A C T A A L I M E N T A R I A P O L O N I C A
Vol. V (XXIX), No. 4 1979

TADEUSZ WOLSKI

# FEED PROTEIN FROM VASTE KERATIN MATERIALS

Department of Basic Chemical Sciences of the Medical Academy, Lublin

Key words: feater keratin, dimethylsulphoxide, fodder protein.

A method of solving chicken feather keratin proteins in dimethylsulphoxide (DMSO) was developed. Two fractions of modified keratin were obtained: one which dissolves in DMSO, that is precipitated after acetone is added, and another one which does not dissolve in DMSO. The soluble fraction contains 90.5% protein. This protein contains approximately 7% of sulphuric amino acids and its real digestibility is 76.7. The method described in this paper is a continuous dissolving method which also encures the circulation of solvents in a closed circuit.

The implementation of the outlines of programs for rational utilisation of protein requires the development and introduction of new production methods and for the utilisation of wastes and by-products in many branches of the food industry [11, 18, 19, 22]. This goal may be achieved by modifying the keratin structure, improving solubility and assimilability and enriching the low-value keratin protein with the lacking exogenic amino acids, mainly lysine, methionine and additionally, tryptophan and histidine.

The amounts of non-edible by-products for various birds of terrestial habit are different [18]. Thus, for example, 17.5 kg of post-drawing offals, 3.5 kg of blood and 7 kg of feathers — all in all 28 kg of by-products [18] are obtained for every 100 kg of live broilers. Many attempts were being made with the purpose to utilise the feathers as fodder e.g. after enzymatic or chemical hydrolysis. Today, the production of meals by thermohydrolysis is one of the industrial methods of processing soft offals, feathers and blood. This process depends on heating wet feathers, with some blood added, at a temperature of 120-130°C during 0.5-2 hours under a pressure of 2-3 atmospheres [11, 18, 19].

*) This study was prepared within the framework of PR-4 program (subproblem 2.5) co-ordinate by the Institute of Zootechnics in Cracow.

Nevertheless, the degree of utilisation of the wastes in the egg poultry industries is still low. As a result, great amounts of wastes are left non-utilised. Thus, for example some 35% of egg wastes and 75% of feathers remain non-utilised [22]. During the next two years the amounts of wastes in the poultry slaughterhouses are to be doubled (compared with 1975). The problem will be acute in 1980, when the annual production of slaughtered poultry will amount to 500 000 tons.

This is why the investigations connected with the problem of utilisation od wastes are important not only from the economic point of view, but also in the environment protection aspect. There are also considerable losses of valuable nutritive ingredients due to incorrect processing.

The properties of keratin proteins depend on their structure and composition. Keratins may contain from 4 to 14% suiphuric amino acids (cystine and cysteine) which form the disulphide — S — S — bridges between the protein polypeptide chains. The presence of disulphide bonds in keratin protein is responsible for its great resistance to the effect of physical, chemical and enzymatic factors. Keratin does not dissolve in water or in water salt solutions and in the majority of organic solvents.

A change or modification of keratin structures may be obtained treating the keratin protein with reducing agents, oxidisers, concentrated urea solutions or water solutions of acids or alkalines, and with some polar solvents having strong electron-donor properties, such as dimethyl-formamide (DMF), dimethyl-sulphoxide (DMSO) and dimethyl-acetamide (DMA) [1-3, 5, 9, 23].

There are no data in the literature which would deal with fundamental researches and the kinetics of dissolving keratin proteins in organic solvents.

The application of dimethyl formamide in order to obtain keratin protein preparations for use as additives to foodstuffs for human consumption [9] has lead the author to the idea of the usefulness of DMSO for these purposes as a solvent of similar physico-chemical properties, but much less toxic and widely used in industrial practice, and having also some therapeutical properties [6, 17]. The attempts of using DMSO instead of DMF gave interesting results [23].

Therefore the author decided to investigate the effect of various parameters on the chicken feather keratin protein dissolving in dimethyl-sulphoxide.

