



Influence of storage conditions on quality and safety of eggs collected from Lebanese farms

Ghenwa Saleh^a, Nada El Darra^{a,*}, Samer Kharroubi^b, Mohammad T. Farran^b

^a Beirut Arab University, Faculty of Health Sciences, Tarik El Jedidah, Beirut, P.O.Box: 115020, Riad EL Solh, 1107 2809, Lebanon

^b Faculty of Agriculture and Food Sciences, American University of Beirut, Riad El-Solh, Beirut, 1107 2020, Lebanon

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ABSTRACT

Egg is considered a nutritionally complete food and an excellent source of protein. However, storing eggs for a prolonged period of time under uncontrolled temperature results in egg quality deterioration. The objective of this study is to determine the effect of storage conditions (time & temperature) on the egg's internal and external quality parameters as well as the microbiological load of eggs. For that purpose, a total of 2160 (white, brown vaccinated and brown non-vaccinated for salmonella) eggs were collected from Lebanese egg farmers in Bekaa valley and stored at 7 °C, 18 °C, 24 °C and 33 °C/20 °C (cyclic) for 2, 4 and 6 weeks. At each time point and temperature setting, 30 eggs were analysed for external and internal quality traits as well microbiological testing. Results showed that brown eggs had significantly higher weight ($P < 0.001$), shell thickness ($P < 0.01$), and darker yolk colour than white eggs. As the storage temperature and time increased, a decrease in Haugh unit (HU) and yolk colour was observed ($p < 0.05$). In addition, a decrease ($p < 0.001$) in the egg weight, specific gravity and shell thickness. For the eggs microbial analysis, a total absence was observed for eggs stored at 7 °C at all-time points. The results suggest that the interaction between temperature, time, and group significantly affect the eggs internal and external quality, by causing significant deterioration in HU, yolk colour, weigh, specific gravity, and shell thickness. This work has concluded that eggs should be stored at a refrigerated temperature (7 °C) for a period not exceeding 4 weeks.

1. Introduction

Eggs are inexpensive highly nutritious food that play a significant role in the diets of children, pregnant women and elderly, who are at risk of low-nutrient intakes (NATOLI, MARKOVIC, LIM, NOAKES, & KOSTNER, 2007). They are a convenient source of high quality protein, and are loaded with large variety of unsaturated fatty acids, vitamins, and minerals, required to build and maintain body tissues (Feddern et al., 2017).

Egg quality is defined as the food properties that affect the acceptance or rejection of the food by the consumer (Yang & Lee, 2019). It was described by Ogunwale and colleagues (2015) as these interior and exterior characteristics of eggs that make them acceptable to consumers (Ogunwale, 2015). The internal quality focuses on the albumen and the yolk quality; whereas, the external quality encompasses the egg cleanliness, egg weight, shell thickness and strength as well as specific gravity (A Şekeroğlu & Duman, 2014). It is to be noted that the egg shell prevents the leakage of yolk and albumen prior to cooking (Aygün,

2017). Egg quality traits are illustrated by Haugh unit, pH, egg weight, yolk colour, shell thickness, and specific gravity (Lee, Cho, Choi, & Sohn, 2016). Storing eggs for a prolonged period of time results in egg quality deterioration. Moreover, eggs' storage at high temperature causes further damage to the eggs' quality (Akter, Omar, & Sazili, 2005). Some packaging technique, such as vacuum packing, was shown to extend the egg shelf life around 42 days compared with eggs control at 5°C and 22 °C (Aygün & Sert, 2013).

Until recently, most studies investigated the hatching eggs quality; however, only few focused on bacterial contamination of table eggs (Kebede Senbeta, Zeleke, & Getachew Molla, 2015). Contamination of table eggs in the process of marketing is a major health concern, associated with foodborne illnesses and food poisoning. Bacterial contamination can occur via two routes. It is either prior to oviposition, due to an infection of the reproductive organs or during or after oviposition, due to a *trans-shell* penetration (Kebede Senbeta et al., 2015).

Reducing egg-borne infection cases requires on-farm intervention strategies and recommendations for the handling and eating of eggs and

* Corresponding author. Department of Nutrition & Dietetics Faculty of Health Sciences P.O. Box: 11 5020 Beirut, Lebanon.

E-mail addresses: n.aldarra@bau.edu.lb, healthsciences@bau.edu.lb (N. El Darra).

URL: <http://www.bau.edu.lb> (N. El Darra).

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egg products by the consumer. Temperature control is considered a major priority to prevent bacterial growth inside egg shells and minimizes the incidence of foodborne incidents (Okamura et al., 2008; Samli, Agma, & Senkoylu, 2005), since egg preservation depends mainly on the storage temperature and time management (Jones and Musgrove, 2005; Samli et al., 2005; Yuceer & Caner, 2014). The recommendations for consumers include avoiding raw, undercooked eggs or homemade foods containing raw eggs such as Caesar salad, mayonnaise, and ice cream (FDA, 2018).

For the quality of an edible egg to be preserved, it is recommended to be held between 0 °C and 4 °C immediately following its collection (Guedes et al., 2016). Previous studies reported that refrigerating eggs maintains its quality even after 20 days of storage. Table eggs are capable of maintaining higher quality if they are displayed for a shorter period under a more stable environment (Serrano et al., 2016).

With respect to egg storage temperature regulations, a difference was noted between European Union (EU) and United States (US). In the US, eggs should be washed and sprayed with chemicals sanitizers before selling to reduce the risk of infection. For this, the USDA (United States Department of Agriculture Food Composition) recommend the eggs to be refrigerated at all times to reduce the risk of bacterial contamination (USDA, 2016). However, in the EU, EFSA (European Food Safety Authority) have banned washing hen eggs, because the process is thought to destroy the cuticle and transfer harmful bacteria like salmonella from the outside to the inside of the egg. Though Europe takes a different approach; according to Regulation (EC) No 853/2004, 'eggs must be stored and transported until sale to the final consumer at a temperature, preferably constant' with no obligation of refrigeration (EFSA, 2014).

