A C T A A L I M E N T A R I A P O L O N I C A
Vol. VII (XXXI), No. 3-4 1981

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STUDIES ON THE SOLUBILITY OF FEATHER KERATIN IN WATER SOLUTIONS OF UREA *)

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Key words: feather keratin, urea-water, amino acid compositions, bisulphide bridges.

Systematic studies on the solubility of feather keratin in the urea-water system, serving for the optimization of composition of keratin-urea granulates, were carried out. The dependence of keratin solubilization on the concentration of urea and time of heating was examined. From the amino acid compositions determined it may be assumed that solubilization of keratin results from the break of disulphide bridges. The quantitative amino acid composition of keratin urea granulates reveals negligible changes in relation to feather keratin what may be an evidence that the technological process of production of granulates does not cause any losses.

Keratin proteins belong to the group of proteins which are insoluble either in water or water-salt solutions and in organic solvents. The object of the study are soluble proteins which may be obtained from keratin material as a result of splitting disulphide bridges by oxidation or reduction. Oxidation of the disulphide bridges of cystine to radicals of cysteic acid gives proteins, called keratoses. When reducing them to cystein radicals, we obtain fractions, called kerateins. In order to prevent any possible secondary reactions, kerateins are subjected to carboxymethylation by the resulting -SH groups, producing S-carboxymethyl-kerateins. The main fractions of keratin proteins obtained by reduction or oxidation can be divided into smaller fractions, e.g. keratoses may be divided into a soluble fractions, containing α and γ keratoses and an insoluble β-keratose fraction, both differing in amino acid composition [1, 3, 5]. The soluble fractions of keratin contain always more sulphur than na-

*) This study was prepared within the framework of PR-4 program (sub-problem 2.5), coordinated by the Institute of Zootechnics, Cracow.

tural protein A. Fraction with a low sulphur content is extracted with 8 M urea [4, 8, 9].

The solubility of keratin increases with heating and with rise in pH of the environment [2].

The present work is a continuation of studies on obtaining modified keratin proteins for animal feeding purpose, and on their properties [10, 11, 12]. The investigations conducted on the production of keratin-urea granulates [10] require basic studies on the behaviour and solubility of chicken feather keratin in the urea-water system, under the effect of increased temperature.

EXPERIMENTAL

MATERIALS AND METHODS

The object of the studies were chicken feathers (I) derived from the Poultry Plant in Lublin and feed urea manufactured in the Nitrogen Fertilization Plant in Puławy. For preparation of the urea solution, distilled water was used. The kinetics of dissolving feather keratin was studied in water solutions of urea at a concentration of 10 to 80%. 0.2 g of whole, previously defatted feathers were weighed on an analytical balance to a testtube with ground stopper and solutions of urea with various concentrations (10-80%) in an amount of 10 g were added. The tubes were placed in a boiling water bath for 1-4 h. In order to obtain reproducible results each sample was analysed three times. When heating of the sample was stopped, it was filtered quantitatively in a Büchner funnel with a hard and dried to constant weight. The obtained solution of protein and urea was dissolved in a measuring flask to a defined volume and protein was quantitatively determined acc. to Lowry method [6] and urea was determined with the use of p-dimethylaminobenzaldehyde [7].

In order to determine the amino acid composition of the insoluble fraction (II) and of soluble fraction (III), the chicken feather keratin (I) wastreated with a saturated solution of urea (80%) at about 100°C, and divided into insoluble (II) and soluble (III) fractions.

The keratin — urea granulate (IV) was obtained acc. to the method described earlier (10). Keratin — urea granulate contained 7.5% water, the dry solids had the following composition: 72.4% by weight urea and 27.6% by weight keratin protein. The content of total nitrogen in the granulate was about 38.45%. The quantitative determination of amino acid in the examined preparations (I-IV) was made with the use of an automatic amino acid analyser, produced by Carlo Erb company.

RESULTS AND DISCUSSION

For better characteristics of the conditions for dissolving chicken feathers in a water-urea system, the proportions of these components, have been presented on the triangular diagram of Gibbs in Fig. 1.

