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Effect of Heat Treatment on Protein Fractions of Edible Poultry Eggs

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ABSTRACT

Poultry eggs are highly important in human nutrition due to their content of essential amino acids, vitamins, minerals, and enzymes. Eggs of edible poultries such as hen, turkey, quail, and goose may have some differences in their nutritional composition. Various heat treatments applied before consumption lead to some alterations in their nutrients, especially proteins. The purpose of this study was to investigate compositional and structural changes in the protein fractions of hen, quail, turkey, and goose eggs when exposed to soft- and hard-boiling (11-16 min and 18-19 min), and frying (2-7 min). Electrophoresis and spectroscopy were used to determine the effects of these heat treatments on egg white and yolk proteins separately. It was observed that the heat degradation of proteins in egg white was higher than that in egg yolk. As expected, protein degradation was increased when heat exposure was extended. Hard-boiling treatment completely denatured egg white proteins almost in all poultry species. Ovomucoid was the most resistant fraction against heat denaturation in white proteins, while livetins in yolk. Soft-boiling under the given conditions resulted in mostly retained profiles of proteins in egg yolk of all species. Relevant to protein degradation, remarkable structural changes were detected in the protein secondary structure of hard-boiled and fried egg samples. Significant data obtained in this research revealed the influence of heat treatment on the protein content of edible eggs. Those findings are expected to help in developing the processes and consumption methods of egg products for dietary purposes and improvement of human health.

Keywords: Egg, Protein, Poultry, Electrophoresis, Spectroscopy

Isıl İşlemin Yenilebilir Kanatlı Yumurtalarındaki Protein Fraksiyonlarına Etkisi

ÖZ

İçerisinde barındırdığı esansiyel amino asitler, vitaminler, mineraller ve enzimler ile yumurta insan beslenmesinde oldukça önemli bir yere sahiptir. Tavuk, bıldırcın, hindi ve kaz gibi yenilebilir kanatlı yumurtaları besin bileşiminde bazı farklılıklara sahiptir. Tüketimden önce uygulanan çeşitli ısıl işlemler özellikle proteinler olmak üzere yumurtaların içerisinde barındırdığı besinlerde bazı bileşimsel ve yapısal değişikliklere yol açabilmektedirler. Bu çalışmanın amacı rafadan (11-16 dk.), tam haşlanmış (18-19 dk.) ve sahanda pişirilmiş (2-7 dk.) tavuk, bıldırcın, hindi ve kaz yumurtalarının protein fraksiyonlarındaki bileşim ve yapısal değişikliklerin araştırılmasıdır. Bu ısıl işlemlerin yumurta beyazı ve sarısı proteinleri üzerine etkileri elektroforez ve spektroskopi kullanarak tespit edilmiştir. Yumurta beyazında sarısına oranla daha fazla ısıl degradasyon gözlenmiştir. Beklendiği gibi ısıl maruziyet süresi uzadıkça protein degradasyonu artmıştır. Tam haşlama hemen hemen bütün kanatlı türlerinin yumurta beyazı proteinlerini denatüre etmiştir. Yumurta sarısında livetin fraksiyonları iken yumurta beyazında ovomukoid ısıl denatürasyona karşı en dayanıklı fraksiyondur. Rafadan haşlamada bütün türlerin yumurta sarılarındaki protein fraksiyonları çoğunlukla denatürasyondan korunmuştur. Protein yıkımı ile bağlantılı olarak tam haşlanmış ve sahanda yumurta örneklerinde protein ikincil yapısında dikkat çekici farklılıklar tespit edilmiştir. Bu çalışmada elde edilen önemli veriler ısıl işlemin yenilebilir yumurta proteinleri üzerine etkisini ortaya koymuştur. Bu bulguların beslenme ve insan sağlığını iyileştirme

amacına yönelik olarak yumurta içeren ürünlerin üretim ve tüketim yöntemlerini geliştirmede katkı sağlayacağı beklenmektedir.

