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Internal Quality of Conventional and Omega-3-Enriched Commercial Eggs Stored under Different Temperatures

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ABSTRACT

The internal quality of conventional and ω-3 enriched commercial eggs stored at different temperatures was evaluated. Eggs derived from Isa Brown layers fed two different diets. In Group 1, 432 hens were fed throughout their productive life with a diet based on corn and soybean meal (production of conventional eggs). In Group 2, starting at 22 weeks of age, other 432 hens were fed a diet containing 1.5% of marine algae substrate and 1.8% of fish oil (production of ω-3 enriched eggs). The following parameters were evaluated: Haugh unit, yolk index, albumen pH, and yolk pH. There were no significant differences between conventional and enriched with ω-3 eggs as to internal quality parameters. Only the interaction between storage time and temperature was significant, and therefore their effects were evaluated, independent of egg type (conventional eggs and ω-3 enriched eggs). Eggs stored at 25ºC presented lower Haugh units and yolk index, and higher albumen pH and yolk pH as compared to those kept at 5ºC. In addition, internal quality was reduced when eggs were stored for 7, 14, and 21 days, particularly when maintained at 25°C. It was concluded that conventional and ω-3 enriched eggs have good internal quality; however, to maintain this quality, eggs must be stored under refrigeration.

INTRODUCTION

Egg quality is understood as the set of factors that are responsible for its acceptance by the consumer, and in compliance with legislation requirements (Brasil, 1991). Quantitative and qualitative factors must be considered when determining final quality attributed of commercial eggs. Egg weight is the main quantitative factor, whereas qualitative factors include both external and internal quality traits, such as eggshell physical status, air cell size, albumen viscosity, and yolk consistency. Internal egg quality characteristics encompass measurements of the physiological conditions of the albumen and the yolk, as egg with thick and abundant albumen and centralized yolk are fresher, and are favorably perceived by the consumer.

Physical-chemical changes in the egg start soon after lay, worsening internal quality, and eventually causing deterioration. When stored under high environmental temperatures, eggs undergo chemical reactions that accelerate the deterioration process due to the action of carbonic acid (H_2CO_3) present in the egg - a mechanism known as buffering system. High environmental temperatures accelerates the activity of the enzyme carbonic anhydrase, which dissociates H_2CO_3 into H_2O and CO_2 that leave the egg through the eggshell pores, thereby increasing internal egg pH (Stadelman & Cotterill, 1995; Keener *et al.*, 2006). This alkalinization results in a series of physical-chemical changes in the egg, such as albumen liquefaction, movement of liquids among structures,

distension and flaccidity of the yolk membrane, and yolk disruption (Alleoni & Antunes, 2005).

There are several methods to preserve internal egg quality, and refrigeration is the most commonly used. Refrigeration reduces the water and CO_2 through the eggshell pores, thereby contributing for the maintenance of the buffering system (Solomon, 1991; Leandro *et al.*, 2005).

The goal of the egg distribution chain is to maintain the original quality of the eggs that reach the consumers' table; however, this is rarely achieved (Moreng & Avens, 1990). Previous research studies have shown that the quality of marketed eggs, particularly when eggs go through wholesale channels, is greatly reduced, not only due to the long distribution time, but also to the lack of use of preservation methods that take into account weather changes, such as temperature (Morais *et al.*, 1997).

During the last few years, the food industry has developed products that, in addition to their natural characteristics, include other factors in their nutritional composition that may benefit human health. These are called functional foods or nutraceutics. An example in the egg market are the eggs enriched with omega-3 polyunsaturated fatty acids (ω-3 PUFA), also known as PUFA eggs. The content of these fatty acids in the yolk can be changed by supplying lipid-rich sources, such as fish substrates and oilseed derivates in layer feeds (Leandro *et al.*, 2005).

Studies have shown that the addition of fatty acids in layer feeds influence internal egg quality. According to literature, this induces a reduction in yolk volume and weight, as these lipids reduce the concentration of the plasma estradiol required for yolk synthesis. Some authors associate an oil-rich diet to reduced albumen quality, as there would be a higher deposition of fat in the oviduct, thereby impairing protein secretion and deposition by this structure (Whitehead *et al.*,1993; Brugalli *et al.*, 1998).

The objective of the present study was to evaluate internal quality aspects of conventional and ω-3 enriched eggs stored under different temperatures.

MATERIAL AND METHODS

The experiment was carried out at the Egg Analysis Laboratory of the Animal Science Institute of the Rural Federal University of Rio de Janeiro (UFRRJ), Brazil, during 21 days, in August, 2006.

