DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY, AMOUNT OF VITAMINS AND AMINO ACIDS WITH SOME PEPTIDES IN DIFFERENT TYPE OF EGGS

MASTERS THESIS

Aminu Haruna TELA

Chemistry of Science

Supervisor: Prof. Dr. Fikret KARATAŞ

February - 2017

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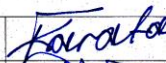
Thesis Supervisor	**Prof. Dr. Fikret KARATAŞ (F.Ü)**	
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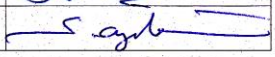
	Prof. Dr. Süleyman AYDIN (F.Ü)	

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DEDICATION

This research work is dedicated to my beloved parents who are my greatest inspiration and to my dearest wife, who always brings out the best in me.

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ÖZET

Değişik Yumurtalardan Total Antioksidan Kapasitesinin Vitaminler, Amino Asit ve Diğer Peptitlerin Miktarının Belirlenmesi

Çiftlik tavuk, Köy tavuk, ördek, bıldırcın ve kaz yumurtalarında A, E, C vitaminleri, MDA, beta-karoten, likopen, glutatyon ve grelin miktarları HPLC ile belirlendi. Aynı yumurtalarda Toplam fenolik, Flavonoid madde miktarları Gallik asit miktarı bakımından ve BHT cinsinden DPPH radikal giderme aktivitesi ise spektrofotometre ile tayin edildi.

A vitamini bakımından çiftlik yumurtası en yüksek(6.07±0.70 µg/g) iken,en düşük ise köy tavuk yumurtası(2.33±0.33µg/g) olarak belirlendi.Yine çiftlik tavuk yumurtası E vitamini bakımından en yüksek (3.33=0.42µg/g) iken,en düşük ise bıldırcın yumurtası (0.32±0.05 µg/g) olarak belirlendi. Bıldırcın yumurtası C vitamini bakımından en yüksek (1.50±0.30 µg/g) iken en düşük ise kaz yumurtası (0.13±0.03µg/g) olarak belirlendi. Beta-karoten bakımından köy tavuk yumurtası en yüksek (0.41±0.07 µg/g) iken, en düşük ise kaz yumurtası (0.19±0.03 µg/g) olarak belirlendi. Yine Köy tavuk yumurtası likopen bakımından en yüksek (0.10 ± 0.01 µg/g) iken en düşük ise ördek yumurtası (0.06 ± 0.01 µg/g) olarak belirlendi. Bıldırcın yumurtası grelin bakımından en yüksek (3.77 ± 0.34 µg/g) iken, en düşük ise çiftlik yumurtası (0.17 ± 0.03 µg/g) olarak belirlendi. Gaz yumurtası GSH bakımından en yüksek (659.25 ± 66.94 µg/g) iken en düşük ise ördek yumurtası (50.33±8.65 µg/g) olarak belirlendi.

Bıldırcın yumurtası GSSG bakımından en yüksek (529.25±77.19 µg/g) iken, en düşük ise köy yumurtası (0.80±0.08 µg/g) olarak belirlendi. Bıldırcın yumurtası MDA bakımından en yüksek (5.48 ± 0.72 µg/g) iken, en düşük ise kaz yumurtası (0.84 ± 0.13 µg/g) olarak belirlendi.Çiftlik tavuk, Köy tavuk, ördek, bıldırcın ve kaz yumurtalarının sarılarının etanol ekstraktlarındaki fenolik madde miktarının µmol gallik asit/g ekstrakt cinsinden değerleri sırasıyla; 1.28 ± 0.08; 2.10 ± 0.09; 2.49 ± 0.11; 2.88 ± 0.11 ve 3.08 ± 0.24 olduğu belirlendi.

Çiftlik tavuk, Köy tavuk, ördek, bıldırcın ve kaz yumurtalarının sarılarının etanol ekstraktlarındaki toplam flavonoid madde miktarının µmol gallik asit/g ekstrakt cinsinden değerleri sırasıyla; 1.00 ± 0.08; 1.88 ± 0.10; 1.62 ± 0.10; 1.13 ± 0.06 ve 1.63 ± 0.07 olarak belirlenmiştir. Çiftlik tavuk, Köy tavuk, ördek, bıldırcın ve kaz yumurtalarının sarılarının etanol ekstraktlarındaki BHT DPPH radikal giderme aktiviteleri sırasıyla; 4.67 ± 1.32; 6.15 ± 2.50; 5.03 ± 1.20; 5.37 ± 1.61 ve 4.83 ± 1.12 µg/mg sarı olarak belirlenmiştir.

Key Words: Eggs, vitamin A, vitamin E, vitamin C, β-carotene, lycopene, Ghrelin, MDA, Glutathione,

SUMMARY

Vitamin A, E, C, MDA, beta-carotene, lycopene, glutathione and ghrelin levels of Farm Chicken, duck, quail and goose eggs were determined by HPLC. In the same eggs total phenolics, flavonoids content, gallic acid content, BHT and DPPH radical scavenging activity were all measured by spectrophotometer In terms of vitamin A farm chicken eggs are the most highest (6.07 ± 0.70 μg/g) whereas organic eggs are the lowest (2.33 ± 0.33 μg/g) were determined respectively. However, the highest in terms of vitamin E (3.33 ± 0.42 μg/g) are farm eggs, whereas the quail eggs are the lowest (0.32 ± 0.05, μg/g), respectively. In terms of vitamin C quail eggs are the highest (1.50 ± 0:30 μg/g), whereas goose eggs are the lowest (0.13 ± 0.03 μg/g), respectively. The highest in terms of Beta-carotene are the organic eggs (0.41 ± 0.07 μg/g), whereas the lowest are the goose eggs (0.19 ± 0.03 μg/g), respectively. However, in terms of lycopene organic eggs found to be the highest one (0.10 ± 0.01 μg/g), whereas duck eggs are the lowest (0.06 ± 0.01 μg/g), respectively. In terms of gherilin quail eggs found to be the highest (3.77 ± 0.34 μg/g), whereas farm chicken eggs are the lowest (0.17 ± 0.03 μg/g), respectively.

In terms of GSH goose eggs are found to be the highest (659.25 ± 66.94 μg/g), whereas duck eggs are the lowest (50.33 ± 8.65 μg/g), respectively. Quail eggs are found to be highest in terms of GSSG (529.25 ± 77.19 μg/g), whereas organic eggs are the lowest (0.80 ± 0.08 μg/g), respectively.

The phenolic content were determined from the egg yolk extract (ethanol) of farm chicken eggs, organic, duck, goose and quail eggs in μmol of gallic acid/g values; 1:28 ± 0.08; 2:10 ± 0.09; 2:49 ± 0.11; 2.88 ± 0.11 and 3.08 ± 0.24 respectively.

The total amount of flavonoid content were determined from the egg yolk extract (ethanol) of farm chicken eggs, organic, duck, goose, and quail eggs in μmol of gallic acid/g 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 BTH and DPPH radical scavenging activity were determined from the egg yolk extract (ethanol) of farm chicken eggs, organic, duck, and quail eggs with respected values; 4.67 ± 1.32; 6.15 ± 2.50, 5.03 ± 1.20; 5.37 ± 1.61 and 4.83 ± 1.12 μg/mg.

Key Words: Eggs, vitamin A, vitamin E, vitamin C, β-carotene, lycopene, Ghrelin, DPPH, MDA, Glutathione.

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SYMBOLS AND ABBREVIATION

GSSG : Glutathione oxide

GSH : Glutathione reduce

MDA : Malondialdehyde

β-carotene : Beta carotene

µg/g : Microgram per gram

µg/mL : Microgram per millilitre

mL : Millilitre

µL : Micro litre

µm : Micrometre

cm : Centimetre

mm : Millimetre

QE : Quercetin equivalent

GAE : Gallic acid equivalents

DW : Dry weight

DPPH : 2, 2-Diphenyl-1-picrylhydrazyl

HPLC : High Performance Liquid Chromatograph

SUPELCOSIL LC-18: Supelcosil Liquid Chromatography-18

IgY : Yolk in immunoglobulins

SOD : Superoxide dismutase

GSH-Px : Glutathione peroxide

CAT : Catalase

BHT : Butylated Hydroxyl Toluene

ROS : Reactive oxygen species

γ-GCS : γ-glutamylcysteine synthetase

GST : Glutathione S-transferase

PPP3 : Phosvitin phosphopeptides

RBP : Riboflavin binding protein

kDa : Kilo Dalton

G : Globulins

pH : Potential of hydrogen

RNA : Ribonucleic acid

DNA : Dioxy ribonucleic acid

COX : cyclooxygenase

HDL : high-density lipoproteins

LDL : low-density lipoproteins

VLDL : Very low-density lipoproteins

Se : Selenium

Met : Methionine

$Fe^{2}+$: Iron(II) ion

$Cu^{2}+$: Copper(II) ion

Na_2CO_3 : Sodium carbonate

$HClO_4$: Hydrogen perchloride (perchloric acid)

KH_2PO_4 : Potassium dihydrogen phosphate

H_2O_2 : Hydrogen peroxide

H_3PO_4 : Phosphoric acid

C_6H_{14} : n-Hexane

CH_3OH : Methanol

$NaClO_4$: Sodium perchloride

SH : Hydrogen sulphur (thiol group)

pI : Iso-electric point

rpm : Rotate per minutes

V/V : Volume per volume

RVT : Refrigerated vapor Trap

FÜ : Firat University

Hr : Hour

1. GENERAL INTRODUCTION

Egg traditionally has been used for breakfast, preparation of home meal, baking and it is extremelly highly nutritious. It gives major amount of complete, provides high quality protein and important amounts of many vitamins **[1].** 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. The children meet the nutritional needs of the body in the period of rapid growth and development of eggs provide a significant contribution. Egg has the highest quality protein and is rich in essential amino acids **[2].** In addition to their excellent nutritional value, egg proteins, also has many unique biological activity **[3].** 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 comon in the world [**4**]. 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, flavor, structure, moisture and nutrition for much prepared foods, from soups and sauces to breads and pastries [**4**].

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 [**5**]. Besides, a lot of people more especially in Asian and middle east countries consumed quail eggs are reported previously that, eggs are packed with vitamins and minerals infact with their small size, their nutritional values are much greater than chicken eggs [**6**]. 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 stablize. They help with anemia by increasing the level of hemoglobin in the body while removing toxins and heavy metals [**6**].

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 [**7**]. Moreover, eggs contain substances with biological functions and activities, i.e. immune proteins, enzymes, e.t.c, 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 [**8**]. In addition to positive effects on the health and nutritional impact of known biologically active compounds since they contain eggs of animal origin and is defined as a natural functional food **[9].**

Egg yolk Lipovitell, lipovitell's, phosvit's, livet's, yolk in immunoglobulins (IgY) are reported to be found, such as small peptides [**10**]. The main proteins in albumin and yolk are ovalbumin, ovotransferin, lysozyme, ovomucin and immunoglobulin Y, the ovalbumin is the most abundant of these proteins, which accounts for more than half of the protein in egg white [**11**]. The highest individual amino acid content in egg was lysine (509 mg/g) whereas the lowest was cysteine (128 mg/g) according to [**12**]. In addition, there are some individual amino acids such as arginine, serine, cysteine and iso-leucine in egg showed values higher than that of other animal products. Among the essential amino acids in egg white, the highest content of amino acid was the combination of phenylalanine and tyrosine whereas tryptophan showed the lowest value. As reported by [**13**]. In eggs A, D, E, K and B group vitamins, other vitamins and unsaturated fatty acids, cholesterol, choline, iron, calcium, phosphorus, selenium, are reported that has a high content of zinc [**14-16**]. Vitamin B that enables cell growth and division. Deficiencies of folate can course increase risk of cancer, dementia and mental debility in older populations, as well as an increased risk of neural tube defects in new born babies. There is a difference in bio-availability and safety between supplemented folic acid and folate from food sources. In some studies, recently demonstrated that folic acid added to laying hen diets is deposited in the egg as natural folate (95 % of this is found in the yolk) and that laying hens fed 4 ug of folic acid per kg of feed, can produce eggs with about 40 ug of folate per egg. This is approximately a threefold increase that elevates eggs to an “excellent” source of folate. Antioxidants are macronutrients that protect against the effects of free radicals, which are molecules produced when the body breaks down food, or is exposed to environmental stressors such as tobacco or radiation. Free radicals can damage cells and have been linked to some diseases such as cancer and heart disease. In some recent research suggested that eggs contain significant quantities of antioxidants. The study investigated the total antioxidant capacity of yolk extracts and the effect of it activity. The study concluded that the antioxidant activity of an egg yolk is almost twice that of an apple **[17].** Eggs are also good antioxidant carotenoids, lutein and zeaxanthin are the source **[18].** Egg antioxidant properties, such as antibacterial and

Angiotensin converting enzyme inhibitory effect has been proven that they have biological activity **[19, 20].**

Nowadays, low cholesterol eggs with different diets, omega-3 fatty acid enriched eggs, conjugated linoleic acid enriched eggs are indicated vitamin-enriched egg and mineral-enriched egg production were carried out **[9].** Eggs are the main ingredient in many dishes and pastry. Eggs, difficulty in chewing certain foods and is a valuable nutrient in the diet of elderly people whose need to lower calories. Being inexpensive, easy to be used in the preparation of various forms and egg cakes reasons of high nutritional value is included as in a healthy diet **[16].** 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 **[21, 22].** According to the xanthophylls and carotenes carotenoid structure are divided into two grades. Xanthophylls lutein, zeaxanthin, β-cryptoxanthin, canthaxanthin while carotenes, β-carotene, α-carotene and lycopene are made from astaxanthin [**23].** Indicated that the eggs of chicken feed are reported to participate in antioxidants to increase the amount of antioxidants in yellow [**24, 25**].

