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Internal quality of commercial eggs stored under conditions that simulate storage from laying to consumption

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Abstract

This study evaluated the effects on the internal quality of eggs of various storage environments through which eggs may pass between being laid and being consumed. Commercial eggs (N = 648) from Dekalb White hens were used. Treatments consisted of T1: 28 days at 4 °C; T2: 28 days at 20 °C; T3: 7 days at room temperature (27 °C ± 2 °C) (humidity 55%) and 21 days at 4 °C; T4: 7 days at room temperature and 21 days at 20 °C; T5: 14 days at room temperature and 14 days at 4 °C; T6: 14 days at room temperature and 14 days at 20 °C; T7: 21 days at room temperature and 7 days at 4 °C; T8: 21 days at room temperature and 7 days at 20 °C; and T9: 28 days at room temperature. The characteristics that were evaluated consisted of Haugh unit (HU), yolk index (YI), colour (L*, a* and b*), albumen pH, yolk pH and lipid oxidation. Eggs stored 28 days were darker (L*), and had greater yolk pH and lipid oxidation than fresh eggs. Eggs stored under T1 and T3 conditions had greater HU and YI than eggs stored in the other environments. The albumin pH of eggs stored at room temperature (T9) was highest of the treatments. Yellowness was increased in eggs stored under T4, T6, T8, and T9 conditions. Eggs should be stored under refrigeration as this promotes maintenance of internal quality and mitigates negative effects of previous storage conditions.

Keywords: fresh eggs, Haugh unit, laying hens, oxidation, storage, yolk index
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Introduction

Brazilian production of commercial eggs has increased by 30% over the last seven years, with 49 billion units produced in 2019. Consumption increased by 27% in the same period, and reached 230 eggs per capita per year (ABPA, 2020). Eggs are known traditionally as one of the most complete foods. They are an important source of fat, carbohydrates, vitamins, minerals and protein in particular, and can contribute to food security in resource-poor settings (Iannotti *et al*., 2014). Eggs are highly perishable and start to deteriorate soon after laying (Akyurek & Okur, 2009). Albumen quality is an important indicator of an egg's freshness, and is important to the egg processing industry (Jin *et al.*, 2011).

Because of market demand and the growth of the poultry industry, sanitary technologies and alternative systems of storage have enhanced product quality, ensured consumer satisfaction and provided economic returns to the production sector (Scatolini-Silva, 2010). Because eggs are perishable, with various physical and chemical changes occurring inside the egg during the storage period (Tabidi, 2011), the way in which they are stored is important. Brazilian Resolution 35, 17 June 2009 (Brazil, 2009) states that packages containing eggs should recommend their storage under refrigeration, but this resolution is not mandatory

Eggs that are packaged improperly or stored at high temperatures and low humidity suffer accelerated biochemical changes to their albumin, making them prone to contamination by pathogens, which reduces their shelf life. This deterioration in quality is associated mainly with the loss of water and carbon dioxide during storage, with the rate of degradation being proportional to the ambient temperature (Leandro *et al.*, 2005). Internal egg quality may be quantified with mathematical expressions that describe the differences between fresh and stored eggs and the changes that occur from storage conditions. Quality changes that are easily observable include those related to the ageing of the albumen and yolk and to the flattening of the yolk (Tabidi, 2011). Thus, the aim of this study was to evaluate the effects of storage under different

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environmental conditions on the internal quality of commercial eggs. Treatments were chosen to mimic the storage situations through which eggs may pass between the time they were laid and consumption.

Material and Methods

This study was developed at the Laboratory of Analyses of Animal Products of the School of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil). Briefly, the eggs (N = 648) were obtained from a commercial farm. Forty-one-week-old Dekalb White hens were housed in galvanized steel cages and fed a diet based on corn and soybean, which was balanced according to their nutritional requirements.

Eggs were taken from the first collection of the day and were transported to the laboratory on that same day. Eggs were randomly assigned to one of these treatments to simulate conditions such as household refrigerator storage, transport in a refrigerated vehicle, storage at room temperature in cities with a mild climate, storage at room temperature during the warmer seasons, and non-refrigerated transport. Thus, the treatments were as follows, T1: 28 days at 4 °C, T2: 28 days at 20 °C, T3: 7 days at room temperature and for 21 days at 4 °C, T4: 7 days at room temperature and 21 days at 20 °C, T5: 14 days at room temperature and for 14 days at 4 °C, T6: 14 days at room temperature and for 14 days at 20 °C, T7: 21 days at room temperature and 7 days at 4 °C, T8: 21 days at room temperature and 7 days at 20 °C, and T9: eggs stored at room temperature for 28 days. Eggs not stored (fresh eggs) were used as a control treatment.

