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CHARACTERISTICS OF KERATIN PREPARATION FROM HEN FEATHERS

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Certain physico-chemical properties of keratin preparation from hen feathers were determined. The analyzed preparation has a limited, pH dependent, solubility in water solutions. It is a non-homogenous protein whose main component's molecular weight is ca 8000 daltons. The preparation is susceptible to proteolytic enzymes. Its aminoacid composition is only slightly different from the aminoacid composition of full proteins from feathers.

A wide utilization of keratin products as a cheap source of nutritional preotein is hampered mainly by the complex physicochemical structure of keratin and its very poor solubility. Keratin has attracted considerable attention, as evidenced by numerous studies and analyses [2,5,14]. Among different keratin material the most susceptible to chemical modification and extraction is feather keratin. The processing methods in use so far were based on treating bird feathers with strong reducing agents (tioglycolic acid, Na_2S, $NaHSO_3$, etc) or with oxidants. Other methods in use involve either acid or alkaline hydrolysis. Strong reducing agents plus detergents and high temperature rendered 80 to 90° feather proteins in soluble form [21]. Most of the methods lead to solubilization and production of chemically modified molecules of keratin: derivatives of S-carboxymethyl (SCM); m.w.ca 10,000 daltons [24]. Such keratin preparations were not utilized in nutrition.

The rendering of homogenous preparations of keratin protein and their adaptation for nutritional purposes is described in a U.S. patent [8].

Also a French patent is known in this regard [17]. It describes a procedure for production of keratin protein utilized in fodder. Our own method of solubilization of feather keratin proteins [23]; a patent pending makes it possible to render a protein preparation, whose characteristics are the object of present analyses.

MATERIALS AND METHODS

Experimentation was conducted with hen feathers obtained from a poultry processing plant in Lublin as well as with protein preparations, soluble in dimethylosulphoxide (DMSO) from the BDH Co. The preparations were obtained from the feathers by the process given in the patent description [23].

Lowry et al.method [13] was used for determining protein in the DMSO solution and in water solutions. Calibration was carried out on protein from crystalline albumin of serum.

Electrophoresis in polyacrylamid gel followed the method given by Peacock et al [16] and Loening [12]. In the course of electrophoresis 8% gel was used at different levels of pH. 50-100μg analyzed protein was put on single gel. The protein was dissolved in DMSO or in diluted hydrochloric acid. Electropheresis was continued for 2 to 3 hours in a tris buffer — HCL 0.1 n at pH 7.2; in acidic acetate buffer (pH 3.5), and in a basic glycin buffer (pH 9.5). The presence of protein in the gel was made visible by immersing in a Serva 'Stain-All' colouring solution. Excess of the latter was removed by multiple rinsing in water. Separation of an acidic solution of the keratin preparation in polyacrylamid gel with addition of SDS was also performed, according to the method reported by Weber and Osborn [22]in a phosphate buffer (pH 7.0). Here, visualization of proteins was attained by colouring for 30 min. in the Commasi Brilant Blue solution [22].

The molecular weight was roughly estimated with the method of thin layer filtration on Sephadex G-200, as described by Andrews [1]. The standard proteins with determined molecular weight were from Serva Co. Both the analyzed preparation and whole feathers were subjected to full hydrolysis in presence of 6 n HCL in sealed glass ampulsat 110°C for 24 hours. Analysis of the aminoacid composition of the hydrolysates was carried out in a Joel auto-analyzer.

The measurement of absorption was performed in 1 cm quartz vessels with the use of a Hilger spectrophotometer. For the enzymatic analyses the following preparations were used: 1. Pronase (Koch-Light, England), 78.400 PUK/gm; 2. Trypsin ("Biomed"), 150-200 units/g; 3. Pepsin ("Bacutil") 4000 units/g. All other reagents had the standard degree of purity for analytical purposes.

RESULTS

The protein preparation obtained from hen feathers was dissolved in DMSO and precipitated by addition of 2 volumes of acetone to 1 volume of the protein solution. The developing white cheese-like sediment was separated by centrifuging at 3000 rpm and twice eluated with acetone, then with distilled water, until there was no further reaction to organic agents. After drying at room temperature a straw-coloured granulated protein preparation was obtained. The preparation contained 9.86% water, 14.7% total nitrogen and 0.5 to 1.0% lipid components. The ash contents after burning was 0.21%. The next test was on solubility of the keratin preparation, precipitated from DMSO, in water solutions. Samples of 10 mg of the sediment were mixed with 2ml 0.2 M buffer solutions, prepared after Colovick and Kaplan [4] with the pH value range from 1.0 to 12.0.

