Physicochemical studies and electrophoretic profile of freeze-dried quail eggs

Svetla Dyankova[1,*], *Maria* Doneva[1], *Ayten* Solak[1], *Daniela* Miteva[2], *Iliana* Nacheva[1], *Kamelia* Loginovska[1], and *Nikolay* Solakov[1]

[1] Department of Cryobiology and Biologically active substances, Institute of Cryobiology and Food Technologies, Agricultural Academy, 1407 Sofia, Bulgaria
[2] Department of Food Technology, Institute of Cryobiology and Food Technologies, Agricultural Academy, 1407 Sofia, Bulgaria

Abstract. The aim of the research is a comparative physicochemical and electrophoretic analysis of lyophilized egg white (albumen), egg yolk and whole egg (mélange) from quail eggs, obtained by processing at three different freezing temperatures before lyophilization. The conditions of freezing and the parameters of lyophilization, in which products with the best physicochemical properties are obtained, have been established. The protein content varies from 87.76% (egg white) to 31.09% (yolk), and the amount of lipids is 0.21% and 52.94%, respectively. All egg powders have low residual moisture – from 1.92% to 3.49%. The content of essential amino acids is significantly higher in the lyophilized egg white - 44.07g/100g than in the lyophilized yolk - 16.29g/100g. The results of the electrophoretic analysis (SDS–PAGE) showed a reliable match in the protein profile of the raw and freeze-dried quail eggs. Therefore, the proteins in the egg white and yolk remain unchanged after freeze-drying. This technology is a suitable approach for the utilization of unrealized quail eggs while preserving the composition, nutritional value and beneficial biological qualities. In addition to the functional advantages, freeze-dried egg powders are convenient to transport and have a significantly extended shelf life.

1 Introduction

Poultry eggs have been included in the human diet since ancient times. They contain high-quality nutrients, are easily digested and can provide a significant part of the nutrients necessary for the growth and maintenance of the tissues in the body. Eggs are also known as one of the best sources of high-quality protein that contains all the essential amino acids [1].

Globally, chicken eggs are the most common, but a steady rate of growth is observed in quail eggs, which are already in second place on the market [2]. The trend is similar in Bulgaria [3].

Selected quail breeds for cultivation and farming are most often based on the Japanese quail (*Coturnix coturnix japonica*) [4]. The weight of their eggs is about 1/5 of the weight of chicken eggs. They have a fine and soft flavor and have been considered a delicacy in many Asian countries for centuries. They can be consumed boiled, fried, marinated or in dishes with added specific spicy sauces.

Scientific studies have established that the consumption of quail eggs is beneficial for human health due to the functional properties of their nutritional components. They are thought to contain a more favorable ratio of nutrients than traditionally consumed alternatives and can support a healthier diet. Despite their small size, their nutritional value is three to four times higher than that of chicken eggs. Quail eggs have a higher protein content, almost three times more vitamin B1 and two times more vitamins B2 and A, compared to chicken eggs. In addition, they contain large amounts of calcium and phosphorus and five times more potassium and iron than chicken eggs [5, 6]. Significant differences were also observed with regards the component composition of proteins and phospholipids, compared to chicken eggs [7, 8].

Due to the greater thickness of the shell and its lower permeability, quail eggs are less susceptible to microbial contamination during storage. They have been found to retain their quality after refrigerated storage for up to 90 days [9].

In traditional Chinese medicine, quail eggs have been used for centuries as a medicinal food to aid in the treatment of allergies and asthma and to prevent diabetes. Modern research also proves that the consumption of quail eggs helps in the treatment of some allergic diseases, bronchial asthma and diabetes. In the 1970s, a French general practitioner successfully used raw quail eggs in the treatment of allergies in children and adults [10]. Later on, several clinical trials were conducted that proved the anti-allergic effectiveness of quail eggs [11].

* Corresponding author: svetla.diankova@ikht.bg

A randomized, double-blind, placebo-controlled trial observed the effect of a proprietary quail egg dietary supplement on allergic rhinitis. It has been proven that the product is effective in alleviating allergy-related symptoms. Improvement in nasal inspiratory flow as evidenced by nasal function testing has been reported, as well as a reduction in other nasal and ocular allergic symptoms. Relief of allergic symptoms was obtained 15 minutes after taking the product, which demonstrates a rapid effect of the application. No adverse side effects were observed [12].

