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Research

MODERNIZATION OF THE EXPERIMENTAL TECHNOLOGICAL LINE FOR THE PRODUCTION OF ENZYME HYDROLYSATES OF DOWN AND FEATHER RAW MATERIAL

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ABSTRACT

At this stage of the work, a pilot line was prepared for the enzymatic splitting of down and feather primary products including a pre-treatment system for removing impurity inclusions from down and feather raw material, a press for pressing the feather, a transporter of down and feather raw material, a preliminary sterilization unit for down and feather raw material, and an experimental fermentative reactor. In order to optimize the process, the main parameters of the enzymatic splitting of down and feather raw material are selected in the pilot line: irrigation module-8; duration of fermentation-2 hours; the dose of protease enzymatic agent No. 6230/2256-15 unit/g of raw material; the dose of Protolad B enzymatic agent-30 unit/g of raw material. The testing of various variants of the equipment arrangement in the technological line was carried out, as a result of which three preproduction lots of enzymatic hydrolysates with a protein content of 87.5% to 97.6% were obtained. It is determined that all three tested equipment layouts in the pilot line lead to the production of enzymatic hydrolysates from down and feather raw material that meet the requirements of the current regulatory documentation according to physical, chemical, microbiological and safety indicators.

INTRODUCTION

In the Russian Federation, with the development of the agricultural sector, the volume of organic waste increases significantly, the utilization of which is an important problem (Prosekov, *et al*., 2016; Toldrá, *et al*., 2016): about 25 million tons of meat and poultry processing waste is produced annually in the food and processing industry, as well as grain distillery stillage, brewers' draff and brewers' yeast, and other types of secondary raw materials. This is due to the presence of a great number of large agricultural enterprises engaged in both livestock breeding and plant growing (Gulyayeva, *et al*., 2016; Arunkumar, *et al*., 2013).

Nowadays, there is no generally accepted way of recycling such wastes in the Russian Federation. Most of the waste is thrown into nearby areas or exported to fields (Arunkumar, *et al*., 2013). At the same time, the waste of the agricultural sector is an easily renewable cheap and affordable source of raw materials for new high-quality and nutritious feeds, which acquire high nutritional and fodder properties when they are properly processed (Babich, *et al*., 2011; Cao, *et al*., 2011).

The problem of waste utilization is of particular importance for the poultry processing industry (Lasekan, *et al*., 2013). There are about 450 million birds in all categories of farms in the Russian Federation. The poultry meat production in Russia is constantly growing due to the increased demand for poultry meat, major investments in the creation

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of new poultry farms and the reduction in imports (Nikolaev, *et al*., 2008; Endo, *et al*., 2010). The share of poultry meat in total meat production has reached 42% against 18% in 1990 which corresponds to the world trends. The leader in the production of meat from various species of birds is chicken meat-more than 97% of production (Ibatova and Motavina, 2014).

With the increase in poultry meat production, the volume of waste from poultry farms is significantly increased. Special difficulties arise in the processing of feather waste which is 7.5% of the live weight of the processed raw materials (Podsoblyaeva, *et al*., 1981) and 30% of the weight of all wastes (Belova, 2009). Such raw materials are distinguished by a high content of keratin protein (Gupta, *et al*., 2013; Prosekov, *et al*., 2016) which determines the value of this product (Eslahi, *et al*., 2014). However, the technologies used for processing keratin-containing raw materials for feed purposes do not always allow obtaining high-quality products (Ibatova and Motavina, 2014; Shevkunov, *et al*., 1980).

Depending on the type of animal raw material, high-grade protein may be 15% to 20% by weight of the potentially recovered protein waste. However, at the current level, the degree of extraction of protein from animal raw materials does not exceed 50% on the average (Yatsyshin, *et al*., 1970). In addition, the existing methods for extracting animal protein from meat and poultry processing waste, namely, severe temperature or acid treatment, do not allow the extraction of the most labile amino acids-methionine and tryptophan (Lasekan, *et al*., 2013; Shevkunov, *et al*., 1980).