Anahtar Kelimeler: Yumurta, Protein, Kanatlı, Elektroforez, Spektroskopi

INTRODUCTION

Eggs contain high quality of nutrients including proteins, phospholipids, and various micromolecules such as vitamins A, B, D and E, and minerals Ca, P, K, Mg, Fe, and Zn. This rich nutrient content is quite beneficial for every living mammalian. Eggs also contain antioxidants and antimicrobial compounds providing health benefits for the human being. Carotenoids such as lutein, zeaxanthin, and choline as well are found in significant amounts in eggs [1]. Besides these beneficial effects, one adverse effect is associated with allergic reactions in the human body due to egg proteins [2, 3]. In comparison to hen and quail eggs, turkey and goose eggs' proteins can exhibit higher allergic potential since they are big and comprising of the high amount of protein fraction [4]. A whole egg is composed of proteins (~10-14%), carbohydrates (~0.5-2.2 %), water (~88%), fat (~10-13%) , and ash (~1%) [5]. From the outer to the inner layer, it is composed of three main parts including shell, egg white (albumin), and egg yolk. In general, based on average weight, 11 % of an egg corresponds to the shell and the inner membrane, 58% to the egg white, and 31% to egg yolk [6]. Both egg white and yolk are rich in high-quality protein. Ovalbumin (54%) conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%) are the most abundant proteins in the egg white, whereas livetins (38%), phosphitin (8%), and apoproteins (high-density lipoproteins, HDL of 36% and low-density lipoproteins, LDL of 17%) are the protein fractions of egg yolk [6, 7].

Electrophoresis is a common tool used for separation and identification of proteins from various sources. Electrophoretic mobilities of protein fractions greatly differ through their molecular weight and charges. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is mostly used to elucidate food proteins and facilitate comparison of their profiles after various treatments.

Animal-based proteins are essential for human nutrition. Egg protein has the highest biological value due to its rich essential amino acid content and high digestibility with 95% when compared to milk (85%) and meat (74%) [8]. Leucine, isoleucine, methionine, and lysine are the most important essential amino acids existing in egg white and yolk proteins [9].

Protein denaturation induced by heating can facilitate the action of digestive enzymes, thus providing higher assimilation of cooked egg proteins [10, 11]. Egg proteins are also widely used as ingredients in the food products including bakery, dressings, and meat formulations. Egg white is commonly used in food processing owing to its functional properties such as emulsifying, foaming, and gelling [12]. These functional properties of egg proteins might be changed desirable or undesirably during processing according to process conditions. For example, controlled heat treatment can induce protein denaturation thus may alter their functionality such as enhanced gelation ability. Both direct consumption and industrial use require the heat processing of eggs through different treatments.

Edible poultry (hen, quail, turkey, goose, etc.) eggs are consumed via different cooking treatments such as boiling and frying. Cooking techniques with varying temperatures and time periods can lead to denaturation of protein fractions at different levels. Some egg proteins are heat resistant such as ovomucoid, whereas some are heat- labile such as lysozyme and ovotransferrin [11]. The length and temperature of heat treatment are critical parameters determining the extend of protein denaturation. The eggs of four different poultry species used in this study are hen, turkey, quail, and goose to follow the effect of different cooking methods on their protein fractions. Eggs of each species differ in size, weight, the proportion of constitutional parts, and their macro- and micro molecular contents [13]. When compared to the other constituents the relative protein amount of the eggs of these species is mostly stable about 13% [11].

Protein denaturation via heat or other factors resulted in destabilization of hierarchical quaternary and tertiary structures unfolded to secondary structure, then followed by conformational alterations in secondary structure elements through breakage of hydrogen bondings and hydrophobic interactions. Fourier Transform Infrared Spectroscopy (FT-IR) is a widely used versatile technique for the determination of structural changes in protein secondary structure via major vibrational modes known as amide I and amide II stretching bands [14].

It has been well-stated that edible poultry eggs have critical importance in nutrition and the preparation methods are highly effective in their constituents regarding compositional and structural features. Since proteins are the fundamental nutrients in eggs, different cooking styles might significantly alter the structure and conformation of protein fractions, potentially leading to altered bioabsorption. There is still lack of comprehensive and comperative analyses regarding the protein structure and bioavailability of various poultry eggs when exposed to thermal treatments. The present work aimed to investigate compositional and structural changes in protein fractions of soft-boiled, hard-boiled, and fried eggs of different poultry species using gel electrophoresis and infrared spectroscopy.

