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Stability and sensory evaluation of eggs produced by addition of different amount of linseed oil into feed

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Sažetak

Cilj ovog istraživanja bio je procijeniti utjecaj povećanja udjela ω -3 masnih kiselina u jajima, postignuta dodatkom različitih količina lanenog ulja u krmu, na kakvoću jaja tijekom skladištenja. Promjene parametara kakvoće među pokusnih skupina su ispitivane na jajima s različitim sadržajem ω -3 masnih kiselina koja su čuvana 7, 14, 21, 28, 35 i 42 dana u hladnjaku (10 jajapo grupi u svakom eksperimentu). Utjecaj povećanja udjela ω -3 masnih kiselina na senzorska svojstva ocijenjen je u dva panel testa. Vrijeme skladištenja je značajno utjecalo na parametre kakvoće jaja. Gubitak težine je najveća u prvom tjednu skladištenja a promjene boje su najveće nakon 28 dana skladištenja u svim pokusnim skupinama. Povećani udjel ω -3 masnih kiselina u jajima nije utjecao na parametre kakvoće jaja. Nadalje, rezultati oba panel testa pokazuju da razlika u udjelu ω -3 masnih kiselina nije utjecala na promjene senzorskih karakteristika jaja.

Ključne riječi: kakvoća jaja, ω-3 masne kiseline, senzorske karakteristike, stabilnost

Summery

The aim of this study was to evaluate the impact of increased amount of ω -3 fatty acids in eggs achieved by addition of different amounts of linseed oil into feed on egg quality during storage. The changes in quality parameters between experimental groups were studied on eggs containing different amount of ω -3 fatty acids stored for 7, 14, 21, 28, 35 and 42 in refrigerator (10 eggs per group in each experiment). The influence of increased amount of n-3 fatty acids on sensory characteristics was evaluated in two panel tests. Egg quality parameters were significantly (P<0.05) influenced by the storage time. Weight loss was significantly higher in the first week of storage, and colour changes were highest after 28 days of storage in for all experimental groups. The increased amount of ω -3 fatty acids in eggs did not affect egg quality parameters. Furthermore, the difference in ω -3 fatty acids content did not cause changes in sensory characteristics of eggs as rated by both panels.

Key words: Egg quality, ω -3 fatty acids, sensory characteristics, stability parameters.

Introduction

Hen eggs are an important contributor of high quality proteins, essential fatty acid (FA), vitamins and minerals, which nutritionally makes them the most complete food. They are constituents of various foods because of their wide acceptance as human diet (Watkins, 1995; Stadelman and Schmieder, 2002). Furthermore, due to their balanced macronutrient composition, eggs increase satiety in overweight and obese persons (Vander Wal et al., 2005).

Hen eggs are a good commodity for enriching the diet with different nutrients that are usually insufficient in human nutrition. Eggs enriched with selenium, conjugated linoleic acid, ω -3 polyunsaturated fatty acid (PUFA) and vitamin E are on the market in developed countries (Patterson et al., 2001; Hidalgo et al., 2008). The main reason for reduction of ω -3 PUFA content in the diet is their limited consumption, and researchers have encouraged production of eggs as functional food with higher level of ω -3 PUFA. Simopoulos (2002) has

stated that the main reason for the lack of ω -3 PUFA is changes in human diet through the centuries of human history. This has especially taken place in last 150 years as a result of application of new agricultural practices and replacement of animal fat with vegetable oils. Consequently, the ratio between ω -6 PUFA, predominant in many vegetable oils and cereals, and ω -3 PUFA has been decreasing dramatically. The European Food Safety Agency (EFSA) has documented this (EFSA, 2005; EFSA, 2009) and pointed out that ω -6 PUFA intake is higher than the recommended daily intake, and that ω -3 PUFA intake is equal or lower than the recommended daily intake.

