



Physicochemical Properties and Oxidative Stability of Hen Egg-Yolk Oils Based on Different Laying Periods

Erman DUMAN^{1,*}

¹ Department Food Engineering, Faculty of Engineering, Afyon Kocatepe University, Afyon, Turkey

*Corresponding author E-mail: eduman@aku.edu.tr

HIGHLIGHTS

- > Nutritional value of hen egg-yolk oils based on different laying period was studied
- > Palmitic, stearic, oleic, and linoleic acids were determined at the highest levels in all periods
- > Cholesterol and campesterol were the two dominant sterols.

ARTICLE INFO

Received : 09.30.2020
Accepted : 11.26.2020
Published : 12.15.2020

Keywords:

LOHMANN white,
egg yolk oil,
fatty acid,
sterol composition,
oxidative stability,
physicochemical properties

ABSTRACT

The aim of this study was to investigate and compare the physicochemical properties and nutritional value of hen egg-yolk oils based on different laying periods. Hen egg-yolk oils were extracted using solvents from double yolk (18 weeks), guide (24 weeks), pullet (30 weeks), and jumbo (80 weeks) eggs. Weight and fat ratios of the hen eggs, as well as the free fatty acid content, peroxide value, iodine value, oxidative stability, fatty acid composition, and sterol composition of the yolk oils were determined in the study. According to the results of the analyses of white-shell eggs, the fat contents of the boiled egg (both the white and yolk), fat contents of the egg yolk, free fatty acid values, peroxide values, and oxidative stability values were between 7.85 and 11.25%, 18.58 and 34.71%, 1.03 and 2.32%, 8.58 and 9.54 meq O₂/kg, and 8.0 and 10.2 h, respectively. Palmitic and oleic were determined at the highest levels in all samples in the fatty acids. Cholesterol and campesterol were the two dominant sterols among the 10 sterol components. We determined that the physicochemical properties of white-shell egg-yolk oils varied according to different laying periods in terms of animal fat, and based on the 80-week laying period, egg-yolk oils were suitable for both internal and external consumption; the most ideal egg-yolk oils were extracted during the 30-week (pullet) period.

Contents

1.	Introduction	13
2.	Materials and Methods	13
2.1.	Materials	13
2.2.	Preparation of Egg Yolk Samples	13
2.3.	Oil Content.....	13
2.4.	Physico-chemical Analysis	13
2.5.	Fatty Acid Composition Analysis	13
2.6.	Sterol Composition Analysis	13
2.7.	Oxidative Stability Analysis	14
2.8.	Statistical Analysis.....	14
3.	Results and Discussion	14
4.	Conclusion.....	15
	Acknowledgement	15
	References	15

Cite this article Duman E. Physicochemical Properties and Oxidative Stability of Hen Egg-Yolk Oils Based on Different Laying Periods.

International Journal of Innovative Research and Reviews (INJIRR) (2020) 4(2) 12-16

Link to this article: <http://www.injirr.com/article/view/60>



Copyright © 2020 Authors.

This is an open access article distributed under the [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits unrestricted use, and sharing of this material in any medium, provided the original work is not modified or used for commercial purposes.

1. Introduction

Hen (*Gallus* sp.) eggs are produced by ovulation in sexually mature hens without copulating with a cock. Eggs are produced in the ovaries as a result of more than 30 natural hormones and the physiological effects of light stimulation. It has been reported that the time that hens begin to lay according to their genetic structure and feeding, for instance in the LOHMANN white types, laying can begin after 16 weeks of age [1–3].

An egg is a natural food containing macro- and micronutrients, is widely consumed throughout the world, and is used as a preservative against various diseases. Ninety-four percent of an egg has nutritional value for humans because it contains all the essential nutrients needed for an adequate and well-balanced diet. Eggs, which have the highest protein quality among all animal products, are rich in vitamins A, D, E, K, and B and in minerals iron and phosphorus [4–8].