The most promising way to extract protein from animal waste is bio-conversion under the influence of microorganisms (Brandelli, 2008). The use of bio-conversion makes it possible to cheapen and speed up the process of manufacturing the finished products, as well as to reduce the costs for protection of soil, water basin and air from pollution (Babich, *et al*., 2011; Ibatova and Motavina, 2014).

The known technologies for bio-conversion of down and feather raw material are characterized by a number of significant drawbacks: low degree of bio-conversion; low yield of protein; labour intensity and the process comprising multiple stages, etc. (Gulyayeva, *et al*., 2016; Arunkumar, *et al*., 2013). In this connection, there was a need to improve bio-conversion technologies, to study more deeply the fundamental aspects of the processes based on the bio-catalytic transformation of keratin-containing waste components, and using new knowledge to develop new technological solutions (Cao, *et al*., 2011; Prosekov, *et al*., 2016).

The purpose of this paper is to choose various variants of the layout of the line equipment to optimize the process of obtaining the enzymatic hydrolysis of down and feather raw material.

MATERIALS AND TECHNIQUES

The object of the research was

Down and feather raw material from different breeds of chicken (ROSS-708, Hisex White and Cross Smena) grown in the territory of OOO Kuzbass Broiler (Kemerovo Region, Russia);

Multi-enzyme composition based on multi-enzyme agents such as Protease No. 2630/2256, Protolad B in the ratio 1:2.

When analyzing the obtained enzymatic hydrolysates of down and feather raw material with the multi-enzyme composition, the following methods were used:

Weight fraction of ash, insoluble in hydrochloric acid, was determined in accordance with GOST 13496.14-87 (Feed compound, all-mash primary produce, feed. Method for determining ash that is insoluble in hydrochloric acid).

Weight fraction of fat was determined in accordance with GOST 32905-2014 (Feed, feed compound, all-mash primary produce. Method for determining the content of raw fat).

Weight fraction of crude protein was determined by ashing technique with sulfuric acid in the presence of a catalyst, followed by alkalization of the reaction product, stripping and titration of the released ammonia according to GOST 32044.1-2012 (Feed, feed compound, all-mash primary produce. Determination of the weight fraction of nitrogen and calculation of the weight fraction of crude protein).

Weight fraction of the total protein was determined by the Dumas method using the RAPID N Cube aluminous nitrogen analyzer.

Weight fraction of moisture was determined according to GOST 17681-82 (Flour of animal origin. Test methods).

The number of mesophyll aerobic and optional-anaerobic microorganisms (QMA&OAMO) was determined in accordance with GOST 10444.15-94 (Food products. Methods for determining the

amount of mesophyll aerobic and optional-anaerobic microorganisms), GOST 25311-82 (Feed flour of animal origin. Methods of bacteriological analysis).

The number of bacteria of the group of intestinal rods (Coliform bacteria) was determined in accordance with GOST 30518-97 (Food products. Methods for detecting and determining the number of bacteria of the Coliform group (Coliform bacteria), GOST 26670-91 (Food products. Methods of cultivating micro-organisms).

The presence of pathogenic and toxin-forming microorganisms was determined in accordance with GOST 25311-82 (Feed flour of animal origin. Methods of bacteriological analysis); the presence of salmonella was determined in accordance with GOST 30519-97 (Food products. Method for the detection of bacteria of *Salmonella* group).

The presence of bacteria of *Proteus* group was determined according to GOST 28560-90 (Food products. The method for the detection of bacteria of *Proteus*, *Morganella*, *Providencia* groups).

The content of arsenic was determined in accordance with GOST 26930-86 (Raw materials and food products. Method for the determination of arsenic).

Toxic elements (lead, cadmium) were determined according to GOST 30178-96 (Raw materials and food products. Atomic absorption method for detection of toxic elements).

The mercury content was determined in accordance with GOST R 53183-2008 (Food products. Determination of trace elements. Determination of mercury by the method of atomic absorption spectrometry of cold vapor with preliminary mineralization of the sample under pressure).

The content of zinc was determined in accordance with GOST 26934-86 (Raw materials and food products. Method for the determination of zinc).