Eggs were stored in a 250 W Eletrolab EL101 BOD incubator (Eletrolab, São Paulo, SP, Brazil) at 4 °C or 20 °C. The temperature of the uncontrolled environment ranged from 25 °C to 29 °C with an average relative humidity of 55%. Haugh units and pH were used to measure albumen quality and colour (L*, a*, b*). pH, yolk index and lipid oxidation were used to assess yolk quality.

Haugh units (Haugh, 1937) were measured as described by Card & Nesheim (1978). Eggs were weighed individually on an analytical balance and broken on a special glass table. The height of dense albumen was measured in three positions with an Ames micrometer (model S-6428, Ames, Waltham, MA). Haugh units were calculated as:

$$HU = 100\log(H + 7.57 - 1.7W^{0.37})$$

where H = the average height (mm) of the dense albumin and W = the weight (g) of the egg.

The pH of albumen and yolk were determined in samples that had been slightly homogenized with a glass rod, using a Testo 205 pH meter (Testo Inc., Sparta, NJ, USA). The yolk was measured with an Ames micrometer (S-6428 model, Ames, Waltham, MA) to determine its height (YH) (mm) and digital callipers (150 mm 6" Zaas Precision) were used to establish its diameter (YD) (mm). The yolk index (YI) was calculated as:

$$YI = YH/YD.$$

Colour was measured with a Minolta CR-400 colorimeter (Konica Minolta, Inc., Osaka, Japan) to evaluate lightness (L*) (0 = white, 100 = black), redness (a*) and yellowness (b*). Lipid oxidation was determined by a method that was adapted from Vyncke (1970). Ten gram aliquots of yolk were put into 50 ml tubes, to which 25 ml of trichloroacetic acid 7.5% solution was added. The samples were homogenized for 1.5 minutes with a Ultra Turrax MA 102 (Marconi Equipamentos Para Laboratórios Ltda., Piracicaba, São Paulo, Brazil) at 7000 rpm. The homogenate was filtered through qualitative filter paper, and duplicate 5 ml aliquots of the filtered homogenate were transferred to test tubes. Five ml 0.02 M thiobarbituric acid was added to each of the test tubes. Samples were placed in a water bath that was maintained at a temperature of 100 ºC for 40 minutes. Samples were analysed in a spectrophotometer (Shimadzu® UV-1800, Kyoto, Japan) with 532 nm wavelength, and the results were expressed as mg of malonaldehyde per kg of sample.

Data originated from two periods of storage and nine storage conditions with three replicates of 12 eggs each. Four eggs were randomly selected from each replicate to assess the response variables. Analysis of variance, appropriate to a 2 x 9 factorial arrangement of treatments, was done using the general linear model procedure of Statistical Analysis System (SAS Institute Inc. 2002-2003, Cary, North Carolina, USA). Means were compared with Tukey's test with significance set at P <0.05.

Results and discussion

The main factors that are involved in the deterioration in quality of stored eggs are the elevated temperature of the environment and dehydration. Improper storage may result in quality changes in dense albumen, an increase in the yolk diameter, enlargement of the air cell, and absorption of off-odours and off-flavours (Tabidi, 2011). By simulating various storage conditions to which eggs could be subjected until the time of consumption, a significant interaction was revealed between storage time and types of storage

affecting HU and YI (Table 1). All the fresh eggs had similar HU and YI. After 28 days of storage, eggs stored in T1, T3, and T5 had greater HU than those stored in the other environments.

Table 1 Means for interaction between time in storage and type of storage affecting Haugh units and yolk index of eggs stored under different environmental conditions

	Haugh units		Yolk index	
Conditions of storage	0 days	28 days	0 days	28 days
T1	93.58^{Aa}	87.57^{Aa}	0.47^{Aa}	0.46^{Aa}
T2	94.70^{Aa}	77.57^{Bb}	0.48^{Aa}	0.36^{Cb}
T3	91.55^{Aa}	83.68^{Aa}	0.43^{Aa}	0.42^{Ba}
T4	89.02^{Aa}	73.61^{Bcb}	0.49^{Aa}	0.34^{CDb}
T5	86.23^{Aa}	75.78^{Ba}	0.46^{Aa}	0.33^{CDb}
T6	92.05^{Aa}	71.91^{CDb}	0.47^{Aa}	0.31^{Deb}
T7	90.91^{Aa}	70.79^{CDb}	0.45^{Aa}	0.28^{Eb}
T8	90.37^{Aa}	66.46^{CDb}	0.46^{Aa}	0.27^{EFb}
T9	95.69^{Aa}	65.14^{Db}	0.49^{Aa}	0.23^{Fb}