In vitro studies have shown that protein fractions from quail eggs, including ovomucoids and ovoinhibitors, act as inhibitors of serine proteases. Some external and internal antigens such as pollen, mold, animal dander and house dust mites contain proteolytic enzymes. Upon contact with the endothelium of the nasal cavity, these proteases can injure the tissues and induce a transient IgE-mediated allergic inflammatory response. By inhibiting proteases, quail egg bioactive substances help reduce allergic reactions [13]. Studies by Hao et al. [14] found that quail egg ovomucoid could inhibit basophilic and eosinophilic cell activation, while chicken egg ovomucoid is a major allergen called Gal d1. The difference in sensitization with quail egg ovomucoid compared to chicken egg ovomucoid may be due to differences in amino acid sequence and alteration of antigenic epitopes [14].

The increased market demand for quail eggs leads to an increase in their production, which at certain times can lead to the accumulation of overproduction with a short shelf life. Unrealized quail eggs could be processed by vacuum freeze-drying and thus their valuable biological qualities could be preserved unchanged for a long period of time.

Freeze-drying (lyophilization) is a process of separating water from the solid matrix of the frozen product at a pressure lower than the triple point pressure of water. The method allows to preserve the structure, composition and nutritional value of the product to the greatest extent. The obtained lyophilizates have a low moisture content, fine structure and consistency and much lighter weight, with preserved nutritional properties, biologically active substances and a flavor-aromatic complex, supplying in digestible form the nutrients and biologically active substances necessary for the body. In addition to functional advantages, freeze-dried food products are very convenient to store and transport, possess significant stability and a long shelf life at room temperature.

The aim of the present study is a comparative physicochemical and electrophoretic analysis of lyophilized egg white (albumen), egg yolk and whole egg (melange) from quail eggs, obtained by processing at three different freezing temperatures before lyophilization.

2. Material and methods

2.1 Materials

The quail eggs (*Coturnix coturnix japonica*) were obtained from a certified farm - GREEN LIVING FARM, Zlatusha village, Sofia region, Bulgaria. Hexane, acetic acid, sulfuric acid and hydrochloric acid were supplied by Merck, ethanol and glycerol by Valerus (Bulgaria). The chemicals and reagents for SDS-PAGE were purchased from BIO-RAD, protein markers - from Sigma-Aldrich. All reagents and chemicals used are analytical grade.

2.2 Freeze-drying

Pretreatment of quail eggs included washing, disinfection and weighing. The eggs were carefully deshelled and egg white and yolk were manually separated, weighed and homogenized (Fig. 1). In addition, whole egg (mélange) samples were also prepared and homogenized.

The obtained egg samples were poured into stainless steel trays and frozen in three modes: (-25°C), (-45°C) and (-196°C, liquid nitrogen).

The frozen samples were lyophilized in a freeze dryer LYOBETA 6PL from Telstar (Spain).

Fig.1. Quail eggs. Separation of egg white and yolk

2.3 Physicochemical analyzes of raw and lyophilized egg samples

2.3.1 Moisture content

The moisture content was determined by express method with Sartorius Thermo Control YTC 01L balance.

2.3.2 Total protein content

The total protein content was determined by the Kjeldahl method (N x 6.25) following the AOAC n. 925.31 (AOAC, 1995), with VELP Scientifica apparatus.

2.3.3 Total fats content

The total fats content was determined by extraction with hexane in a “Soxtec 2055” apparatus;

2.3.4 Total ash

The total ash was determined by sample mineralization in a muffle oven, according to ISO 936:1998

2.3.5 pH measurement

The pH of egg samples was measured with pH meter Jenway 3000.