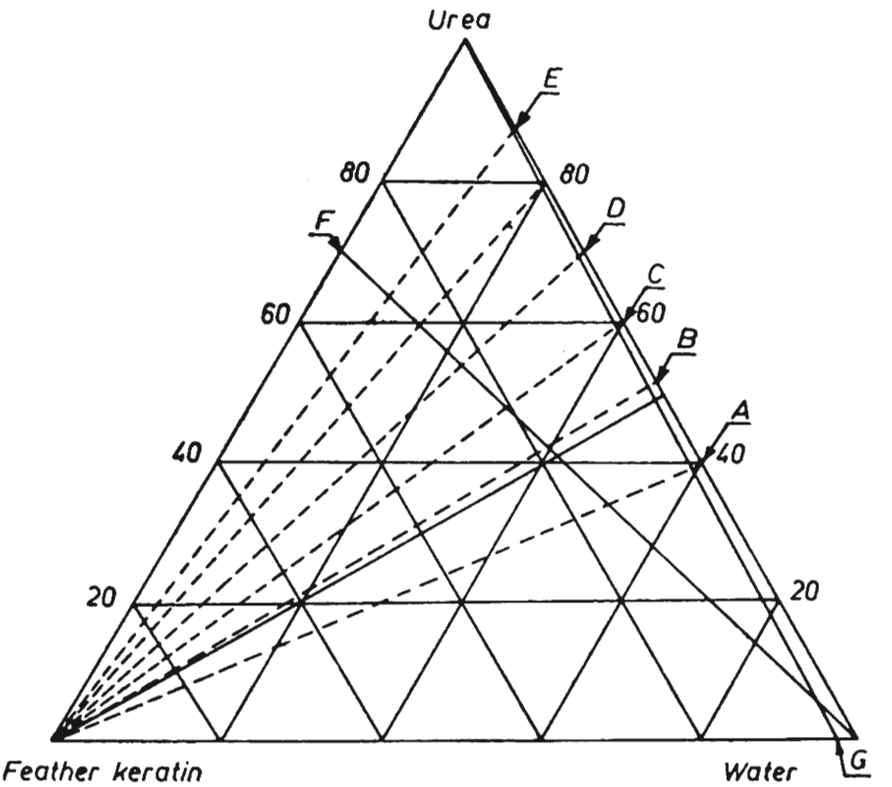

Fig. 1. Ratio of constituents in three-component system: keratine — urea — water; expressed in weight percent; points A-E show the compositions of two-component mixtures of saturated urea solutions and water, depending on the temperature of dissolving (data in Table 1); point F shows: urea 70%, keratin 30%, point G : keratin 2%, water 98%

The keratin — urea — water system is a very complicated model of a three-component system with two solid components and one liquid; one of the two solid constituents, keratin, is a high-molecular substance with a complex structure. The solubility of keratin in water solutions of urea will be always analysed at a boiling temperature of the solution which is dependent on the concentration of urea in the solution (Table 1). The points presented on the sides of the triangle, marked with letters from A to E, illustrate the composition of two-component mixtures: water — urea and their detailed characteristic is given in Table 1.

Point F shows the composition of a two-component mixture: keratin 30% by weight; urea 70% by weight. Point G has the following composition: keratin 2% by weight; water 98% by weight. The tests on the solubility of chicken feather keratin in the urea-water system can be analysed in various possible modifications.

The first possibility of studying keratin solubility is to maintain a constant ratio of two components: keratin and water, the amount of added urea being a variable component (Fig. 1 point G and line G — Urea).

Table 1. Solubility of urea in water, depending on temperature of dissolving and temperature of boiling of these solutions under normal pressure conditions

Point on diagram no 1	Temp. of dissolving in °C	Solubility		Boiling temperature in °C at normal pressure
		in molar fraction	in % weight	
A	0	0.167	40.00	105.4
B	20	0.241	51.14	107.7
C	35	0.309	59.85	110.8
D	60	0.429	71.10	115.2
E	95	0.668	87.00	126.0

The second possibility for a determination of chicken feather keratin solubility is the addition of varying weight quantities of keratin to urea solutions having a constant concentration (dashed lines, constant weight ratio: water — urea). In the third modification, a constant weight proportion of urea and feather keratin was established (Fig. 1 point F) and the amount of added water was variable (line F — Water). Being a constituent of a three-component system, having a very large own volume, feather keratin, may be examined only at certain intervals of weight ratio

