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Protein profile in freeze-dried chicken embryo eggs with different periods of development
Perfil protéico em ovos embrionados e liofilizados de galinha com diferentes dias de desenvolvimento

Célia Maria Teixeira de CAMPOS[1];
Antônio José Simões HAMAD[1];
Edna Regina AMANTE[1];
Jean-Louis THAPON[2];
Françoise NAU[2];
Catherine GUERIN-DUBIARD[2]

1 - Universidade Federal de Santa Catarina (CIF/CCS) - Campus Universitário, Trindade - SC
2 - École National Supérieure Agronomique (ENSA) – Département Agro-Alimentaire - Rennes – França

Correspondência para:
CÉLIA MARIA TEIXEIRA DE CAMPOS
Universidade Federal de Santa Catarina
UFSC - CIF/CCS
Campus Universitário Trindade
Caixa Postal: 476.
88010-970 - Florianópolis - SC
celinha@ccs.ufsc.br.

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Abstract

This article presents the protein profile in freeze-dried chicken embryo eggs with different development periods (0, 3, 5, 7, 9, 11 days). The protein profile was determined through HPLC-reverse phase, electrophoresis SDS-PAGE and IFE-electrophoresis. Protein profile of these eggs changes according to the period of development and the change is more evident after the 5th day, where there was an increase in ovalbumin, ovotransferrin, apoLDL, apoHDL and lysozyme concentration, and a decrease in ovomucin and ovostatin concentration. The hypothesis that the change in the protein profile is due to protein biosynthesis and disintegration that happens during incubation process is discussed.

Key-words:
Embryo eggs.
Protein profile.

Introduction

Since ancient times chicken embryo eggs, fertile eggs and unfertilized eggs have been used by humanity as food and also in the treatment of diseases. From the nutrition point of view, eggs were always one of the most complete foods available for man.[1] Besides vitamins and mineral elements eggs can provide three essential elements for a good diet: proteins, lipids and carbohydrates.[2] Many researchers have shown that eggs, besides being a source of several nutrients, can provide active substances for therapeutic and diagnostic uses.[3,4]

It is known that there are more than 40 proteins in the egg. Some egg white proteins are enzymes and others are nutrients. The main egg white proteins have been studied in details: Ovoalbumin is the most abundant of white egg proteins.[5] Although no biological function has been identified to ovalbumin it supposedly has a role in nutrition as well as participation in the immune and allergic (to humans) properties of the egg white. Ovoalbumin has foaming and gelling properties.[6]

Ovotransferrin is also known as conalbumin and represents 13% of egg white proteins. It has two functions: to transport iron to storage cells and protect the embryo from bacterial infections.[6,7] This protein can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.[7]

Ovomucin represents 1,5% of the total of egg white and has high viscosity.[5,6] Ovomucin is rich in sialic acid, which is an antiviral active substance.[8,9] Ovomucoid, ovoinhibitor, cystatin and ovostatin play a role against bacterial proteinases.[5] Avidin is the most studied of the vitamin binding proteins. Avidin is responsible for the nutricional human syndrome caused by consuming uncooked egg white.[5]

Ovoglobulins were originally divided into three classes G1, G2 and G3. Ovoglobulin G1 is known as lysozyme and its role is to protect the embryo against bacterial invasion as G2 and G3 do not have this propierty.

Materials and Methods

Preparation of sample units

Chicken embryo eggs (*Gallus gallus domesticus* L.) were obtained from *Isabrown* fertile eggs, incubated at 39.0°C ± 0.5°C, for .a period of 0, 3, 5, 7, 9, and 11 days. The *Isabrown* hens were bred in intensive outdoor raise system, according to Salatin.[10]

The shell of incubated eggs was removed aseptically in sterile plates and the stage of development of the embryo was verified. The remaining content was homogenized, frozen and freeze-dried using a BagMixer (Interscience, France), a plate freezer (Frigostrella, Brazil) and E-C Micromodulyo Freeze Dryer (E-C Apparatus, USA). The freeze-dried product was packed in 30g plastic flasks, sealed and labeled.

Samples of the flasks were selected for analysis using random numbers.

Protein profile by HPLC – reverse phase

Protein characterization in freeze-dried chicken embryo eggs with different periods of development was determined by HPLC– reverse phase according to Nau et al.[11], using a chromatograph Spectra-physics (Fremont, California, USA), equipped with an inert pump for binary gradients model Spectra SERIES P200, with a valve of manual injection, 200m⇨L inert injector and helium as carrier gas (He). Detection was carried out at 280 nm with absortion UV/ Vis detector, model Spectra SERIES UV 100. The chromatograms were processed with Azur v2.0 (1999 – 2001) in Windows software.

