

UTILIZATION OF POULTRY FEATHERS IN FOOD AND FEED. 3. CHEMICAL EVALUATION OF PROTEIN ISOLATES, PREPARED BY TWO METHODS FROM FEATHERS OF DIFFERENT POULTRIES

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Abstract

Protein isolate was prepared from feathers of chicken, goose and duck by NaOH hydrolysis (NaOH-F.P.I., Treatment 1) or KOH hydrolysis (KOH-F.P.I., Treatment 2) followed by isoelectric precipitation with orthophosphoric acid. The moisture content ranged from 4.87 to 6.61 % on dry weight basis (D.W.) protein, fat ash and carbohydrate ranges were 90.15-94.02%, 0.56-0.80%, 2.87-6.11% and 1.89-3.86% respectively. Energy value on WW and DW were 357-368 and 379-391 cal/100gm. Highest protein and best physical characteristics were found for chicken KOH-F.P.I. The main source of energy is the protein which constituted more than 94% of the total energy, while fat plus carbohydrate contributed less than 6% of the total energy. Essential amino acids (EAA) concentration and amino acids scores (A.S.) were higher for chicken than goose or duck F.P.I. and KOH-F.P.I. showed a better profile of EAA composition than NaOH-F.P.I. Chicken KOH-NaOH was deficient in 5 EAA, first limiting EAA was lysine. This sample showed highest calculated essential amino acid index (E.A.A.I.), biological value (B.V.) and protein efficiency ratio (PER) values. (When compared with F.P.I., Hydrolyzed feather meal (HFM) products which was deficient in 6-8 EAA could be considered as either protein concentrate (not less than 70% protein or defatted meal (not less than 50% protein) but never considered P.I. (not less than 50% protein). E.P.I. is recommended to be used in man's diet, while be used in human food as meat substitute.

INTRODUCTION

The production of protein isolated from cereals received the attention many decades ago, where protein is extracted at an alkaline pH, followed by precipitation of protein at an acid pH subsequent removal of the bulk of water to obtain protein isolates (P.I.) containing not less than 90% protein (NX 6.25) on dry weight basis. These P.I. are widely used in different foods even in infant milk formulations (Smith and Circle 1980). Nevertheless P.I. were also prepared from slaughter house by-products as well as from fish wastes and offals to be used in human diet. Thus, protein isolates for man's diet were prepared from animal bones (Young, 1976), hydrolyzed keratins (Dulard and Paivot, 1975), fish wastes (Rekhina *et al.* 1978 and Wafaa Z. Abd El-Wahed *et al.* 1989) as well as from buffalo horns (Ghoneim *et al.* 1982).

This work was conducted to prepare and evaluate isoelectric protein isolates from feathers (F.P.I.) of chicken, goose and duck using alkaline hydrolysis with NaOH or KOH.

MATERIALS AND METHODS

Feathers of adult chickens, geese and ducks were collected after slaughter, washed with running water, air dried, then cut into small pieces. Feathers of each kind of poultry were subjected to alkaline hydrolysis at 100°C for 6-8 hours in 4% NaOH or KOH solution, after 2-4 days of soaking in the same solutions at room temperature. Both hydrolyzates were filtrated through cheese cloth then the filtrates (each alone) were acidified to pH 4.5 by 1% orthophosphoric acid. After 1-2 hours liquid phase was withdrawn carefully and precipitated protein washed for 2-4 hours with a very weak flow of running water. After washing water reached pH 7.0 the liquid was withdrawn and precipitate was vacuum dried (500 mm) at 100°C to obtain protein isolate or feathers protein isolate (P.I. or F.P.I.).