According to CODEX Alimentarius Commission Standards, fresh eggs are not permitted to be washed or cleaned and should be stored at a temperature $\geq +5$ °C and $< +20$ °C, and collected on daily basis (UNECE, 2010).

Regarding Lebanese market, it is still unclear whether they abide by the US or EU regulations; however, (LIBNOR) stated eggs should be maintained in market at a temperature not exceeding 25 °C. But eggs sold in Lebanese supermarkets are mostly displayed under room temperature conditions. It is common that eggs are not refrigerated to reduce cost, due to the fact that Lebanon suffers from a shortage in power supply, moreover, there is no food safety regulation demanding egg refrigeration in markets.

Therefore, the present study has been undertaken to evaluate the quality and the microbiological contamination of white and brown (vaccinated and non-vaccinated for salmonella) table eggs at different storage conditions and periods of time. To our knowledge, this is the first study in Lebanon to determine the effect of four different storage temperatures (7 °C, 18 °C, 24 °C, and cyclic 33/20 °C) at different time intervals (2, 4 and 6 weeks) on the internal and external quality as well as microbial safety of eggs. The main objective is to preserve egg quality and to reduce salmonella poisoning by optimizing time/temperature storage conditions.

2. Materials and methods

2.1. Egg collection and storage

A total of 2160 fresh eggs were collected from three Lebanese egg producers situated in Bekaa valley in the east of Lebanon. From the totality of the eggs, 720 were white eggs, 720 were brown originating from vaccinated hens for salmonella and 720 were brown from unvaccinated hens. The eggs were transported in cardboard trays containing 30 eggs each to the laboratories of Advancing Research Enabling Communities Centre (AREC) of the Faculty of Agricultural and Food Sciences (FAFS) at the American University of Beirut (AUB), Bekaa Valley.

Eggs were stored in chambers at four different temperatures: constant 7 °C, 18 °C, 24 °C and cyclic temperature 33 °C/20 °C (cyclic). The

storage conditions were selected to encompass all the conditions recommended worldwide. In the US, eggs are refrigerated at all times with a storage temperature not exceeding 7.2 °C (USDA, 2016). While Europe takes a different approach though; according to Regulation (EC) No 853/2004, eggs must be stored and transported with temperature not greater than 18 °C (EFSA, 2014). However, regarding Lebanese market, it is still unclear whether they abide by the US or EU regulation and most eggs are displayed in Lebanese supermarkets under room temperature conditions (24 °C). A cyclic temperature of 33/20 °C was also used to imitate the Lebanese day/night weather during summer.

90 eggs (30 white Eggs, 30 brown vaccinated and 30 unvaccinated brown eggs) from each of the four temperature conditions totalling 360 eggs were collected at week 2, 4 and 6 then analysed for the following variables (egg weight, egg shell thickness, specific gravity, yolk colour and Haugh unit) at AREC. 1080 eggs were used in 36 treatments (3 different egg types x 3 storage periods x 4 storage temperatures) with 30 eggs examined at each point. Similarly, another 360 eggs were transported at each time point, totalling 1080 eggs, to the food microbiology laboratories at the Lebanese Agricultural Research Institute (L.A.R.I.) in Fanar to conduct microbiological analysis.

2.2. External egg quality analysis

2.2.1. Egg weight

The eggs were numbered and individually weighed on digital weighing scale, then recorded.

2.2.2. Egg specific gravity

Specific gravity is an indirect indicator of external eggshell quality. It was determined by submerging the eggs in NaCl solutions of varying specific gravity to determine which salt solution concentration causes the eggs to float (Feddern et al., 2017). Five NaCl solutions were prepared ranging from 0.060 to 1.100 SG. Each egg was then immersed in the different salt solutions. The specific gravity of the solutions in which the egg floats, is the specific gravity of the egg (Bennett, 1993; Guedes et al., 2016).

2.2.3. Egg shell thickness(EST)

Eggs were broken and their contents (yolk and albumen) were separated from the shells. Egg shells were then cleaned and the egg shell thickness was measured using a Thickness Gauge (Japan).

2.3. Internal egg quality analysis

2.3.1. Haugh unit

The Haugh unit (HU), proposed by Haugh in 1937, is considered the most common indicator of albumen quality, thus determining the HU score is a gold standard to assess the egg's internal quality during storage (Serrano et al., 2016). The HU is expressed as a function of egg weight and albumen height of a broken egg (Figueiredo et al., 2013).

To determine the albumen quality, the egg is cracked open on a flat surface followed by measuring the albumen height using a Tripod micrometer (U.S.A) and a weighing balance. This height is then converted into HU by using the following formula (Haugh, 1937).

$$HU = 100 \log_{10} (H-1.7 W^{0.37} + 7.56)$$

Where H, albumen height in mm and W, egg weight in grams.

2.3.2. Yolk colour (YC)

Each egg was broken onto a flat surface and yolk colour was determined using a DSM Yolk Colour Fan (Switzerland), which was developed for judging egg yolk colour by Vuilleumier, 1969. The YC fan has 15 segments with colour intensity ranging from pale yellow with score 1 to deep orange with score 15.

Table 1

Effect of storage temperature and time on egg weight, shell thickness (ST), specific gravity (SG), egg haugh unit (HU) and yolk colour (YC), respectively for brown vaccinated (BV), brown non-vaccinated (BNV) and white non-vaccinated (WNV) eggs.

