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Determination of Total Antioxidant Capacity in Different Type of Eggs

Aminu Haruna TELA[1], Ebru CÖTELİ[2], Fikret KARATAŞ[3]*

[1] University Putra Malaysia, Department of Food Science, Putrajaya, MALAYSIA
[2] Kırşehir Ahi Evran Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Kırşehir, TÜRKİYE
[3] Firat University, Department of Chemistry, Faculty of Science, 23200 Elazığ, TÜRKİYE
*Corresponding author: fkaratas@firat.edu.tr

Abstract

Total phenolics, flavonoids and, gallic acid content of different egg types (Farm chicken, duck, quail goose eggs) and radical scavenging activity 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were determined. The phenolic substance content in the ethanol extract of farm chicken egg, organic, duck, goose and quail egg yolk were determined as 1.28±0.08; 2.10±0.09; 2.49±0.11; 3.08±0.11 and 2.88±0.24 µmol gallic acid/g extract, respectively.

The total amount of flavonoid content was determined from the egg yolk from ethanol extract of farm chicken eggs, organic, duck, goose, and quail eggs in µmol of gallic acid/g extract values as; 1.00 ± 0.08; 1.88 ± 0.10; 1.62 ± 0.1; 1.13 ± 0.06 and 1.63 ± 0.07 respectively.

The DPPH radical scavenging activity were determined from the egg yolk extract (ethanol) of farm chicken eggs, organic, duck, and quail eggs with respected values; 3.67 ± 0.32; 5.15 ± 0.50, 4.03 ± 0.41; 4.37 ± 0.46 and 3.83 ± 0.37µg/mL. While the total phenolic substance amounts in farm chicken, local chicken, duck and quail egg yolks are different from each other ($p<0.05$), there is no difference in quail and goose eggs ($p>0.05$). The flavonoid substance in farm chicken and quail, and in duck and goose eggs is statistically no different from each other ($p>0.05$), while the others are different from each other ($p<0.05$). In terms of IC50, farm chicken, goose, duck and quail eggs are statistically no different from each other ($p>0.05$).

From these results, it can be said that the best egg is gas egg in terms of total phenolic substance, local chicken in terms of flavonoid amount and farm chicken in terms of total antioxidant capacity.

Keywords: Eggs, phenolic substance, flavonoid substance, total antioxidant capacity

1. Introduction

Eggs are among the most important animal protein; it comprises all the essential amino acids needed for human body. Egg is a balanced and natural food source for people of all ages. **[1].** In addition to their excellent nutritional value, egg proteins, also has many unique biological activity **[2].** Egg is defining as a main source of food improved from most poultry animals, commonly chicks. Eggs, mostly chicken eggs, which define as an excellent food for humans due to their high protein content, cheapest and being common in the world. They are extremely variable and are used throughout the kitchen, both by serving alone or by using as ingredients in the preparation of meal in order to supplies texture, flavour, structure, moisture and nutrition for much prepared foods, from soups and sauces to breads and pastries [**3**]. Egg consumption is a popular choice for good nutrients which they are variety of chicken (farm chicken egg, local chicken egg), duck and quail, but by a wide margin the egg most often humanly consumed is the

chicken egg, especially unfertilized [**4**]. Consumption of quail eggs regularly helps fight against many diseases which is a natural fighting against digestive tract disorders for example a stomach ulcer. Quail eggs make the immune system stronger, promote memory health, increase brain activity and make the nervous system to stabilize. They help with anemia by increasing the level of hemoglobin in the body while removing toxins and heavy metals [**5**]. Chinese use quail eggs in the treatments of some disease such as tuberculosis, asthma, and even diabetes. Quail eggs also help to prevent people that are suffering from kidney, liver, or gallbladder stones and remove these types of stones. The nutritional value of quail eggs is much higher than those offered by other eggs (farm chicken, goose, organic chicken) which they are rich sources of antioxidants, minerals, and vitamins, and give us a lot of nutrition than any other foods [**6**]. Moreover, eggs contain substances with biological functions and activities, i.e. immune proteins, enzymes, etc., characterized by antiadhesive and antioxidant properties, antimicrobial activities, immunomodulatory, anticancer, and antihypertensive activities, protease inhibitors, nutrient bioavailability, and functional lipids, highlighting the advantages of egg and egg components in human health and treatment of disease and prevention [**7**]. Egg antioxidant properties, such as antibacterial and angiotensin converting enzyme inhibitory effect has been proven that they have biological activity **[8].**

