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HYDROLYSIS OF KERATIN MATERIALS DERIVED FROM POULTRY INDUSTRY

HYDROLIZA MATERIAŁÓW KERATYNOWYCH POCHODZĄCYCH Z PRZEMYSŁU DROBIARSKIEGO

Abstract: In 2011, the European Union produced about 45 million tons of meat and in this time Poland occupied the fourth place in Europe in the production of poultry and pork meat and seventh in production of beef. The consumption of poultry meat grows year by year, which results in an increase of its production. Feathers consist in 90% of keratin and they make up 5-7% of the total weight of adult chickens what causes that feathers waste is the main source of keratin. Feathers are discarded in the process of the converting of poultry as a waste product and contribute to environmental pollution. The development of the poultry industry in the world has led to the generation of more than 4 million tons per year of waste feathers. Average farm size in Poland produces about 7 tons of chicken feathers a day. Nationally, during each year 77,000 tons of waste is produced. Keratin has a high immunity to physical and chemical factors and it is the reason of searching for new methods of keratin waste conversion. It would help to avoid a problem with storage of feather wastes. Application of alkaline hydrolysis is one of the ways of feathers utilizations. During this hydrolysis the keratin swells and is subject to degradation. The increase in solubility of keratin in bases is caused by peptide and disulfide bond cleavage and it leads to increase of keratin plasticity and decrease of its strength. The aim of this study was to determine the effect of process parameters on the degree of degradation of keratin and optimize the process to achieve its maximum value.

Keywords: keratin, feathers, hydrolysis

Introduction

In 2011, the European Union produced about 45 million tons of meat and in this time Poland occupied the fourth place in Europe in the production of poultry and pork meat and seventh in production of beef.

The consumption of poultry meat grows year by year, which results in an increase of its production [1]. A considerable amount of waste are produced during slaughter of chickens. The main waste are: feathers, skin, blood, heads and legs and they belong to the III waste category and they may be used for feed and fertilizer after heat treatment. Poultry processing plants currently convert feather waste into low-nutritive animal feed [2]. Industrial methods of processing this waste are based on chemical (acid, base, catalyst) or enzymatic hydrolysis. Chemical hydrolysis requires more aggressive conditions of reaction (high temperature and pressure) and carries a greater risk to the environment. The product has a low nutritional value, because it contains small amounts of the essential amino acids. Enzymatic hydrolysis is more expensive and requires a longer time of operation.

The nutritional value of the product obtained by hydrolysis is determined by amino acids contained in it. These amino acids are methionine and histidine and their number decreases with age of chicken [3, 4]. In some countries legal regulations prohibit the use of waste feathers into animal feed and in most such cases, feathers are stored [5]. Odors

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formed from the degradation of substances contained in the biomass and greenhouse gases such as methane and carbon dioxide are the main problem of storing [6]. In addition, non-volatile products of decomposition can penetrate along with the water into the soil and contaminate groundwater [7].

Feathers consist in 90% of keratin and they make up 5-7% of the total weight of adult chickens what causes that feathers waste is the main source of keratin [8]. Feathers are discarded in the process of the converting of poultry as a waste product and contribute to environmental pollution. The development of the poultry industry in the world has led to the generation of more than 4 million tonnes per year of waste feathers. Average farm size in Poland produces about 7 tonnes of chicken feathers a day. Nationally, during each year 77,000 tonnes of waste is produced [7, 9].

Keratin is a biopolymer having a three-dimensional, fibrous structure with hierarchical character. It consists of small nano-amino acids which are polymerized in the known sequence to the molecular weight of protein order of 10-100 nm. The molecular weight of the feather keratin is approximately 10,500 Da. The content of cysteine/cystine in the amino acid sequence is about 7%. Keratin has in its structure about 40% hydrophilic and 60% hydrophobic chemical groups according to the amino acid sequence. Molecules of protein may be accumulated in the structure of the α -helix, β -sheet or random, unordered macrostructure. Keratin fibers in feather consist of 41% α -helix, 38% β -sheet, and 21% random structure [7, 10].

The structure of α -helix are intramolecular hydrogen bonds between the carbonyl group of one amino acid and the amino group of another amino acid. The structure β -sheet is characterized by the interchain hydrogen bonds between the amino and carbonyl groups. A hydrogen bond may be combined with the water bound in the structure of the protein. Keratin is characterized by a high stability, because of intermolecular bonds that occur between the polar and non-polar amino acids, and poor solubility caused by the presence of S-S bonds between cysteine amino acids [7, 10].

Keratin is a popular product in the pharmaceutical, medical, cosmetic, and biotechnological industry. Materials obtained from wool keratin may be converted into porous foam of different shapes, sponges, mats, coatings, gels, microfibers and materials of high molecular weight. Non-antigenic keratin has a positive effect on wound healing and tissue reconstruction. Implantation layer, construction or keratin biomaterial may be absorbed by the surrounding tissue [7, 11].