The endogenous ligand for the growth hormone secretagogue receptor are known as ghrelin, and it has powerful growth hormone releasing activity. The oxyntic mucosa of the stomach is place where endocrine cells are mainly manufactured ghrelin **[2**6**, 27].** There are two subtypes of ghrelin: an acylated form, deacylated form **[28].** The ghrelin has the follwing functions as follows. First, ghrelin has an appetite stimulatory effect is associated with both central regulation and peripheral signals, ghrelin encourages food intake. **[29-33].** Secondly, ghrelin acts in the central nervous system to stimulate gastric acid secretion and regulates gastric acid secretion. **[34].** Thirdly, promotes gastric emptying and induces the migrating motor complex. Lastly, the effect of gastroprotective in the context of the generation of nitric oxide and prostaglandins also have reported as a function of ghrelin **[35, 36].**

1.2. Antioxidants

Antioxidants are molecules that protect biological systems either by inhibiting or preventing the oxidation of substrate by free radicals **[37].** Antioxidants are divided into two parts, those are enzymatic and non-enzymatic. Enzymatic antioxidants include the most important intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx), which protects against low levels of oxidative stress and catalase (CAT), and the non enzymatic

antioxidants like ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids and flavonoids **[38].** Antioxidant peptides from egg proteins have also been reported **[39].** Apart from proteins, other antioxidant compounds in animal tissues like ascorbic acid and vitamin E are well-known for their antioxidant properties **[40].** Salmon and shrimp are among the aquatic animals that contain high amounts of carotenoids with strong antioxidant properties. Fish and shrimp are also found to have high concentrations, by showing strong singlet oxygen and radical scavenging ability, which was much greater than Alpha-tocopherol activity **[41].** The activity was mainly attributable to the presence of hydroxyl and keto endings on each ionone ring in the structure of astaxanthin **[42].**

1.2.1 Beta-carotene

The orange yellow-color of the egg yolk are course by carotenoids which are lipid soluble compounds. To date more than 600 carotenoids have been identified and it was suggested that about 50 of them can occur in our diets and 14 of them in human blood **[43, 44]**. Carotenoid does not synthesize by human body and therefore must be get from the diet. For this reason, the type and bioavailability of dietary carotenoids are very important to be consider. The green leafy vegetables have low bioavailability compared to egg carotenoids because of the solubility of yolk lipids that makes eggs excellent and major bioactive carotenoids **[45]**. Beta-caroten and lycopene, are carotenoids with important components of antioxidant defense against lipid peroxidation in living cells **[46]**. Colour of egg yolk is an important factor in consumer's acceptance of a product. Colour of egg yolk is an important factor in consumer's acceptance of a product. Desirable egg yolk colour varies between markets, but yellow to golden colours are usually considered as an indication of better egg quality. Natural colour of egg yolks is a result of carotenoid accumulation. Hens are not able to synthesise colour pigments, but they have ability to transport pigments to the yolk from the ingested feed and, therefore, carotenoid profile of yolk reflects carotenoid profile of diets**[47]**.

Yellow corn grain is usual dietary base for laying hens diet, e.g., its content in hen's diet in Croatia reach up to 70 %. Carotenoids responsible for the colour of corn grain are also the pigments for egg yolks colour. Yellow corn grain is source of lutein, zeaxanthin, β-cryptoxanthin and β-carotene **[48]**. Their contents in total carotenoids content of corn grain depend on corn hybrid, but the major substances are lutein and zeaxanthin **[48]**. Although monohydroxyl and monoketocarotenoids (β-cryptoxanthin and β-carotene) could be converted to vitamin A**[49]**.Lutein and zeaxanthin are effectively transferred to egg yolk **[47]**.

Carotenoid have major interest currently, that are found in vegetables and plants, not only because of their provitamin A activity, however by scavenging oxygen radicals and reducing oxidative stress in the organism are all due to their antioxidant action **[50]**.

The strong correlations between carotenoid intake and a reduced risk of some diseases are showed by many researchs, such as cancer, aterogenesis, bone calcification, eye degeneration and neuronal damages **[51]**. Lipid peroxidation can also prevent by carotenoids and act a preventive in carcinogenesis **[52]**. Even though useful at average concentration, A pro-oxidant are act due to high doses of supplementation of carotenoids **[53]**. The most important antioxidant in preventing lipid peroxidation are consider to be vitamin E. Another class of lipid soluble compounds with antioxidant properties are carotenoids. The main mechanisms are singlet oxygen quenching, reacting with free radicals and delocalizing the unpaired electrons with the aid of unsaturation and resonant stabilization **[53, 54]**. The main protective mechanism of eye macular against blue light-induced oxidative damage are moved by singlet oxygen scavenging ability of lutein and zeaxanthin **[55, 56]**.

1.2.2 Lycopene

Lycopene; the red colour in tomato, watermelon and other fruits are caused by lycopene compound and it is also used as a colour ingredient in so many types of food formulations **[57]**. Lycopene, is a compound of hydrogen and carbon, has received particular attention as a result of studies indicating that it has highly efficient antioxidant and free radical scavenging capacity **[58]**. Lycopene is a lipophilic compound that is insoluble in water, but soluble in organic solvents, and it has a quenching constant double that of beta-carotene and 10 times alpha tocopherol **[58]**. A great interest has recently been focused on lycopene due to its preventive activity against several pathologies, such as cardiovascular disease **[59]**. First separated from Tamus communis L, lycopene is one of carotenoids and commonly found in fruits and vegetables **[60]**. Some carotenoids have cyclic structures and are the precursor for the synthesis of vitamin A **[61]**. The family of carotenoids are all lycopene. Due to the long chain of conjugated double bonds, it structure consists of two open end rings. The longest structure of all carotenoids is lycopene. Lycopene with a molecular formular of ($C_{40}H_{56}$, molecular weight 536.85) is a hydrocarbon carotenoid with 13 carbon-carbon double bond, 11 of which are not carbo-carbo double bond and are arranged in straight. The vibrant red color of lycopene are caused by conjugated double bonds **[60-62]**.

1.2.3 Flavonoids

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 **[63]**. They are present in food as glycosides and largely processed to their aglycones before absorption. From the most prominent flavonoids quercetin, e.g., is a flavonol, catechin a flavanol, genistein an isoflavone, and cyanidin an anthocyanidin **[63]**. Flavonoids have already showed that, it has anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity **[64-67]**. The flavonoids that are effects by broad therapeutic will be largely connected to their antioxidant property. Moreve flavonoid compounds may exert care against heart disease through the obstruction of cyclooxygenase and lipoxygenase activities in platelets and macrophages by antioxidant effect **[66]**.

1.2.4 Vitamins

Birds are naturally well adapted to cold due mainly to their highly efficient insulation provided by feathers **[68]**. However, production efficiency of poultry goes down at low ambient temperatures. Increase in feed intake cause low ambient temperatures, but also result in decreased egg production and feed efficiency in laying hens **[69]**. Decreases in serum concentrations of some vitamins like minerals, and insulin also cause by such cold conditions, and increases in serum of poultry and humans too **[70]**. The stress in animals that reduced ascorbic acid, retinol and α-tocopherol concentrations in plasma and blood cells are due to environmental temperature found **[71]**. But due to an increased production of free radical's MDA levels were found high in plasma and tissues **[72]**. Moreover, the dietary requirement of these vitamins can increases the absorption of vitamins A, E and C due to ambient temperature **[73]**. The increased fortification of infant formulae with fat and water-soluble vitamins has led to the need for rapid methods of vitamin determination **[74]**. Vitamins are a large group of compounds, that are different in their chemical composition, physiological action and nutritional importance. From a nutritional point of view an adequate intake of vitamins has a great importance. The legislation and labelling guidelines for retinol, tocopherols and vitamin D_3 will also reflected. For these, a proper methods of analysis for vitamins that are needed will be taken **[74]**. Vitamins have a key role in the metabolism as part of enzymes, so-called coenzymes, as antioxidants to prevent undesired oxidative processes in the body and as hormones. The deficiency syndromes of some B vitamins such

as beriberi, pellagra, oral genital lesions and peripheral neu-ropathies, that are larger public health problems in some parts of the world. It is known that the human body needs an enough supply of 13 different vitamins up to now. **[74]**. Vitamins are group of macronutrients or organic substances found only in living things, plants and animals. Vitamins were discovered by **[75]**. Who won the novel prize in physiology and medicine in 1929. For normal metabolism and good health small amounts of vitamins are necessary for normal metabolism. Vitamins doesn't have calories therefore are not sources energy, but assist in nutrients, food metabolism, and are not valuable to keep body run smoothly. It is possible to make some vitamin to be digest, and absorbs other nutrients to metabolized by the body **[75]**.

Base on their solubility vitamins are devided into two. The fat-soluble vitamins which are vitaminS A.D.E.K. The water-soluble vitamins are vitamin B12, B9, B7, B3, B2, B1 and vitamin C (ascorbic acid). Fat soluble vitamins contain only carbon, hydrogen, and oxygene, while water-soluble vitamins contain these three elements plus nitrogen and sometimes sulphur. The body can store Fat-soluble vitamins in perceptible amounts **[75]**. Even thought their chemicals structures are not related, a considerable number of papers have been published describing different physical, biological and chemical methods to analyze the vitamins **[76]**.

1.2.5 Vitamin A

Vitamin A (retinol retinal, retinoic acid) are group of unsaturated organic compound is an essential nutrient required in small amounts by an organism for the normal functioning of the visual system such as growth and development, maintenance of epithelial cellular integrity, immune function, and reproduction**[77].** Retinol is carried to ocular tissue and to the retina of the eye by intracellular binding and transport protein in which it plays an important role in the generation of rhodopsin. When there is a deficiency amount of retinol, synthesis of rhodopsin may be occur and the resultant will be night blindness **[78**]. Vitamin A also serves as mediators of cell signaling, regulators of cell and tissues, growth and differentiation. Vitamin A occurs naturally in two different forms; preformed vitamin A which occurs in animal foods as retinyl esters of fatty acids associated with membrane bound cellular lipid, fat containing storage cell and provitamin A are carotenoids in foods vegetable orogin which are associated with cellular lipids but are embedded in complex cellular structures such as the pigment containing a portion of chloroplast **[79**].

1.2.6 Vitamin E

Vitamin E has for long been considered as an antioxidant only. For this reason, it has been tested for its protective effect in the prevention of diseases connected to oxidative stress, mainly atherosclerosis. Numerous experiments with different animal models did not show a consistent inhibitory effect of vitamin E. Results were contradictory, depending on the model used and the feeding modus. In most of the large clinical trials vitamin E consistently failed to prevent atherosclerosis (reviewed in **[80]**. In addition, there is little evidence that compounds with antioxidant properties in a test tube also inhibit oxidative reactions *in vivo* **[81]**. Future studies with vitamin E should consider novel functions of vitamin E which include regulation of gene activity. Identifying the vitamin Eregulated genes and the biological processes depending thereon will definitely help to understand the real physiological role of vitamin E and to develop strategies how to use the vitamin to keep an organism healthy **[81]**.

1.2.7 Vitamin C

Vitamin C is also mainly considered as an antioxidant. It is an electron donor and therefore a reducing agent. Electrons are donated sequentially, i.e. vitamin C is first converted to a radical, the semidehydroascorbic acid or ascorbyl radical. Since the ascorbyl radical is fairly unreactive and relatively stable, ascorbate can react with a more reactive and thus more harmful radical and, therefore, may be considered to be a good free radical scavenger. Loss of a second electron results in dehydroascorbic acid **[82]**. Vitamin C is, however, required for the function of a number of enzymes. The most important are prolyl and lysyl hydroxylases which hydroxylate collagen, a process required for the adequate formation of the collagen molecular structure. Vitamin C deficiency prevents proper collagen formation, thus leading to scurvy **[82]**.

In vitro, vitamin C prevents oxidation of LDL, proteins and DNA. In animal experiments it has been shown to have pro- and antioxidant effects, depending on the experimental design **[82]**. Beneficial effects of supplemental vitamin C have been noted in small studies, while large, placebo-controlled and prospective studies have failed to show benefit in conditions believed to result from oxidative stress. To increase vitamin C plasma levels to putatively protective concentrations corresponding to in vitro studies is not possible, since the plasma concentrations are tightly controlled and excess vitamin C is excreted **[82]**.

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 **[83]**.

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 **[84]**. It has been shown that peptides derived from egg white obtained after the isolation of lysozyme and cystatin exhibit antioxidant activity against free radicals, 2,2 - diphenyl-1-picrylhydrazyl (DPPH) **[85].** It has been reported that egg-yolk hydrolysates show antioxidant capacities in a linoleic acid oxidation system **[86]**. DPPH scavenging activity and suppression of discoloration of β-carotene have also been observed **[86, 87].** The hydrolysis of egg yolk protein phosvitin with trypsin also leads to obtain a peptide fraction with an ability to in hibit the oxidation of linoleic acid, DPPH free radical scavenging and chelating iron (II) ions **[88].**

Antioxidant properties of peptides often translate into a reduction of the risk of cancer **[89]**. Demonstrated that the consumption of egg yolk protein hy-drolysates inhibits tumor cell proliferation in the colon. Studies have shown that this effect results primarily from an improvement of antioxidant protective systems in the mucosa of the colon. This can result from the fact that phosphooligopeptides from phosvitin have the ability to modulate the secretion of antioxidant enzymes such as catalase and glutathione reduce **[89].** Furthemore, it was demonstrated that these phophooligopeptides have the ability to increase the activity of intracellular GSH and regulate the expression of γ-glutamylocysteine in intestinal epithelial cells, which catalyzes the synthesis of GSH **[90]**. Peptides showing a variety of properties are known as multifunctional **[84]**.