The copper content was determined by the polarographic method in accordance with GOST 26931-86 (Raw materials and food products. Methods for determining copper).

The content of strontium-90 was determined in accordance with GOST 32163-2013 (Food products. Method for determination of strontium Sr-90 content).

The content of cesium-137 was determined in accordance with GOST 32161-2013 (Food products.

Method for determination of cesium content Cs-137).

RESULTS

The technological scheme of the enzymatic hydrolysis of down and feather raw material in instrumentation is shown in Fig. 1.

The pilot line for the enzymatic hydrolysis of down and feather raw material with the productivity of 300 kg/h to 500 kg/h makes it possible to obtain a high-protein feed supplement with the improved biological value and digestibility.

The main element of the line is a universal twin-screw fermentative reactor which allows grinding the feather raw material in flux, as well as completing sterilization of the mass and hydrolysis of the protein (keratin) contained in the raw material.

Several working sections are allocated in the production line.

Section 1-installation for dehydration of down and feather raw material (preparation of down and feather raw material) includes the press for releasing moisture, the turner of down and feather raw material and the conveyor.

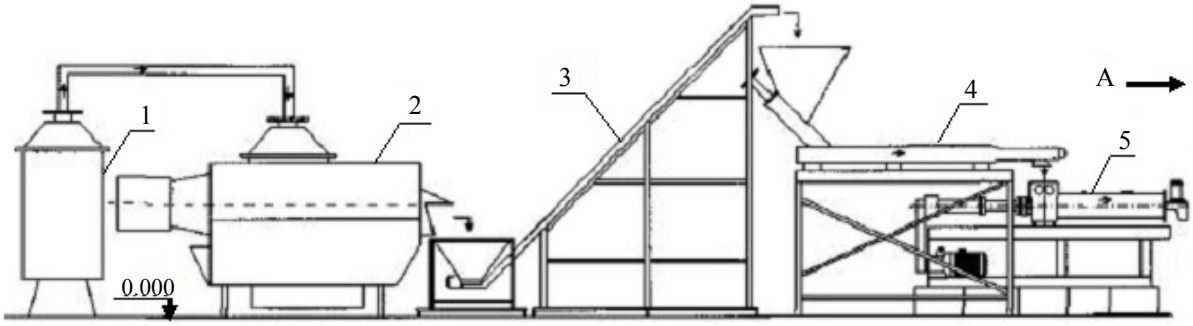

Fig. 1 Technological scheme of the enzymatic hydrolysis of down and feather raw material in instrumentation: 1-preliminary purification system of down and feather raw material from impurity inclusions, 2-press for pressing feather, 3-conveyor of down and feather raw material, 4-preliminary sterilization unit of down and feather raw material, 5-fermentative reactor

Tremesa press for releasing moisture is intended for water-feather pumpable slurry from the killing line and water removal up to 50% to 55% of humidity of feather. Humidity of down and feather raw material is controlled by the air pressure in the air cylinder.

After the excess moisture is squeezed out, the raw materials are transferred to the turner. It breaks lumps which can form in the press for releasing moisture. Then, the distended down and feather raw material is conveyed to the sterilizer bin.

Section P includes the conveyor, the pre-sterilization unit for the first down and feather raw material (sterilizer), the universal fermentative reactor, and the heating system (the thermal oil system).

The sterilizer is designed for preheating and steaming of down and feather raw material while maintaining its moisture content within 50%. The temperature of vapor at the exit from the sterilizer can vary between 80°C to 120°C. The preliminary heating of down and feather raw material essentially changes its physical and mechanical properties-it reduces the strength. In addition, the preheated raw material in the sterilizer is heated more quickly in the fermentative reactor to the desired temperature and the time of the presence of raw material in the reactor increases at a high temperature. Thus, a high-temperature hydrolysis of feather is more complete. The sterilizer in the process of work destroys and eliminates the pathogenic microflora providing the final product with a guaranteed high quality. The temperature of down and feather raw material is established by an automatic pilot valve of direct action. The valve automatically maintains the steam supply for heating the feather raw material at a predetermined temperature. The heated down and feather raw material from the sterilizer is sent to the fermentative reactor where the enzymatic hydrolysis and grinding of the raw material takes place in a continuous mode.