An increase in the content of PUFAs in diery products may lead to additional lipid oxidation and induce negative effect on organoleptic characteristics. Higher number of double bonds enlarges the possibility of hydroperoxide formation even at low temperature. Then secondary oxidation products occur at elevated temperature or during long-term storage causing a rancid aroma and lower acceptability. Furthermore, some authors reported significant changes of sensory proper-



ties of foods when fish oil was added to a feed as an ω -3 PUFA source (Hoffman et al., 2005; Ceylan et al., 2011). On the other hand, linseed oil as an alternative source of ω -3 FA has shown less negative effect (Scheideler et al., 1997; Lopez-Ferrer et al., 1999).

The objectives of this study were to observe the effects of increased amount of linseed oil on egg quality parameters, and to monitor deterioration of these parameters during storage. Furthermore, sensory characteristics (appearance, colour, taste, flavour, off-flavour, texture and overall acceptability) were evaluated between groups over storage time. This information could be of great interest to producers of ω -3 enriched eggs emphasizing their quality aspects.

Materials and methods

Samples

Egg samples were collected from Lohmann Brown laying hens fed with control diet (mark K in tables and figures) and four experimental diets containing 1-4% of linseed oil added into control diet: group 1 (G1) 1% of linseed oil added to fed, group 2 (G2) 2% of linseed oil added to fed, group 3 (G3) 3% of linseed oil added to fed, and group 4 (G4) 4% of linseed oil added to fed. Each group hed 30 hens housed in standard cages. The ratio between ω -6 and ω -3 polyunsaturated fatty acids in eggs decreased in first five weeks and then remained stable until the end of the experiment for all experimental groups. Different contents of linseed oil in feed highly influenced the ω-6/ ω -3 ratio (P<0.0001). Detailed description of the production parameters and fatty acid profile of eggs was described previously (Petrović et al., 2012). The samples for stability testing and sensory evaluation were collected after 8 weeks of feeding when all production parameters and fatty acid composition of egg yolk were stabilized.

Stability testing

The changes of egg quality parameters during storage have been evaluated on control and four experimental groups having different ω -6/ ω -3 ratio, ten eggs per group. The initial values of egg quality parameters were tested on the day of collection and the rest of the samples were stored in a refrigerator at 5-8°C prior to analysis. Quality parameters were evaluated on ten eggs per group and the mean values were calculated. A set of samples for weight loss determination was weighted for each egg separately at the day of collection, each egg separately. Eggs were stored in refrigerator and weighted after day 7, 14, 21, 28, 35 and 42. Weight loss for each egg was calculated by subtracting the weight of the egg after a storage time from initial egg weight. Yolk weight, yolk percentage, Haugh Units, yolk index and yolk colour were evaluated on the day of collection and after 7, 14, 28 and 42 days of storage in refrigerator. Each egg was broken onto a flat surface and egg parameters were measured: height of the albumen midway between the yolk and the edge of the tick albumen, yolk height and yolk diameter. Haugh units (HU) were calculated using the formula: $HU = 100 \log(H + 7.57 \text{ to } 1.7W^{0.37})$ where H is the height of the albumen and W is the weight of the egg

(Haugh, 1937; Silversides and Villeneuve, 1994). Thereafter the yolk was rolled over a blotting paper and weighted. Then yolk percentage and height/diameter ratio were calculated. Yolk colour was evaluated by comparison with the 15-points Roche Yolk Colour Fan (DSM, 2005-HMB, 51548, Switzerland). Closest matching colour with the colour fan was given as a yolk colour and averaged value has been given as a group result.

