Effect of Vitamin *E* Supplementation on Egg Yolk Quality and Oxidative Stability

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ABSTRACT— ***This study was carried out to evaluate the effect of dietary vitamin E supplementation on oxidative stability in cooked egg yolk. A total of 240 ISA hens were used and divided into two uniform groups: water with a vitamin E supplementation (450 ppm) was used in the experimental group, while for the control group only pure water (100%) was used. Eggs were collected daily after 30 days of feeding and stored at 4°C. Lastly eggs were boiled in water at 100°C for 10 minutes and egg quality was immediately tested (0 days). The advance of the oxidative state was further assessed at 6 days of storage at 4°C after cooking. The obtained results show that the colour and α-tochopherol concentration of egg yolk were significantly different between the two groups, at 0 days. The malondialdehyde (MDA) value and hexanal % were higher at the two storage time after cooking in the control group than the experimental one, probably due to antioxidant activity of vitamin E. During storage, the oxidative stability decreases in both groups.***

Keywords— Egg yolk, antioxidant, oxidation; α-tocopherol, volatile compounds.

1. INTRODUCTION

Eggs are considered as one of nature's perfect foods that have been consumed for centuries all over the world. Egg yolk is a rich source of both nutritive and non-nutritive compounds important to human health. It is well known that hens' diet influences yolk composition. In fact, through dietary manipulation, certain substances with important health benefits can be enriched in egg yolk (Surai and Sparks, 2001). Egg yolk, 28–29 % of whole egg, consists mainly of lipids (63 %, dry basis), followed by proteins and traces of carbohydrates. Neutral lipids (NL, 65 %) and phospholipids (PL, 30 %) are main components, whose fatty acids are more unsaturated than those of most animal lipids. The egg is an excellent source of essential fatty acids mainly belonging to the n-6 series (linoleic and arachidonic acids) and also contains moderate amounts of n-3 polyunsaturated fatty acids (PUFA), which are essential for many biological functions (Lesckanich and Noble, 1997). The double bonds of unsaturated fatty acids are particularly sensitive to oxidative deterioration and potentially responsible for the formation of peroxides and off-flavors, changes of taste, texture and color, loss of nutrients, and production of toxic compounds (Eriksson, 1987). The susceptibility of lipid to oxidation depends on a number of factors, besides the level of polyunsaturated fatty acids, among which the pro-oxidant and anti-oxidant concentrations. Vitamin E has been reported as an excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals (Leeson, 2007). In animal cells, vitamin E is mainly found in the hydrocarbon part of the membrane lipid bilayer towards the membrane interface and in close proximity to oxidase enzymes which initiate the production of free radicals. Tocopherols may also provide health benefits mainly in preventing cancer and coronary diseases (Diplock, 1991;), so that the incorporation of vitamin E to the egg may both increase the oxidative stability and provide a source of tocopherols useful for human nutrition and health. In this regard, vitamin E was included into animal feed to improve performance, or strengthen immunological status, to improve quality of animal-originated food, and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of human (Flachowsky, 2000). As animal, poultry cannot synthesize vitamin E; therefore, vitamin E requirements must be met from dietary sources. Incorporation of vitamin E into poultry diets tends to bring about oxidative stability of their eggs and reduces the development of off-flavors while it seems to increase egg production (Ajuyah et al., 1993; Sahin et al. (2001, 2002) reported that broilers supplemented with dietary vitamin E had a significant reduction in malondialdehyde (MDA) values, an indicator of lipid peroxidation, in serum and tissue. On the contrary, Grobas et al. (2001) report that no effect of dietary treatment was observed on yolk oxidation and other egg quality characteristics in ISA brown hens after dietary dl-α-tocopherol acetate and vitamin A supplementation. Similarly the supplementation of the diet with extra amounts of vitamin E and C, to increase egg production or egg quality, was not

justified as reported by Irandoust et al. (2012). Although there are numerous knowledge of the positive effect of the dietary vitamin E integration into the animal feed, there are contrasting results regarding its effect on cooked egg quality.