## MATERIALS AND METHODS

Chicken feathers from the Poultry Plant in Lublin were used for solubility tests. Dimethylsulphoxide (DMSO) produced by International Enzymes Ltd., Britain with a water content of 0.2% and purity in accordance with literature data [17] was used as the solvent. Feathers solubility was tested in a three-neck round-bottom 1.5 $dm^3$ flask heated electrically and provided with a stirrer, a common two thermometers (a common one of a contact-type) and a reflux condenser 20 g dry feathers were placed in the flask and then 1000 g DMSO were added. The whole contents were heated, with the stirrer reflux condenser and heater engaged, so as to maintain the temperature within 100-105°C for four hours. The hot reaction mixture was then filtered of in the Büchner funnel with the help of a water pump. The sediment in the funnel, i.e. the insoluble remanings of the flask content was washed and dried. 6.5 g dry matter obtained as a result, could easily be powdered into meal. The solution of protein in DMSO (1 $dm^3$) was cooled to room temperature and then mixed with 2 $dm^3$ acetone to precipitate white, cheese-like sediment which, after falling down and after the solvent had been decanted, was filtered and washed until the negative reaction to acetone and DMSO. The water from washing the sediments precipitate was collected and used afterwards for the regeneration of DMSO. The mixture of solvents consting of acetone and DMSO was separated by distillation for repeated use: DMSO for solving the feathers and acetone to precipitate protein. The white cheese-like precipitate obtained, with the teste of skimmed-milk cottage cheese, was dried at a temperature of 60-80°C. In this way 12.6 g dry powder of light-yellow colour were obtained from one batch.

The kinetics of chicken feathers dissolution was tested in the following way: 0.2 g of whole feathers was weighed and then transferred quantitatively to test-tube with ground stoppers. Then a present amount of DMSO was added to the test-tube from a burette, and the content was heated in a boiling water bath for 1-8 hours. Afterwards heating was stopped and protein was determined quantitatively by Lowry et coll, method [16]. The calibration of the method was carried out with the protein of crystalline serum albumin. In this way the optimum feathers to solvent ratio and the effect of temperature on the kinectics of solving were determined, by heating the test-tubes at 40, 60, 80 and 100°C.

Keratin protein obtained by this method was then subjected to digestion with proteolytic enzymes [4] and its biological value and total digestivity were determined by the balance-sheet method on the rats for dry preparation compared with other similar protein preparations [10].

## RESULTS OF INVESTIGATIONS

Investigations of the solubility of chicken feathers were started with comparing their solubility in polar organic solvents having electron donor properties during heating at a temperature of approximately 100°C. The kinetics of chicken feather dissolving in DMSO, DMF and DMA expressed as the percentage of soluble protein as function of the heating time is presented graphically in Fig. 1.

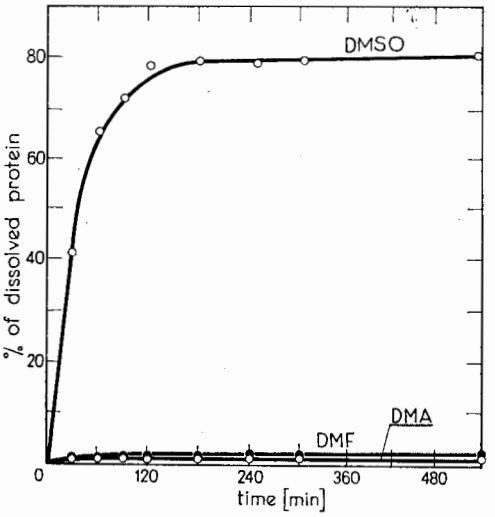


Fig. 1. Solubility of chicken feather keratin during heating at 100°C in pure organic solvents: dimethylsulphoxide (DMSO), dimethyl formamide (DMF) and dimethylacetamide (DMA). The feather ratio to DMSO, DMF and DMA was 1:50

To show the effect of temperature on chicken feathers solubility in DMSO, the percentage of soluble proteins was determined depending on the time of heating for 40, 60, 80 and 100°C. The dependence between these values is shown in Fig. 2.

One of the basic relationships which decisively affect the yield of the technological process is the raw material to solvent ratio. Thus the optimum weight ratio of feathers to DMSO, at which solubility degree is the highest, had to be determined. The results are illustrated in Fig. 3.

