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Comparative study of chicken shell eggs stored under different storage conditions

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Abstract
The current study was conducted to evaluate the effect of storage time and temperatures (4±1 °C and 21-32 °C) on the quality parameters of shell eggs. Immediately after collection, the fresh unwashed eggs were labelled and weight was measured. Fresh eggs were evaluated within 2-3 h of laying. The effects of the storage at 4±1 °C and at room temperature on quality parameters, microbiological qualities and the lipid peroxidation of egg yolk were evaluated. The results showed that the eggs stored at 4±1 °C have significantly higher (p< 0.05) Haugh unit values, Yolk Index and lower air cell depth, albumen and yolk pH than those eggs stored at room temperature. Refrigeration temperature was found responsible to slow down the Total plate count and lipid peroxidation of the yolk. The results helped to conclude that the storage temperature plays a significant role in the maintenance of the quality parameters of chicken shell eggs.

Keywords: External quality, internal quality, lipid peroxidation, yolk index, haugh unit

Introduction

Hen's eggs have the optimum balance of essential nutrients, besides being generally considered an important constituent of human food (Opaliński, 2017) [12]. For the same reasons it is very much perishable food items as well, which could lose its quality rapidly during the period between it is laid and consumed. Eggs provide a unique and well-balanced source of protein which contains almost all the essential amino acids in right proportion to maintain life and support growth even when used as a sole source of food protein (Ricketts, 1981) [12]. Farm eggs might be of good quality, but poor handling and storage conditions on farm and in distribution channels may result in the egg quality losses (Al-Obaidi *et al*. 2011) [2]. The main difference between freshly laid eggs and the stored eggs are related to internal qualities such as albumen quality, which is influenced by genetic (*viz*., breed) and the environmental factors such as storage temperature, duration and humidity (Samli *et al.* 2005) [14]. The shelf life of shell eggs, during which they are acceptable, is influenced by the inner carbon dioxide content (Keener *et al.* 2001) [6]. Therefore, proper storage of eggs is very much essential to preserve the quality and the functional properties. Poor storage conditions can reduce grade of eggs within few days in normal storage conditions. High storage temperatures and the dehydration of shell eggs are the primary quality degrading factors. During storage the carbon dioxide gas is lost through the shell as the egg ages, and the inner contents of the eggs become more alkaline, causing the albumen to become transparent and watery (Okeudo *et al.* 2003) [11]. At the higher temperatures, the loss of carbon dioxide gas is faster and the albumen quality deteriorates at faster rate. Albumen quality is not only an important indicator of egg freshness but it also significant for the egg processing industries, as it is the standard measure of different egg and egg products.

At the present health-conscious people are very concerned about the quality of eggs they consume. So, the researchers are emphasising to maintain the quality of eggs for longer duration for its better acceptability during marketing. Therefore, the present study was designed with the objective to evaluate the effect of storage time and temperature on the external and internal quality parameters, functional properties of eggs, microbiological qualities and the lipid peroxidation of egg yolk, during the storage at 4±1 °C and at room temperature (21-32 °C).

Materials and Methods
The experiments were conducted at the Division of Livestock Products Technology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh. The freshly laid, unwashed, chicken (*Gallus domesticus*) shell eggs were procured from a local poultry farms in Bareilly and used for the experiments. The eggs immediately after collection were labelled and weight was measured. Fresh eggs were analysed within 2-3 h of laying. To study the effect of storage on the external and internal quality parameters, the eggs were stored at 4 °C and room temperature (21-32 °C) and different quality parameters were evaluated at every 0, 3, 6, 9, 14, 21, 27 and 30 days.

Analytical procedures
Thiobarbituric acid value of the egg yolk sample was measured according to method described by Witte *et al.* (1970) [19]. Total plate count (TPC) were determined by APHA (1992) using pour plate method.

Measurement of external quality
The individual eggs were weighed on a digital balance to the nearest of 0.01 g accuracy. The shape index was calculated by multiplying the ratio of breadth to length with 100. The inner shell membrane was removed from the shells and the shells were kept for drying in the open air for 24 h. All the dried shells (devoid of shell membrane) were weighed and divided by the egg weight to get the total shell quantity %.