Sensory analysis

Sensory analysis was performed by a panel of nine assessors selected and trained according to international standards (ISO 8586:2012). Sensory analyses of eggs were carried out by two sensory tests. Sensory test using response scale where the obtained numerical values indicates the quality of studied sensory properties (ISO 4121:2003) evaluated characteristics of hard-boiled eggs. Panellists received hardboiled eggs of control and four experimental groups in five working sessions: freshly collected eggs and eggs after 7, 14, 28 and 42 days of storage in refrigerator. Samples were boiled for 10 minutes in water and cooled to room temperature. After separation of shells, eggs were cut into 5 mm tick rings and given to each panellist on a plastic plate divided into five sections. Each panellist was asked to evaluate each group of eggs for appearance, colour, taste, flavour, off-flavour, texture and overall acceptability using a numeric unipolar discrete response scale (1=very bad, 10=excellent). Between each testing the panellists used unsalted bread and water to refresh their senses. The mean values were calculated for each group.

In addition, ranking was carried out to evaluate hedonic preference of control and experimental groups of egg samples. Each group was prepared separately on three different ways: by boiling for 10 minutes, by frying of homogenised eggs on teflon frying pan for 5 minutes without any oil addition and preparing in microwave oven at 600-700 W for 4 min. The tests were conducted on freshly collected eggs and on eggs that had been refrigerated for 28 days. Panellists evaluated the samples presented in random order and place them in rank order of preference (ISO 8587:2006).

Sensory analysis took a place in sensory laboratory which is in compliance international standard for test rooms (ISO 8589:2007).

Statistical analyses

Analysis of variances for all quality parameters and sensory evaluations were performed by using Statistica software 10.0 (StatSoft, Tulsa, OK, USA). Means were compared using Turkey's test for multiple comparisons. A probability level of P<0.05 was considered statistically significant. Each data point for statistical analysis was represented as a mean value \pm standard deviation of ten separated measurements.

Results and discusion

Egg weight during storage

The average value of weight loss for the control and experimental groups after 42 days of refrigeration are shown in Table 1.

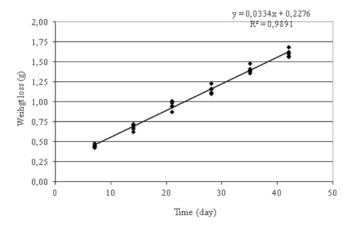


Parameter		Gro		P-value ^d		
	1 2		3	4	Control	r-value
Weight loss (g)	-1.60±0.16	-1.58±0.12	-1.57±0.18	-1.69±0.10	-1.62±0.12	0.9237
Yolk mass (g)	0.82±0.11	0.91±0.13	0.75±0.15	1.05±0.21	0.71±0.19	0.5289
Yolk (%)	1.81±0.21	1.59±0.16	1.64±0.12	1.92±0.20	1.79±0.16	0.9501
Haugh units	-16.27±0.68	-17.03±0.81	-16.73±0.62	-16.37±0.87	-15.74±0.59	0.6762
Yolk index	-0.039 ±0.003	-0.043 ±0.003	-0.038 ±0.004	-0.042 ±0.004	-0.042 ±0.003	0.9386
Colour index	-1.3±0.3	-1.0±0.2	-1.0±0.2	-1.1±0.2	-1.4±0.2	0.9338

Table 1. Average changes of parameters obtained after 42 days of storage in refrigeratora.

Weight loss of eggs was highest in the first week of storage and was not significantly different between groups (P>0.05) while the difference between weeks was significant (P<0.0001). Evaporation of water was highest in the first week of storage, which caused the greatest loss of weight. Repeated ANOVA without values for the first week was also significant (P=0.0054). A possible reason for this is that the samples were not analysed each time at the same time of the day causing small differences in results between weeks. Average values of total weight loss after 42 days of storage were not significantly different (P=0.9237). Correlation coefficient of weight loss cumulative values (Figure 1) with the storage time was significant (r=0.9891, P=0.05). It can be concluded that egg weight was influenced by storage time and that differences in ω -3 FA content did not impact weight loss of eggs during storage.

Figure 1. Cumulative weight loss of egg in the control and experimental groups during storage.