Therefore, the objective of this study was to evaluate the effects of laying hen diet enriched with vitamin E, supplemented in drinking water, on oxidative stability and egg quality in cooked egg yolk.

2. MATERIALS AND METHODS

2.1 Animal Care and Dietary Treatments

The project has been performed in a small breeding at Mosciano Sant'Angelo (Teramo, Italy). A total of 240 ISA laying hens, 32 weeks old (at the third month of egg deposition), were randomly distributed into two groups of 120 hens each and were maintained in individual cages for 30 days in a windowed poultry house, at a regimen of 16 h light and 8 h dark. The hens were assigned to two dietary treatments: water with a vitamin E supplementation (450 ppm) was used in the experimental group (EG), while for the control group (CG) only pure water (100 %) was used. The drinking water supplemented with Vitamine E was provided each two days in a tank sited on the top of the battery cage. Feed was a standard solution for laying hens (SaGeM, Roseto Degli Abruzzi, TE, Italy) composed by maize, soy extraction meal, calcium carbonate, wheat bran, sunflower extraction meal, soybean vegetable oil, dicalcium phosphate, sodium chloride, sodium bicarbonate and magnesium oxide. It was offered once a day and pure water was provided ad libitum. The chemical composition of the diets is reported in Table 1.

2.3 Sample collection and egg quality

Eggs were collected daily during the experimental time, after 30 days of controlled feeding, and stored at 4 °C.

After four weeks of administration to the experimental group of the drinking water supplemented with 450 ppm of vitamin E, all the laid eggs of the two groups were collected (310 per group) during three consecutive days and stored in a storage room of the same farm.

The collected eggs were weighted and 40 per group were located in one boiler and covered with 40-45 °C warm water. The boiler was then switched on and the boiling temperature was reached in 25-30 minutes and held for 10 minutes. Afterwards, the boiler was turned off and immediately emptied, and the eggs were cooled to room temperature in around ten minutes, pouring cold tap water.

Once room temperature was reached (T0), colour, malondialdehyde (MDA) and volatile compounds were analysed on ten egg yorks per group. The oxidative status and MDA levels were evaluated subsequently after 2, 4 and 6 days of storage at 4 °C. Volatile compounds, aldehyde and ketone amounts were evaluated again at the end of storage (T6).

The levels of fat and fatty acids of ten fresh eggs per group were also analysed.

2.3 Analytical Determination

2.3.1 Reagents

All chemicals were reagent grade commercial products and were used without any further purification. TBA: 2-thiobarbituric acid (Sigma-Aldrich, Italy) in acetic acid 90 % (Carlo Erba, Italy); TCA: trichloroacetic acid (Carlo Erba, Italy) in distilled water; BHT: butylated hydroxytoluene (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); standard solution (STD solution): 1,1,3,3-Tetramethoxypropan 99 % (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); KOH: potassium hydroxide (Titolchimica, Roma, Italy); chloroform (Carlo Erba, Italy); n-exane (Carlo Erba, Italy); isopropanol (Carlo Erba, Italy); ethanol (Carlo Erba, Italy); (±)-α-Tocopherol (Sigma-Aldrich, Italy).

2.3.2 Egg weight and colour

The egg weight was measured on fresh eggs using an analytical balance. The colour of the yolk was evaluated according to the Roche scale in which 14 graded colours are available for comparison with egg yolk samples (Vuilleumier, 1969). Each fan blade (1-14) contains a colour measured objectively and then reproduced in the yolk.