To obtain greater quantities of keratin protein by dissolving chicken feathers in DMSO, a process with the closed circulation of solvents was used, as shown in the following figure (Fig. 4).

Table gives the digestion data determined by the balance-sheet method on the rats and determining the biological value and total digestivity of dry keratin preparation obtained by dissolving feathers in DMSO in comparison to other preparations of this type, which is described in detail

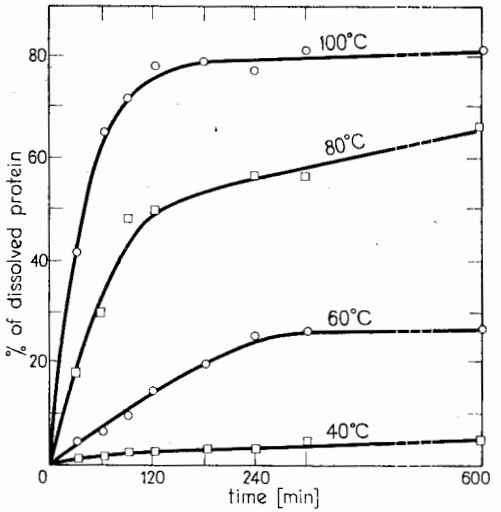


Fig. 2. Solubility of chicken feathers in dimethylsulphoxide (DMSO) during heating, for the following temperatures: 40, 60, 80 and 100°C. Feather to DMSO ratio was 1:50

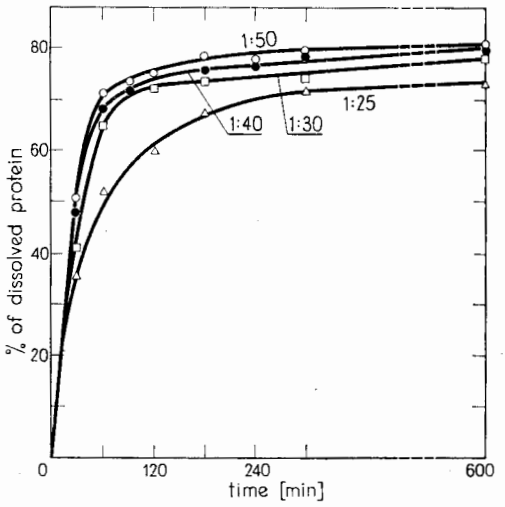


Fig. 3. Solubility of chicken feathers in dimethyl sulphoxide (DMSO) during heating at 100°C for the feathers to DMSO solvent ratios of 1:25, 1:30, 1:40, 1:50

in a separate publication [10]. Dried preparation contained 9.5% water. 14.65% total nitrogen, 0.2 ash and 0.3% lipids. The wet protein preparation obtained by precipitation from DMSO solution, and washed with water, dissolves completely in an acid medium (pH = 1) and in alkaline medium (pH = 12). Dried powder is difficult to dissolve both in acid and alkaline media. Total digestivity is 76.7, and sulphur aminoacid contentabout 7%.