Eggs comprise three parts—the shell, albumen (the white), and yolk—with the highest fat content found in the yolk. It has been reported that the fat content of egg yolks varies between 22.96 and 25.09% depending on the species (duck, hen, quail). In hen egg yolks, this has been reported to be 25.09% [9]. Hen egg-yolk oil is rich in palmitic acid as a saturated fatty acid and oleic and linoleic acids as mono- and polyunsaturated fatty acids, respectively. These fatty acids are crucial to human nutrition for cardiovascular health and cell-membrane permeability. Egg yolks also contain 400 mg/100 g cholesterol, which, contrary to previous beliefs, does not increase the amount of cholesterol in the blood and is an excellent animal food source with very high nutritional value. Eggs are recommended to be included in the daily diets of all consumers, including growing children, young and elderly people, pregnant women and breastfeeding mothers, obese people and diabetics, and even those with cholesterol problems in limited amounts. In traditional medicine, egg-yolk oil is used to treat dermatological diseases, especially wounds, which has led to an increase in commercial sales [10–14].

Previous studies have concentrated on the egg's nutritional components, such as its carbohydrates, proteins, fatty acids, vitamins, and minerals. The aim of the present study was to investigate the changes in the physicochemical properties of commercially obtained egg-yolk oils based on the laying periods between 18 and 80 weeks. These properties included acidity, peroxide value, oxidative stability, and fatty acids and sterol composition in terms of the technical use of animal fat and the egg's nutritional value.

2. Materials and Methods

2.1. Materials

The white-shell eggs used in this study were obtained from three LOHMANN white hens reared on a farm in Afyonkarahisar, Turkey; the hens were observed for 80 weeks. The eggs were gathered from the weeks 18 (double yolk), 24 (guide), 30 (pullet), and 80 (jumbo) laying periods.

2.2. Preparation of Egg Yolk Samples

The yolk and fat extracted from the eggs were analyzed using a 4 × 6 × 3 trial pattern and the following methods. First, white-shell eggs obtained during different laying periods were grouped for the laboratory studies. To prepare them for analyses, the eggs were boiled for 10 min, after which the yolks were separated from the albumen and dried in an oven at 105 °C for 2 h.

2.3. Oil Content

Oil amounts of the dried egg yolks were determined using the soxhlet method. Accordingly, 10 grams of crushed egg yolk sample from each sample was weighed into a soxhlet cartridge and extracted with n-hexane for 3 hours at 85 °C. The n-hexane in the extract was removed under vacuum at 45 °C in a rotary evaporator. After cooling in the desiccator, the amount of oil was determined as a percentage by using the weight differences [15].

2.4. Physico-chemical Analysis

Egg yolk oils; free fatty acidity, peroxide number, iodine number were analyzed according to AOCS methods [16].

2.5. Fatty Acid Composition Analysis

Fatty acid methyl esters were prepared by treating oils with potassium hydroxide and n-Heptane, and then determined by gas chromatography. Gas chromatography with SHIMADZU-2025 GC (Kyoto, Japan) brand was used in the analysis. Flame Ionization Detector (FID) is used on the device. The column is the brand of RTX-2330 and has a length of 60 m, a diameter of 0.25 mm and a film thickness of 0.20 μm [17]. In Table 1, Operating conditions of Gas chromatography are given.

Table 1 Gas Chromatography operating conditions

Stationary phase		10% Diethylene Glycol Succinate (DEGS)
Support material		Chromosorb W (AW-DMCS) (60-80 mesh)
Detector		FID
Temp	Column	180 °C
	Injection	200 °C
	Detector	200 °C
Flow rate	Carrier gas (N ₂)	30 ml/min
	Flammable gas (H ₂)	28 ml/min
	Dry air	220 ml/min
Injection amount		1 μl

2.6. Sterol Composition Analysis

Sterol compositions for egg yolk oils were determined using a method as described by Lencher et al., (1999). SGE BP-5 column (30m length × 0.25 mm i.d., 0.25 μm film thickness) and a Perkin Elmer Boston, MA, USA GC autosystem were used. All gas (He, He-2 and air) flow rates were 45 mL min⁻¹.

