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CHARACTERISTICS OF MODIFIED PROTEINS OF KERATIN IN CHICKEN FEATHERS

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Key words: keratin, modified proteins, chicken feathers, composition of aminoacids.

Investigations of modified proteins of keratin in chicken feathers versus non-modified keratin in feathers were carried out. Determinations included the following parameters: dry mass, total protein, ether extract, fiber, quantitative composition of aminoacids and contents of alpha-aminic nitrogen.

Keratin, the main component of feathers, is very insensitive to proteolytic enzymes, so that it may be digested only by some animals possessing high concentrations of such enzymes. Consequently, when trying to utilize feather keratin for feed and nutrition it must be processed into a meal in order to modify the keratin structure.

So far processes of modification of keratin structures are performed with some popular organic solvents: dimethyloformamide (DMF) and dimethylosulphoxide (DMSO) [1, 8, 9] and others, or by applying the so-called thermohydrolytic process [2, 4, 5]. Effects of modification of keratin proteins can also be obtained by heating them with urea or its concentrated solutions [10, 11].

The aim of investigations in this part of the project was to arrive at physico-chemical characteristics of chicken feather keratin proteins modified by chemical agents — DMSO and urea and by physical parameters — temperature and pressure, which is of special significance in a large-scale production of keratin meals as compared to non-modified keratin of the feathers.

To obtain information on modification of keratin structures the following parameters were determined: dry mass, total protein, ether extract, fibers, ash, quantitative and qualitative composition of aminoacids as well as alpha-amino nitrogen.

MATERIALS AND METHODS

The object of investigations consisted of chicken feathers (I), from the Lublin Poultry Plant, keratin meal from feathers (II) obtained by dissolving feathers in DMSO, shaking and drying [8, 9], keratin meal from feathers (III) manufactured according to a Polish technological process at the Waste Processing Plant in Wrocław [2, 4], keratin meal from feathers (IV) produced with an imported license at Leszczyna [2, 4], and a granulate of urea and keratin (V), obtained according to the method described earlier [10, 11].

The following reagents were used in the procedures: hydrochloric acid 36.5% (5.7N) from the Tarnów Nitrogen Works, a set of aminoacid standards produced by Merck, Darmstadt, FRG; ninhydrine from the Polytechnical Institute of Silesia; acetone from VEB Labor. Chemie Apolda, GDR; Whatman filter paper No. 3; glacial acetic acid; n-butanol; $CuCl_2 \cdot 2H_2O$; $Na_3PO_4 \cdot 12H_2O$; sodium hydroxide; $Na_2B_4O_7 \cdot 10H_2O$; potassium iodide; potassium thiosulphate; soluble starch; ethyl alcohol 96% from Gliwice. Water solutions of the aminoacid standards had a concentration of 0.01 M, except for tryptophane (0.005) and thyrosine (0.0025). All aminoacids were protected by an addition of crystalline thymol. Ninhydrine solution (0.2g/100cc acetone) was used for development of chromatographs.

Hydrolysis of particular keratin preparations (I-V) was performed by weighing 0.1 g of an approariate preparation and placing it in a glass ampule to which 2 cc 5.7 N HCl was added. The sealing of ampules was done in ambient nitrogen. Sealed ampules were seated in an SUP-2 laboratory drier at a temp. of 105°C for 20 hrs.

The contents of alpha-amino nitrogen in individual preparations was determined with the copper method of Poppe-Stevens [3]. To obtain a preliminary comparison of the qualitative composition of aminoacids present in particular preparations paper chromatography was used. Chromatograms were developed in the ascending technique [3]. Quantitative aminoacid composition in preparations from I to IV was determined

Table 1. Chemical composition of keratin meals from feathers; %

No. of meal	Dry mass	Total protein	Ether extract	Fiber	Ash
I	95.3	94.5	0.4	0.2	0.2
II	91.3	90.5	0.3	0.3	0.2
III	93.0	86.9	3.1	0.7	2.3
IV	92.7	85.6	4.6	1.0	1.5
V	92.8	92.6 in that 71.2 urea	—	0.1	0.1

