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Qualitative characteristics of fatty acid profile in fresh and boiled n-3 PUFA enriched eggs

Kvalitativne značajke profila masnih kiselina u svježim i kuhanim n-3 PUFA obogaćenim jajima

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ABSTRACT

Eggs are a foodstuff of animal origin that are widely consumed by human population. Quality of eggs depends on many factors, such as laying hens' feed composition. Modification of laying hens' diet by supplementing different fat sources affects the fatty acid profile in egg yolks. This paper presents the comparison of the fatty acid profile (FA) in fresh and boiled n-3 PUFA eggs. There is the question asked if the values referring to daily consumption of specific fatty acids in fresh eggs are credible. The portion of fatty acids in omega-3 fresh eggs is the following: SFA 30.76%, MUFA 41.97%, n-6 PUFA 22.24%, n-3 PUFA 4.96%, and the n-6/n-3 PUFA ratio is 4.51. Comparison of FA profile in fresh and thermally processed n-3 PUFA eggs (boiled for 10 min. at 95 °C) shows that there are differences in the content of specific FAs, but they are not statistically significant ($P>0.05$), except for myristoleic fatty acid ($P<0.05$). There results justify the presentation of fatty acids profile in eggs on the basis of fresh eggs analysis results, as there are no differences in the fatty acid profile between fresh and cooked eggs, which was also shown by some other authors.

Keywords: boiled eggs, fatty acid profile, fresh eggs, n-3 PUFA

SAŽETAK

Jaja su namirnica životinjskog podrijetla koje ljudi često konzumiraju. Kvaliteta jaja ovisi o mnogim čimbenicima, kao što je primjerice sastav hrane za kokoši nesilice. Modificiranje sastava obroka kokoši nesilica, dodatkom različitih izvora masnoća, utječe na profil masnih kiselina u žumanjku. U radu je prikazana usporedba profila masnih kiselina (MK) u svježim i kuhanim n-3 PUFA jajima. Postavlja se pitanje jesu li vrijednosti koje se odnose na dnevnu potrošnju specifičnih masnih kiselina iz svježih jaja vjerodostojne. Udio masnih kiselina u omega-3 svježim jajima je sljedeći: SFA 30,76%, MUFA 41,97%, n-6 PUFA 22,24%, n-3 PUFA 4,96%, a omjer n-6/n-3 PUFA je 4,51. Usporedba FA profila u svježim i toplinski obrađenim n-3 PUFA jajima (kuhana 10 minuta na 95 °C) pokazuje da postoje razlike u sadržaju pojedinih MK, ali one nisu statistički značajne ($P>0,05$), osim za miristoleinsku masnu kiselinu ($P<0,05$). Rezultati ovog istraživanja, kao i rezultati nekih drugih autora, opravdavaju prikaz profila masnih kiselina u jajima na temelju rezultata analize svježih jaja, jer ne postoje razlike u profilu masnih kiselina između svježih i kuhanih jaja.

Ključne riječi: kuhana jaja, n-3 PUFA, profil masnih kiselina, svježa jaja

INTRODUCTION

Table eggs can be considered as a functional product if they are enriched with n-3 polyunsaturated fatty acids (PUFA). Studies have shown that eggs enriched with α-linolenic (ALA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) have beneficial effects on human health (Singh et al., 2012). Essential polyunsaturated fatty acids, α-linolenic (ALA; n-3 PUFA) and linoleic acid (LA; n-6 PUFA) are precursors of long-chain polyunsaturated fatty acids and are mutually competitive in the elongation process to the enzyme Δ^6 desaturase. Excessive consumption of n-6 PUFA over n-3 PUFA causes deficiency of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. EPA and DHA influence the prevention of cardiovascular diseases (Delgado-Lista et al., 2012; Lovegrove and Griffin, 2013). Simopoulos (2008) stated that the n-6/n-3 PUFA ratio in the western countries was unfavorable, by being approximately 15-16. The increase of the n-3 PUFA in poultry feed enables the enrichment of eggs with long-chain n-3 PUFA (Douny et al., 2015; Kralik et al., 2017; Kralik, G. et al., 2018; Kralik, Z. et al., 2018). Eggs are usually consumed after being thermally processed (Hayat et al., 2010). Consequently, there is the question of what happens with oxidative processes and if the concentration of long-chain acids in thermally processed eggs is reduced when compared with fresh eggs. Oxidation of polyunsaturated fatty acids is affected by thermal processing, as well as by storage of eggs. Increased lipid peroxidation increases the risk of atherosclerosis and other inflammatory diseases (Štefan et al., 2007). This paper presents the comparison of fatty acid profile in fresh and boiled omega-3 eggs.

