



BIOCHEMICAL CHARACTERISATION OF EGG SHELL MEMBRANE OF COLUMBA LIVIA

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Abstract

Egg shell membrane is dietary supplement that contain naturally occurring food materials for maintaining health. It also protect the contents of the egg from the physical and microbial environment. It provides calcium for embryonic development. They are composed of protein fibres and have high percentage of certain amino acids which are essential for body development like maintaining healthy joint and connective tissues. They have antimicrobial substances and are effective in treatment for arthritis and fibromyalgia.

(Index Terms- Natural egg shell membrane, amino acids, lipids, minerals, biochemical properties)

INTRODUCTION

The eggshell is essential for propagation of all avian species. It is a sophisticated structure¹ whose properties reflect perfectly their cultural functions in reproduction. These functions are basically to protect the contents of the egg from the microbial and physical environment to control the exchange of water and gases through pores during the extra-uterine development of the bird embryo and to provide calcium for embryonic development once the yolk stores are depleted. Just below the egg shell there are two membranes – outer egg shell and inner egg shell membrane.

Numerous authors have investigated the nature of the constituents of the shell membrane²⁻⁴. The fibrous material was initially identified as ovokeratin but the amino acid composition and the use of specific antibodies did not support the hypothesis. It is noteworthy that intact egg shell membranes are a prerequisite for shell calcification in laying birds by the detrimental effect that disruption of egg shell membrane crosslinking by copper deficiency or aminopropionitrile has on egg shell structure⁵.

Egg shell membranes are composed of protein fibres between egg white and inner surface of eggshell. Egg shell membrane protein has high percentage of certain amino acids such as arginine, glutamic acid, methionine, valine, cysteine and proline⁶.

Sugaro reported that the egg shell membrane protein can be used to grow human skin fibroblasts for severe burn situations⁷. Egg membrane protein is also being used in many cosmetics in Japan. Egg lecithin combines with vitamin B₁₂ may show the progress and possibly prevent Alzheimer's disease. In addition shell membranes provide a barrier to prevent inward mineralization. It also contains naturally occurring glycosaminoglycons and proteins essential for maintaining healthy joint and connective tissues. It is a possible new effective and safe therapeutic option for the treatment of pain and inflexibility associated with joint and connective tissue disorders. Keeping this biochemical property in mind we have done comparative study of

chemical composition of inner and outer shell membrane of *Columba livia* and reported amino acid, mineral composition and lipids in these membranes.

EXPERIMENTAL

Eggs of birds were obtained from villages Jasoli and Eroli Tehsil – Mantt, Dist – Mathura (UP) during July – Aug 2010. The procedure for locating eggs was to walk slowly through the study area where reproductive pairs and incubating females were previously observed. While looking under bushes and herbs most of the eggs were found isolated and dispersed in the field. Each egg was broken into half. Sample of inner and outer shell membranes were collected from the half shell containing the air cell⁸. These membranes were separated from each other by applying air pressure through a small hole made on the inner membrane of the air cell⁸. All sample membrane were washed and deionized with water and dried in acetone. Sample of egg shell membranes were powdered using a blender and stored at 4⁰C until analyzed.

Moisture was determined by keeping 1.0 grams of the air dried membrane in an aluminum cup in an electric oven at 11⁰C for 16 hours and then weighing the oven dried membrane.

Ash content was determined by putting 1.0 gram egg shell membrane powder in a high silica crucible and placed in a muffle furnace at 650⁰C for 4 hours.

Sodium was estimated by flame photometer, Magnesium was estimated colorimetric after removing calcium as calcium sulphate precipitate using Erichrome Black T reagent. Calcium is determined volumetrically using standard KMnO₄ solution and phosphorus was determined by alkalimetric method. The crude fat was extracted in a soxhlet extractor using petroleum ether.

Amino acid separation and identification were carried out using high performance liquid chromatographic amino acid analyzer.

RESULTS AND DISCUSSION

Identification and quantification of component amino acids by high performance liquid chromatography.

Using HPLC we found twenty amino acids in outer and inner shell membrane in different amounts (Table I & II) Egg shell membranes are composed of protein fibres between egg white and inner surface of egg shell. Egg shell membrane proteins has high percentage of certain amino acids. The presence of hydroxyproline in hydrolysates of membrane layers suggest that membrane layers fibre structure consists of collagen as component⁹. Egg shell membrane primarily contains type I collagen, type V collagen and type X collagen¹⁰. All the three binds of collagen can be used in various field. It has been determined that about 10 % of total proteinaceous constituent in eggshell membrane structure is collagen.