MATERIALS AND METHODS

Materials

Four different poultry eggs were used in this study. Hen and quail eggs produced by Kor Agro Organic Gıda AŞ. (Turkey) were purchased from a local market, and goose and turkey eggs were kindly supplied by villagers in Uşak, Turkey. Commercially produced twenty day-old hen eggs with approximately 62 g in weight, and thirty day-old quail egg with 14 g in weight were used. Also, village-grown, twenty to thirty-day old turkey and goose eggs with 75 g and 180 g in average weight, respectively, were used in this study. Electrophoresis chemicals and the standard marker were purchased from Bio-Rad Laboratories Inc (Hercules, CA, USA). Other chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA).

Methods

Proteins extracted from soft-boiled, hard-boiled, and fried egg white and yolk samples were analyzed by SDS-PAGE for protein profiling and FT-IR for determination of conformational changes in protein structure.

Sample Preparation

Eggs of all species were subjected to heat treatment for different time periods as given in Table 1. Soft- and hard-boiling conditions were set due to preliminary studies. Various trials were conducted to determine cooking time for obtaining the desirable appearance of egg white and yolk. In case of soft-boiling, the white was not fully set (still soft) and the yolk was thick but runny. In case of hard-boiling, both the white and yolk were fully set. For the boiling procedure, one-litter glassware containing 500 ml water and eggs were placed onto a heating plate adjusted to 350 ℃ (not pre-heated) and exposed to heating via time schedules indicated in Table 1. Hen and quail eggs were boiled two by two, whereas goose and turkey eggs were boiled one by one. Then the eggs were taken out and placed in water at room temperature (~ 24℃) to cool down. The egg white and yolk were separated prior to protein extraction to be explained in the following section. White and yolk parts of the eggs to be fried were separated first, then exposed to frying in a non-stick pan with a diameter of 20 cm, on the heating plate at 350 °C as scheduled in Table 1. The heating plate was not pre-heated when the first sample, the hen's egg white was fried, however, it was already pre-heated for the other samples. The treatment was achieved when the whites and yolks were totally set. No oil was used for the frying treatments in order to eliminate its effects on the changes in protein structure. Two eggs were cooked for each poultry type and the experiments were conducted in dublicate. Uncooked or raw egg samples were also used for comparison in the experiments.

Table 1. Cooking periods applied to the poultry eggs

Type of poultry eggs	Soft-boiling (min)	Hard-boiling (min)	Frying (min)	
			Yolk	White
Hen	15	18	3	7
Quail	11	18	2	5
Turkey	15	18	2	3
Goose	16	19	4	3

Protein Extraction

The extraction of protein fractions in the eggs was achieved according to a previous study with minor modifications [15]. One hundred milligrams of samples from uncooked, boiled and fried eggs were put into Eppendorf© tubes and mixed quite well with 100 µL ultrapure water. It was stored at +4°C overnight. To unfold protein, 1 ml of 90% trichloroacetic acid (TCA), v/v, was added and vortexed until mixed quite well. Then the samples were kept on ice for one and half an hour and centrifuged at 12000 rpm and +4°C for 5 minutes. Non-proteinous parts and other residues within the supernatant were discarded by three sequential washing steps. After the last washing, diethyl ether/ethanol (50/50 %, v/v) solution was added and mixed well. The samples were centrifuged at 12000 rpm and +4°C for 5 minutes. By discarding supernatant two sequential washing steps were carried out. The resultant pellets were dissolved in 500 µL ultrapure water and stored at +4°C for further analyses.

SDS-PAGE Analysis

Proteins in the fractions were analyzed by SDS-PAGE according to Laemmli [16]. Samples were mixed with sample buffer (1:1) and boiled for 5 minutes to denaturate proteins. Twenty microliters of each sample were loaded to each well. Electrophoresis was carried out on a 4-12 % polyacrylamide gel. It was run at 75 V in the first 15 minutes, then at 150 V for 1 hour. Following protein staining performed using Coomassie Brillant Blue R250 for 30 min, destaining was carried out for a few hours and protein bands were examined.

FT-IR Spectroscopy

Infrared measurements were carried out using a Perkin-Elmer 100 FTIR Spectrometer (Wesseley, MA, USA) equipped with a horizontal ATR sampling accessory, at room temperature. Nearly, 100 µl samples were placed onto a crystal surface and the measurements were taken in the range of 4000-400 cm^{-1}. The resolution was 4 cm^{-1} and 32 scans were recorded per each spectrum with a scan speed of 1 cm/sec. A background was recorded before each measurement.

