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COMPARATIVE STUDIES OF EGG ALBUMIN FROM DIFFERENT SOURCES

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Received: 11/03/2018 ; Accepted: 18/04/2018

ABSTRACT: This work describes the use of different methods to study egg white proteins from hen, duck and quail. For this purpose, protein content determination, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Urea-polyacrylamide gel electrophoresis (Urea-PAGE), amino acids composition and Fourier Transform Infrared (FTIR) Spectroscopy were used to compare egg white proteins from these poultry species. Protein content, ranging from 71.31 to 72.36%. Quail egg white showed the lowest protein concentration (71.31%). The highest protein concentration was observed in duck egg white, which reached 72.36%. The analysis by SDS-PAGE, egg white proteins presented a wide range of relative molecular masses. The main egg white proteins are ovalbumin, ovotransferrin, ovomucoid and lysozyme. The contents of the hydrophobic amino acids residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) are 34.54%, 34.95% and 34.94% for egg albumin from hen, duck and quail, respectively. The migration in Urea-PAGE into cathode direction indicated that duck egg white protein and quail egg white protein were much faster than hen egg white protein referring to bigger positive charges. Also, the secondary structure of protein was observed by FTIR.

Key words: Egg albumin, hen, duck, quail, amino acids, SDS-PAGE, FTIR.

INTRODUCTION

Eggs have been an important part of the human diet throughout the world. Eggs have a variety of applications as an ingredient in food and feed, and also many other usages in shampoos, bacterial culture media, fertilizers, and vaccines (Jing *et al.*, 2011). Main applications are the consumption of table eggs and addition of egg components in food products for both nutritional and functional values. Eggs are considered a source of protein, essential fatty acids, vitamins, and minerals (Gray and Griffin, 2009). Egg white provides not only many essential nutrients supporting the development of new life but also bioactivities for the protection of embryo against microorganisms (Cook *et al.*, 2005; Alabdeh *et al.*, 2011). Egg white is considered as a rich source of high quality proteins with various bioactive peptide fractions (Nimalaratne *et al.*, 2015). Egg white protein is not only used as a good source of dietary protein but also extensively used as a functional food ingredient in food processing for its excellent functional

properties, such as foaming, emulsification and gelling properties (Yin *et al.*, 2014). Egg white protein is a major raw material for the food and pharmaceutical industries because of its technological properties, especially foaming and gelling. Some of the egg white proteins could be interesting for nonfood applications, such as health benefit (lysozyme and ovotransferrin). These proteins show antimicrobial and antiviral activity (Kovacs-Nolan *et al.*, 2005). Egg white presents about 58% of the entire egg mass and has a protein content of 10–12%, comprising mainly ovalbumin, ovotransferrin, ovomucoid, globulins and lysozyme (Mine, 2002). Proteins in chicken egg white are glycoproteins such as ovalbumin (Harvey *et al.*, 2000; Raikos *et al.*, 2006), ovotransferrin (Spik *et al.*, 1988), ovomucin (Donovan *et al.*, 1970), ovoglycoprotein (Haginaka, 2001) and α_1 -acid glycoprotein (Küster *et al.*, 1998). Ovalbumin, ovotransferrin (conalbumin) and ovomucoid from egg white have been reported to exist in multiple forms (Huang and Richards, 1997). Many studies have focused on egg white proteins especially

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from chicken because chicken eggs constitute one of the major protein sources of human diet (Miguel *et al.*, 2005).

Egg white proteins from other poultry species, such as quail and duck are proving to be very useful, in some cases, present certain advantages over hen egg white proteins (Hytönen *et al.*, 2003; Valuev *et al.*, 2003). Interspecies variability may explain particular characteristics concerning technological, functional and biological performance of egg proteins from different avian origins. Comparative studies of egg proteins from these poultry species are, therefore, necessary, in order to define qualitative and quantitative differences between them.