Eggshell membrane may be an alternative potential important source of collagen for application in foods, cosmetics and biomedical materials. In China eggshell is constituent available as by-product from food industry that is about four million tonnes per year. Eggshell membrane collagen has been proved to be of very low autoimmune and allergic reaction¹¹. According to the result of research on characteristic of eggshell membrane and biosafety, the possibility of application in functional foods, cosmetics and other industries is great.

Identification and quantification of crude fat by Soxhlet extractor

Crude fat of outer and inner shell membrane are given in Table III & IV. The result indicates that crude fat of outer shell membrane is found in greater amount than the inner shell membrane of egg whereas total nitrogen and crude protein (Table III & IV) of outer and inner shell membrane are found in the same amount. This can be attributed to the greater absorption of fat in the outer shell membrane. Obviously supply of fat to the inner shell membrane will be low as the inner shell membrane itself deposit less fat than the outer shell membrane.

Identification and quantification of mineral constituents by instrumentation

Total mineral, moisture and ash contents of pigeon are given in table V and VI respectively. Moisture content is found in less amount but ash content is slightly greater amount in the outer shell membrane than the inner shell membrane. Ash unaccounted for is greater in the case of outer shell membrane than inner shell membrane.

Minerals magnesium, phosphorus, sodium and their corresponding oxides are found in traces in the outer and inner shell membranes. Minerals magnesium, phosphorus are found more amount in outer shell membrane than inner one and their corresponding oxides. Mineral sodium is found in less amount in outer shell membrane than inner one and so their corresponding oxides. Calcium is the main mineral element in the outer and inner shell membranes. Outer shell membrane contains slightly more calcium than the inner shell membrane and so their mineral oxide.

TABLE I**Component amino acids of outer shell membrane of egg of Pigeon (*Columba livia*)**

(Values are expressed as gram/100 gram of the dry matter)

S. No.	Amino Acid	Values
1	Cystine	3.9
2	Lysine	1.3
3	Histidine	3.5
4	Arginine	4.6
5	Hydroxyproline	1.2
6	Cysteine	0.0
7	*Serine	6.7
8	Aspartic acid	1.5
9	Glycine	6.8
10	Glutamic acid	7.1
11	*Threonine	1.3
12	Alanine	1.1
13	Tyrosine	1.2
14	Valine	1.0
15	Proline	10.9
16	Methionine	3.5
17	Isoleucine	0.92
18	Leucine	2.90
19	Phenylalanine	1.5
20	Tryptophan	0.0
	Total	60.92

N – Terminal residue not determined

-CONH₂ Not determined

* Corrected for the loss during hydrolysis.