Reverse-phase (RP) chromatography was performed on a Vydac protein C4 column (Touzart & Matignon, Les Ulis, France). Reagents: trifluoracetic acid (TFA) (Sigma, Steinhein, Germany) and HPLC-grade acetonitrile (ACN) (Carbo Erba Reagenti, Rodano, Italy). The linear gradient elution was made using water– acetonitrile 0.025% TFA, at a flow-rate of 0.8 mL/min.

Sample units of freeze-dried chicken embryo eggs were re-hydrated with water purified by Milli-Q System (0.08 g of powder for 10 mL of water) (Millipore, Molsheim, France), centrifuged and the supernatant was filtered through a 0.20m⇨m membrane before injection in chromatograph. Liquid egg white supernatant (200 m⇨L) was processed as a control.

The identification of proteins HPLC–RP was made through the comparison of their retention times with those of proteins from liquid egg white supernatant.

Protein profile by SDS-PAGE electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli[12], using a 15% acryl amide separating gel containing 0.1% of SDS. Solution (40m⇨L) from sample units of embryo eggs with different development days at 2.5 m⇨g/m⇨L was used. Electrophoresis was carried out at constant current (150 V) for 45 min in a TRIS-glycine buffer pH 8.3 containing 0.5% SDS. The gel was stained for proteins by the Coomassie blue method.[13]

Protein profile by isoelectric focusing electrophoresis – IFE

Isoelectric focusing electrophoresis (IFE) was conducted according to Desert et al.[14], using a 7.5% acrylamide gel and 3-7 ampholytes. 5 m⇨L and 10 m⇨L of solution from samples units of freeze-dried embryo eggs with different periods were used corresponding to 10 and 20 m⇨g of protein, respectively. Electrophoresis was

carried out at 100V (amperage 9 mA) during 1 hour, 250 V (amperage 20 mA) during 2 hours and 30 minutes and at 500 V (amperage 9 mA) during 2 hours and 30 minutes. Migration buffer was cathode: lysine 20 mM-arginine 20 mM, and anode: H_3PO_4 10 mM. After migration, the gel was stained for protein by the silver staining method.

Results and Discussion

Protein concentration of embryo eggs changes according to development time, and this variation is more expressive since the 5th day because of protein biosynthesis and disintegration that happens during incubation process (Figure 1).

The two highest peaks, identified as OVT and OVA, correspond to ovotransferrin and ovoalbumin proteins, respectively, because their retention times are coincident with those in liquid egg white.

The peak marked with OVA, corresponds to that found by Croguennec et al.[8], where pure ovoalbumin was eluted with a retention time of about 15 minutes in RP-HPLC using a Vydac protein C4 column.

According to Nau et al.[11], ovoalbumin peaks have several contaminants in low quantities, such as ovomucoid, ovoglobulin, cystatin, ovoflavoprotein, because these proteins have pI values that are very close to that of ovoalbumin.

Protein profile analysis by SDS-PAGE electrophoresis of samples units from in freeze-dried chicken embryo eggs with different incubation days were compared with egg white liquid, with yolk liquid and with markers of molecular weight (Figure 2).

Figure 2 indicates that there are differences in protein characterization in freeze-dried chicken embryo eggs with different incubation days, and the difference was more evident following the 5th day of development, where there was an increase in ovalbumin, ovotransferrin, apoLDL, apoHDL, lysozyme concentration and there was a decrease in ov omucin and ovostatin concentration.

Bands shown in figure 2 correspond to protein fractions: ovomucin/ ovostatin, apoLDL, apoHDL, a⇨-livetin, ovotransferrin, apoLDL, ovoinhibidor, ovoalbumin, phosvitin, b⇨-livetin, ovomucoid + flavoprotein, apoHDL and lysozyme, respectively. These bands are coincident with molecular weights published by Stevens[4] and with data of Awandé et al.[6]

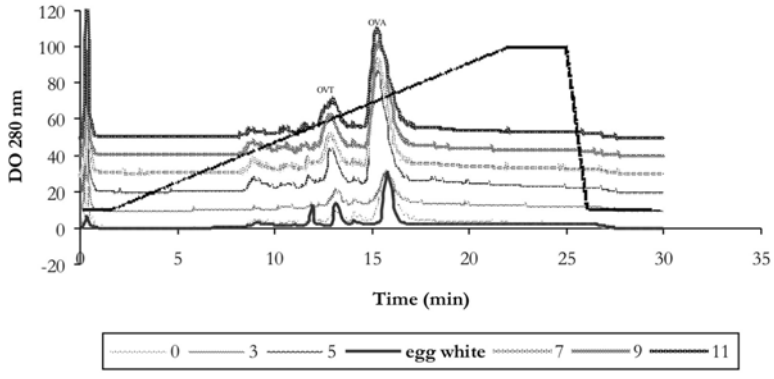

Figure 1
RP-HPLC chromatogram of freeze-dried chicken embryo eggs proteins with different incubation days