The column temperature was increased from 0 to 60 °C (2min) and then from 60 to 220 °C (18 min) and finally held at 220 °C (35 min) [18].

2.7. Oxidative Stability Analysis

In this study, the induction time was determined with Metrohm 743 Rancimat device (Methrom) and ransimat device by using 3 grams of egg oil obtained from each period for oxidation stability. Oxidative stability was measured at an airflow rate of 10L / hour set at 110 degrees. Conductivity of 0.055 µs ultra pure water was used in the study [19].

2.8. Statistical Analysis

The data obtained in the study were analyzed using the SPSS (Statistical Package for Social Sciences) Windows 22.0 program. In the evaluation of the data, as descriptive statistical methods, mean standard deviation and comparing quantitative continuous data between more than two independent groups, *One-Way* Anova test and Duncan multiple comparison test were used [20, 21].

3. Results and Discussion

Table 2 shows the fat contents in the parts of the eggs (albumen + yolk) based on the different laying periods tested. These were determined to be between 7.85 and 11.25%; with the fat ratios in the yolks between 18.58 and 31.17%. The lowest fat content in the egg yolk (18.58%) was observed in the 18-week double yolk; the highest (31.17%) was observed in the 24-week guide egg. Various studies have been conducted on the total fat content of eggs (albumen + yolk). Decker and Cantor (1992) [22] have determined that the total fat content of eggs is 11.2%. Liu et al. (2005) [23] have found that the fat content in different hen egg yolks is between 26.65 and 46.33%. Other researchers have observed similar values for fat content in the yolks [9, 22–24].

Table 2 Physicochemical properties egg-yolk oils from different laying periods

Period –	18.week	24.week	30.week	80.week
Commercial Name	Double yolk	Guide	Pullet	Jumbo
Albumen + Yolk Fat (%)	8.14c ± 0.26	11.25a ± 0.50	9.28b ± 0.71	7.85d ± 0.33
Yolk Fat (%)	18.58d ± 0.69	31.17a ± 1.02	29.28b ± 0.94	26.33c ± 0.80
Acidity (%)	1.84b ± 0.55	1.40c ± 0.61	1.15d ± 0.47	2.15a ± 0.79
Peroxide Value (meqO ₂ /kg)	8.66a ± 0.48	8.58a ± 0.35	8.62a ± 0.51	9.57b ± 0.85
Iodine Value (mgI ₂ /100g)	71.1c ± 0.47	72.5b ± 0.88	72.3b ± 0.16	76.6a ± 0.96
Oxidative Stability (h)	10.2a ± 0.55	9.5b ± 0.61	9.3b ± 0.47	8.0c ± 0.79

^{a-d} values marked with different letters are statistically different from each other ($p < 0.005$)

We observed that the free fatty acidity of egg-yolk oils varied between 1.15 and 2.15%, with the lowest observed in the 30-week pullet and the highest in the 80-week jumbo. Liu et al. (2005) [23] have determined that the free fatty acidity values

of hen egg-yolk oils extracted using various methods were between 1.20 and 14.83 mg KOH/g [23].

The iodine value was observed to vary between 71.1 and 76.6, with the lowest observed in the 18-week double yolk and the highest in the 80-week jumbo. Based on these values, egg-yolk oil was determined to be part of the group of nondrying oils, such as palm, coconut, and butter [25, 26].

We observed that the peroxide values varied between 8.58 and 9.57 meq O₂/kg, with the lowest observed in the 24-week guide egg and highest in the 80-week jumbo. The oxidative stability of the egg-yolk oils varied between 8.0 and 10.2 h, with the lowest observed in the 80-week jumbo and the highest in the 18-week double yolk egg. We did not find any studies regarding the peroxide value and oxidative stability of egg-yolk oils that indicated their resistance to oxidation, especially under storage conditions.