Indicated that the eggs of chicken feed are reported to participate in antioxidants to increase the amount of antioxidants in yellow [**9**]. Antioxidants are molecules that protect biological systems either by inhibiting or preventing the oxidation of substrate by free radicals **[10].**

The eggs are added to the feed to increase the nutrient content of various contributions. Ingestion involved in natural antioxidants are vitamin C, vitamin E, β-carotene, lycopene, lutein and other carotenoids, polyphenols, flavonoids, flavones and flavonols **[11].**

Phenolic substances are important for human health due to their antimicrobial and antioxidant effects and enzyme inhibition [**12**]. Flavonoids are generally responsible for colour, the taste, prevention of fat oxidation, and the preservation of vitamins and enzymes in foods [**13**].

Flavonoids are divided into several classes according to the degree of oxidation of the chroman heterocycle and the position of the attached benzene ring; flavones, flavonols, isoflavons, flavanones, flavanols, anthocyanins, and proanthocyanidins **[14]**. Flavonoids have already showed that, it has anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The flavonoids that are effects by broad therapeutic will be largely connected to their antioxidant property. Moreover, flavonoid compounds may exert care against heart disease through the obstruction of cyclooxygenase and lipoxygenase activities in platelets and macrophages by antioxidant effect **[15]**.

Phenolics are found as secondary metabolites in plants, consisting various compounds such as complex flavonoids, simple flavonoids, phenolic acids and anthocyanins **[16].** Phenolic compounds and their largest group flavonoids are highly potent antioxidants given their aromatic rings bearing hydroxyl groups. They cause the colorization of fruits and vegetables and some phenolic compounds play important roles in the activity of some enzymes **[13].**

Antioxidants protect cells not only by scavenging the deleterious free radicals, but also regulating the gene expression by modulating the signal pathways, regulating normal cell cycle, restraining the neoplastic cell proliferation, hindering tumor invasion and angiogenesis, activating the immune system, reducing inflammatory oxidative conditions, and thereby promoting immunity **[17]**. Therefore, antioxidants, especially of natural origin, for example food-derived peptides are greatly appreciated at present. Antioxidant peptides may be released from numerous plant and animal origin proteins, such as whey protein, peanut kernels, rice bran or milk casein, mackerel, egg yolk and white **[18]**.

The majority of the antioxidant capacity of dietary food such as eggs, fruit and vegetable may be from compounds other than vitamin E, vitamin C, or β-carotene. For example, some flavonoids that are frequently components of the human diet demonstrated strong antioxidant activities those includes catechin, isocatechin anthocyanins, flavonones, flavones and isoflavones **[19].** Therefore, the total antioxidant capacity of dietary food such as fruit, egg and vegetable having much more interest to measure their antioxidant capacity.

In this study, it was aimed to determine the total phenolic and flavonoid contents and total antioxidant capacities of different eggs (Local chicken eggs (organic), Duck eggs, Quail eggs, Goose eggs, Farm chicken eggs) in both egg yolk and albumin and to compare them with each other according to egg types.

2. Materials and Methods

In this study, fresh eggs were purchased from a local market in Elazığ province. Five types of eggs were used throughout the experiment: farm-raised chicken eggs, local chicken eggs, duck eggs, and quail eggs. Analyses were performed on egg yolks. Fresh egg whites and yolks were separated by various methods and prepared appropriately by removing the outer shells. All samples were homogenized using a vortex and centrifuge to obtain representative samples for all analyses.