Keratin was used in a narrow range, because of the insolubility in water and a limited number of available methods for the extraction and processing. In the past it was used as a biomaterial in regenerative medicine. The interest in keratin and its modifications has increased in the past few years, especially popular has become keratin obtained from wool. Yamauchi and Tachibana described the increasing of degree of fibroblasts distribution on the keratin and keratin-chitosan coating [11]. Fujii studied the effect of proteins derived from human hair on the rat mast cells. Verma described the preparation of the protein structure of a human hair to its use in tissue engineering. Van Dyke research group conducted the study of hydrogels and porous structure based on keratin from hair and their interactions with cells and tissues. Recently tests based on the use of keratin biomaterials for biomedical applications were carried out. The literature also attempts to use the keratin coating on ocular surface reconstruction [7, 12].

Fibers of chicken feather are composed of a hydrophobic keratin, which is a protein having similar strength to nylon one, and a smaller diameter than the wood fiber. A surprising feature of the chicken feather fibers are semi-crystallinity and cross-linked structure, which enhances the resistance of composites based on polymers to mechanical stress and causes a relatively high modulus of elasticity (GPa 3.4-5). In addition, fibers of chicken feather have a high elongation coefficient. These mentioned properties suggest that there are possibilities of successful application of chicken feather fiber as a polymeric reinforcement [7, 13].

Non-woven composites formed by combination between synthetic and chicken feathers (PP, PES) have also a good sorption and mechanical properties. The wide range of excellent sorption of hydrophobic hydrocarbon and selectivity (absorb liquids immiscible with water) is typical for that kind of materials. Non-woven is cheap, lightweight, easy to store and may be used in a variety of weather conditions and surfaces (on the surface of water, concrete or asphalt). Non-woven composites are prepared by papermaking method and in the presence of water [14].

The selection and optimization of process parameters of alkaline hydrolysis of waste feathers was the aim of the study. Method of chemical hydrolysis of feathers proposed in the project reduces the temperature, pressure and shortens the time of process. Moreover, it leads to a decrease in energy consumption of the process, what saves a considerable amount of its cost and the resulting product has a high nutritional value.

Experimental part

Waste feathers provided from poultry plant were the object of the research.

During the study, the moisture content was determined using the analyzer RADWAG WPS 210s at 105°C and 5 s sampling time.

In order to provide greater surface area for heat exchange hydrolysis was carried out in a laboratory autoclave that was set in a horizontal position. Alkaline hydrolysis with calcium hydroxide was carried out in two stages. The first step was to sterilize the material according to the law that determined the parameters of the hydrolysis process. According to the law, animal by-products must be heated during the sterilization to a temperature above 133°C for at least 20 min at a pressure (absolute) of at least 3 bars produced by saturated steam [15]. In the second stage, the reactor temperature was lowered to 90-110°C and the pressure was lowered to ambient pressure. The removal of moisture from the material followed under these conditions. Digestible protein and total protein was determined in the product (dry up in dryer in 40°C) by the Kjeldahl method (PN-75/A-04018+Az3:2002+I-01/PN-75/A-04018:1975).

The response surface was determined using the central-compositional plan and it allowed for the optimization of the hydrolysis process and the application of created model to predict the dependent variable (total protein content and digestible in the product).

Results and discussion

A set of parameters characterizing the object of study was determined (qualitative model of research object) in order to select of the test plan and these parameters were reviewed and classified into value of input, output, and constant interference.

Weight of calcium hydroxide added to the feathers (0.05-0.75 g), the mass of water (2.0-5.0 g), the temperature of the second stage of the process (90-110°C), its duration (60-240 min) were input quantities that describe the test object (the process of alkaline hydrolysis feathers). The feather mass of 15 g and its humidity of 60% were adopted. Input values determine the composition of feather meal - content of protein and digestible protein [%]. The optimal test plan was central compositional plan with twice-repeated of the experiment at a central point.

The parameters of process and results of product's analysis are summarized in Table 1. Decomposition has been an object of research and it involved the creation of two objects of researches characterized with one the value of the output (the percentage of total protein and digestible protein).

