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Optimization of Parameters of Enzymatic Hydrolysis of Feather-and-Down Raw Material in a Pilot Installation

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Abstract

As a result of the present work, modes of the feather-and-down raw material enzymatic hydrolysis in a pilot installation have been determined with the aim of producing high-protein feed additives with their quality indicators meeting the requirements to enzymatic hydrolysates: the degree of fineness of the feather-and-down raw material should be 1-2 mm; the concentration of sodium sulphite - 0.5%; pH - 7.2; temperature - 55°C; duty water curve - 8; duration of fermentation - 10 hours; the dose of the Protease No. 6230/2256 enzyme preparation – 15 U/g of raw material; the dosage of the Protolog B enzyme preparation – 30 U/g of raw material. It has been found that by the microbiological and chemical safety indicators, enzymatic hydrolysates of feather-and-down raw material obtained at the pilot installation in optimized modes of hydrolysis satisfy the requirements to enzymatic hydrolysates.

Keywords: feather-and-down raw material, enzymatic hydrolysis, pilot installation, process parameters, technological process, optimization.

INTRODUCTION

The problem of waste disposal in poultry-processing industry is especially urgent [1]. In the Russian Federation, in all categories of farms there are about 450 million birds. Production of poultry meat in Russia is growing due to the increasing demand for poultry meat, major investment into new poultry farms' construction, and the reduced import. Some regions that were not leaders in poultry production earlier are now becoming the largest producers (for example, the Belgorod region). The share of poultry in the total meat production has reached 42% against 18% in 1990, which is in line with global trends. The leader of meat production from various poultry species is chicken meat – more than 97% of production.

With the growth of poultry meat production, the amount of birds' evisceration wastes significantly increase as well [2]. Special difficulties arise in processing feather waste, which reaches 7.5% of the live weight of processed raw material. Such raw material features high content of protein named keratin, which determines the value of this product. However, the technologies used for reprocessing keratin-containing raw materials for obtaining fodder do not always allow obtaining high quality product.

Depending on the type of animal raw material, complete protein may reach 15-20% by weight of the potentially recoverable protein waste [3, 4]. However, at this level, the degree of protein extraction from animal raw material does not exceed 50%. Besides, the currently existing methods of extracting animal protein from the waste of meat and poultry processing industry, namely, hard thermal or acid treatment, do not allow extracting the most labile amino acids – methionine and tryptophan. Therefore, the currently used technologies allow obtaining biologically incomplete products of poor or unsatisfactory quality [5-8].

In the framework of the first and second phases of the project "Organization of high-tech production of high-protein feed supplements and biofertilizers based on the complex technology of reprocessing feather-and-down raw materials and other low-value wastes of the poultry-processing industry", the technology of feather-and-down raw material hydrolysis with a multienzyme composition (Protease No. 2630/2256 and Protolab B) has been optimized in a laboratory using the methods of full-fledged experiment with three varying parameters, including the dosage of enzyme preparation, the duration of fermentation, and the mode duty water curve; and the modes of enzymatic hydrolysis of feather-and-down raw materials in the pilot installation have been perfected.

This work is aimed at optimizing the parameters of enzymatic hydrolysis of feather-and-down raw materials in a pilot installation with the purpose of obtaining high-protein feed additives that correspond to enzymatic hydrolysates in terms of quality and safety.

MATERIALS AND METHODS

The object of the research was feather-and-down raw materials from poultry of species "ROSS-708", "Hisex white" and "Cross Smena" from poultry farm LLC Kuzbassky broiler (the Kemerovo region, Russia).

Proteolytic activity of the multienzyme complex was assessed according to the modified Anson's method. The modified Anson's method is based on determining the sodium caseinate enzymatic hydrolysis reaction rate with the studied enzyme preparation down to peptides and amino acids, with their subsequent determination by the colorimetric reaction with Folin's reactant [9, 10]. The unit of proteolytic activity is the enzyme's ability to transform in 1 minute's time at the temperature of 30° C sodium caseinate into unsettleable in trichloroacetic acid state in the amount corresponding to 1 μmole of tyrosine. The proteolytic activity is expressed by the number of specified units in 1 g of the test preparation.

The method of determining proteolytic activity of the multienzyme complex is as follows. In the first phase, the solution of the substrate was kept at the studied temperature. After that, a solution of preliminarily temperature-controlled multienzyme complex was added to the substrate; the test tubes were shaken and left at the studied temperature for 10 min. After 10 min, the enzymatic reaction was stopped, and protein and high-molecular products of hydrolysis were precipitated by adding trichloroacetic acid. After thermostating for 20 min, the reaction mixture was filtered, and sodium carbonate solution and Folin's reactant were added. As a result of the reaction, solutions acquired blue color, the intensity of which was measured by a spectrophotometer relative to the reference. The reference experiment was prepared by adding the reactants in the reverse order: a solution of the substrate was added after the solution of the multienzyme complex. Optical density of the analyzed solutions was measured at the wavelength of 670 nm in cuvettes with the absorbing layer 10 mm thick.