These results are in good agreement with previous reports (Scott and Silversides, 2000; Silversides and Scott, 2001; Akyürek and Okur, 2009; Jirangrat et al., 2010). Scott and Silversides (2000) showed in their study that the main reason for weight loss is the reduction in egg albumen during storage. Egg, shell, albumen and yolk weight, albumen pH and HU at room temperature were monitored for 10 days. They observed a reduction in average egg weight, albumen weight and HU,

while the yolk weight and albumen pH increased significantly during storage at room temperature for 10 days. The obtained results showed good correlation between the egg weight loss and albumen weight loss. Akyürek and Okur (2009) studied the influence of hen's age and storage time on egg quality parameters at two different temperatures: 4°C and 20°C. The obtained results for weight loss at 4°C were very similar to the values obtained in our experiment while the results obtained after storage at 20°C for 14 days were significantly higher than our results obtained after 42 days of storage in refrigerator.

Our results and the results of other studies indicate significant weight loss of eggs during storage. The mean weight loss after 28 days of storage in refrigerator was 1.20 g. The mass of eggs during sorting must be adjusted for the value because this may cause a change of egg class within the validity period. Jirangrat et al. (2010) studied the influence of mineral protective coating on the quality of eggs stored for 15 weeks in refrigerator at 7°C. The weight loss of the group with a mineral coating was significantly lower. Both groups showed a linear weigh loss during storage, which is consistent with our results.

Yolk weight and yolk percentage during storage

Yolk weight and yolk percentage were increasing during storage and there was no significant difference (P>0.05) between groups (Table 1). During the entire egg production (Petrović et al., 2012), the yolk weight of the experimental group fed with the addition of 1% of linseed oil into feed was the largest since, and during the storage as well the average egg weight in that group was the largest. This had no effect on yolk weight and yolk percentage during storage, and the correlation between changes of yolk percentage and storage time was significant (r=0.962, P=0.05).

Akyürek and Okur (2009) did not achieve significant differences in the weight of yolk, albumen and shell during storage, which is unusual considering that they obtained the significant loss of egg weight. The results of these authors are inconsistent with our results and the results of other studies (Scott and Silversides, 2000; Silversides and Scott, 2001; Silversides and Budgell, 2004). Silversides and Budgell (2004) monitored the changes of egg quality parameters, weight of eggs, egg yolks and egg shells depending on hen's age and

^a Each value is the mean value ± standard deviation (n=10) of the difference between value obtained at the start and after 42 days of storage in refrigerator. ^b The group number is equivalent to the percentage of linseed oil added to the feed. ^c Negative value indicates a decrease of the parameter during storage. ^d Statistical significance of the difference between groups.



storage time at room temperature. The relative increase in yolk weight after 10 days of storage at room temperature corresponded to the values achieved in our experiment after 28 days of storage in a refrigerator.

Changes in HU during storage

Values of HU showed a significant decrease during storage and the average changes of HU for control and four experimental groups is shown in Table 1. The difference among groups was not significant (P>0.05). The correlation coefficient of HU relative to addition of linseed oil in feed was not significant (r=0.219, P=0.05) indicating that changes in the ratio of n-6/n-3 did not affect HU of stored eggs. The correlation coefficient of changes in HU relative to storage time was r=0.951, which was statistically significant ($r_{crit=0.950}$, P=0.05). However, the correlation coefficient of quadratic equation was much better (r = 0.978) indicating the possible influence of other factors apart from storage time on HU (e.g. weight loss), and the effect of the addition of linseed oil in feed was not significant.