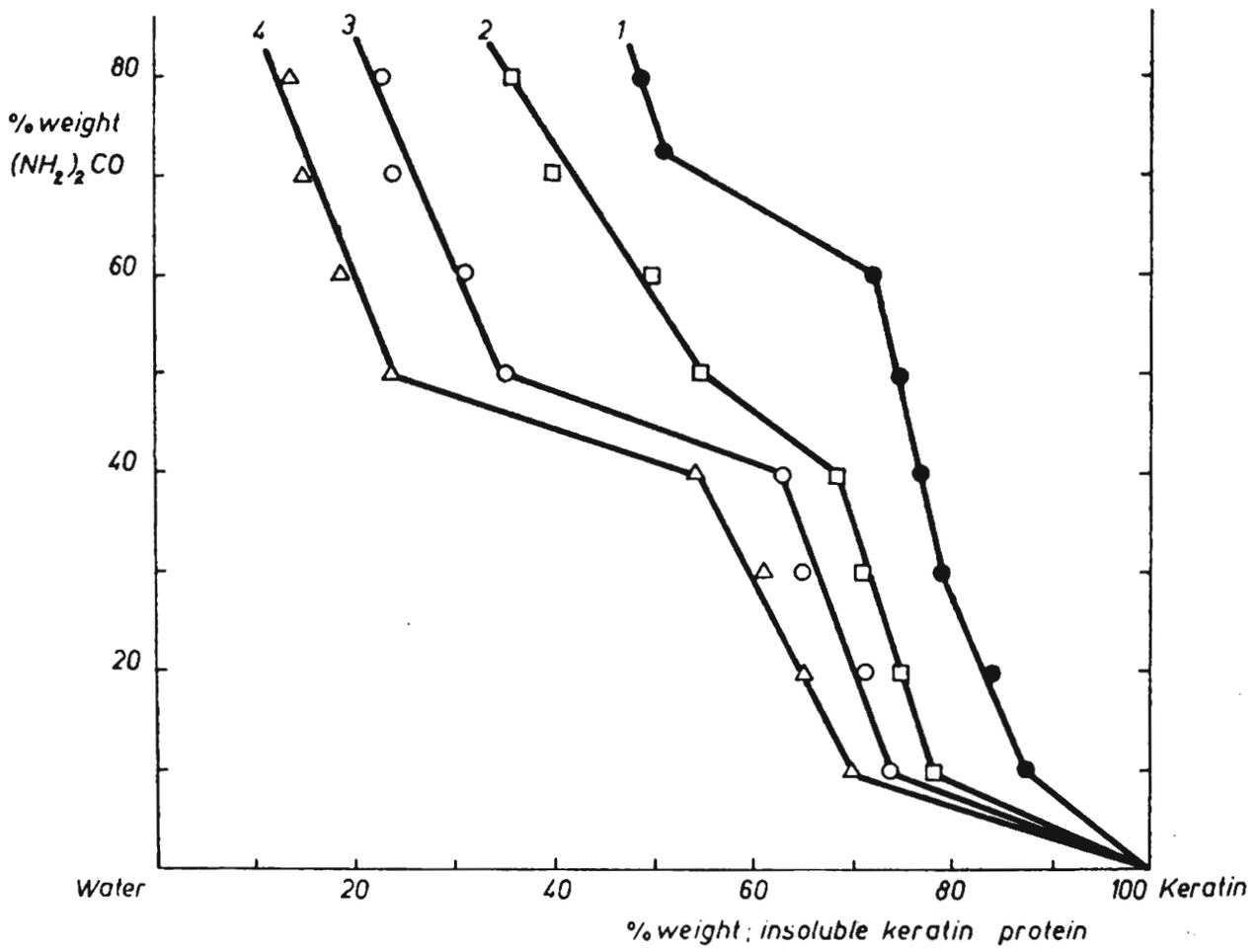

Fig. 2. The percentage content of insoluble parts of chicken feather keratin depending on the concentration of urea in water (10-80% weight) during heating from 1 do 4 hours (curves 1-4)

Table 2. The percentage of insoluble parts of feather keratin during heating for 1 to 4 h (curves 1, 2, 3 and 4) with urea solutions at a concentration of 10 to 80% weight

Concentration of urea in water in % weight	% by weight of insoluble parts of feather keratin after heating during:			
	1 h	2 h	3 h	4 h
10	87.0	78.0	74.0	70.0
20	84.0	75.0	71.0	65.0
30	79.0	71.0	65.0	61.0
40	77.0	69.0	63.0	54.0
50	75.0	55 0	35.0	24.0
60	72.0	50.0	31.0	19.0
70	51.0	40.0	24.0	15.0
80	48.0	36.0	23.0	14.0

Table 3. Quantitative amino acid composition of chicken feather keratin (I) in comparison to amino acid compositions of insoluble fraction (II) and soluble fraction (I II) obtained by treatment with saturated (at 100°C) urea solution, and to the compo sition of keratin — urea granulate (IV).

