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Physicochemical Properties and Indices of Chemical and Microbiological Safety of Enzyme Hydrolysates from Feather-and-down Raw Material

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Abstract

This article studies physiochemical properties of enzyme hydrolysates obtained from feather-and-down raw material during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment. Content of protein and dry matters in enzyme hydrolysates from feather-and-down raw material varies in the range of 1.80-3.00% and 2.6-4.4%, respectively. Protein weight percentage in terms of moisture content in this case is 67.7-70.6%. Ash weight percentage in terms of moisture content in all samples of enzyme hydrolysates does not exceed 0.43%, fat weight percentage is 0.74%, crude fiber weight percentage - 1.28%, sodium chloride weight percentage - 1.92%, weight percentage of impurities insoluble in hydrochloric acid - 0.77%. Microbiological properties of enzyme hydrolysates from feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment have been determined. Indices of chemical safety of enzyme hydrolysates from feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment have been measured.

Keywords: enzyme hydrolysates, feather-and-down raw material, safety indices, microbiological properties, physicochemical properties.

INTRODUCTION

Poultry operation in Russia has been steadily increasing in the recent five years, annual growth is from 13 to 15%, this is a good guarantee for processing industry [1, 2].

The amount of low cost byproducts accumulated upon poultry processing at Russian facilities is about 1.0 million tons. This is a headache for the industry. At the same time the byproducts are valuable resources of animal protein and fat. The content of animal protein in the byproducts is about 200.0 thousand tons, of fat - 43.0 thousand tons. Disposal of poultry processing wastes becomes more and more important considerably increasing prime costs of poultry products [3].

Both Russian and western conventional techniques make it possible to produce feeding flour with low content of digestible protein (protein digestibility from 25 to 50%) on the basis of poultry wastes, herewith, from 50 to 75% of available protein is lost due to severe long thermal processing. In addition, conventional processing techniques significantly consume energy and pollute environment require. Unfortunately, despite obvious disadvantages, Russian producers actively purchase such equipment forgetting that in competitive environment the winner is who operates present-day techniques or even those of tomorrow [4, 5].

Analysis of achievements in this field showed that possible approaches to eliminate the mentioned drawbacks could be as follows: processing intensification of raw materials, its continuous processing, elimination of microbiological spoilage of raw material prior to processing and combination of processing techniques [6].

At present agricultural raw materials are processed using advanced techniques which preserve as much as possible useful properties and biological value, as well as improve them upon manufacturing of ready food and feeding products. Such approaches include *high-temperature short-time processing in thin layer* (HTST). About 50% of protein is contained in guts, blood, byproducts of poultry wastes, hence, conversion of protein of poultry wastes into digestible form is highly important in terms of mobilization of reserves of native protein and environmental safety [7, 8, 9].

Nowadays technological development should be based on power and material saving production of high quality items and decrease in negative environmental impacts. Such approach can be applied to production of feeding additives.

Feeding products, high protein feed additives are complex multicomponent compositions which can vary their physicochemical properties, microbiological indices and toxicological characteristics upon storage, treatment, processing and handling. Therefore, it is highly important to analyze the aforementioned properties and quality indices.

This research stage is devoted to analysis of physicochemical properties of enzyme hydrolysates from feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment.

The enzyme hydrolysates were made of feather-and-down raw material after short time hydrothermal treatment. Preliminary hydrothermal treatment makes it possible to comminute raw feathers together with decrease in keratin packing density due to partial breakage of hydrogen bonds and decrease in hydrophobic interactions. The obtained substrate is more easily exposed to fermentation attack.

This work is aimed at determination of physicochemical properties and indices of chemical and microbiological safety enzyme hydrolysates from feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment.

MATERIALS AND METHODS

The subject matter of the experiments was feather-and-down raw material after short time hydrothermal treatment from poultry grown at OOO Kuzbass Broiler (Kemerovo oblast, Russia), as well as enzyme hydrolysates from feather-and-down raw material.