1.2.8 Ghrelin

Currently ghrelin is the only known gastrointestinal hormone that increase food intake. Plasma ghrelin levels are inversely related with body weight and increase weight loss in humans **[91**]. Ghrelin (hunger hormone) is a peptide hormone breeded by ghrelinergic cell in gastrointestinal tract **[92].** That functions as neuropeptide in the central nervour system(CNS**) [93].** Furthermore it regulate apetite, ghrelin also plays an important role in

regulating the rate use of energy distribution **[94**]. Ghrelin was composed by 28 amino acids with an acyl side chain attached to the third position of serine residue. This acyl group is very important for ghrelin's orexigenic and growth hormone releasing action **[95].** The idea of Kojima's paper and some other research show that De-acyle ghrelin should be accepted as degredation product of ghrelin without biological activities**[95].**

1.2.9 Glutathione

Glutathione, are substantial antioxidant that are soluble in water, is the amino acids such as glutamate, cysteine, glycine that synthesized it. Glutathione directly eliminate lipid peroxides that is ROS, also plays an important act in metabolism of xenobiotic **[96]**. The glutathione and vitamin C work interactively to quench free radicals as suggested by some researchers and that they have a sparing effect upon each other **[96].** Glutathione are L-glutamyl-L-cysteinylglycine, is a tripeptide that soluble in water can react with ROS using its thiol group and form glutathione disulfide (GSSG) by oxidation which then convert back to GSH by the action of NADPH as a cofactor and GRx **[97, 98]**. The regeneration of ascorbate was involved by GSH **[97]**. The present of coenzyme Q10 in all cells and membranes, is the only endogenous liposoluble antioxidant that synthesized. It is a powerful antioxidant that involved in regenerating vitamin E during the initiation step and prevents lipid peroxidation **[99]**. The metabolic product of purine nucleotide is uric acid, then will be absorbs back into the body when kidney filtration is taken place then into the plasma **[86]**. A hydroxyl radical scavenger and powerful singlet oxygen, the lysis of red blood cells can be prevented by uric acid through peroxidation **[100]**. Poultry nutrition are made up of two 2 essential substances those are selenium (Se) and methionine (Met). The best known of among them is glutathione peroxidase (GSH-Px), which is an essential component of a variety of selenoproteins refers as Se. The GSH-Px family of enzymes is a crucial player in the integrated antioxidant system, neutralizing potential threats to the integrity of cellular macromolecules by eliminating hydrogen peroxide and detoxifying lipid hydro peroxides **[101]**. The diet of the female bird that deposited in the egg are derived from Se. And are distributed among the developing tissues during embryo genesis **[102]**. Consequently, GSH-Px is expressed in the chicken embryo in a tissue and stage-specific manner **[103]**. Supplementary Se in the diet of the hen was shown to increase the concentration of this element in the egg and in the tissues of the chick at hatch, and to elevate the expression of GSH-Px, while reducing the generation of lipid peroxides in the liver of the day-old chick. Have shown that dietary supplementation of

the female chicken with Se increased Se concentrations and GSH-Px activity in blood, liver, and breast of chicks **[104]**. The initiation has been associated with Intestinal oxidative stress and chronic intestinal pathologies propagation like sore bowel diseases example intestinal cancers **[105-107]**. The dietary pro-oxidants ingestion includes heme, lipid hydroperoxides and within the gastrointestinal tract the deleterious effects caused by transitionmetal ions **[108]**. Variety of enzymatic was utilize in response to these threats, mucosal cells and unwanted ROS accumulation were tightly control by nonenzymatic mechanisms. glutathione (GSH) like R-tocopherol, ascorbate is nonenzymatic antioxidants, whereas antioxidative enzymes include catalase (CAT), glutathione reduces (GR), glutathione S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD), act to coordinately detoxify ROS **[109]**. When the oxidative stress occurs, oxidized (GSSG) increases GSH while concentration rapidly decreases by shifting the thiol redox status of the cell as well establishing oxidant that response to transcriptional elements, the rate-limiting enzyme in GSH synthesis will projected to the up-regulation of antioxidant enzymes and γ-glutamylcysteine synthetase (γ-GCS), **[110]**. An excess of reactive oxygen species (ROS) are caused by oxidative stress, that participates in cellular damage by reacting with lipid membranes, proteins, and DNA **[110]**. For example, the study of **[111]** showed that peptides derived from hen egg lysozyme have the ability to neutralize reactive oxygen species (ROS) and inhibit the growth of Bacillus bacteria. Ovoalbumin, with antimicrobial properties, is one of the precursors of peptides. Hydrolysis of this protein with trypsin leads to the release of the penta-hexa and octapeptides exhibiting strong bactericidal activity against B. subtilis **[112]**.

1.2.10 Sources of Antioxidants

The antioxidant properties of the natural sources were attributed during the increased free radical production by either reducing or scavenging the reactive species, quenching singlet oxygen, or by chelating with pro-oxidant metals **[113].** Antioxidants are found in various natural sources such as plants, animals, microbes, etc. Naturally occurring antioxidants usually originate from plant based ingredients such as fruits, vegetables, cereals, and nuts. However, antioxidants also form from animal's source, for example, muscle tissues with carnosine, a dipeptide with metal a chelating and free radical scavenging property **[114].** Certain vitamins, minerals, and enzymes also serve as an antioxidant.

1.3. Composition and physicochemical characteristics of hen egg proteins

Bird eggs are well-considered as an important source of nutrients, such as enzymes, proteins, lipids, and some biological substances, like defence factors and growth promoting factors. Overall, an egg is composing of 27.5% egg yolk, 9.5% eggshell and 63% egg white (Table 1.1). Whole egg comprises of about 13% proteins, which are actually distributed within the egg yolk, white and with a small amunt in the eggshell.

Lipids, that are negligible in white egg, are almost exclusively found in the egg yolk and are associated with proteins to form lipoproteins. Carbohydrates are minor egg ingredient, which are present in the egg as both free carbohydrates and bound to proteins. Approximatively 98% of the free carbohydrates of egg white are glucose. Most of the minerals are found in the eggshell and in the yolk **[115].**

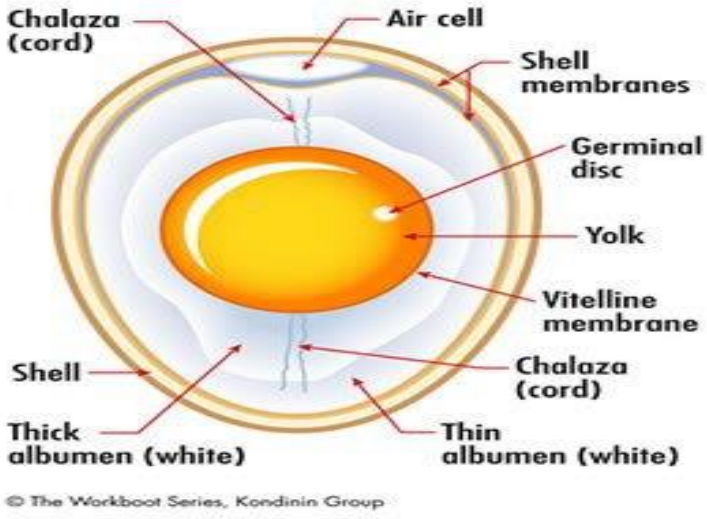

Figure 1.1 Biological structure of an egg.

The poultry eggs primarily consist of the following parts:

i) Shell

ii) Membrane

iii) Albumen

iv) Yolk

The proximate composition of egg is given above. Albumen or egg white is the single largest component of an egg, which represents about 60% of total weight of the egg (Froning 1998) **[116]**. Albumen is high in protein; ovalbumin is the main protein of egg white; which accounts for 54% of total proteins present in albumen (Table 1.1) below **[117]**. Some of the

previous research works show that eggs have antioxidant effect. This property has been attributed to conalbumin (or Ovotranferrin). Research has to be done to determine the true potential of egg white as an antioxidant (Froning 1998) **[116]**. This can be an important property which could have great impact on the food processing industry as they are looking for natural ingredients in order to produce healthy, chemical free foods **[117]**.

Table 1.1 Structure and some composition of eggs.

Nutrients	Whole egg	Egg white	Egg yolk
Energy, kcal.	149	50	358
Water, g	75.33	87.81	48.81
Protein, g	12.49	10.52	16.76
Fat, g	10.02	0	30.87
Carbohydrate, g	1.22	1.03	1.78
Cholesterol, mg	425	--	1,281
Vitamin A, IU	635	--	1,945
Riboflavin, mg	0.508	0.452	0.639
Calcium, mg	49	6	137
Phosphorus, mg	178	13	488

Nutrient Composition of egg (per 100 g) **[117]**

1.3.1 Lipoproteins in egg yolk

Low density lipoprotein (LDL), which contains between 80 and 90% lipids, characterized by its emulsifying capacity. The main protein in egg yolk is LDL, accounting for about 70% of yolk proteins. If LDL is treated with ether, residual fraction is referred to lipovitellenin containing 40% lipid. High density lipoprotein (HDL) or lipovitellin contain about one sixth of egg yolk solids in the granular yolk proteins. It has a molecular weight of $4.0x10^5$ and it constitute about 80% protein and 20% lipid. HDL exists as a complex with a phosphoprotein referred to phosvitin **[118]**. The effect of some ingredient of egg yolk lipoprotein on the growth and immunoglobulin production of human-human hybridoma cells and other human-reproduce cells are studied by Shinohara et al. (1993) **[119]**. The growth and IgM secretion of HB4C5 cells where enhanced by the fractions of rich-LDL. The promoting activity was found in the commercial LDL. The addition of one or two eggs a day to a healthy person's diet does not adversely affect lipoprotein levels, and can actually increase plasma HDL levels **[120]**.

VLDL made up of apoVLDL II and apolipoprotein-B (Burley *et al.*, 1984). ApoVLDL II blood lipoproteins is the only apoprotein that will transferred to yolk without

any alteration and is known as apovitellenin I **[121].** The source of yolk low-density lipoproteins (LDL) is VLDL. During the transfer from blood to yolk, apolipoprotein-B is cleaved into several fragments, reffered as apovitellenin I-VI **[122].** Vitellogenin consists of three species designated vitellogenin I, II and III. Vitellogenin is cleaved into the yolk granule proteins lipovitellin I, II and the phosphoprotein phosvitin. Amino acid analysis indicated the presence of a highly phosphorylated phosvitin in vitellogenin I and II and small phosvettes derived from vitellogenin III **[123].** The granules of egg yolk were separated by subjecting to a centrifugal force of 20 000g and granules consisted of 11-13% of the solids in yolk with phosphoprotein, lipoprotein contained and most calcium of the yolk and the iron **[124]**. This was approved in more detailed enquired by **[125],** The granules represent about 19-23% of the yolk solids on a dry weight16% phosvitin and 12% LDL and consisted of 70% high-density lipoproteins (HDL) that reported **[125].** These authors concluded that low-density lipoproteins is structural constituents of the granules.

1.3.2. Malondialdehyde

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation; Also it can generate during prostaglandin biosynthesis in cells. Amino groups reacts with MDA on proteins and other biomolecules to establish a range of adducts, adducts with DNA bases that are mutagenic when included will be possibly carcinogenic. The measurement of MDA increase levels of lipid peroxidation products, which has been associated with various conditions and pathological states of diseases **[126].** Polyunsaturated fatty acids will produced Lipid peroxides, are not stable and saperate to form a complicated series of compounds, that contained reactive carbonyl compounds, comprises of MDA **[126]**. During cyclooxygenase catalysis in human platelets MDA will be generated, formed from prostaglandin endoperoxide, with the help of catalyzed thromboxane synthase and in liver cells by breaking down of PGH_2 **[127].** Malondialdehyde, MDA, are produced by an extremely reactive three carbon dialdehyde as a byproduct of an organic compound of double or triple bond of fatty acid peroxidation **[128]** and also the synthesis of prostaglandins occur during arachidonic acid metabolism **[129].** Combination of MDA with several functional groups on molecules those molecules include lipoproteins, proteins, DNA and RNA **[130].** Most of biological samples forms MDA those include serum tissues and urine, foodstuffs and plasma, as a result of this lipid peroxidation. MDA were used in determining the distribution

of plasma lipid peroxides in men and women, was found approximatly by a normal distribution. The median level of 10% between 30 and 70 years of age in both sexes, were increased that may be appropriate to the increases prevalence of atherosclerosis with age **[131].** MDA has been reported to be induced in various conditions and states of some chronic disease such as hepatitis C infection, smoking, diabetes and HIV seropositive children **[132].** It has also been reported that chondrocyte-derived lipid peroxidation product MDA mediates oxidation of cartilage collagens and leads to alteration of biochemical and biophysical properties of cartilage collagen fibrils, making them prone to degradation and initiating the changes observed in aging and osteoarthritis **[133].**

1.3.3. Phosvitin peptide

The new functional bioactive peptides derived from egg yolk are known as phosvitin phosphopeptides with molecular mass of 1-3 kDa that are prepared through tryptic hydrolysate by partial dephosphorylation **[134].** The calcium binding capacity was shown by the action of phosvitin phosphopeptides and the formation of non soluble calcium phosphate that are inhibited. for the prevention of osteoporosis of phosvitin peptides as a novel functional peptides are suggested from the results **[134].**

1.4. Biological activities in egg yolk phosvitin and their peptides

1.4.1 Antimicrobial activity of phosvitin

The amino acids in the protein almost nearly 50% of them are serine, of which 96% in the form of phosphoserines were present; a very strong metal-chelating properties were distracted by phosvitin. The antimicrobial properties of phosvitin are responsible for high iron-binding capacity. Incubation of *Escherichia coli* under thermal stress at 50°C in the presence of phosvitin at 0.01% and 0.1% significantly decreased the growth of *Escherichia coli* population, which results the synergic effect of the strong metal chelating ability and the surface properties **[135].**

1.4.2 Enhancement of calcium solubility of phosvitin phosphopeptides

Phosphopeptides prepared from egg yolk phosvitin with 35% phosphate retention have been found to better enhance calcium-binding ability and inhibit the formation of insoluble calcium phosphates than phosphopeptides with 65% and 17.5% phosphate retention, evaluated the effectiveness of phosvitin tryptic hydrolysate for enhancing the intestinal absorption of calcium and accumulation of bones **[136].** The results suggested that phosvitin phosphopeptides increased calcium absorption and enhanced accumulation calcium in bones.