The obtained feather P.I. of different poultries were analyzed for moisture, protein (NX 6.25, Kjeldahl method), fat (hexane solvent, Soxhlet apparatus), ash and carbohydrates (by difference) using the methods described by the A.O.A.C. (1980). Energy value was calculated by multiplying both protein and carbohydrates by 4.0 and fat by 9.0. Amino acids composition was determined after HCl hydrolysis using paper chromatograph method according to Block (1958), while

tryptophan was determined colorimetrically after alkaline hydrolysis by the method of Blauth *et al.* (1963). Amino acids scores (A.S.) were calculated in comparison with the FAO reference proteint (FAO/WHO, 1973) as follows:

$$A.S. = \frac{\text{Amino acid content (g/16 g N) of sample}}{\text{Amino acid content (g/16 g N) of the pattern}}$$

Essential amino acid index (E.A.A.I.) in relation to egg protein was determined as described by Oser (1959) using concentrations (gm/16gm N) of isoleucine , leucine, lysine, threonine, tryptophan, valine, methionine + cystine and phynyalanine + tyrosine:

$$E.A.A.I. = \sqrt{\frac{\text{Isoleucine.P}}{\text{Isoleucine.S}} \times \frac{\text{Leucine. P.}}{\text{Leucine. S.}} \times \frac{\text{Phenylalanine + tyrosine. P}}{\text{Phenylalanine + tyrosine. S}}}$$

where P refers to the experimental protein and S, the standard protein (whole egg protein).

Biological value (B.V.) of protein was calculated as follows : B. V. = 1.09 (E.A.A.I.) - 11.73.

Calculated protein efficiency ratio (PER) of P.I. obtained using amino acid composition (g.16 g/N) by 3 equations described by Alsmeyer *et al.* (1974).

$$PER_1 = - 0.684 + 0.456 (\text{Leucine}) - 0.047 (\text{Proline}).$$

$$PER_2 = - 0.468 + 0.454 (\text{Leucine}) - 0.105 (\text{Tyrosine}).$$

$$PER_3 = - 1.816 + 0.453 (\text{Methionine}) + 0.78 (\text{Leucine}).$$

$$+ 0.211 (\text{Histidine}) - 0.944 (\text{Tyrosine}).$$

RESULTS AND DISCUSSION

Feather protein isolates were prepared separately from chicken, geese and ducks feathers separately, F.P.I. was precipitated from KOH (KOH - F.P.I., treatment 1) or (NaOH - F.P.I., Treatment 2) hydrolyzates by acidifying with orthophosphoric acid.

1 . Proximate composition

The gross chemical composition of F.P.I. (Table 1) revealed that the moisture content was low (4.87 - 6.61%), and regardless the source of used feathers. NaOH-F.P.I. (Treatment 2) tended to have somewhat higher moisture than for KOH - F.P.I. (Treatment 1).

It could be observed that protein content of F.P.I. was high, being 84.19 - 88.79% on WW and 90.15- 94.02 % on DW . Highest protein content was found for chicken F.P.I., followed by geese and ducks. Moreover, KOH-F.P.I. showed higher protein than NaOH-F.P.I. This indicated the superiority of treatment 1, particularly when using chicken feathers. Similarly the fat content of F.P.I. was the highest for treatment 1 than 2 . The fat in F.P.I. was highest for geese, the lowest for chicken while intermediate for ducks production of F.P.I. decreased markedly the fat of feathers , since the final products had 0.52 - 0.76 % fat , while untreated poultry feathers showed 1.52 - 2.28% fat (Abd El - Moaty 1989).

Ash increased in F.P.I. (2.69 - 5.77%) when compared with the untreated feathers (1.03 - 2.40%), (Abd El-Moaty , 1989). This because salts formed due to using alkalines in hydrolysis and acid, for precipitation. In this concern, KOH-F.P.I. showed higher ash than NaOH- F.P.I. The increase of carbohydrates (1.89-3.86%, Table 1) in F.P.I. than the untreated feathers (1.03 - 2.40%, Abd El Moaty 1989), might be ascribed to the loss of fat during processing , NaOH-F.P.I. , while showed lower fat (highest fat loss) had higher carbohydrates than in the case of KOH -F.P.I. for both ash and carbohydrates, samples of F.P.I. could be arranged descendingly as follows : duck goose and chicken, The same mentioned arrangement was found for untreated feathers.