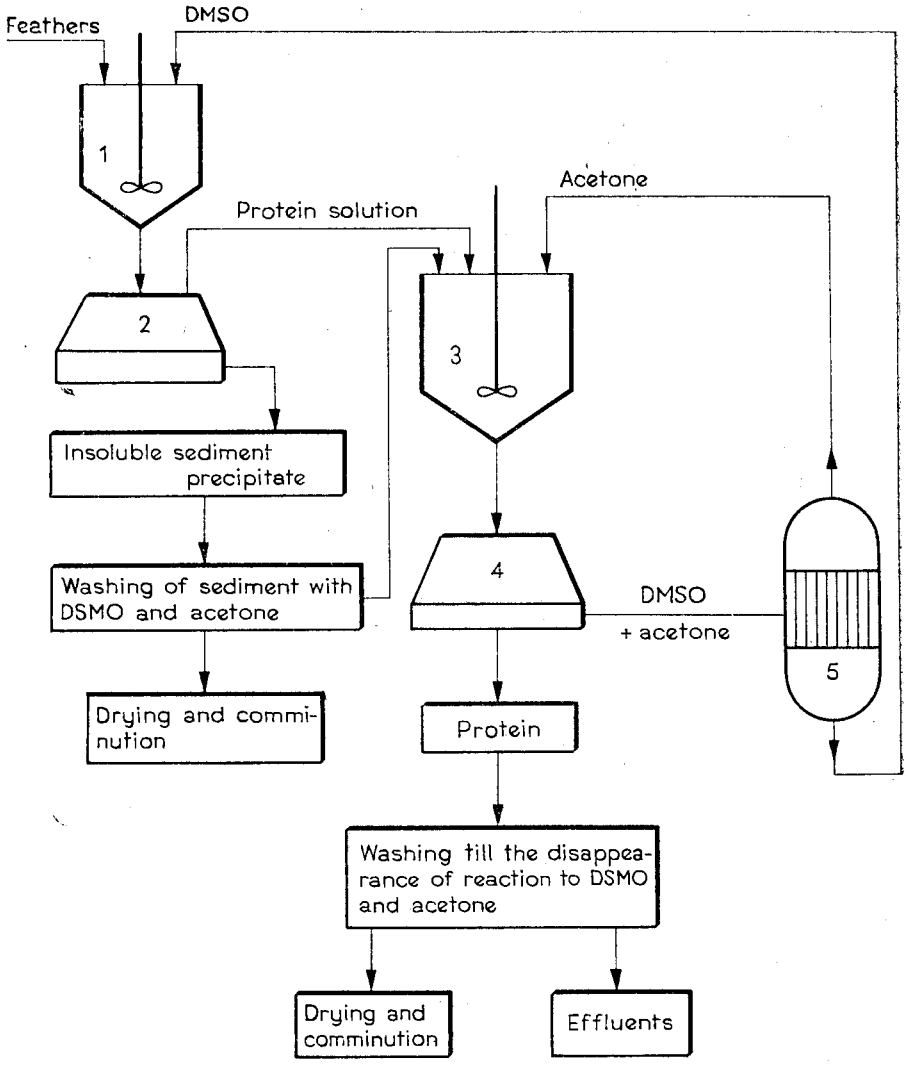


Fig. 4. Technological flow-sheet: 1 — reactor for solving chicken feathers in DMSO, 2 — centrifuge or filtering press for separation of protein solution from insoluble fraction, 3 — reactor for precipitation of proteins from solution, 4 — filtering press for separation of precipitated protein, 5 — unit for distillation of solvents

Table 1. Nutritive values determined by the balance-sheet method on trats

| Protein preparation | Biological value (BH) | Total digestivity (TD) |
|---|---|---|
| Keratin meal obtained from feathers by dissolving them in DMSO, precipitation and drying | 31.1 | 76.7 |
| Keratin meal obtained from feathers as above fish meal (protein 1:1) | 49.1 | 83.4 |
| Fish meal | 70.1 | 94.8 |
| Keratin meal from feathers obtained according to Polish technology — ZPO Wrocław | 42.7 | 90.0 |
| Keratin meal from feathers obtained according to licence technology — ZPO Leszczyny | 42.7 | 94.7 |

## DISCUSSION OF RESULTS

The solubility of feather chickens keratin determined for pure organic solvents determines their ability to modify the keratin structure. From the graph shown in Fig. 1 it follows that DMSO has a very high ability to modify the keratin structure compared with DMF and DMA which hardly affect this structure. Dimethylsulphoxide already after two hours of heating of the samples on a boiling water bath dissolves some 80% of the weighed portion of feathers. Further heating only slightly increases the percentage content of soluble protein.

It follows from the preliminary tests that the feather keratin solubility depends, to great extent, on temperature. The results of measurements on chicken feather keratin protein solubility kinetics shown in Fig. 2 for 40, 60, 80 and 100°C reveal a great span of solubility which for 40°C does not exceed 5% for a time of 10 hours, whereas for 100°C this value increases to more than 80%. At 60°C the maximum percentage of soluble protein is 25%, and for 80% — approximately 65%. From the data shown in Fig. 2 it also results that the highest output of dissolving in the shortest time can be obtained when the temperature of the mixture being dissolved is approximately 100°C. Then the percentage of dissolved protein after two hours of heating will be close to 80%. Further heating and prolongated time of heating only slightly changes this value. It is also interesting that heating of feathers in DMSO for two hours at 80°C gives a protein solubility percentage of nearly 50, whereas the same figure for 60°C is only 15%. By elevating the temperature from 60 to 100°C one can obtain a 5-fold reduction of the heating time.