Temp	Time	group	N	HU	YC	Weight	Loss	ST	SG
7 °C	2wks	BV	30	61.37	12.43 ^a	63.23 ^a	2.39	36.70 ^a	1.080 ^b
		BNV	30	68.07	6.83 ^b	62.29 ^a	1.0	35.23 ^{ab}	1.085 ^a
		WNV	30	62.67	4.27 ^c	51.68 ^b	0.94	34.03 ^b	1.076 ^c
	4wks	BV	30	57.13	13.93 ^a	61.28 ^a	4.34 ^a	38.07 ^a	1.079 ^a
		BNV	30	60.7	7.93 ^b	64.06 ^a	-0.769 ^b	37.10 ^{ab}	1.081 ^a
		WNV	30	63.07	6.93 ^c	53.51 ^b	-0.89 ^b	36.10 ^b	1.078 ^b
	6wks	BV	30	54.53 ^b	12.53 ^a	61.63 ^a	3.996	31.90 ^a	1.060 ^c
		BNV	30	62.07 ^a	7.27 ^b	61.59 ^a	1.701	43.77 ^b	1.081 ^a
		WNV	30	58.07 ^{ab}	6.03 ^c	51.74 ^b	0.88	37.00 ^c	1.063 ^b
18 °C	2wks	BV	30	69.83 ^a	11.33 ^a	60.88 ^a	4.746 ^a	36.33 ^b	1.067 ^b
		BNV	30	49.63 ^b	6.20 ^b	62.86 ^a	0.431 ^b	38.83 ^a	1.071 ^a
		WNV	30	50.7 ^b	4.57 ^c	51.54 ^b	1.08 ^a	35.13 ^b	1.026 ^c
	4wks	BV	30	25.8 ^b	13.07 ^a	61.37 ^a	4.256	35.83 ^b	1.060
		BNV	30	42.1 ^a	7.97 ^b	60.36 ^a	2.931	38.60 ^a	1.061
		WNV	30	28.6 ^b	7.20 ^c	49.64 ^b	2.98	34.07 ^b	1.060
	6wks	BV	30	16.67 ^a	12.37 ^a	61.69 ^a	3.936	30.93 ^b	1.060
		BNV	30	17.83 ^a	7.33 ^b	61.72 ^a	1.571	34.13 ^a	1.060
		WNV	30	9.7 ^b	5.40 ^c	52.37 ^b	0.25	29.17 ^b	1.060
24 °C	2wks	BV	30	10.63 ^a	12.57 ^a	61.59 ^a	4.036	36.90 ^{ab}	1.077 ^a
		BNV	30	21.4 ^b	6.70 ^b	61.58 ^a	1.711	37.33 ^a	1.070 ^b
		WNV	30	56.93 ^c	5.50 ^c	52.36 ^b	0.26	35.13 ^b	1.060 ^c
	4wks	BV	30	0.00	13.20 ^a	59.38 ^a	6.246	36.80 ^{ab}	1.060
		BNV	30	0.23	7.37 ^b	59.77 ^a	3.521	37.63 ^a	1.060
		WNV	30	3.57	6.60 ^c	49.51 ^b	3.11	35.57 ^b	1.060
	6wks	BV	30	2.87	12.20 ^a	60.22 ^a	5.406	31.50 ^a	1.060
		BNV	30	2.8	7.27 ^b	58.66 ^a	4.631	32.60 ^a	1.060
		WNV	30	0.00	6.67 ^b	49.75 ^b	2.87	29.27 ^b	1.060
33/20 °C	2wks	BV	30	2.4	12.73 ^a	60.52 ^a	5.106	36.93 ^a	1.061 ^b
		BNV	30	2.37	7.27 ^b	58.57 ^a	4.721	38.00 ^a	1.064 ^a
		WNV	30	7.33	4.53 ^c	47.73 ^b	4.89	32.63 ^b	1.061 ^{ab}
	4wks	BV	30	0.00	12.53 ^a	54.56 ^a	8.731 ^a	36.37 ^b	1.060
		BNV	30	0.00	7.17 ^b	57.06 ^a	6.231 ^b	38.20 ^a	1.060
		WNV	30	0.00	4.90 ^c	45.75 ^b	6.87 ^b	34.93 ^b	1.060
	6wks	BV	30	0.00	12.63 ^a	50.34 ^a	15.286 ^a	31.10	1.060
		BNV	30	0.00	8.00 ^b	50.47 ^a	12.821 ^a	31.20	1.060
		WNV	30	0.00	6.07 ^c	45.65 ^b	6.97 ^b	30.83	1.060
SEM		2.074	0.208	0.853	1.158	0.541	0.001		
Source of variation									
Temperature		<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Egg Group		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Temperature * Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Temperature * Group		<0.001	<0.001	NS	NS	<0.001	<0.001	<0.001	
Time * Group		<0.001	<0.001	0.018	NS	<0.001	<0.001	<0.001	
Temperature * Time * Group		<0.001	0.008	0.003	NS	<0.001	<0.001	<0.001	

a-c Different letters indicate significant difference among means between groups in each column. NS: Not significant.

2.4. Microbial analysis of the egg shell

Bacterial analysis was conducted on 1080 eggs. At each time point, eggs were cracked open then shells and egg internal contents were separated. Three pooled samples were formed for each treatment by combining crushed egg shells or egg contents from 10 eggs into a sterile sample bag and the egg content were blended in a stomacher (Interscience, France) for 1 min at normal speed. 25 ml of each sample were inoculated with 225 ml of buffered peptone water (1 in 10 dilution) for further processing, then the inoculum was incubated for 30 min at room temperature.

2.4.1. Aerobic plate count

The Aerobic Plate Count (APC) is used to measure organisms that grow aerobically at mesophilic temperatures (25–40 °C). APC do not directly correlate with pathogens existence but used to measure gauge sanitary quality, and the adherence to good manufacturing practices (Fsis, 2012).