The total summation of the concentrations of protein fat and carbohydrates controlled the energy value of F.P.I. Energy value was highest for chicken (F.P.I.), followed by geese and ducks. Such arrangement followed the levels of protein in the F.P. I. (Protein is the major component in F.P.I., thereby considerably affecting the level of energy). In other words most energy in F.P.I. was derived from the protein rather than the fat or carbohydrates. On WW basis energy value was higher for treatment 1 than 2. But on dry weight basis energy value was more for treatment 2 than 1. It seems possible that carbohydrates affected the energy value when calculating on DW basis due to higher carbohydrates content (on DW fat was lower and carbohydrates higher for treatment 2 than 1) . Although difference in the energy

Table 1. Chemical composition of protein isolate from chicken, geese and ducks feathers on wet and dry weight (g/100g)

Components		Chickens feather protein isolate		Geese feather protein isolate		Ducks feather protein isolate	
		(1) KOH 4%	(2) NaOH 4%	(1) KOH 4%	(2) NaOH 4%	(1) KOH 4%	(2) NaOH 4%
Moisture	WW	5.56	6.43	4.87	5.73	5.49	6.61
	DW	—	—	—	—	—	—
Protein	WW	88.79	87.60	86.44	85.38	85.56	84.19
	DW	97.02	93.62	90.87	90.57	90.53	90.15
Fat	WW	0.61	0.52	0.76	0.63	0.64	0.56
	DW	0.65	0.56	0.80	0.67	0.68	0.60
Ash	WW	3.15	2.69	5.46	4.61	5.77	4.78
	DW	3.34	2.87	5.74	4.89	6.11	5.12
Carbohydrates	WW	1.86	2.76	2.47	3.65	2.54	3.86
	DW	1.99	2.95	2.59	3.87	2.68	4.13
Energy value (cal/100gm)	WW	368.21	366.12	362.48	361.79	358.16	357.24
	DW	389.89	391.32	318.04	383.79	378.96	382.52
Colour of meal		Light yellow	Light yellow	Light brown	Light brown	Light black brown	Light black brown
Flavour of meal		Very weak fertilizer	Very weak fertilizer	High fertilizer	Medium fertilizer	High fertilizer	Medium fertilizer

(1), (2) Neutralization was carried out by thophosphirc acid 1%

value were very low, on DW energy should be higher for treatment 1 than 2 because differences between both treatments in fat was similar (0.09), difference in protein was only 0.40% (in favour of treatment 1), but difference in carbohydrates were higher than in protein (being 0.96 (in favour of treatment 2)). The reverse was found on WW basis, because difference in protein were high 1.19% (in favour of treatment 1) and difference in carbohydrates lower (0.87% in favour of treatment 2) difference in fat was also 0.09 i.e. as for calculation on WW basis.

In general KOH (Treatment 1) hydrolysis might be recommended for production of F.P.I., specially from chicken feathers for it is relatively higher protein content and accordingly higher nutritional value. Moreover F.P.I. of chicken feathers was of better colour and flavour as given in Table 1.

2 . Amino acid composition

Amino acids composition of F.P.I. as prepared from the feathers of chicken, geese and ducks using KOH (Treatment 1) or NaOH (Treatment 2) hydrolyzed is given in Table 2.

When the highest concentration (g/16 g N or g/100 g sample) of each of the individual essential amino acids (EAA) was considered, it could be noticed that chicken F.P.I. was mostly the best compared either with geese or ducks F.P.I. (Table 2). This confirms that F.P.I. of chicken feathers was of higher nutritional value due to containing more high quality protein.