The aim of this work is to perform preliminary studies to analyse and compare egg white proteins from different avian species (hen, duck and quail) by protein content, SDS-PAGE, Urea-PAGE, amino acid analysis and FTIR.

MATERIALS AND METHODS

Egg White Sampling

Fresh eggs of different poultry species (hen and quail) were collected from the farm of poultry Department, Faculty of Agriculture, Zagazig University, Egypt. But, the fresh egg of duck was collected from local market from Zagazig city, Egypt and was used in this study.

Protein Extraction

Protein extraction was performed basically according to the method reported by (Omana *et al.*, 2011), with some modification. Egg white was carefully separated from yolk and gently homogenized with a magnetic stirrer for 30 min to reduce the viscosity. The white homogenate was lyophilized and stored at -20°C until used.

Protein Characterization

Protein estimation

Nitrogen contents of different egg white samples were determined by Kjeldahl method (Horwitz and Latimer, 2000) and protein contents were calculated by using a conversion factor of 6.25.

SDS-PAGE

SDS-PAGE was performed on a discontinuous buffered system according to (Laemmli, 1970).

Stacking and separation gels (3% and 15%) were prepared from 30% acrylamide and 0.8% N, N-bis methylene acrylamide solution. The electrode buffer (pH 8.3) contained 0.025 mol Tris, 0.192 mol glycine, and 0.1% SDS. Five milligrams of each sample were dispersed in 1 ml of 0.03 M Tris buffer (pH 8.0) for 15 min with vortexing and the extract was then centrifuged for 10 min at 11000 × *g*. An aliquot of the extract (20 µl) was mixed with 20 µl of SDS-sample buffer, heated at 96°C for 3 min and an aliquot (10 µl) from the final mixture was electrophoresed. After running at 10 and 20 mA (at the stacking gel and running gels respectively) staining was performed with Coomassie Brilliant Blue R-250 dye. The molecular weight of bands was calculated using the corresponding protein marker (10 -500 KD).

Urea-PAGE

Lyophilized egg white proteins from different sources were dispersed (20 mg/ml) in a pH 6.8 buffer containing 0.25 M Tris-base, 50% glycerol and bromophenol blue traces. Samples were centrifuged at 15000 *g* for 5 min at 20°C. Supernatants were analyzed by Urea-PAGE (10 µl of protein / lane) in 3% and 12% stacking and resolving gels, respectively according to (Evans and Williams, 1980).

Amino acid analysis

Total amino acids composition of egg white protein from different sources (hen, duck and quail) were determined by amino acid analyzer apparatus model "Eppendorf LC3000" (Simpson *et al.*, 1976) using the following steps: A known weight (0.2 g) of each sample was received 10 ml 6 N hydrochloric acid in a sealing tube, and then placed in oven at 110°C for 24 hr. Hydrolysates were transferred quantitatively into a porcelain dish and the hydrochloric acid was then evaporated to dryness at 50-60°C on a water bath. Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid and finally the residue was dissolved in 10 ml distilled water and filtrate through 0.45 mm filter. The filtrate was dried under vacuum with a rotary evaporator, then 10 ml of distilled water were added and the samples were dried a second time. One ml of 0.2 N sodium citrate buffer at pH 2.2 was added and the samples were stored frozen in a sealed vial until separation of amino

acids by amino acid analyzer (Column : hydrolysate column Eppendorf LC 3000 (250 × 4.6). The temperature of amino acid analyzer was 47°C; Sample: 20 µl; Buffer system: Sodium acetate, buffer A (pH 3.3), buffer B (pH 3.6), buffer C (pH 4.3) and buffer D (pH 11.0); Flow rate: 0.2 ml/min.). Ninhydrin was used for the detection of amino acids at 440 nm for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction. The peak area and percentage of each amino acid were calculated by computer software AXIOM CHROMATOGRAPHY- 727.