Table 2. Qunatitative aminoacid composition in keratin proteins from feather meals (g/16 g N)

Aminoacid	No. of meal			
	I	II	III	IV
Alanine	3.89	4.24	4.91	2.42
Arginine	6.83	9.23	13.10	6.27
Asparaginic acid	5.24	5.57	6.56	6.46
Cysteine	7.07	6.77	5.10	6.34
Glutaminic acid	8.74	9.91	11.62	10.58
Glycine	6.16	7.99	8.16	7.46
Histidine	0.51	0.07	1.27	0.81
Isoleucine	4.33	5.29	5.00	4.64
Leucine	7.16	8.18	8.33	8.35
Lysine	1.68	0.36	4.71	2.32
Methionine	0.41	trace	0.58	0.49
Phenyloalanine	4.22	5.49	4.42	4.44
Proline	8.86	11.57	10.48	10.13
Serine	10.18	12.21	10.57	10.21
Treonine	4.01	4.32	4.71	4.56
Thyrosine	2.38	4.83	1.44	1.26
Valine	6.60	9.23	8.16	7.79

to observe quantitative proportions of individual aminoacids in relation to the aminoacid composition of the feathers. An automatic analyzer of aminoacids (Carlo Erba, Italy) was used. The basic chemical composition was determined according to commonly used methodology [6].

RESULTS

In order to have a more detailed characteristics of the investigated preparations (I-V) the chemical composition was determined and it follows from it that except for preparation V all other meals contain substantial quantities of protein. The remaining elements of the chemical composition approximate preparations I, II, III and IV. The urea preparation V clearly departs from others in this respect. Table 1 gives detailed data.

Quantitative assessment of changes of contents in aminoacids concerning particular preparations (I-IV) was performed to estimate effect of a method used to produce of a given preparation of modified of keratin structure, as compared with the aminoacid composition of non-modified feather (I). Table 2 presents data on this part of the study.

Determination of alpha-amino nitrogen was the next attempt at defining the degree of modification of keratin proteins in the investigated

Table 3. Contents of alpha-amino nitrogen and the degree of modification

No. of meal	Alpha-amino nitrogen mg		Degree of modification
	per 100 g dry mass	per 94.5 mg protein	
I	0.450	0.450	1.000
II	0.700	0.731	1.624
III	0.420	0.457	1.016
IV	0.420	0.464	1.031
V	0.480	2.120	4.711

preparations. To arrive at comparable data the quantity of alpha-amino nitrogen was converted, in preparations II to V, into total protein content as in preparation I — 94.5%. As a degree of modification, the ratio of contents of alpha-amino nitrogen in preparations I-V (after conversion) to the quantity of alpha-amino nitrogen in preparation I was chosen. Table 3 gives results of determinations of alpha-amino nitrogen and the degree of modification of particular preparations.

DISCUSSION

According to the data in Table 1, keratin meals II-V have lower levels of total protein in comparison with the control preparation I, that is non-modified feathers. Differences in the contents of this protein may be due to diverse technological reasons such as different ways of making particular preparations or effects of physico-chemical parameters which may cause losses in protein (temperature, pressure, solvent). Keratin meals III and IV produced technologically contain considerable amounts of ether extract, ash and fibers. This is related to the production process in which blood and bones are added [2, 4]. The high level of urea (71.2%) in preparation V results from the technology as well in which urea plays the role of a reagent modifying the keratin structure being at the same time a rich source of non-protein nitrogen [10, 11]. The apllied simple method of preliminary qualitative assessment of the aminoacid composition of the investigated preparations [3] showed insignificant changes in it. They mainly pertained to preparation V where presence of asparagine was observed. The latter may develop during the technological process from asparaginic acid. Ninhydrin-positive compounds were not observed to be present. In other preparations the qualitative aminoacid composition was identical. Presence of compounds reacting with ninhydrine in the form of three spots was observed. Probably these are soluble peptides.