It should be noted that hydrolysis of keratin renders such protein available for digestive enzymes due to breakage of disulphide bonds (Liberman and Petrovski, 1973). F.P.I. of feathers, is expected to be available for animals or human subjects because the protein was precipitated from KOH or NaOH hydrolyzate. When F.P.I. of treatment 1 and 2 were compared to concentration of individual EAA (as g/16 g N, using A.S. value or as g/100 g sample), it could be readily seen that KOH-F.P.I. had mostly higher amounts of each of the EAA. This might indicate that EAA survived more the KOH hydrolysis process (as compared with NaOH hydrolysis), or the loss of essential amino acids in the solution was less pronounced during precipitation with orthophosphoric acids from KOH than NaOH hydrolyzate.

Deficiency in EAA of the untreated feathers (Abd El-Moaty 1989) was noticed only for two amino acids (lysine and tryptophan). But when compared with the FAO pattern, F.P.I. was deficient in more number of EAA as indicated by the A.S. value.

Table 2. Amino acid composition and amino acid score of chicken, geese and ducks feathers protein isolate

Amino acid	FAO reference protein (g/16 gN)	Chicken feathers protein isolate						Chicken feathers protein isolate						Chicken feathers protein isolate					
		(1) KOH 4%			(2) KOH 4%			(1) KOH 4%			(2) KOH 4%			(1) KOH 4%			(2) KOH 4%		
		g/16 g/N	A.S	g/16g sample	g/16 g/N	A.S	g/16g sample	g/16 g/N	A.S	g/16g sample	g/16 g/N	A.S	g/16g sample	g/16 g/N	A.S	g/16g sample	g/16 g/N	A.S	g/16g sample
Leucine	7.0	7.73	1.10	6.87	7.10	1.01	6.22	7.63	1.09	6.60	6.99	0.99	5.97	7.65	1.09	6.54	7.06	1.01	5.95
Isoleucine	4.05	4.78	1.20	4.24	4.39	1.10	3.85	4.72	1.18	4.08	4.323	1.08	3.69	4.73	1.18	4.05	4.37	1.09	3.67
Phenylalanine		2.79		2.48	2.71		2.37	2.83		2.06	2.32		1.98	2.76		2.36	2.72		2.29
Valin	5.0	4.41	0.88	3.92	3.79	0.79	3.48	3.59	0.72	3.10	2.95	0.59	2.52	3.08	0.62	2.64	2.98	0.60	2.51
Methionine		0.43		0.38	0.36		0.31	0.39		0.34	0.35		0.30	0.36		0.31	0.33		0.28
Methionine+cystine	3.5	4.01	1.15	3.56	4.03	1.15	3.52	3.78	1.08	3.77	3.76	1.07	3.21	3.54	1.01	3.03	4.28	1.22	3.61
Tyrosine		2.85		2.35	2.93		2.57	3.14		2.71	3.18		2.72	3.17		2.71	3.20		2.69
Phenylalanine+tyrosine	6.0	5.64	0.94	5.01	5.64	0.94	4.94	5.52	0.92	4.77	5.5	0.92	4.7	5.93	0.99	5.07	5.92	0.99	4.98
Proline		6.56		5.82	6.64		5.82	6.63		5.73	6.77		5.78	7.15		6.12	7.23		6.09
Alanine+glutamic		13.14		11.67	13.28		11.63	12.93		11.18	12.98		11.05	13.05		11.17	13.12		11.05
Threonine	4.0	2.49	0.62	2.21	3.26	0.59	2.07	2.69	0.54	2.32	2.45	0.49	2.09	2.80	0.70	2.40	2.56	0.64	2.16
Glycine+aspartic		14.86		13.19	14.94		13.09	14.64		12.65	14.72		12.57	13.63		11.66	13.75		11.58
Serine		7.45		6.61	7.61		6.67	8.01		6.92	8.08		6.90	7.23		6.19	7.29		6.14
Arginine		4.55		4.04	4.59		4.02	4.64		4.01	4.73		4.04	4.92		4.21	4.29		4.18
Histidine		1.28		1.14	1.31		1.15	1.23		1.06	1.25		1.07	1.19		1.02	1.21		1.02
Lysine	5.5	0.98	0.18	0.87	0.93	0.17	0.81	0.88	0.16	0.76	0.82	0.15	0.70	0.95	0.17	0.18	0.91	0.16	0.77
Cystine		3.58		3.18	3.67		3.21	3.39		2.93	3.41		2.91	3.18		2.72	3.95		3.33
Typtophan	1.0	0.39	0.39	0.35	0.37	0.37	0.32	0.32	0.32	0.28	0.28	0.28	0.24	0.25	0.25	0.21	0.21	0.21	0.18
E.A.A.I.		47.86			45.50			45.29			41.30			43.45			41.69		
B.V.		40.44			37.87			37.64			33.29			35.63			33.71		
PER-1		2.57			2.28			2.52			2.22			2.50			2.23		
PER2		2.74			2.45			2.67			2.37			2.67			2.40		
PER3		1.98			1.40			1.60			1.05			1.57			1.07		

