

UTILIZATION OF BIODEGRADABLE KERATIN AND COLLAGEN-CONTAINING WASTES THROUGH ENZYMATIC TREATMENT

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1. ABSTRACT

In the production of chicken meat the main waste are feathers and in the production of leather the main waste that remains after splitting of limed hides is fleshing (i.e., the lowest layer of the skin together with the underlying fatty tissue). Both wastes represent a problem for the industry since they pollute the environment. Their utilization is difficult since the feathers have a very rigid protein structure, and the fleshing contains too much water, lime and fat. But both wastes contain to much protein (feather - 80-90%, fleshing - 30-35% on dry mass basis). In this work, simple technologies for processing waste feathers and fleshing are depicted to produce a protein hydrolysate from feathers and raw gelatin, cattle tallow and protein hydrolysate from fleshing. Both technologies are applicable to a large-scale production.

2. INTRODUCTION

The protein shortage for food and feed oblige us to look for new protein sources, including waste products. Feathers are a bio-resource with a high protein content (more than 750 g kg⁻¹ crude protein). Poultry slaughterhouses produce large amounts of feathers. Further, burning in special installations is economically ineffective. Uncontrolled disposal of feathers is environmentally unacceptable. The solution of the problem is obligatory since poultry production plays a vital role in the protein supply and also in the agricultural economy for many countries in the world. Five percent of the body weight of poultry is feathers; from a slaughterhouse with a capacity of 50 000 birds daily are produced 2-3 tones dry feathers. Because of their high protein content, feathers can be counted as a protein source for food and feed but for this purpose the complete destruction of the rigid keratin structure is necessary.

Keratin is dissolved comparatively easily by means of reducing agents such as mercapto-acetate, alkylation with iodoacetic acid, copper sulfite, ammonia and sodium tetrathionate in the presence of 8M urea, etc (Gorjaev, 1979; Krilova and Popov, 1983; Seifter and Gallop, 1968). These approaches are, however, unsuitable for a large-scale application. In a large-scale process the main requirement is the application of accessible and not toxic reagents to obtain a product with a high biological (nutritive) value.

During the industrial processing of leather, the fleshing is scraped off the skin as a waste product and substantial quantities of it are continuously being thrown away. A middle capacity leather factory which processes about 100 tones of hides daily, produces about 30 tones of this waste each day and this creates serious ecological problems. Different methods have been proposed for processing leather wastes (Mdinaradze, 1987): physical (thermal), chemical (extraction with different reagents) and hydrolytic (treatment with enzymes). Efforts for the utilization of limed wastes are directed to extraction of the collagen after acid treatment with sulfuric acid (Gaidouk *et al.*, 1985), pressing at high temperature for the removal of water and fat (Tkach *et al.*, 1987) or thermal treatment at high pressure.

In the present work, we report on the use of a simple process using alkaline proteinase after pretreatment with dilute sodium hydroxide and mechanical disintegration at moderate temperature to produce feather protein hydrolysate. For the utilization of fleshing a simple wasteless method is presented for processing the material into fractions of high value, namely raw gelatin, cattle tallow and protein hydrolysate.

3. MATERIALS AND METHODS

3.1 Starting material

Feathers were obtained from a poultry slaughterhouse in 'Kostinbrod' near Sofia, as a regular waste product. The water content of the native feathers was about 500 g kg⁻¹ and they were used for processing without drying or without any other pretreatment. The chemical composition of feathers is given on Table 1.

Constituent	Feathers	FPC
Water	495	55
Crude protein	894	95
Fibers	-	6.8
Fat	14.1	13.1
Ash	62.4	87.7
Ca	3.5	3.4
P	1.3	1.1
Na	4.0	14.2
Cl	8.0	22.2

Table 1. Chemical composition of feathers and Feather protein concentrate (FPC), g kg⁻¹ to dry mass.

Fleshing from cattle hides from the regular production of the leather factory 'Lion' Gabrovo was used. It was removed by splitting the limed hides before tanning and contains water soluble chemicals. The chemical composition of fleshing is given in Table 4.

3.2 Reagents

All chemical reagents were technical grade and within the limits of the requirements for fodder products. The enzyme was alkaline proteinase B72 from *Bacillus subtilis* with an activity of 50 000 U g⁻¹, a regular product of the Plant for Enzyme Preparations Botevgrad, Bulgaria.

3.3 Analytical procedures

Standard methods for analysis of foodstuffs were used for determination of protein, fat, minerals, water etc (AOAC, 1984). Amino acid analysis was carried out on an automatic analyzer Microtechna T339 Prague, after hydrolysis of 2 mg of the sample with 4 ml 6N HCl for 24 h at 110°C in a sealed tube (cysteine and methionine were determined after oxidation of the sample using performic acid).