2.3.3 Egg MDA content (TBARS-test)

The susceptibility of egg yolk to oxidation was determined by TBARS-test (Tarladgis et al., 1960) modified, at 0 and 6 storage days after cooking. The egg yolk was weighted (3-3.5 g), added with BHT, homogenised (Ultra-Turrax T25) with 50 ml of TCA 7 % and distilled. Two mL of distilled was mixed with 2 mL of 0.02 M TBA solution prepared in acetic acid 90 %. The mixture was heated in a boiling water bath at 80 °C for 60 min and cooled to room temperature. The absorbance was read at 534 nm with JENWAY 6305 UV/vis Spectrophotometer (Barloworld Scientific, Milano, Italy). Each sample was replicated three times. Standard curve were built through increasing concentrations of standard solution (STD) from 1.25 µg to 20 µg in methanol.

2.3.4 Feed, pasture and egg total fat and fatty acid composition

Total fat for fatty acid analysis, extracted with the method of Folch et al. (1957), was transmethylated into methyl esters (FAME) at room temperature by using KOH 2M in methanol. FAME composition was determined by gas chromatography using Focus GC Thermo Scientific (Milan, Italy) with flame ionisation detection (FID) equipped with a VARIAN column CP-SIL 88 of 100 m (Milan, Italy) the carrier gas was hydrogen. Oven temperature program was as follows: 75 °C held for 6 min; 160 °C at 6 °C/min, held for 13 min; 190 °C at 2.5 °C/min, held for 10 min; 220 °C at 3 °C/min, held for 10 min. Fatty acid identification was carried out with standard mixture and fatty acid values were expressed in percentage.

2.3.5 Tocopherol analysis

At 2 g of cooked egg yolk were added 500 µL of BHT (0.2 % in methanol) and 10 mL of ethanol. The sample was incubated in water bath at 70 °C for 20 min with 1 mL of 10 N KOH. The extraction was performed with 10 mL of n-hexane for three times. The total extract was recovered, dried in a rotavapor and replenished with a volume of 1 mL of methanol. Twenty µL were then injected into the HPLC/ UV-Vis (Perkin-Elmer, series 200 pump equipped with an autosampler sistem, series 200) on a SUPELCOSIL^{TM} LC-18-S column (250 x 4.6 mm internal diameter, 5 µm particles size; Sigma-Aldrich, Italy) at the flow rate of 1 mL/min. The mobile phase was solution of isopropanol/methanol (45/55, v/v). α-Tocopherol was identified using a UV/Vis detector (Perkin-Elmer,series 200) set at wavelength of 295 nm and was quantified using external calibration curves prepared with increasing amounts of pure tocopherols in methanol.

2.3.5 Egg volatile profile

To study the volatile profile immediately after cooking and at 6 days after cooking, 4 g of ground egg yolk were weighed into a 30 mL vial screw-capped with a laminated Teflon-rubber disk. The used fiber was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 µm thickness; Supelco Inc, Bellafonte, PA, USA). Before the SPME fiber was inserted into the vial, the sample was equilibrated for 30 min at the extraction temperature (40 °C). The extraction was carried out for 30 min at 40 °C. Prior to analysis the SPME fiber was preconditioned in the injection port of the gas chromatograph at the temperature of 270 °C and for the time of 60 min as suggested by the manufacturers (Supelco Inc, Bellafonte, PA, USA).

As for the peak identification, a GC-MS analysis was carried out, using an instrument SHIMATZU 2010, coupled with a quadrupole mass spectrometer SHIMAZU QP 2010, equipped with a fused silica capillary column SPB % (5 % diphenyl metilsilicone), 60 m x 0.25 id, 1 µm of film thickness (Supelco Inc, Bellafonte, PA, USA).

Following GC-MS peak identification, the samples analysis were performed using Thermo Focus GC-FID instrument (Thermo Fisher Scientific Inc.), with the same GC-MS condition.