Particular samples were titrated at room temperature for four hours. The undissolved residue was separated by centrifuging. The protein dissolved in a supernatant was precipitated with the double quantity of 10% trichloroacetic acid and the resulting residue was also separated by centrifuging. The contents in the residue was determined quantitatively. The sum total of protein concentrations in the two residues always agreed with the initial concentration of protein. Solubility of the keratin preparation was dependent on the pH value is presented in Fig. 1.

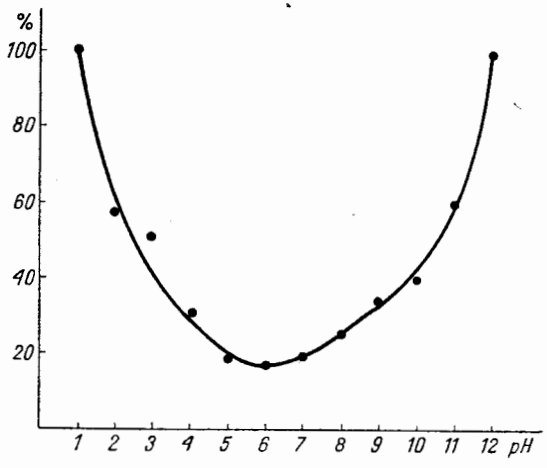

Fig. 1. Solubility of keratine preparation at different pH values

As it follows from the diagram, the analyzed preparation shows the lowest degree of solubilization between pH 5.0 and 7.0. It dissolves rather well in a strongly acidic anvironment 0.01 n HCL and in a basic environment 0.1 n Na OH.

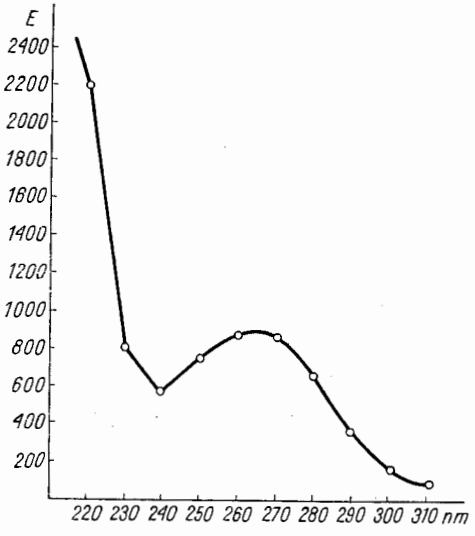

Fig. 2. Spectrum of absorption in UV of aciduous solution of keratine preparation

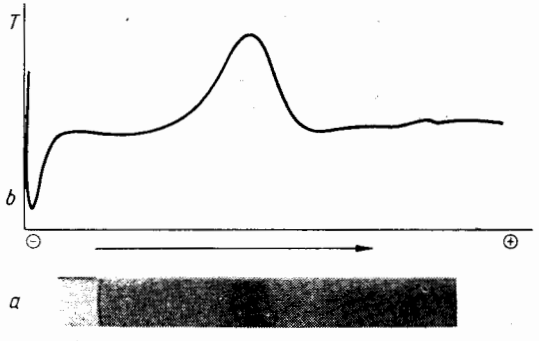

Fig. 3. Electrophoresis of keratine preparation in polyacrylamid gel at pH = 9.5; a) coloured gel, b) densitometric diagram

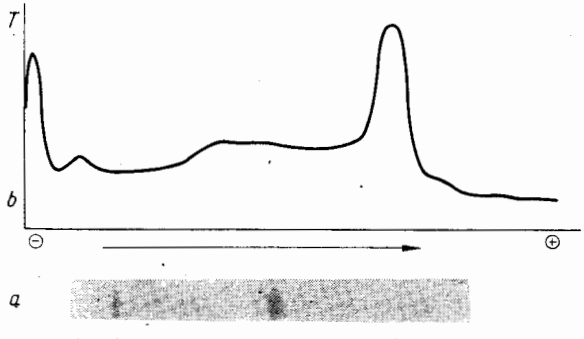

Fig. 4. Electrophoresis of keratine preparation in polyacrylamid gel at pH = 3.5; a) ditto, b) ditto