Assessing the quantitative composition of aminoacids in the investigated preparations (Table 3) from the point of view of contents of exogenous aminoacids, the following order in preparation I can be established: leucine (7.16); valine (6.60); isoleucine (4.33), phenyloalanine (4.22); threonine (4.01), lysine (1.68) and methionine (0.41). It ought to be stressed that in preparations II-IV the contents of exogenous aminoacids increases in comparison with the control preparation I except for methionine and lysine in preparation II. In the case of endogenous aminoacids, i.e., asparaginic and glutaminic acids, glycine, proline, and serine we observe an increase of contents of these aminoacids in preparations comparing to the control preparation I. Other aminoacids showed both increases and decreases of their contents versus the control preparation. The lowering of contents of lysine and methionine in preparation II may be caused by effect of DMSO on these acids. Lysine content, relatively high in preparations III and IV, results of addition of blood to the meal production process. One shall also notice that all the preparations have rather high levels of sulphur aminoacids (cysteine).

The degree of modification of the keratin protein structure in the investigated meals was estimated by comparing the contents of alpha-amino nitrogen in the investigated preparations. It was assumed that the obtained results would be comparable after conversion of the content of alpha-amino nitrogen in dry mass into its quantity in pure protein of the control preparation I (94.5%) In this way it turnedout that the studied preparations contained respectively higher levels of that nitrogen (Table 3). Considering the volume of alpha-aminic nitrogen as 1 in Preparation I, the degree of modification of preparations II-V was calculated. It was determined that the highest level of modification of the keratin structure took place in protein of Preparation V (4.711), then that of Preparation II (1.624). The remaining preparations have values approximating the modification level in Preparation I.

The increasing content of alpha-aminic nitrogen in keratin preparations may suggest conformational changes in the investigated proteins or a partial degradation of polypeptide chains which leads to increasing of the quantity of N-final aminoacids.

CONCLUSIONS

1) The investigated keratin preparations I-IV have a high content of total protein (above 85%).

2) The keratin preparations I-IV are a rich source of cysteine (5% to 7%).

3) Utilization of the preparations for feeding purposes requires supplementation of the aminoacid composition with some exogenous aminoacids, e.g., methionine and lysine.

4) The results of the investigation indicate that the highest level of modification in the structure of keratin protein was obtained for the keratin-urea granulate (V).

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CHARAKTERYSTYKA ZMODYFIKOWANYCH BIAŁEK KERATYNY PIÓR KURZYCH

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Streszczenie

Badano preparaty: I — niezmodyfikowaną keratynę piór kurzych, II — mączkę keratynową otrzymaną przez rozpuszczenie piór kurzych w dwumetylosulfotlenku (DMSO), mączki keratynowe III i IV otrzymywane na skalę przemysłową w Zakładach Przerobu Odpadów we Wrocławiu i w Leszczynach oraz granulat keratynowo-mocznikowy V. Wyznaczono składy chemiczne badanych preparatów i stwierdzono, że wszystkie badane mączki mają bardzo wysoką zawartość białka ogólnego — powyżej 85%. Ponadto wyznaczono składy aminokwasowe metodą jakościową oraz przy zastosowaniu automatycznego analizatora aminokwasów, z których wynika, że badane preparaty I-IV są bogatym źródłem aminokwasów siarkowych. Z przedstawionych w tabeli 2 składów aminokwasowych wynika konieczność ich uzupełniania w niektóre, brakujące egzogenne aminokwasy, w metioninę i lizynę, w przypadku zastosowania do celów paszowych. Wyznaczenie zawartości azotu alfa-aminowego w badanych preparatach, było próbą określenia stopnia modyfikacji struktury białek keratynowych. Z danych przedstawionych w tablicy 3, wynika, że najbardziej zmodyfikowaną strukturę keratynową ma białko w preparacie V, a następnie II, pozostałe preparaty III i IV wykazują minimalny stopień modyfikacji w stosunku do preparatu I.