T1: storage for 28 days at 4 °C, T2: storage for 28 days at 20 °C, T3: storage for 7 days at room temperature and for 21 days at 4 °C, T4:: storage for 7 days at room temperature and for 21 days at 20 °C, T5: storage for 14 days at room temperature and for 14 days at 4 °C, T6: storage for 14 days at room temperature and for 14 days at 20 °C, T7: storage for 21 days at room temperature and 7 days at 4 °C, T8: storage for 21 days at room temperature and 7 days at 20 °C, T9: storage at room temperature for 28 days

^{A,B,C,D} Means within a column followed by the same uppercase letter did not differ in Tukey's test at P =0.05

^{a,b} Means within a trait and row followed by the same lowercase superscript letter did not differ in Tukey's test at P =0.05

Haugh units, which increase with albumin height and decrease with egg weight, are a standard measure of egg quality. Greater HU values represent better quality (Trinidad *et al.*, *2*007). Haugh units are influenced by genetic factors (strain), age of laying hen (Akyurek & Okur, 2009), and environmental factors such as temperature and storage time (Samli *et al.*, *2*005; Akyurek & Okur, 2009; Jin *et al.*, *2*011). During storage, egg quality decreases primarily because of loss of water and carbon dioxide (CO_2), with these losses being proportional to the temperature and humidity conditions (Souza *et al.*, 1997; Akyurek & Okur, 2009). Albumen height is at its maximum immediately after laying, and decreases with storage (Jin *et al., 2*011) because with ageing the proportion of liquid albumen increases at the expense of dense albumen (Leandro *et al.i2*005). On leaving the farm, fresh eggs have between 75 and 85 HUs on average (Coutts & Wilson, 2007). To be of excellent quality for human consumption, the United States Department of Agriculture (USDA) recommends that eggs must have HUs greater than 72 (classified as "AA"), and good quality eggs must have HUs between 60 and 71 (classified as "A") (Menezes *et al.*, 2012). Even after 28 days of storage, the eggs tested in this study could be considered good quality eggs.

Albumen buffering capacity is reduced In the presence of oxygen (Akyurek & Okur, 2009). Carbonic acid (H_2CO_3) in the egg breaks down to produce H_2O and CO_2, which are lost by evaporation through the shell (Gardner, 1995; Scatolini-Silva *et al.*, *2*010; Rocha *et al.*, *2*013). The result is that the flavour of the egg changes because of its increased alkalinity (Moreng & Avens, 1990). Keeping eggs under constant refrigeration can preserve their internal quality and prolong their shelf life. However, when one considers the conditions of the Brazilian market, it seems that about 90% of eggs are commercialized in natura without refrigeration (Scatolini-Silva *et al.*, *2*010).

Only T1 and T3 preserved the height and diameter of egg yolks such that after 28 days of storage they were similar to the yolks of fresh eggs. Eggs in T9 had a reduced YI, which indicated poor yolk quality compared with all other storage conditions. The yolk flattening that becomes evident with increased time in storage is caused mainly by increased concentration of water by osmotic migration from the albumin through the vitelline membrane (Rocha *et al.*, *2*013), which is then weakened (Messens *et al.*, *2*005; Akyurek & Okur, 2009; Jones *et al.*, *2*014) and can break easily (Tabidi, 2011). Researchers concluded that yolk height decreased about 10% with an increase of environmental temperature from 5 °C to 23 °C, and there was only

a slight reduction of yolk height with storage time (Keener *et al.*, *2*006). Thus, albumin height decreases at a more rapid rate than the yolk index.