Values of HU at the beginning of stability experiment were in line with the values obtained by Akyürek and Okur (2009), and were lower than the values obtained in the research of Jones and Musgrove (2005) and Biladeau and Keener (2009). The probable reason for this is that the tests did not use the same hybrids. Scott and Silversides (2000) confirmed the influence of hens breed on albumen height. The highest initial values were achieved by Jirangrat et al. (2010), and the reduction in group HU for eggs coated with mineral oil and for the control group was linear like in our experiment. Biladeau and Keener (2009) studied the influence of protective coating of paraffin wax, mineral oil, soy protein isolate and whey protein isolate on the quality of the eggs kept in the refrigerator. All coatings showed a significant impact on improving the egg quality compared to the control group without coating. The smallest decline in the quality was observed in the group of eggs protected with mineral oil coating. Although the initial

HU value was higher than ours, the reduction obtained in control group is in accordance with the reduction obtained in our experiment. Reduction in HU during storage for the control group in other studies (Jones and Musgrove, 2005, Akyürek and Okur, 2009) was lower than in our experiment, which can also be explained by the difference in hen breed used in the experiments.

Changes in yolk index during storage

The mean values of changes in yolk index during storage in the refrigerator are shown in Table 1. The yolk index decreased during storage and the difference between the control and four experimental groups was not significant (P>0.05). The correlation coefficient of the yolk index related to the addition of linseed oil in the feed was not significant (r=0.509, P=0.05) indicating that a change in the composition of the fatty acids does not affect the index of egg yolk. In contrast, the correlation coefficient of the yolk index related to the storage time was significant (r=0.977, P=0.05).

Akyürek and Okur (2009) and Jirangrat et al. (2010) have also studied the change in the yolk index during storage, and the reduction of the yolk index shown in their study is lower than we have obtained in our experiment. The possible rea-

son for this is small changes in the storage temperature, which was 4°C in their experiment whereas in our experiment it was 5-8°C. The values obtained by Akyürek and Okur (2009) after 10 days of storage at 20°C were lower than the values obtained in our experiment after 42 days of storage at 5-8°C.

Changes in yolk colour during storage

Average changes in yolk colour during storage is shown in Table 1. The ANOVA test of differences in yolk colour during storage showed a significant difference among weeks of storage (P<0.0001). It was found that the colour changes of the last week of storage were the highest. The repeated ANOVA test of the data in the last week of storage showed no significant difference between groups (P=0.5080) which means that colour changes occurred after 28 days, which is the egg shelf life according to the current regulations (EC, 2008). The obtained value of the correlation coefficient between colour changes and the time of storage was not significant (r=0.717, P=0.05). The changes between groups were not significantly different (P>0.05), indicating that the changes in the fatty acid composition do not affect the colour of egg yolks.

Jung et al. (2011) also have not obtained the changes of yolk colour in their experiment in which they add gallic and linoleic acid into feed. The difference in colour was achieved by Yannakopoulos et al. (2005) who added a source of n-3 FA into feed and part of feed replaced with a green mass. Mean colours according to Roche's scale were 10 for n-3 enriched eggs and 12 for the control group. Greater difference in yolk colour was obtained by Dvořák et al. (2011) who compared individual cage rearing with classical cage rearing; the hens in individual cages were fed with the addition of natural green grass as a natural pigment source. Their results have shown a significant improvement in yolk colour in case of hens fed with addition of green grass. It can be concluded that the main reason for these differences was the composition of the feed, which in our experiment contained canthaxanthin, and that the addition of ω-3 fatty acids does not affect the egg colour.

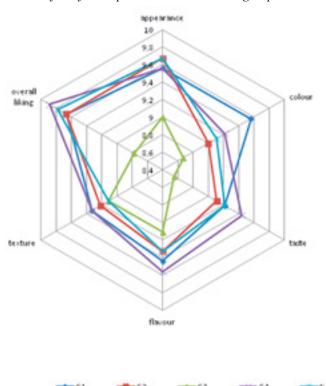
Evaluation of sensory characteristics of stored eggs

The intensity of each sensory characteristics (appearance, colour, taste, flavour, texture and overall liking) for fresh hardboiled eggs and hard-boiled eggs after 7, 14, 28 and 42 days of storage, from four experimental and control group were shown on spider web diagrams in Figures 2 to 6. The intensity of off flavour was also investigated but from technical reasons was not shown. In fresh hard boiled samples only sample G3 had off flavour (average of intensity 0,44). During storage off flavour become pronaunced especially in hard-boiled eggs from all groups after 14 days of storage (average from 1,11 to 1,44). After 42 days of storage off flavour in all groups was in range from 0,11 to 0,56 in average.