MATERIALS AND METHODS

The research was performed on 80 laying hens of the Tetra SL genotype, at the age of 22 weeks. Laying hens were kept in enriched cages. Feeding and watering of laying hens was *ad libitum*. Composition of laying hens' diet used in this research is presented in the Table 1. The diet was fed to laying hens over 4 weeks, after which the laid eggs were collected for the analysis of the fatty acid profile.

Table 1. Composition of diets

Ingredients	%
Maize	49.2
Alfalfa	1.5
Roasted soybean	3.33
Soybean meal	21
Sunflower meal	5
Yeast	0.5
Salt	0.33
Livestock stone	10.68
Mono-calcium phosphate	1.33
Methionine	0.15
[1] Sal-CURB™	0.33
[2] Nanofeed-zeolite	0.33
[3] Premix	1.33
Soybean oil	1
Rapeseed oil	1.33
Linseed oil	1.33
Fish oil	1.33
[4] Chemical composition of diets (g/kg)	
Crude protein	172.6
Crude fat	75
Moisture	81
Crude fibers	26
Ash	158

[1] Sal-CURB™ - an antimicrobial for control of pathogenic microorganisms and their harmful consequences in raw materials and finished feeds (Kemin Industries, Des Moines, IA USA).

[2] Nanofeed-zeolite is a natural mineral functional supplement added to animal mixtures in the amount of 0.2-0.4%.

[3] Premix: Calcium 33%, vit. A 833.34 I.U., vit. D_3 208.34 I.U., vit. E 8.350 mg, vit. K_3 170 mg, vit. B_1 150 mg, vit. B_2 375 mg, Pantothenic acid 590 mg, Niacin 2.100 mg, Choline chloride 33.340 mg, vit. B_6 200mg, vit. B_{12} 960 mg, Biotin 7.100 m, Folic acid 70.5 mg, vit. C 1.900 mg, Iron 2.500 mg, Copper 415 mg, Zinc 5.200 mg, Manganese 5.835 mg, Iodine 75 mg, Selenium yeast 35 mg, Antioxidant (Apo-ester 85 mg, canthaxanthin 250 mg).

[4] Referential methods applied for chemical analysis of feed: HRN ISO 6496:200; HRN EN ISO 5983-2:2010; HRN EN ISO 6865:2001, Modified according to instructions of FOSS Fiber Cap manual; HRN ISO 5984:2004; HRN ISO 6492:2001, Modified according to instructions of extraction system ANKOM XT15; RU-5.4.2-11 (internal method)

Analysis of fatty acids in eggs and hens' feed

Egg weight was 53-63 g, which refers to the M class. The FA profile was determined on 10 fresh and 10 boiled eggs. Boiling of eggs was done so that the eggs were left at room temperature half an hour before boiling. Water was boiled in a pot, eggs were dropped in boiling water with a spoon and cooked for 10 minutes. After cooking, eggs were taken out from the water, cooled, peeled off and egg yolks were prepared for fatty acid profile analysis. The analysis of fatty acid profile in eggs and in the laying hens' diet was carried out as follows: homogenized samples' fat content was extracted with the method of Folch et al. (1957). All solvents used were ultrapure-grade by Sigma-Aldrich (Schnelldorf, Germany), and 100 mg/L butylated hydroxitoluene was added to the extraction mixture (chloroform/methanol 2/1 vol/vol) as antioxidant. After this, fatty acid containing lipids were transmethylated by the base-catalyzed sodium-methoxide method of Christie (1982). Gas liquid chromatography was performed on a Bruker 430-GC apparatus (Billerica, MA, SAD), equipped with a FAMEWAX (RESTEK, Bellefonte, PA, USA) type capillary column (30 m x 0.32 mm internal diameter, 0.25 µm film) and flame ionization detector. Characteristic operating conditions were: injector temperature: 220 °C, detector temperature: 230 °C, helium flow: 25 ml/min. The oven temperature was graded: from 50 to 225 °C: 6 °C/min, 21 min at 225 °C. To identify individual FA in the chromatogram, a FA standard mixture (Supelco 37 Component FAME Mix, SUPELCO® Analytical, Bellefonte, PA, USA) was used. Portions of individual and total fatty acids were shown as a percentage of total fatty acids in the lipids of yolks.