A number of 864 Isa Brown commercial layers was used. Five birds were housed per 45x50x45cm cage (450 cm²/bird) at Shintaku farm, located in Marília, SP, Brazil. Birds were distributed in two groups, called "Group 1" and "Group 2", which were fed two different diets. In Group 1, 432 layers were fed throughout their productive life with a diet based on corn and soybean meal, and the eggs produced by these birds were called "conventional eggs" in the present experiment. The remaining layers were designated to Group 2, and, starting at 22 weeks of age, were fed a diet containing 1.5% marine algae powdered substrate, and 1.8% fish oil (Table 1). The eggs produced by layers in Group 2 were called "omega-3 (ω-3) enriched eggs".

Table 1. Percentage composition of the diets fed to Isa Brown layers in Group 1 and Group 2.

Ingredients (%)	Group 1*	Group 2**
Corn	60.44	59.17
Soybean meal (46%)	25.80	25.98
Dicalcium phosphate (18% P, 21% Ca)	1.93	1.94
Limestone	8.87	8.86
Salt	0.32	0.32
Fish oil	0.00	1.80
Soybean oil	0.71	0.00
Algae substrate	0.00	1.50
Sand	1.50	0.00
Choline chloride (60%)	0.05	0.05
DL-methionine	0.13	0.13
Mineral-vitamin premix[1]	0.25	0.25
Total	100	100
Ether extract (%)	6.09	5.96
Calculated composition[2]		
Metabolizable energy[3] (kcal/kg)	2800	2800
Crude protein (%)	17.18	17.07
Calcium (%)	3.90	3.90
Available phosphorus (%)	0.45	0.45
Sodium (%)	0.17	0.17
Methionine (%)	0.40	0.40
Methionine + cystine (%)	0.69	0.69
Lysine (%)	0.89	0.89

* Production of conventional eggs. ** Production of ω-3 enriched eggs.[1]Supplementation per kg feed: vitamin P (6250 IU); vitamin D (2500 IU); vitamin E (12 IU); vitamin K (0.04 mg); thiamine (0.25 mg); riboflavine (3.40 mg); vitamin B6 (0.25 mg); vitamin B12(20 IU); pantothenic acid (3.80 mg); niacin (9.90 mg); biotin (0.10 mg); folic acid (0.25 mg); copper (6.00 mg); iron (52.50 mg); iodine (0.33 mg); selenium (0.21 mg); magnesium (48.0 mg); zinc (60.23 mg); ethoxiquin (0.31 mg). [2]Calculated composition according to Rostagno *et al*. (2000).

On the day of lay, eggs were transported in a non-refrigerated truck from the farm (Marília, SP) to the food wholesale market of Rio de Janeiro (CEASA), RJ, located at an approximate distance of 524 km, and then transported by car, under no refrigeration, to the Egg Analysis Lab (LAO) of the Animals Science Institute of UFRRJ, located in Seropédica, RJ. The time spent between egg collection at the farm and arrival at the lab was four days.

Eggs (320, being 160 conventional and 160 ω-3 enriched eggs) were packed in polyethylene egg packages identified by production date.

Before evaluating quality parameters, eggs were weighed in a digital scale (0.001g precision), and only eggs weighing between 63.40 and 65.10g were analyzed. Egg quality analyses were carried out at the aforementioned laboratory, where the 320 eggs were identified, and half was stored at environmental temperature (25ºC) and half in a refrigerator (5ºC) for four different storage periods: "Period 1", four-day-old eggs stored immediately upon arrival at the lab; "Period 2", four-day-old eggs stored for seven days, "Period 3", four-day-old eggs stored for 14 days; and "Period 4", four-day-old eggs stored for 21 days, simulating the arrival of four-day-old eggs at the consumer's home.

Temperature and air relative humidity were checked twice daily, at 09:00 am and 03:00 pm, with the aid of a fixed digital thermo-hygrometer, model TEC-HT-210 manufactured by TecnoVip (Valinhos, SP).

Eggs were distributed in a completely randomized experimental design with a 2x4x2 factorial arrangement (2 egg types x 4 storage periods x 2 temperatures), with each egg considered as a replicate.