Measurement of internal quality
The Shell thickness ware calculated by taking average of the four pieces of shells (one from each end, broad & narrow, two from the body of the eggs) with the help of screw gauze. The egg length and breadth were measured with the help of digital vernier callipers. The length and width of the albumen, yolk and air cell depth were measured in mm with the help of a digital vernier calliper. The height of the albumen and yolk were measured at their highest point by using the spherometer on a table glass. Care was taken to balance the table and table glass with the help of dumpy level. The height of the albumen was measured at 3 or 4 locations and average was calculated. The pH of the albumen and the yolk were measured with a pH meter (Hanna Instrument Inc.). The Yolk index was calculated by using the Formula 1.

$$\text{Yolk index} = \frac{\text{Yolk height (mm)}}{\text{Yolk diameter (mm)}} \quad \text{..........Formula 1.}$$

Haugh unit (H.U.) was calculated by using the Formula 2.

$$\text{H.U.} = 100 \log (H+7.57-1.7W^{37}) \quad \text{........................Formula 2.}$$

Where, H is albumen height (mm), measured by Spherometer and W is observed weight of the egg in grams.

Statistical analysis
All the data obtained during the present investigation were analysed statistically by using SPSS (Version 24.0) software. The data obtained were analysed by Randomized Block Design and subsequent one-way ANOVA except storage study parameters were analysed by Complete Randomized Design for two-way ANOVA. Further the significance between the data was compared by Tukey's Post Hoc Test by SPSS-24® software package. A probability value of $p< 0.05$ was described as significant.

Results and Discussion
External egg parameters
All the results of external quality parameter changes are presented in the Table 1. Weight loss (%) of eggs stored at different storage temperature for 0, 3, 6, 9, 14, 21, 27 and 30 days indicated that regardless of storage temperatures, a significant ($p< 0.05$) weight loss was observed. The weight loss were gradually increased with the increase of storage periods and the eggs stored at room temperature showed maximum weight loss (6.54%) as compare to eggs stored at refrigeration temperature (4.04%) at 30th day of storage. Similar observations were made by Wardy *et al.* (2010) [18], in their experiment the weight loss of eggs progressively increased with increasing storage period, from 1.93% after 1 week to 4.80% after 5 weeks of storage at 25±1 °C and to 11.77% after 20 weeks of storage at 4±1 °C.
The shape index did not affect significantly (p>0.05) by the storage time and temperatures. The similar findings also reported by Song *et al.* (2000) [16] and Tilki and Saatci (2004) [17], who found that the storage time and temperature had no effect on the egg shape index. Storage period and the temperature had no effect (p>0.05) on the shell weight %. These results are consistent with the findings of Silversides and Scott (2001) [15] and Akyurek and Okur (2009) [1], who found that storage time, had no effect on egg shell weight. In contrast, Samli *et al.* (2005) [14] showed a significant ($p< 0.05$) change in shell weight during the storage at various time and temperature levels.

Table 1: External parameters of chicken shell eggs during different storage temperature

Parameters		Storage day							
		0 Day	3rd Day	6th Day	9th Day	14th Day	21st Day	27th Day	30th Day
Egg wt. loss (%)	21-32 °C	$0±0.00^h$	$0.52±0.13^g$	$0.96±0.15^f$	$1.69±0.34^e$	$3.23±0.21^d$	$4.69±0.17^c$	$6.03±0.26^{ab}$	$6.54±0.14^a$
	4±1 °C	$0±0.00^h$	$0.31±0.23^g$	$0.78±0.27^f$	$1.09±0.32^e$	$2.37±0.24^d$	$3.26±0.21^c$	$3.87±0.19^{ab}$	$4.04±0.16^a$
Shape index (%)	21-32 °C.	81.03±0.65	80.60±0.43	80.60±0.43	81.80±0.34	81.75±0.53	80.01±0.47	80.53±0.52	80.56±48
	4±1 °C	80.96±0.31	81.41±0.54	81.41±0.54	80.34±0.72	79.96±0.46	80.16±0.27	81.32±0.82	80.11±0.34
Shell weight (%)	21-32 °C.	11.51±0.31	11.51±0.34	11.49±0.26	11.49±0.32	11.48±0.28	11.48±0.25	11.47±0.27	11.47±0.33
	4±1 °C	11.51±0.23	11.52±0.35	11.48±0.26	11.48±0.31	11.47±0.33	11.47±0.37	11.47±0.35	11.46±0.27

a-h Mean + S.E. values with different superscripts in rows differ significantly ($p< 0.05$)