The profile of fatty acids in laying hens' diet is overviewed in the Table 2. The feeding mixture was as follows: ΣSFA 18.11%, ΣMUFA 34.19%, Σn-6 PUFA 38.91% and Σn-3 PUFA 8.78%. Referring to the total n-3 PUFA, ALA was the most represented (8.11%), followed by EPA (0.41%) and DHA (0.26%). Processes of desaturation and elongation in the laying hens' organism supported the deposition of stated fatty acids in egg yolks. In this research, deposition of DHA was particularly efficient, which is considered as desirable (Table 3).

Table 2. Fatty acids profile in laying hens' diet (% of total fatty acids)

Fatty acid	%
Butyric (C14:0)	0.43
Pentadecanoic (C15:0)	0.05
Palmitic (C16:0)	12.2
Heptadecanoic (C17:0)	0.1
Stearic (C18:0)	3.91
Arachidic (C20:0)	0.5
Heneikosanoic (C21:0)	0.33
Behenic (C22:0)	0.34
Lignoceric (C24:0)	0.24
ΣSFA	18.11
Palmitoleic (C16:1)	0.33
Oleic (C18:1n9c)	31.7
Elaidic (C18:1n9t)	1.43
Eicosenoic (C20:1n9)	0.66
Nervonic (C24:1n9)	0.08
ΣMUFA	34.19
Linoleic (C18:2n6)	38.91
Σn-6 PUFA	38.91
α-linolenic (C18:3n3)	8.11
Eicosatrienoic (C20:3n3)	0.41.0.26
Docosahexaenoic (C22:6n3)	8.78
Σn-3 PUFA	4.43
Σn-6 PUFA / Σn-3 PUFA	

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA –polyunsaturated fatty acids

Statistical analysis

Research results are processed in the Statistica for Windows version 13.3. (StatSoft Inc., 2017). The data analysis was performed by using descriptive statistics and analysis of variance (ANOVA), and if the P value was statistically significant, then the differences between groups were tested by the Fisher's LSD test.