1.4.3 Egg yolk phosvitin as natural antioxidant

Egg proteins can act as antioxidants by inhibiting lipid oxidation in food model systems. For example, egg yolk proteins have been found to possess antioxidant activity in a linoleate emulsion, indicating that the granules were the potent antioxidant component in egg yolk **[137].** The amino acids sequence of phosvitin are grouped into numerous phosphoserine, for the iron binding capacity that are responsible and could be a strong natural antioxidant. Both Fe2+ and Cu2+ has been shown to inhibit catalyzed phospholipid oxidation by phosvitin and the iron binding capacity and antioxidant potential of phosvitin were not affected by pasteurization treatment **[138].**

The antioxidant activity of phosvitin and phosvitin-galactomannan conjugate has been evaluated in a powder oil model system produced a novel macromolecular antioxidant by conjugating phosvitin with galactomannan which could withstand a sterilization treatment at 121 ℃ for 15 min **[139].**

1.4.4 Ovalbumin

Ovalbumin is a predominant protein contributing to the functional properties of egg white **[140].** Ovalbumin is a monomeric phosphoglycoprotein with a molecular weight of 44.5 kDa and an isoelectric point of 4.5. Ovalbumin are made up of 385 amino acids is also glycoprotein and totally constitutes of 54% of egg white protein approximately. It composes of six cysteine residues containing one single disulfide bond and ovalbumin is the only egg white protein that has free thiol (SH) groups **[121].** The ability of ovalbumin to act in redox regulation and binding metal ions is because of the thiol groups therefore exert antioxidant

properties **[121].** The key reference protein in biochemistry is known as ovalbumin. As a stabilizer, carrier, standard or blocking agent, has served the fundamentalists as well as the food industry are served by highly purified ovalbumin. For long the subject of chemical and physical studies has been used as a convenient protein model **[141].**

It has credited that ovalbumin, perticularly its unphosphorylated form, for the developing embryo amino acids serves as a source. By considering the high investigations support on ovalbumin, remains normally without knowing its function. The only egg white protein that has free sulfhydryl groups is ovalbumin. Whole amino acid sequence of hen ovalbumin that inclose of almost 385 residues and its crystal structure have reported **[141].**

1.5. Egg white proteins

Egg white consists of a solution of proteins, containing the major proteins such as ovalbumin, ovotransferrin, ovomucoid, ovomucin and lysozyme which account for > 83% of total egg white proteins. Other minor proteins are also found at low concentration and account for < 17% of egg white proteins. The physicochemical characteristics of some egg white proteins are listed in Table 1.1 Egg white proteins are predominantly globular proteins having an acidic pI, the exception being lysozyme and avidin **[142].**

Well-known biological functions of egg white proteins are the prevention of microorganisms' penetration into the yolk and supply of nutrients to the embryo during the late stages of development. The egg white proteins Mostly appear to have antimicrobial properties or specific physiological functions to step in with the growth and spread of attack microorganisms. Egg white proteins are mostly soluble and can be isolated easily. Approximately 40 different proteins that egg white contains. Unique functional properties were possessing by egg white proteins, like vitamin binding, enzymatic and anti-enzymatic, antimicrobial, metal binding, cell growth stimulatory and immunological activities **[143].**

1.5.1. Globulins

Egg proteins are first classified into yolk proteins and egg white proteins. Egg white proteins are further divided into albumins and globulins. The three globulins G1, G2 and G3 from egg white have been separated by moving-boundary electrophoresis **[144].** Lysozyme was identified as globulin G1 while on the G2 and G3 globulins small work has been

published. Previous studies, the present of globulin fractions in egg white were thought. They are ovoglobulins G1, G2 and G3 macroglobulin, and other two globulins. On the other hand, the two globulins were also catagorised as ovoglobulin G1 was identified as lysozyme and ovoinhibitors. Now the name ovoglobulin is dedicated only to ovoglobulins G2 and G3, which have molecular weights of 45 and 36 kDa. These proteins their biological function has not been clearly elucidated, but they have important in the foaming capacity of egg white in their appearences **[118].**

1.5.2. Ovoflavoprotein

The acidic protein with a molecular weight of 32-36 kDa and contains a galactose and glucosamines, 7-8 phosphate groups and 8 disulfide bonds, carbohydrate moiety (14%) made up of mannose are refers to ovoflavoprotein. After the transportation from the blood to the egg white, the egg white bound to an apoprotein mostly store Vitamin B2 and is called flavoprotein. One mole of stored vitamin B2 (flavoprotein) binds one mole of riboflavin, but when the protein is exposed to a pH below its isoelectric pH 4-2 this binding ability can easily lose **[118].**

Ovoflavoprotein, also referred to riboflavin binding protein (RBP), is a phosphoglycoprotein which bound riboflavin at pH above 4.3 with an association constant of 7.9 x 108 M. Constituted of 219 amino acids **[145].**

1.5.3. Ovoglycoprotein

Ovoglycoprotein is an acidic glycoprotein with an isoelectric point (pI) of 3.9 and a molecular weight of 24.4 kDa. It contains 13.6% hexoses, 13.8% glucosamine and 3% sialic acid. In common with many other glycoproteins, ovoglycoprotein remains soluble even after heat treatment at 100°C or by trichloroacetic acid treatment **[146].**

1.6. Antioxidant activity of natural hydrolyzed phosvitin

When phosvitin was subjected to tryptic hydrolysis following partial alkaline dephosphorylation, phosphopeptides with molecular weight value of 1-3 kDa were generated **[147].** Then, phosphopeptides with different phosphorus content were prepared using anion-exchange HPLC. Three fractions named PPP1, PPP2 and PPP3 were separated and the

phosphorus contents were 0, 7.2 and 18.9%, respectively. PPP3 showed antioxidant activity against oxidative stress in human intestinal epithelial cells in an *in vitro* assay using caco-2 cells. Pretreatment of cells with PPP3 resulted in an inhibition of hydrogen peroxide induced IL-8 secretion and decreased malondialdehyde levels while phosvitin did not show any protection effect. Oligophosphopeptides from egg yolk phosvitin can up regulate cellular glutathione biosynthesis-associated enzymes activities and also enzymes imply in antioxidative stress activities **[148].** It was shown that phosvitin peptides had stronger antioxidant activity in a linoleic acid system when compared to phosvitin, which could be attribute to the changes of phosphorus content and the amino acid sequence **[149].** Recently, egg yolk oligophosphopeptides from commercial delipidated egg yolk proteins were produced using a combination of alcalase and different bacterial proteases. Crude egg yolk phosphopeptides were evaluated *in vitro* and *in vivo* for antioxidative stress properties **[150].** Antioxidant egg yolk phosphopeptides were produced using a combination of alcalase and protease N hydrolysis. These peptides exhibited antioxidative stress properties in an *in vitro* hydrogen peroxide induced Caco-2 intestinal cell culture. *In vivo*, egg yolk phosphopeptides induced glutathione synthesis, increased antioxidative enzymes activities stress, as well as, reduced lipid and protein oxidation. Further studies have been conducted to elucidate and sequence egg yolk-derived phosvitin phosphopeptides (PPP3) and pepsin- and pancreatin-digested PPP3. Limited proteolysis of PPP3 was observed after simulated gastrointestinal digestion and the contiguous serine and phosphoserine sequences may influence bioactivity **[151].**

According to literature, antioxidant activity seems to be related to common characteristics of bioactive peptides. Composition in amino acids plays an important role in activity of bioactive peptides **[152].** Reported six peptides from enzyme hydrolysed soy β-conglycinin yielded antioxidative activity against the peroxidation of linoleic acid in an aqueous system, synthesized a smaller peptide LLPHH as model in order to characterize antioxidative properties. The peptides with the sequence of Leu-Leu-Pro-His-His showed a strong inhibition of lipid peroxidation, and the His-His segment played an essential role in the antioxidant activity. Deletion of Leu residue at N-terminal position does not affect antioxidant activity **[153].** However, deletion of His residue at C-terminal position decreased activity and the activity was further improved by the addition of Pro, His to the N-terminus. Similar results were obtained by **[153].** Two peptides isolated from egg yolk lecithin hydrolysate were composed of 10-15 amino acid residues with Leu at the N-terminal position.

1.6.1 Total Antioxidant Capacity

Ascorbate and Alpha-Tocopherol are potent water-soluble and fat-soluble antioxidant vitamins which interact and work synergically to form a non-enzymatic antioxidant system operating in the aqueous phase (cytosol) and the lipid phase (plasma membrane and membrane of cell organelles) of aerobic cell respectively. Hydroxyl radical and superoxide radical are potentially neutralized by Ascorbate while lipid peroxide are neutralized by Alpha-tocopherol hydrogen peroxide are neutralized by anti-oxidants like Ascorbate, Glutathione, Beta carotene, Tocohperol and Tocotrienols **[154].** Nutrient that derived antioxidants like ascorbic acid, carotenoids, tocopherols and tocotrienols (vitamin E) and other low molecular weight compounds example like lipoic acid and glutathione **[96].**

Currently there is a great emphasize on measuring "Total Antioxidant Capacity" in biological fluid for the co-ordination among antioxidants. Total antioxidants capacity represents total strength of different antioxidants to combat reactive oxygen species (ROS) attack in many ways and serum/plasma level is representative of cell/tissue status. The consumption of dietry food such as eggs, fruits and vegetables has been associated with lower risk and lower mortality rates of cancer in several community and case control studies for all common cancer sites **[155].** The majority of the antioxidant capacity of dietry food such as eggs, fruit and vegetable may be from compounds other than vitamin E, vitamin C, or a-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 **[156].** Therefore, the total antioxidant capacity of dietry food such as fruit, egg and vegetable having much more interest to measure their antioxidant capacity.

1.7. Aim

Both egg yolk and the albumen of different eggs; Local chicken eggs (organic), Duck eggs, Quail eggs, Goose eggs, Farm Chicken eggs, were used throughout the experiments.

These work are aimed at solving the problems mentioned below with a quite simple preparation of samples, the selection of solvent mixtures (extraction) were more compatible with mobile phase and short run times, by developing a suitable, reliable, rapid and simple HPLC and spectrophotometrical method for the analysis of the following;

1) Water-soluble and fat-soluble vitamins,

2) The amount of certian biological important of peptide were it determined using High Performance Chromatography (HPLC).
3) Total antioxidant capacity was determined using spectrophotometer.
4) The findings obtained were compared with each other according to the different types of eggs collected, which have very different content of water-soluble and fat-soluble vitamins, essential and non-essential amino acids, amount of biological peptides, total antioxidant capacity.

2. MATERIALS AND METHODS

In this study, fresh eggs were purchased from local supermarket of Elazig city, Turkey. Five type of eggs were used, namely Multiyolk eggs, Farm Chicken eggs, Local chicken eggs, Duck eggs and Quail eggs were used throughout the experiment. The analysis was done in both the yolk and albumen of the eggs. Fresh egg white and egg yolk were separated differently and prepared as appropriate by removal of the outer shell. All samples were homogenized by vortext and centrifuge machine to obtain representative sample for all analysis.

2.1. Chemical Analysis

Analytical-grade solvents used for sample preparation was re distilled before use as impurities in the solvent were interfered with the sample peak in the HPLC chromatogram. Solvents for liquid chromatography were of HPLC grade. All solvents used as the mobile phase in HPLC were filtered through an individual membrane filter and degassed using an ultra-sonic bath.

All radical testing chemicals, retinol, β-carotene, lycopene, α-tocopherol, pantothenate or vitamin B_5 (vitamins B), vitamin A, quarcetine (dihydrate), DPPH (2,2-Diphenil-1-picrylhydrazil), Amonium Thiocyanide, Iron II cloride, Potasium persulphate, Sodium nitrate, Aluminium chloride, Gallic acid, Butylated hydroxy toluene, Flavone, Perchloric acid, and all other Standard reagents were purchased from Sigma Aldrich (Sigma Chemical Co., İstanbul, Turkey). Other Chemicals used were of analytical grade.

2.2. Determination of Vitamins A, E and β-Carotene content in Eggs

The instability of vitamin A, E and beta carotene, the preparation of a sample for analysis during the process of extraction, handling, and elimination of organic solvents makes an extremely delicate task, often requiring successive and complex procedures to ensure that all the vitamin A, E and beta carotene in eggs are extracted. Content of vitamin E, A and β-carotene in egg yolk were determined by High Performance Liquid Chromatograph (HPLC) using method described with minor modifications below.

A fresh egg yolk samples (1.5 g) were mashed in a homogenizer and 1.5 g homogenate sample were taken for extraction of vitamins A, E, β-carotene. Parameter for determining the amount of fat-soluble vitamins, the egg yolk was disintigrated thoroughly, from the homogenizer the sample were weighed approximately 1.5 g were taken into the

polyethylene tube, each tube upon addition of 4.5 mL of ethyl alcohol was vortexed. Then the mixture was centrifuged at 4500 speed for 15 minutes and the samples were rinsed by the addition of 0.50 mL of n-hexane. Thus, retinol, retinoic acid, β-carotene, lycopene, α-tocopherol and n-hexane phase was extracted. This extraction procedure was repeated twice. The resulting extracts were combined to dryness by passing nitrogen gas through the medium. The residues in the tube was dissolved in 0.20 mL of methanol and ready for HPLC analysis. Supelcosil LC-18 column (25.0 cm x 4.60 mm x 5.0 μm) was used in the determination of retinol, retinoic acid, α-tocopherol, lycopene and β-carotene consisting the mixture of acetonitril: methanol: water (63:33: 0.4 v/v) in 1litre as mobile phase. The mobile phase flow rate was set at 0.80 ml / min. The α-tocopherol 296.00 nm, retinol and retinoic acid 326 nm, lycopene 475 nm and β-carotene 470 nm were determined respectively by **[157-159].**

2.3. Determination of vitamins C and MDA content in Eggs

One g from egg yolk was taken and it vortexed by the addition of 0.5 mL from 0.5 M $HClO_4$, that mixture was then vortexed 4500 rpm / min for 15 min, the protein precipitate was formed by the centrifugation, extracts been centrifugated was then passed through Whatman's No. 1 filter paper**.** Utisil-XB-C-18 column was used in the determination of vitamin C, 3.7 mM KH_2PO_4 (pH = 4), as mobile phase in HPLC with flow rate: 0.8 mL/min and wavelength of 245 nm **[160].** 20 μL of crystalline part of the centrifugated sample was taken carefully and injected into the HPLC machine. For the determination of MDA; 20 ml from the top of the centrifuged filtrate was taken carefully and injected into HPLC. Inertsil ODS-4 (5 mm in 4.6 × 150 mm) HPLC column, KH_2PO_4 30 mM and methanol mixture (65% to 35%) mobile phase adjusted to pH of 4 with H_3PO_4 were used. MDA were determined by adjusting the mobile phase flow rate of 0.5 ml / min at 254 nm. The diluted samples were multiplied by the dilution factor of 1:10 **[161].**

2.4. Determination of peptides content in Eggs

2.4.1. Identification of peptides content in eggs

Both the yolk and the albumen of eggs were weighed approximately 1.2 g from both was taken into 6.1 gram of white polyethylene tube and vortexed by the addition of 0.5 mL from 0.5 M $HClO_4$. The mixture was then vortexed and centrifuged at 4500 rpm/min for 15 min, and the protein precipitate was occured by the centrifugation. 20 μL of crystalline part

of the centrifugated sample were taken carefully and injected into HPLC**.** Analytical HPLC ULTISIL-XB-C-8 column, 50 mM $NaClO_4$ solvent were prepared using 0.1 % H_3PO_4 solution and was used as a mobile phase. GSH and GSSG amounts were determined at 215 nm **[162 154].**

2.4.2. Determination of acylated and desacylated ghrelin in eggs

The supernatants of eggs centrifugated extracts was then passed through Whatman's No. 1 filter paper, the were analyzed by High Performance Lipid Chromatography (HPLC) and a ULTISIL-XB-C-8 column with whole system were set at room temperature **[136].** The mobile phase (pH 4.0) consisted of 50 mM $NaClO_4$ and 1% H_3PO_4 with a flow rate of 0.8 mL/ min monitored on-line (215 nm absorbance) were used **[163].**

2.5. 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, 50 g of each pulverized eggs (egg yolk and egg albumen) were extracted in 1000 mL of ethanol, water and acetone separately. Maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, Essex, UK) for about 48hr.