Internal parameters
All the results of internal quality parameter changes are presented in the Table 2. The air cell depth found increasing in both the storage temperatures significantly ($p< 0.05$) throughout the storage period. The eggs stored at refrigeration temperature showed less increase of air cell depth (6.69 mm) at 30th day as compared to the eggs stored at room temperature (10.01 mm). As per the findings of Samli *et al.*

(2005) [14], the air cell depth exceeds 4mm in two days at 21 °C storage temperature. Alsobayel and Albadry (2011) [3] observed that the eggs stored under the unfavourable temperature and humidity, have significant adverse effects upon the air cells depth and increase in size of air cell as the result of long-term storage. Shell Thickness was not affected (p>0.05) by the storage period and temperature. Similar observation has been made by Dudusola (2009) [4], who did not find any effect of storage time and temperature on the shell thickness.

Yolk weight of eggs showed significant ($p< 0.05$) changes during storage, and increased linearly with the increasing storage time, which might be due to the diffusion of water from the albumen to the yolk. On the other hand, when the storage temperature was higher, the rate of increase of yolk weight was significantly ($p< 0.05$) higher than in refrigeration (4 °C). These results are supported by the findings of Leandro *et al.* (2009) [8], who reported that the most important factors that affects egg quality during storage are temperature and the relative humidity.

The results showed that the albumen weight was significantly ($p< 0.05$) affected by storage time and the temperature. The lowest albumen weight was recorded on the 30th day of storage in both the temperatures. Consequently, albumen weight loss was significantly ($p< 0.05$) higher at room temperature than at refrigeration on the 30th day of storage. This reason behind this loss might be due to the ingress of solvents from albumen to yolk, which resulted in the decrease the weight of the albumen and the increase the weight of yolk. Similar results were reported by Akyurek and Okur (2009) [1], they observed that the water loss from eggs has been influenced by the storage time, temperature, relative humidity and the porosity of the shell as well.

In this study, the yolk index values of all eggs shows significant decreased significantly ($p< 0.05$) with increasing storage period. These changes were more pronounced at the room temperature as compared to the refrigeration temperature. After 30 days, the yolk index decreased from 0.46 to 0.28 at room temperature compared to that of 0.46 to 0.37 at refrigeration temperature. The decreased yolk index indicated the progressive liquefaction of the yolk caused mainly by the water diffusion from the albumen to yolk (Obanu and Mpieri, 1984) [10].

The Haugh unit demonstrates the albumen quality of eggs as a function of thick albumen height and the egg weight. In this present study the Haugh unit found to be significantly declined ($p< 0.05$) with the increasing storage period both at room and refrigeration temperature (Figure 1). At room temperature, the units decreased from 81.48 on the 0 day to 30.56 on the 30th day of storage. Whereas, at refrigeration temperature the units decreased from 81.48 on the 0 day to 64.78 on the 30th day. The evaporation of carbon dioxide and water through egg shell pore leads to the increase in the pH of the albumen to alkaline state which leads to the egg white to change its gel structures and lose its strength and the white became watery causing the decrease in Haugh unit (Yimenu *et al.* 2017) [20]. The reduction in Haugh unit was due to the decrease in thick albumen height, because during storage, the ovomucin-lysozyme complex breaks down, due to increase the in pH of eggs.

The results revealed that the pH of egg yolk increased significantly ($p< 0.05$) on the 30th day of storage (Figure 2). The pH of the yolk was significantly higher ($p< 0.05$) in room temperature than in refrigeration. This occurred might be due to the loss of carbon dioxide from egg by diffusion resulting to increase the yolk pH. The present results are in agreement with the findings of Samli *et al*. (2005) [14] and Akyurek and Okur (2009) [1], they observed that the increases in yolk pH were significantly ($p< 0.05$) affected by the storage time.

Albumen pH is important parameter of egg freshness and the quality (Silversides and Scott, 2001) [15]. The changes in albumen pH during storage are presented in Figure 3. The albumen pH found significantly ($p< 0.05$) higher at room temperature than the refrigeration temperature during the whole storage period, which may be due to higher rate of evaporation from eggs. Increase in albumen pH with the increasing storage period was also reported by Moula *et al*. (2009) [9].