Table 3 Fatty acid compositions (%) of LOHMANN white white-shell egg-yolk oils from different laying periods

Fatty Acid Composition (%)	Period – Commercial Name			
	18.week Double Yolk	24.week Guide	30.week Pullet	80.week Jumbo
Myristic	0.40	0.26	0.28	0.31
Palmitic	27.78	24.73	25.60	24.26
Stearic	8.16	9.17	8.26	6.86
Behenic	0.12	0.18	0.10	0.09
Saturated Fatty Acids	36.46 ± 1.15 ^a	34.34 ± 0.99 ^b	34.24 ± 1.05 ^b	31.52 ± 0.7 ^c
Palmitoleic	3.49	2.98	3.30	3.29
Oleic	40.48	42.51	43.69	45.50
Linoleic	17.65	18.03	16.91	18.38
Linolenic	0.96	0.75	0.90	0.98
Arachidonic	0.97	1.38	0.95	0.37
Unsaturated Fatty Acids	63.54 ± 0.97 ^c	65.66 ± 0.97 ^b	65.86 ± 1.19 ^b	68.52 ± 1.7 ^a

^{a-d} values marked with different letters are statistically different from each other ($p < 0.005$)

As seen in Table 3, the percentage of total saturated fatty acid of white-shell egg-yolk oils varied between 31.52 and 36.46%, with the lowest observed in the 80-week jumbo commercial egg and the highest in the 18-week double yolk egg. The total unsaturated fatty acid content varied between 63.54 and 68.52%, with the lowest observed in the double yolk egg and the highest in the jumbo egg. In addition, the dominant saturated fatty acids in white-shell egg-yolk oils were observed to be palmitic (24–27%) and stearic (6–9%), while the dominant unsaturated fatty acids were palmitoleic (2–3%), oleic (40–45%), and linoleic (16–18%).

Nyberg (2017) [27] and Sehu et al. (2012) [28] have found oleic acid to be the dominant fatty acid in egg yolk. It has been reported that most of the fatty acids (53–56%) in an egg are unsaturated [27–29]. In their study on the fatty acid composition of eggs, Stibilj et al. (1999) [30] have determined the following ratios: myristic acid (0.28%), palmitic acid (21.67%), palmitoleic acid (3.58%), stearic acid (9.80%), oleic acid (43.86%), and linoleic acid (14.92%).

Table 4 Sterol compositions (%) of LOHMANN white white-shell egg-yolk oils from different laying periods

Sterol Composition (%)	Periods (Commercial Name)			
	18.week (Double yolk)	24.week (Guide)	30.week (Pullet)	80.week (Jumbo)
Cholesterol	95.91 ± 1.20 ^c	95.53 ± 1.36 ^c	97.83 ± 1.51 ^a	96.37 ± 1.33 ^b
Brassicasterol	0.31 ± 0.09 ^a	0.39 ± 0.09 ^a	0.37 ± 0.05 ^a	0.31 ± 0.03 ^b
Campesterol	3.19 ± 0.67 ^{ab}	3.35 ± 0.82 ^a	1.29 ± 0.48 ^c	2.87 ± 0.80 ^b
Stigmasterol	0.46 ± 0.06 ^b	0.56 ± 0.08 ^a	0.51 ± 0.03 ^a	0.45 ± 0.01 ^b
Δ5,23- Stigmastadienol	nd.	nd.	nd.	nd.
Δ5,24- Stigmastadienol	nd.	nd.	nd.	nd.
Δ5-Avenasterol	nd.	nd.	nd.	nd.
Δ7-Avenasterol	nd.	nd.	nd.	nd.
Δ7-Stigmastenol	0.12 ± 0.03 ^a	0.12 ± 0.05 ^a	nd.	nd.
β-sitosterol	nd.	0.063 ± 0.07 ^a	nd.	nd.