RESULTS AND DISCUSSION

Protein Profiling

Hen, quail, goose, and turkey's eggs were subjected to boiling and frying treatments and resultant varying degree of denaturation in protein structure was investigated. Protein profiles of raw and heat-treated egg samples recorded using SDS-PAGE analysis were given in Figure 1. Egg yolk and white proteins belonging to each poultry species were well-fractionated on the gels in consistency with the previous studies [4, 7, 15, 17, 18]. Based on their molecular weights, the identification of the protein fractions in egg white and yolk were carried out by the help of marker proteins (M) run in the corresponding lanes of each gel.

Major protein fractions in egg white are ovalbumin (~ 45 kDa) [19], ovotransferrin (~ 70 kDa) [20], ovomucoid (~ 28 kDa) [21], and lysozyme (~ 14 kDa) [22], whereas in egg yolk are LDL (~20-220 kDa) [7], HDL (~30-110 kDa) [23], livetins (~ 25-203 kDa) [17], and phosvitin (~45 kDa) [18]. In raw egg white of all poultries four major protein fractions were detected, however higher band intensities were observed in some fractions such as ovalbumin and ovotransferrin due to their higher amounts and solubilities when compared to the others [7]. The sorted profiles of heat-treated egg white and yolk samples were examined separately by comparing them to the corresponded untreated samples. Qualitative differences were detected in the protein fractions of different poultry eggs investigated in this study.