2.4 SDS-PAGE of raw and lyophilized egg samples

Electrophoretic analysis of egg samples was performed according to Laemmli [15] with slight modification [1]. Raw egg white was diluted with distilled water in a ratio of 1:9, homogenized and 500 µl of the resulting solution was mixed with 500 µl of sample buffer (0.2 M Tris HCl, pH 6.8, containing 2% SDS, 16% glycerol, and 10mM DTT). Raw egg yolk was diluted with distilled water (1:4) and the mixture were subjected to centrifugation at 12,000 x g at 10°C for 20 min. The collected precipitate was suspended in distilled water and mixed with equal volume of sample buffer. Lyophilizates were defatted by hexane, air-dried at room temperature and rehydrated with distilled water (10 mg/ml). 500 µl of the obtained solutions were mixed with 500 µl of sample buffer. Electrophoresis was performed on a 12% TGX Stain-Free running gel and 4% stacking gel using the Mini-PROTEAN Tetra Cell (Bio-Rad), at a constant voltage of 200 V. SigmaMarker low range, was used as standard protein mixture. The gel was visualized and densitometered using GelDoc Go Imaging system and Image Lab 6.1 Software.

2.5 Amino acid analysis

The analytical method for determining the amino acid composition of proteins in lyophilized quail egg white and yolk involved preliminary hydrolysis of egg proteins with 6.0 M hydrochloric acid, dilution to a protein concentration of 1.0-1.5 mg/ml, derivatization, and quantitative and qualitative determination of amino acids by high performance liquid chromatography. The lyophilized yolk samples were previously defatted with hexane. Ortho phathalaldehyde (OPA) was used as derivatization reagent. Agilent 1260 infinity II apparatus was used, with UV/Vis-DAD, Poroshell 120 HPH-C18 column 4.6 × 100mm, 2.7µm particles and flow rate: 2ml/min, max 600 bar.

Mobile phases: Mobile phase A: 10mM Na2HPO4 – 10mM Na2B4O7, pH 8.2; Mobile phase B: acetonitrile: methanol: water (45:45:10). The quantity of amino acids was calculated in g per 100 g of lyophilizate.

2.6 Statistical analysis

Assays were performed with 3 replicates. Data are presented as mean ± standard deviation (SD). Statistical evaluation of the results was performed with the Excel 2016 software package. A one-way analysis of variance was performed to determine the differences.

3 Results and discussion

3.1. Physicochemical analyses of the raw material

The results of measuring the weight of the different parts of quail eggs (n=20) showed the following values (± SD):

- Egg white: 7.38 ± 1.12 g
- Egg yolk: 4.69 ± 0.86 g
- Whole egg (without shell): 12.07 ± 0.78 g

The results for the weight of quail eggs parts are close to those reported by Genchev [16]. The egg white occupies 61% of the mass of the egg, which is also confirmed by the observations of other authors [7, 17]. The pH values of egg white and yolk were 9.01 and 6.41, respectively (Table 1).

Table 1. Proximate compositions of raw quail eggs

Sample	pH	Dry matter, %	Fat, %	Protein, %	Ash, %
Egg white	9.01±0.18	13.75±1.24	0.26±0.01	13.27±0.32	0.57±0.06
Egg yolk	6.41±0.08	53.70±2.25	25.67±1.65	15.03±0.55	1.10±0.10
Egg melange	7.93±0.33	29.73±0.97	8.58±0.85	10.57±0.01	0.89±0.17

Values are given as means ± SD (n = 3).

In a study by Northcutt et al. [9], the pH value of egg white was 8.91 ± 0.08 in the initial period and increased to 9.22 ± 0.03 at 30 d of storage at 4°C. Changes in albumen pH are often used as a key indicator of degraded egg quality. Our results are indicative of the freshness and proper storage of the quail eggs.

Regarding the physicochemical composition of the eggs, there are no significant differences from the values reported by other authors [6, 16].

3.2 Technological trials for freezing and lyophilization of quail eggs

The experiments carried out to determine the proper freeze-drying regime for egg white, yolk and melange included pre-dilution with distilled water in a ratio of 1:1, homogenization and freezing at three different temperatures: (-196°C) - liquid nitrogen; (-45°C); (-25°C). The lyophilization of the experimental series was carried out under the following parameters: sublimation temperature: (-45°C); desublimator temperature: (-65°C); maximum working vacuum: 2.10^{1}Pa; secondary drying temperature: from +25°C to 30°C; duration of the process – 38-40 h.

One of the main indicators of a correctly selected freeze-drying mode is the low residual moisture of the obtained lyophilizates. Fig. 2 graphically presents the results for the moisture content (MC) of the lyophilized samples of quail eggs at different freezing temperatures.