4 RESULTS AND DISCUSSION

4.1 Processing of feathers

The feathers were processed in an experimental reactor which was a rust-free steel vessel with a working volume of 80 liters, water-jacketed for temperature control. The reactor was supplied with a mechanical stirrer, which could be regulated between 60 and 180 rpm. To the reactor a disintegrating 'knife' was fitted running at about 2000 rpm. The reactor was supplied with a movable cover to avoid evaporation and to observe the content.

The reactor was charged with 30 liters 0.3M NaOH and the temperature was raised to 80-85°C. Under continuous stirring at 180 rpm 40 kg waste feathers were added in portions. The amount of material was calculated to give a dry weight of 500 g kg⁻¹, so if the water content of feathers were very different from normal the amount of the feathers might be such that the end concentration of NaOH was 0.2N. After adding the whole amount of feathers the temperature dropped to 70-80°C and at this temperature the alkaline pretreatment was carried out for 30 min under continuous stirring at 60 rpm. In this time the disintegrating knife was activated for 10-15 s every fifth minute. At the end of the alkaline pretreatment stage the reaction mixture was a thick homogeneous mass. Its pH was then brought to 8.0-8.3 with 10% HCl and 100 g of alkaline proteinase was added. The temperature was maintained at 55°C and enzymatic digestion was carried out for 2h with stirring at 120 rpm. At the end of the process the pH was adjusted to 7.0 with 10% HCl. For destroying the enzyme activity the temperature was raised to 95°C for 15 min. At this stage of the process the reaction mixture was a thick and turbid solution. After spray-drying the product was a grayish powder with a mass of 19 300 g. This was 'Feather protein concentrate' (FPC). The scheme of the process is shown on Figure 1.

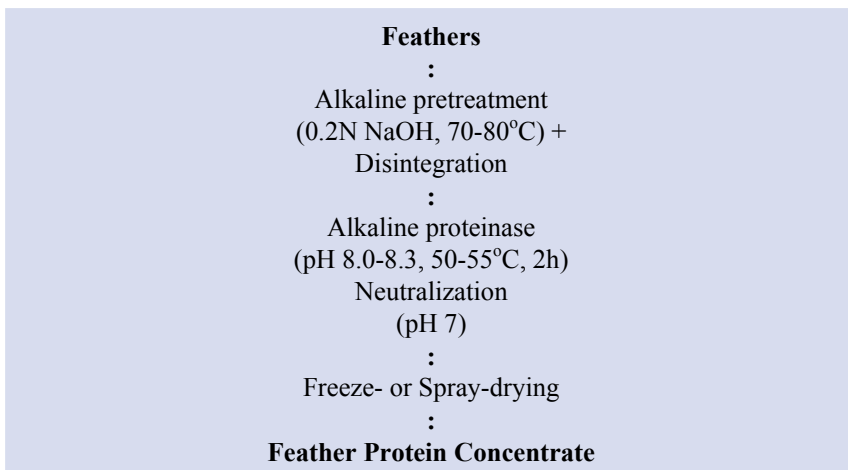


Figure 1. Scheme of feather processing.

4.2. Processing of fleshing.

For processing of fleshing same type reactor was used. 100 kg of fleshing were cut into pieces (3 - 5 cm), put in a rotating perforated cylinder and rinsed continuously with 200 liters of tap water, 200 liters of 0.2N HCl and then again with 200 liters of water. The washed fleshing was heated in the reactor at 80-90°C with continuous stirring for 2h. Under these conditions the material was liquefied to become a thick dark viscose mass with some insoluble particles in it, which however did not hinder its agitation. Because of the high water content of the fleshing, addition of more water for the first extraction was not necessary. After separation in a three-stage, centrifugal separator, three fractions were obtained: a light fraction (fat), a water fraction (raw gelatin solution), and an insoluble residue. The main part of the fleshing protein was extracted in the water phase, but a considerable part remained as particles —i.e., an insoluble residue. Water was added to it (50 liters each time) and the extraction was repeated twice for 30 min at the same conditions. The extracts were gathered, concentrated by vacuum evaporation and dried. This product was the "raw gelatin". The complete solubilisation of the insoluble residue was done by using alkaline proteinase according to the following procedure: 25 kg of the wet insoluble residue was mixed with 50 kg water, the pH was adjusted to 8.0-8.5, the temperature was raised to 50-55°C, and 100 g alkaline proteinase was added. The mixture was stirred (120 rpm) for 3h at this temperature and then was dried in a vacuum dryer. The resulting product was the protein concentrate.