To determine aerobic plate count (APC), the pour plate technique was used according to ISO4833-1:2013. 1 ml of each pooled sample was serially diluted by ten-fold with 9 ml sterile saline-peptone solution. 1 ml of each dilution was transferred into an empty Petri dish (plastilab, Lebanon), where 15 ml of plate count Agar (PCA)

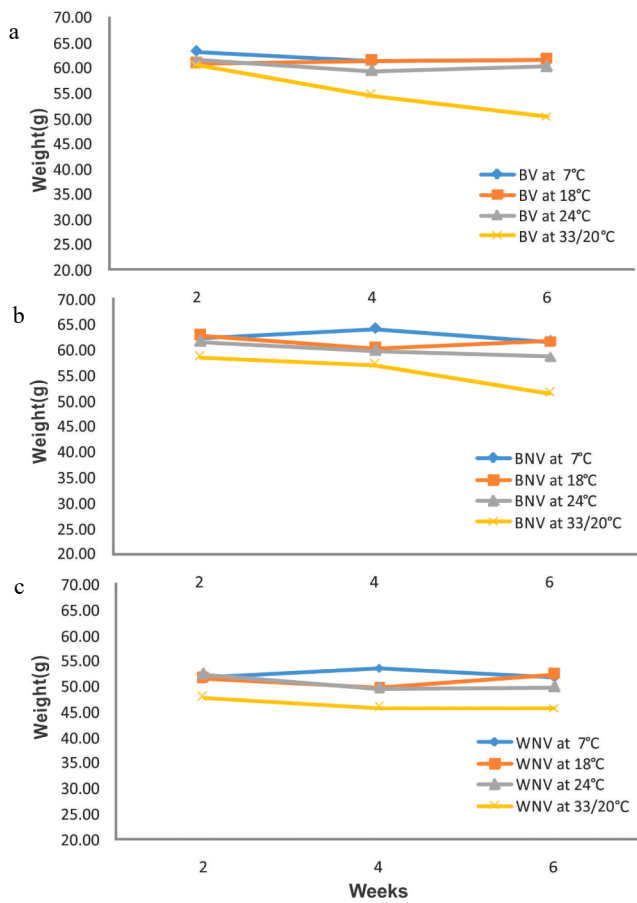


Fig. 1. Effect of storage temperature ($^{\circ}\text{C}$) and storage time (weeks) on the weight of (a) brown vaccinated (BV) eggs, $n = 30$; (b) brown non-vaccinated (BNV) eggs and (c) white non-vaccinated (WNV) eggs; $n = 30$.

(SCHARLAU, Spain) were poured over and mixed well with the inoculum by gentle rotations to set. When the medium is solidified, extra layer 4 ml were added, to avoid colony invasion. The Petri dishes were then inverted and incubated at 30°C for 72 h. PCA plates showing colonies between 15 and 300 were selected and counted.

2.4.2. *Staphylococcus aureus*

The technique used to determine *Staphylococcus aureus*, is according to ISO6888-1:1999. 1 ml of the inoculum was added on the surface of 3 small agar plates (Baird Parker Agar; SCHARLAU, Spain). The inoculum was quickly spread, and allowed to dry for 5 min. The prepared dishes were inverted and then stored in an incubator for 48 h at 37°C . If typical colonies appeared, black or grey colonies surrounded by a clear zone, the sample is suspected. For confirmation, 1 colony was transferred to brain heart infusion broth and incubated at 37°C for 24 h. 0.5 ml of the culture was added to 0.5 ml Rabbit plasma tubes then incubated at 37°C . Afterwards, the clotting of the plasma was examined after 4–6 h. If clotting occurred, this means the results are positive.

2.4.3. *Enterobacteriaceae*

Referring to ISO 21528-2:2004, 1 ml of inoculum was transferred to an empty Petri-dish, then poured 15 ml of the Violet Red Bile Glucose agar (SCHARLAU, Spain). After solidification, 15 ml of the same medium were added to prevent spreading growth and to achieve anaerobic conditions. After solidification, plates were inverted and incubated at 37°C for 24 h. Typical colonies, pink to red or purple with or without precipitation haloes, were selected and streak onto a glucose agar, then incubate at 37°C for 24 h. Glucose agar tubes must turn from purple to yellow if positive.

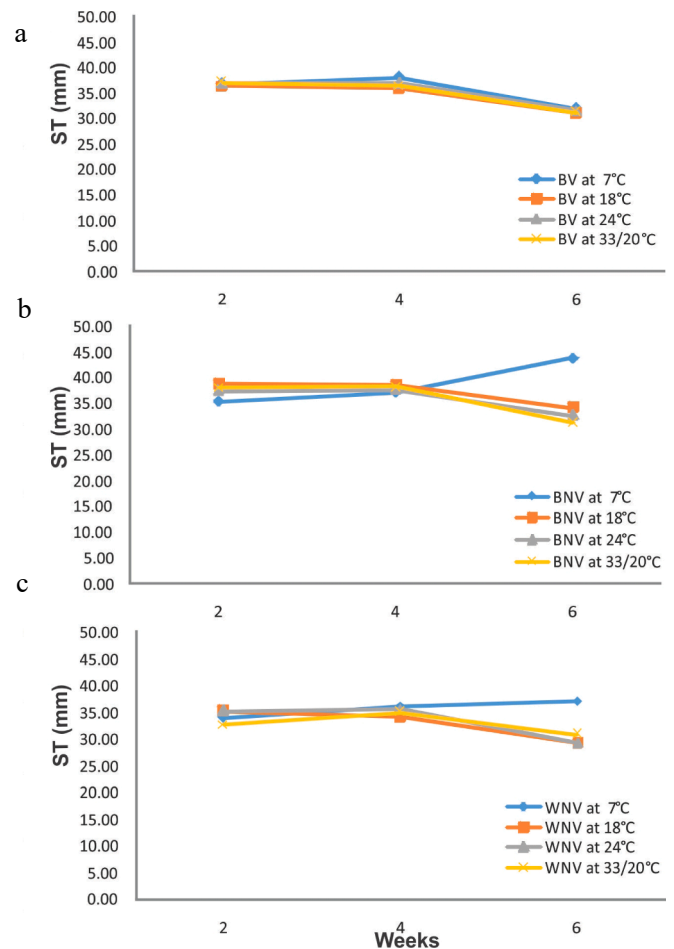


Fig. 2. Effect of storage temperature ($^{\circ}\text{C}$) and storage time (weeks) on the shell thickness of (a) brown vaccinated (BV) eggs, $n = 30$; (b) brown non-vaccinated (BNV) eggs and (c) white non-vaccinated (WNV) eggs; $n = 30$.