No.	Amino acid	Preparation no.			
		I	II	III	IV
1.	Alanine	3.89	7.15	5.48	4.23
2.	Arginine	6.83	4.36	5.75	7.82
3.	Aspartic acid	5.24	5.85	5.36	5.97
4.	Cystine	7.07	1.09	8.65	7.24
5.	Glutaminic acid	8.74	9.36	8.70	10.90
6.	Glycine	6.16	10.35	11.68	6.98
7.	Histidine	0.51	0.35	0.58	0.71
8.	Isoleucine	4.33	4.73	5.10	5.27
9	Leucine	7.16	8.25	7.38	7.06
10.	Lysine	1.68	1.25	1.58	2.25
11.	Methionine	0.41	0.35	0.48	0.53
12.	Phenylalanine	4.22	3.78	4.32	4.54
13.	Proline	8.86	7.95	8.26	10.34
14.	Serine	10.18	10.60	9.58	11.04
15.	Treonine	4.01	4.70	3.35	11.19
16.	Thyrosine	2.38	1.75	2.10	2.49
17.	Valine	6.60	8.15	7.28	4.53

with respect to other constituents of the system discussed. Such a limit for the preparation of keratin-urea granulates had the following ratio: urea 70%; feather keratin 30% (Fig. 1 point F).

Taking the first possibility for the study of solubility, i.e. constant weight ratio of feather keratin and water (Fig. 1 point G), the kinetics of dissolving keratin in the urea-water system was determined. Fig. 2

illustrates the percentage of insoluble parts of keratin protein, depending on the concentration of urea in % weight in water during heating for 1-4 h.

The data for this diagram have been collected in Table 2.

The quantitative determination of amino acid composition in the particular preparations (I-IV) allows to evaluate the influence of the conditions of dissolving feather keratin in water solutions of urea at boiling temperature of water-urea solution in comparison to the quantitative composition of amino acid in chicken feathers untreated with urea or its water solutions (I). Table 3 shows the quantitative compositions of amino acids in preparations I-IV.

DISCUSSION

The Gibbs phase diagram presented in Fig. 1 indicates the possibility of selecting the ratio of particular components in the system. The limiting parameter for the investigations on the solubility of feather keratin in the water-urea system, specific the large is volume of feather keratin, in comparison with other components in the system. For this reason, the systematic studies on the solubility of keratin during the production of keratin-urea granulates, could be carried out within certain limiting proportions, e.g. urea 70% weight and feather keratin 30% by weight; point F and keratin 2% by weight and water 98% by weight, Fig. 1, point G. The data presented in Table 1 show that together with the rise in water temperature, the solubility of urea in water and the boiling temperature of these solutions are increased. This has probably a considerable effect on the process of solubilization of keratin. The second important factor which may effect the process of keratin solubilization (under the conditions: water + high temperature) is the process of urea amonolysis as a result of which the alcalinity of the solution increases to pH 8-9. It is well known that an increase in pH of the medium in the alkaline direction may cause a transfer of disulphide bonds to radicals of sulphinyl acids and thiol groups [2] which results in an increased solubilization. Such an argumentations is supported by the amino acid composition of the soluble part of keratin (III) which contains a very high level of sulphuric amino acids, over 8-times more than the insoluble fraction of keratin (II) (Table 3).

The kinetics of solubility of feather keratin presented in the rectangular system of coordinates in Fig. 2 shows the percentage dependence of insoluble parts of feather keratin in the urea-water system. The diagram shows that the solubilization of keratin proteins depends on the percent concentration of urea in water and heating time of the sample.

Obtaining a 50% solubilization of feather keratin during 1 hour heating requires use of urea solutions with concentration about 70% weight.

Prolongation of heating time from 2-4 h allows to reach a considerably higher degree of solubilization than with a lower concentration of urea. Solutions of urea with concentration of 50% weight during heating the feather keratin for 4 hours, give an almost 80% solubilization, and increase in the concentration of urea to 80% weight changes only insignificantly the percentage of solubilization: about 10%, like when heating for 2 and 3 hours.

It follows the data presented in Fig. 2 and in Table 2 that to achieve a solubilization degree within 50% during a short heating time, it is necessary to use urea with concentration 70-80% weight. Prolongation of heating time (2-4) allows to obtain an equally high percentage of keratin solubilization, using urea solutions with concentration 40-50% by weight.