The separated extracts were then filtered through Whatman's No. 1 filter paper. The filtrated ethanol, water and acetone were separately concentrated to dryness *in vacuo* using a rotary evaporator, the solvents were removed. The filtrate that obtained from the aqueous was froze at −40 °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, water and acetone. The homogenates were also helded by ultrasonic water bath for about 1.0 hr at a high level, the solvent content of the cell were passed. The homogenate was filtered after some period through filter paper (No. 2). The filtrate was used to determined the total phenolic and flavonoid contents at 4 °C.

2.5.1. Analysis of Phenolics

Total phenolic compounds content was assayed using the Folin–Ciocalteu reagent, followed Singleton and Rossi method **[164].** An aliquot (0.1 mL) of diluted sample extract water, acetone and ethanol. (0.3 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 hr, the absorbance at 760 nm were read versus the prepared blank. Total phenolic content of egg extract was expressed as micrograms of gallic acid equivalents per gram of dried weight (µg GAE/g DW) through the calibration curve with microgram gallic acid and catechol equivalents per gram of dried weight (µg GAE/g DW) (Figure 3.11, 3.12).

2.5.2. Determination of total flavonoids content

Determination of the flavonoids content was achieved by the used of the method described by Huang et al. **[165].** The aluminum chloride reagent was added to the solution containing the extract. The absorbance was read at 510 nm and concentrations of flavonoids were deduced from a standard curve and were calculated in µg gallic acid equivalent (GAE)/g dry weight (DW) (Figure 3.13).

2.6. Determination of DPPH free radical scavenging activity

The scavenging activity on α, α-diphenyl-β- picrylhydrazyl (DPPH) free radicals was measured according to the method of Liyana-Pathiana and Shahidi **[166].** Was used for the determination of scavenging activity of DPPH free radical in the extract solution. A solution of 0.135 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared and 1.0 mL of the solution were mixed with 1.0 mg of extract in methanol containing 0.2–1.0 mg/mL of the extract. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm BHT was used as standard. The scavenging ability of the eggs extract was calculated by the used of the equation below:

DPPH Scavenging activity (%) = [(Abscontrol − Abssample)] / (Abscontrol)] × 100 ... (1)

Where;

Abscontrol as the absorbance of DPPH + methanol;

Abssample as the absorbance of DPPH radical + sample extract or standard.

3. RESULTS

Calculation for each parameters were done using the working graph. Information gathered from HPLC chromatogram were calculated by two ways, according to the peak area (Figure 3.1, 3.2, 3.4, 3.5, 3.6 and 3.7) or the peak height (Figure 3.3, 3.8, 3.9 and 3.10).

For instance the equation of the graph y = 7889x + 66.18 was used in calculating the amount of vitamin A in the egg sample. Chromatogram of vitamin A in respect of retention time peak area in the sample was calculated by writing vitamin A instead of x in the correct equation.

Provided that the dilution occurred, the result were multiplied by the dilution factor (Figure 3.1).

For example, the determination of beta-carotene and ghrelin parameters was done based on calculation in the chromatogram peak height.

The value of x was determined from the working graph related to beta-carotene by writing the value of chromatogram peak height in place of y in the equation y = 2506x + 0.446.

In the case of chromatogram that are not compatible with the retention time maternal standards method were applied.

All calculations for the working graph below were utilised by each parameters and findings were calculated from the correct equation.

Calculations of the amount of vitamin A, E, C, MDA, beta-carotene, lycopene, glutathione reductive (GSH), glutathione oxide (GSSG) and Ghrelin in different eggs, the studied chart of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG were used in the equation. Therefore, y = 7889x + 66.18, where; y is a values of peak area in the chromatogram of the vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in egg samples. Vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG values were calculated as x for each samples. Similarly, calculations of radical scavenging activity and Total phenolic content were made by the created graphs (Figure 3.11, 3.12 and 3.13).

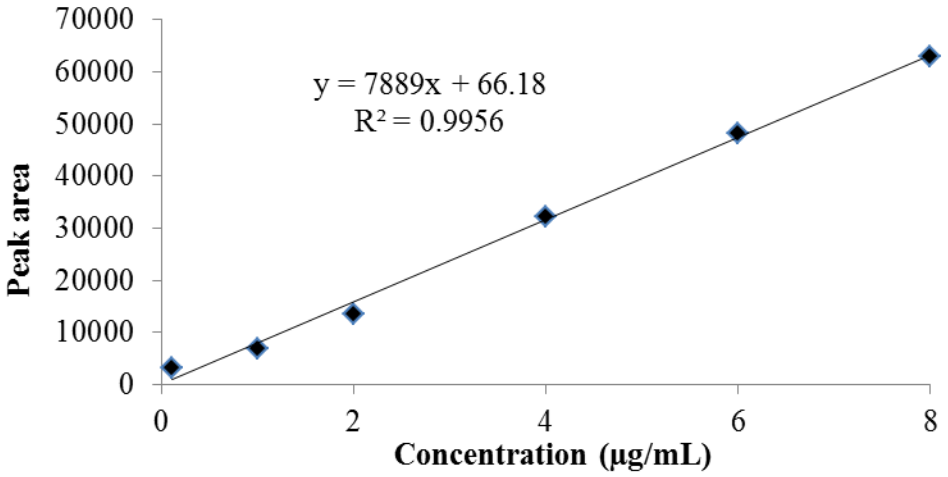

Figure 3.1. Working graph for the correct equation of vitamin A

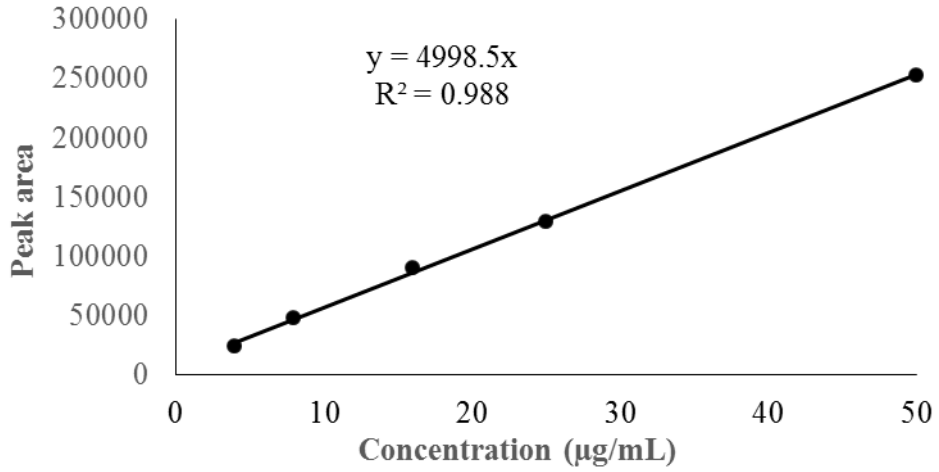

Figure 3. 2. Working graph for the correct equation of vitamin E

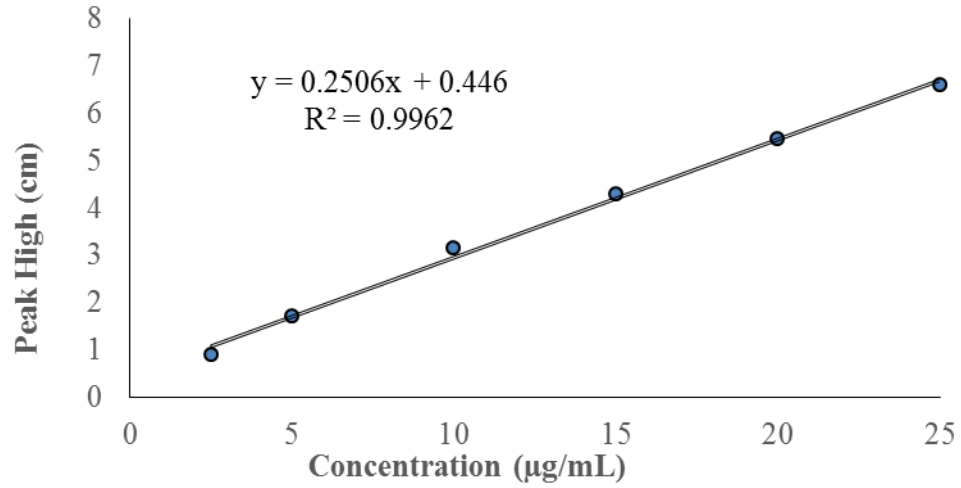

Figure 3. 3. Working graph for the correct equation of beta-carotene

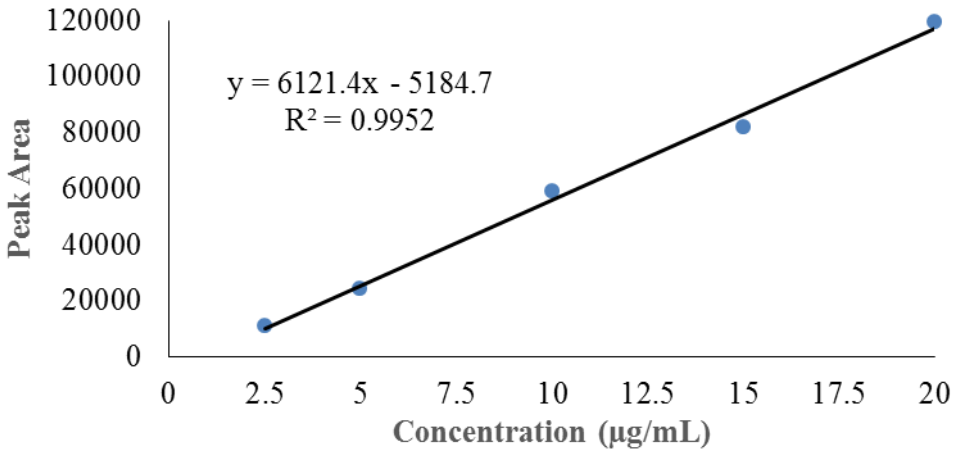

Figure 3. 4. Working graph for the correct equation of lycopene

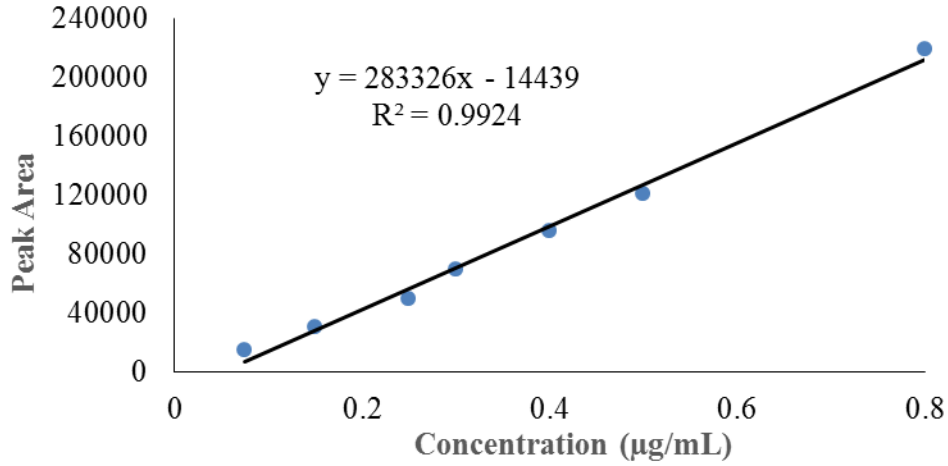

Figure 3. 5. Working graph for the correct equation of vitamin C

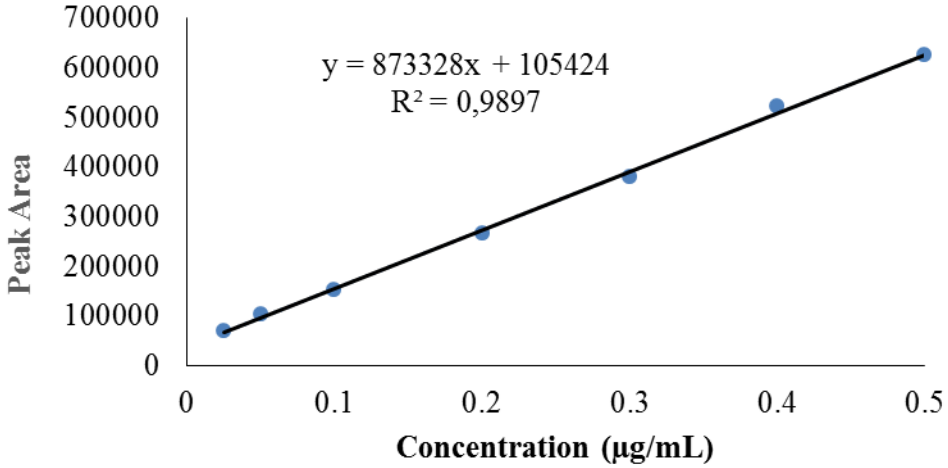

Figure 3. 6. Working graph for the correct equation of MDA

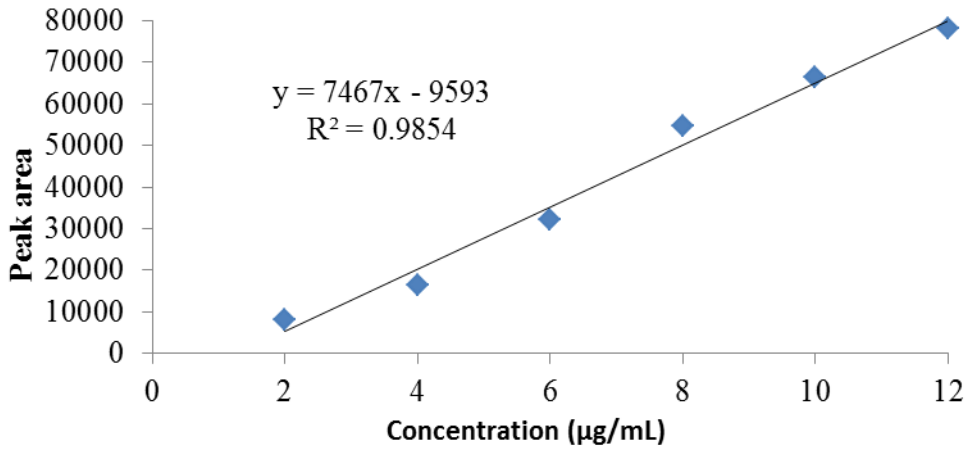

Figure 3. 7. Working graph for the correct equation of glutathione reduce (GSH)