The fermentative reactor consists of two sections, they both rotate two screws. Each section has jackets for heating raw materials with heat-the supplied thermal oil. Heating of the thermal oil to the temperature of 300°C to 320°C is carried out in the thermal oil system. The pumping of the thermal oil is carried out by the pump.

The fermentative reactor works in conjunction with the heating system-TS-8 thermal oil system (thermal station).

The thermal station is designed for autonomous heat supply of production and process equipment. Therminol-72, Siltherm 800, Dowtherm A (G) high-temperature organic heat carriers with an operating temperature of at least 350 °C are used as heat carriers.

The thermal station is mounted on the frame where the four-section welded shell and the surge tank are fixed. There are blocks of tubular heating element in each section inside the shell; they are fixed through the copper gasket on the flanges of each section of the shell. One of the blocks usually located in the lower section is adjustable.

The pilot line for carrying out the enzymatic hydrolysis of down and feather raw material has a centralized control system. The managing software and hardware complex is assembled on the basis of a laptop.

The technological process of carrying out the enzymatic hydrolysis of down and feather raw material includes the following operations:

1. **Formation of a multi-enzyme composition that performs the enzymatic hydrolysis of down and feather raw material (Protease No. 2630/2256 and Protolad B in 1:2 ratio).**

Protolad B (protease B) enzymatic agent is an alkaline protease synthesized by *B. licheniformis*-60. The enzymatic agent is provided by All-Russian Research Institute of Nutrition and Biotechnology. Protease B has a nonspecific endopeptidase activity and a significant keratinase activity. *B. licheniformis*-60 VKM B-2366 D bacterial strain was secured using a multistage genetic selection with effective mutagenesis from *B. licheniformis*-78 BKM B-2184 D strain by irradiation with ultraviolet light and simultaneous action of strong chemical mutagens.

The enzymatic agent of protease No. 2630/2256 is a mixture of 1:1 Agroport (No. 2630) enzyme agent and protease B (No. 2256). Agroport (No. 2630) is an acidic protease produced by *Penicillium canescens* RN-11-7-7.

These enzymatic agents were developed by scientists of the Department of Chemical Enzymology of the Moscow State University named after Lomonosov, as well as the Institute of Biochemistry named after Bach.

2. **The enzymatic hydrolysis of pre-treated feather raw material with a multi-enzyme composition.**

Previously, it was found that the optimal parameters of the process of the enzymatic hydrolysis of down

and feather raw material in the pilot line are the following:

- Irrigation module (weight ratio of water and raw materials) 8;
- Duration of fermentation, h 2;
- The dose of Protease No. 6230/2256 enzymatic agent, unit/g of raw material 15;
- The dose of Protolad B enzymatic agent, unit/g of raw material 30.

To carry out the hydrolysis, water is added to the fermentative reactor in a volume of 3.0 m^3 in accordance with the set mode of the irrigation module, and preliminary grinded raw material is added. After loading, the temperature of the mixture of raw materials and water is risen up to 55°C. A solution of the multi-enzyme composition (Protease No. 2630/2256 and Protolade B) is added to the mixture heated to 55°C. The enzymatic hydrolysis is carried out at a temperature of 55°C while constant stirring at a speed of 24 rpm for 2 hours.

3. **Pasteurization of biomass**

After the end of the enzymatic hydrolysis, the temperature of the mass increases to 90°C to 95°C; the mass is kept at this temperature for 30 minutes and sent for further processing.

4. **Preliminary cleaning of biomass from the remains of raw materials**

The pasteurized mass is sent to the decanter for cleaning from "dense" inclusions, then the "liquid phase" is sent to the receiving container and then to the separator for de-fatting and further purification.

5. **Preparation of protein broth**

The protein semi-finished product is sent to the separator from the receiving container for de-fatting; the temperature of the protein semi-finished product sent to the separator is not less than 80°C.