Table 2: Internal parameters of chicken shell eggs during different storage temperature

Parameters		Storage day							
		0 Day	**3rd Day**	**6th Day**	**9th Day**	**14th Day**	**21st Day**	**27th Day**	**30th Day**
Air Sac(mm)	21-32 °C	0.17 ± 0.04	0.79 ± 0.05^{g}	2.26 ± 0.03^{ef}	2.57 ± 0.08^{e}	3.97 ± 0.06^{d}	6.59 ± 0.03^{c}	6.83 ± 0.05^{b}	10.01 ± 0.03^{a}
	4±1 °C	0.19 ± 0.16^{h}	0.72 ± 0.18^{g}	1.78 ± 0.17^{f}	1.94 ± 0.16^{e}	2.32 ± 0.18^{d}	4.21 ± 0.16^{c}	5.12 ± 0.15^{b}	6.69 ± 0.18^{a}
Shell thickness (mm)	21-32 °C	0.40±0.004	0.40±0.003	0.40±0.005	0.40±0.002	0.40±0.004	0.39±0.003	0.39±0.005	0.39±0.003
	4±1 °C	0.40±0.002	0.40±0.006	0.40±0.004	0.39±0.003	0.39±0.002	0.39±0.005	0.38±0.002	0.38±0.004
Yolk wt.(g)	21-32 °C	16.12 ± 0.17^{gh}	16.24 ± 0.17^{g}	17.23 ± 0.16^{f}	17.98 ± 0.18^{e}	19.24 ± 0.1^{d}	20.42 ± 0.16^{c}	21.27 ± 0.18^{b}	21.43 ± 0.16^{a}
	4±1 °C	16.22 ± 0.09^{gh}	16.24 ± 0.07^{g}	17.43 ± 0.08^{f}	18.12 ± 0.07^{e}	19.78 ± 0.07^{cd}	20.10 ± 0.08^{c}	21.10 ± 0.07^{ab}	21.29 ± 0.08^{a}
Albumen wt. (gm)	21-32 °C	31.10 ± 0.23^{a}	31.02 ± 0.21^{ab}	30.67 ± 0.21^{c}	29.49 ± 0.24^{d}	28.89 ± 0.23^{e}	28.23 ± 0.20^{f}	27.47 ± 0.23^{g}	27.52 ± 0.21^{gh}
	4±1 °C	30.10 ± 0.26^{a}	29.91 ± 0.24^{ab}	29.47 ± 0.25^{c}	29.29 ± 0.23^{d}	29.14 ± 0.25^{de}	29.05 ± 0.24^{f}	28.78 ± 0.23^{g}	28.52 ± 0.25^{gh}
Yolk index	21-32 °C	0.46 ± 0.05^{a}	0.42 ± 0.04^{ab}	41.54 ± 0.05^{c}	0.40 ± 0.04^{d}	0.38 ± 0.06^{e}	0.34 ± 0.04^{f}	0.32 ± 0.05^{g}	0.28 ± 0.06^{h}
	4±1 °C	0.46 ± 0.04^{a}	0.45 ± 0.05^{ab}	0.44 ± 0.06^{c}	0.43 ± 0.05^{d}	0.42 ± 0.04^{e}	0.40 ± 0.06^{f}	0.38 ± 0.04^{g}	0.37 ± 0.05^{h}
TPC (CFU/ml)	21-32 °C	3.66 ± 0.02^{h}	3.69 ± 0.02^{g}	4.38 ± 0.01^{f}	4.78 ± 0.02^{e}	5.61 ± 0.01^{d}	6.36 ± 0.02^{c}	6.67 ± 0.03^{b}	7.50 ± 0.02^{a}
	4±1 °C	3.66 ± 0.02^{gh}	3.65 ± 0.04^{h}	3.69 ± 0.02^{f}	3.76 ± 0.01^{e}	4.14 ± 0.02^{d}	4.25 ± 0.02^{c}	4.32 ± 0.04^{b}	4.38 ± 0.02^{a}

$^{a-h}$ Mean + S.E. values with different superscripts in rows differ significantly ($p< 0.05$)