Volatiles were separated using a fused silica capillary column (60 m x 0.25 mm i.d., film thickness 0.25 µm, Perkin Elmer, Monza, Italy). The SPME fiber was desorbed and maintained in the injection port at the used temperature. Oven temperature program was as follows: 40 °C held for 10 min; 200 °C at 5 °C , held for 0 min; 250 °C at 20 °C, held for 30 min. *n*-Alkanes were run under the same chromatographic conditions as the samples to calculate the Kovats indices (KI) of detected compounds (Kovats, 1958). The gas chromatography conditions were:

Carrier gas: helium (He), 60 kPa pression and 1.3 mL/min flow;

Oven temperature program: 40 °C held for 10 min; 200 °C at 5 °C/min, 250 °C at 20 °C/min; held for 10 min. Injection temperature: 270 °C.

2.3.6 Statistical Analysis

All data were statistically analysed by one-way ANOVA using SPSS 9.0 for windows. ANOVA analysis was carried out to determine the main effects (α-Tocpherol supplementation) and its interaction on egg yolk parameters. The significant differences were determined using t-Test at the level of $P < 0.05$.

3. RESULTS

The results of egg weight, colour and oxidative parameters at T0 are reported in Table 2. These data show that the egg size was not significantly different between EG (experimental group) and CG (control group) ($P > 0.05$). Instead, the yolk colour seems to have a slightly higher value ($P < 0.05$) in the EG than in the CG (12.47 vs 12.28 Roche scale). The evaluation of the oxidative status at T0 shows that MDA concentration was significantly highly in CG than in EG (0.43 ± 0.05 vs 0.24 ± 0.03 mg/Kg). As predictable, the york α-tocopherol level was significantly higher in EG than in CG ($P < 0.05$): 153.63 vs 15.16 µg/g of yolk (table 2).

The results of TBARS test performed at 2, 4 and 6 days of storage at 4 °C after cooking confirmed that CG egg yorks had always MDA contents significantly higher ($P < 0.05$) when compared with those of the EC (Figure 1): 1.09 ± 0.24

mg/Kg vs 0.60 ± 0.05 mg/Kg at T2, 1.05 ± 0.19 mg/Kg vs 0.58 ± 0.22 mg/Kg at T4 and 1.01 ± 0.23 vs 0.57 ± 0.06 mg/Kg at T6.

In Table 3 are reported the obtained percentages of total fat and fatty acids. Although water with a vitamin E supplementation was used in the experimental group, no significant differences were observed between groups for total fat and fatty acids composition.

Table 4 shows area % of selected volatile compounds at 0 and 6 days after cooking. A total of five volatile compounds were tentatively identified in this study. Identified compounds belonged to the following chemical groups: alcohols (1 compound, 1-Hexen-3-olo), aldehydes (3 compound, Hexanal, Decanal and Nonanal), and ketones (1 compound, 2-Butanone). Hexanal and 1-hexen-3-olo increased during storage (3.83 % vs 8.19 % in the CG and 1.82 % vs 4.66 % in the EG; 3.69 % vs 5.80 % in the CG and 3.80 % vs 7.73 % in the EG, at 0 and 6 days after cooking respectively), while decanal, nonanal and 2-butanone decreased (25.55 % vs 19.44 % in the CG and 23.78 % vs 17.22 % in the EG, 40.18 % vs 39.51 % in the CG and 22.65 % vs 15.59 % in the EG; 22.65 % vs 15.59 % in the CG and 17.40 % vs 10.84 % in the EG, at 0 and 6 days respectively). Aldehydes were the most significant flavour compounds both in the control and in the experimental group at 0 and 6 days after cooking. Aldehydes were present in lowest percentages in the experimental group probably due to the antioxidant effect of vitamin E. In our case, nonanal was the most abundant aldehyde identified in egg yolk, both in the control and in the experimental group. 2-Butanone, an aliphatic ketone formed by lipid autoxidation, was present in major content in the control group at 0 days after cooking (22.75 % vs 17.40 %, respectively) but was not statistically significant ($P > 0.05$). This compound decreases during storage both in the control and in the experimental group probably due to the oxidation process.