The quantitative composition of amino acids in feather keratin (I) as a control preparation has been evaluated in previous publications [11]. When determining the amino acid compositions in the preparations obtained as a result of heating feather keratin with urea solutions (I-IV), it has to be stated that the process of solubilization affects, to a certain degree the quantitative composition of amino acids. This is especially visible in the composition of insoluble fraction II and soluble fraction III. They differ in cystin content, the nearly total amount of which was found in the soluble fraction, which may explain the mechanism of solubilization. The other differences between preparation II and III are small while both preparations differ from the control preparation (I) in the level of alanine glycine and valine. All these amino acids are found in preparations II and III in greater quantities than in the control preparation (I). The composition of amino acids in keratin — urea granulate IV is similar to that of feather keratin (I) being the control preparation. The main difference lies in the considerably higher level of treonine and a certain rise in the level of glutamic acid, lysine and proline and small decrease in valine content. It is worth mentioning that the content of sulphuric amino acids in keratin — urea granulate is similar to that of the control preparation (I) i.e. feather keratin. This may be an indication of the fact that the technological process of production of keratin-urea granulates does not have any unfavourable effect on the change in the composition of amino acids. It includes also losses in sulphuric amino acids which are usually lost in the process of alkaline hydrolysis or therm hydrolysis of keratin raw material.

CONCLUSIONS

1. The systematic studies on the solubilization of feather keratin (I) in the water — urea system (at boiling temperature), conducted in various possible modifications, according to variable ratios of the particular

components presented in the triangular phaseous diagram of Gibbs, will serve as data for the optimization of the production process of keratin — urea granulates for feeding purpose.

2. The results of investigations presented in this paper, point to the dependence of the degree of keratin solubilization (I) on the concentration of urea in water and time of heating the sample. Shortening of the heating time requires an application of very high concentrations of urea, about 80% by weight and a prolongation of time above 2 hours gives similar effects of solubilization at a concentration of urea about 50% by weight.

3. A comparison of the quantitative compositions of amino acids present in the preparations examined (I-IV) makes it possible to assume that the process of solubilization occurs mainly due to the break of disulphide bridges. This is supported by the high level of cystine in the soluble fraction (III).

4. A quantitative evaluation of the amino acid composition of the keratin — urea granulate (IV) in comparison to that of feather keratin (I) reveals only insignificant changes. This indicates that the technological process of producing keratin-urea granulates does not cause losses in amino acids content.

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Manuscript received: July, 1980
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ROZPUSZCZALNOŚĆ KERATYNY PIÓR W WODNYCH ROZTWORACH MOCZNIKA

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Streszczenie

Badania nad rozpuszczalnością keratyny piór stanowią kontynuację badań nad otrzymywaniem białek keratynowych na cele paszowe oraz ich właściwościami. W pracy wykonano systematyczne badania nad solubilizacją keratyny piór (I) w układzie: mocznik — woda w temperaturze wrzenia roztworów. Badania powyższe realizowane były w różnych wariantach, według zmiennych proporcji poszczególnych składników, co przedstawiono na trójkątnym diagramie fazowym Gibbsa (rys. 1). Wyniki tych badań posłużą jako dane do optymalizacji procesu otrzymywania granulatów keratynowo-mocznikowych na cele paszowe. Badanie kinetyki rozpuszczalności keratyny piór (I) pozwoliło określić stopień solubilizacji keratyny (I) od stężenia mocznika i czasu ogrzewania próbki (rys. 2).

Skrócenie czasu ogrzewania keratyny z roztworami mocznika wymaga stosowania bardzo wysokich stężeń mocznika ok. 80% wag. Podobne efekty solubilizacji keratyny (I) można uzyskać stosując roztwory mocznika o mniejszym stężeniu (ok. 50% wag.) w wydłużonym powyżej 2 h czasie ogrzewania (rys. 2 i tab. 2). Ocena składów ilościowych aminokwasów badanych preparatów (I-IV) (tab. 3), pozwala przypuszczać, że proces solubilizacji keratyny piór (I) przebiega głównie wskutek rozrywania mostków dwusiarczkowych, czego dowodem jest różnica w zawartości cystyny we frakcji rozpuszczalnej (III) oraz frakcji nierozpuszczalnej (II) (tab. 3). Z porównania składów aminokwasowych granulatu keratynowo-mocznikowego (IV) i keratyny piór (I) wynika, że proces technologiczny otrzymywania granulatów keratynowo-mocznikowych wpływa nieznacznie na zmiany składu ilościowego aminokwasów. Zawartość cystyny w granulatach (IV) i w keratynie piór (I) (tab. 3) dowodzi, że proces technologiczny nie powoduje strat tych aminokwasów traconych często w trakcie hydrolizy alkalicznej lub termohydrolizy.