The following reagents and materials of Russian and foreign manufacture were used:

– sodium chloride according to State standard GOST 450-77;
– sulfuric acid according to State standard GOST 2184-2013;
– distilled water according to State standard GOST 6709-72;
– calcined sand according to State standard GOST 8735-88;
– petroleum ether according to State standard GOST 11992-66;
– silver nitrate according to State standard GOST 1277-75;
– chloroform according to State standard GOST 20015-88;
– silver nitrate according to State standard GOST 1277-75;
– meat-peptone broth according to State standard GOST 20730-75;

– microbiological agar according to State standard GOST 17206-96;
– edible gelatin according to State standard GOST 11293-89;
– glycerol according to State standard GOST 6259-75;
– calcium carbonate according to State standard GOST 4530-76;
– sucrose according to State standard GOST 5833-75;
– anhydrous sodium sulfite according to State standard GOST 195-77;
– lead nitrate, chemically pure, according to State standard GOST 4236-77;
– aqueous ammonia, chemically pure, 5 wt % solution according to State standard GOST 3760-79;
– cadmium metal, reagent grade according to State standard GOST 1125;
– phenolphthalein according to Specifications TU 6-09-5360.

Crude protein weight percentage was determined by ashing with sulfuric acid in the presence of catalyst with subsequent alkalization of reaction product, stripping and titration of extracted ammonia. Nitrogen weight percentage was calculated in terms of crude protein weight percentage by multiplication of the obtained result by conversion factor of nitrogen weight percentage to crude protein weight percentage equaling to 6.25 [2, 10].

Ash weight percentage insoluble in hydrochloric acid was determined using splitting of organic matters upon calcination, treatment of the obtained residue by hydrochloric acid with subsequent filtration, drying, calcination and weighing [9].

Mositure weight percentage was determined by drying at 130°C. Two vessels were dried with opened covers in drying oven in 30 min at 130°C, cooled in desiccator and weighed with the accuracy of two decimal places. A considered sample was placed into preliminary weighed and dried vessels. Then, the vessels with sample in opened state and covers were placed into a drying oven preheated to 130±2°C. The sample was dried in 40 min. The process duration was counted after reaching 130°C in the drying oven. After 40 min the vessels were removed from the drying oven using crucible tongs, quickly closed with covers and placed into charged desiccator in order to cool to ambient temperature for about 20 min [4, 11].

Total protein weight percentage was analyzed by the Dumas method using a RAPID N Cube analyzer of protein nitrogen.

Fat weight percentage was determined according to State standard GOST 32905-2014 [12, 13].

Crude fiber weight percentage in enzyme hydrolysates was determined according to State standard GOST 13496.2-91.

The following equipment was used during the analysis:
–VK 600.1 analytical balance (Massa-K, Russia);
– ShSvL-80 electric drying oven with the operating temperature range from 0°C to 160°C and main error of temperature stabilization equaling to ±2°C (OAO GRPZ, Russia);
– electric muffle furnace maintaining from 0°C to 550°C inside furnace and main error of temperature stabilization equaling to ±20°C;
– electric vacuum pump, water jet or Komovsky pump;
– SNOL 6/1 electric chamber laboratory furnace (Umega, Lithuania);
– laminar box. class 2/type A (Laminar Systems, Russia);
– LSI-3016R shaking incubator (Labtech, Korea);
– DGM-80 autoclave (DGM, Switzerland);
– desiccator 2-50 according to State standard GOST 25336;
– TKh 25-11.15.92-81 BS.W39 redistiller (OAO Khimlabpribor, Russia);
– HI 96801 refractometer (HANNA, Germany);
– AxioScope A1 upright microscope (Carl Zeiss AG, Germany);
– ShSvL-80 dry air laboratory cabinet (Kasimov, Russia);
– 5430 centrifuge (Eppendorf, Ukraine);
– UV 1800 spectrophotometer (Shimadzu, Japan);
– AAS Solaar MK II atomic absorption spectrometer (Germany);
–RAPID N Cube protein nitrogen analyzer;
– Sevew Compact pH meter (Mettler Toledo, USA);

RESULTS AND DISCUSSION

A sample of hydrothermally treated raw material was obtained in accordance with the following process variables of hydrothermal hydrolysis:

– initial moisture content of feather – 55%;
– heating temperature – 190-200°C;
– heating duration – 90 s.