A. S. : Amino acid score.

For F.P.I. deficiency was found mainly in 5 EAA, being valine, phenylalanine + tyrosine, threonine, lysine and tryptophan. This was found for chicken, goose and duck F.P.I. prepared of KOH or NaOH hydrolyzate exception, goose F.P.I. prepared of NaOH hydrolyzate was also deficient in leucine (i.e. deficient in 6 EAA) as indicated by A.S. values (Table 2). A.S. value below 1.0 indicate the deficiency in EAA when compared with the FAO reference protein. Therefore F.P.I. were deficient in 5-6 of the essential amino acids. A.S. value were higher for KOH than NaOH samples, and for chicken than geese or ducks F.P.I. This confirms the aforementioned observation about the relatively higher nutritional value of KOH chicken F.P.I. Such sample showed highest E. A. A. I., B. V. and PER values.

Comparison between hydrolyzed feather meals (HFM) and F.P.I. :

According to Smith and Circle (1980) soy products such as defatted meal, protein concentrate and protein isolate should have not less than 50, 70 and 90% protein respectively on dry weight basis. Accordingly Abd El -Moaty (1989) reported that NaOH hydrolyzed feather meal (HFM) of chicken (71.52% protein DW) represents a protein concentrate, chicken KOH - HFM (54.95%, geese NaOH-HFM (57.12%) and duck NaOH - HFM (53.40%) were in the range of protein content of defatted soy products. None of the HFM had protein content amounting to soy P.I. (not less than 90%) . On the contrary, KOH - F.P.I. of chicken contained higher protein level (94.02%) , and for other F.P.I. products lowest protein content was 90.15% (Table 1) . Therefore all F. P.I. Products (Table 1) are considered true protein isolates. As given by Abd El -Moaty (1989) HFM products were deficient in 6-8 EAA (Chicken) , 7 - 8 EAA (goose) and 8 EAA(duck, the deficiency in 8 EAA means that all individual EAA were low. On the other hand only NaOH-F.P.I. of geese was low in 6 EAA and other 5 F.P.I. products were low in 5 EAA only. This might indicate that the nutritional value of F.P.I. was probably higher than for the HFM because of higher protein contents and less number of deficient EAA. Because F.P.I. was prepared from NaOH or KOH hydrolyzates (as for HFM) , and number of deficient EAA was less for F.P.I. than the H.F.M., it should be concluded that drying process was markedly damaging for EAA in HFM, while the drying process during production of F.P.I. was of less damaging effect. Moreover, during processing of HFM the hydrolyzate was neutralized, the whole bulky mixture was dried which consumed more time when compared with drying of precipitated and washed, lower amounts of wet F.P.I. Longer periods of drying might increase the damage and loss of EAA in the form of volatile NH_3 -N or volatile sulfur compounds (as H_2S from methionine). Physical characteristics of F.P.I. were much better than in the case of HFM. For example in