Conditions: samples of embryo eggs were re-hydrated in H_2O Milli-Q, egg white liq as pattern; column: Vydac protein C4; elution with water-acetonitrile 0.025% TFA (dashed lines indicate the concentration of acetonitrile in the phase); flow rate: 0.8 mL/min; detection: 280 nm.
Due to the graphic line have been put upon, 10 points were added in DO (ordinates axis), for every incubation day (starting from 3rd day), for a better visualization of the cromatogram.

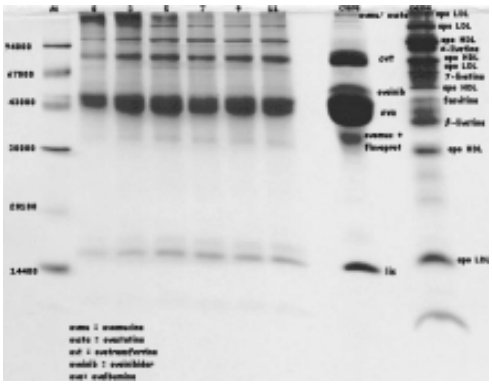

Figure 2
Characterization of proteins in freeze-dried chicken embryo eggs with different incubation days by SDS-PAGE electrophoresis.

Coomasie blue staining method. Deposit: 40 m⇨L to 2.5 m⇨g/m⇨L. M = markers of molecular weight. 0, 3, 5, 7, 9 and 11 = embryo eggs with different development periods.

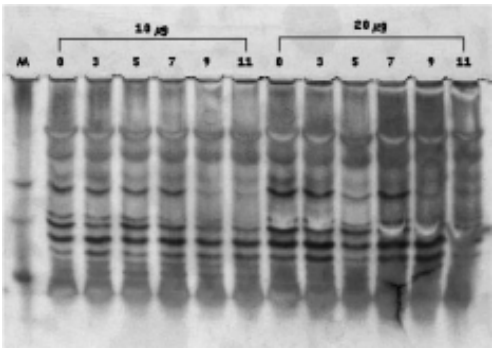

Figure 3
Characterization of freeze-dried chicken embryo eggs proteins with different development periods by IFE-electrophoresis (pH 3-7).

Silver staining method. Deposit: 10 m⇨L and 20 m⇨g of protein.migration parameters: 1 h (100 V), 2:30 h (250 V) and 2:30 h (500 V).
M = markers of pI.
0, 3, 5, 7, 9 and 11 = embryo eggs with different development periods.

and Croguennec et al..[8]

Figure 3 indicates that there are differences in protein characterization in freeze-dried chicken embryo eggs with different incubation days. Isoelectric focusing electrophoresis – IFE showed to be the most sensitive separation methodology to evidence concentration differences of protein fractions between fertile and embryo eggs at different periods of development.

Czuka, Novy and Jirosová[15] also found differences between protein profile with 7 incubation days embryo eggs and unfertile eggs in essays with others electrophoresis separation methods. These authors mention the occurrence of protein biosynthesis and disintegration during incubation process.

Concentration differences of protein fractions are more evident since 5th incubation because they are associated to the development of tissues and specific organs. According to Noble and Cocchi[16], at the 5th incubation day most of the organs and tissues can be identified and at the 10th day the embryo has its appearance recognized. It is remarkable that carbohydrate and proteins metabolism are predominant during two first weeks of chicken embryo development.[17,18]

Resumo

Este artigo mostra o perfil protéico em ovos de galinha embrionados e liofilizados com diferentes períodos de desenvolvimento (0, 3, 5, 7, 9, 11 dias). O perfil protéico foi determinado por CLAE-fase reversa, eletroforese SDS-PAGE e eletroforese de focalização isoelétrica. O perfil protéico destes ovos não é igual, sendo que a diferença fica mais evidente a partir do 5º dia de desenvolvimento onde há um aumento da concentração das proteínas ovalbumina, ovotransferrina, apoLDL, apoHDL e lisozima, e diminuição da concentração da ovomucina e ovostatina, por estarem associadas ao desenvolvimento de tecidos e órgãos embrionários.

Palavras-chave:
Ovos embrionados.
Perfil protéico.

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