2.4.4. *Salmonella*

Referring to ISO6579:202, Inoculum was incubated at 37°C for 18 h. Two Broth were prepared; 0.1 ml of inoculum added to 10 ml of Rappaport-Vassiliadis Soy Broth (SCHARLAU, Spain) and incubated at 41.5°C for 24 h. 1 ml of inoculum added to 10 ml of Muller-Kauffmann Tetrathionate-Novobiocin Broth (SCHARLAU, Spain) at 37°C for 24 h. Each broth over Xylose Lysine Deoxycholate agar (XLD) and Salmonella Shigella agar (SS) plates (SCHARLAU, Spain) were isolated, using 10 μl capacity loop and incubated at 37°C for 24 h. Loop strike colonies with black centre surrounded with clear zone are cultured on Tryptone Soya Agar (SCHARLAU, Spain) and incubated for 37°C for 24 h. Bacteria pure without colour undergo a Serological and biochemical confirmation.

2.5. Statistical analysis

Data were checked and entered into the statistical package for social sciences (SPSS 24 for Windows) for data analysis. Descriptive statistics were presented to summarize the study variables of interest as counts and percentages for the categorical variables and as means and standard deviations for the continuous ones. The chi-square (χ^2) test or Fisher's exact test were used to calculate the association between two categorical variables. Independent t-tests and Mann-Whitney tests were used to chart comparisons for normal and non-normal continuous variables. Normality of the variables were evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. If both tests are statistically significant, indicating that the variables of interest

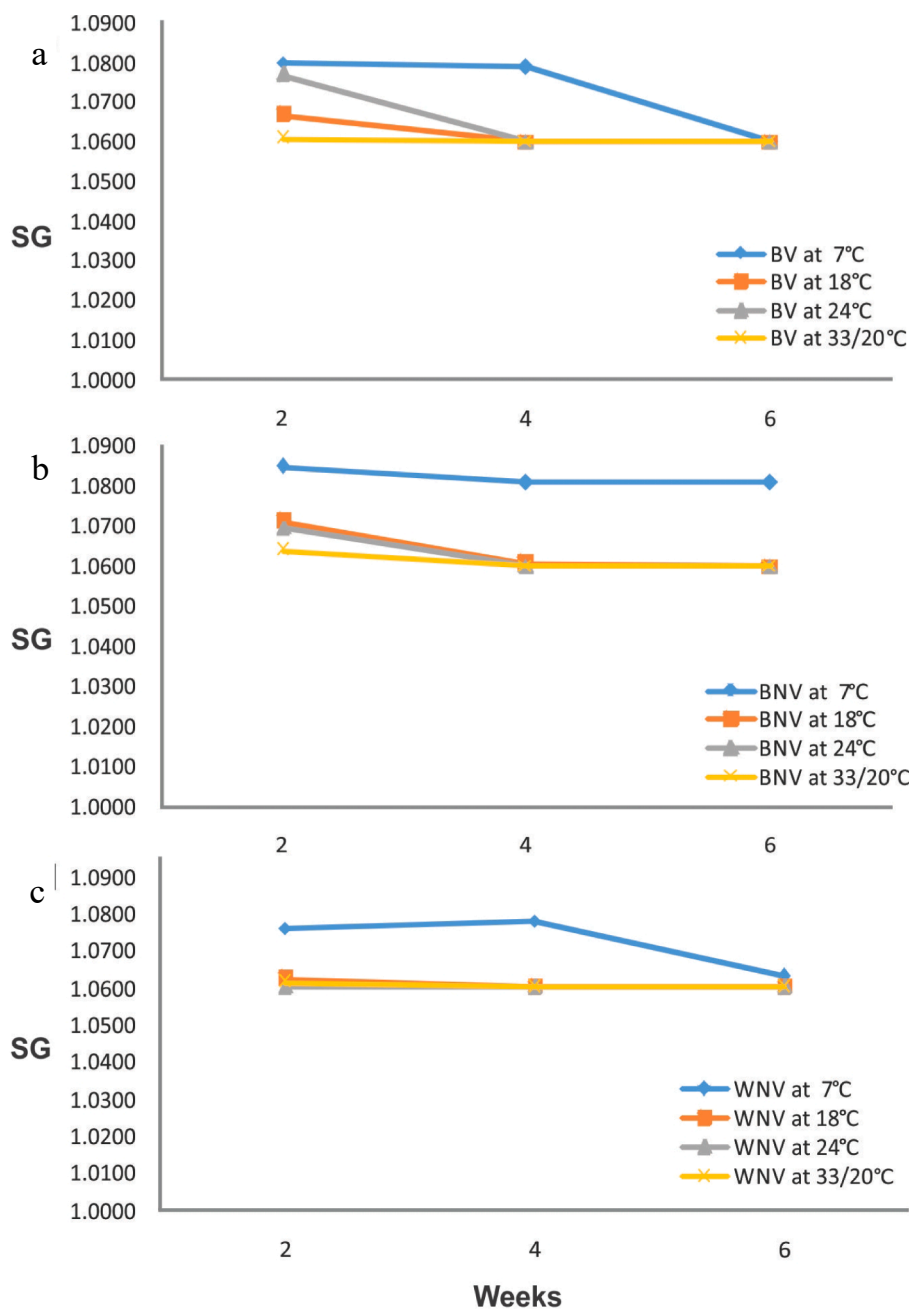


Fig. 3. Effect of storage temperature ($^{\circ}\text{C}$) and storage time (weeks) on the specific gravity of (a) brown vaccinated (BV) eggs, $n = 30$; (b) brown non-vaccinated (BNV) eggs and (c) white non-vaccinated (WNV) eggs; $n = 30$.

