producing protein, dead cells were not detected. The most active cell growth is observed in samples 2 and 3, in which the same indicator is higher than 2–3 times compared to sample 1. However, to obtain a sample of nutrient medium 2, a smaller dose of protease enzymes was applied compared to sample 3.

Therefore, from the point of view of decreasing the cost of ancillary materials and the provision of the most active cell growth, it is important to prepare wort, as a nutrient medium for isolation and cultivation of a culture of microorganisms, with a dose of bacterial protease 0.50 unit g^{-1} of raw material and fungal protease 0.30 activity unit g^{-1} of raw material.

For the cultivation of microorganisms, such basic groups of substances as carbohydrates and available nitrogen-containing substances are needed. Based on this, an analysis of the carbohydrate composition and free amino acids in the filtrate and the grains was carried out. The data are presented in Tables 2 and 3, % of absolutely dry substance.

Statistically (at 0.05 significance), the concentration of reducing substances of filtrate and grains are significantly different.

From the data presented in Tables 2 and 3, a significant part of sugars and amino acids that were contained in the raw material was transferred to the filtrate, thereby reducing their concentration in the grains. Therefore, in the preparation of a nutrient medium for the cultivation of a strain of a microorganism producing protein, a partial addition of the filtrate to the mass of the grains (pellet) is required. In order to establish the quantitative fraction of return of the filtrate to the mass of the pellet (grains), nutrient media were prepared with the addition of different concentrations of the filtrate to the grains, exactly 10%, 20%, 30% and 40% by weight. In the obtained samples of nutrient media was carried out the cultivation of a strain of the microorganism. The results of the experiments are presented in Figs 2–5.

Table 2. The carbohydrate composition of the filtrate and grains of rye wort (% of absolutely dry substance)

Sample	Reducing substances, %	Total reducing substances, %
The filtrate of rye wort	84.41 ± 1.10	-
Grains of rye wort	4.78 ± 0.01	6.07 ± 0.01

Table 3. The content of free amino acids in the filtrate and grains of rye wort, % of absolutely dry substance

Name of amino acid	Free amino acid content (%)	
	Grains (Pellet)	Filtrate
Aspartic acid	0.0390	0.1365
Serine	0.0449	0.1486
Threonine	0.1807	0.6466
Glutamic acid	0.0770	0.3332
Proline	0.1496	0.6466
Glycine	0.0196	0.0643
Alanine	0.0587	0.1526
Valine	0.0414	0.1526
Methionine	0.0219	0.0763
Isoleucine	0.0426	0.1446
Leucine	0.1001	0.3334
Tyrosine	0.0311	0.1205
Phenylalanine	0.0702	0.2369
Histidine	0.0679	0.2249
Lysine	0.0564	0.1647
Tryptophan	0.2048	0.4900
Arginine	0.0875	0.2431
Total amount of free (unbound) amino acids	1.2934	4.3454

According to the data presented in Figs 2 to 5, the most active cell growth was observed in the first 24 hours, and the maximum of budding cell count was reached. The greatest cell count is at 24 hours of cultivation,after which comes the stage of attenuation of the vital activity of microorganisms. The total duration of cultivation was 48 hours.

Thus, by cultivating the strain of the microorganism of the protein producer on the nutrient medium, dry samples of the protein product were obtained from grains and filtrate of the wort. To determine the nutritional value of the samples, the crude protein content was determined. The results are shown in Fig. 6.

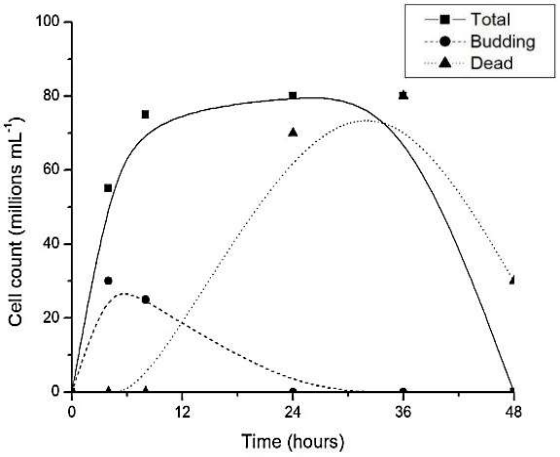

Figure 2. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in the amount of 10% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.

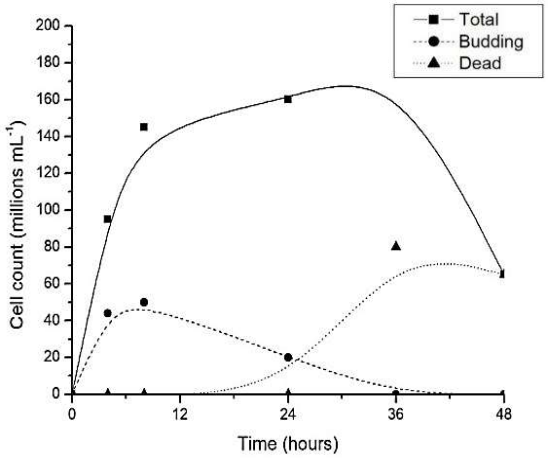

Figure 3. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in an amount of 20% by weight of the used solid fraction; cell growth, million mL^{-1}.