RESULTS AND DISCUSSION

The Table 3 presents the portions of fatty acids, as well as the ratio of n-6/n-3 PUFA in fresh and boiled eggs. The data indicate that palmitic acid was the most represented in the ΣSFA (21.92% and 21.98%), followed by stearic acid (7.78% and 7.97%). The differences in portions of specific fatty acids, as well as inΣSFA, were not statistically significant (P>0.05). In the ΣMUFA (41.97% and 41.96%) there were also no statisticallysignificantdifferences, except for the portion ofmyristoleic acid (0.03% and 0.01%, P<0.05). The most represented were oleic and elaidic acids (39.81% and 31.92%). In the Σn-6 PUFA (22.24% and 22.37%), the highest concentration was proved for linoleic acid, both in fresh and boiled eggs (20.18% and 20.41%, respectively; P>0.05). Content of arachidonic acid was lower in boiled than in fresh eggs (1.62% and 2.6%, respectively; P>0.05). Furthermore, boiled eggs also had lower portions of αLNA, eicosatrienoic acid and DHA, as well as the Σn-3 PUFA than fresh eggs(P>0.05). Fresh eggs exhibited more favorable ratio of n-6/n-3 PUFA thanboiled eggs (P>0.05). Murcia et al. (1999) stated that boiled eggs had higher portions of LA and arachidonic acid than fresh eggs (P>0.05). Douny et al. (2015) also confirmed that boiling of eggs did not have significant influence on the portions of n-3 PUFA. The percentage of ALA and DHA in total fatty acids of enriched egg yolks was reduced from 12.7% to 12.4%, i.e. from 1.4% to 1.3%. The lowering of the ratio was not confirmed for conventional eggs, which is explained by the fact that PUFA with 3 double bonds (enriched eggs) are more susceptible to oxidation than PUFA with 2 double bonds. Saturated fatty acids, as well as fatty acids that containone double bond, are more resistant toward the free radical activity than PUFA. The research in vitro proved that DHA had 5 times better ability of oxidation than LA (Štefan et al., 2007). Cortinas et al. (2003) confirmed significant lowering of DHA, but not of ALA in boiled eggs after feeding laying hens with fish oil. Van Elswyk et al. (1992) reported that boiling of eggs did not change the fatty acid profile of eggs enriched with omega-3. These authors concluded that the portions of omega-3 fatty acids did not change significantly after thermal processing of eggs. The results of this research are also in accordance with this conclusion. According to Douny et al. (2015), the analysis confirmed significantly higher portions of EPA and DHA in boiled eggs than in fresh eggs. The findings of present research showed that only EPA was more present in boiled eggs (0.26%:0.32%, P>0.05), than in fresh eggs. As of the research of Douny et al. (2015), the ratio of n-6/n-3 in eggs was 1.4:1.0 in processed and in fresh omega-3 eggs, respectively. In the conducted research, this ratio was 4.51 and 4.71 (P>0.05) in fresh and in boiled eggs, respectively. In n-3 PUFA enriched eggs, Botsoglou et al. (2012) determined significant lowering of DHA (but not of ALA) in fried eggs, which is also in accordance with the research results referring to portions of eicosatrienoic acid and DHA.The portion of ALA in omega-3 eggs is higherthan the one reported by Botsoglou et al. (2012), Halle and Schöne (2013), as well as by Meynier et al. (2014). The results of the present research proved slightly higher portion of DHA in n-3 PUFA eggs than it was in the research of above-mentioned authors. Samman et al. (2009) determined 4.5% of ALA and 2.1% of DHA in the Austrian omega-3 eggs. Kralik et al. (2010) investigated the nutritive composition of eggs produced by two producers present on the Croatian market. They confirmed that omega-3 eggs contained 2.6-5.62 times higher portion of n-3 PUFA than conventional eggs. The ratio of n-6/n-3 PUFA in conventional eggs was 11.09-16.86, and the same ratio in eggs enriched with n-3 PUFA was 2.79-4.10. The research into enriching of table eggs with functional ingredients performed by Kralik G. et al. (2018) proved that the ratio of n-6/n-3 PUFA in the experimental group was more than twice as favorable as in the control group (5.91 vs. 13.34; P<0.001). By designing of diets fed to laying hens, Kralik Z. et al. (2018) increased the n-3 PUFA content in designed eggs when compared to eggs from the control group (conventional feeding of hens). The above-mentioned authors emphasized the increase of n-3 PUFA content for two times. More precisely, the omega-3 content in conventional eggs was 366.53 mg/100 g of egg yolk, and in the designed eggs it was 878.62 mg/100 g of egg yolk.

Table 3. Fatty acid composition (% of total FA) in fresh and boiled n-3 PUFA enriched eggs