4. DISCUSSION

Our results show that the vitamin E supplementation in the diet of hens positively influences the quality of the cooking egg yolk. Indeed, this vitamin is able to modify functional properties of egg and can improve its quality, also at different days after cooking. The results show that the colour of egg yolk was higher in the EG than the CG ($P < 0.05$). Vitamin E is a natural antioxidant, which has shown its beneficial effects in egg yolk colour stabilization when added to poultry (Jackson et al., 1978). Because yolk pigments are associated with the lipid molecules of the yolk membranes, probably vitamin E in its role as an antioxidant was also expected to stabilize the yolk pigments.

The oxidative stability was higher in the EG than the CG, probably due to antioxidant activity of vitamin E. During storage the oxidative stability decreases in both groups, but the content of MDA is always higher in the CG than the EG. Marshal et al. (1994) suggested that there is a formation and degradation of TBA-reactive substances with storage. However, marked oxidation and accumulation of oxidation products (including cholesterol oxides) may take place when eggs are processed under prooxidative conditions (Lai et al., 1995), which is particularly important when processed eggs are stored prior to consumption. In these cases, it is essential to prevent oxidation, and therefore it may be necessary to customize the vitamin E concentration in egg yolk by dietary intervention.

These results support the hypothesis of Franchini et al. (2002) that, since the shell egg is a closed system, little exposed to oxygen and oxidant agents and thereby little involved in oxidative reactions, vitamin E is not consumed in preventing oxidative processes. In fact when ordinary table eggs were stored in refrigerated conditions, the development of oxidative products was negligible. On the contrary, the storage at room temperature increased TBARS results and reduced the content of vitamin E presumably consumed in oxidative processes.

About volatile profile, the results are not easy interpretable. Lipids, proteins and carbohydrates, the major structural components of living cells are also the major source of flavour in foods. Generally, the negative qualities of food flavour are associated more closely with lipids than with proteins and carbohydrates. Strecker degradation of methionine, phenylalanine and proline, as well as autoxidation of phospholipid-bound linoleic and arachidonic acids are proposed as the major factors for egg yolk flavour formation. According to literature search (Cherian et al., 2002), results of volatile profile of samples showed remarkable variability in the same group. Especially, 1-hexen-3-olo showed no difference between the groups, and did not significantly increase during storage. Hexanal, decanal and 2-butanone had lower concentrations in the experimental group, probably due to effect of vitamin E. In fact, this chemical group can be produced by scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the present fatty acids, the formed hydroperoxide isomers and the stability of the decomposition products. The most abundant aldehydes identified in egg yolk flavor were hexanal, decanal and nonanal. The content of these compounds are suggested to be good indicators of oxidation (Ahn et al., 1998). In fact, hexanal is the primary oxidation product of linoleic acid (Meynier et al., 1999). The autoxidation of linoleic acid generates 13-hydroperoxide of linoleic acid. Cleavage of 13-hydroperoxide will lead to hexanal. Whereas the amount of linoleic acid in the egg yolk ,between the two groups, was similar (15.25% vs 14.74%), we can assume an antioxidant effect of vitamin E on egg yolk, given the lower concentration of hexanal found in the EG when compared to CG. Decanal and nonanal are the primary oxidation products of oleic acid. Hexanal increased during storage too, while nonanal, decanal and 2-butanone decreased. Being fat-soluble, vitamin E probably tends to intervene more rapidly in lipid oxidation and thus to decrease the

concentration of nonanal and 2-butanone than 1-hexen-3-olo. However the contribution of alcohols to flavors of foods has been reported to be minor compared to aldehydes and ketones (Heath and Reineccius, 1986); indeed ketones in foods, have been implicated with off-flavors referred to as "perfume" rancidity (Stokoe, 1928). Furthermore, the hexanal derives from the oxidation of linoleic acid, the more easily oxidizable acid, due to more unsaturations when compared to oleic acid.