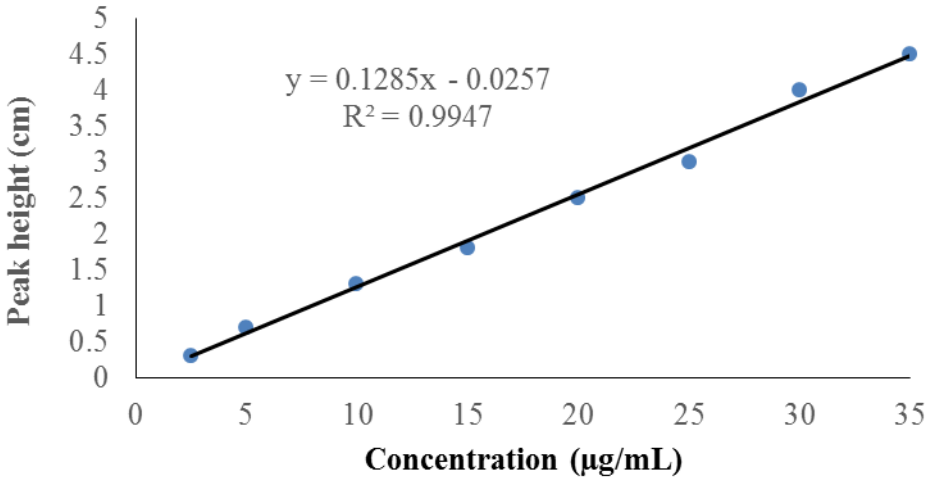

Figure 3. 8. Working graph for the correct equation of glutathione oxide (GSSG)

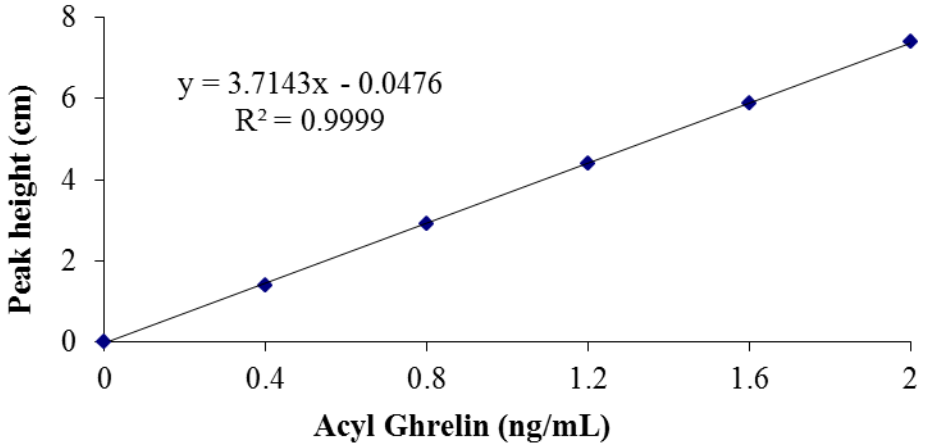

Figure 3. 9. Working graph for the correct equation of Acyl ghrelin (AG)

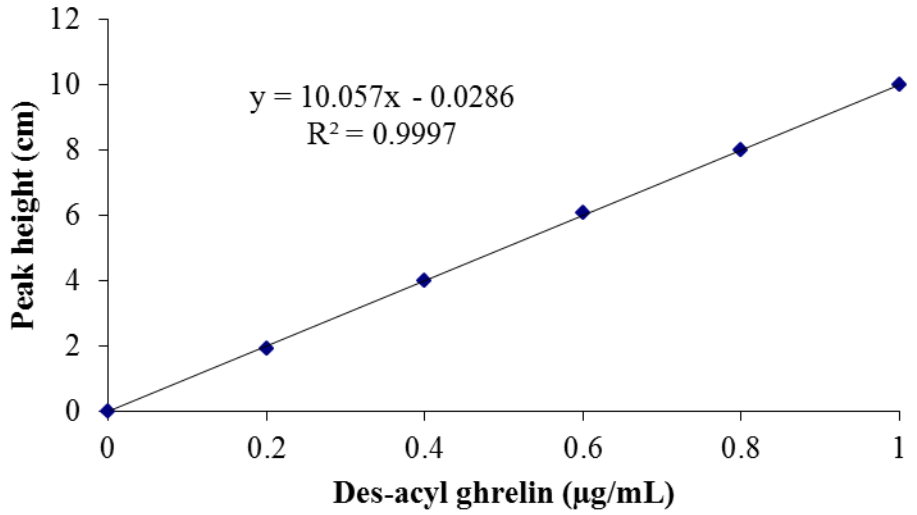

Figure 3. 10. Working graph for the correct equation of Des-acyl ghrelin (DAG)

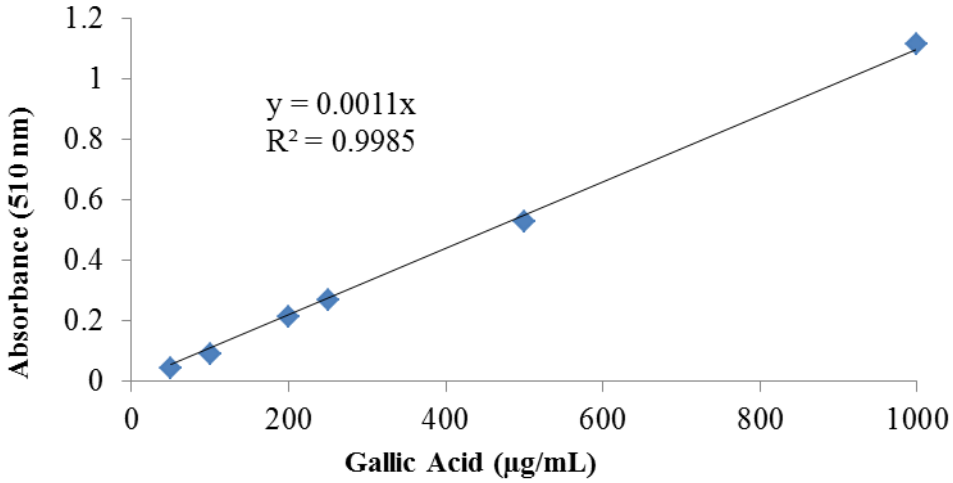

Figure 3. 11. Study chart of gallic acid for the determination of total flavonoids content

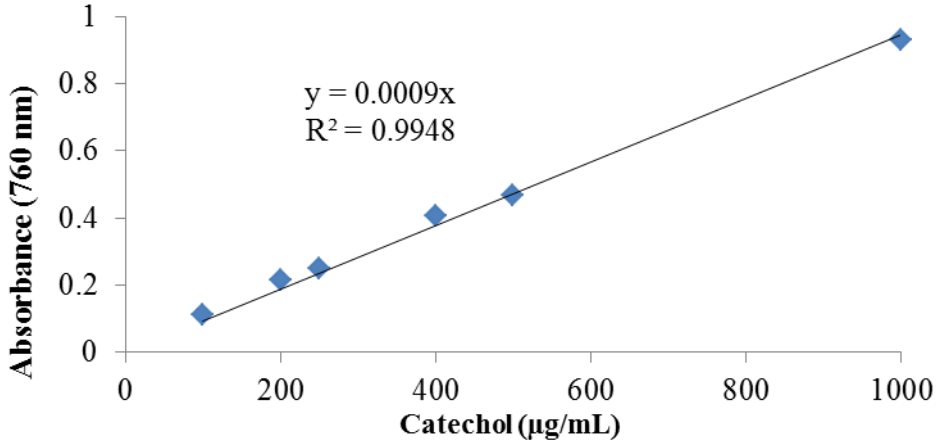

Figure 3. 12. Study chart of catachol for the determination of total phenolics

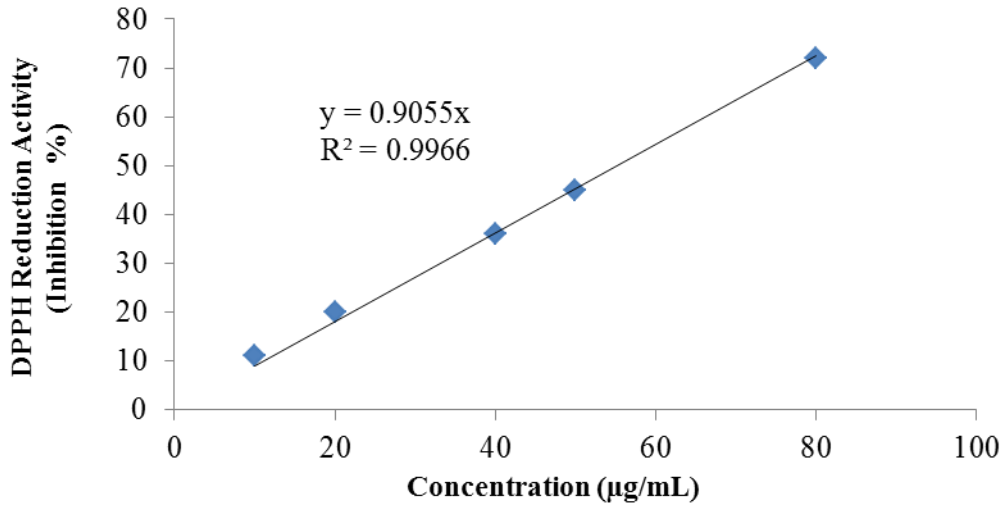

Figure 3. 13. Study chart of DPPH radical scavenging activity

Table 3. 1. The amounts of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in Farm Chicken eggs.

Farm Chicken eggs.	No. of eggs sample (n)	Egg White (Albumen)	Egg yolks
Vitamin A (µg/g)	8	-	6.07 ± 0.70
Vitamin E (µg/g)	8	-	3.33 ± 0.42
Vitamin C (µg/g)	8	0.11 ± 0.03	0.16 ± 0.06
β-Carotene (µg/g)	8	-	0.35 ± 0.03
Lycopene (µg/g)	8	-	0.10 ± 0.01
Ghrelin (µg/g)	8	0.08 ± 0.01	0.17 ± 0.03
GSH (µg/g)	8	94.73 ± 11.95	98.03 ±12.60
GSSG (µg/g)	8	22.10 ± 2.90	20.71 ± 2.50
MDA (µg/g)	8	0.58 ± 0.10	0.85 ± 0.11

All parameters in the same column chart are given according to some parameters and standard deviation were multiplied or divided by 10 or even 100 coefficients. For example; while creating the column chart of villages eggs; Vitamin C, beta-carotene, lycopene, ghrelin and MDA values and standard deviations are multiplied by 10 coefficient given, GSH and GSSG graphics were created from the divided value of 10 coefficient.

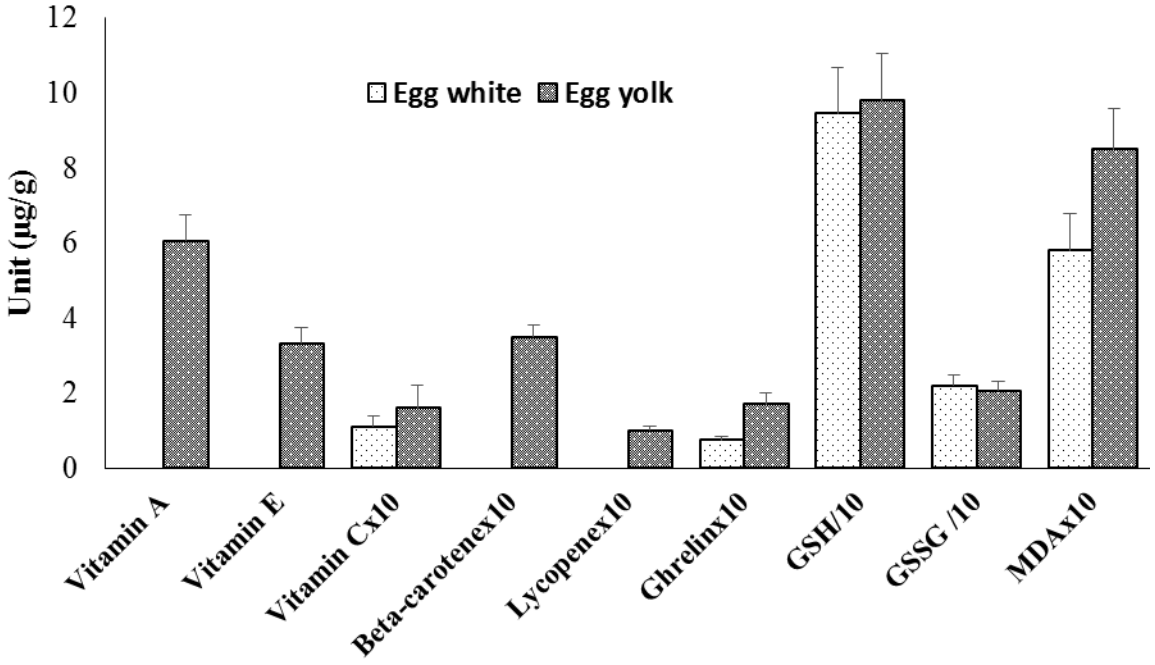

Figure 3. 14. The column chart of parameters found in Farm chicken eggs

Table 3. 2. The amounts of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in Local chicken eggs (organic).