Fourier transform infrared (FTIR) spectroscopy

Egg white protein from different sources (hen, duck and quail) was prepared with potassium bromide (KBr) pellet method (Souillac *et al.*, 2002). Infrared spectra were measured with a FT-IR spectrometer (NICOLET NEXUS 470, DTGS, Thermo Scientific, Waltham, MS, USA) at 25°C. For each spectrum 256 interferograms were collected with a resolution of 4 cm⁻¹ with 64 scans and a 2 cm⁻¹ interval from the 4000 to 400 cm⁻¹ region. The system was continuously purged with dry air. Second derivation spectra were obtained with Savitsky–Golay derivative function soft (Surewicz and Mantsch, 1988). The relative amounts of different secondary structure of egg white protein were determined from the infrared second derivative amide spectra by manually computing the areas under the bands assigned to a particular substructure.

RESULTS AND DISCUSSION

Protein Characterization

Determination of protein contents (Table 1) showed that all species present a similar protein content, ranging from 71.31 to 72.36%. Quail egg white showed the lowest protein concentration (71.31%). The highest protein concentration was observed in duck egg white, which valued 72.36%. These results agree with those reported by Miguel *et al.* (2005).

Different electrophoretic methods, SDS-PAGE (Fig. 1) and Urea-PAGE (Fig. 2) were used to analyze egg white proteins of various poultry species. When analyzed by SDS-PAGE (Fig. 1), egg white proteins presented a wide range of relative molecular masses, present in very different concentrations. The main egg white

proteins, ovalbumin, ovotransferrin, ovomucoid and lysozyme, constitute, respectively, 54, 12, 11 and 3.4% of total hen egg white proteins, these results are in agreement with those obtained by Mine (1995). SDS-PAGE revealed that the electrophoretic profile is qualitatively and quantitatively very similar in hen, duck and quail egg white.

The amino acids composition of egg white from different sources (hen, duck and quail) were listed in Table 2. The contents of the hydrophobic amino acids residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) are 34.54%, 34.95% and 34.94% for egg white from hen, duck and quail, respectively. The contents of the acidic amino acid residues (asp + glu, are 22.25%, 21.32% and 22.35% for egg white from hen, duck and quail, respectively) and there are lower than that of the basic amino acids (arg + lys + his) which represented 25.82%, 30.76% and 28.19% for egg white from hen, duck and quail, respectively). These results agree with those reported by Abdel-Shafi *et al.* (2016).

Urea-PAGE

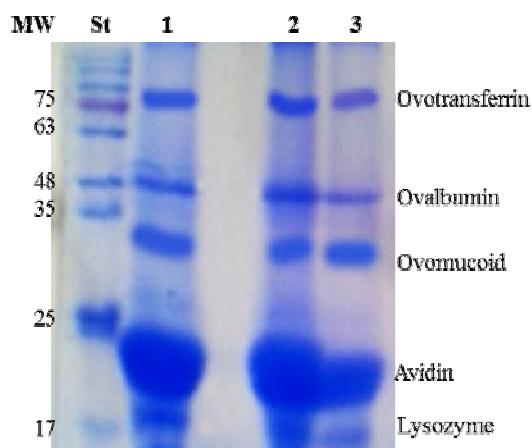
The migration in Urea-PAGE into cathode direction (Fig. 2) indicated that duck egg white protein and quail egg white protein were much faster than their respective hen egg white protein referring to bigger positive charges. hen egg white protein indicated further lower migration rate than duck egg white protein and quail egg white albumin protein in accordance with the fact that the content of the acidic amino acid residues since the contents of asp + glu are 22.25%, 21.32% and 22.35% for egg albumin white from hen, duck and quail, respectively and those are lower than that of the basic amino acids (arg + lys + his which amounted 25.82%, 30.76% and 28.19% for egg white albumin from hen, duck and quail, respectively). These results agree with those reported by Abdel-Shafi *et al.* (2016).