Table 1
Summary of input variables (independent) and the content of total and digestible protein in feather meal

	Mass Ca(OH) ₂ [g]	Mass H ₂ O [g]	Temperature 2 nd stage [°C]	Time 2 nd stage [min]	Total protein [%]	Digestible protein [%]
1	0.05	3.5	100	150	88.5	70.2
2	0.05	5.0	110	240	91.1	78.3
3	0.75	2.0	90	240	84	89.0
4	0.75	3.5	100	150	76.5	71.5
5	0.75	2.0	110	240	75.5	65.3
6	0.40	3.5	100	60	83.7	69.9
7	0.40	3.5	100	150	86.1	78.5
8	0.75	5.0	90	60	78.7	57.6
9	0.40	5.0	100	150	83.3	74.1
10	0.40	3.5	100	150	83.5	42.3
11	0.40	3.5	90	150	82.6	70.7
12	0.05	2.0	90	60	89.5	67.5
13	0.40	3.5	110	150	82.8	67.9
14	0.40	3.5	100	240	83.9	46.1
15	0.40	2.0	100	150	82.8	76.7
16	0.40	3.5	100	150	84.2	63.7
17	0.75	5.0	110	60	76.3	60.0
18	0.05	2.0	110	60	89.4	62.9
19	0.05	5.0	90	240	89.1	72.1

Feather hydrolysis tests performed on various parameters of the second stage of the process has shown the variability of output parameters of the process. The content of total protein is in the range 75-91%, and 42-89% of digestible protein. The lowest content of total protein (75.5%) was obtained for the parameters of process: temperature 110°C, duration of process 240 minutes and the addition of 2 g of water and 0.75 g of calcium hydroxide. It is the highest concentration (91.1%) reported for the test conducted at the same temperature and for the same period of time, but with 0.05 g of Ca(OH)₂ and 5 g of H₂O. The amount of calcium hydroxide added during the hydrolysis process was the main factor contributing to content of the total protein of the meal. This is due to its content in the final product after the reaction because Ca(OH)₂ remains in the system and enters into the composition of the product. The greater number of calcium hydroxide makes the lower

content of total protein in the meal. In the case of digestible protein a greater difference between the extreme results is observed. The lowest content of this protein (42.3%) is noticed in the test carried out during 150 min and at 100°C with the addition of 0.4 g of calcium hydroxide and 3.5 g of H₂O. The highest amount (89%) is for a process carried out at a temperature of 90°C and during 240 minutes with 2 g of H₂O and 0.75 g of Ca(OH)₂. The amount of digestible protein in the final product depends on the degree of degradation of the keratin. The environment of the process (pH, temperature, time) influences the degree of degradation of that biopolymer. An increasing of keratin solubility in the bases is caused by peptide and disulfide bonds cleavage. The solubility is dependent on the concentration of base in the system, temperature and process time.

Tests performed during the waste feathers conversion into feather meal showed that the content of total and digestible protein depends on many parameters of the process. The mass of calcium hydroxide (0.05 g), water (5.0 g), the temperature of the second stage of the process (110°C) and duration (200 min) which were admitted as optimal parameters of the hydrolysis process.

Conclusions

Elaborated method of hydrolysis of feathers allows to obtain feather meal, which may be used as an additive to animal feed. The content of total and digestible protein in a received product was higher than those in commonly used animal feed. It is a result of high content of amino acids significant for food.

This method of hydrolysis is environmentally and economically advantageous because it is carried out under mild conditions of temperature and pressure, and the shorter the duration.

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Abstrakt: W 2011 roku w Unii Europejskiej wyprodukowano około 45 mln ton mięsa. W tym czasie Polska zajmowała czwarte miejsce pod względem produkcji mięsa drobiowego oraz wieprzowego, była również siódmym producentem wołowiny. Spożycie mięsa drobiowego wzrasta z roku na rok, co skutkuje zwiększeniem jego produkcji. Pióra zbudowane są w 90% z keratyny i stanowią 5-7% całkowitej masy dorosłych kurczaków, wskutek czego są głównym źródłem odpadów keratynowych. W trakcie procesu przetwórstwa drobiu pióra są odrzucane, a jako produkt odpadowy przyczyniają się do zanieczyszczenia środowiska. Rozwój przemysłu drobiarskiego na świecie doprowadził do generowania ponad 4 milionów ton odpadowych piór rocznie. Średniej wielkości ferma w Polsce generuje około 7 ton pierza kurzego w ciągu doby, a w skali kraju rocznie wytwarzane jest 77 000 ton tego odpadu. Keratyna posiada wysoką odporność na czynniki fizyczne oraz chemiczne, co jest powodem poszukiwania nowych metod przetwarzania odpadów keratynowych. Pomoże to uniknąć problemów ze składowaniem odpadowego pierza. Zastosowanie hydrolizy zasadowej jest jednym ze sposobów utylizacji piór. Podczas tej hydrolizy keratyna pęcznieje i ulega degradacji. Rozerwanie wiązań peptydowych i disiarczkowych powoduje wzrost rozpuszczalności keratyn w zasadach oraz prowadzi do zwiększenia plastyczności keratyny i spadku jej wytrzymałości. Celem badań było określenie wpływu parametrów procesu na stopień degradacji keratyny oraz optymalizacja procesu w celu uzyskania jego maksymalnej wartości.

Słowa kluczowe: keratyna, pióra, hydroliza