For technological reasons it is necessary to determine the optimum proportion of the raw material to DMSO. The raw material such as chicken feathers has a large volume and therefore in the course of

heating one should add solvent so as to keep all the raw material in a wet condition. From the authors tests (Fig. 3) it results that the optimum ratic of material and the solvent (DMSO) is 1:50. If the quantity of the solvent is reduces by a half, the percentage solubility of keratin protein distincly decreases. This is due to the incomplete covering of feathers by DMSO.

The basic tests carried out and determining the kinetics of dissolving keratin in chicken feathers as shown in Figs. 1-3 have made it possible to develop a flow-sheet and to introduce it on an pilot — plant scale.

From the flow-sheet shown in Fig. 4 it results that the solvent, DMSO, used to dissolve chicken feathers keratin may be used in a closed circuit. It flows from reactor [1] where the keratin dissolving process takes place, through centrifuge or filtering press [2] to tank [3] where it is mixed with acetone and where protein is precipitated. Then it flows again through the filtering device [4] to the distillation unit [5] where acetone (boiling temperature 56°C) it easily separated from DMSO (boiling temperature 189°C under normal pressure). The solvents separated by distillation return: DMSO to reactor [1] to dissolve feather keratin there, and acetone to reactor [3] where is precipitates protein. The insoluble parts of the keratin fractions, separated in the filtering unit [2] are washed, dried and easily powdered into keratin meal. The precipitated keratin protein of a consistence of cottage cheese, separated in the filtering unit [4], is cleaned from solvents used in the technological process and used in the wet condition, or it is dried and powdered.

## CONCLUSIONS

From the author's investigations the conclusions are as follow:

1. Dimethylsulphoxide (DMSO) is a good solvent for the modification of keratin structure of the chicken feathers. In this process a solution of protein fractions precipitated e.g. by acetone and an insoluble fraction (in the form of keratin meal) is obtained. No other comparable organic solvents had such ability.

2. Protein obtained by this method is well digisted in vitro [4] and it also shows good total digestivity in vivo [10]. The relatively low biological value of this protein results from the very low content of some exogenic amino acids, mainly lysine and methionine, and histidine and tryptophan [10] keratin meals from feathers can be obtained by adding other organic wastes, (e.g. blood to the raw material).

3. The described method of obtaining keratin proteins may be carried out in a continuous way. It is also ensures the possiblity of regeneration of solvents used for the process, i.e. acetone and DMSO.

4. Protein solutions in DMSO have long service life and they can be

stored for a long time. Precipitated protein may be used wet or dried. Dried protein may easily be powdered and it can be easily transported and stored due to its long shelf life.

5. The chicken keratin dissolving process should be carried out at a temperature of approximately 100°C, because then the output is the highest and heating time is the shorest. Heating for more than 4 hours slightly improves the effect of dissolving.

6. The optimum raw material to solvent ratio is 1:50.

7. To ensure a more economical dissolving process, water from solution in DMSO should be used to precipitate protein DMSO regeneration should be carried out by distillation under reduced pressure.

8. As a results of the investigations described in this paper a flowsheet for feather keratin dissolving and for obtaining soluble and insoluble fractions, has been developed.