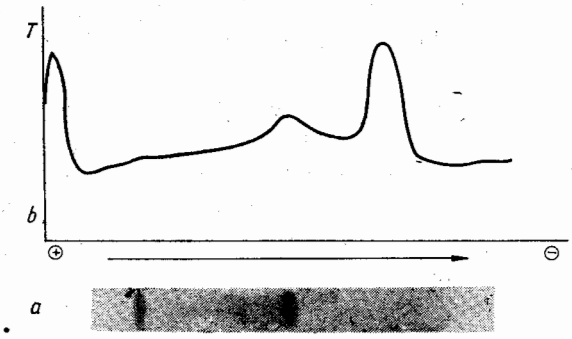

Fig. 5. Electrophoresis of keratine preparation dissolved in DMSO in polyacrylamid gel at pH = 3.5; a) ditto, b) ditto

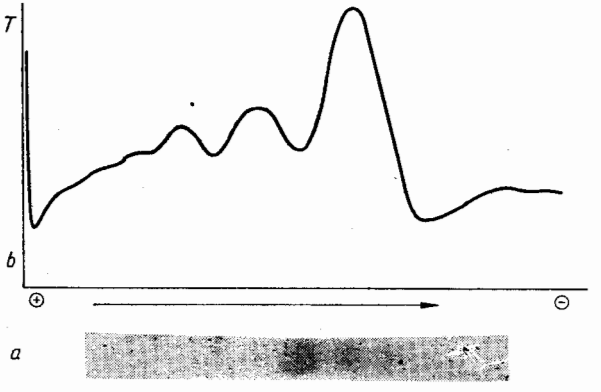

Fig. 6. Electrophoresis of keratine preparation in polyacrylamid gel in presence of SDS at pH = 7.4

Absorption of acidic water solutions of the keratin preparation was determined at different wavelengths of the light wave. The absorption curve is given in Fig. 2. The maximum levels of absorption for the given preparation stayed within the limits of 260-270 nm. The DMSO keratin solution and the water acidic solution of the keratin preparation were subjected to electrophoresis in polyacrylamid gel at varying levels of pH. The results of the analyses are illustrated by the photographs of the gel and the densitometric diagrams drafted with the assistance of a KIPP & ZONEN densitometer. (Figs. 3,4,5,6)

As it follows from the above quoted data from electrophoresis, the keratin preparation forms a single fraction at pH 9.5, while at pH 3.5, next to the main fraction and an insignificant quantity of protein remaining at the loading point, two additional fractions appear. They become even more conspicuous if the electrophoresis is extended over the solution of keratin in DMSO. Distinct heterogeneity is shown by the analyzed

preparation if its separation was carried out in presence of SDS. Next to the main fraction two other small, less mobile in the electric field, subfractions also occur.

Considering the presence of one main fraction in the analyzed keratin preparation, test were carried out to determine the molecular weight by means of the thin-layer filtration. The picture of migration of the acidic keratin preparation, as compared with standard proteins, is given in Fig. 7. The molecular weight of the main fraction determined under these conditions is ca 8.000 daltons.

Fig. 7. Thin-layer molecular filtration of keratine preparation along matrix proteins; A — 20 μg keratine preparation, B — 10 μg keratine preparation, C — crystalline aldolase, D — blood serum albumin, E — chymotrypsin

The next stage of the experiment focused on determination of susceptibility of the keratin preparation to proteolytic enzymes. Samples of the keratin protein residue resulting from precipitation from 1 ml solution in DMSO were added with 4.5 ml of the following compounds: 0.1 n HCl, 0.1 n $NaHCO_3$, or 0.01 n tris buffer — HCl (pH 7.2) and, following mixing, the mixture was incubated for 10 minutes at 37°C. Subsequently, 0.5 ml of an acidic 0.02% water solution of pepsin was added with a basic solution of 0.5 ml of 0.02% solution of trypsin. The neutral solution was added with 0.5 ml of 0.2% solution of crystalline pronase. After varying incubation times the samples were centrifuged, the supernatant was added with 10% TCA and the developing sediment was separated by centrifuging. Protein was quantitatively determined in the resulting sediments. Results of the proteolysis are presented in Table 1.