Physicochemical properties of the obtained sample of hydrothermally treated feather-and-down raw material were determined as follows: density, hygroscopy, moisture weight percentage, fat weight percentage, ash weight percentage, protein weight percentage, crude fiber weight percentage, sodium chloride weight percentage, calcium weight percentage, weight percentage of mineral impurities insoluble in hydrochloric acid. The physicochemical properties of initial feather-and-down raw material after hydrothermal treatment are summarized in Table 1.

The physicochemical properties of feather-and-down raw material after hydrothermal treatment summarized in Table 1 meet the requirements of Specifications TU 9219-094-23476484-09 Feeding hydrolyzed flour. Protein concentrated product made of feather.

Then the physicochemical properties of six samples of enzyme hydrolysates from hydrothermally treated feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment (with increasing antioxidant capacity with regard to peroxy radical) were studied: GP-1, GP-2, GP-3, GP-4, GP-5, GP-6. The obtained results are summarized in Table 2.

The data in Table 2 demonstrate that the content of protein and dry matters in enzyme hydrolysates from feather-and-down raw material varies in the range of 1.80-3.00% and 2.6-4.4%, respectively. The protein weight percentage in terms of moisture weight percentage in this case is 67.7-70.6%. The calcium weight percentage in terms of moisture weight percentage in the considered samples varies in the range from 12.6 to 13.8%, the phosphorus weight percentage – in the range from 12.9 to 14.9%. The ash weight percentage in terms of moisture weight percentage in all samples of enzyme hydrolysates does not exceed 0.43%, the fat weight percentage – 0.74%, the crude fiber weight percentage – 1.28%, sodium chloride weight percentage – 1.92%, the weight percentage of impurities insoluble in hydrochloric acid – 0.77%. The data in Table 2 also demonstrate that minimum content of protein in terms of moisture weight percentage is observed in enzyme hydrolysates with hydromodule equaling to 4. Contents of dry matters and protein in enzyme hydrolysates from feather-and-down raw material correlate with each other. Probably, this can be attributed to high relative protein portion in dry matter of enzyme hydrolysates from feather-and-down raw material. Moisture and ash contents of enzyme hydrolysates from feather-and-down raw material correspond to the range of these parameters for other enzyme hydrolysates of animal origin.

Manufacture of products of poultry meat is at present the leading field of agriculture and has significant impact on production of food in other fields. Great attention is paid to safety of foods for poultry.

Development of extended feed base is an important factor for products of animal origin. An important task upon development of new feed additives is provision of maximum quality control and safety of produced additives and feeds. Sanitary quality of such products has significant influence on reproducibility, output and health of animals, as well as

directly on their biological value. Sanitary quality of foods is determined by their bacterial content.

The highest attention in evaluation of feed additives is paid to such indices as total bacterial content, existence of pathogenic organisms and toxins.

High content of such microorganisms as salmonella, clostridium, etc. leads to infections and disease progress in animals and poultry, their premature death and, hence to significant economic loss.

Veterinary and sanitary experts perform microbiological study of feeds of animal and vegetable origin with regard to total bacterial content, salmonella, coliform bacteria, proteus, and toxin forming anaerobes.

Salmonella in feed additives is a reason of infectious salmonellosis. Growing stock, including poultry, animals and humans, suffer from salmonellosis. Salmonellosis is accompanied by fever and profuse diarrhea, it also can lead to lung diseases. During pregnancy salmonellosis can cause spontaneous miscarriage.

Bacterial content in poultry feed is the main reason of poisoning and initiation of various infectious diseases. Further researches are devoted to microbiological indices and hydrothermally treated feather-and-down raw material. The obtained results are summarized in Table 3.

Table 3 shows that the microbiological properties of feather-and-down raw material meet the requirements of Specifications TU 9219-094-23476484-09 Feeding hydrolyzed flour. Protein concentrated product made of feather.