the case of HFM dark black colour might appear, while worst colour for F.P.I. was only light black. Besides, all HFM were of glue flavour, while the flavour of F.P.I. was amild ranging between weak to developed fertilizer flavour which is not objectionable. Therefore HFM is suggested to be used for animal ration, while the F.P.I. (of better eating qualities) is suggested to be utilized as meat substitute during production of meat products, particularly the processing of F.P.I. is more expensive due to elimination of more fat, ash and carbohydrates (less yield) when compared with HFM. Nevertheless the glue flavour of HFM was not rejected completely and could be masked using a proper spices mixture when used as meat substitute.

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الاستفادة من ريش الدواجن في الطعام وفي العلائق: ٣- التقييم الكيماوي للبروتين المفصول المجهز بطريقتين من ريش الدواجن المختلفة

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جهاز البروتين المفصول من ريش الدجاج والاوز والبط بالتحليل القولي للريش باليوتاسا الكاوية (بوايد ، معاملة ١) أو بالصودا الكاوية (ص أيد ، معاملة ٢) ثم الترسيب عند نقطة التعادل الكهربائي بحامض اورثوفوسفوريك . وقد كان محتوس الرطوبة في مجال ٨٧،٤ ، ٦١،٦٪ . وعلي أساس وزن جاف كان محتوى البروتين والدهن والرماد والكربوهيدرات ٩٠،٣ - ٩٤،٠٪ ، ٥٦،٠ - ٨٠،٠٪ ، ٢،٨٧ - ٦،١١٪ ١،٨٩ - ٣،٨٦٪ علي التوالي . وعلي اساس وزن رطب وجاف كان محتوى الطاقة ٣٥٧ - ٣٦٨ ، ٣٧٩ - ٣٩١ كالوري / ١٠٠ جم وقد كان أعلى محتوى بروتين وأفضل خواص طبيعيه للبروتين . المفصولن ريش الدجاج بمعامله ١ . وقد كان البروتين هو المصدر الرئيسي للطاقة في العينات (تؤول عن أكثر من ٩٤٪ من الطاقة) بينما الدهن مع الكربوهيدراتسأهم بأقل من ٦٪ من الطاقة . وقد كانت تركيزات الأحماض الأمينية الأساسية وكذلك تركز كل حامض أميني أساس بالنسبة لمثيله في البروتين النموذجي لهيئة الأغذية والزراعة أعلي في حالة البروتين المفصول من ريش الدجاج بالمقارنه بريش الأوز أو البط . وأعلي في معاملة ١ (بوايد) عن معاملة ٢ (ص أيد) والبروتين المفصول من ريش الدجاج بالمعاملة ١ كان ناقصا في ٥ أحماض أمينية أساسية (الحامض المحدد الأول هو الليسين) . هذه العينة أعطت أيضا أعلي قيم محسوبه لدليل أو سر للأحماض الأمينية الأساسية والقيمة البيولوجيه ونسبه كفاءة البروتين . وبالمقاونه ببروتين الريش المفصول فان مسحوق الريش المحلل (حيث العينات ناقصة في ٦ - ٨ حامض أميني أساسي) كان علي الأقصي مركز بروتين (لا يقل محتواه عن ٧٠٪ بروتين) أو كسب منزوع الدهن لا يقل محتواه عن ٥٠٪ بروتين) ولكن لا يعتبر بروتين مفصول (لا يقل محتواه عن ٩٠٪ بروتين) . وينصح بالاستفادة ببروتين الريش المفصول في غذاء الإنسان بينما مسحوق الريش المحلل فيقترح الاستفادة منه كمصدر للبروتين في اعلاف الحيوان ومن الجائز امكان الاستفادة منه في طعام الإنسان كبديل للحم.