As follows from the presented table, pronase is most effective as regards decomposition of the keratin preparation. On the hand, however, a high level of proteolysis is obtained in effect of activity of the alimentary tract enzymes. After 24 hours trypsin induces 97.7% decomposition

Table 1. Enzymatic hydrolysis of tested keratin preparation

Enzyme	Digestion time min	Protein mg				% decomposition
		subject to digestion	unolisolved	precipitated TCA	digested	
Pronase	60	8.8	0.8	0.7	7.3	82.9
Pepsin	90	8.8	0.2	4.6	4.0	45.4
	180	8.8	0.1	4.1	4.6	52.2
Trypsin	90	8.8	1.8	1.7	5.3	60.2
	180	8.8	1.0	0.6	7.2	81.8
	24 hrs	8.8	0.2	0	8.6	97.7

of protein. A comparative enzymatic digestion process of the keratin preparation and the serum crystalline albumin was also carried out. The effect of proteolysis performed with pepsin and trypsin is illustrated in Fig. 8.

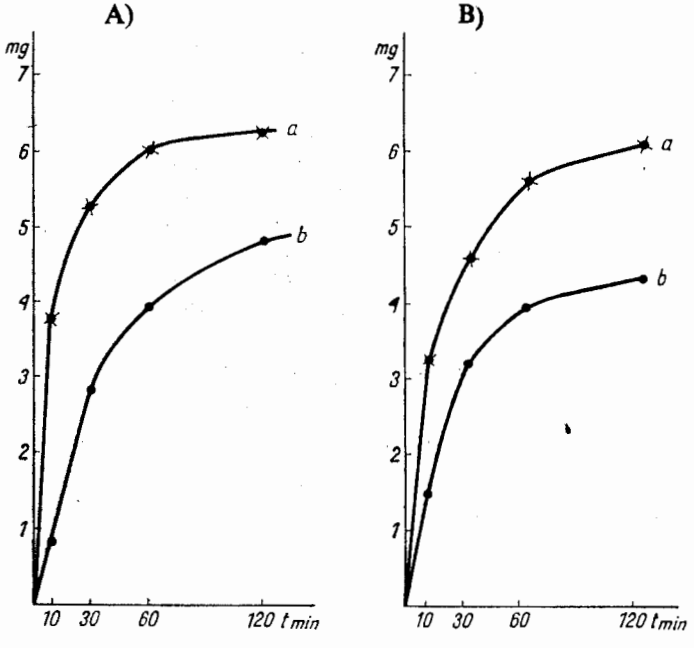

Fig. 8. Kinetic of enzymatic hydrolysis of keratine preparation (a) and crystalline albumin (b) with use of pepsin (A) and trypsin (B)

As follows from the diagrams, the kinetics of digestion of the keratin preparation does not differ from the kinetics of digestion with the same enzymes of serum proteins. Consequently, it can be surmised that the

analyzed keratin preparation is susceptible to proteolytic enzymes of the alimentary tract. Additional test decompositions of the keratin preparation with the stomach juice of a healthy human being were also completed. According to the experimental data, a 10 mg protein sample is decomposed in 89.7% within 1.5 hour.

The aminoacid composition of the soluble keratin preparation was compared with the aminoacid composition of whole feathers after their complete acidic hydrolysis. Results of the analysis are illustrated in Table 2.

Table 2. Amino-acid composition of protein hydrolysates of full feathers and of keratin preparation soluble in DMSO

Aminoacid	Protein whole feathers	Keratin preparation
Cysteine	56.6	83.2
Aspartic acid	70.2	57.0
Treonine	45.1	36.2
Serine	93.8	83.0
Glutamic acid	101.4	90.4
Proline	120.5	133.0
Glycine	125.6	145.8
Alanine	57.5	66.5
Valine	118.6	131.0
Isoleucine	39.6	44.4
Leucine	79.3	81.2
Tyrosine	2.4	1.4
Phenyloalanine	38.7	14.0
Lysine	17.5	3.4
Arginine	32.7	29.8

It follows from the presented data that the soluble keratin preparation has a different quantitative aminoacid composition as compared with the aminoacid protein composition in whole feathers. It has higher concentrations of cysteine, proline, glycine, alanine, valine as well as somewhat lower concentrations of tyrosine, phenyloalanine and lysine. The remaining aminoacids occur in roughly the same concentration.