follow a normal distribution. A three-way ANOVA (Analysis of Variance) was performed to assess the effects of the three variables (storage temperature, storage time and group of eggs) and their interactions on the internal quality (haugh unit, yolk colour) and external quality (weight, shell thickness, specific gravity) of eggs. This was followed by testing for statistically significant difference between variables using Tukey's HSD post-hoc test. All reported p -values were based on two-sided tests and were compared with a significance level of 5%.

3. Results and discussion

3.1. Effect of storage temperature and time on external egg quality parameters

The results of the effects of storage temperature and time on the

external quality (weight, shell thickness and specific gravity) of brown vaccinated (BV), brown non-vaccinated (BNV) and white non-vaccinated (WNV) eggs are presented in Table 1. The analysis revealed that each of the variables (temperature, time and group) had a significant influence on all external quality parameters ($p < 0.01$). A significant three-way interaction ($p < 0.01$) between temperature, time and group was also shown in Table 1. The results revealed also that egg weight was significantly higher ($p < 0.001$) in brown eggs (BV and BNV) when compared to white eggs (WNV). This finding is in line with the study of (Soria, Bueno, & Bernigaud, 2013), who showed that among 5424 eggs (3475 white and 1949 brown) collected from 113 supermarkets in Entre Rios, Argentina; brown eggs presented greater egg weight than white eggs (Soria et al., 2013). This was attributed to the fact that brown eggs have more shell and albumen than white ones (Scott & Silversidest, 2000).

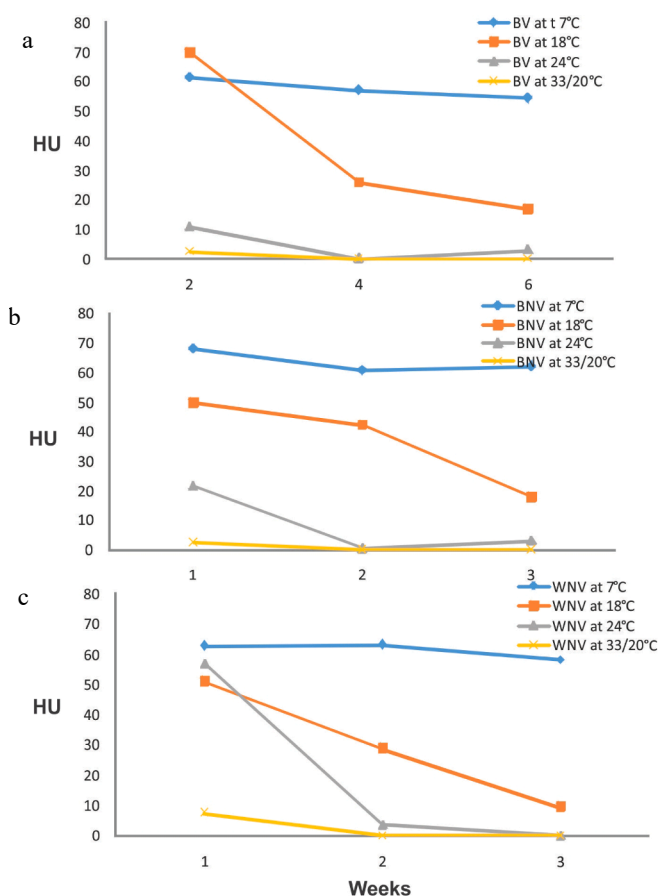


Fig. 4. Effect of storage temperature (°C) and storage time (weeks) on the Haugh unit in (a) brown vaccinated (BV) eggs, $n = 30$; (b) brown non-vaccinated (BNV) egg sand (c) white non-vaccinated (WNV) eggs; $n = 30$.

Fig. 1 presents the effect of storage temperature (°C) and storage time (weeks) on the weight of BV, BNV and WNV eggs. For all egg groups, eggs stored at 7 °C, 18 °C and 24 °C showed no significant difference in weight when stored for 6 weeks (Fig. 1). However, when storage temperature was increased to 33/20 °C, the egg weight significantly (p -value??) decreased after from 63.2 g to 60.5 g, 62.3 g–58.6 g and 51.7 g–47.7 g for BV, BNV and WNV, respectively, at 2 weeks of storage. Similar results were obtained by Jin and Lee (2011), which showed that increasing storage temperature to 29 °C led to drastic increase in egg weight loss from 1.74% to 3.67% at 5 and 10 days of storage time, respectively. Sert et al. (2011) has shown that storage time affects the egg weight; since eggs stored for 10 days, regardless of the storage temperatures, present lower weight compared with fresh eggs (Sert, Aygun, & Demir, 2011). Another study by Sert, Aygun, Torlak, and Mercan (2013) has shown an increase in weigh loss of eggs between 0.42 and 4.64% with storage time, passing from day 1 till day 14. The reduction in weight as temperature increases to 33/20 °C could be due to fact that at such a high temperature, the cuticle plugging the shell pores will dry faster and thus begin to shrink allowing moisture, ammonia, carbon dioxide and nitrogen to escape from the egg content through the shell pores by evaporation (Addo, Hamidu, Ansah, & Adomako, 2018; Akter, Sazili, & Omar, 2014; Eke, Olaitan, & Ochefu, 2013).