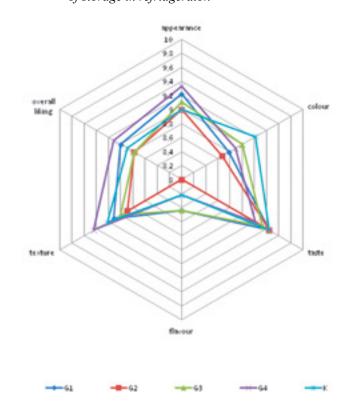


Note: G1-G4 - four experimental groups and K - control group

Figure 2. Sensory characteristics of fresh hard-boiled eggs from four experimental and control group.

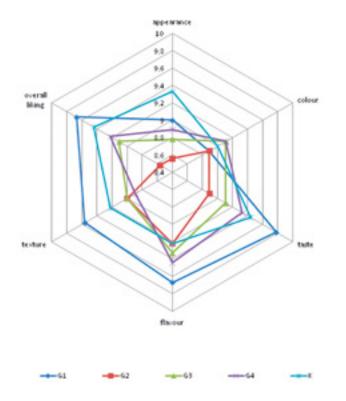


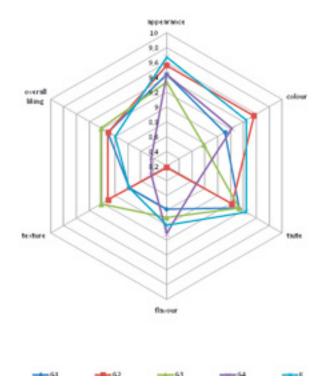
Note: G1-G4 - four experimental groups and K - control group *Figure 4.* Sensory characteristics of hard-boiled eggs from four experimental and control group after 14 days of storage in refridgerator.



Note: G1-G4 - four experimental groups and K - control group *Figure 3.* Sensory characteristics of hard-boiled eggs from four experimental and control group after 7 days of storage in refridgerator.

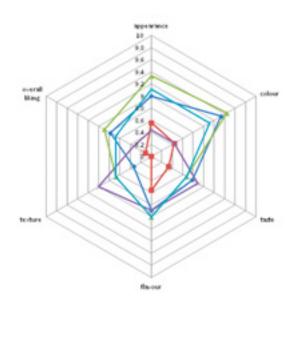
Note: G1-G4 - four experimental groups and K - control group *Figure 5.* Sensory characteristics of hard-boiled eggs from four experimental and control group after 28 days of storage in refridgerator.







Note: G1-G4 - four experimental groups and K - control group *Figure 6.* Sensory characteristics of hard-boiled eggs from four experimental and control group after 42 days of storage in refridgerator.



The average scores of sensory characteristics of hard-boiled eggs obtained in four experimental and control group together with changes in sensory characteristics during storage are shown in Table 2. The statistical significance is also shown in Table 2 together with correlation of scores with storage time and correlation of scores with addition of linseed oil into feed.

Table 2. Average values of sensory scores for hard-boiled eggs in the control group and four experimental groupsa.

Time (days) ^b		Group ^c				n volvo	-
	1	2	3	4	- Control	p-value	r
0	9,57	9,47	9,01	9,60	9,51	0,0053 ^d	-0,254
7	9,53	9,00	9,09	9,34	9,43	0,0847	-0,430
14	9,34	9,11	9,21	9,29	9,18	0,6753	0,141
28	9,13	9,35	9,24	8,97	9,29	0,2821	-0,552
42	9,16	8,65	9,14	8,83	9,11	0,0810	-0,404
r	-0,923 ^d	-0,644	0,598	-0,980 ^d	-0,835		

^a Each value is the mean of scores obtained by 9 panellists.