To calculate the proteolytic activity, a calibration curve was preliminarily built for tyrosine, and it was used to calculate tyrosine equivalent – the value of optical density that would be provided by 1 μmol of tyrosine in 1 cm^3 of the standard solution.

The monitored physico-chemical properties of enzymatic hydrolysates of feather-and-down raw materials were the mass fraction of moisture, the mass fraction of protein, the mass fraction of fat, the mass fraction of crude fiber, the mass fraction of sodium chloride, the mass fraction of calcium, the mass fraction of phosphorus, the mass fraction of mineral additives

insoluble in hydrochloric acid, the mass fraction of ash, and digestibility. Of these indicators, the determinative ones were: the mass fraction of protein, the mass fraction of calcium, the mass fraction of phosphorus, and digestibility.

The mass fraction of moisture was determined by drying at 130 ^{o}C. For this purpose, two sample bottles were dried for 30 min with the lids open in a drying cabinet at 130 ^{o}C, then cooled in a desiccator, and weighed with the accuracy up to the second decimal digit. The studied sample was placed into weighed and dried sample bottles. After that, the opened sample bottles with the sample and lids were placed into a drying cabinet heated to 130±2 ^{o}C. The sample was dried for 40 minutes. The process started after the temperature in the drying cabinet reached 130 °C. After 40 min., the test bottles were removed from the drying cabinet with crucible tongs, lids were quickly closed, and bottles were placed into a filled desiccator for cooling to room temperature for about 20 min [11].

The mass fraction of crude protein was determined by ashing with sulfuric acid in the presence of a catalyst, followed by alkalizing the product of the reaction, stripping and titration of released ammonia. The mass fraction of nitrogen was calculated from the calculation of the mass fraction of crude protein by multiplying the result by the conversion factor of nitrogen mass fraction to crude protein mass fraction, which was equal to 6.25.

The mass fraction of ash insoluble in hydrochloric acid was determined by the method based on organic substances decomposition during calcination, treating the residue with hydrochloric acid, followed by filtration, drying, calcination and weighing [12].

The mass fraction of total protein was studied by Duma's method using a RAPID N Cube protein nitrogen analyzer.

The mass fraction of fat was determined according to GOST 32905-2014.

Table 1 shows specifications of the equipment for enzymatic hydrolysis.

Table 1. Specifications of the equipment for enzymatic hydrolysis.

Position	Name and technical characteristic	Q-ty
1	The system for precleaning feather-and-down raw materials from foreign impurities	1
2	Press for feather squeezing	1
3	Feather-and-down raw materials transporter	1
4	Feather-and-down raw material presterilization unit	1
5	Experimental enzymatic reactor	1

The pilot installation for enzymatic hydrolysis of feather-and-down raw materials with the capacity of at least 300 kg/h consists of the system for feather-and-down raw material precleaning from foreign particles, a press for pressing feathers, a feather-and-down raw material transporter, a feather-and-down raw material presterilization unit, and an experimental enzymatic reactor.

This line is intended for studying and optimizing the processing modes of such raw materials with the aim of obtaining a high-protein feed additive with improved biological value and digestibility.

This line is unique, and has no analogues in Russia and in the world, both in terms of its layout, and in terms of functional purposes of its individual elements. The main components of the line are state-of-the-art world-class units and devices, which allow simulating and studying the process of raw materials' dehydration and preparation before feeding into the universal enzymatic reactor.

The main element of the line is the universal enzymatic reactor that allows grinding down raw materials, complete sterilization of the mass, and hydrolysis of protein (keratin) contained in the raw material in the flow. The line can be used to develop various modes of processing feather-and-down raw materials, allowing to obtain the end product - a high-protein feed additive that is well-balanced in terms of amino acids with protein content of not less than 95%, and the share of physiologically available protein in the resulting product of not less than 95%.

Perfection of the optimal technological modes for processing feather-and-down raw materials on the basis of the line allows eliminating the risk of scaling the technology of producing high-protein feed additive on industrial scale.