The rate of extraction of the fleshing depended on the amount of water in the material and on the temperature and time of extraction. At the conditions of our experiment the first extraction removed 59% of the protein of fleshing, 18.5% more was extracted in the second one, 6.2% in the third one, and after the enzyme treatment only 5% of the protein remained in the insoluble residue. This complicated procedure is worth consideration. It could however be stopped after the second or after the third extraction when 67.5% or 73.7% of the initial protein, respectively, was extracted. This would reduce the yield of the gelatin fraction but it would raise the yield and protein content of the residue that is a protein concentrate of good nutritional value and could be used as a fodder product. Using the third extract as a solvent for the second extraction instead of water the process could be optimized. The scheme of the process is shown on Figure 2.

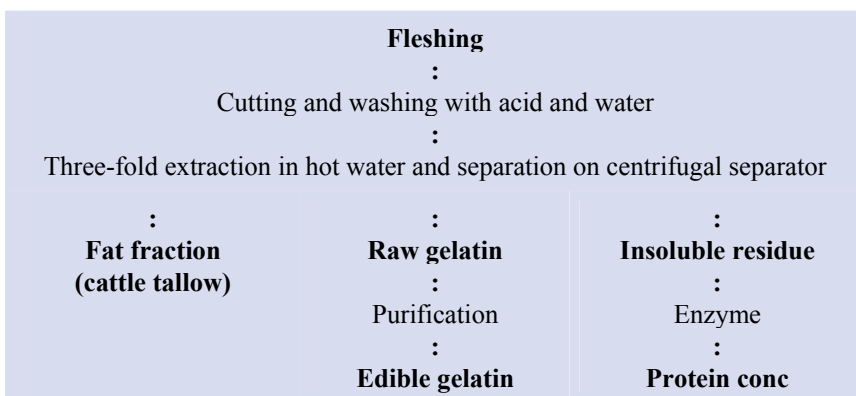


Figure 2. Scheme of fleshing processing.

4.3 Characteristics of the product of feather processing

The only product of feather processing was the feather protein concentrate (FPC). As a bulk material LPC was a powder with a grayish color and a density of 0.334 g cm^{-3} . Its solubility in water, at pH 7.0, was 65 - 85%. The taste was salty but not unpleasant. The smell was specific. The data for the chemical characteristics are shown in Table 1. As shown the difference in the data for feathers and FPC is negligible. According to the protein content, FPC can be added to the group of 'protein concentrates' of animal origin such as fishmeal (710 g kg^{-1} protein), blood meal (820 g kg^{-1}), etc. FPC considerably exceeded in protein content all protein concentrates of vegetable origin as soy bean grouts (480 g kg^{-1} protein content), sunflower grouts (390 g kg^{-1}), etc. The content of crude fiber was comparatively low so this would not be a limiting factor for application in a mixed feed for broilers where a low fiber content is very important. Ash content is slightly higher than the native feathers, resulting from the neutralization with HCl and it could not be a limiting factor as salt is an indispensable component of broiler feeds. The other minerals in FPC are as much as in feathers. A great difference between feathers and FPC in chemical composition would not be expected. The same is true for amino acid composition as well (Table 2).

Amino acid	Feather	FPC	Amino acid	Feather	FPC
Ala	56.6	59.2	Leu	84.3	86.5
Arg	67.4	70.7	Lys	22.5	23.9
Cys	46.1	44.8	Phe	52.2	50.8
Glu	102.5	103.8	Pro	90.8	89.6
Gly	76.6	76.8	Ser	114.4	112.9
His	14.4	13.9	Thr	48.9	44.9
Ile	49.2	51.8	Tyr	24.1	24.5
Val	76.1	70.1			

Table 2. Amino acid composition of feather and FPC (g kg^{-1} protein).

As can be seen on the Table 2 the amino acid composition of FPC did not differ considerably from that of the feathers. The main essential amino acid, which is a basis for balance of combined feed for broilers, is Lysine. The feathers and FPC exceed considerably in Lysine (23.9 g kg^{-1} lysine content), all cereals such as maize, wheat, and its content is very similar to that of green pea (18.0 g kg^{-1}), sunflower grouts (15.0 g kg^{-1}) and peanut grouts (16.0 g kg^{-1}). However, this is lower than in fishmeal (52.0 g kg^{-1}) and soy grouts (59.0 g kg^{-1}). Methionine is also an essential amino acid of great importance. Its content is three times higher than in cereal fodder, it is almost equal to that of soy grout but twice or three times lower than in fishmeal. Nevertheless, the complete amino acid composition of FPC characterizes the product as a good source for preparation of mixed feed in combination with other protein products or with mixtures of synthetic amino acids.