FTIR

In order to study proteins, the analysis of the secondary structure of protein are often required by FTIR in recent years. FT-IR spectroscopy has been proven to be a powerful tool for providing conformational and structure dynamics information of proteins. Comparison of FT-IR spectra of the egg white protein from different poultry species was shown in Fig. 3.

Table 1. Protein contents of lyophilized egg white samples from different poultry species

Species	Protein content (%)
Hen	71.92
Duck	72.36
Quail	71.31

**Fig. 1. SDS-PAGE of egg white protein from different poultry species, Lane (1) hen, lane (2) duck and lane (3) quail compared to standard molecular weight (St)****Table 2. Amino acid composition of egg albumin from different sources (hen, duck and quail)**

Amino acid	Concentration (%)		
	Hen egg white (HEW)	Duck egg white (DEW)	Quail egg white (QEW)
Aspartic	9.39	9.73	10.10
Threonine	2.97	2.14	1.92
Serine	5.44	4.96	5.41
Glutamic	12.86	11.59	12.25
Proline	0.11	0.06	0.07
Glycine	7.56	7.63	7.00
Alanine	7.30	8.17	8.96
Valine	3.58	3.25	3.36
Methionine	4.14	2.72	2.63
Isoleucine	1.64	1.83	2.00
Leucine	6.10	6.52	6.10
Tyrosine	4.87	3.17	4.55
Phenylalanine	8.25	7.49	7.45
Histidine	4.12	3.99	3.72
Lysine	21.58	26.54	24.33
Arginine	0.12	0.23	0.14

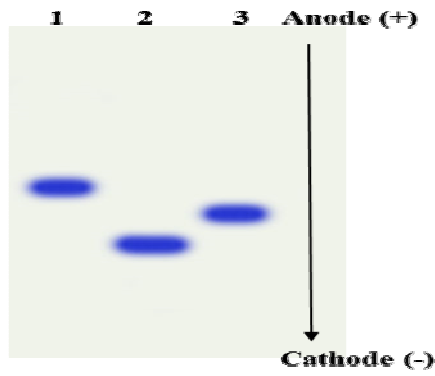


Fig. 2. Urea-PAGE of egg white protein from different poultry species, Lane (1) hen, lane (2) duck and lane (3) quail