The obtained results indicate that there was no significant difference in sensory characteristics of fresh and stored eggs between groups (P>0.05). A significant difference was obtained only with fresh eggs where the eggs of group 3 differed considerably from other groups. The obtained correlation coefficients between average scores and the addition of linseed oil in the feed were not significant ($r<r_{crit}$, r_{crit} =0.878 with 3 degrees of freedom and P=0.05). The correlation between average sensory scores and storage time showed negative trend in all groups except in group 3. The correlation coefficient of groups 1 and 4 was significant ($r>r_{crit}$, r_{crit} =0.878 with 3 degrees of freedom and P=0.05), while group 2 and control group was not significant. Group 3, unlike others, had a positive trend and a significant difference from other groups of fresh eggs studied.

Hedonic preference of fried, microwaved and hard-boiled eggs (Table 3) showed no significant differences between groups and control samples (P=0.0699) of fresh eggs and eggs refrigerated for 28 days. Correlation coefficients of the achieved sum of sensory scores and linseed oil addition in feed were not significant (r<r_{crit} r_{crit}=0.878 with 3 degrees of freedom and r=0.05) same as in the first sensorial test.

^bEgg samples were stored in refrigerator at 5-8 °C.

^c The group number is equivalent to the percentage of linseed oil added to the feed.

^d Statistically significant difference between samples



Table 3.	Rank sums	for d	ifferently	prepared eggs.

Samples ^a		Gro	Control	r _c		
Samples	1	2	3	4	Control	
Fried eggs (Test 1)	17	15	17	10	17	-0,730
Microwaved eggs (Test 1)	21	29	22	18	22	-0,274
Hard-boiled eggs (Test 1)	15	20	22	19	15	0,550
Fried eggs (Test 2)	19	22	21	15	21	-0,566
Microwaved eggs (Test 2)	13	23	19	16	16	0,251

^aFresh eggs were used in test 1 and in test 2 eggs stored at 5-8°C for 28 days were used.

The results of our study are in correspondence with Scheideler et al. (1997) who studied the influence of fish oil or linseed oil addition in feed on fried eggs sensory characteristics. The authors evaluated appearance, texture, flavour, off-flavour and overall acceptability and they did not prove the differences in sensorial characteristics. Similary, Mazalli et al. (2004) did not observed any changes in sensory characteristics of hen eggs. In their study the feed was enriched with 3% of linseed or fish oil. A significant influence on sensory characteristics of hen eggs was recorded in survey of Jiang et al. (1992). They added 15% grinded linseed in hens feed. A sensorial evaluation showed an off-flavour, which was described as a fish tasting flavour. Plagemann et al. (2011) studied volatile flavours in row eggs yolk of hens fed with different diets. Changes in flavour were analysed by gas chromatography, and in group fed with laying meal with the added cabbage and onion a significant amount of compound responsible for onion-like odour has been detected, which confirms the potential impact of hen diet on the sensory characteristics of eggs.

Conclusions

The impact of the increased amount of omega-3 fatty acids, obtained by addition of linseed oil into fed, on egg quality during storage was evaluated by monitoring the physical characteristics of the eggs refrigerated at 5-8°C and by testing sensory characteristics. The changes of egg weight, yolk weight, yolk percentage, Haugh units and yolk colour between experimental groups were significantly influenced by the storage time. The obtained results proved that the increased amount of n-3 FA in hen feed, resulting in an increase of n-3 FA in eggs, did not affect the egg quality parameters or changes of these parameters during refrigeration.

The averaged sensory scores over storage time showed negative trend among all panellists, but the increased amount of n-3 FA in eggs did not cause lower acceptability of eggs which is good cognition for producers of enriched eggs.

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^bThe group number is equivalent to the percentage of linseed oil added to the feed.

^cSamples were not statistically different.



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