The following parameters were analyzed: Haugh units, yolk index, albumen pH, and yolk pH. For internal quality evaluation, eggs were broken on a flat glass surface, and thick albumen height was measured using an Ames tripod micrometer model S-6428. Haugh unit was calculated using the formula proposed by Card & Nesheim (1966), $HU = 100 \log (H + 7.57 - 1.7W^{0.37})$, where H = thick albumen height (mm) and W = egg weight (g). Yolk height was measured after separation from the albumen using the same apparatus used to measure albumen height, and yolk diameter was measured using a Mitutoyo analogical micrometer. Yolk index was calculated as the ratio between yolk height and diameter (Sharp & Powell, 1930). In order to measure pH, albumen and yolk were individually placed in lidded plastic flasks immediately after egg physical parameters were evaluated, which took around two hours, and submitted to the Food and Beverages Analyses Lab of UFRRJ, where pH was measured using a Toledo pH meter model 320.

The obtained results were submitted to analysis of variance, and means were compared by the test of Tukey at 5% significance level, using Sisvar 4.3 software program (Ferreira, 2003).

RESULTS AND DISCUSSION

Average daily temperature and air relative humidity during the experimental period were 25.1ºC and 61%, respectively, when eggs were stored under environmental conditions, and 5ºC and 43%, respectively, when eggs were stored under refrigeration.

No significant differences ($p>0.05$) were observed between conventional and ω-3 enriched eggs as to Haugh units, yolk index, albumen pH, or yolk pH (Table 2). These results are consistent with those reported by Mazalli *et al.* (2004), who included different polyunsaturated fatty acid sources in layer diets to produce ω-3 enriched eggs, and also did not find any differences in Haugh units or yolk index between control (conventional) and ω-3 enriched eggs.

Table 2. Internal egg quality parameters of conventional and ω-3 enriched commercial eggs (Haugh units, yolk index, albumen pH, yolk pH) stored for four different periods (0, 7, 14, 21 days) under two different temperatures (5 and 25 ºC).

	Haugh unit	Yolk index	Albumen pH	Yolk pH
Egg type (ET)				
Conventional	46.45ª	0.35ª	8.87ª	5.74ª
ω-3 enriched	48.92ª	0.34ª	8.84ª	5.75ª
Storage period (P)				
1¹	60.27a	0.36a	8.58a	5.16a
2²	51.97b	0.35a	8.73b	5.30a
3³	45.29c	0.35a	8.78b	6.00b
44	33.23d	0.33b	9.34c	6.52b
Temperature in degrees Celsius (T)				
5ºC	57.94a	0.40a	8.79b	5.78b
25ºC	33.43b	0.29b	8.92a	5.91a
Source of variationP values				
Egg type (ET)	0.623	0.333	0.872	0.789
Storage period (P)	0.039	0.025	0.009	0.043
Temperature (T)	0.021	0.008	0.020	0.035
ET x P x T	0.502	0.354	0.489	0.323
ET x P	0.089	0.187	0.155	0.300
ET x T	0.205	0.073	0.489	0.894
P x T	0.016	0.006	0.024	0.049

Treatment means followed by different letters in the same column are significantly different ($p < 0.05$) by the test of Tukey.¹four-day-old eggs at arrival; ²four-day-old eggs stored for seven days; ³four-day-old eggs stored for 14 days; 4four-day-old eggs stored for 21 days

In the present study, only the interaction between storage period and temperature was significant (Table 2), and therefore, their effects on internal egg quality parameters, independent of egg type, were analyzed (Table 3).

Haugh units

Table 2 shows that, in both egg types, the highest Haugh unit (HU) mean was observed in the non-stored

Table 3. Internal egg quality parameters of commercial eggs (Haugh units, yolk index, albumen pH, yolk pH) stored for four different periods (0, 7, 14, 21 days) under two different temperatures (5 and 25 ºC).

	Haugh unit		Yolk index		Albumen pH		Yolk pH	
Period	25º C	5º C	25º C	5º C	25º C	5º C	25º C	5º C
1[1]	59.47Aa	61.07Aa	0.36Aa	0.36Ba	8.52Ca	8.62Ba	5.23Ba	5.09Ba
2[2]	45.58Ab	58.36Aa	0.32ABb	0.39Aba	8.84BCa	8.64Bb	5.42Ba	5.17Ba
3[3]	32.71Bb	57.87Aa	0.28Bb	0.41Aa	9.08Ba	8.72Bb	6.16Aa	5.83Ba
4[4]	11.78Cb	54.50Aa	0.22Cb	0.43Aa	9.59Aa	9.07Ab	6.60Aa	6.40Aa
CV(%)	13.24		9.30		2.88		10.30	