Local chicken eggs (organic)	No. of eggs sample (n)	Egg white (Albumen)	Egg yolks
Vitamin A (µg/g)	8	-	2.33 ± 0.33
Vitamin E (µg/g)	8	-	2.88 ± 0.46
Vitamin C (µg/g)	8	0.14 ± 0.03	0.16 ± 0.03
β-Carotene (µg/g)	8	-	0.41 ± 0.07
Lycopene (µg/g)	8	-	0.07 ± 0.01
Ghrelin (µg/g)	8	0.33 ± 0.03	0.42 ± 0.06
GSH (µg/g)	8	82.16 ± 10.00	147.88 ± 14.06
GSSG (µg/g)	8	1.45 ± 0.20	2.8 ± 0.50
MDA (µg/g)	8	0.64 ± 0.07	2.03 ± 0.32

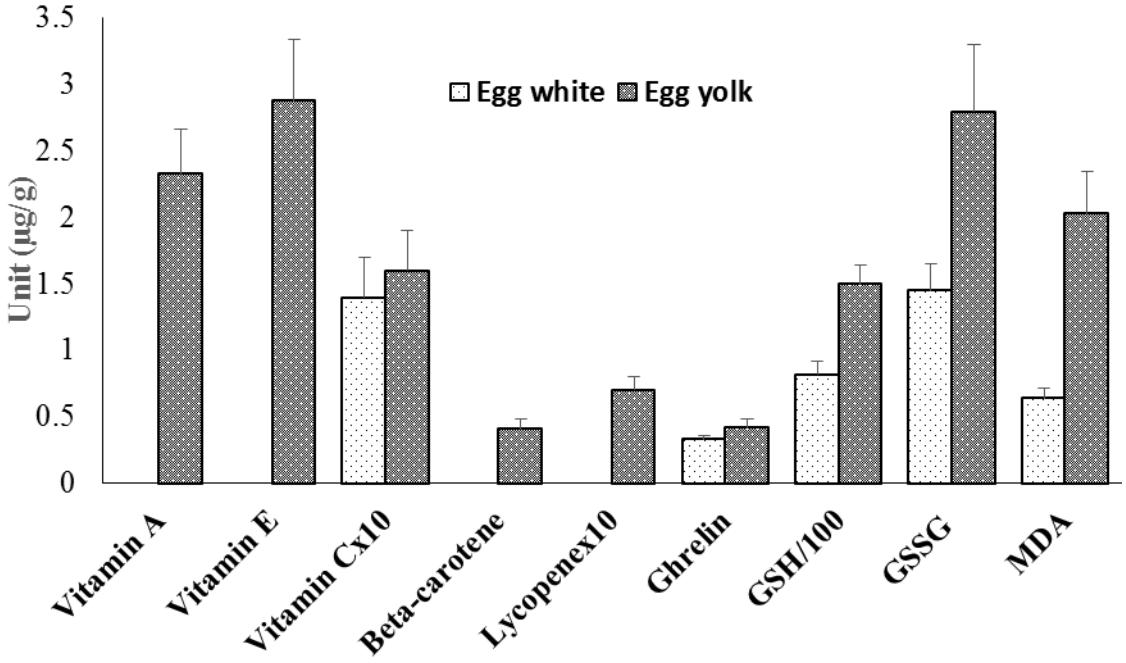

Figure 3. 15. The column chart of parameters found in Local chicken eggs (organic)

Table 3. 3. The amounts of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in Duck eggs

Duck eggs	No. of eggs sample (n)	Egg white(Albumen)	Egg yolks
Vitamin A (µg/g)	8	-	2.77 ± 0.39
Vitamin E (µg/g)	8	-	0.40 ± 0.07
Vitamin C (µg/g)	8	0.17 ± 0.05	0.24 ± 0.04
β-Carotene (µg/g)	8	-	0.34 ± 0.04
Lycopene (µg/g)	8	-	0.06 ± 0.01
Ghrelin (µg/g)	8	1.78 ± 0.39	1.36 ± 0.16
GSH (µg/g)	8	43.02 ± 7.43	50.33 ± 8.65
GSSG (µg/g)	8	14.89 ± 1.67	22.50 ± 2.80
MDA (µg/g)	8	0.46 ± 0.08	1.32 ± 0.23

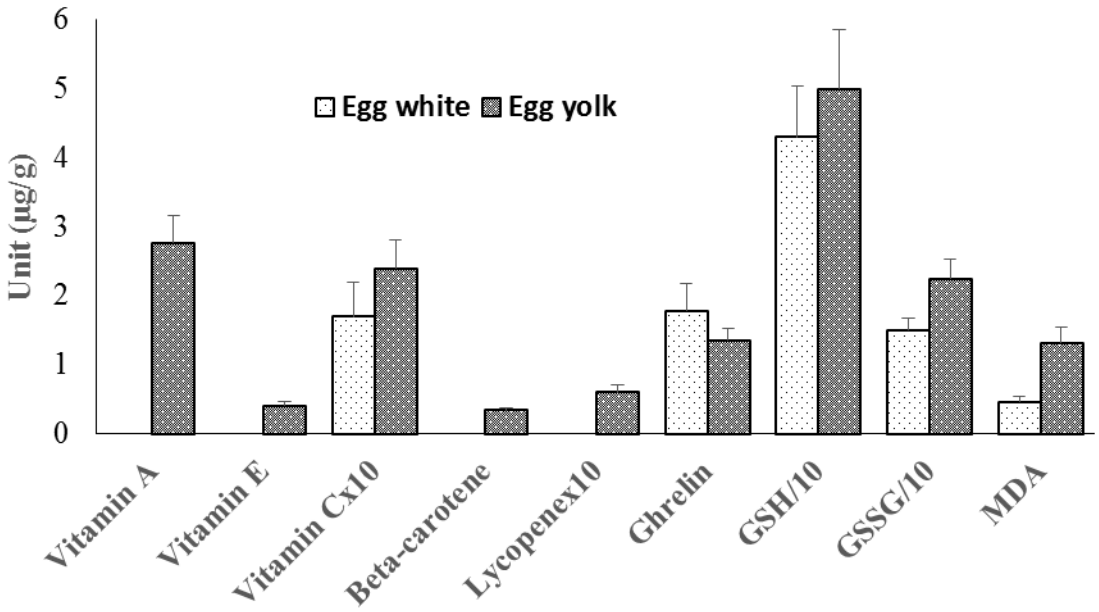

Figure 3. 16. The column chart of parameters found in Duck eggs

Table 3. 4. The amounts of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in Quail eggs.

Quail eggs	No. of eggs sample (n)	Egg white (Albumen)	Egg yolks
Vitamin A (µg/g)	8	-	3.62 ± 0.47
Vitamin E (µg/g)	8	-	0.32 ± 0.05
Vitamin C (µg/g)	8	0.20 ± 0.04	0.50 ± 0.10
β-Carotene (µg/g)	8	-	0.40 ± 0.05
Lycopene (µg/g)	8	-	0.08 ± 0.01
Ghrelin (µg/g)	8	0.87 ± 0.10	3.77 ± 0.34
GSH (µg/g)	8	206.74 ± 16.68	290.75 ± 20.13
GSSG (µg/g)	8	5.46 ± 0.67	529.25 ± 77.19
MDA (µg/g)	8	2.94 ± 0.41	5.48 ± 0.72

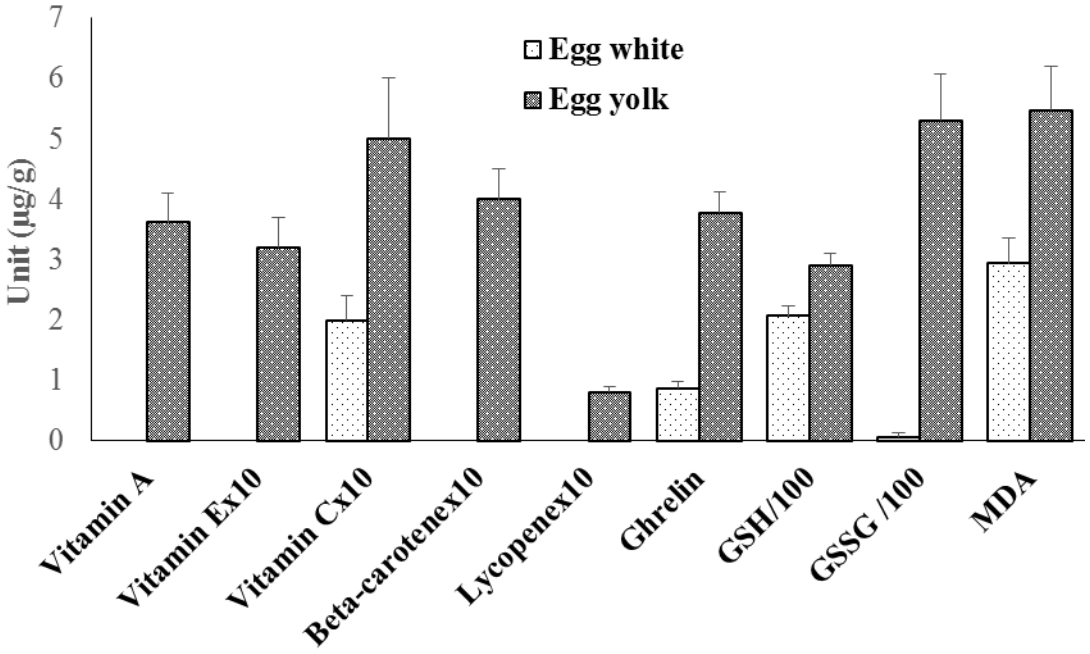

Figure 3. 17. The column chart of parameters found in Quail eggs

Table 3. 5. The amounts of vitamin A, E, C, β-carotene, lycopene, Ghrelin, MDA, GSH and GSSG in Goose eggs

Goose eggs	No. of eggs sample (n)	Egg white (Albumen)	Egg yolks
Vitamin A (µg/g)	8	-	4.64 ± 0.60
Vitamin E (µg/g)	8	-	0.55 ± 0.12
Vitamin C (µg/g)	8	0.02 ± 0.004	0.04 ± 0.008
β-Carotene (µg/g)	8	-	0.19 ± 0.03
Lycopene (µg/g)	8	-	0.08 ± 0.01
Ghrelin (µg/g)	8	0.14 ± 0.02	0.92 ± 0.16
GSH (µg/g)	8	75.20 ± 12.15	659.25 ± 66.94
GSSG (µg/g)	8	18.55 ± 2.58	475.50 ± 16.15
MDA (µg/g)	8	0.75 ± 0.12	0.84 ± 0.13

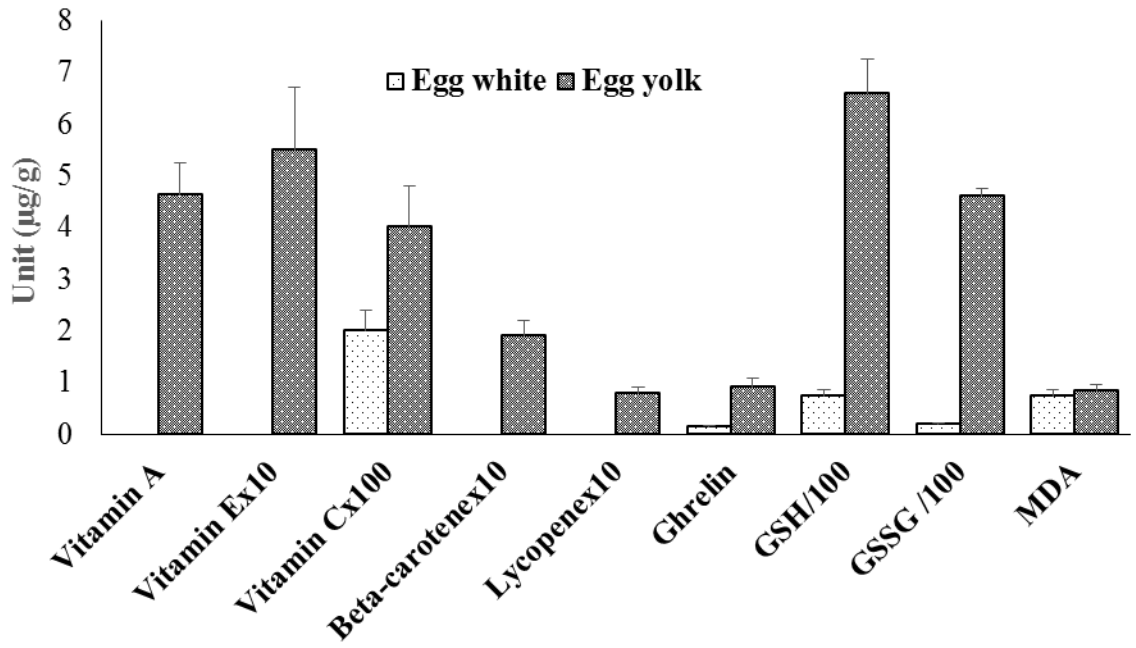

Figure 3. 18. The column chart of parameters found in Goose eggs

Table 3. 6. 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/mg extract)
Farm Chicken	1.28 ±0.08	1.00 ± 0.08	4.67±1.32
Local chicken	2.10 ±0.09	1.88 ± 0.10	6.15±2.50
Duck	2.49 ±0.11	1.62 ± 0.10	5.03±1.20
Quail	2.88 ±0.11	1.13 ± 0.06	5.37±1.61
Goose	3.08 ±0.24	1.63 ± 0.07	4.83±1.12