2.1 Preparation of the Extract

The fresh eggs constant was weighed in the laboratory. The material was dried and then pulverized using an electric blender (Pye Unicam, Cambridge, England) and it was stored in an air-tight container for further use. The slices were wrapped in aluminium foil, lyophilised with liquid nitrogen and freeze-dried. The weight of the freeze-dried sample was recorded, and the sample was stored in a desiccator at 20°C until ready for extraction. For the extraction, 15 g of each pulverized eggs (egg yolk and egg albumen) were extracted in 300 mL of ethanol separately. The separated extracts were then filtered through Whatman's No. 1 filter paper. The filtrated ethanol was separately concentrated to dryness *in vacuo* using a rotary evaporator, the solvents were removed. The filtrate that obtained from the aqueous was froze at −20 °C and dried for about 48 hr by the used of freeze dryer Savant Refrigerated Vapor Trap. Preparation of egg extracts; The egg samples (yolk and albumen) 1:10 (g/mL) were homogenised by 80% of ethanol. The homogenates were also held by ultrasonic water bath for about 1.0 hr at a high level, the solvent content of the cell was passed. The homogenate was filtered after some period through filter paper (No. 2). The filtrate was used to determine the total phenolic and flavonoid contents at 4 °C.

2.2. Analysis of Total Phenolics

The spectrophotometer was used to determine the total phenolic content according to the Folin Ciocalteu method modified by Dewanto et al. **[20]**. An aliquot (0.1 mL) of diluted sample extract ethanol (0.30 mg/mL) was added to 500 µL of the Folin–Ciocalteu reagent and 6.0 mL of water. The mixture was shaked and allowed to stand for 5 min, before the addition of 1.5 mL of Na_2CO_3 (20%). An aliquot of 1.9 mL of distilled water was added and mixed thoroughly. After incubation in dark for 2.0 h, the absorption was measured at 760 nm by a UV-Visible spectrophotometer. A working graph of gallic acid solutions prepared at different concentrations was established. The total phenolic content of the samples was determined and the results were given as µg gallic acid per g dry weight sample (µg GAE/g DW).

2.3. Determination of total flavonoids content

The total flavonoid substance was determined by UV-visible spectrophotometer as described by Huang et al. **[21].** 0.100 g sample or gallic acid, 1250 µL distilled water and 75 µL 5% sodium nitrite solution, 150 µL 10% solution of aluminum chloride were mixed in a glass tube and allowed to stand for 5 minutes then 500 µL 1.0 M sodium hydroxide solution was added and total volume was completed to 2500 µL with distilled water followed by measurement of absorbance at 510 nm. A working graph was formed with gallic acid solutions prepared in different concentrations. The total phenolic content of the samples was determined using the working graph and the results were given as µg GAE /g DW.

2.4. Determination of DPPH free radical scavenging activity

The antioxidant capacity was measured according to the method based on the free radical scavenging activities of the stable DPPH as described by Nile et al. [**22**]. The assay is based on the loss of violet color of DPPH solution when reduced by an antioxidant. A solution of 25 µg/mL DPPH in CH_3OH was prepared, and the absorption of DPPH solution was measured at 510 nm. Then, different amounts of the sample extracts were added to DPPH solution and kept in dark for 30 min before absorbance measurement at 510 nm. The percentage of DPPH scavenging effect was calculated using the equation:

$$DPPH\ Scavenging\ effect\ (\%)\ or\ Percent\ Inhibition = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0 is the absorbance of the sample free solution, and A_1 is the absorbance of the sample containing solution after 30 min. The results of DPPH were given as IC_{50} (µg/mL). The IC_{50} values indicate the concentration of the antioxidant substance which inhibits 50 % of the DPPH radical in the medium, low IC_{50} values indicate high antioxidant activity or vice versa.

The results obtained after the measurements were plotted and the DPPH half-maximum inhibitory concentration (IC_{50}) values (mg/mL) were calculated. Butylated hydroxytoluene (BHT) was used as the standard in this study.