Treatment means followed by different letters in the same column are significantly different ($p < 0.05$) by the test of Tukey. [1]four-day-old eggs at arrival; [2]four-day-old eggs stored for seven days; [3]four-day-old eggs stored for 14 days; 4four-day-old eggs stored for 21 days

eggs (Period 1). It must be noted that the observed HU values in non-stored eggs (59.47 and 61.07) are according to the description of the Egg-Grading Manual, which classifies 4- to 6-day-old eggs stored at room temperature as A (HU between 72 and 55), that is, good quality eggs (USDA, 2000). In the present study, eggs were analyzed four days after collection due to the time required to transport the eggs from the production site (Marília, SP) to the Egg Analysis Lab of UFRRJ (Seropédica, RJ). At the temperature of 25ºC, there was a 80.19% decrease in HU values after 21 days of storage, reducing egg quality from good at Period 1 (59.47 HU) to poor (11.78 HU) at 21 days, at the end of the experimental period. When eggs were stored at 5ºC, HU score reduction was less pronounced, of only 10.76%, and HU was not significantly different ($p>0.05$) among storage periods. Therefore, eggs stored under refrigeration for 0, 7, 14, and 21 days presented good internal quality (Table 3). These results show that refrigeration at 5ºC aids the conservation of the internal egg quality of commercial eggs (Stadelman & Cotterill, 1995). Similar results were reported by Farrel (1998), who compared the internal quality of conventional eggs and eggs enriched with ω-3 by feeding layers with fish oil and linseed oil, and found that, independently of egg type, eggs stored at 5ºC for 30 days presented higher Haugh units as compared to those stored at 25ºC for the same period.

Yolk index

There was no significant difference ($p>0.05$) in yolk index between egg types (Table 2). Therefore, when conventional and ω-3 enriched eggs were analyzed together, yolk index means of eggs stored at 5ºC at any storage period remained within the standard limit of 0.30 to 0.50 estimated for fresh chicken eggs (Romanoff & Romanoff, 1949). However, in eggs stored at 25ºC, lower values were observed after 14 days of storage (Table 3). Studies show that storing eggs at high environmental temperatures increases yolk membrane permeability, allowing water from the albumen to enter the yolk, which looses its spherical shape, becoming elliptical, thereby reducing the yolk index and increasing the probability of breaking the yolk when the egg is manipulated (Stadelman & Cotterill, 1995).

Albumen pH

Storage temperature presented similar influence ($p>0.05$) on albumen pH of conventional and ω-3 enriched eggs (Table 2). Albumen pH significantly increased ($p<0.01$) during storage, and was higher when eggs were maintained at 25ºC as compared to 5ºC. At 25ºC, mean albumen pH of eggs at arrival (8.52) was significantly lower than those stored for seven (8.84) and 14 days (9.08), increasing 1.07, in average, at 21 days of storage. When eggs were stored at 5ºC, albumen pH increased less (0.43), and there were no significant differences among eggs at arrival (8.62), stored for seven (8.64) or 14 days (8.72). However, mean albumen pH of eggs stored for 21 days (9.07) was significantly higher as compared to the other storage periods (Table 3). These results are consistent with literature findings, which indicate a direct relationship between albumen physiological changes and pH increase during storage at high temperatures. This alkalization is due to an imbalance in the carbonic acid (H_2CO_3) buffering system caused by the continuous release of H_2O and CO_2 through the eggshell pores. Refrigeration contributes for the maintenance of the buffering system by reducing the activity of the enzyme carbonic anhydrase, thereby promoting pH stability (Stadelman & Cotterill, 1995).

Similar results were obtained by Pappas *et al.* (2005), who observed higher albumen pH in eggs stored longer (14 days), independently of egg type (conventional or enriched eggs).

Yolk pH

There was no significant difference ($p>0.05$) in yolk pH between the two types of eggs studied (Table 2).

Independently of egg type, there was no effect of storage temperature ($p>0.05$) on yolk pH of eggs at arrival or of those stored for seven days. However, mean yolk pH of eggs stored for 14 and 21 days at 25ºC was significantly higher than of those maintained at 5ºC for the same periods of time (Table 3). These results are consistent with those of other authors, who reported that yolk pH slowly increases during storage, and no considerable changes are observed until the end of the first week of storage, independently of storage environment. However, after seven days, the yolk pH of eggs stored in hot environments becomes more alkaline than of those kept under refrigeration as cold storage temperatures slow the rate of egg yolk quality deterioration (Solomon, 1991; Jordão Filho *et al.*, 2006).

CONCLUSIONS

Eggs enriched with ω-3 fatty acids presented good internal quality, as well as the conventional eggs, but both depend on refrigerated storage environment (5ºC) to maintain this quality.

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