^{a-d} values marked with different letters are statistically different from each other ($p < 0.005$). nd. Not determined

As shown in Table 4, among the 10 sterol components of the egg-yolk oils obtained from the different laying periods, cholesterol and campesterol were observed to be dominant. The cholesterol in white-shell egg-yolk oils varied between 95.52 and 97.83%, with the lowest observed in the 24-week guide and the highest in the 30-week pullet. The campesterol content varied between 1.29 and 3.35%, with the lowest observed in the 30-week pullet and the highest in the 24-week guide (3.35%). In white-shell egg-yolk oils, except for those sterols, brassicasterol, stigmasterol, Δ7-stigmastenol, and β-sitosterol were found in different proportions. These sterols in the egg-yolk oils are important for human health and nutrition because they have been reported to provide protective effects against cardiovascular diseases by reducing the risk of heart attack by 15–45% and against certain cancers and by strengthening the immune system [31–34]; therefore, these biologically active substances have been extensively studied for neutrocytic and the production of functional foods [35]. Liu et al. (2005) [23] have stated that the amount of cholesterol in egg oils varies between 28.51 and 38.15 mg/g. Shahid et al. (2015) [36] and have indicated that the cholesterol levels in egg yolks are 11.65 to 19.27 mg/g [23, 36]. Faitarone et al. (2013) [37] have determined that the cholesterol in egg yolks obtained from different laying periods is 792–1061 mg/100 g. Beyer and Jensen (1989) [38] have stated that 5.2% of egg lipids consist of cholesterol, and that the cholesterol content in the egg is ~213 mg. The studies have focused mainly on the amount of cholesterol in the egg and yolk; no study was found on the sterol composition of egg-yolk oil. Several researchers have reported that feeding, maintenance, environmental conditions, age, and climate may have an effect on the macro- and micronutrient composition of hen eggs [39–41].

4. Conclusion

The fat ratios in the whole egg and egg yolk, as well as the free fatty acidity, peroxide value, iodine value, oxidative stability, total saturated and unsaturated fatty acids, and sterol composition of egg-yolk oils varied physico-chemically based on the laying periods with a statistically significant level of $p < 0.05$. These results also suggested that egg-yolk oil is a good source of nutrients in terms of percent fat amount, saturated and unsaturated fatty acids, and sterol composition. Based on the 80-week laying period, the results suggested that the egg-yolk oils were suitable for both internal consumption and external application with the most ideal egg oils extracted from the 30-week pullet. Further studies are needed on the results from different extraction methods, such as cold press and CO₂, for the consumption of egg-yolk oil as an animal fat, which has increased as a trade throughout the world.

Acknowledgement

This work was funded by AKU BAP (16:Fen.Bil.44)