5. CONCLUSION

This study was carried out to evaluate the effect of dietary vitamin E supplementation on oxidative stability in cooked egg yolk. Indeed, through dietary manipulation, certain substances with important health benefits can be enriched in egg yolk (Surai and Sparks, 2001). Since lipids are one of the main egg components, whose fatty acids are mostly polyunsaturated, egg is very sensitive to lipid oxidation and then potentially exposed to peroxides and off-flavors development; further, egg is also susceptible to other modifications such as changes of texture and colour. The susceptibility of lipid to oxidation depends on a number of factors, besides the level of polyunsaturated fatty acids, among which the pro-oxidant and anti-oxidant concentrations. Vitamin E has been reported as an excellent antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals. Vitamin E is not synthesized in animal metabolisms and must be necessarily provided by diet. However, it is not well established if a supplementation of laying hen diet with vitamin E brings about oxidative stability of their eggs and/or other modifications.

The results of this study show that the diet implementation with 450 mg/Kg of vitamin E has several effect on egg properties, very likely attributable to a higher oxidative stability.

It was indeed observed that the colour of egg yolk was higher in the experimental group than the control one, and yolk pigments are highly associated with the lipid molecules of the yolk membranes. Besides, york colour increased during the storage in both groups.

In addition, MDA concentration was significantly lower in the EG, at different days after cooking, indicating a greater oxidative stability. The oxidative stability decreases in both groups during storage, but the MDA levels were always higher in the CG than in the EG. MDA is a three-carbon compound formed by scission of peroxidized PUFAs, mainly arachidonic acid, and is one of the main secondary products of lipid peroxidation (Fernández et al., 1997). Thus, aldehydes are produced in substantial quantities during lipid oxidation, so their detection has a relevance in studies to examine lipid peroxidation.

Table 1. Formulation and chemical composition of standard diet.

Diet			
Composition (% fresh matter)		fatty acid %	
Crude protein	16	C12:0	0,03
Ether extract	3	C14:0	0,24
Cellulolose	3.8	C15:0	0,05
Ash	13.50	C16:0	15,37
Lysine	0.85	C18	3,66
Methionine	0.38	SFA	15,69
Calcium	4.10	C16:1	0,27
Phosphorus	0.48	C18:1 ω9	23,78
Sodium	0.14	C18:1 ω7	0,63
Additives kg		MUFA	24,68
Vitamin A UI	9000	C18:2 ω6	50,15
Vitamin D3 UI	3000	C18:3 ω3	3,22
Vitamin E mg	24	PUFA	53,37
Trace elements tot[a] mg	382.69	Others	2,60

[a]Added per kg: ferrous carbonate 114.89 mg, manganous oxide 67.73 mg, manganous sulphate monohydrate 161.70 mg, zinc oxide 111.60 mg, sodium selenite 0.66 mg, cupric sulfate pentahydrate 23.58 mg, potassium iodide 2.36 mg, potassium basic carbonate monohydrate 0.27 mg.

The york α-tocopherol level was also increased by vitamin E supplementation in diet, as it was always higher in the EG then in the CG. Indeed, tocopherols increase oxidative stability. In addition, an incorporation of tocopherol to the egg may provide a source of tocopherol useful for human nutrition, as it may provide health benefits, mainly preventing cancer and coronary diseases.

As for the volatile profile, aldehydes were the most significant off-flavour compounds detected both in the CG and in the EG at 0 and 6 days after cooking. Nonanal was the most abundant aldehyde identified in egg yolk in both the control and experimental groups. Hexanal, decanal and 2-butanone had lower concentrations in the experimental group, probably as an effect of vitamin E. Hexanal and 1-hexen-3-olo increased during storage, while nonanal and 2-butanone decreased. 2- Butanone, an aliphatic ketone, was present in greater content in the CG at 0 days after cooking but decreased during storage both in the CG and in the EG, probably due to the oxidation process.