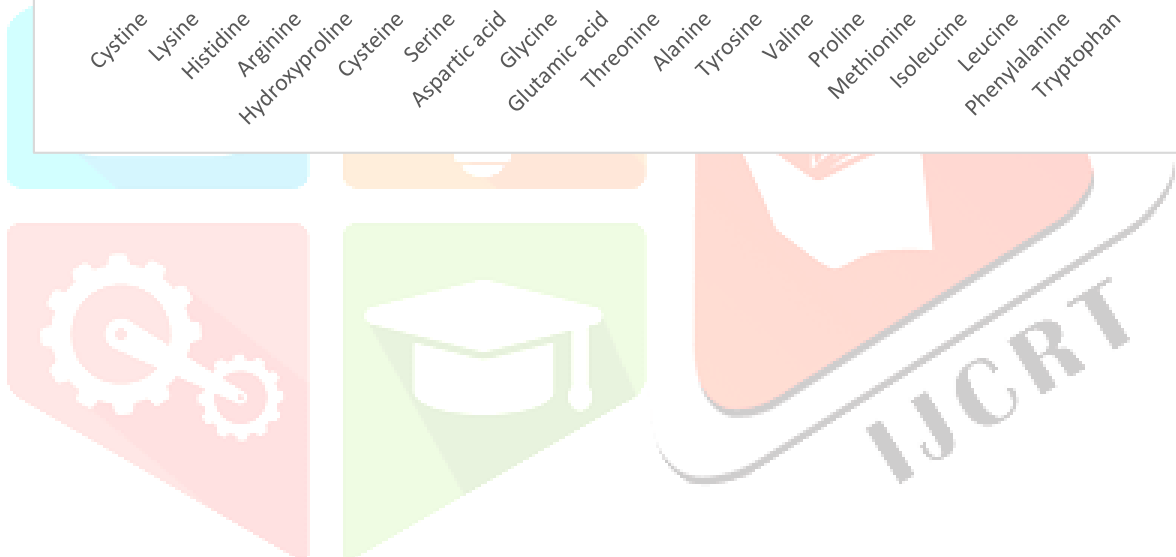
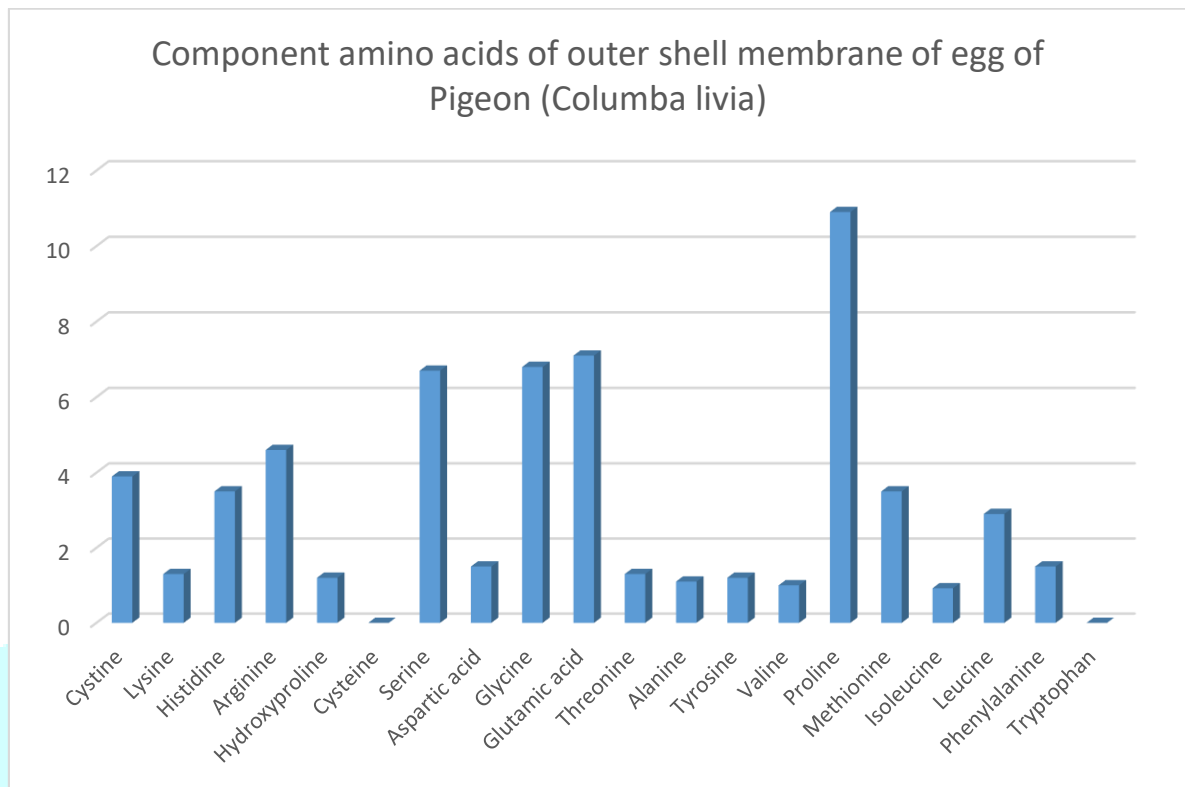


TABLE II**Component amino acids of inner shell membrane of egg of Pigeon (*Columba livia*)**