References

- [1] Sah N, Mishra B. Regulation of egg formation in the oviduct of laying hen. *World's Poultry Science Journal* (2018) **74**(3):509–522.
- [2] Nys Y, Bain M, van Immerseel F. *Improving the Safety and Quality of Eggs and Egg Products: Volume 1: Egg Chemistry, Production and Consumption*: Elsevier (2011).
- [3] Jonchère V, Réhault-Godbert S, Hennequet-Antier C, Cabau C, Sibut V, Cogburn LA, et al. Gene expression profiling to identify eggshell proteins involved in physical defense of the chicken egg. *BMC genomics* (2010) **11**(1):57.
- [4] Aydın D, Rashid S, Aydın R. Tavuk yumurtası ve kolesterol gerçeği [Chicken eggs and the truth of cholesterol]. *Tarım ve Doğa Dergisi* (2014) **17**(3):26.
- [5] Ali MA. A Comparative Study on Nutritional Value of Quail and Chicken Eggs. *مجلة البحوث في مجالات التربية النوعية* (2019) **2019**(22):39–56.
- [6] Açıköz Z, Önenç SS. Fonksiyonel Yumurta Üretimi [Functional Egg Production]. *Hayvansal Üretim* (2006) **47**(1):36–46.
- [7] Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P, Rodriguez JA, Lamas A, et al. Egg and egg-derived foods: effects on human health and use as functional foods. *Nutrients* (2015) **7**(1):706–729.
- [8] Senji Sakanaka, Kouichi Kitahata, Takayuki Mitsuya, Miguel A. Gutierrez, Lekh R. Juneja. Protein Quality Determination of Delipidated Egg-yolk. *Journal of Food composition and Analysis* (2000) **13**(5):773–781. doi:10.1006/jfca.2000.0914.
- [9] Özcan MM, Al Juhaimi F, Uslu N, Ghafoor K, Babiker EE, Mohamed Ahmed IA, et al. Effect of boiling on fatty acid composition and tocopherol content of hen, duck, and quail egg oils. *Journal of Food Processing and Preservation* (2019) **43**(7):e13986.
- [10] McNamara DJ. The fifty year rehabilitation of the egg. *Nutrients* (2015) **7**(10):8716–8722.
- [11] López Sobaler AM, Aparicio Vizuete A, Ortega RM. Papel del huevo en la dieta de deportistas y personas físicamente activas [Role of the egg in the diet of athletes and physically active people]. *Nutricion hospitalaria* (2017) **34**(Suppl 4):31–35. doi:10.20960/nh.1568.
- [12] Pelletier X, Thouvenot P, Belbraouet S, Chayvialle JA, Hanesse B, Mayeux D, et al. Effect of egg consumption in healthy volunteers: influence of yolk, white or whole-egg on gastric emptying and on glycemic and hormonal responses. *Annals of nutrition and metabolism* (1996) **40**(2):109–115.
- [13] Beynen AC. Serum and liver cholesterol in rats fed cholesterol-free of high-cholesterol diets differing in type and amount of fat. *Nutrition reports international* (1987) **35**(6):1327–1332.
- [14] Çelebi Ş, Karaca H. Yumurmanın besin değeri, kolesterol içeriği ve yumurtayı n-3 yağ asitleri bakımından zenginleştirmeye yönelik