The peculiarities of the modernization of experimental production line are its components: modern instruments and devices allowing to model different variants of the fractionation of biomass obtained after enzymatic treatment of down and feather raw material, to assess the depth and the parameters of the separation of biomass into liquid and solid phases, to provide quality degreasing and product clarification and prepare the final protein product (broth) for subsequent processing (sterilization, concentration and drying), as well as availability of manual and automatic control mode. It allows operators to vary the rate of flow, the speed of rotation of the screw in the decanter centrifuge, control the quality of phase separation and the degree of removal of impurities and fat in the separator.

The testing of various variants of the arrangement of equipment (refiner, decanter and separator) was carried out. They are intended for the modernization of the experimental technological line for obtaining enzymatic hydrolysates of down and feather raw material providing mechanical purification and degreasing of the primary broth. The most optimal equipment configuration was chosen that fully meets the requirements of the technological process.

The raw material for hydrolysates was the down and feather raw material which had undergone preliminary hydrothermal treatment.

To optimize the technological parameters of hydrothermal processing of down and feather raw material, a high-temperature laboratory device (AVTOL) was designed. The down and feather raw material comes to this device with a moisture content of 50% to 55%. Further, it is densified and heated in the blending and chopper device during continuous supply at a pressure of 0.4 MPa to 10 MPa and raw material temperature of 60°C to 120°C, followed by hydrothermal treatment of the raw material at a temperature of 190°C to 200°C for 90 seconds with a simultaneous thin grinding and abrasion. Then the processed raw material is taken out to the atmospheric pressure zone. The grinding process and the enzymatic hydrolysis of keratin are combined and carried out in a thin layer up to 20 millimeters.

The applied regimes of hydrothermal treatment of down and feather raw materials lead to complete decontamination of the protein concentrate ensuring the almost complete preservation of thermolabile amino acids.

The variant of the equipment arrangement No. 1

Fermenter → Pump → Refiner → Receiving tank → Pump → Separator → Pump → Receiving tank.

The fermented mass after pasteurization with a temperature of 80°C to 90°C from the fermenter is sent by the pump to the refiner-a flow-through filter of primary refining. There are 2 fractions-"solid" and "liquid" (broth + fat) at the exit from the flow-through filter of primary refining.

The filter screen was selected with a diameter of 0.5 mm which allowed the fermented mass to be filtered at all supply rates.

The change in the pitch of the blades has a significant

effect on the foaming process. It is determined that the smaller the angle of inclination of the blades the less the protein-fatty emulsion is formed. To test the technology of obtaining enzymatic hydrolysates, the angle of inclination of the blades was chosen equal to 30°, which made it possible to minimize the foaming process.

After the flow-through filter of primary refining, the "liquid" fraction is sent to the receiving tank, and then it is sent to the separator by the pump to separate the fat and clarify the broth.

The supply rate of the enzymatic mass to the separator was chosen to be 0.6 m^3/h, whereby the process of fat separation and partial clarification of the broth were observed.

The choice of the optimum washing time for the drum was determined as the time required for the timely removal of the sludge. In the current situation, the sludge (emulsion) from the drum space of the separator was removed every 5 minutes which was not technologically sufficient.

The variant of the equipment arrangement No. 2

Fermenter → Pump → Decanter → Receiving tank → Pump → Separator → Pump → Receiving tank.

The fermented mass after pasteurization at a temperature of 80°C to 90°C from the fermenter is pumped into the decanter-a horizontal centrifuge for separating the suspended particles. There are 2 fractions to be obtained-"solid" and "liquid" (broth + fat) on exit from the centrifuge.

The "liquid" fraction from the centrifuge is sent to the receiving tank, then to the separator to separate the fat and clarify the broth. Since there was no coarse mechanical cleaning with this arrangement of equipment, the accumulation of bone mass took place in the decanter centrifuge, as a result of which both its productivity and the productivity of the technological line were decreased as a whole.

The variant of the equipment arrangement No. 3

Fermenter → Pump → Refiner → Receiving tank → Pump → Decanter → Receiving tank → Pump → Separator → Pump → Receiving tank.