Fig. 2 presents the effect of storage temperature and storage time on the shell thickness of BV, BNV, and WNV. As shown in Fig. 2, the shell thickness (ST) was significantly ($p < 0.01$) higher in brown eggs (BV; 36.70 mm and BNV; 35.23 mm) compared to white eggs (WNV; 34.03 mm) as shown in Fig. 2. These results are in accordance with the study by Soria et al (2013) that demonstrated brown eggs to have

greater shell thickness than white eggs (Sert et al., 2011).

With the exception of storage of eggs at 7 °C, all other temperatures caused a decrease in the shell thickness after 4 weeks of storage. This was in agreement with Alsobayel and Albady (2011) who reported that prolonged storage period had an adverse effect on shell thickness (Alsobayel & Albady, 2011).

The effect of storage temperature and storage time on the specific gravity of BV, BNV, and WNV is presented in Fig. 3. While assessing the specific gravity (SG), eggs stored at 7 °C presented a SG that decrease gradually after 4 weeks of storage with the exception of BNV where SG decreased significantly ($p < 0.01$) after only 2 weeks of storage. However, the SG declined sharply when eggs were kept at 18 °C, 24 °C and 33/20 °C. The greatest decline in SG was seen when eggs were stored at 33/20 °C regardless of time by 1.97%. Eggs stored at 7 °C showed gradual decline in SG after 4 weeks of storage. It is worth mentioning that SG was almost 1.06 when stored at 33/20 °C at all-time points. This was in accordance with a study conducted by Samli et al. (2005), on eggs ($n = 35$) stored for 2, 5, and 10 days at 5 °C, 21 °C, and 29 °C, that shows a significant decline in specific gravity from 1.08 to 1.063, as time and temperature increased (Samli et al., 2005). This decline in SG could be due to the increase in the size of the air cell as storage time and temperature increases (Akter et al., 2014).

3.2. Effect of eggs' storage temperature and time on internal egg quality parameters

The effect of storage temperature and time on internal egg quality parameters such as haugh unit (HU) and Yolk colour (YC) in BV, BNV and WNV eggs is presented in Table 1. Storage time, temperature and egg group (BNV, BV, WNV) significantly affected the eggs' HU and YC ($p < 0.001$). Significant three-way interaction among storage time, temperature and egg group were also observed for both HU ($p < 0.001$) and YC ($p < 0.01$).

The effect of storage temperature and storage time on the HU of BV, BNV, and WNV is presented in Fig. 4. With the exception of storage at 7 °C, there was a drastic reduction in HU with increasing storage temperature and time for all egg groups. At 18 °C, HU was 69.8 for BV, 49.6 for BNV and 50.7 for WNV for 2 weeks of storage; however, HU declined rapidly ($p < 0.05$) to 25.8, 42.1 and 28.6 for BV, BNV and WNV, respectively at week 4. Regardless of the egg group, even after only 2 weeks of storage, there was considerable deterioration in HU, which reached almost zero after 4 weeks of storage at storage temperatures of 24 °C. Our results are in agreement with Jin, Zhang, Boyd, and Tang (2007) who reported that eggs stored at 21 °C and 29 °C demonstrated a dramatic deterioration in HU; however, no decline was observed at storage temperature of 5 °C up to 10 days of storage (Jin et al., 2007). Storage time and temperature are among the crucial factors affecting the haugh units. It was hown that HU decrease during storage (Sert et al., 2013). This decline in HU could be attributed to the loss of carbon dioxide from the egg albumin leads to its watery structure and thus to deterioration in haugh unit as the HU is calculated from the albumen height and weight of egg (Eke et al., 2013).

It is worth mentioning that when eggs were kept at 33/20 °C, the HU was drastically decreased regardless of storage time and egg group (Fig. 4).

The effect of storage temperature and storage time on the YC of BV, BNV, and WNV is presented in Fig. 5. While assessing the YC, there was significant changes ($p < 0.001$) between the three groups. Regardless of storage time and temperature, the brown eggs showed darker yolk colour that white eggs. BV yolk colour reached the score of 13.9 (dark orange), followed by BNV with a score of 8 and WNV with a score of 7.2 (pale yellow). This result was consistent with a study by Soria et al (2014) which demonstrated a YC range of 2–11 for white eggs and 2–14 for brown eggs (Sert et al., 2011). Our results also showed a significant ($p < 0.01$) increase in YC from 2nd to 4th week of storage, followed by a decline at 6 weeks. This was in agreement with other studies showing