Fatty acid	Fresh eggs (n=10) $\bar{x}$ ± sd	Boiled eggs (n=10) $\bar{x}$ ± sd	P-value
Butyric (C4:0)	0.09±0.01	0.1±0.01	0.441
Tridecanoic (C13:0)	0.01±0.002	0.01±0.002	0.067
Myristic (C14:0)	0.27±0.02	0.27±0.04	0.967
Pentadecanoic (C15:0)	0.07±0.01	0.08±0.01	0.678
Palmitic (C16:0)	21.92±0.56	21.98±0.53	0.894
Heptadecanoic (C17:0)	0.21±0.01	0.21±0.02	0.628
Stearic (C18:0)	7.78±0.5	7.97±0.46	0.651
Arachidic (C20:0)	0.1±0.07	0.03±0.002	0.138
Heneikosanoic (C21:0)	0.24±0.08	0.18±0.01	0.32
Behenic (C22:0)	0.03±0.002	0.02±0.001	0.47
Tricosanoic (C23:0)	0.05±0.03	0.02±0.001	0.109
ΣSFA	30.76±0.49	30.87±0.59	0.822
Myristoleic (C14:1)	0.03^{a}±0.01	0.01^{b}±0.002	0.047
Palmitoleic (C16:1)	1.63±0.15	1.57±0.09	0.663
Heptadecenoic (C17:1)	0.22±0.04	0.18±0.02	0.263
Oleic+elaidic (C18:1n9c+C18:1n9t)	39.81±2.29	39.92±0.26	0.937
Eicosenoic (C20:1n9)	0.29±0.04	0.28±0.01	0.657
ΣMUFA	41.97±2.07	41.96±0.17	0.996
Linoleic (C18:2n6c)	20.18±0.73	20.41±0.15	0.614
Linolelaidic (C18:2n6t)	0.05±0.02	0.05±0.005	0.985
γ-linolenic (C18:3n6)	0.18±0.06	0.1±0.07	0.22
Eicosadienoic (C20:2n6)	0.24±0.11	0.18±0.01	0.484
Arachidonic (C20:4n6)	2.6±0.04	1.62±0.07	0.695
Σn-6 PUFA	22.24±0.95	22.37±0.13	0.833
α-linolenic (C18:3n3)	2.6±0.41	2.39±0.11	0.435
Eicosatrienoic (C20:3n3)	0.1±0.03	0.07±0.02	0.317
Eicosapentaenoic (C20:5n3)	0.26±0.04	0.32±0.05	0.141
Docosahexaenoic (C22:6n3)	2.01±0.2	2±0.36	0.968
Σn-3 PUFA	4.96±0.63	4.79±0.49	0.721
Σn-6 PUFA / Σn-3 PUFA	4.51±0.38	4.71±0.52	0.626

$\bar{x}$ - arithmetic means; sd - standard deviation;
[a,b] P<0.05; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA –polyunsaturated fatty acids.

Juhaimi et al. (2017) reported that the method of thermal processing had influence on the content of lipids ineggs. The authorscomparedthe composition of fatty acids in eggs processed in different ways: boiled in water, thermally processed in electric oven, in microwave oven and in a frying pan. They determined that eggs boiled in water contained less arachidonic acid, which was also confirmed in this research.

Based on research results (Table 4), 100 g of fresh n-3 PUFA eggs contain 17.82 mg EPA and 140.43 mg DHA. In the same amount of boiled n-3 PUFA eggs, there is 22.45 mg EPA and 139.64 mg DHA. Since the differences in the content of fatty acids between fresh and boiled eggs are not statistically significant, it is justified that the authors present the profile of fatty acids only for fresh eggs, thus making the procedure simpler.

The Table 5 shows the results of the confidence interval estimation under the conditions t=2.62 and n=10. By using the confidence interval, there are values of the arithmetic mean of basic fatty acids groups presented for fresh and boiled eggs.

Table 4. Effect of cooking on the fatty acid composition (mg FA/100 g of egg*) in fresh and boiled n-3 PUFA enriched eggs

Fatty acid[1]	Fresh eggs (n=10) $\bar{x}$ ± sd	Boiled eggs (n=10) $\bar{x}$ ± sd	P value
ΣSFA	2,142.44 ± 37.44	2,151.35 ± 40.25	0.793
ΣMUFA	2,928.67 ± 142.03	2,926.98 ± 14.01	0.985
Σn-6 PUFA	1,553.2 ± 68.43	1,559.9 ± 8.85	0.875
Σn-3 PUFA	346.07 ± 43.81	333.78 ± 34.12	0.721
EPA (C20:5n3)	17.82 ± 2.55	22.45 ± 3.57	0.141
DHA (C22:6n3)	140.34 ± 14.13	139.64 ± 25.18	0.968

$\bar{x}$ - arithmetic means; sd - standard deviation; 1SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; n-6 PUFA - omega-6 polyunsaturated fatty acids; n-3 PUFA - omega-3 polyunsaturated fatty acids; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; *edible part.