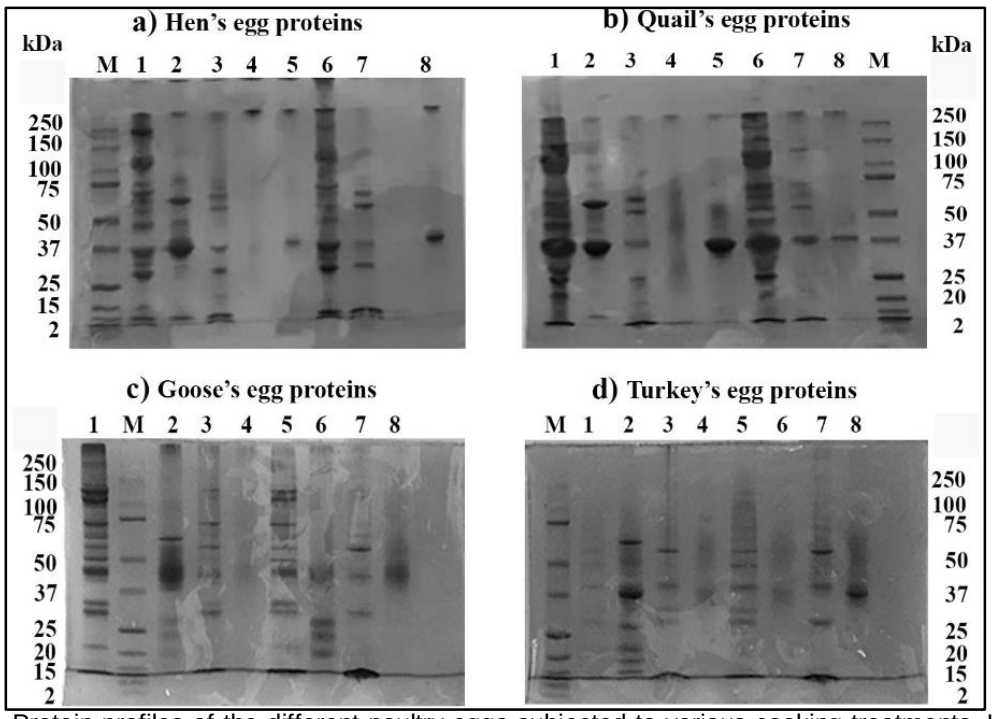

Figure 1. Protein profiles of the different poultry eggs subjected to various cooking treatments. Lane M represents the protein standard. a) Hen's egg proteins, lane a1: raw egg yolk; lane a2: raw egg white; lane a3: hard-boiled egg yolk; lane a4: hard-boiled egg white; lane a5: soft-boiled egg white; lane a6: soft-boiled egg yolk; lane a7: fried egg yolk; lane a8: fried egg white. b) Quail's egg proteins, lane b1: raw egg yolk; lane b2: raw egg white; lane b3: hard-boiled egg yolk; lane b4: hard-boiled egg white; lane b5: soft-boiled egg white; lane b6: soft-boiled egg yolk; lane b7: fried egg yolk; lane b8: fried egg white. c) Goose's egg proteins, lane c1: raw egg yolk; lane c2: raw egg white; lane c3: hard-boiled egg yolk; lane c4: hard-boiled egg white; lane c5: soft-boiled egg yolk; lane c6: soft-boiled egg white; lane c7: fried egg yolk; lane c8: fried egg white. d) Turkey's egg proteins, lane d1: raw egg yolk; lane d2: raw egg white; lane d3: hard-boiled egg yolk; lane d4: hard-boiled egg white; lane d5: soft-boiled egg yolk; lane d6: soft-boiled egg white; lane d7: fried egg yolk; lane d8: fried egg white.

Table 2 represents the detected protein fractions in raw, soft- and hard-boiled, and fried egg white and yolk's of each poultry species based on SDS-PAGE images given in Figure 1. As it is clearly seen in the gel images and the table protein denaturation induced by heat treatment resulted in lowered band volume intensities and/or disappeared bands. Egg yolk proteins seemed to be more resistant to heat denaturation than egg white

fractions in all species. Protein profiles of soft-boiled yolks were very similar to that of raw yolks with lowered band intensities most probably arisen from partial denaturation. Ovomucoid fraction in egg white showed remarkable resistance to boiling and frying treatments. Similarly, α-, β- livetin, and apovitellenin III exhibited heat resistance among other yolk fractions. In comparison to LDL proteins, HDL proteins in egg yolk were observed as much more heat-labile. A band corresponding to phosvitin fraction remained in goose and turkey egg yolks after heat treatments including hard-boiling and frying.

The protein patterns of hen's and quail's eggs were very similar in both white and yolk after treatments. It is remarkably observed that most of the whole egg white proteins of all species were denatured in case of hard-boiling. In a previous study investigating the thermostability of egg yolk granules when exposed to heat up to 79°C [23]. They reported that LDL and some HDL fractions were heat-sensitive while phosphitin and some other HDL fractions were resistant to heat in prepared egg yolk granules. Our findings were in agreement with those to some extend since here the egg samples were subjected to a higher temperature during boiling and frying treatments, thus strongly inducing protein denaturation.

Table 2. Protein fractions in white and yolk's of eggs cooked with different treatments

Major protein fractions in egg white	Mwt (kDa)	Egg white															
		Hen				Quail				Goose				Turkey			
		R	S	H	F	R	S	H	F	R	S	H	F	R	S	H	F
Ovalbumin	~ 45	+	-	-	-	+	-	-	-	+	+	-	+	+	-	-	-
Ovotransferrin (conalbumin)	~ 76	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Ovomucoid	~ 28	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+	+
Lysozyme	~ 14	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Major protein fractions in egg yolk	**Mwt (kDa)**	**Egg yolk**															
		Hen				Quail				Goose				Turkey			
		R	S	H	F	R	S	H	F	R	S	H	F	R	S	H	F
Livetins	α- livetin	~ 55, 73	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
	β- livetin	~ 33, 36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	γ- livetin	~ 203, 25	+	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-
Phosvitin		~ 45	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+
LDL apoproteins	Apovitellenin VIa	~ 220	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
	Apovitellenin Va	~ 120	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-
	Apovitellenin IV	~ 68	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-
	Apovitellenin III	~ 62	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
HDL apoproteins	Apovitellin 3+4	~ 110	+	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-
	Apovitellin 5+6	~ 78	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
	Apovitellin 8	~ 31	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Apovitellin 8	~ 31	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R: raw, S: soft-boiled, H: hard-boiled, F: fried, Mwt: molecular weight, kDa: kilo dalton, LDL: low density lipoproteins, HDL: high density lipoproteins