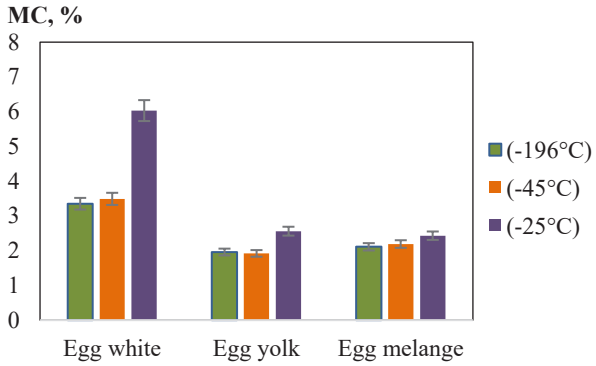

Fig. 2. Moisture content (MC) of egg lyophilizates, obtained at different freezing temperatures

In the samples frozen at (-25°C), the moisture content was higher with a significant difference ($p < 0.05$). In the lyophilizates obtained in the other two freezing modes, no reliable differences in moisture content are reported, i.e. freezing at (-45°C) ensures good quality of lyophilized egg powders with less energy consumption, compared to liquid nitrogen treatment. Photographs of the obtained lyophilizates are presented in Figure 3, and Table 2 shows the results of the physicochemical analysis.

Fig. 3. Photographs of quail egg lyophilizates

Table 2. Proximate compositions of freeze-dried quail egg powders

Sample	pH (10% solution)	MC, %	Fat, %	Protein, %	Ash, %
Egg white	9.28±0.08	3.49±0.25	0.21±0.06	87.76±0.21	2.39±0.56
Egg yolk	6.54±0.07	1.92±0.21	52.94±0.23	31.09±0.34	3.78±0.31
Egg melange	8.42±0.20	2.19±0.28	35.86±0.15	47.89±0.78	2.98±0.23

Values are given as means ± SD (n = 3).

Lyophilization leads to significant changes in the physicochemical parameters of the three types of egg samples. Dry matter increased to values from 96.51 g/100g to 99.08g/100g, which proportionally affects the content of fat, total protein and ash. As can be expected, the protein content is the highest in the lyophilized egg white - 87.76%.

3.4 SDS-PAGE of raw and lyophilized egg samples

Figure 4 presents an SDS-PAGE image of the proteins in raw and lyophilized egg white and yolk samples. In the egg white (lines 1 and 2), six bands between 16 and 90 kDa appear. The bands with molecular mass 16 kDa, 44 kDa and 73 kDa were identified as lysozyme, ovoalbumin and ovotransferin, respectively. The electrophoretic profile of egg white is similar to that reported by Segura-Campos et al. [18] for lyophilized quail egg white. For the egg yolk (lines 3 and 4), seven protein bands were appeared. The 70 to 60 kDa bands most likely correspond to the different livetin fractions. The protein fraction with Mw about 48 kDa corresponded to yolk phosvitin. The pale bands with Mw ranged from 30 to 40 kDa most likely correspond to HDL apoproteins [1]. A reliable match was observed in the protein profile of the raw and lyophilized egg white and yolk samples. Therefore, the proteins in the egg white and yolk retain their molecular mass and structure after the conducted freeze-drying.

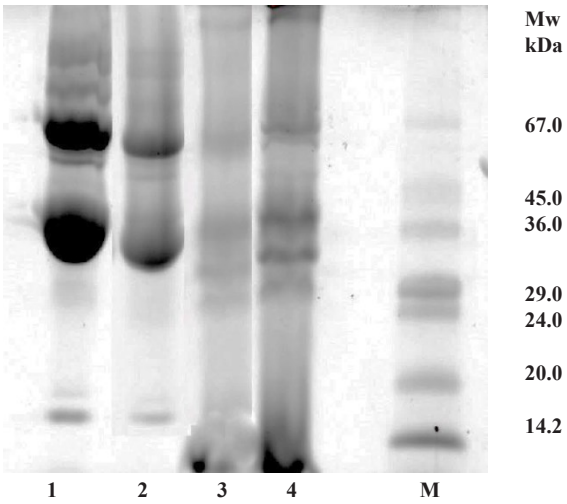

Fig. 4. Photograph of the SDS–PAGE of quail egg samples: 1 - raw egg white; 2- freeze-dried egg white; 3 - raw egg yolk; 4-freeze-dried egg yolk; M- Sigma Marker low range