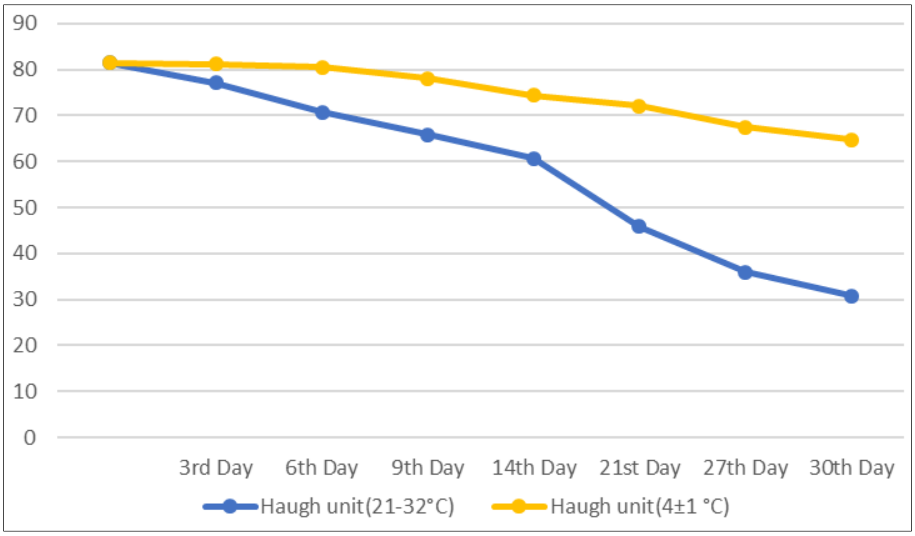

Fig 1: Effect of different storage temperature on Haugh Unit

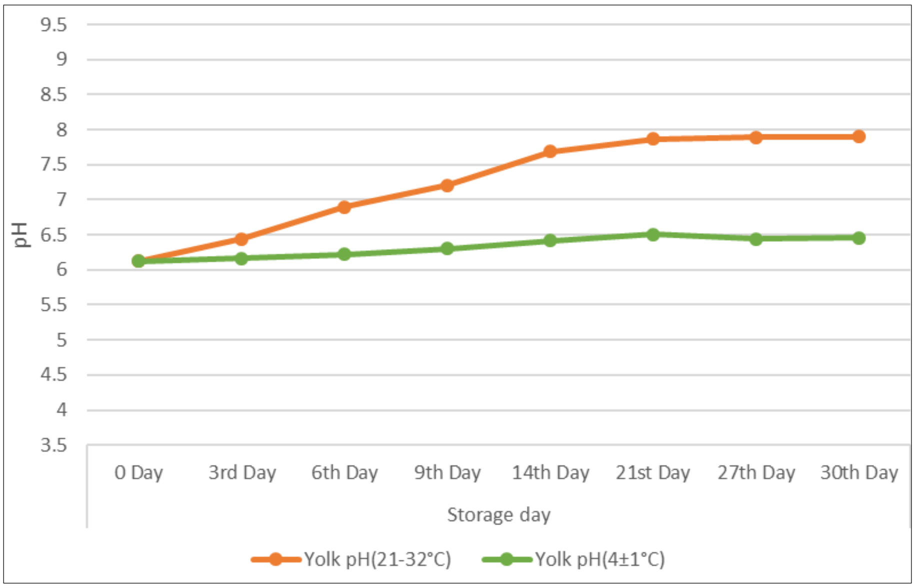

Fig 2: Effect of different storage temperature on Yolk pH

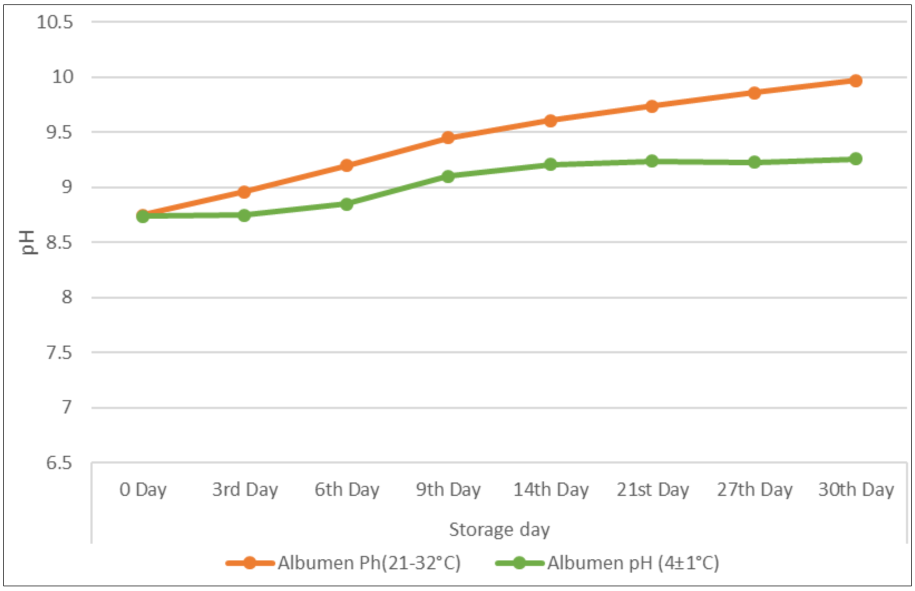

Fig 3: Effect of different storage temperature on Albumen pH

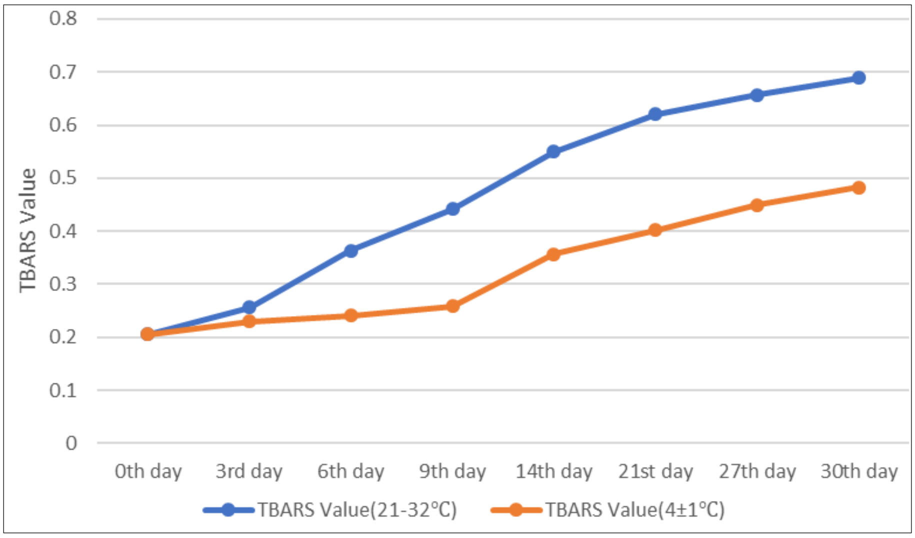

Fig 4: Effect of different storage temperature on TBARS Value

Thiobarbituric acid (TBA) Value

The changes in TBA values of eggs are presented in Figure 4. Eggs stored under refrigeration temperature showed significantly ($p< 0.05$) lowered TBA value as compared to the room temperature. Although, the values increased in both the conditions significantly ($p< 0.05$) throughout the period. Eggs stored under refrigeration temperature showed the lower TBA value than the room temperature. This result agrees with the findings of Lakins *et al*. (2009) [7] that the TBARS values increased when the eggs were stored for 30-90 days at 4 °C.

Total plate count (TPC)

The TPC of refrigerated eggs was significantly ($p< 0.05$) lower than those stored under room temperature. The TPC increasing in both the storage temperatures significantly ($p< 0.05$) throughout the storage period. The eggs stored at refrigeration temperature showed less increase count throughout the storage period as compared to the eggs stored at room temperature. Eggs those are stored in room temperature exceed the FAO recommended maximum permissible limit (<10^6 CFU) of TPC at 14th day. The present results are in agreement with the findings of Eke *et al*. (2013) [5] showed higher bacteria count on those stored under ambient condition with the initial count of 3.69 at 1st week and 7.44 at the 4th week of storage.

Conclusion

The observation in the present study has shown that the quality characteristics of eggs were not adversely affected when eggs were stored at refrigeration temperature. The storage of shell eggs at room temperature found to negatively influence the egg quality by increasing the weight loss, yolk weight, yolk pH, albumen pH, yolk lipid peroxidation, TPC and by reducing the HU and albumen weight. The findings help to conclude that the egg should be kept at refrigeration temperature (4±1 °C) for up to 30 days and at room temperature (21-32 °C) for up to 14 days.

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