2.5. Equipment and Chemicals

Measurements were made by SECOMAM BP 106 spectrophotometer. The All the chemical reagents used in the analysis were analytical grade and obtained from Merck (Darmstadt, Germany). Double distilled water was used throughout the work.

2.6. Statistical Analysis

All measurements were triplicated. Mean standard deviation was determined and the results were subjected to Analysis of Variance. The SPSS 10.0 for Windows was used for variance analysis and LSD multiple comparison test was performed at $p<0.05$ level.

3. Results

Table 1. The amount of flavonoids, phenolic substances and DPPH scavenging activities of ethanol extracts from five different egg yolk

Eggs	Phenolic Content of Gallic Acid (µmol/g extract)	Flavonoids Content of Gallic Acid (µmol/g extract)	DPPH activity of BHT (µg/mL)
Farm Chicken	1.28 ±0.08	1.00 ± 0.08	3.67±0.32
Local chicken	2.10 ±0.09	1.88 ± 0.10	5.15±0.50
Duck	2.49 ±0.11	1.62 ± 0.10	4.03±0.41
Quail	2.88 ±0.11	1.13 ± 0.06	4.37±0.46
Goose	3.08 ±0.24	1.63 ± 0.07	3.83±0.37

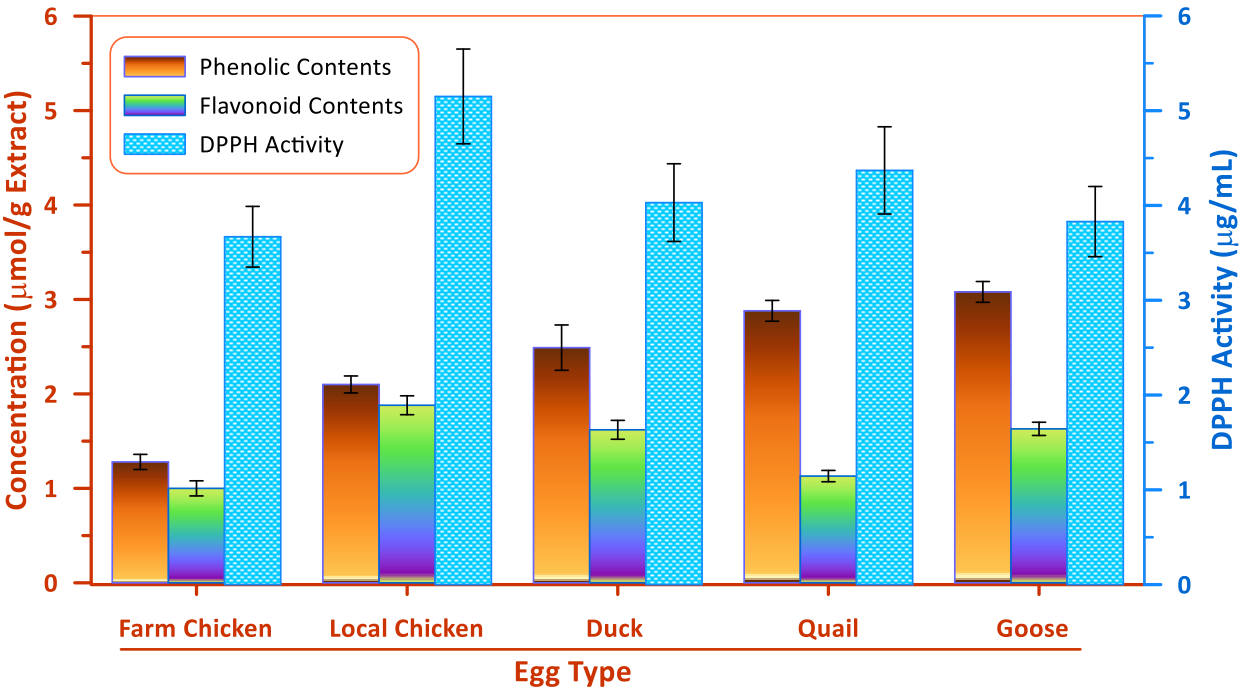

Figure 1. Total phenolic and flavonoid contents and IC_{50} values of different egg yolks

4. DISCUSSION

Phenolic compounds happened to be the most known secondary metabolites found in plants and their distribution is seen throughout the metabolic processes. These phenolic substances consist of different kinds of compounds that include: complex flavonoids, simple flavonoids, phenolic acids and anthocyanins **[16]**. The total phenolic contents of gallic acid 3.83 3.49 µg/g were determined from the chicken's egg yolk feeds diets contained wheat and corn **[23]**.