The fermented mass after pasteurization at a temperature of 80°C to 90°C from the fermenter pump is sent to the refiner. There are 2 fractions to be obtained-"solid" and "liquid" (broth + fat) on exit from the refiner. After the refiner, the "liquid" fraction is sent to the receiving container; then with the help of pump it is sent to a "decanter" centrifuge for further purification from solid particles.

There are 2 fractions to be obtained-"solid" and "liquid" (broth + fat) at the exit from the decanter.

After the decanter, the "liquid" fraction is sent to the receiving tank, and then pumped to a separator to separate the fat and clarify the broth.

Thus, using the pilot technological line, three preproduction lots of enzymatic hydrolysates of down and feather raw material (three variants of the equipment layout) were obtained, for which physicochemical quality indicators were analyzed, as well as chemical and microbiological safety indicators Table 1.

DISCUSSION OF RESULTS

The presented data show that all preproduction lots of enzymatic hydrolysates of down and feather raw material obtained as a result of different arrangements of the equipment of the processing line are distinguished by a high protein content (87.5% to 97.6% on dry basis), and low content of fat (not more than 1.7%) and ash (not more than 0.31%). Enzymatic hydrolysates of down and feather raw materials meet the requirements of the current regulatory documentation in accordance with chemical, microbiological and safety indicators.

CONCLUSION

Based on the above information, it is concluded that all three tested equipment configurations in the pilot line lead to the production of enzymatic hydrolysates from down and feather raw material that meet the requirements of the current regulatory documentation and can be used to develop a production line for the production of high-protein feed additives based on enzymatic hydrolysates of down and feather raw material. With the help of the line, it is possible to develop various modes for processing the down and feather raw material which make it possible to obtain the amino acid-balanced high-protein fodder supplement as the final product with a protein content of at least 95% and the proportion of physiologically available protein in the products-not less than 95%.

This line is unique and has no analogues in Russia and in the world, both in terms of its layout and functional purpose of the separate elements. The main components of the line are modern world-class instruments and devices that allow simulating and studying the process of dehydration and preparation of raw materials before supplying them to the

Table 1. Indicators of quality and safety of preproduction lots of enzymatic hydrolysates of down and feather raw material

Name of indicator	Indicator value		
	Preproduction lot No. 1	Preproduction lot No. 2	Preproduction lot No. 3
Moisture content, %	96.0 ± 9.6	95.8 ± 9.6	97.2 ± 9.7
Mass fraction of protein, %	3.5 ± 0.4	4.1 ± 0.4	2.6 ± 0.4
Relative protein content, % of solids content	87.5 ± 8.8	97.6 ± 9.8	92.8 ± 9.3
Mass fraction of ash, %	0.22 ± 0.02	0.31 ± 0.03	0.25 ± 0.03
Mass fraction of fat, %	1.5 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
QMA&OAMO, CFU in 1.0 g, not more than	$2.0 \cdot 10^3$	$1.5 \cdot 10^2$	$3.4 \cdot 10^1$
Product weight (g), CGB (coliforms) are not allowed	Not found	Not found	Not found
Mass of product (g), pathogenic microorganisms are not allowed incl. Salmonella	Not found	Not found	Not found
Mass of product (g), anaerobes (toxin-forming) are not allowed	Not found	Not found	Not found
Mass of product (g), *Proteus* is not allowed	Not found	Not found	Not found
Lead, mg/kg	0.12 ± 0.01	Less than 0.1	Not found
Cadmium, mg/kg	Less than 0.01	0.050 ± 0.005	0.020 ± 0.002
Arsenic, mg/kg	0.080 ± 0.008	Not found	Less than 0.1
Mercury, mg/kg	Not found	Not found	Not found
Copper, mg/kg	10.0 ± 0.1	8.5 ± 0.9	12.0 ± 1.2
Zinc, mg/kg	20.0 ± 2.0	25.0 ± 2.5	14.0 ± 1.4
90Sr, Bq/kg	Not found	Not found	Not found
137Cs, Bq/kg	15.0 ± 1.5	12.0 ± 1.2	10.0 ± 1.0

universal fermentative reactor.

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