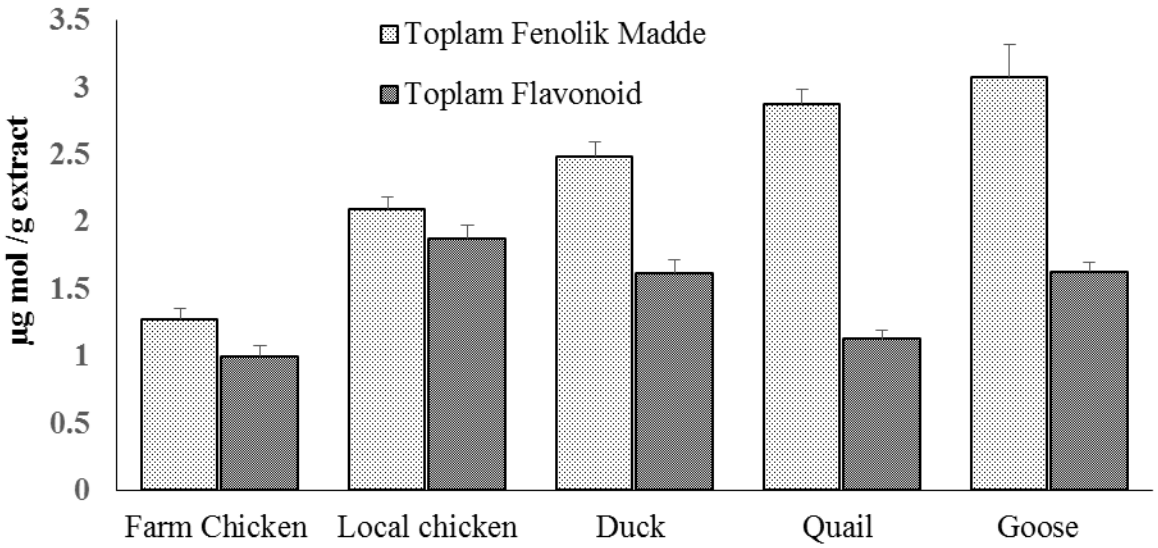

Figure 3. 19. The column chart of the Total phenolic and flavonoid compounds of egg yolk ethanol extracts in terms of gallic acid

4. DISCUSSION

Vitamins are organic compounds that occur in metabolism deficiency disorders. large majority of vitamins metabolism must be taken outside. Eggs are said to be rich in many vitamins **[167].** Irie et al (2010) in their study of chicken, quail and duck eggs the amount of retinol in the their egg yolk 4.97 ± 0.72; 8.96 ± 0.57 and 2.67 ± 1.01 µg/g were reported **[168].** Akdemir et al (2012) mentioned that vitamin A in egg yolk 10:43 µg/g **[169].** The results of vitamin A our farm. organic. duck. quail and goose eggs yolk 6:07 ± 0.70; 2.33 ± 0.33; 2.77 ± 0.39; 0:47 ± 3.62 and 4.64 ± 0.60 µg/g were determined respectively (Table 3.1-3.5 and Figure 3.14-3.18). It appears to be consistent with the literature value of vitamin A in different eggs. In terms of farm eggs vitamin seems to be higher than other eggs. Vitamin A in eggs may be due to the differences in type of animal or it nutrition.

Akdemir et al (2012) mentioned that vitamin E 137.2 µg/g in egg's yolk **[169]**. Murcia et al (1999) repoted that vitamin E 6.1 ± 0.08/ 100g in eggs sample. whereas the activity of vitamin E are reported to be 6.98 **[170].**

The results of vitamin E in in egg yolk of our farm, organic, duck, quail and goose; 3.33 ± 0.42; 2.88 ± 0.46; 0.40 ± 0.07; 0:32 ± 0.05. and 0.55 ± 0.12 µg/g were determined respectively. (Table 3.1-3.5 and Figure 3.14-3.18). In accor dant with the literature our results Farm chicken eggs seems to be high in Vitamin E.

Although. generally stated that no poultry eggs can synthesis vitamin C **[171]** studies indicate that increase in egg production and quality of vitamin C is present in animal feed **[172].**

The results of vitamin C in egg yolk of farm chicken, organic, duck, quail and goose eggs were 0.16 ± 0.06; 0:16 ± 0.03; 0:24 ± 0:04; 0.50 ± 0.10 and 0.04 ± 0.008 µg/g while. Vitamin C from the same egg white were determined (Table 3.1-3.5 and Figure 3.14-3.18). 0.11 ± 0.03; 0.14 ± 0.03; 0:17 ± 0:05; 0:20 ± 0.04 and 0.02 ± 0.004 µg/g respectively. The low amount of vitamin C in our results, especially in goose eggs were the lowest. It can be explained that the present vitamin C in animal eggs probably by the consumption of green vegetables. It was observed that the amount of vitamin C in the egg yolk were lower then in egg white.

As it known that Beta-carotene is a precursor of vitamin A, it reported that poultry they coold not synthesized carotenoids but can be reservoir of oxide carotenoid **[167**].

Akdemir et al (2012) have indicated that the amount of beta-carotene in egg yolk 172 μg/g **[169]**. However, our results, amounts of beta-carotene in egg yolk of farm chicken, organic chicken, duck, quail and goose eggs were determined (Table 3.1-3.5 and Figure 3.14-3.18). 0.35 ± 0.03; 0:41 ± 0:07; Was 0.34 ± 0.04; 0:40 ± 0.05. and 0.19 ± 0.03 μg/g respectively. It is seen from the results that the goose eggs have the lowest beta-carotene (Table 3.5 and Figure 3.8).

Differences in the amount of carotenoids in egg can vary depending on the nutrition and animal species Jung-Woo (2008) **[173]** chicken feeds with added lycopene reported that the amount 1:57 μg/g in egg yolk **[174]**. Akdemir et al (2012) have declared that the amount of lycopene 6:53 μg/g in egg yolk were found within 90 days 5g/kg dose of chicken feeds with lycopene tomatoes powder **[169]**.

However. Sahin et al (2008), stated that the quail eggs without lycopene diet lycopene may not be determiend in their egg yolk. The quail eggs with lycopene diets mg/kg 22:42 μg/g lycopene were reported in their egg yolk **[175]**.

The results of lycopene in egg yolk of our farm chicken, organic chicken, duck, quail and goose determined 0.08 ± 0.01; 0.07 ± 0.01; 0:06 ± 0.01; 0:08 ± 0.01 and 0.08 ± 0.01 μg/g respectively. (Table 3.1-3.5 and Figure 3.14-3.18). As can be seen from the results the amount of lycopene in eggs were relatively low and are quite close together. The low value of lycopene compared to the literature may be due to the animals feeds with added lycopene.

Lipopeptide hormones that are associated with appetite are known as ghrelin. That function is to controlling the energy balance and enabling the stimulation of growth hormone release. The body fluids contained many peptide hormone has been reported **[176]**. Furthermore, the total level of this hormone has been reported. Even thought is in two major forms (desacylated and acylated) with several functions. The active form of the hormone is the acylated form, bearing an *n*-octanoic acid on its third amino acid (ser-3). This change is essential for connecting to growth hormone secretagogue receptor-1a, a necessary for the growth hormone-releasing impact of ghrelin **[177]**. However, the desacylated form of ghrelin, is missing in this change (*n*-octanoic acid) even though this form is noted as the inactive form. it has an affect in cell proliferation and adipogenesis **[178]**. Yoshimura et al (2009) the amount of ghrelin in egg yolk 95 ± 5 μg/g was reported **[179].**

The results of the amount of ghrelin hormone in egg yolks of our farm chicken, organic chicken, duck, quail and goose eggs were determined 0:17 ± 0.03; 0:42 ± 0.06; 1.36 ± 0.16; 3.77 ± 0.34 and 0.92 ± 0.16 μg/g respectively. The amount of ghrelin in the same egg white were observed (Table 3.1-3.5 and Figure 3.14-3.18) 0:08 ± 0.01; 0.33 ± 0.03; 1.78 ±

0.39; 0.87 ± 0.10 and 0.14 ± 0.02 µg/g respectively. Our results shows that the amount of ghrelin were higher than the literature value. This case can be explained by the diets or animal species.

The heat stress applied on nutritional metabolism reduced production of duck egg. also decreased the amount of plasma GSH and increased the amount of MDA of these ducks **[180]**. The results of GSH levels in egg yolks of our farm chicken, organic chicken, duck, quail and goose were determined 98.03 ± 12.60; 147.88 ± 14.06; 50.33 ± 8.65; 20:13 ± 290.75 and 659.25 ± 66.94 µg/g respectively. The GSH levels in the same egg white were determined 94.73 ± 11.95; 82.16± 10:00; 43.02 ± 7.43 206.74 ± 16.68 and 75.20 ± 12:15 µg/g respectively. The amount of GSSG in egg yolk of farm chicken, organic chicken, duck, quail and goose were determined 20.71± 2:50; 0.80 ± 0.08; 22:50 ± 2.80; 16:15 ± 77.19 and 475.50 ± 529.25 µg/g respectively. The amounts of both GSH and GSSG in whole eggs are seen that are much more likely in egg yolk than in egg white. The redox level of glutathione was reported due to the reduced and the ratio of oxidized levels (GSH / GSSG) **[181]**.

It was stated that due to stress the basal levels of GSH / GSSG ratio is high while the situation in the oxidative stress decreased **[182]**. Additionally, environmental and physical factors influence those ratios **[183,184]**. Malondialdehyde (MDA) is the most important of reactive carbon compounds, widely used as a marker of lipid peroxidation **[185].**. Bakalivanov et al (2008) studied the amount of MDA depending on their storage time for frozen and dried eggs **[186]**. The amount of egg depending on their storage time ranged from 0.1-0.2 µg/g were reported. Akdemir et al (2012) mentioned that the amount of MDA in egg yolk 0.335 µg/g **[169]**.

However. Sahin et al (2008) reported that the amount of MDA in quail egg yolk 0.86 µg/g of non lycopene diet. while the quail eggs contained lycopene diet of 100 mg / kg it amount of MDA in egg yolk were decreased to 0.79 µg/g **[175]**. The results of the amount of MDA in egg yolk of our farm chicken, organic, duck, quail and goose eggs 0.85 ± 0.11; 2:03 ± 0.32; 1:32 ± 0.23; 5:48 ± 0.72 and 0.84 ± 0.13 µg/g were designated respectively. The amount of MDA in the same egg white 0:58 ± 0.10; 0.64 ± 0.07; 0.46 ± 0.08; 2.94 ± 0.41 and 0.75 ± 0.12 µg/g were observed respectively. Our results were slightly higher than the literature valued. Particularly MDA in quail eggs considerably was found to be higher (2.94 ± 0.41. µg/g). the quail are said to be the stressed animals when both MDA and GSH/GSSG ratio were compared. The total phenolic contents of gallic acid 3.83 3.49 µg/g were determined from the chickens egg yolk feeds diets contained wheat and corn **[187]**. The phenolic substance in ethanol extract µmol gallic acid/g extract value terms in farm chicken,

organic chicken, duck, quail and goose eggs were determined 1:28 ± 0.08; 2:10 ± 0.09; 2:49 ± 0.11; 3.08 ± 0.11 and 2.88 ± 0.24 respectively from their egg yolk. The total amount of flavonoid compounds in ethanol extracts μmol gallic acid/g extract value terms in farm chicken, organic chicken, duck, quail and goose eggs were determined 1.00 ± 0.02; 1.88 ± 0.02; 1.62 ± 0.01; 1.13 ± 0.02 and 1.63 ± 0.02. respectively from their egg yolk.

DPPH scavenging activity and suppression of discoloration of β-carotene have also been observed **[86, 89**]. The hydrolysis of egg yolk protein phosvitin with trypsin also leads to obtain a peptide fraction with an ability to in hibit the oxidation of linoleic acid. DPPH free radical scavenging and chelating iron ions (II) **[88**].

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 **[187**].

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; 4.67 ± 1.32; 6.15 ± 2.50. 5.03 ± 1.20; 5.37 ± 1.61 and 4.83 ± 1.12 μg/mg respectively from their egg yolk.

All results were profiled in the form of a chromatogram where the peak height and peak area under each was proportional to the amount of standard vitamin A. E. C. lycopene. beta-carotene. GSH, GSSG, MDA, Total phenolic, flavonoid compounds, DPPH scavenging activities and ghrelin used as their standard. Showed in (Figure 3.1, 3.2, 3.4, 3.5, 3.6, 3.7) and (Figure 3.3, 3.8, 3.9, 3.10).

An essential step will be taken to conduct more clinical trials to prove the health benefits of those substances that are enriched eggs and unenriched for all the above-mentioned substances. The egg yolk protein phosvitin that hydrolysis with trypsin leads to obtain a peptide fraction with anability to inhibit the oxidation of linoleic acid. Chelating iron ions (II) and DPPH free radical scavenging **[88].** In addition. The consumer must have knownlage about the beneficial effects to establish the credibility of the health claims. An additional field of research is required to isolate and purify egg components with pharmaceutical effects, like bioactive proteins example, immunoglobulin antibodies (IgY) from egg yolk with antibiotic activity and lysozyme from egg white **[188].**

Conclusions

Eggs are a good source of nutrients such as protein, vitamins, pro-vitamin, hormone, antioxidant and other compounds (such as A, E, C, Beta-carotene, Lycopene, Ghrelin, Glutathione, flavonoid and malondialdehyde) and functional substances like lutein, bioactive proteins and special fatty acids. Furthermore these components are highly bioavailable from eggs.

Vitamin A, E, C, β-carotene, lycopene, grelin. glutathione and phenolic substance, amount of flavonoid and DPPH activity when compared to the eggs.

It can be said that farm chicken eggs are high in vitamin A, E and lycopene whereas Organic eggs were high in Beta-carotene. amount of Flavonoid and DPPH radical scavenging activity.

It can be seen that the goose eggs are high in terms of GSH and Phenolic substance, in terms of vitamin C, Grelin, GSSG and MDA are high in quail eggs.

In addition, MDA and GSSG in quail eggs are very high because they are more stressful. MDA and GSH / GSSG ratio are used as stress indicators.

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