FT-IR Analysis of Protein Fractions in Eggs

Figure 2 shows the FT-IR spectra of protein fractions in yolk and white of hen, quail, and goose eggs. Structural changes in their proteins were tracked in soft- and hard-boiled, and fried eggs of these species. Raw eggs of these poultries were also analyzed for comparison. Qualitative assessment of secondary structure changes revealed denaturation and aggregation in egg proteins leading to alterations in their functional and bioactive properties [24]. The amide I band defined between ~1700 and 1620 cm^{-1} due to the C=O stretching is the well-recognized region to determine the changes in the protein secondary structure. Other vibrational regions involved in the identification of the elements in protein structure are the amide II band between ~1560 and 1520 cm^{-1} due to combination of N-H bending and C-N stretching vibrations and the amide III band between ~1320 and 1220 cm^{-1} due to combination of N-H bending and C-N stretching vibrations [25-27].

The major peaks detected in each infrared spectra were analyzed by considering the given polypeptide vibration band assigned to the secondary structure elements with the reported literature works [27-29]. The stretching bands at 1697 cm^{-1} and 1636/1626 cm^{-1} correspond to β-sheet, and those at ~1687/1670/1969 cm^{-1} correspond to β-turn structures. The peaks belonging to those structural elements were tracked in all egg samples (Figure 2). It was observed that β-sheet structures assigned to the bands at around 1697/1687/1636 cm^{-1} mostly retained during heat treatments with noted partial losses in case of hard boiling and frying. The α-helix structure is mostly attributed to the bands at 1664/1663/1650 cm^{-1} were observed with lowered peak intensities indicating partial degradation due to boiling and frying treatments. The random coil structures were also observed due to the peaks detected at ~1646/1645 cm^{-1}. In the amide II region, the peaks at ~1558 cm^{-1} assigned to α-helical and ~1525 cm^{-1} to β-sheet structures were detected in both yolk and white samples before and after cooking treatments. The peaks

between ~1315 and 1280 cm^{-1} in the amide III region attributed to α-helix and some random structures were detected in yolk and white fractions of eggs as well. The detected peaks and corresponding structural elements are comparable to the previous reports [17].

In hen's, quail's, and goose's eggs, the spectra of raw and soft-boiled yolks resembled with the lowered intensities in the latter case (Figure 2A1, 2B1 and, 2C1). In the amide I region, decreases and decreases in the corresponding bands showed lowered α-helix content and increased β- and random structures due to cleavage of protein structure via heat. Hard-boiling and frying resulted in remarkable changes in protein conformation especially in the amide I region. In case of hard-boiling irreversible denaturation seemed to enhanced protein aggregation and thus buried helical or beta structure patches.

Overall, many shifts, losses, and newborn peaks detected especially in the amide I region exhibited some structural changes in the egg yolk and white fractions of the investigated species. Conformational alterations such as the loss in the helices and increase in the β-, random, and aggregated structures indicated thermal denaturation and reconstitution due to aggregation of cleaved peptide fragments. As the heat exposure prolonged, eg. during hard-boiling, or various reactions taken place, eg. due to water loss during frying, the hydrogen bonds mostly holding the structural elements of egg proteins were broken down and tend to form new bindings leading to an aggregation network.