LITERATURE

1. Anker Ch. A.: US Pat. No. 3.464.825, 1969.
2. Blazej A., Galatik A., Minarik L., Humpa D.: CSR Pat. No. 147.548, 1973.
3. Blazej A., Soncek J., Galatik A.: CSR Pat. No. 157,930, 1975.
4. Borkowski T., Wolski T., Pasternak K.: Acta Alimentaria Polonica 1978, **4**, (3), 275.
5. Brokes P., Tachyna J.: CSR Pat. No. 157.859, 1975.
6. Chem. Eng. News: 1978, **56**, (13), 6.
7. Chomyszyn M. Moczniki i inne związki azotowe niebiałkowe w żywieniu przeżuwaczy, PWRiL, Warszawa 1960.
8. Dąbkowski Z.: Przem. Spoż., 1975, 29, (11), 419.
9. Goodwin W. D.: US Pat. No. 2.250.864, 1973; No. 3.773.745, 1973; No. 3.970.614, 1976.
10. Hanczakowski P., Wolski T.: Roczn. Naukowe Zootechniki (in press).
11. Jeske J., Doruchowski W., Wcisło H.: Przem. Spoż., 1976, 30, (7), 255.
12. Kluk T.: Przem. Spoż., 1975, **29**, (2), 49.
13. Kluk T.: Przem. Spoż., 1975, **29**, (12), 459.
14. Kluk T.: Przem. Spoż., 1976, **30**, (1), 2.
15. Looski J. K., McDonald J. W.: Związki azotowe niebiałkowe w żywieniu przeżuwaczy, PWRiL, Warszawa 1971.
16. Lowry O. H., Rosenbrough H. J., Faar A. L., Randall R. J.: J. Biol. Chem., 1951, **193**, 265.
17. Martin D., Hauthal H. G.: Dimethylsulfoxid, Akademie Verlag, Berlin 1971.
18. Niewiarowicz A.: Niejadalne produkty uboczne drobiu. WNT, 1977, 367.
19. Pietrowska J., Doruchowski W., Konarkowski A.: Przem. Spoż., 1978, **32**, (5), 176.
20. Prędecka K., Sztarbałło W.: Przem. Spoż., 1975, **29**, (11), 425.
21. Rutkowski A.: Przem. Spoż., 1977, **31**, (5), 174.
22. Sokołowska A.: Przem. Spoż., 1977, **31**, (10), 384.
23. Wolski T., Borkowski T., Soczewiński E. et all: Pat. PRL No. 100, 847, 1978.
24. Życki W.: Przem. Spoż., 1975, **29**, (3), 97.

*Manuscript received: December, 1978*
*Author address: 20-209 Lublin, Mełgiewska 2*

*T. Wolski*

# OTRZYMYWANIE BIAŁKA PASZOWEGO Z ODPADOWYCH SUROWCÓW KERATYNOWYCH

Instytut Chemii Podstawowych, Akademia Medyczna, Lublin

Streszczenie

Przeprowadzono badania nad rozpuszczalnością keratyny piór kurzych w dwumetylosulfotlenku (DMSO) w porównaniu z dwumetyloformamidem (DMF) oraz dwumetyloacetamidem (DMA) (rys. 1), które wykazały, że DMSO jest dobrym rozpuszczalnikiem modyfikującym strukturę keratynową piór. Rozpuszczalną w dwumetylosulfotlenku (DMSO) frakcję białka można wytrącić przez rozcieńczenie DMSO np. acetonem. Zbadano również wpływ temperatury na kinetykę rozpuszczania piór kurzych, co przedstawiono na rys. 2. Stwierdzono, że w temperaturze 100°C zachodzi prawie maksymalne rozpuszczenie już po upływie ok. 2 godz. ogrzewania. Na rys. 3 wyznaczono optymalny stosunek surowca — piór do rozpuszczalnika — DMSO, który wynosi 1:50. Opracowano ciągłą metodę otrzymywania rozpuszczalnych białek keratynowych z możliwością regeneracji rozpuszczalników, które cyrkulują w obiegu zamkniętym. Schemat technologiczny rozpuszczania piór w DMSO podany jest na rys. 4. Otrzymane roztwory białek, jak również wysuszone po wytrąceniu białko keratynowe, są trwałe i mogą znaleźć zastosowanie, zarówno do celów paszowych, jak i do produkcji odczynników biochemicznych. Otrzymane tą metodą białko keratynowe, stosowane do celów paszowych należałoby wzbogacać w niektóre brakujące egzogenne aminokwasy, a także stosować mieszanki obu frakcji rozpuszczalnej i nierozpuszczalnej.