Table 5. Confidence interval $\bar{x} \pm t_{\bar{x}} * S ; t = 2{,}62$

Fatty acid[1]	Fresh eggs	Boiled eggs
ΣSFA	30.76 ± 0.73	30.8 ± 0.89
ΣMUFA	41.97 ± 3.14	41.96 ± 0.26
Σn-6 PUFA	22.24 ± 1.44	22.37 ± 0.21
Σn-3 PUFA	4.96 ± 0.94	4.79 ± 0.73
EPA	0.26 ± 0.05	0.32 ± 0.08
DHA	2.01 ± 0.31	2 ± 0.55
ALA	2.6 ± 0.6	2.39 ± 0.18
Σn-6/Σn-3 PUFA	4.51 ± 0.58	4.71 ± 0.79

[1] SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; n-6 PUFA - omega-6 polyunsaturated fatty acids; n-3 PUFA - omega-3 polyunsaturated fatty acids; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; ALA - alpha-linolenic acid.

It is expected that EPA in fresh eggs will be in the range from 0.21% to 0.31%, and in boiled eggs from 0.24% to 0.4% of total fatty acids. DHA in fresh eggs will be presented from 1.7% to 2.32%, and in cooked eggs from 1.45% to 2.55%. The percentage of ALA in fresh eggs will be from 2% to 3.2%, and in boiled eggs, it will be 2.21% to 2.57% of total fatty acids.

CONCLUSION

This paper presents the research into the fatty acid profile of fresh and boiled omega-3 eggs. Eggs were laid by the Tetra SL laying hens fed with diet containing 8.78% n-3 PUFA in total fatty acids. There were no statistically significant differences (P>0.05) determined in the content of SFA, MUFA (except for myristoleic acid, P<0.05), n-6 PUFA, n-3 PUFA, EPA and DHA betweenfresh and boiled omega-3 eggs. Results of present research proved that feeding laying hens with diet that contains high concentration of n-3 PUFA positively affected the deposition ofthesefatty acids in egg yolk lipids. It is justified to present the qualitative characteristics of fresh eggs' fatty acid profile, which is agreed upon also by other authors.

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REFERENCES

Botsoglou, E., Govaris, A., Ambroisiadis, I., Fletouris, D. (2012) Lipid and protein oxidation of α-linolenic acid - enriched pork during refrigerated storage as influenced by diet supplementation with olive leaves (Olea Europa L.) or α-tocopheryl acetate. Meat Science, 92 (4), 525-532. DOI: https://doi.org/10.1016/j.meatsci.2012.05.022

Christie, W.W. (1982) A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. Journal of Lipid Research, 23 (7), 1072-1075.

Cortinas, L., Galobart, J., Barroeta, A.C., Baucells, M.D., Grashorn, M.A. (2003) Change in α-tocopherol contents, lipid oxidation and fatty acid profile in eggs enriched with linolenic acid or very long-chain ω3 polyunsaturated fatty acids after different processing methods. Journal of the Science of Food and Agriculture, 83 (8), 820-829. DOI: https://dx.doi.org/10.1002/jsfa.1418

Delgado-Lista, J., Perez-Martinez, P., Lopez-Miranda, J., Perez-Jimenez, F. (2012) Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. British Journal of Nutrition, 107 (2), 201-213. DOI: https://dx.doi.org/10.1017/S0007114512001596

Douny, C., El Khoury, R., Delmelle, J., Brose, F., Degand, G., Moula, N., Farnir, F., Clinquaart, A., Maghuin-Rogiste, G., Scippo, M.L. (2015) Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. Food Science and Nutrition, 3 (2), 140-152. DOI: https://dx.doi.org/10.1002/fsn3.197

Folch, J.M., Lees, M., Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226 (1), 495-509.