CONCLUSION

Physiochemical properties of enzyme hydrolysates obtained from feather-and-down raw material during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment have been studied. Content of protein and dry matters in enzyme hydrolysates from feather-and-down raw material varies in the range of 1.80-3.00% and 2.6-4.4%, respectively. Protein weight percentage in terms of moisture content in this case is 67.7-70.6%. Ash weight percentage in terms of moisture content in all samples of enzyme hydrolysates does not exceed 0.43%, fat weight percentage - 0.74%, crude fiber weight percentage - 1.28%, sodium chloride weight percentage - 1.92%, weight percentage of impurities insoluble in hydrochloric acid - 0.77%.

Table 1. Physicochemical properties of hydrothermally treated feather-and-down raw material.

Property	Actual value	Method of analysis
Moisture weight percentage,%	6.3	State standard GOST 17681-82
Protein weight percentage,%	84.1	State standard GOST 32044.1-2012
Fat weight percentage,%	2.7	State standard GOST 32905-2014
Ash weight percentage,%	1.0	State standard GOST 32933-2014
Crude fiber weight percentage,%	0.8	State standard GOST 13496.2-91
Sodium chloride weight percentage,%	1.6	State standard GOST 13496.1-98
Calcium weight percentage,%	1.2	State standard GOST 26570-85
Phosphorus weight percentage,%	1.5	State standard GOST 26657-97
Weight percentage of mineral impurities insoluble in hydrochloric acid,%	0.5	State standard GOST 25555.3-82

Table 2. Physicochemical properties of enzyme hydrolysates from feather-and-down raw material obtained during multifactor experiment on optimization of enzymatic hydrolysis of feather-and-down raw material in laboratory environment.

Property	Samples					
	GP-1	GP-2	GP-3	GP-4	GP-5	GP-6
Moisture weight percentage,%	95.7	96.9	97.4	97.3	95.6	96.1
Protein weight percentage,%	2.98/69.3	2.19/70.6	1.80/69.2	1.87/69.3	3.00/68.2	2.64/67.7
Fat weight percentage,%	0.02/0.43	0.01/0.3	0.005/0.19	0.005/0.19	0.01/0.23	0.01/0.26
Ash weight percentage,%	0.01/0.23	0.002/0.06	0.002/0.08	0.02/0.74	0.003/0.07	0.005/0.13
Crude fiber weight percentage,%	0.05/1.16	0.03/0.98	0.03/1.15	0.02/0.74	0.03/0.68	0.05/1.28
Sodium chloride weight percentage,%	0.06/1.39	0.05/1.61	0.05/1.92	0.04/1.48	0.08/1.82	0.05/1.28
Calcium weight percentage,%	0.54/12.6	0.40/12.9	0.34/13.1	0.35/13.0	0.60/13.6	0.54/13.8
Phosphorus weight percentage,%	0.62/14.4	0.40/12.9	0.36/13.8	0.38/14.1	0.65/14.8	0.58/14.9
Weight percentage of mineral impurities insoluble in hydrochloric acid,%	0.02/0.46	0.02/0.65	0.02/0.77	0.01/0.37	0.03/0.68	0.02/0.51*

* numerator: as is basis; denominator: in terms of moisture weight percentage

Table 3. Microbiological properties of hydrothermally treated feather-and-down raw material.

Microbiological properties	Norms	Tests	Regulatory documents
Count of aerobic mesophilic and facultative anaerobic microorganisms, CFU in 1.0 g, not higher than	$5.0 \cdot 10^5$	$1.0 \cdot 10^2$	State standard GOST 10444.15-94
Product bulk (g) without detected Coliform bacteria	50.0	50.0	State standard GOST 25311-82, State standard GOST 30518-97
Product bulk (g) without detected pathogenic germs, including Salmonella	50.0	50.0	State standard GOST 25311-82, State standard GOST 30519-97
Product bulk (g) without detected anaerobic bacteria (toxin forming bacteria)	50.0	50.0	State standard GOST 25311-82, State standard GOST 29185-91
Product bulk (g) without detected *Proteus*	1.0	1.0	State standard GOST 28560-90

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