- çalışmalar [Studies on nutritional value, cholesterol content of eggs and enrichment of eggs in terms of n-3 fatty acids]. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi* (2006) **37**(2):257–265.
- [15] Kaushik, N., Kumar, K., Kumar, S., Kaushik, N., Roy, S. Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*Jatropha curcas* L.) accessions. *Biomass and Bioenergy* (2007) **31**:497–502.
- [16] Al Juhaimi F, Uslu N, Özcan MM. Oil content and fatty acid composition of eggs cooked in drying oven, microwave and pan. *Journal of food science and technology* (2017) **54**(1):93–97.
- [17] Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International journal of molecular sciences* (2015) **16**(6):12871–12890.
- [18] Lechner M, Reiter B, Lorbeer E. Determination of tocopherols and sterols in vegetable oils by solid-phase extraction and subsequent capillary gas chromatographic analysis. *Journal of Chromatography A* (1999) **857**(1-2):231–238.
- [19] Coppin EA, Pike OA. Oil stability index correlated with sensory determination of oxidative stability in light-exposed soybean oil. *Journal of the American Oil Chemists' Society* (2001) **78**(1):13–18.
- [20] Sosyal Biometrinin temel prensipleri [Basic principles of biometry]. *Trakya Üniversitesi Tekirdağ Ziraat Fakültesi Yayınları* (1998)(95):15–35.
- [21] Nelson LS. Introduction to Statistical Quality Control. *Journal of Quality Technology* (1987) **19**(4):233–236.
- [22] Decker EA, Cantor AH. Fatty acids in poultry and egg products. In: Chow CH, editor. *Fatty Acids in Foods and Their Health Implications*. New York, NY: Marcel Dekker, Inc (1992). p. 137–167.
- [23] Liu Z, Wu G, Bryant MM, Da Roland Sr. Influence of added synthetic lysine in low-protein diets with the methionine plus cysteine to lysine ratio maintained at 0.75. *Journal of applied poultry research* (2005) **14**(1):174–182.
- [24] Aletor, O., Famakin, F. M. *Vitamins, amino acids, lipids and sterols of eggs from three different birds genotypes. J. of Environ. Sci., Toxicology and Food Techn* **11** (3 Ed.) (2017). 41–47.
- [25] Caballero B, Trugo LC, Finglas PM. *Encyclopedia of food sciences and nutrition*: Academic (2003).
- [26] Gunstone FD, Harwood JL, Dijkstra AJ. *The Lipid Handbook*. Boca Raton, Florida: CRC Press (2007). 68 p.
- [27] Nyberg J. *Analysis of fatty acids in egg yolks of various production systems*. Master's Thesis. Swedish University of Agricultural Sciences. Uppsala (2017).
- [28] Sehu A, Kucukersan S, Coskun B, Koksall BH, Citil OB. Effects of dietary glycerol addition on growth performance, carcass traits and fatty acid distribution in cloacal fat in broiler chickens. *Revue de Médecine Vétérinaire* (2012) **163**(4):194–200.
- [29] Demirulus H. Yumurta tüketiminin kan kolesterolü üzerindeki etkisi [The effect of egg consumption on blood cholesterol]. *Uluslar arası Tavukçuluk Fuarı ve Konferansı, İstanbul* (1999):3–6.
- [30] Stibilj V, Rajčič MK, Holcman A. Fatty Acid Composition Of Eggs Enriched With Omega - 3 Fatty Acids On The Market. *Ljubljana University Biotechnical Faculty Zootechnical Dept* (1999) **74**(2):27–36.
- [31] Bouic PJD. The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. *Current Opinion in Clinical Nutrition & Metabolic Care* (2001) **4**(6):471–475.
- [32] Adeniyi PO, Obatolu VA, Farinde EO. Comparative evaluation of cholesterol content and storage quality of chicken and quail eggs. *World J Nutr Health* (2016) **4**(1):5–9.
- [33] Law M. Plant sterol and stanol margarines and health. *Bmj* (2000) **320**(7238):861–864.
- [34] Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. *The Journal of nutrition* (2000) **130**(9):2127–2130.
- [35] Hendriks HF, Weststrate JA, van Vliet T, Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition* (1999) **53**(4):319–327.
- [36] Shahid S, Chand N, Khan RU, Suhail SM, Khan NA. Alternations in cholesterol and fatty acids composition in egg yolk of Rhode Island Red x Fyoumi Hens fed with hemp seeds (*Cannabis sativa* L.). *Journal of Chemistry* (2015) **2015**.
- [37] Faitarone AB, Garcia EA, Roça RdO, Ricardo HdA, Andrade EN de, Pelicia K, et al. Cholesterol levels and nutritional composition of commercial layers eggs fed diets with different vegetable oils. *Brazilian Journal of Poultry Science* (2013) **15**(1):31–37.
- [38] Beyer RS, Jensen LS. Overestimation of the cholesterol content of eggs. *Journal of Agricultural and Food Chemistry* (1989) **37**(4):917–920.
- [39] Réhault-Godbert S, Guyot N, Nys Y. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrients* (2019) **11**(3):684.
- [40] Eseceli, H. Kahraman, R. Ayçiçek ve Balık yağı içeren yumurta tavuğu Rasyonlarına E ve C Vitaminleri İlavasının Performansa Etkisi [The Effect of Addition of Vitamins E and C to Laying Chicken Diets Containing Sunflower and Fish Oil on Performance]. *Gıda ve Yem Bilimi-Teknolojisi* (2003) **4**:13–22.
- [41] Scheideler SE, Froning GW. The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens. *Poultry Science* (1996) **75**(10):1221–1226.