4.4 Biological (nutritional) characteristics of FPC

The high nutritional value of FPC can be expressed in percentage through the relation of the essential amino acids in the FPC protein and in the protein standard of FAO/WHO (1973). This relation has been named 'chemical score' (CS) or 'amino acid number' (AAN) and it is calculated through the formula $CS=(a/b) \times 100$ where a is quantity of the amino acid (in mg/g) in the protein investigated and b is the quantity of the same amino acid (in mg/g) in the protein standard. The theoretical model suggested by the General Committee of WHO experts is considered as a protein standard. The scale of the essential amino acids of the protein standard and the estimated values of CS of the essential amino acids in FPC are shown in Table 3. Lysine excepted, the CS of all essential amino acids of FPC are above 100%. Remarkably high is the CS for the sulphur-containing amino acids. All this means that FPC could be used as a constituent of feeds, where a correction of sulphur content was necessary, as well as a constituent of all kinds of feeds.

Amino acid	Protein standard	CS	Amino acid	Protein standard	CS
Ile	40	129.5	Thr	40	112.2
Leu	70	123.5	Val	50	140.2
Lys	55	43.4	Met + Cys	35	294.2
Phe + Tyr	60	125.5			

Table 3. Scale of the essential amino acid (mg g^{-1} protein) for the protein standard and CS of FPC (%).

4.5 Characteristics of the products of the fleshing processing

The yields and characteristics of these products are given on Table 4. The fat fraction amounts 4-12% of the total mass of the fleshing. It has a light yellow color and is solid at room temperature (20°C). Its melting point is $40-45^{\circ}\text{C}$ and its acid number is 10-20. It meets the Bulgarian State Standard for 'Cattle tallow and can be applied as a fodder additive or as technical fat. The yield of protein concentrate fraction depends on the number of extractions and this product could reach 1-6% of fleshing. It has a comparatively high protein content which determines its nutritional value.

Products	Yield	Water	Protein	Fat	Ash	Sugars
Fleshing	100	750-870	350-600	100-350	-	100-140
Raw gelatin	5-9	30-60	910-930	20-30	60	10-20
PCF	1-3	40-80	350-450	30-80	90	250-350
Tallow	4-8	20-60		930-950	2-4	

Table 4. Chemical composition of fleshing and the products following its processing (kg kg^{-1} to dry mass) and yields (% to dry mass).

Amino acid	Fleshing Skin	Raw gelatin	PCF
Asp	8.67; 6.66	7.34	7.05
Thr	3.83; 2.11	2.37	6.29
Ser	5.05; 4.16	5.52	6.49
Glu	10.76; 11.04	10.49	10.76
Pro	11.67; 15.48	13.15	12.38
Gly	17.36; 26.14	22.88	19.01
Ala	8.82; 9.75	9.46	8.63
Val	4.83; 2.32	3.08	4.26
Met	1.19; 1.03	1.31	1.39
Ile	3.19; 1.50	2.13	2.67
Leu	5.17; 3.42	3.68	4.74
Tyr	2.58; 0.89	1.42	2.67
Phe	3.83; 2.24	2.50	3.08
His	2.43; 0.74	2.13	4.80
Lys	4.71; 3.82	3.92	3.69
Arg	5.99; 8.71	8.62	8.09

Table 5. Amino acid composition of protein in fleshing, raw gelatin fraction, PCF and skin collagen (Fitton-Jackson, 1964) ($\text{g}/100 \text{g}$ protein).

The presence of fat and carbohydrates raise its energetic value. The amino acid composition of its protein shows that the proteins are mainly collagens (Table 5). The high content of Threonine and Methionine is notable. In experiments with mice (made in the Institute for Fodder Industry, Bulgarian Agricultural Academy) this product showed 76% digestibility and absence of toxic effects. So this fraction, named 'Protein concentrate from Fleshing', PCF) could be recommended as a protein source for fodder. After freeze-drying the raw gelatin fraction represents a yellow powder

containing 91-93% protein with a typical amino acid composition for collagen - mainly of short amino acid (Table 5). It meets the requirements for animal glue according to the Bulgarian State Standard (relative viscosity of 7% solution at 40°C not less than 4.5, gluing ability over 110 kg cm⁻², and resistible to microbial growth for at least 72h). However we succeeded to purify this product to obtain edible gelatin through active coal and flocculation with calcium phosphate (Tzokov *et al.*, 1996). The resulting product meets the Bulgarian State Standard for "Edible gelatin".

5. CONCLUSIONS

The proposed methods for processing waste feathers and fleshing give possibilities for the complete utilization of these wastes of industry, thus solving both economical and environmental problems and in the same time giving products that can be of commercial interest for food and feed.

6. ACKNOWLEDGEMENTS

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