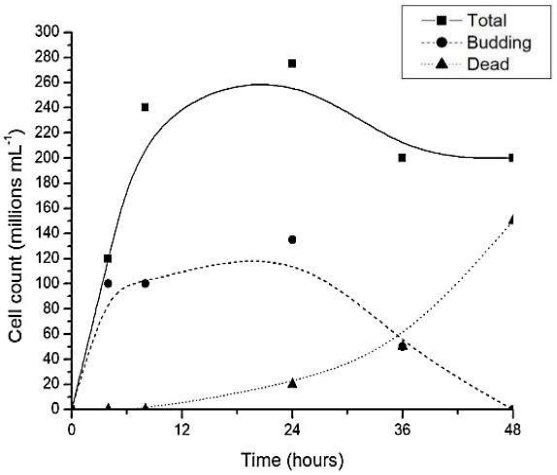

Figure 4. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in an amount of 30% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.

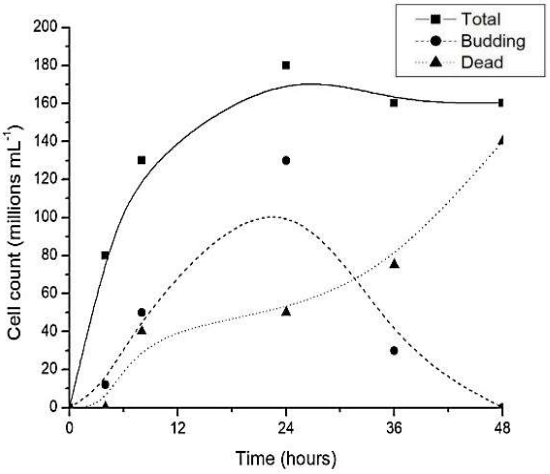

Figure 5. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in the amount of 40% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.

Where 1 is a sample of grains; 2 – a sample of the protein product, obtained in a nutrient medium with a 10% addition of rye wort filtrate by weight of the solid fraction, % in terms of a.d.s.; 3 – a sample of the protein product obtained in a nutrient medium with a 20% addition of rye wort filtrate from the mass of the solid fraction,% in terms of a.d.s.; 4 – sample of a protein product obtained in a nutrient medium with 30% addition of rye wort filtrate by weight of the solid fraction,% in terms of a.d.s.; 5 – a sample of

the protein product obtained in a nutrient medium with 40% of the filtrate of rye wort from the mass of the solid fraction, % of absolutely dry substance.

As shown in Fig. 6, there is an increase in the content of crude protein in samples 2, 3 and 4 with an increase in the amount of nutrient media in the wort filtrate. The maximum content of crude protein is in sample 4, where this parameter is above 40% of a.d.s. Thus, under these cultivation conditions, the greatest accumulation of crude protein can be achieved with a 30% addition of the filtrate to the culture medium.

The biological value of both food and feed products is assessed by their amino acid composition, exactly, the content of essential amino acids. In order to establish the nutritional value of the obtained protein product, the amino acid composition of sample 4 was examined. The results of the analyses are presented in Table 4.

According to the data presented in Table 4, the protein product has a complete amino acid composition. The total content of essential amino acids in the protein product, which includes valine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and phenylalanine, is more than three times higher than that of the grains (pellet). And the content of lysine, which is considered the main limiting amino acid in the diet of pigs, is higher in the protein product more than 3 times than in the grains. The resulting product can be recommended as a protein

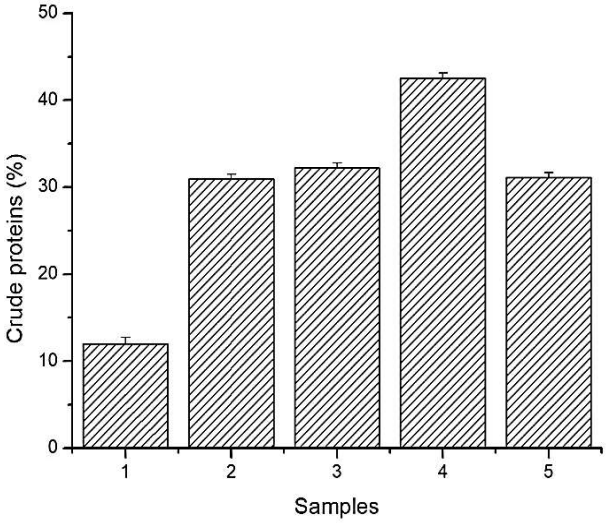

Figure 6. The content of crude protein in samples of the protein product, % in terms of absolutely dry substance, *a.d.s (absolutely dry substance).

Table 4. The amino acid composition of grain mash and protein product grains obtained on a nutrient medium with 30% addition of rye wort filtrate by weight of the solid fraction, mg g^{-1}

The name of the amino acid	The total content of amino acids, mg g^{-1}	
	Protein product	Grains (pellet)
Aspartic acid	15.79	4.99
Serine	10.26	3.37
Threonine	8.48	2.51
Glutamic acid	26.12	11.87
Proline	13.21	6.42
Glycine	10.20	3.61
Alanine	11.16	3.71
Valine	8.11	2.80
Methionine	1.74	0.61
Isoleucine	7.61	2.01
Leucine	13.00	4.39
Tyrosine	3.37	0.84
Phenylalanine	8.09	2.97
Histidine	4.75	1.90
Lysine	7.66	2.28
Tryptophan	15.12	5.71
Arginine	8.96	2.72
The total content of essential amino acids	69.81	23.28
Total quantities	173.63	62.71

supplement in the diet of farm animals. A fine particle size distribution of such a feed additive may be promising in the poultry industry (Rimareva et al., 2001).

CONCLUSIONS

As a result of the experiments, it was found that for the enrichment of the protein components of the grain obtained from the wort prepared from rye processed at the SDA-installation, it is necessary to add an acidic and neutral protease in the process of preparing the wort. It is also advisable to carry out the enrichment of the grain protein yeast strain Candida.

The results of cultivation of Candida yeast strain on this nutrient medium makes it promising to search for new microorganisms of protein producers with high biomass yield and non-pathogenic for animals and humans.

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