Halle, I., Schöne, F. (2013) Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk. Journal für Verbraucherschutz und Lebensmittelsicherheit, 8 (3), 185-193. DOI: https://dx.doi.org/10.1007/s00003-013-0822-3

Hayat, Z., Cherian, G., Pasha, T.N., Khattak, F.M., Jabbar, M.A. (2010) Oxidative stability and lipid components of eggs from flax-fed hens: effect of dietary antioxidants and storage. Poultry Science, 89 (6), 1285-1292. DOI: https://dx.doi.org/10.3382/ps.2009-00256

Juhaimi, F.A., Uslu, N., Özcan, M.M. (2017) Oil content and fatty acid composition of eggs cooked in drying oven, microwave and pan. Journal of Food Science and Technology, 54(1), 93-97. DOI: https://dx.doi.org/10.1007/s13197-016-2439-x

Kralik, G., Škrtić, Z., Kralik, Z., Hanžek, D. (2010) Fatty acid profile in lipids of table eggs available on Croatian market. XIIIth European Poultry Conference, Tours, France, 23-27 August 2010.

Kralik, G., Kralik, Z., Strakova, E., Grčević, M., Hanžek, D. (2017) Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans. Acta Veterinaria Brno, 86 (3), 293-301. DOI: https://dx.doi.org/10.2754/avb201786030293

Kralik, G., Kralik, Z., Grčević, M., Kralik, I., Gantner, V. (2018) Enrichment of table eggs with functional ingredients. Journal of Central European Agriculture, 19 (1), 72-82. DOI: https://dx.doi.org/10.5513/JCEA01/19.1.2025

Kralik, Z., Kralik, G., Grčević, M., Kralik, I., Gantner, V. (2018) Physical-chemical characteristics of designer and conventional eggs. Brazilian Journal of Poultry Science, 20 (1), 119-126. DOI: https://dx.doi.org/10.1590/1806-9061-2017-0631

Lovegrove, J.A., Griffin, B.A. (2013) The acute and long-term effects of dietary fatty acids on vascular function in health and disease. Current Opinion in Clinical Nutrition and Metabolic Care, 16 (2), 162-167. DOI: https://dx.doi.org/10.1097/MCO.0b013e32835c5f29

Meynier, A., Leborgne, C., Viau, M., Schuck, P., Guichardant, M., Rannou, C., Anton, M. (2014) n-3 fatty acid enriched eggs and production of egg yolk powders: An increased risk of lipid oxidation? Food Chemistry, 153 (15), 94-100. DOI: https://dx.doi.org/10.1016/j.foodchem.2013.12.028

Murcia, M.A., Martinez-Tome, M., del Cerro, I., Sotillo, F., Ramirez, A. (1999) Proximate composition and vitamin E levels in egg yolk; losses by cooking in a microwave oven. Journal of the Science of Food and Agriculture, 79 (12), 1550-1556. DOI: https://dx.doi.org/10.1002/(SICI)1097-0010(199909)79:12<1550::AID-JSFA402>3.0.CO;2-3

Samman, S., Kung, F.P., Carter, L.M., Foster, M.J., Ahmad, Z.I., Phyjal, J.L. (2009) Fatty acid composition of certified organic, conventional and omega-3 eggs. Food Chemistry, 116 (4), 911-914. DOI: https://dx.doi.org/10.1016/j.foodchem.2009.03.046

Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ration in cardiovascular disease and other chronic diseases. Experimental Biology and Medicine, 233 (6), 674-685. DOI: https://dx.doi.org/10.3181/0711-MR-311

Singh, V.P., Pathak, V., Akhilesh, K.V. (2012) Modified or enriched eggs: A smart approach in egg industry: A review. American Journal of Food Technology, 7 (5), 266-277. DOI: https://dx.doi.org/10.3923/ajft.2012.266.277

StatSoft, Inc. (2017) STATISTICA (data analysis software system), version 13.3. Tulas, OK: StatSoft Inc. Available at: http://www.statsoft.com

Štefan, L., Tepšić, T., Zavidić, T., Urukalo, M., Tota, D., Domitrović, R. (2007) Lipidna oksidacija – uzroci i posljedice. Medicina, 43 (2), 84-93.

Van Elswyk, M.E., Sams, A.R., Hargis, P.S. (1992) Composition, functionality, and sensory evaluation of eggs from hens fed dietary Menhaden oil. Journal of Food Science, 57 (2), 342-344. DOI: https://dx.doi.org/10.1111/j.1365-2621.1992.tb05490.x