Further studies are being carried out for a better understanding of the role of the vitamin E in the aroma and in the quality of cooking egg yolk.

Table 2. Physical and chemical parameters of yolk egg in the Control Group (CG) and Experimental Group (EG) at 0 days after cooking.

		CG	EG	ES
Egg weight	g	68.26	66.83	1.36
Colour	Roche scale	12.28[a]	12.47[b]	0.10
MDA	ppm	0.43[b]	0.24[a]	0.08
α-tocopherol	µg/g of yolk	15.16[a]	153.63[b]	4.59

n = 20 per group
MDA: malondialdehyde.
[a, b] P < 0.05 on the same row

Table 3. Total fat (%) and fatty acids (%) in egg yolks of the Control Group (CG) and Experimental Group (EG) at 0 days after cooking.

	CG	EG	SE
Lipid (%)	19.22	21.50	2.25
C14:0	0.19	0.24	0.50
C16:0	23.99	25.35	1.17
C16:1	1.25	1.53	0.25
C18:0	6.80	6.62	0.50
C18:1ω9	45.13	45.53	2.35
C18:1ω7	1.25	1.45	0.13
C18:2ω6	15.25	14.74	1.79
tot SFA	30.98	32.16	1.58
tot USFA	62.87	63.26	3.10

n = 20 per group
SFA: saturated fatty acids; USFA: unsaturated fatty acids

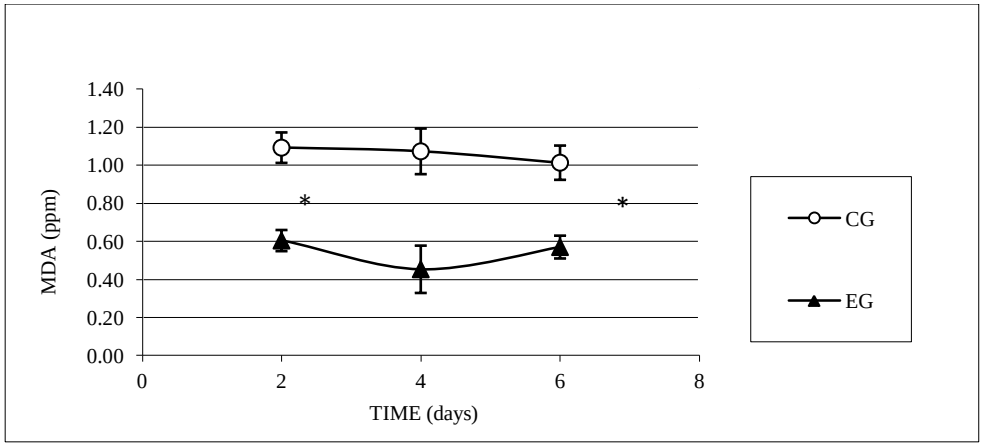

Figure 1. MDA content (ppm) in egg yolks of the Control Group (CG)and Experimental Group (EG).
***** P < 0.05

Table 4. Volatile compounds (mean area %) in egg yolks of the Control Group (CG) and Experimental Group (EG) at 0 and 6 days after cooking.

	0 days *			6 days			SE between days	
	CG	EG	SE	CG	EG	SE	CG	EG
Hexanal	3.83^{b}	1.82^{a}	0.51	8.19^{A}	4.66^{B}	1.91	1.13	2.26
Decanal	25.55	23.78	9.64	19.44	17.22	1.76	5.21	4.64
1-Hexen-3-olo	3.69	3.80	1.16	5.80	7.73	1.81	1.00	2.26
Nonanal	40.81	52.68	5.31	39.51	40.81	7.50	8.38	7.32
2-Butanone	22.65	17.40	5.77	15.59	10.84	5.54	8.47	3.67

n = 20 per group
a, b P < 0.05 on the same row into the 0 days
A, B P < 0.05 on the same row into the 6 days

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