The amount of phenolic substances in the ethanol extracts of farm chicken, organic chicken, duck, quail and goose egg yolks were determined as (1.28 ± 0.08; 2.10 ± 0.09; 2.49 ± 0.11; 3.08 ± 0.11 and 2.88 ± 0.24) µmol gallic acid/g extract, respectively. While the total phenolic substance amounts in farm chicken, local chicken, duck and quail egg yolks are different from each other ($p<0.05$), there is no difference in quail and goose eggs ($p>0.05$).

The total amount of flavonoids in the ethanol extracts of farm chicken, organic chicken, duck, quail and goose egg yolks were determined as 1.00 ± 0.02; 1.88 ± 0.02; 1.62 ± 0.01; 1.13 ± 0.02 and 1.63 ± 0.02 µmol gallic acid/g extract, respectively. The flavonoid substance in farm chicken and quail, and in duck and goose eggs is statistically no different from each other ($p>0.05$), while the others are different from each other ($p<0.05$).

Omri et al. **[24]** reported that the total phenolic content in the yolk of fresh eggs was 1.86 mg GAE/g as gallic acid and the flavonoid content was 1.92 mg CE/g as catechin.

Total antioxidant capacity in sumac samples was determined by the DPPH methods. High IC_{50} values calculated in the DPPH method indicate low antioxidant capacity. Total antioxidant capacity is a measure of the specific amount of free radicals scavenged by a sample. Antioxidant capacity measurements yield the amount of a heterogeneous mixture of antioxidants, which determines the total scavenging ability of the sample **[25].**

DPPH scavenging activity and suppression of discoloration of β-carotene have also been observed **[26**]. The hydrolysis of egg yolk protein phosvitin with trypsin also leads to obtain a peptide fraction with an ability to inhibit the oxidation of linoleic acid. DPPH free radical scavenging and chelating iron ions (II) **[27**]. Scavenging activity in dried egg yolk of chickens feed with wheat and corn that 13.2 ± 0.2; 26.8 ± 0.9 µmol TE/g were reported **[23**].

In addition, DPPH free radical scavenging activities were determined in eggs, BHT, DPPH radical scavenging activity in ethanol extracts of farm chicken, organic chicken, duck, quail and goose were determined; 3.67 ± 0.32; 5.15 ± 0.50. 4.03 ± 0.41; 4.37 ± 0.46 and 3.83 ± 0.37 µg/mL respectively from their egg yolk. In terms of IC_{50}, farm chicken, goose, duck and quail eggs are statistically no different from each other ($p>0.05$).

Omri et al. **[24]** reported that the antioxidant activity in the yolk of fresh eggs was in terms of ascorbic acid 4.48 mg AAE /g and gallic acid 3.14 mg GAE /g.

Bardakçı Yılmaz and Boyacıoğlu **[28]** reported that the EC_{50} values of standard vitamin C and torolox as well as quercetin, curcumin, vitamin E and resveratrol by the DPPH method were 1.697, 1.729, 1.722, 2.800, 3.123 and 3.970 µg/mL, respectively.

5. Conclusions

Eggs are a good source of nutrients such as protein, vitamins, pro-vitamin, hormone, antioxidant and other compounds total phenolic and flavonoid substance, functional substances like lutein, bioactive proteins and special fatty acids. Furthermore, these components are highly bioavailable from eggs. Phenolic substance, amount of flavonoid and DPPH activity when compared to the eggs. It can be said that Organic eggs were high in amount of Flavonoid and DPPH radical scavenging activity.

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