As a result of the research, the optimum conditions of feather-and-down raw materials' enzymatic hydrolysis in the pilot plant have been determined:

- ✓ sodium sulfite concentration - 0.5%;
- ✓ pH - 7.2;
- ✓ temperature - 55°C;
- ✓ duty water curve - 8;
- ✓ duration of fermentation - 4 h;
- ✓ the dosage of Protease No. 6230/2256 enzyme preparation – 15 U/g of raw material; and
- ✓ the dosage of Protolab B enzyme preparation – 15 U/g of raw material;

However, these process parameters for scaling the technological process of enzymatic hydrolysis of feather-and-down raw material require clarification. In addition, the most important parameter of the fermented feather-and-down raw material is fineness degree (particle size). Given the above, at this stage of work, parameters of enzymatic hydrolysis of feather-and-down raw materials in the pilot installation were optimized.

Technological operation "Preparation of multienzyme composition for enzymatic hydrolysis of feather-and-down raw material" has been perfected in the pilot plant beforehand, since this process operation makes a significant contribution to the process of obtaining high-protein feed additive based on feather-and-down raw material.

During the experiments for optimizing enzymatic hydrolysis of feather-and-down raw material in the laboratory, two enzymes were chosen for preparing the multienzyme composition: Protease No. 6230/2256 and Protolab B. At this stage, the process of preparing the multienzyme composition for hydrolysis of feather down raw materials was perfected at the pilot installation. The varying process parameters were the following: enzymes' shares, temperature, and the pH value. During the first stage of the research, the process temperature and the pH value were kept constant, 55°C and 7.2, respectively. The experiment was performed with the following ratios of proteolytic enzymes Protease No. 6230/2256 / Protolab B: 1:1; 1:2; 1:3; 1:4; 2:1; 3:1; 4:1. The monitored parameter was proteolytic activity of the obtained multienzyme complex. The results of the experiments are shown in Figure 1.

The data shown in Figure 1 indicate that the maximum value of proteolytic activity was achieved when the ratio of enzymes Protease No. 6230/2256 / Protolab B was 1:2 (1,445 U/g) and 1:3 (1,425 U/g). However, due to the economic feasibility, the ratio of enzymes 1:2 was chosen for further studies.

After that, the optimum temperature was chosen for preparing the multienzyme composition at the pilot installation. For this purpose, the experiment was made with the ratio of enzymes Protease No. 6230/2256 / Protolab B of 1:2, pH value equaled to 7.2, and the process temperature was varied between 25°C and 60°C in 5°C increments. The results of the research are shown in Figure 2.

Figure 2 shows that the process of preparing the multienzyme composition should be performed at the temperature of 55°C, at which the maximum proteolytic activity of the multienzyme complex of 1,445 U/g is reached.

The third series of experiments was devoted to choosing the optimum pH value in preparation of the multienzyme composition

consisting of Protease No. 6230/2256 and Protrude B in the pilot installation. The process was performed with the ratio of enzymes Protease No. 6230/2256 / Protolab B of 1:2 and at the temperature of 55°C. The pH value was varied between 6.0 and 8.0 in 0.2 increments. The results are shown in Figure 3.

Figure 3 shows that the maximum proteolytic activity of the Protease N 6230/2256 / Protolab B multienzyme complex has been achieved at pH equal to 7.2. This pH value has been chosen for preparing the multienzyme composition that performs enzymatic hydrolysis of the feather-and-down raw material.

Thus, the following parameters of preparing the Protease No. 6230/2256 / Protolab B multienzyme composition that performs enzymatic hydrolysis of feather-and-down raw materials have been chosen: the ratio of the Protease No. 6230/2256 / Protolab B enzymes is 1:2, the temperature is 55°C, and the pH value is 7.2.

The varying parameters of feather-and-down raw material enzymatic hydrolysis were the following: raw materials' fineness, sodium sulfite concentration, pH value, temperature, duty water curve, and duration of fermentation.

The monitored physico-chemical characteristics of the obtained enzymatic hydrolysates of feather-and-down raw materials were the mass fraction of moisture, the mass fraction of protein, the mass fraction of fat, the mass fraction of crude fiber, the mass fraction of sodium chloride, the mass fraction of calcium, the mass fraction of phosphorus, the mass fraction of mineral additives insoluble in hydrochloric acid, the mass fraction of ash, and digestibility. Among these indicators, the determinative ones were the following: the mass fraction of protein, the mass fraction of calcium, the mass fraction of phosphorus, and digestibility.