Eggs stored for 28 days had greater L* (were darker), yolk pH, and lipid oxidation than fresh eggs (Table 2). There was no effect of the type of storage on the lightness (L*), redness (a*), yolk pH, and lipid oxidation. The increase in L* occurs because of the movement of water from albumen to the yolk that results from a difference in osmotic pressure (Moreng & Avens, 1990; Rocha *et al.*, *2*013). Significant increases in the yolk pH were observed in studies of the effects of storage time, and not the influence of storage temperature (Samli *et al.*, *2*005; Akyurel & Okur, 2009; Jin *et al.*, *2*011). In natura eggs are considered resistant to lipid oxidation, though the yolk lipids can undergo oxidation during storage (Cherian *et al.*, *2*007, Giampietro *et al.*, *2*008; Rocha *et al.*, *2*013). Carotenoids, resulting from feeding xanthophylls to laying hens that are deposited in the yolk, have an important antioxidant role and can protect yolk nutrients during storage (Rocha *et al.*, *2*013). The lipid oxidation (TBARS) results in this study were considered low, with no signs of rancidity, which can be perceived by sensory analysis and can cause problems to consumer health.

Table 2 Mean values of lightness (L*), redness (a*), yellowness (b*), albumen pH, yolk pH, and lipid oxidation (mg malonaldehyde/kg) of eggs stored for 28 days under different environmental conditions

	L*	a*	b*	albumen pH	yolk pH	TBARS
			Storage periods (S)			
0 days	61.45^{B}	-5.13	37.94	8.35	6.09^{B}	0.177^{B}
28 days	64.45^{A}	-4.82	48.27	9.53	6.34^{A}	0.215^{A}
			Storage treatment (T)			
T1	63.40	-4.94	41.74	8.96	6.12	0.204
T2	62.85	-4.51	43.28	8.92	6.19	0.208
T3	62.04	-5.04	41.34	8.83	6.18	0.197
T4	63.71	-5.01	42.18	9.07	6.11	0.190
T5	63.78	-5.19	43.03	8.98	6.22	0.201
T6	63.79	-5.97	44.93	9.02	6.29	0.184
T7	62.58	-5.98	41.87	8.76	6.17	0.190
T8	62.51	-5.49	43.40	8.87	6.39	0.195
T9	61.87	-4.63	46.20	9.04	6.27	0.191
P-value (S)	<0.0001	0.1503	<0.0001	<0.0001	<0.0001	<0.0001
P-value (T)	0.6753	0.6196	0.2908	0.0381	0.3507	0.4858
P-value (SxT)	0.1367	0.3814	0.0025	0.0019	0.8829	0.2390
CV (%)	3.84	18.10	8.94	2.01	3.59	5.97

^{A,B} Storage periods differed significantly if the means have different superscript letters
TBARS: 2-thiobarbituric acid reactive substances; MDA, malonaldehyde; T1: storage for 28 days at 4 °C; T2: storage for 28 days at 20 °C; T3: storage for 7 days at room temperature and for 21 days at 4 °C; T4: storage for 7 days at room temperature and for 21 days at 20 °C; T5: storage for 14 days at room temperature and for 14 days at 4 °C; T6: storage for 14 days at room temperature and for 14 days at 20 °C; T7: storage for 21 days at room temperature and 7 days at 4 °C; T8: storage for 21 days at room temperature and 7 days at 20 °C; and T9: storage at room temperature for 28 days.

Significant interactions between storage period and type of storage were observed for albumen pH and yellowness (b*) (Table 3). Fresh eggs were similar in albumen pH and yellowness (b*) of yolk. After 28 days of storage, an increase in albumen pH was observed in eggs stored under all conditions, with the eggs in T9 having the greatest albumen pH value. Fresh eggs have a neutral pH, and albumen that is transparent, consistent, dense, and consists of only a small fluid portion (Murakami *et al.* 1994). The albumin pH of recently laid eggs is approximately 7.6 (Coutts & Wilson, 2007; Akyurek & Okur, 2009) and increases to about 9.0 during storage (Akyurek & Okur, 2009). The yolk pH of freshly laid eggs has been reported to be about 6.0 (Coutts & Wilson, 2007), which is consistent with the current results (Table 2). Bakst andf Holm (2003) reported that during storage the yolk remains slightly acidic, with pH of approximately 6.5, which was slightly greater than the current observations.