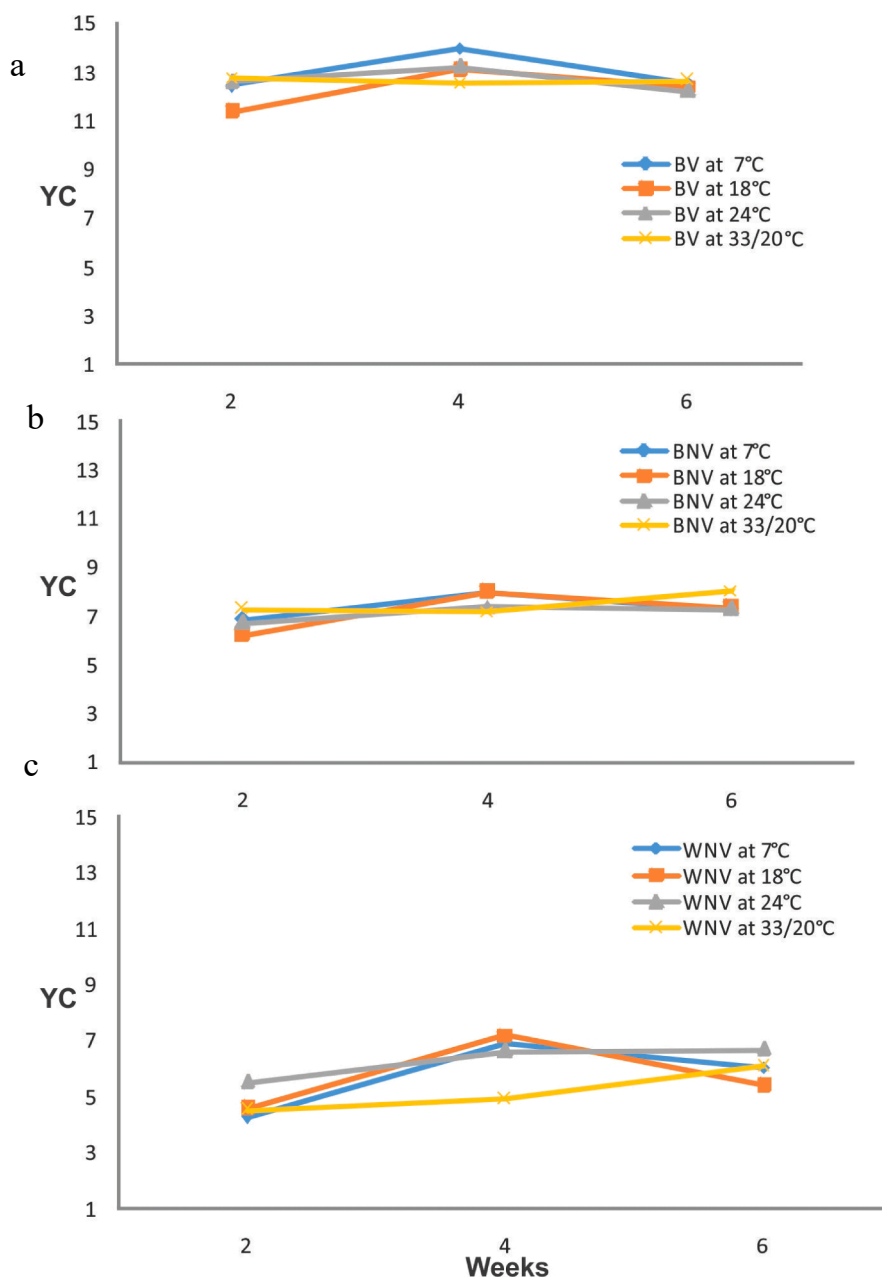


Fig. 5. Effect of storage temperature ($^{\circ}\text{C}$) and storage time (weeks) on the yolk colour of (a) brown vaccinated (BV) eggs, $n = 30$; (b) brown non-vaccinated (BNV) eggs and (c) white non-vaccinated (WNV) eggs; $n = 30$.

that with increased storage time, yolk colour gets darker up to 3 weeks of storage et al., 2016). The increase in storage temperature and time (more than 4 weeks) is associated with the collapse of the albumen's protein structure and the vitelline membrane that surround that albumen from the yolk. This will cause the albumen ingredients (protein and water) to enter the yolk, dilute its pigment and thus reduce the yolk colour (Jin & Lee, 2011).

3.3. Microbiological properties of eggs

All collected eggs (1080) showed 100% absence of salmonella, when stored at 7°C , 18°C , 24°C , and $33/20^{\circ}\text{C}$ for 7, 14, and 21 weeks. Moreover, the presence of *Enterobacteriaceae* was not confirmed when stored at 7°C at all time points. However, *Enterobacteriaceae* was found in pooled samples stored at 18°C and 24°C for the WNV and RV, in both the yolk and the shell, at 2 and 4 weeks respectively. The results

are similar to those of Pereira et al. who observed total absence of salmonella in eggs (Pereira, Santos, & Coelho, 2014). Oliveira and Taham (2012) study on eggs sold in Brasília, also showed no microorganisms in 67% of the samples (Oliveira & Taham, 2012).

The negligible amount of microorganism in the eggs content and shell, might be due to the fact that the farms, where the eggs were collected implement good farming practices and hygienic measures or due to the exposure of poultry to antibiotics. Furthermore, the absence of bacteria could be due to the high pH of the egg white, that limits the bacterial growth. In addition, the shell matrix present proteins with antimicrobial properties controlling the invasion of microorganisms (Aygün, 2017). The proteins in egg albumen including lysozyme and ovotransferrin may conduct an antimicrobial activity against the egg associated microorganisms (Abdel-Shafi, Osman, Enan, El-Nemer, & Sitohy, 2016).

4. Conclusion

This study assessing the effect of storage temperature and time on quality and safety of eggs is of great importance, as it informs retailers about best storage conditions that can offer eggs free from microorganisms with high internal and external quality characteristics. It is the first to be done in Lebanon about the effect of temperature, time and group interaction on commercial eggs' quality and safety. The findings showed that storage period and temperature and the interaction between the two significantly affected all of the internal (HU, YC) and external egg quality parameters (Weight, ST, SG). Moreover, results showed statistically significant quality differences in shell thickness, weight and yolk colour between brown and white eggs. This work has concluded that eggs should be stored at a refrigerated temperature (7 °C) for a period not exceeding 4 weeks. The results could generate a substantial recommendation for LIBNOR, the Lebanese Standards Institution, which still has no clear regulation about the optimum storage conditions for eggs.

Author contribution

Ghenwa Saleh is the graduate student who conducted the experimental work. She participated in the data acquisition, data analysis and writing the manuscript. **Nada El Darra** is the corresponding author and the main supervisor of the student. She participated in the conceptualization of the idea, the design of the methodology, data interpretation, the writing of the original manuscript draft, and the final review of the manuscript. **Samer Kharroubi** participated in the data acquisition, data analysis, data interpretation, manuscript drafting, critical revision, and the final review of the manuscript. **Mohammad T. Farran** is the co-supervisor of the student. He provided the laboratory facility and participated in the design of the methodology, data interpretation, manuscript drafting and the final review of the manuscript.

Declaration of competing interest

The authors declare that they have no relevant or material financial interests that relate to the research described in this paper. Authors have no conflicts of interest.

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