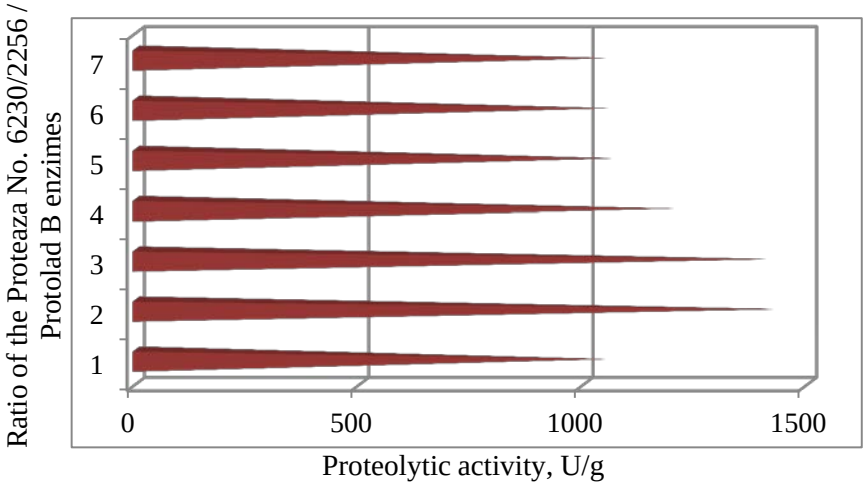

Figure 1. Multienzyme complex' proteolytic activity dependence on the ratio of enzymes Protease No. 6230/2256 / Protolab B: 1 – 1:1; 2 – 1:2; 3 – 1:3; 4 – 1:4; 5 – 2:1; 6 – 3:1; 7 – 4:1.

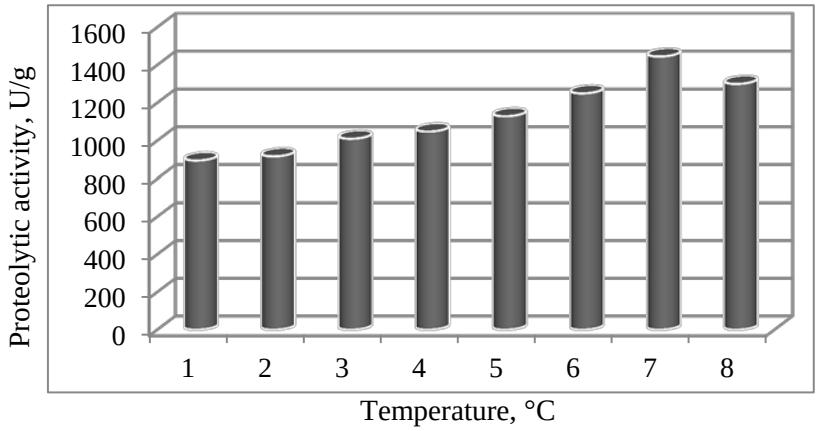

Figure 2. Dependence of proteolytic activity of the Protease N 6230/2256 / Protolab B multienzyme complex on temperature: 1 – 25°C; 2 – 30°C; 3 – 35°C; 4 – 40°C; 5 – 45°C; 6 – 50°C; 7 – 55°C; 8 – 60°C.

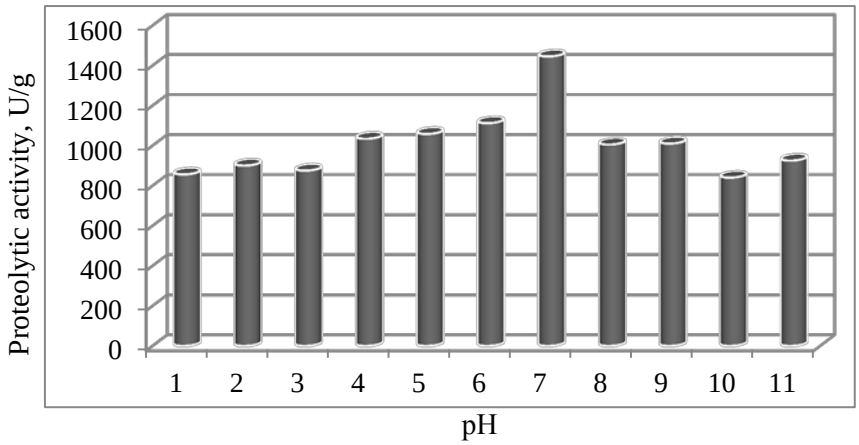

Figure 3. Dependence of proteolytic activity of the Protease N 6230/2256 / Protolab B multienzyme complex on the pH value: 1 – 6.0; 2 – 6.2; 3 – 6.4; 4 – 6.6; 5 – 6.8; 6 – 7.0; 7 – 7.2; 8 – 7.4; 9 – 7.6; 10 – 7.8; 11 – 8.0.

CONCLUSIONS

The performed experiments for optimizing the parameters allowed to select the modes of feather-and-down raw materials' enzymatic hydrolysis at the pilot installation aimed at obtaining high-protein feed additives:

- duration of fermentation - 10 h;
- the dosage of Protease No. 6230/2256 enzyme preparation – 15 U/g of raw material;
- the dosage of Protolab B enzyme preparation – 30 U/g of raw material.

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