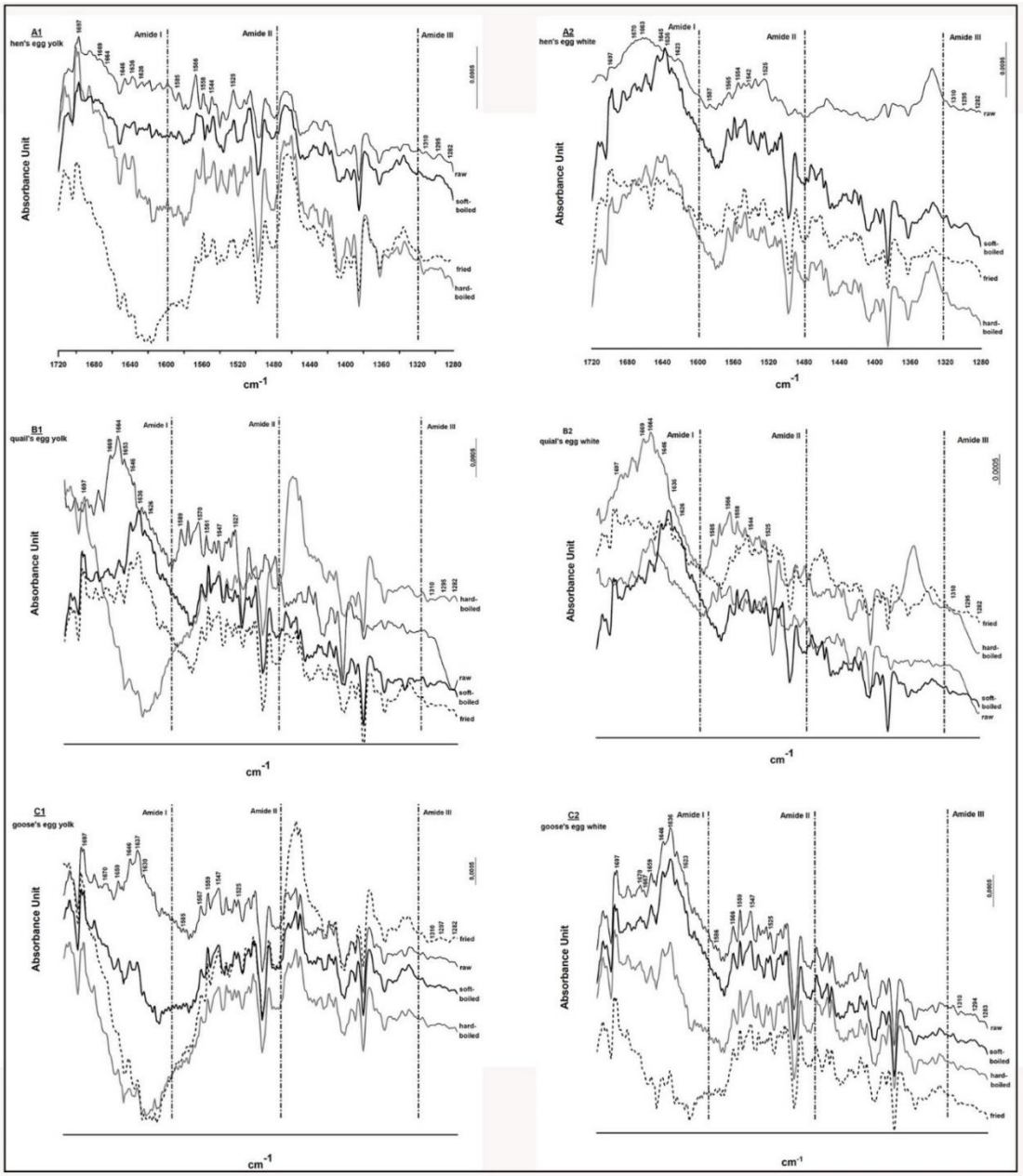

Figure2. FT-IR spectra of poultry eggs. A1 and A2 represent hen's egg yolk and white; B1 and B2 represent quail's egg yolk and white; C1 and C2 represent goose's egg yolk and white for raw, soft-boiled, hard-boiled, and fried eggs. Peaks of α-helices, β-sheets, turns and random coils were assigned based on literature.

CONCLUSION

Egg proteins are quite valuable for human nutrition. The eggs of different poultry species can be consumed via different cooking styles including boiling and frying. These treatments strongly lead to protein denaturation and thus change its technological properties and nutritive value due to digestibility in the body. Here, protein profiles and structural conformations of the egg white and yolk proteins were studied using electrophoresis and spectroscopy. SDS-PAGE analysis revealed that some protein bands corresponding to well-known fractions in raw egg yolk and white were mostly retained in soft-boiling, but disappeared in hard-boiled and fried yolk samples due to denaturation. According to IR findings, hard boiling and frying treatments resulted in conformational changes in the protein structure, especially in egg yolk. Quail's egg white was mostly retained it's conformational structure when exposed to boiling. In each poultry species soft-boiling led to less protein denaturation and thus structural deformation in both egg white and yolk. However, hard-boiling resulted in significant protein denaturation and aggregation.

Overall, the native structure of the existing protein fractions in the egg was degraded in varying degrees based on the cooking technique. Additionally, the protein secondary structure was mostly retained with some conformational changes. To better emphasize the time and temperature dependency of the egg protein denaturation, another research is underway. Moreover, the effect of these treatments on the bioavailability of essential amino acids will also be investigated in the future.

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