Table 3 Means for the interactions between storage period and type of storage that affected albumen pH and yellowness (b*) of eggs stored for 28 days under different environmental conditions

Conditions of storage[1]	Albumen pH		b* (yellowness)	
	0 days	28 days	0 days	28 days
T1	8.53^{Ab}	9.40^{Ba}	41.46^{Aa}	42.02^{Da}
T2	8.22^{Ab}	9.63^{ABa}	38.57^{Aa}	47.99^{BCDa}
T3	8.19^{Ab}	9.47^{ABa}	38.85^{Aa}	43.84^{CDa}
T4	8.59^{Ab}	9.57^{ABa}	35.94^{Ab}	48.43^{BCa}
T5	8.59^{Ab}	9.37^{Ba}	37.40^{Aa}	48.66^{BCa}
T6	8.46^{Ab}	9.58^{ABa}	38.87^{Ab}	50.99^{Aba}
T7	8.12^{Ab}	9.41^{Ba}	36.76^{Aa}	46.97^{BCDa}
T8	8.14^{Ab}	9.60^{ABa}	36.29^{Ab}	50.50^{Aba}
T9	8.35^{Ab}	9.79^{Aa}	37.35^{Ab}	55.04^{Aa}

T1: storage for 28 days at 4 °C, T2: storage for 28 days at 20 °C, T3: storage for 7 days at room temperature and for 21 days at 4 °C, T4:: storage for 7 days at room temperature and for 21 days at 20 °C, T5: storage for 14 days at room temperature and for 14 days at 4 °C, T6: storage for 14 days at room temperature and for 14 days at 20 °C, T7: storage for 21 days at room temperature and 7 days at 4 °C, T8: storage for 21 days at room temperature and 7 days at 20 °C, T9: storage at room temperature for 28 days

^{A,B,C,D} Means within a column followed by the same uppercase letter did not differ by Tukey's test at P =0.05

^{a,b} Means within a trait and row followed by the same lowercase superscript did not differ by Tukey's test at P =0.05

The increase in albumen pH during storage is unavoidable because of CO_2 and water losses through the eggshell pores (Feddern *et al*., 2017). Most of the changes in the egg quality are related to humidity and these CO_2 losses (Jin *et al.*, *2*011). The pH increase is related to the deterioration of the dense albumen (reduced HU), which becomes progressively liquefied and diluted, becoming liquid albumen, which occurs because of the changes in the ovomucin-lysozyme complex from the pH increase over time (Tabidi, 2011). When they evaluated the effects of temperature and storage time on the quality of eggs from laying hens at peak production, Jin *et al.* (2011) observed a rapid increase in albumen alkalinity, regardless of storage temperature, with most of the increase occurring during the first five days of storage. The albumen pH rises rapidly in the first three days of storage and then has little change, whereas the yolk pH rises slowly during prolonged storage (Linden & Lorient, 1996; Barancelli *et al.*, 2012).

The impermeability of the vitelline membrane is important for food safety (Messens *et al.*, *2*005). It decreases with storage time, which can allow nutrients in the yolk to become available for any microorganisms that are present in albumen (Kirunda & McKee, 2000; Keener *et al.*, *2*006; Akyurek & Okur, 2009). However, with increasing albumen pH, the environment within the egg becomes unfavourable to the development of pathogens such as Salmonella (Humphrey, 1994), which require pH between 6.0 and 7.5 for multiplication (Banwarth, 1989). Eggs also contain enzymes that impede the growth of bacteria (Oliviera & Silva, 2000).

The yellow colour of the yolk is related directly to a diet that is rich in carotenoid pigments, usually from xanthophylls such as corn (Rocha *et al.*, *2*013). In this study, there was greater yellowness of the yolks from eggs that were subjected to T4, T6, T8, and T9, compared with the colour of fresh egg yolks, perhaps because of some increase in the products of oxidation. Being unsaturated, carotenoids are subject to oxidation in the presence of light, heat and pro-oxidant compounds, which can result in discoloration of the yolk (Ribeiro & Seravalli, 2004; Melendez-Martinez *et al.*, *2*004). Jin *et al.* (2011) observed a reduction in yolk colour with storage time. The authors attributed this to the possible dilution of the yolk pigments caused by the breakdown of the vitelline membrane. In contrast, Giampietro-Ganeco *et al*. (2012) suggested that the reduction of internal quality in commercial eggs causes liquid waste to migrate from the yolk to the albumen, resulting in an increased concentration of the carotenoid pigments and thus a more intense yellow colour.

Conclusion

Egg quality is influenced by room temperature and storage time. Eggs should be stored under refrigeration as this maintains internal quality and mitigates the negative effects of previous conditions. The transportation of eggs for later marketing should be carried out in a refrigerated vehicle to preserve product

quality. More studies are needed to verify the extent of lipid oxidation and free radical production in stored eggs.

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Authors' Contributions

ASR and BH designed the study, and all of the authors collaborated in the collection of data. ASR performed the statistical analysis and wrote the article. All authors read the final article and approved its contents.

Conflict of interest declaration

The authors declare that they have no conflict of interest relative to this work.

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