DISCUSSION

The method patented by the present authors utilizes DMSO for solubilization of protein preparations from feather keratin. The mechanism of effects of DMSO on keratin structure is not quite clear yet. Friedman and Koenig [7] suggested that interaction occurs between keratin sulphhydryl gropus and DMSO which leads to structural changes

in keratin fibres. The results of analyzes presented here are not oriented on explaining the DMSO effects on keratin but serve as chracteristics of the protein preparation soluble in DMSO. It follows from our investigations that the keratin preparation soluble in DMSO constitutes a non-homogenous fraction of proteins completely soluble in water only in acidic or alkaline media. The main fraction molecular weight was ca 8,000 daltons; the other two had higher m. weight. Different techniques of chemical modification of keratin rendered keratin preparations with molecular weight varying between 10,000 and 11,000 daltons [9, 10]. The SCM derivatives of keratin from feathers were also non-homogenous in the electrophoretic analysis [3, 11]. Comparison of the molecular weight of the preparation we analyzed with the chemically modified preparations obtained by other authors suggests an assumption, that regardless of methods of solubilization, keratin structure undergoes destruction in effect of breaking up of disulphide bridges. This, in turn, leads to development of protein molecules of similar molecular weight.

The higher susceptibility of feather keratin to solubilizing agents is probably due to its beta structure versus the alfa structure of keratin in mammals [6, 15].

Particular morphological structures of feathers show certain differences in the aminoacid composition [9, 18]. In our investigations we were not interested in the morphological differences but we tried to determine the differences in the composition of aminoacids in full protein of feathers and in the obtained soluble keratin preparation. It turned out that the soluble keratin preparation has a different composition of aminoacids and the differences concerned mainly the concentrations of sulphur and aromatic aminoacids.

Thus, it follows from the analysis that during solubilization of feather keratin we receive a fraction that differs from full keratin in feathers mainly by a higher contents of the aminoacid of cystein. The aminoacid composition of the preparation analyzed by the present authors approximated the compositions of various keratin preparations obtained other authors [18, 19, 20].

Our keratin preparation had the properties of a fully nutritional protein, owing to its aminoacid composition. We tried, therefore, to determine its susceptibility to proteolytic enzymes. The experiments indicated that both pepsin and trypsin catalize degradation of the protein equally well as in the case of the serum albumin, analyzed for comparision. Thus it can be assumed that a soluble preparation of feather keratin may constitute a new source of nutritional protein. Wide introduction of the preparation to alimentation calls, however, for additional nutritional investigations on laboratory animals. Results of such studies will appear in our successive publication.

CONCLUSIONS

1. A feather keratin fraction, soluble in dimethylosulphoxide, precipitates quantitatively from solutions in effect of a double volume of acetone. The protein dissolves entirely in an acidic environment (pH = = 1.0) and in basic environment (pH = 12.0). The lowest solubility appears at pH = 5 to 7.

2. The analyzed preparation consists of one main fraction (m.weight ca 8,000 daltons) and a small quantity of non-differentiated fragments slowly travelling in electrophoresis on polyacrylamid gel.

3. The soluble keratin preparation is susceptible to proteolytic enzymes. Within 1 hour 65% protein undergoes proteolysis in effect of reaction with pepsin and trypsin, and 80% — in effect of pronase.

4. In comparison with full proteins of feathers the analyzed preparation shows higher concentrations of cystein, proline, glycine and valine while being less rich with tyrosine, phenyloalanine and lysine.

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CHARAKTERYSTYKA PREPARATU KERATYNOWEGO OTRZYMANEGO Z PIÓR KURZYCH

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Streszczenie

Rozpuszczalna w dwumetylosulfotlenku frakcja keratyny piór wytrąca się ilościowo z roztworu pod działaniem dwóch objętości acetonu. Białko to rozpuszcza się całkowicie w środowisku kwaśnym (pH — 1,0) i środowisku zasadowym (pH — 12,0). Najniższą rozpuszczalność wykazuje w obszarze pH 5-7. Analizowany preparat składa się z jednej głównej frakcji o ciężarze cząsteczkowym ok. 8000 daltonów oraz niewielkich ilości niezróżnicowanych fragmentów wolno wędrujących w elektroforezie na żelu poliakrylamidowym.

Rozpuszczalny preparat keratynowy jest podatny na działanie enzymów proteolitycznych. W ciągu jednej godziny 65% białka ulega proteolizie pod wpływem trypsyny i pepsyny, zaś 80% pod działaniem pronazy. W porównaniu ze składem aminokwasowym białek piór całkowitych badany preparat wykazuje wyższe stężenie cysteiny, proliny, glicyny i waliny a uboższy jest w tyrozynę fenyloalaninę oraz lizynę.