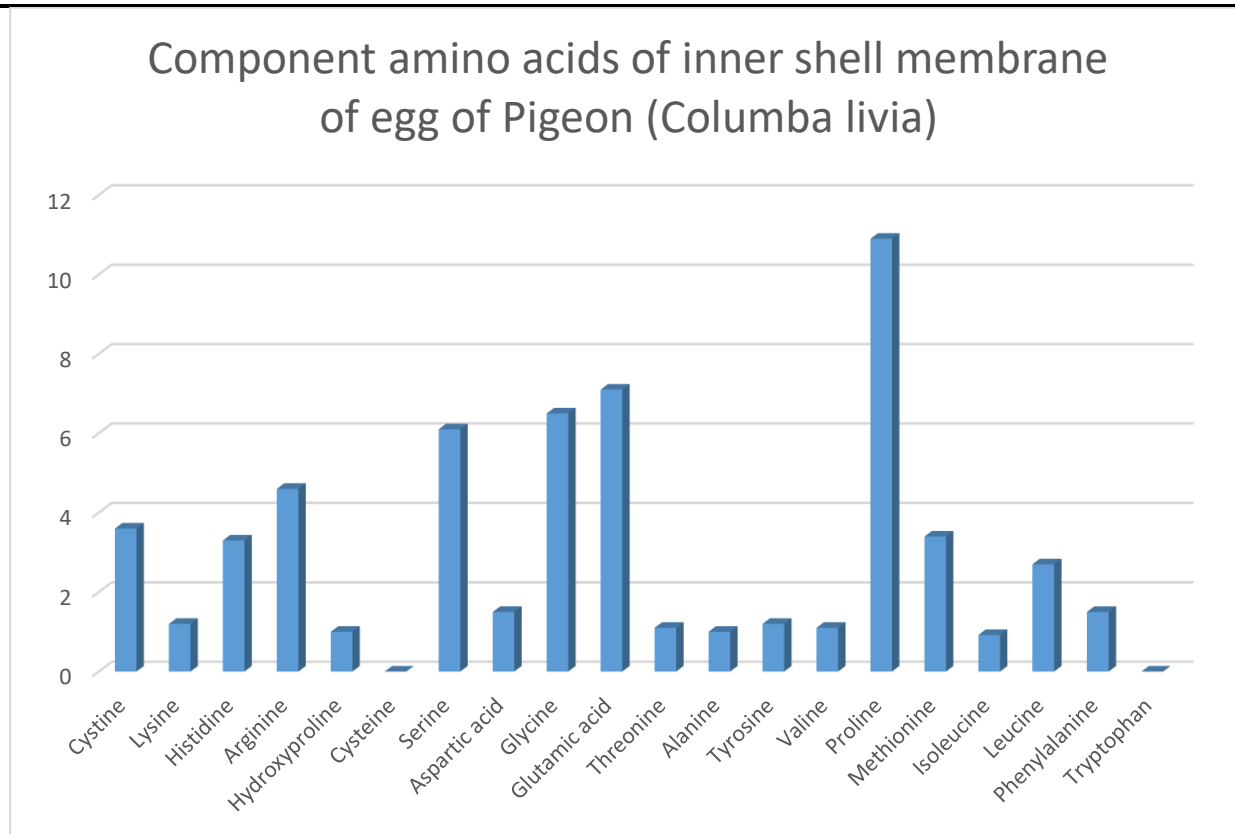
(Values are expressed as gram/100 gram of the dry matter)

S. No.	Amino Acid	Values
1	Cystine	3.6
2	Lysine	1.2
3	Histidine	3.3
4	Arginine	4.6
5	Hydroxyproline	1.0
6	Cysteine	0.0
7	*Serine	6.1
8	Aspartic acid	1.5
9	Glycine	6.5
10	Glutamic acid	7.1
11	*Threonine	1.1
12	Alanine	1.0
13	Tyrosine	1.2
14	Valine	1.1
15	Proline	10.9
16	Methionine	3.4
17	Isoleucine	0.92
18	Leucine	2.70
19	Phenylalanine	1.50
20	Tryptophan	0.0
	Total	58.72

N – Terminal residue not determined

–CONH₂ Not determined

* Corrected for the loss during hydrolysis.

**TABLE III**

Crude fat, total nitrogen content and crude protein of outer shell membrane of egg of Pigeon (*Columba livia*)

(Values are expressed as gram/100 gram of the dry matter)

Crude Fat	Total Nitrogen	Crude Protein (N×6.25)
0.70	15.64	97.75

Total of Ash + Crude fat + Crude protein = 100.01

TABLE IV

Crude fat, total nitrogen content and crude protein of inner shell membrane of egg of Pigeon (*Columba livia*)

(Values are expressed as gram/100 gram of the dry matter)

Crude Fat	Total Nitrogen	Crude Protein (N×6.25)
0.69	15.64	97.75

Total of Ash + Crude fat + Crude protein = 99.98

TABLE V**Mineral Composition of outer shell membrane of egg of Pigeon (*Columba livia*)**

(Values are expressed as gram/100 gram of the dry matter)

Moisture	Ash	Minerals	Values	Mineral Oxide	Values	Ash Unaccounted for
15.80	1.56	Ca	0.5764068	CaO	0.8069695	0.7300260
		Mg	0.0055525	MgO	0.0092542	
		P	0.0058169	P ₂ O ₅	0.0133226	
		Na	0.0003173	Na ₂ O	0.0004277	
				Total	0.8299740	

TABLE VI**Mineral Composition of inner shell membrane of egg of Pigeon (*Columba livia*)**

(Values are expressed as gram/100 gram of the dry matter)

Moisture	Ash	Minerals	Values	Mineral Oxide	Values	Ash Unaccounted for
16.20	1.54	Ca	0.5637966	CaO	0.7893152	0.7283751
		Mg	0.0052203	MgO	0.0087005	
		P	0.0057424	P ₂ O ₅	0.0131519	
		Na	0.0003393	Na ₂ O	0.0004573	
				Total	0.8116249	

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