3.4 Amino acid composition of the lyophilized quail egg white and yolk

The results for the amino acid composition of the lyophilized quail egg white and yolk are presented graphically in Figures 5 and 6. Eighteen amino acids were identified in both types of samples, but in different percentages. In egg white, the total amount of amino acids is 87.70 g/100g lyophilizate. Of these, the sum of essential amino acids is 44.07 g/100g, with the largest amount being leucine, phenylalanine, lysine and isoleucine. Of the non-essential amino acids, the content of aspartic acid is highest, followed by glycine, glutamic acid, alanine and cysteine. The values obtained by us are slightly lower than those reported by Kudre et al. [1], which may be due to differences in the breed, laying period and feeding regime of the quails.

In the lyophilized yolk, the total amount of amino acids is 31.06 g/100g. Of these, the essential amino acids are 16.29 g/100g, with the highest in content being lysine, followed by phenylalanine, isoleucine, leucine and valine.

Of the non-essential amino acids, the largest amount are aspartic acid, glutamic acid, serine and proline.

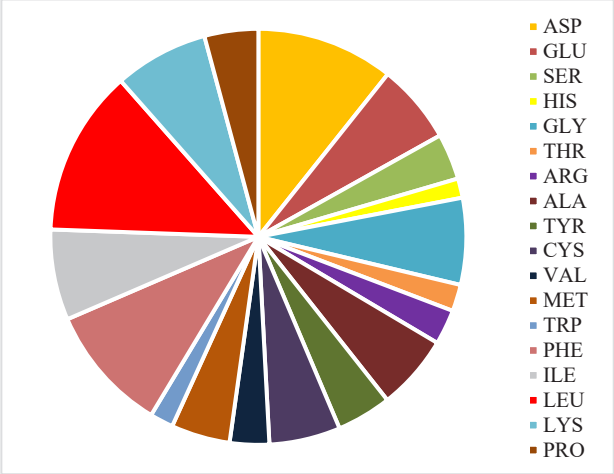

Fig. 5. Amino acid compositions of freeze-dried egg white

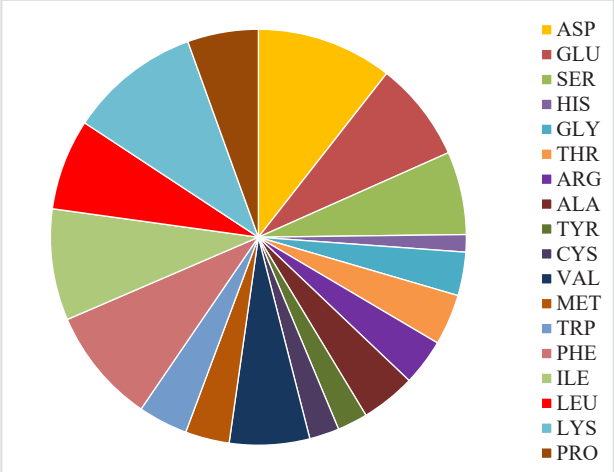

Fig. 6. Amino acid compositions of freeze-dried egg yolk

4 Conclusion

Technological trials were carried out for freeze-drying of quail egg white, yolk and whole egg (mélange). It was established that preliminary homogenization of the samples with water (1:1) and freezing at (- 45°C) ensures obtaining of lyophilizates with low residual moisture and good physicochemical parameters. Analyzes of the lyophilized egg powders showed a protein content from 87.76% (egg white) to 31.09% (yolk), respectively. The content of essential amino acids is significantly higher in the lyophilized egg white - 49.89g/100g than in the lyophilized yolk - 16.29g/100g. The results of the electrophoretic analysis showed a reliable match in the protein profile of the raw and lyophilized egg white and yolk samples. Therefore, the proteins in the egg white and yolk retain their molecular mass and structure after freeze-drying. Lyophilization was found to be a suitable approach for the utilization of unrealized quail eggs while preserving the composition, nutritional value and biological properties. In addition to the functional advantages, freeze-dried egg powders are convenient to transport and have a significantly extended shelf life.

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