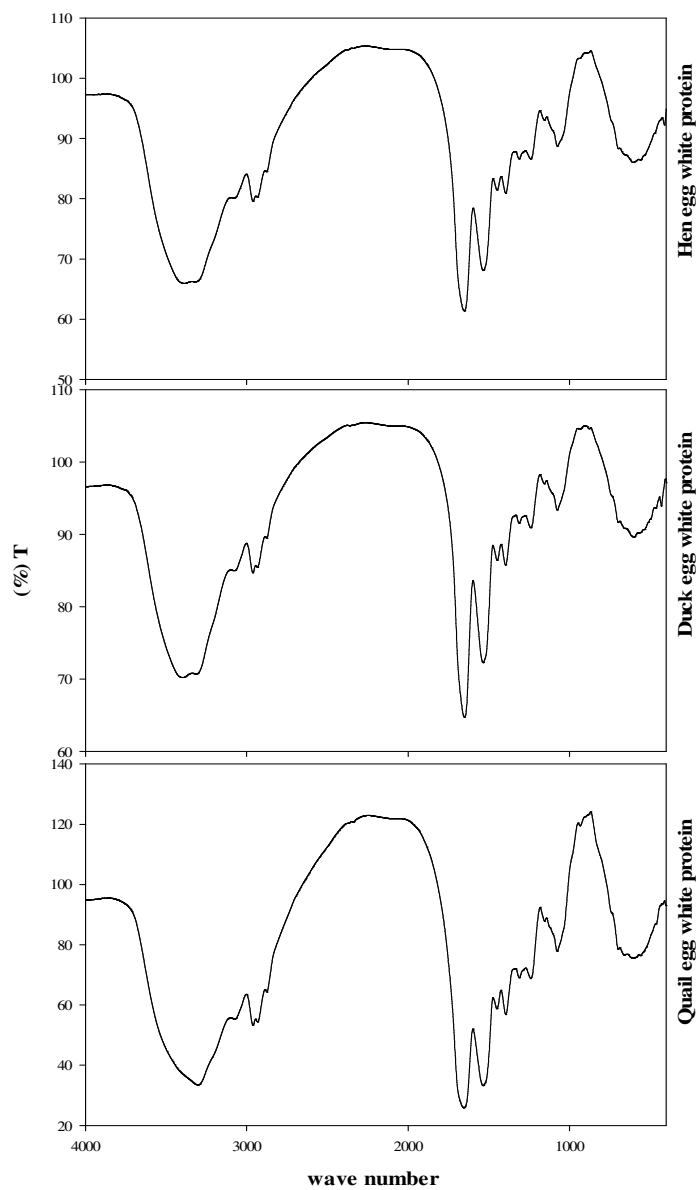


Fig. 3. FTIR spectra of egg white protein from different poultry specie

The secondary structure of the protein were commonly based on the amide I band analysis (1700 – 1600 cm^{-1}). Amide I band peaks identified method is more mature now, Amide I is the most intense absorption band of the polypeptides. ν (C=O) has a predominant role in amide I, ν (C-N) follows. There are also some in-plane NH bending contribution to Amide I. The secondary structure of proteins are reflected by these bands as follows: 1610 ~ 1640 cm^{-1} for the β -sheet; 1640 ~ 1650 cm^{-1} for the random coil; 1650 ~ 1658 cm^{-1} for the α -helix; 1660 ~ 1700 cm^{-1} for the β -turn (Zhang *et al.*, 2003).

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دراسات مقارنة لألبومين البيض من مصادر مختلفة

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قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة الزقازيق – مصر

في هذا البحث تم دراسة تركيب بروتينات بياض البيض لعدة مصادر مختلفة (الدجاج – البط – السمان) عن طريق تقدير محتواها من البروتين والتفريد الكهربى على SDS-PAGE للمكونات الجزئية لألبومين البيض لمعرفة أوزانها الجزيئية والتفريد الكهربى على Urea-PAGE للتعرف على شحنتها النهائية وكذلك دراسة محتواها من الأحماض الأمينية والتحليل الطيفى لها بالأشعة تحت الحمراء وقد أظهرت النتائج أن محتوى البروتين فى الأنواع الثلاثة يتراوح ما بين ٧١,٣١% - ٧٢,٣٦% وكان أقل تركيز للبروتينات فى بياض السمان، وبالتفريد الكهربى لها تبين وجود عدة بروتينات ذات أوزان جزيئية مختلفة تمثل البروتينات الأساسية لبياض البيض وهى أوفوالبيومين – أوفوترانس فيرين – أوفوميوسين – ليزوزيم، أظهرت نتائج تحليل الأحماض أن نسبة الأحماض الأمينية الهيدروفوبية (برولين – جليسين – ألانين – فالين – أيزوليوسين – ليوسين – فينيل ألانين) لكل من بروتينات بياض الدجاج والبط والسمان على التوالى تمثل ٣٤,٥٤%، ٣٤,٩٥% و ٣٤,٩٤% من إجمالى الأحماض الأمينية على الترتيب، تشير نتائج التفريد الكهربى إلى اتجاه الكاثود على جيل اليوريا أن بروتينات بياض البط والسمان كانت أسرع من بروتينات بياض الدجاج وذلك يرجع لزيادة معدل الشحنة الموجبة كما تمت دراسة التركيب الثانوى للبروتين باستخدام التحليل الطيفى بالأشعة تحت الحمراء حيث يعكس النطاق من ١٦١٠ إلى ١٦٤٠ سم^{-١} الصفائح بيتا والنطاق من ١٦٥٠ إلى ١٦٥٨ سم^{-١} للحلزون ألفا.

المحكمون:

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